Jerzy Bourski

Program
Abstracts
List of participants

Institute of Bioorganic Chemistry
Polish Academy of Sciences

INTERNATIONAL CONFERENCE

NUCLEIC ACIDS AND THEIR CONSTITUENTS: Chemical Evolution Underlying Biological Evolution

Dedicated to Professor Maciej Wiewiórowski on the occasion of his 80th birthday

Poznań, May 10-13, 1998

Program
Abstracts
List of participants

Scientific Publishers OWN
Poznań 1998

International Conference
Nucleic Acids and their Constituents:
Chemical Evolution Underlying Biological Evolution

Poznań 10-13 May, 1998
Science Center, Polish Academy of Sciences
Wieniawskiego 17/19, 61-713 Poznań, Poland

ISBN 83-87671-25-8

Published by: Scientific Publishers OWN
Polish Academy of Sciences
Wieniawskiego 17/19, 61-713 Poznań
tel.: +4861 8528503 fax: +4861 8520671
e-mail: own@man.poznan.pl

Technical Editor: Andrzej Wójtowicz

Printed in Poland (TotalDruk, Poznań)

CONFERENCE ORGANIZERS

Institute of Bioorganic Chemistry
Polish Academy of Sciences

Polish Chemical Society

Committee of Chemical Sciences
Polish Academy of Sciences

Faculty of Chemistry
Adam Mickiewicz University

SCIENTIFIC COMMITTEE

Jerzy Boryski
Henryk Koroniak
Tadeusz M. Krygowski
Andrzej B. Legocki (chairman)
Bogdan Marciniec
Elżbieta Wyrzykiewicz

ORGANIZING COMMITTEE

Andrzej B. Legocki (chairman)

Elżbieta Adamczyk
Jerzy Boryski
Małgorzata Dąbkiewicz
Katarzyna Kulińska
Urszula Muszyńska
Joanna Stępkowska
Józef Żarnowski

Conference sponsored by
the State Committee for Scientific Research
SIGMA-ALDRICH

Program

SUNDAY, MAY 10

17.00 **Opening**
Andrzej B. Legocki

17.15 **Keynote lecture**

Nelson J. Leonard, Pasadena
Solutions to two structural puzzles via pseudo-DNA:
1) Modified Hoogsteen base pairing;
2) Terminal A-T bonding or frying

18.00 **Get together party**

MONDAY, MAY 11

Nucleotides and DNA synthesis – Part I
Chair: Jyoti Chattopadhyaya

8.30-9.00 **Jacek Stawiński,** Stockholm
H-Phosphonate chemistry in the synthesis of nucleotides and their analogues

9.00-9.30 **Jan Michalski**, Łódź
Unconventional chemistry and stereochemistry of phosphorus compounds directed towards biophosphates and their structural analogues

9.30-10.00 **Yoshiharu Ishido,** Tokyo
Oligonucleotide synthesis using 2-(levulinyloxymethyl)-5-nitrobenzoyl (LMNBz) group for the 5'-hydroxyl groups of nucleosides 3'-phosphoramidite derivatives

10.00-10.30 **Adam Kraszewski,** Poznań
Nucleoside aryl-H-phosphonates – synthesis and applications in nucleotide and oligonucleotide chemistry

10.30-11.00 Coffee

11.00-11.30 **Wolfgang Pfleiderer,** Konstanz
Synthesis and biological activities of 2'-5'-oligoadenylates – a new group of antivirally active compounds

11.30-12.00 **Paul Agris,** Raleigh
Nucleoside structure/function relationships and the design of new nucleic acids

12.00-12.30 **Wojciech Markiewicz,** Poznań
Synthetic oligonucleotide combinatorial libraries and their applications

12.30-13.00 **Stanisław Penczek,** Łódź
High-molecular-weight macromolecules with phosphodiester bonds – by one step polymerization

13.00-14.00 Lunch

Chemical Evolution and its Implications
Chair: Volker A. Erdmann

14.00-14.30 **Jens Nyborg,** Aarhus
Possible evolution of factors involved in protein biosynthesis

14.30-15.00 **Dieter Söll**, New Haven
Archaeal aminoacyl-tRNA synthesis: unique determinants of a universal genetic code?

15.00-15.30 **Włodzimierz Zagórski**, Warsaw
Potato spindle tuber viroid population studies

15.30-16.00 **Andrzej B. Legocki**, Poznań
Evolution of plant-microbe interactions

16.00-16.30 Tea

16.30-18.30 **Round Table Discussion:** ***Ethic aspects of modern science***
Moderator: Jacek Kozioł, Poznań

20.00 Concert and Conference Reception

TUESDAY, MAY 12

RNA and Protein Synthesis
Chair: Jacek Augustyniak

8.30-9.00 **Volker A. Erdmann**, Berlin
The Berlin Network for RNA-technologies

9.00-9.30 **Uttam RajBhandary**, Cambridge
tRNA - protein interactions in initiation of protein synthesis

9.30-10.00 **Mathias Sprinzl**, Bayreuth
Aminoacylated RNA a link between RNA and protein world

10.00-10.30 **Brigitte Wittmann-Liebold,** Berlin
Fine structure analysis of the RNA-protein interaction in the ribosome

10.30-11.00 Coffee

11.00-11.30 **Douglas Turner**, Rochester
Molecular recognition in RNA

11.30-12.00 **Michael W. Holmes,** Richmond
Conformational changes in tRNA (quanosine m-1) methyltransferase and tRNA accompany RNA substrate binding and recognition

12.00-12.30 **Jan Barciszewski**, Poznań
Plant aminoacyl-tRNA synthetases

12.30-13.00 **Tomasz Twardowski,** Poznań
Biosynthesis of lupin proteins: ferritine and LDC; regulatory mechanisms

13.00-14.00 Lunch

Nucleotides and DNA Synthesis – Part II
Chair: Paul Agris

14.00-14.30 **Shigenori Iwai,** Osaka
Synthesis of oligonucleotides containing the (6-4) photoproduct and its application to the study of UV-induced mutations

14.30-15.00 **Bożenna Golankiewicz,** Poznań
Modified nucleosides for human health

15.00-15.30 **Jerzy Boryski**, Poznań
Novel application of transglycosylation reactions in nucleoside synthesis

15.30-16.00 **Zbigniew Leśnikowski**, Łódź
Carboranylmethylphosphonate (CBMP) oligonucleotides

16.00-16.30 Tea

16.30-17.00 **Jyoti Chattopadhyaya**, Uppsala
Why has nature chosen pentofuranose not hexopyranose in the construction of DNA and RNA?

17.00-17.30 **Barbara Ramsay-Shaw,** Durham
Boranophosphates: a new DNA backbone

17.30-18.00 **Lech Łomozik**, Poznań
Interactions in the systems with nucleosides and nucleotides including metal ions and polyamines

18.00-18.30 **Tadeusz M. Krygowski**, Warsaw
Aromaticity – what does it mean?

18.30-20.00 **Poster Session**

WEDNESDAY, MAY 13

Biological Aspects of Nucleic Acids Structure and Function
Chair: Wolfram Saenger

8.30-9.00 **David Shugar,** Warsaw
Viral and host-cell protein kinases as potential antiviral targets

9.00-9.30 **Susumu Nishimura**, Tsukuba
8-Hydroxyguanine in DNA formed by oxygen radicals: its repair and implication in mutation/carcinogenesis

9.30-10.00 **Włodzimierz J. Krzyżosiak**, Poznań
RNA structure of trinucleotide repeats

10.00-10.30 **Jerzy Ciesiołka**, Poznań
Pathways for RNA folding

10.30-11.00 Coffee

11.00-11.30 **Antoni Rafalski**, Wilmington
Electronic Northerns: Gene expression information from high throughput EST Sequencing

11.30-12.00 **Upendra Pandit**, Amsterdam
Inspirations derived from the thymidylate synthase reaction

12.00-12.30 **Marek Figlerowicz**, Poznań
Genetic recombination in (+)RNA viruses

12.30-13.00 **Krzysztof Szyfter,** Poznań
Tobacco smoke-induced DNA damage in human tissues

13.00-14.00 Lunch

Conformation of nucleic acids and proteins
Chair: Jerzy Pawełkiewicz

14.00-14.30 **Wolfram Saenger**, Berlin
Tetrocycline-repressor acts as a molecular switch regulated by tetracycline binding

14.30-15.00 **Kazimierz L. Wierzchowski**, Warsaw
Effects of promoter DNA bending on transcription initiation by E. coli RNA polymerase

15.00-15.30 **Ryszard W. Adamiak,** Poznań
The RNA hydration. Two examples

15.30-16.00 **Hanna Sierzputowska-Gracz**, Raleigh
Structure of IRE studied by cobalt(III) hexamine binding, molecular modeling and NMR spectroscopy

16.00-16.30 Tea

16.30-17.00 **Thomas Haertlé**, Nantes
Engineering of novel proteolytic specificities of trypsin

17.00-17.30 **Kazimierz Grześkowiak**, Los Angeles
Sequence dependent structural and biochemical properties of DNA

17.30-18.00 **Andrzej Joachimiak,** Argonne
Rapid protein crystal structure determination using 3rd generation synchrotron source

18.00-18.30 **Mariusz Jaskólski,** Poznań
Molecular recognition motifs in nucleoside salts

18.30-19.10

Special session on alkaloids:
Towards the roots of nucleic acids chemistry in Poznań
Chair: Krzysztof Gulewicz

Włodzimierz Boczoń, Poznań
Bis-quinolizidine alkaloids – new derivatives
Waleria Wysocka, Poznań
Lupin alkaloids at present

19.10-20.00 **Closing Session**
Chair: Andrzej B. Legocki

Antoni Rafalski, Wilmington
Summary of the Conference

Conference is dedicated to
Professor Maciej Wiewiórowski on his 80th birthday
by his friends, co-workers and pupils

SOLUTIONS TO TWO STRUCTURAL PUZZLES VIA PSEUDO-DNA: 1) MODIFIED HOOGSTEEN BASE PAIRING; 2) TERMINAL A-T BONDING OR FRYING

Nelson J. Leonard

Division of Chemistry and Chemical Engineering, The California Institute of Technology, M/C 164-30, Pasadena, CA 91125, USA

The timing is right for another international conference in Poznań on nucleic acids, this one dedicated to Professor Maciej Wiewiórowski. The program, which includes writers of definitive articles and texts on all aspects of DNA and RNA, is indicative of the richness and variety of current research on evolution, synthesis, structure, function, and medical applications. Structures of aminoacyl tRNA synthetases have been determined by X-ray, some with bound tRNA. New structural RNA motifs have been observed by X-ray and NMR, and new affinities and functions are being sought in an „RNA world". Triple helices are coming of age with the development of polyamides, enhanced by appropriate spacers and linkers, that show sequence specificity in DNA binding, permeate cells in culture, travel to the nucleus, and block the expression of a targeted gene. New classes of drugs can be envisaged that will block the expression of genes whose products are responsible for the development of disease.

Within this framework, my research at the Unviersity of Illinois has dealt with the solution - or partial solution - together with Balkrishen Bhat, Andrew H.-J. Wang and Howard Robinson, of two modest problems by means of pseudo-DNA. The first question posed was how the structural isomer of deoxyadenosine, namely 2'-deoxy-3-isoadenosine, hydrogen bonds when embedded in a double-helical DNA. The answer is that the isomer forms modified Hoogsteen-type base pairs in a $d(CG[IA]TCG)_2$ duplex. The second question addressed relates to the degree of stabilization of hydrogen bonding in a terminal dA-dT base pair, i.e. fraying vs. bonding, held proximal by an adjacent, covalently-linked dimensional analog of dA-dT.

H-PHOSPHONATE CHEMISTRY IN THE SYNTHESIS OF NUCLEOTIDES AND THEIR ANALOGUES

Jacek Stawiński

Department of Organic Chemistry, Arrhenius Laboratory, Stockholm University, S-106 91 Stockholm, Sweden.

In the last decade, the emergence of antisense/antigen technology for modulation of gene expression using synthetic oligonucleotides, invigorated the interest in chemical synthesis of phosphate analogues.

Although most of oligonucleotide surrogates bearing modifications at the phosphorus center are accessible *via* various variants of phosphoramidite and phosphotriester methods, in the middle of the 80's a new synthetic methodology, based on mono- and diesters of phosphonic acid (H-phosphonate method), began gaining impetus. In contradistinction to phosphite triesters, mono- and di-substituted derivatives of phosphorous acid exist almost entirely in the phosphonate form (presence of the P-H bond, lack of a lone electron pair on phosphorus, tetracoordinated tetrahedral structure), that dominates chemistry of this class of compounds.

The growing interest in H-phosphonate chemistry is probably due to the fact that it combines advantages of the most important methodologies for the preparation of phosphorus-containing natural products. H-Phosphonate ($\lambda^3\sigma^4$) derivatives preserve the most fundamental and synthetically useful property of P(III) phosphorus compounds, *i.e.* an ability to be oxidatively converted into P(V) derivatives, but they are more stable and easy to handle than tricoordinated phosphite ($\lambda^3\sigma^3$) derivatives. The major advantages of methods based on H-phosphonate intermediates are: (i) starting materials are stable, easy to handle and resistant to air oxidation; (ii) high reactivity of various types of mixed anhydrides generated from these compounds during condensation; (iii) no need for protection of the phosphorus centre, which facilitates synchronisation of other protecting groups used in a synthesis; (iii) various phosphate analogues can be obtained from one precursor by changing oxidation conditions; (iv) the synthetic procedures are usually time- and cost-effective by comparison with other approaches.

UNCONVENTIONAL CHEMISTRY AND STEREOCHEMISTRY OF PHOSPHORUS COMPOUNDS DIRECTED TOWARDS BIOPHOSPHATES AND THEIR STRUCTURAL ANALOGUES

Jan Michalski, Wojciech Dąbkowski and Izabela Tworowska

Centre of Molecular and Macromolecular Studies, Polish Academy of Sciences, 90-363 Łódź, Sienkiewicza 112,

The synthetic challenge of preparing different types of tricoordinate phosphorus compounds containing a P-F bond derived from alcohols of biological interest will be examined. Then our progress in finding novel activating reagents in the synthesis of biophosphates *via* the phosphoroamidite route will be presented.
Finally, the potential for using P(III)-F intermediates in the synthesis of P-modified nucleotides will be discussed and evaluated.

OLIGONUCLEOTIDE SYNTHESIS USING 2-(LEVULINYLOXXYMETHYL)-5-NITROBEZOYL (LMNBz) GROUP FOR THE 5'-HYDROXYL GROUPS OF NUCLEOSIDES 3'-PHOSPHORAMIDITE DERIVATIVES

K. Kamaike, H. Takahashi, K. Morohoshi, N. Kataoka, T. Kakinuma, and Y. Ishido

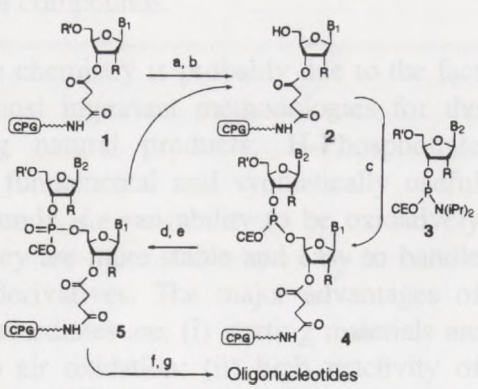
Laboratory of Pharmaceutical Chemistry, School of Pharmacy
Tokyo University of Pharmacy and Life Science
Horinouchi, Hachioji, Tokyo, Japan 192-0392

In view of the research activity seeking for a base-labile protecting group for the 5'-position of nucleosides 3'-phosphoramidite derivatives in oligonucleotides synthesis, the authors undertook an investigation on developing a novel protecting group for that purpose and recently proposed the LMNBz group (K. Kamaike, H. Takahashi, T. Kakinuma, K. Morohoshi, and Y. Ishido,*Tetrahedron Lett.*, **1997**, *39*, 6857 - 6860).

The authors initiated the present investigation with a comparative study of 2-(levulinyloxymethyl)benzoyl (LMBz) and LMNBz protecting groups based on a discussion on the excellence of (1) levulinyl group for the 5'-position, which is easily removable by treating with 0.5 M hydrazine hydrate in 1:4 acetic acid - pyridine at room temperature for 2 min (liquid-phase approach[a,b]) or 10 - 15 min (solid-phase appraoch[c,d]) [a) J. H. van Boom and P. M. J. Burgers, *Tetrahedron Lett.*, **1976**, 4875 - 4878; b) J. H. van Boom, P. M. J. Burgers, C. H. M. Verdegaal, and S. Wille, *Tetrahedron*, **1978**, *34*, 1999 - 2007; c) S. Iwai and E. Ohtsuka, *Nucleic Acids Res.*, **1988**, *16*, 9443 - 9456; d) S. Iwai, T. Sasaki, and E. Ohtsuka, *Tetrahedron*, **1990**, *46*, 6673 - 6688], and (2) the modified 2-(hydroxymethyl)benzoyl groups for an exocyclic amino groups, which are removable under basic conditions [J. H. van Boom, C. Christodoulou, C. B. Reese, and G. Sindona, *J. Chem. Soc., Perkin Trans. 1*, **1984**, 1785 - 1790; C. B. Reese, *Nucleosides & Nucleotides*, **1987**, *6*, 121 - 129; C. Christodoulou, S. Agrawal, and M. Gait, *ibid*, **1987**, *6*, 341 - 344].

2-(Levulinyloxymethyl) benzoic acid and its 5-nitrobenzoic acid were prepared from phthalide in 3 and 4 stes, respectively, and the introduction of LMBz and LMNBz groups to 5'-position of nucleosides using the acids (1.1 mol. equiv.) and 2,4,6-triisopropylbenzenesulfonyl chloride (2.2 mol. equiv.) in pyridine afforded the corresponding 5'-acylates in 60 - 72% yields.

The superiority of LMNBz group over LMBz group in oligonucleotide systhesis was confirmed by performing the reaction cycle for assembly of oligonucleotides (See Scheme 1) with respect to TpTpT, TpTpTpT, UpCpApGpUpUpGpG in combination with Thp protection for the 2'-position, Gp-A(2'-*O*-β-D-ribofuranosyl)pG, CpCpA bearing exocyclic amino groups with ^{15}N-labeling , and TpsT; all the reaction cycles were performed by manual, and not automated, synthesis.



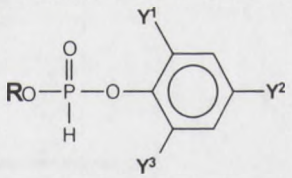
Scheme 1 Reaction Cycle for Assembly of Oligonucleotides
Conditions; (a) 0.5 M NH_2NH_2 H_2O,1:4 CH_3COOH - pyridine,15 min; (b) 0.5 M imidazole, CH_3CN, 5 min; (c) 0.05 M **3**, 0.25 M 1*H*-tetrazole, CH_3CN, 10 min; (d) $(CH_3CO)_2O$, 2,6-lutidine, 1-methylimidazole, THF, 1 min; (e) 0.1 M I_2, H_2O - pyridine - THF,1 min; (f) conc. NH_4OH, r. t., 3 h - 55 °C, 6 h; (g) *p*H 2 HCl aq., r. t.,1 day.

NUCLEOSIDE ARYL H-PHOSPHONATES - SYNTHESIS AND APPLICATIONS IN NUCLEOTIDE AND OLIGONUCLEOTIDE CHEMISTRY

Adam Kraszewski

Polish Academy of Sciences, Institute of Bioorganic Chemistry, Noskowskiego 12/14, 61-704, Poznań, Poland

Nucleoside H-phosphonates are useful synthons in the preparation of nucleotides, oligonucleotides and their analogues. They are stable, easy to handle compounds, but become exceedingly reactive upon activation with various condensing agents, *e.g.* pivaloyl or adamantanecarbonyl chloride, bis(pentafluorophenyl) carbonate, diethyl chlorophosphate, 2-chloro-5,5-dimethyl-2-oxo-1,3,2-dioxaphosphinane. Irrespective of the coupling agent used, reactive species generated during the activation process are of type of mixed anhydrides (phosphono-acyl, phosphono-carbonate or phosphono-phosphoric). These have two electrophilic centers, which may lead to some side reactions if the attack of a nucleophile is not completely chemoselective.



R = nucleoside-3'- or 5'-y
Y = various substituents

Quite different type of "active" H-phosphonate derivatives represent nucleoside aryl H-phosphonates (above). In these compounds only one electrophilic center (located on phosphorus) is present that, in principle, alleviate problem of chemoselectivity during the nucleophilic substitution. An additional advantage of using aryl H-phosphonates is that their reactivity can be modulated by substituents on the aromatic ring. This is clearly apparent from comparison of reactivity of nucleoside 2,4,6-trimethylphenyl and nucleoside 2,4,6-trichlorophenyl H-phosphonates towards 5'-unprotected nucleosides. The later derivative is very reactive and produces the corresponding dinucleoside H-phosphonate in less than 3 min, while for the former one, the reaction is not complete even after 60 hours.

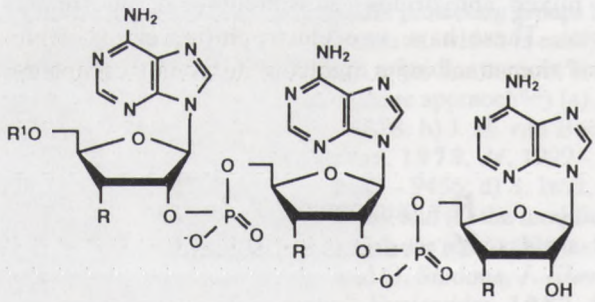
Aryl H-phosphonates are also superior to mixed anhydrides when nucleophiles other than alcohols are used for the reaction. Recently, we have demonstrated synthetic utility of this class of compounds (i) in the synthesis of nucleotide analogues bearing P-N non-bridging bond, (ii) for the functionalization (*e.g.* introduction of aminoalkyl residues) of support-bound oligonucleotides and (iii) in the preparation of synthetically useful alkyl-H-phosphonates.

SYNTHESIS AND BIOLOGICAL ACTIVITIES OF 2'-5'-OLIGOADENYLATES A NEW GROUP OF ANTIVIRALLY ACTIVE COMPOUNDS

Wolfgang Pfleiderer[1], Ramamurthy Charubala[1], Marita Wasner[1], Cornelia Hörndler[1] Earl E. Henderson[2] and Robert J. Suhadolnik[2]

[1]Fakultät für Chemie, Universität Konstanz, Postfach 5560, D-78434 Konstanz
[2]Department of Biochemistry, Temple University, Philadelphia PA 19140 USA

Recent investigations have shown that one way to counteract a cellular virus infection is manifested by the so-called 2-5A-synthase/RNase L pathway leading to a degradation of viral RNA and subsequent inhibition of protein synthesis. This interferon initiated cascade produces from ATP a series of most interesting 2'-5'-connected oligoadenylate-5'-triphosphates, the trimer (**1**) of which posseseses the best features to activate the latent RNase L responsible for the hydrolysis of single-stranded RNA. This low-molecular activator, however, is quickly deactivated by cellular phosphodiesterases.



	R	R'
1	OH	HO-P(O⁻)-O-P(O⁻)-O-P(O⁻)-
2	H	H
3	H	H_2O_3P
4	H	H_2O_2SP

Chemical syntheses of structurally modified analogues have been performed by changing the base and sugar moieties as well as the phosphodiester backbone of a broad variety of oligonucleotides [1] to obtain enzymatically more stable but still active derivatives. Especially the cordycepin trimer (**2**) and its 5'-monophosphate (**3**) and 5'-monophosphorothioate (**4**) built-up from 3'-deoxyadenosine turned out to be good candidates against a broad variety of viruses and retro-viruses. Their half-life of enzymatic degradation has increased drastically. More detailed studies indicated that cordycepin-trimer (**2**) is a most versatile antiviral agent inhibiting also reverse transcriptase of HIV-1 by interaction with the primer binding site normally occupied by tRNA Lys3.
The antiviral activity of 2'-5'-oligonucleotides can also been improved by the formation of conjugates which carry either at the 2'- or the 5'-terminal end of the molecule directly or via a spacer a hydrophobic function such as cholesterol and lipids, respectively, or vitamins of the type vitamin E and folic acid, respectively.
The chemical syntheses of the various 2'-5'-oligonucleotides and their conjugates as well as the results of the biochemical screening experiments will be discussed in detail.

[1] R. Charubala and W. Pfleiderer in "Progress in Molecular and Submolecular Biology", Ed. W.E.G. Müller and G. Schröder, Springer Verlag Berlin, 1994, Vol. 14, 114.

NUCLEOSIDE STRUCTURE/FUNCTION RELATIONSHIPS AND THE DESIGN OF NEW NUCLEIC ACIDS

Paul Agris[1], Richard Guenther[1], Mufeed Basti[2], Salman Ashraf[1], Elzbieta Sochacka[3], Winnell Newman[1], John Stewart[1], Karl Koshlap[1], Robert Cain[1], Andrzej Malkiewicz[3]

[1]Biochemistry, North Carolina State University, Raleigh, NC 27695-7622, USA
[2]Chemistry, North Carolina Agricultural and Technical University, Greensboro, NC, 27411, USA
[3]Institute of Organic Chemistry, Technical University, 90-924 Lodz, Poland

The nucleic acid chemist typically has but four chemistries with which to design nucleic acids having particular structures and functions. However, the 100s of naturally-occurring and non-natural modified nucleosides provide a riches of chemistries not to be ignored. There are a number of challenges to understanding the physicochemical contributions of modified nucleosides to nucleic acid function before one attempts to apply this understanding to new structure/function relationships. Our approach is to synthesize modified nucleosides, study them as monomers and then incorporated at site-specifically into increasingly larger nucleic acids. The nucleic acid sequences we choose have biological functions that can be readily assessed. We have incorporated a large number of natural and non-natural, and stable isotope labeled modified nucleosides into biologically active RNAs and DNAs of as large as 150-160 residues. The labilities of modified nucleosides to the phosphoramidite chemistries of the automated nucleic acid synthesizer were over-come with the use of a variety of protecting groups and alterations to the standard instrument and deprotection protocols. The result has been mg quantities of biologically active RNAs from micromole syntheses.

Our particular research interests are in the structure/function relationships of naturally-occurring modified nucleosides of the various structural elements, domains of tRNAs. Transfer RNAs are excellent models for the study of modified nucleoside contributions because of the large number and great variety of modified nucleosides in tRNAs. Our approaches include both biochemical and biophysical techniques. We have determined the roles of some modified nucleosides in the anticodon and T stem/loop domains and the invariant U_{33} in tRNA anticodons and have led to the design and synthesis of the first DNA analogue that requires Mg^{2+}, as does the tRNA, to bind the ribosome in response to codon, and effectively compete with tRNA in translation. We have determined the structure of the $tRNA^{Lys}_{SUU}$ anticodon and with it, explained a number of biochemical observations: $tRNA^{Lys}$ mis-reading codons, pre-maturely terminating protein synthesis, frameshifting and the human $tRNA^{Lys}_{SUU}$, being recruited by HIV as primer for reverse transcription. [Supported by NIH, NSF and the Polish Committee for Scientific Research]

SYNTHETIC OLIGONUCLEOTIDE COMBINATORIAL LIBRARIES AND THEIR APPLICATIONS

Wojciech T. Markiewicz

Institute of Bioorganic Chemistry, Polish Academy of Sciences, Noskowskiego 12, PL-61704 Poznań, Poland

The elucidation of the complex „network" of mutual interactions of biological (macro-) molecules is of utmost importance to the understanding of processes in living systems. Chemical interactions of isolated biomolecules are more easy to analyse and can model biorecognition systems. Furthermore, they are a means to understand specific interactions of biomolecules with non-biological analogues and organic compounds in general. Recent advances in preparative techniques have made it possible to look not only at the chemorecognition of two molecular partners, but to screen the interaction of a given „acceptor" partner with a whole library of structurally variant „donor" (macro-)molecules, i.e. to set up a complete *chemoselection* system.

The concept of using a chemical synthetic approach to generate molecular diversity in order to understand the nature of intermolecular interactions has become tremendously important [1-8].

Results of studies with dispersed Synthetic Oligonucleotide Combinatorial Libraries (SOCL) will be presented.

References

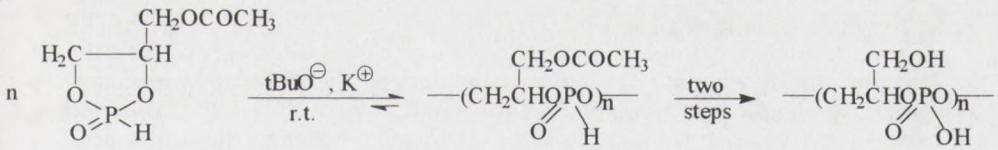
1. Southern, E. M., Maskos,U., and Elder, J .K. (1992) *Genomics*, **13**, 1008.
2. Furka, A., Sebestyen, F., Agesdom, M., & Dibo, G. (1988) *Abstr. 14th Int. Congr. Biochem.*, **47**.
3. Gallop, M. A., Barrett, R. W., Dower, W. J., Fodor, S. P. A., and Gordon, E. M. (1994) *J. Med. Chem.*, **37**, 1233; Gordon, E. M., Barrett, R. W., Dower, W. J., Fodor, S. P. A. and Gallop, M. A. (1994) *J. Med. Chem.*, **37**, 1385.
4. Lowe, G. (1995) *Chem Soc Rev.*, 309.
5. Früchtel, J. S. and Jung, G. (1996) *Angew. Chem.* **108**, 19.
6. Seliger, H., Bader, R., Hinz, M., Astriab, A., Markiewicz, M., and Markiewicz, W. T. (1997) *Nucleosides, Nucleotides*, **16**, 703-710
7. Markiewicz, M., Astriab, A., and Markiewicz, W. T. (1996) *Collection Czechoslov. Chem. Commun. Spec. issue*, **61**, 120; Markiewicz, W. T., Markiewicz, M. and Astriab, A. (1996) *ibid.*, 315.
8. Markiewicz, W. T., Godzina, P., Markiewicz, M. and Astriab, A. *Nucleosides and Nucleotides,* in press.

HIGH-MOLECULAR-WEIGHT MACROMOLECULES WITH PHOSPHODIESTER BONDS - BY ONE STEP POLYMERIZATION

Stanisław Penczek, Grzegorz Łapienis, Julia Pretula, and Krzysztof Kałużyński

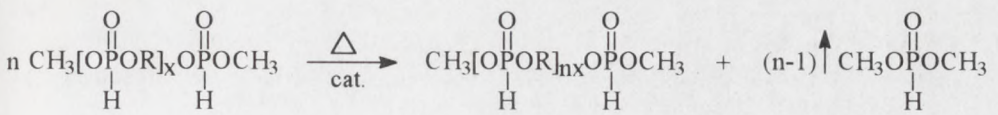
Polish Academy of Sciences, Centre of Molecular and Macromolecular Studies
90-363 Łódź, Poland

Three methods have been elaborated, leading directly to the high-molecular-weight macromolecules, mimicking chains of biomacromolecules, like nucleic acids or teichoic acids.
This presentation will summarise our previous and more recent work on the ring-opening ionic polymerization of cyclic esters of phosphoric and phosphorous acids, polyaddition of H_3PO_3 or monoesters of H_3PO_4 to diepoxides as well as polyaddition / polytransesterification of dialkyl- (diaryl) H-phosphonates with the corresponding diols.
Ring-opening polymerization of e.g. five membered, properly substituted rings with ring-strain of approx. 20 kJ mol^{-1}, leads to the simplest models of teichoic acids, namely poly (1,2- (1,3-) glycerol phosphates):
e.g.:



$\overline{M}_n \sim 30\,10^3$

1,3-Dioxaphospholanes with chiral carbon atoms fully retain chirality during polymerization. However, direction of the ring-opening could not yet be controlled.
Models of teichoic acids prepared this way were applied in estabilishing mechanisms of active transport of Ca^{+2} and Mg^{+2} cations in biomembranes.
Addition of H_3PO_3 or monoesters of phosphoric acid to bis-oxiranes allowed direct preparation of poly(alkylene phosphates) with pendant hydroxyl groups. The major secondary reaction, namely addition of oxiranes to hydroxyl groups formed during polyaddition could be eliminated by blocking hydroxyl groups in situ.
Finally, the transesterification of the low-molecular-weight oligomers of the general structure:



allowed preparation of the high-molecular-weight poly(H-phosphonates) in one step and with $\overline{M}_n$ up to $30\,10^3$. In spite of the extensive dealkylation this method leads also to the structurally uniform macromolecules. The kinetics and mechanism of transesterification proceeding with alcoholate "active species", and their interconversion into acidic species (due to dealkylation), will briefly be described.

POSSIBLE EVOLUTION OF FACTORS INVOLVED IN PROTEIN BIOSYNTHESIS

Jens Nyborg[1], Poul Nissen[1], Ole Kristensen[1], Morten Kjeldgaard[1], Søren Thirup[1], Galina Polekhina[1], Ludmila Reshetnikova[2] and Brian F.C. Clark[1].

[1]Institute of Molecular and Structural Biology, University of Aarhus, Gustav Wieds Vej 10C, DK-8000 Aarhus C, Denmark
[2]Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, 32 Vavilov Str., 117984 Moscow, Russia

The structure of the ternary complex of yeast Phe-tRNA, T. aquaticus EF-Tu and the GTP analog GDPNP has been determined (1). When the model of this complex is compared to the structure of EF-G:GDP (2) an unexpected macromolecular mimicry is observed. The biological implications of this mimicry, as well as some views on evolution of translation factors in protein biosynthesis will be discussed. Elongation factors EF-Tu and EF-G catalyze the elongation step of prokaryotic protein biosynthesis. Their actions are controlled by GTP. EF-Tu:GTP forms a ternary complex with all aminoacylated tRNAs, protects the amino acid ester bond and assists in placing the cognate aminoacylated tRNA into the ribosomal A site. Inactive EF-Tu:GDP is released from the ribosome. A peptide bond is formed between the aminoacylated tRNA and the peptide on peptidyl tRNA in the P site. EF-G:GTP translocates the newly formed peptidyl tRNA into the P site and at the same time advances the mRNA one codon. Structures of E. coli EF-Tu:GDP (3) as well as of T. thermophilus and T. aquaticus EF-Tu:GDPNP (4) have been determined earlier. Recently the structures of T. aquaticus and E. coli EF-Tu:GDP (5) is published. They reveal a large conformational rearrangement of domains and of the socalled switch regions of EF-Tu upon activation. This rearrangement is supported by the structure of the EF-Tu:EF-Ts complex (6). The structure of the quaternary complex of the antibiotic kirromycin and the ternary complex of EF-Tu is being determined (7). It indicates the mode of action of this antibiotic. The visualisation of the quaternary complex on the surface of the ribosome using cryo-EM gives the first picture of the ribosome in action (8).

(1) P. Nissen et al., Science (1995), 270, 1464.
(2) J. Czworkowski et al., EMBO J. (1994), 13, 3661; A. Ævarsson et al., EMBO J. (1994), 13, 3669.
(3) M. Kjeldgaard and J. Nyborg, JMB (1992), 223, 721.
(4) H. Berchtold et al., Nature (1993), 365, 126; M. Kjeldgaard et al., Structure (1993), 1, 35.
(5) G. Polekhina et al., Structure (1996), 4, 1141; K. Abel et al., Structure (1996), 4, 1152.
(6) T. Kawashima et al., Nature (1996), 379, 511; Y. Wang et al., Nature Struct. Biol. (1997), 4, 650.
(7) O. Kristensen et al., FEBS Lett. (1996), 399, 59.
(8) H. Stark et al., Nature (1997), 389, 403.

ARCHAEAL AMINOACYL-tRNA SYNTHESIS: UNIQUE DETERMINANTS OF A UNIVERSAL GENETIC CODE?

M. Ibba, I. Celic, A.W. Curnow, H.-S. Kim, J. Pelaschier, D. Tumbula, U.C. Vothknecht, and D. Söll

Department of Molecular Biophysics and Biochemistry, Yale University, New Haven CT 06520-8114, USA

The accurate synthesis of aminoacyl-tRNAs is essential for faithful translation of the genetic code and is assumed to be one of the most orthologous processes in biology. This dogmatic view has been called into question by the sequencing of a number of microbial genomes; for example, the genomic sequence of *Methanococcus jannaschii* does not contain open reading frames encoding homologs of the asparaginyl-(AsnRS), cysteinyl-(CysRS), glutaminyl-(GlnRS) and lysyl-tRNA synthetases (LysRS). The use of two-step (indirect) aminoacylation pathways for the formation of Asn-tRNA^{Asn} and Gln-tRNA^{Gln} in some organisms circumvents the need for the enzymes which catalyze one step formation of these molecules, AsnRS and GlnRS (1-3). While no adequate explanation yet exists for the apparent absence of CysRS, it has recently been shown that several members of the Archaea, including *M. jannaschii*, contain a functional LysRS with no resemblance to known bacterial or eukaryal LysRSs (4). Continued genomic sequencing efforts suggested this novel LysRS may also be found in some Bacteria, as was confirmed by the cloning of a gene encoding a functional archaeal-type LysRS from *Borrelia burgdorferi* (5). Thus, non-orthologous replacement of essential enzymes such as AsnRS, GlnRS and LysRS is widespread in both Archaea and Bacteria, indicating that while the process of translation is orthologous its constituents are not.

(1) Ibba, M., Curnow, A.W. and Söll, D. (1997) *Trends Biochem. Sci.* **22**, 39-42.
(2) Curnow, A.W., Ibba, M. and Söll, D. (1996) *Nature* **382**, 589-590 .
(3) Curnow, A.W., Hong, K.W., Yuan, R., Kim, S.I., Martins, O., Winkler, W., Henkin, T.M. and Söll, D. (1997) *Proc. Natl. Acad. Sci. USA* **94**, 11819-11826
(4) Ibba, M., Morgan, S., Curnow, A.W., Pridmore, D.R., Vothknecht, U.C., Gardner, W., Lin, W., Woese, C.R. and Söll, D. (1997) *Science* **278**, 1119-1122.
(5) Ibba, M., Bono, J.L., Rosa, P.A. and Söll, D. (1997) *Proc. Natl. Acad. Sci. USA* **94**, 14383-14388.

POTATO SPINDLE TUBER VIROID POPULATION STUDIES

Anna Góra , Wojciech Podstolski, Włodzimierz Zagórski

Institute of Biochemistry and Biophysics Polish Academy of Sciences
ul. Pawińskiego 5a, 02-106 Warszawa, Poland.

Potato spindle tuber viroid (PSTVd) is nonencapsidated, circular single stranded RNA molecule of approximately 360 nt. causing specific disease symptoms when inoculated on plants. Pathogen RNA does not code for any proteins, so its replication is completely dependent on host cell enzymatic machinery. Since mutation rate in RNA genomes is high, propagation of unique PSTVd molecules in host plants often leads to appearance of a new sequence variants creating *de novo* a population. This phenomenon fits to quasi species theorem formulated by Eigen, because PSTVd behaves as a complex, self-perpetuating population of diverse , related entities acting as a whole.

Indeed local field PSTVd isolates analysed in our study where found to be a mixture of a coexisting molecular variants each able to cause disease symptoms of defined severity when inoculated separately on plants. The overall symptoms of disease caused by mixture of variants are determined by the most pathogenic molecules asking the presence of milder ones. The analysis of progeny derived from different parental sequence variants showed that degree of variability is different for each molecule since progenies of parental S27 sequences analysed where found to diverge, while parental S23 sequence was found to be genetically stable. Based on collected sequence data for S27 variant progeny it is possible to hypothesise a mutation series events leading to phenotype conversions.

Nearest neighbour analysis allows to identify sequence variations leading to phenotype conversions. Group assignment based on sequence analysis of specific viable variants in PSTVd population allows to weight out frequences and population number of molecular variants.

It seems that a specific variant S23 is conservative and point mutations in this particular sequence are lethal.

Molecular mechanisms responsible for observed differentiation and stability remain unclear and require further investigation.

EVOLUTION OF PLANT-MICROBE INTERACTIONS

Andrzej B. Legocki

Institute of Bioorganic Chemistry, Polish Academy of Sciences,
Poznań, Noskowskiego 12/14, Poland

Upon interaction with symbiotic (beneficial) or pathogenic microorganisms the plant reacts in superficially different ways leading to either an endosymbiotic association or defense against the intruder. The molecular events that constitute plant-microbe interactions involve ligand-receptor recognition and induction of signal transduction pathways in the plant, leading to a specific response. Most likely the response mechanisms were adapted to specific needs in the course of evolution.

Recent discoveries concerning signal exchange between plant and microorganisms have shown that conserved lipo-chitin molecules might function as universal plant regulators. It is likely that they are part of the endogenous growth regulatory system. The legume *Rhizobium* symbiosis provides a useful model for studies of plant-microbe relationship. It originated 85-90 million years ago and made legumes the only plant family which is autotrophic for external nitrogen. Because this association is highly beneficial, legumes have evolved a set of organ-specific genes whose expression is required for the development and function of a root nodule - a new organ where binding and reduction of atmospheric nitrogen occurs. Closer examination of the expression patterns of certain PR (pathogenesis-related) legume genes and the fact that they are susceptible to microbial signals suggest that the defense and symbiotic mechanisms are conserved and evolutionarily related.

THE BERLIN *Netzwerk* FOR RNA TECHNOLOGIES

Volker A. Erdmann

Institute for Biochemistry, Free University of Berlin, Thielallee 63, D-14195 Berlin, Germany

A new and very dynamically evolving field is that of RNA-Technologies. These RNA-Technologies show great promises for the medicine and biotechnology of the 21st century. The technologies are based upon the inherent properties of RNA-molecules to function in a hitherto unforeseen manner. **Antisense RNA-molecules** are already used in clinical tests for the treatment of specific forms of leukemia. Based on their hydrolytic properties so called **ribozymes** may be used as enzymatically active RNA-molecules. **High affinity RNAs** (aptamers) are characterized through their specific binding properties to proteins, lipids, polysaccharides and even such small molecules like amino acids and nucleotides. Therefore and because of their broad application they are comparable with monoclonal antibodies. The **protein bioreactor** allows the *in vitro* -synthesis of large amounts of proteins in a cell free system using a mRNA-template and thus generating tailor-made proteins with predicted properties.
In Berlin potentials of the RNA-Technologies for the field of molecular medicine have been early recognized. To foster the RNA-Technologies the **Inter-disciplinary Research Association** („Interdisziplinärer Forschungsverbund - IFV RNA-Technologien") has been funded through the Berlin Senator for Science, Research and Culture. The aim of this IFV „RNA-Technologies" is the organization of all activities in the region Berlin-Brandenburg connected with basic and applied RNA research. The current membership list includes about 100 members from different institutions in the region Berlin-Brandenburg. One of the most important goals of the IFV „RNA-Technologies" is its role of shifting the region's potentials from basic research to applied research and to establish these new technologies in the industry of Berlin-Brandenburg. For this purpose 38 projects out of the IFV „RNA-Technologies" were selected for a major grant application called *Netzwerk* „RNA-Technologies" to be submitted to the Federal Ministry for Education, Science, Research and Technology (BMBF). This major initiative is so far supported by 20 different industrial partners in Germany. The idea behind the *Netzwerk* „RNA-Technologies" is to have a core facility („Knotenpunkt") in which the protein bioreactor is developed and the design and synthesis of the different ribozymes and high affinity RNAs are carried out. The testing or application of these molecules is then performed in the laboratories of the 38 partners. It is therefore one of the major concepts of the Netzwerk to develop the RNA-Technologies in such a way that they can be applied in the fields of biotechnology and medicine.

tRNA-PROTEIN INTERACTIONS IN INITIATION OF PROTEIN SYNTHESIS

Uttam L. RajBhandary, Ramesh Vaidyanathan, Sadanand Gite, Yan Li, Xin-Qi Wu and Dev Mangroo

Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA

Initiator tRNAs possess a number of specific properties different from those of elongator tRNAs. For eubacterial initiator tRNAs, these include, (i) the formylation of Met-tRNA to fMet-tRNA by the methionyl-tRNA formyltransferase, (ii) the recognition of the fMet-tRNA by the initiation factor IF2, (iii) the direct binding of the fMet-tRNA to the ribosomal P site, (iv) exclusion of the initiator tRNA from the ribosomal A site, and (v) the unique resistance of fMet-tRNA to peptidyl-tRNA hydrolase. Using a combination of in vivo and in vitro studies, we previously identified the sequence and the structural features important for specifying these distinctive properties and showed that most of these features cluster in two regions: the end of the tRNA acceptor stem and the last three base pairs of the anticodon stem. Interestingly, a single structural feature, unique to the initiator tRNA accounts, for three of its specific properties.

Our more recent work has focussed on use of the mutant tRNAs generated during the above work for identifying some of the steps of protein synthesis initiation in vivo. The results suggest (i) that IF2 can act as a carrier of the fMet-tRNA to the ribosome and (ii) that at least under certain conditions, the 30S ribosome may bind first to the initiator fMet-tRNA and then to the mRNA.

In further studies on the molecular mechanisms of interaction of the initiator tRNA with methionyl-tRNA formyltransferase, we have used chemical crosslinking of the enzyme to periodate oxidized tRNA and the isolation and analysis of suppressor mutations in the enzyme. This work has led to identification of lysine 206 as the amino acid in the enzyme that comes close to the 3'-end of the tRNA and to the suggestion that a sixteen amino acid insertion module in the enzyme plays an important role in specificity of recognition of the tRNA.

This work was supported by grant GM17151 from the National Institutes of Health.

AMINOACYLATED RNA A LINK BETWEEN RNA AND PROTEIN WORLD

Andreas Schußter[1], Barbara Nawrot[1,3], Stefan Limmer[1], Rolf Hilgenfeld[2], and Mathias Sprinzl[1]

[1]Laboratorium für Biochemie, Universität Bayreuth, D-95440 Bayreuth, Germany, [2]Abteilung Strukturbiologie und Kristallographie, Institut für Molekulare Biotechnologie D-07708 Jena, Germany, [3]present address: Polish Academy of Sciences, Centre of Molecular and Macromolecular Studies, Department of Bioorganic Chemistry, ul. Sienkiewicza 112, PL - 90-363, Lodz, Poland

Several enzymes, protein factors and ribosomes interact with aminoacyl-tRNA or peptidyl-tRNA during protein biosynthesis. These macromolecules have to specifically recognise the aminoacyladenosine on the 3'-end of aminoacyl-tRNA. In the case of interaction with elongation factor Tu differences in the equilibrium dissociation constants between tRNA and aminoacyl-tRNA amount to up to five orders of magnitude. This raises the question about the structural differences between aminoacyl-tRNA and non-aminoacylated tRNA, that provide the basis for this discrimination.

Anthraniloyl group attached to the adenosine 76 of tRNA mimics the structure of the 3´-end of aminoacyl-tRNA. 3'-0-Anthraniloyladenosine (3'-ant-Ado), an analogue of the 3'-terminal aminoacyladenosine, was crystallised and its structure was determined by X- ray crystallography. In the crystal, the sugar ring exhibits the 2'-*endo* conformation. By NMR spectroscopy a predominant 2´-endo conformation of the ribose ring was also demonstrated for aqueous solutions of 2'(3')-ant-adenosine, 2'(3')-ant-AMP and CpCpA- which carries an anthraniloyl group at the 3'-adenosine residue. The crystal structure of the ternary complex formed by 3'-ant-AMP, EF-Tu and GppNHp was determined. It shows that the 3'-0-anthraniloyladenosine-5-phosphate binds to the binding pocket of EF-Tu in 2´-*endo* conformation, near the interface between domains 1 and 2. This binding site is identical to the location of the aminoacyl residue in the aminoacyl-tRNA.EF-Tu.GppNHp ternary complex (1). Thus, the ant-AMP lacking the whole tRNA sequence possess all structural elements to recognised by the correct binding site on the EF-Tu. 2'-*endo* conformation of the sugar ring is necessary to place the adenine ring into hydrophobic binding pocket and the anthraniloyl group into the cleft for the aminoacyl residue.

A fluorescent analogue of several tRNAs in which formycin replaces the adenosine 76 has been prepared and used to monitor the conformational changes of 3'-terminus upon enzymatic aminoacylation and hydrolytic deaminoacylation. A 30% increase of the relative fluorescence of aminoacyl-tRNA-CCF in comparison to the relative fluorescence of non-aminoacylated tRNA-CCF demonstrates that aminoacylation results in partial destacking of the 3´-terminal base moiety. This destacking is the consequence of changes in the puckering of the 3´terminal ribose caused by aminoacylation and interaction between the ester carbonyl and vicinal hydroxyl group. We can demonstrate, using analogues of aminoacyl-tRNA, that this subtle conformational change is also important to define the structure of substrates and products during aminoacylation of tRNAs by aminoacyl-tRNA synthetases.

1) P. Nisen et al. (1995) Science **270**, 1454-1472

FINE STRUCTURE ANALYSIS OF THE RNA-PROTEIN INTERACTION IN THE RIBOSOME

Bernd Thiede and Brigitte Wittmann-Liebold

Max-Delbrueck-Centrum für Molekulare Medizin, Research Group Protein Chemistry, Robert-Roessle-Str. 10, D-13125 Berlin-Buch, Tel: 49-30-2875; Fax: 49-30-3869; email: liebold@mdc-berlin.de

RNA-protein contact sites in the 30S and 50S subunit of eubacterial ribsomes (*E. coli* and *B. stearothermophilus*) were isolated as peptide oligonucleotide cross-linked complexes employing a new approach (1). Their contact points were unambigously determined on the amino acid and ribonucleotide level by direct amino acid sequencing of the peptide moiety within the complex and by partial sequencing of the nucleotide part by MALDI-mass spectrometry (2-4). The data accumulated allow, for the first time, to study precisely the structural elements and sequence motifs associated with the ribosomal RNA-protein interaction. The comparison of our results with known three-dimensional structures of r-proteins and available rRNA-models show that mainly loop and bulge structures, respectively, are involved in the peptide-oligonucleotide cross-linking sites. Hence it is of interest to investigate whether stable structures may be formed upon binding. This would explain the highly specific interaction of each of the proteins to the respective part of the rRNA. Furthermore, our data are the basis for and stimulate considerably the correct incorporation of r-protein structures into the present day's structural models of the 16S and 23S RNA of the bacterial ribosome. Similarly, studies are ongoing to investigate the human ribosomal RNA-protein interaction and to extend these studies to other RNP particles of the cell, e.g. snRNPs (Urlaub and Lührmann, in progress).

References

1. Urlaub, H., Kruft, V. and Wittmann-Liebold, B.: Purification scheme for isolation and identfication of peptides cross-linked to the rRNA in ribosomes. In: *Methods in Protein Structure Analysis* / ed. by Atassi, M.Z. and Apella, E. - New York : Plenum Press, 1995. - 275-282. - (*Proceedings of the Tenth International Conference)*
2. Urlaub, H., Kruft, V., Bischof, O., Müller, E.-C. and Wittmann-Liebold, B.: Protein-rRNA binding features and their structural and functional implications in ribosomes as determined by cross- linking studies. *EMBO J.* 14 (1995) 4578-4588
3. Urlaub, H., Thiede, B., Müller, E.-C., Brimacombe, R. and Wittmann-Liebold, B.: Identification and sequence analysis of contact sites between ribosomal proteins and rRNA in Escherichia Coli 30S subunits by a new approach using matrix assisted laser desorption ionization mass spectrometry combined with N-terminal microsequencing. *J. Biol. Chem.* 272 (1997) 14547-14555
4. Urlaub, H., Thiede, B., Müller, E.-C. and Wittmann-Liebold, B.: Contact sites of peptide-oligoribonucleotide cross-links identified by a combination of peptide and nucleotide sequencing with MALDI MS. *J. Protein Chem.* 16 (1997) 375-383

MOLECULAR RECOGNITION IN RNA

Douglas H.Turner[1], David H. Mathews[1], Tianbing Xia[1], Michael Zuker[2], and Stephen M. Testa[1]

[1] Department of Chemistry, Box 270216, University of Rochester, Rochester, NY 14627

[2] Institute for Biomedical Computing, Washington University, St. Louis, MO 63110

RNA is increasingly becoming a target for therapeutics. This trend is likely to continue due to the explosion in sequence information. Two approaches to facilitating targeting of RNA will be discussed.

One approach is prediction of RNA secondary structure from sequence by free energy minimization. This facilitates identification of motifs important for function and for structural studies. Recent results on Watson-Crick and non-Watson-Crick motifs will be presented that illustrate the importance of hydrogen bonding in formation of secondary structure. Inclusion of these effects in Michael Zuker's folding algorithm, MFOLD, appears to improve predictions of secondary structure from sequence.

The second approach to improving targeting of RNA is to increase specificity by taking advantage of molecular recognition involving tertiary interactions in binding of short oligonucleotides. Recent results will be discussed for small natural and unnatural oligonucleotides targeting the internal guide sequence of a group I intron from mouse *Pneumocystis carinii*.

CONFORMATIONAL CHANGES IN tRNA (QUANOSINE M-1) METHYLTRANSFERASE AND tRNA ACCOMPANY RNA SUBSTRATE BINDING AND RECOGNITION

Joanna Gabryszuk, Ruth Tyler-Cross, J. Neel Scarsdale, and W. Michael Holmes

The Institute for Structural Biology and Drug Discovery, Medical College of Virginia of Virginia Commonwealth University, Richmond, VA 23219

We have utilized circular dichroism spectroscopy (CD), chemical and enzymatic probing of tRNA-enzyme complexes, and Multidimensional NMR to examine the interactions of *Escherichia coli* tRNA (quanosine m-1) methyltransferase (1MGT) with tRNA1leu, the minimal substrate GpG, and S-Adenosyl Methionine analogues. Titration of 1MGT with GpG results in a marked reduction of negative ellipticity at wavelengths between 205 and 240 nanometers. This indicates that this minimal substrate triggers substantial changes in enzyme structure. GpG also contributes to the thermal stability of 1MGT. Moreover, tRNA structure appears to be altered in complex as well. We have obtained multidimensional ^{15}N and ^{1}H, HSQC spectra of 1MGT in the absence and presence of GpG and the AdoMet analogue, Sinefungin. In the presence of GpG or Sinefungin, one tryptophan residue shows a marked change in environment as evidenced by a pronounced chemical shift. Using chemical probes we previously observed that the target residue G37, which is methylated by 1MGT, is surprisingly, not protected against lead cleavage. Here we show that occupancy of the AdoMet binding site with Sinefungin now causes the protection of G37. We propose that occupancy of the AdoMet binding site is required for the movement of G37 into the active site of the enzyme.

Another analog N-Methyl AdoMet also triggers protection of the G36pG37 residues, but also causes increased cleavage at several sites near the crucial G37 residues and at other distant sites. Taken together, it is clear that substantial changes in RNA and protein structure accompany complex formation and may be required for recognition.

PLANT AMINOACYL-tRNA SYNTHETASES

Jan Barciszewski

Institute of Bioorganic Chemistry, Polish Academy of Sciences, Noskowskiego 12/14, PL-61704 Poznań, Poland,

The accuracy of protein biosynthesis generally rests on a family of 20 aminoacyl-tRNA synthetases, one for each amino acid. In eubacteria, archaea and eukaryotic organelles the formation of Gln-tRNA^{Gln} is prevalently accomplished by a transamidation pathway: aminoacylation of tRNA^{Gln} with Glu by glutamyl-tRNA synthetase (GluRS; EC 6.1.1.17) followed by a tRNA-dependent transamidation of Glu from Glu-tRNA^{Gln}. A few bacterial species, such as *Escherichia coli*, do possess a glutaminyl-tRNA synthetase (GlnRS; EC 6.1.1.18), responsible for Gln-tRNA^{Gln} formation. Phylogenetic analysis of the GlxRS (GluRS or GlnRS) family suggested that GlnRS has a eukaryotic origin and was horizontally transferred to a restricted set of eubacteria. We have now isolated an additional GlnRS gene from the plant lupin *Lupinus luteus* and analysed in more detailed the modular architecture of the paralogous enzymes GluRS and GlnRS, starting from a large data set of 33 GlxRS sequences. Our analysis suggests that the ancestral GluRS-like enzyme was solely composed of the catalytic domain bearing the class-degining motifs of aminoacyl-tRNA synthetases, and that the anticodon binding domain of GlxRSs was independently acquired in the eubacteria and archaea branches of the universal tree of life, the eukarya sub-branch arising as a sister group of archaea. The transient capture of UAA and UAG codons could have favored the emergence of a *bona fide* GlnRS in early eukaryotyes.

BIOSYNTHESIS OF LUPIN PROTEINS: FERRITIN AND LDC; REGULATORY MECHANISMS

Tomasz Twardowski, Joanna Smól, Maciej Astriab

Institute of Bioorganic Chemistry PAS
ul. Noskowskiego 12
Pl-61-704 Poznań, Poland
twardows@ibch.poznan.pl

Two stress proteins from lupine were purified to homogeneity: lysine decarboxylase [LDC] and ferritin. LDC is a key enzyme in quinolizidine alkaloids biosynthesis and ferritin is a protein responsible for iron housekeeping.
For both of them molecular characteristics were established. We researched the mechanisms of their activities and particularly the regulatory functioning. For LDC we found the presence of a specific inhibitor. This inhibitor is responsible for the specific activity of the enzyme. In the case of ferritin we strongly emphasized the posttranslational regulation of biosynthesis process. This mechanism limits the amount of ferritin available for iron chelating.

SYNTHESIS OF OLIGONUCLEOTIDES CONTAINING THE (6-4) PHOTOPRODUCT AND ITS APPLICATION TO THE STUDY OF UV-INDUCED MUTATIONS

Sligenori Iwai[1], Hiroyuki Kamiya[2], Yoshie Fujiwara[1], Hiroshi Kasai[2], and Morio Ikehara[1]

[1] Biomolecular Engineering Research Institute, 6-2-3 Furuedai, Suita, Osaka 565-0874, Japan

[2] Institute of Industrial Ecological Sciences, University of Occupational and Environmental Health, 1-1 Iseigaoka, Yahatanishi-ku, Kitakyushu 807-8555, Japan.

Ultraviolet (UV) light causes two major types of photolesions at dipyrimidine sites in DNA, namely cyclobutane pyrimidine dimers and pyrimidine(6-4)pyrimidone plotoproducts. It has been reported that the (6-4) photoproduct is much more mutagenic than the cyclobutane dimer. UV-irradiated plasmid DNAs have been used in biochemical experiments to study mutagenesis and repair of this photolesion, but since various base damages are induced by exposure of DNA to UV radiation, it is doubtful whether one can discuss this particular DNA damage using the data derived from such experiments. The only method of preparing „pure" DNA containing the (6-4) photoproduct was UV-irradiation of a very short olgionucleotide containing a single TT site, followed by HPLC purification. However, this procedure suffered from limitations in chain length, sequence, and yield. Therefore, we synthesized a dinucleotide building block of this photoproduct and incorporated it into oligonucleotides. Even long oligomers, such as 30- and 49-mers, were synthesized successfully and purified by a single HPLC separation.

In SOS-induced *Escherichia coli*, the T→C transition has been reported with an extremely high frequency at the 3' pyrimidone of the (6-4) photoproduct of thymidylyl(3'-5')thymidine. In order to gain insight into the mechanism of this mutation, base pair formation of this photoproduct was analyzed thermodynamically using synthetic oligonucleotides. The duplexes were designed to be used as models for template-primer systems, and the thermodynamic parameters were determined from the thermal melting curves. The duplex was stabilized only when guanine was opposed to the 3' pyrimidone of the (6-4) photoproduct. This result provided evidence of base pair formation between the 3' pyrimidone and the opposite guanine, which can explain the 3' T→C transition in *E. coli*. By contrast, this mechanism did not agree with the mutation spectra in mammalian cells reported previously. So we carried out similar mutation experiments using the synthetic oligonucleotides. A plasmid containing the photoproduct at a defined site was transfected into simian COS-7 cells, and sequence determination of the replicated DNA revealed that the (6-4) photoproduct of TT mainly induced 3' T→C transition again in mammalian cells.

MODIFIED NUCLEOSIDES FOR HUMAN HEALTH

Bożenna Golankiewicz

Institute of Bioorganic Chemistry of the Polish Academy of Sciences,
Noskowskiego 12/14, 61-704 Poznań, Poland

In the search for structure modifications of the potent antiherpetic drugs, acyclovir, 9-[(2-hydroxyethoxy)methyl]guanine and ganciclovir, 9-[(1,3-dihydroxy-2-propoxy)methyl]guanine, aimed to expand the range of their applications, we transformed their guanine moiety into the tricyclic 3,9-dihydro-9-oxo-6-R-5*H*-imidazo[1,2-*a*]purine system. We have found that this modification, natural prototype of which occurs in $tRNA^{Phe}$ in nucleosides of the wyosine series, allows to shape physical, chemical and biological properties of the parent compounds. 6-Substitution (R = alkyl or aryl) is crucial for modulation of the chemical reactivity of the tricyclic system and for the obtainment of more selective or fluorescent antivirally active analogues.

Discussion of the synthetic aspects of this investigation will particularly focus on the introduction of aromatic substituents, which may render the tricyclic analogues fluorescent. This property opens the perspectives for the tricyclic analogues to be used in the noninvasive diagnosis of herpesvirus infections and studies on the mechanism of phenomena connected with VDEPT (virally directed enzyme prodrug therapy) employing herpes simplex virus 1 thymidine kinase gene/ganciclovir system

NOVEL APPLICATIONS OF TRANSGLYCOSYLATION REACTIONS IN THE NUCLEOSIDE SYNTHESIS

Jerzy Boryski

Institute of Bioorganic Chemistry, Polish Academy of Sciences,
ul. Noskowskiego 12/14, Pl-61704 Poznań, Poland

The 6-oxopurine nucleosides (*i.e.* derivatives of guanine, hypoxanthine, etc.) readily undergo an exceptional, fully reversible 7 $\rightleftarrows$ 9 isomerization in the presence of acidic catalysts or under thermal conditions.[1] The isomerization is an intermolecular reaction and proceeds *via* unstable 7,9-diglycosylpurine intermediates.[2] This unique property creates new possibilities in the nucleoside synthesis, because either sugar substituents or the purine portion can be easily exchange in order to get new nucleoside analogues. Thus, reaction of tetra-acetylguanosine with an excess of fully acetylated sugars or their analogues yields the guanine nucleosides having modified sugar portions. That approach, called "transpurination", has been especially useful in the synthesis of biologically active acyclonucleosides, *e.g.* acyclovir and ganciclovir.[2,3] Even more promissing is a counter approach - the "intermolecular transglycosylation", which has been applied to a stereoselective synthesis of 2'-deoxy-β-D-ribonucleosides. In this method, 2'-O-acetyl-3',5'-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-6-oxopurine derivatives reacts with an equimolar amount of other heterocyclic bases to get new β-D-ribonucleosides in a quantitative yield. The products of transglycosylation may be then transformed to the respective 2'-deoxy derivative by using the Barton's 2'-deoxygenation procedure, or they may be further modified at the 2'-position.

This study was supported by the Komitet Badań Naukowych, Project No 4 PO5F 007 12.

REFERENCES

1. J. Boryski, *Nucleosides Nucleotides*, **15**, 771 (1996); and references cited therein.
2. J. Boryski, *J. Chem. Soc., Perkin Trans. 2*, 649 (1997).
3. J. Boryski, B. Golankiewicz, *Nucleosides Nucleotides*, **8**, 529 (1989).

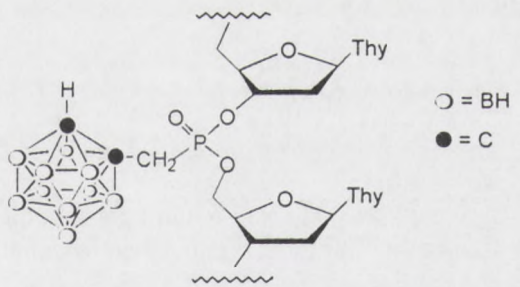
CARBORANYLMETHYLPHOSPHONATE (CBMP) OLIGONUCLEOTIDES

Zbigniew J. Lesnikowski[1], Raymond F. Schinazi[2]

[1] Center of Microbiology and Virology, Polish Academy of Sciences, Laboratory of Molecular Virology and Biological Chemistry, Lodz 93-232, Poland;

[2] Veterans Affairs Medical Center, and Laboratory of Biochemical Pharmacology, Department of Pediatrics, Emory University School of Medicine, Atlanta, Georgia 30322, USA.

o-Carboran-1-yl-methylphosphonate (CBMP) oligonucleotides [1,2] are novel class of oligonucleotide analogues potentially useful as boron rich carriers for boron neutron capture therapy (BNCT), as anisense agents for antisense oligonucleotide technology (AOT), and as new probes for the molecular diagnostics of infectious and genetic diseases.



CBMP group containing dodecathymidylic acid d[$(T_P)_{11}$T] manifested marked increases in lipophilicity and resistance to digestion by 3'-exonuclease from snake venom (SVPDE), and 5'-exonuclease from bovine spleen (SPDE) as compared to the corresponding unmodified oligomer. The thermostability of the duplexes formed by CBMP oligonucleotides with complementary strand poly r(A) was in general higher than for the unmodified oligomer. Phosphorylation of CBMP oligonucleotides with T4 polynucleotide kinase was observed for oligomers bearing modification in the middle or at the 3'-end, but not for the 5'-modified oligomer. Comparison of CBMP and methylphosphonate oligonucleotides demonstrated advantage of CBMP oligomers in their physicochemical and biological characteristics over the corresponding methylphosphonate counterparts [2].

[1] Z.J. Lesnikowski, R.F. Schinazi, *J. Org. Chem.*, 58, 6531 (1993).

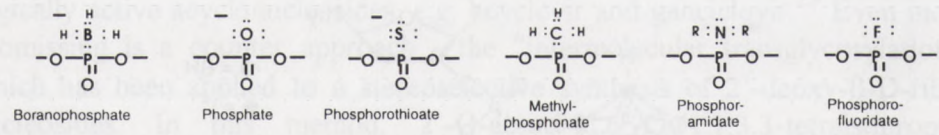
[2] Z.J. Lesnikowski, R.F. Schinazi, *Nucleosides & Nucleotides*, 16, 1503 (1997)

BORANOPHOSPHATES: A NEW DNA BACKBONE

Barbara Ramsay Shaw

Duke University, Durham, NC 27708-0346., (919) 660-1551; Fax: (919) 660-1618

The boranophosphates are isoelectronic and isoionic analogs of the naturally occurring O-phosphate esters. Boranophosphates possess properties that may make them uniquely useful as antisense agents. Boronated DNA dimers are 18-fold more lipophilic than natural nucleotide dimers, and orders of magnitude more resistant to exo and endonucleases. Boronated oligomers form stable duplexes and support RNAse H mediated cleavage of mRNA. Boronated DNA may provide an exceptionally useful addition to the repertoire of nucleic acid mimetics and gene targeting drugs.



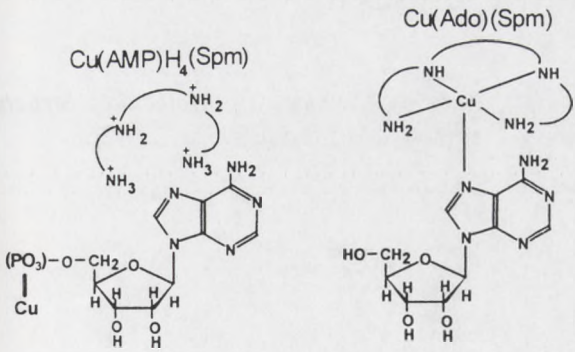
Isoelectronic analogs of natural phosphodiesters.
Only the first three retain a negative charge.

INTERACTIONS IN THE SYSTEMS WITH NUCLEOSIDES AND NUCLEOTIDES INCLUDING METAL IONS AND POLYAMINES

Lechosław Łomozik and Anna Gąsowska

Faculty of Chemistry, A. Mickiewicz University, Poznań, Poland

A main coordination sites in the complexes formed by metal ions with nucleotides are N(3) atoms of pyrimidine bases and N(7) or/and N(1) nitrogen atoms of purine bases, as well as phosphate groups from nucleotides. In the Ado/Cu binary systems a coordination dichotomy occurs (binding *via* N(1) or N(7) donor atoms from bioligand) in an acidic medium. At pH above 7, the dichotomy disappears and the major coordination site is N(7) atom of the purine base. The introduction of polyamine (PA) into the systems where complexes between nucleotides or nucleosides and metal ions are formed, changes the character of interactions. For example, the presence of putrescine (Put) - $NH_2(CH_2)_4NH_2$ results in an extension of the pH range of the occurrence of coordination dichotomy in the copper(II)/adenosine system, whereas the presence of spermidine (Spd) - $NH_2(CH_2)_3NH(CH_2)_4NH_2$ or spermine (Spm) - $NH_2(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$ in the system causes the disappearance of the dichotomy. The N(1)/N(7) dichotomy was also observed in the systems containing Co(II) or Ni(II) and adenosine monophosphate (AMP), however, Co(II) coordinates the phosphate group in the whole pH range studied, while Ni(II) only at a high pH. An interesting difference was found between nucleotides and nucleosides in the ternary system with copper(II) including spermine. It has been observed that in the Cu(Ado)(Spm) complex, Cu(II) binds four nitrogen atoms of Spm in equatorial arrangement and N(7) atom of the Nuc in the axial position. On the other hand, in the system with AMP, Cu(II) is coordinated to the nucleotide phosphate group, but Spm is involved in a non-covalent interaction with the purine ring which results in the formation of a molecular complex as shown in the scheme. It was found in the Nuc(NMP)/PA (metal-free) systems that molecular complexes are formed as a result of the non - covalent interaction between $-NH_x^+$ groups of PA and deprotonated N(1) and N(7) atoms of adenosine and N(3) atom of cytidine as well as phosphate groups of nucleoside monophosphates. Adducts are formed only in the pH range where Ado or Cyd are deprotonated and PA protonated. The tendency to form adducts increases with the chain length and number of amino groups of PA (e.g., $AdoH_2Spm$ is more stable than $AdoH_2Spd$) indicating that not only the charge, but also the structural factor influences the molecular complex formation. Centers involved in the ion-dipol interactions between bioligands play also the role of the sites at which the reaction between metal and ligand proceeds, thus metal ions should be regarded as a factor which influences genetic processes.



AROMATICITY - WHAT DOES IT MEAN?

Tadeusz Marek Krygowski

University of Warsaw, Department of Chemistry, 02 093 Warsaw, Pasteura 1

The term aromaticity (aromatic character) is one of the most often used in organic chemistry. However the term is not uniquely defined. Historically the energetic criterion (resonance energy) has been first accepted as a deciding factor. Since then magnetic properties (^{1}H-NMR) and geometric (bond length averaging) ones have been in use. A short review of the quantitative approach to the term aromaticity will be presented. The most accessible property of molecules is their geometrical pattern[1]. Bond lengths may be used effectively to define the aromatic character of any π-electron system either as a whole molecule or its particular fragment. The idea is given by a scheme:

aromaticity index = 1 - GEO - EN

where **GEO** is a term which describes the dearoamtization due to the bond length alternation, whereas **EN** is the part of dearomatization due to the bond lengthening (i.e. decreasing stability of the system, or its resonance energy). In this model, a fully aromatic character has the aromaticity index = 1 and the increase of bond length alternation of the system or extension of the mean bond length in the system leads to a decrease of its value, i.e. indicates a dearomatization of the system in question.

During this presentation, an application of the model to various molecular systems - carbocyclic or heterocyclic will be provided. The problem of multidimensionality of aromaticity will be also discussed.

1. Krygowski, T.M. and Cyrański, M., in *Advances in Molecular Structure Research*, ed. M. Hargittai and I. Hargittai, JAI Press, **1997** p. 227-268

VIRAL AND HOST-CELL PROTEIN KINASES AS POTENTIAL ANTIVIRAL TARGETS

David Shugar

Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw

Despite the multitude and variety of targets available for development of antiviral agents, most of the current successes are based on the use of nucleoside analogues, which are activated by phosphorylation by viral-encoded and/or host-cell nucleoside kinases, to be illustrated by several specific examples. During the past five years considerable progress has been made in the identification of both viral-encoded and host-cell protein kinases involved in viral replication. Taken together with current progress in development of potent and specific inhibitors of cellular protein kinases, it will be shown that the protein kinases involved in viral replication are promissing targets for development of effective antiviral agents.

8-HYDROXYGUANINE IN DNA FORMED BY OXYGEN RADICALS: ITS REPAIR AND IMPLICATION IN MUTATION/CARCINOGENESIS

Susumu Nishimura

Banyu Tsukuba Research Institute (Merck), Okubo 3, Tsukuba 300-26, Japan

8-Hydroxyguanine (7,8-dihydro-8-oxoguanine, abbreviated as 8-OH-G or 8-Oxo-G) is one of the major DNA oxidation products implicated on mutagenesis induced by oxygen radical-forming agents, including ionizing radiation. It is also believed to be involved in spontaneous mutation induced by metabolically produced oxygen radicals.

8-OH-G was discovered by Kasai & Nishimura in 1983, during the course of study on isolation of mutagenic and carcinogenic heterocyclic amines produced by cooking or broiling foods. Importance of 8-OH-G in biological systems was already suggested in 1986 by its decrease in DNA during incubation of tissue culture cells after γ-ray irradiation. Misreplication of DNA by the presence of 8-OH-G was first reported by Kuchino *et al.* Later, Grollman *et al.* showed that 8-OH-G specifically induces G to T transversion.

The presence of repair system for a DNA adduct is a key indication for its biological significance. We isolated the enzyme from *E. coli* responsible for specific excision of 8-OH-G from DNA, and later found that it is the same as FPG protein. Subsequently all picture on repair systems in *E. coli* has been clarified by the effort of many workers; namely, the three mutator genes such as *mutM*, *mutT* and *mutY* were identified. *MutM* (the gene for FPG) is a glycosylase/AP-lyase (aprinic, apyrimidic lyase) that hydrolyzes the glycosidic bond of 8-OH-G and then further processes the resulting abasic site by cleaving the phosphodiester bond. The 8-OH-G:A mispair, a poor substrate for *mutM*, is repaired by *mutY*, a monofunctional DNA glycosylase that cleaves the misincorporated A residue. The product of *mutT* is 8-OH-dGTPase, which hydrolyzes 8-OH-GTP and eliminates it from the nucleotide precursor pool. The presence of extensive repair systems for 8-OH-G in *E. coli* suggested that the repair of 8-OH-G is crucial for the survival of *E. coli*.

An important question raised is whether similar repair systems for 8-OH-G exist in mammalian cells. In the case of *mutT* and *mutY*, similar genes and their corresponding proteins have been identified. Recently *mutM* homologue, *ogg1* gene and its protein (OGG1) have been identified from *Saccharomyces cerevisiae*. An attempt was made by many laboratories to identify human *mutM* homologue by similarity search using the human EST database. Aburatani and his group in collaboration with us and others also adopted the similar approach, and a mammalian homologue of 8-OH-G glycosylase/AP-lyase (*mutM* homologue, MMH) has been identified. The human MMH protein (hMMH, the same as hOGG1), 34% identical to the yeast OGG1 protein, is a member of the DNA repair protein superfamily. The *hMMH* gene was composed of seven exons, with the alternate last exon, exon 8, producing three major alternative splicing isoforms, because splicing of the sixth intron was optional. The hMMH protein was expressed in *E. coli* by transfection of the expression vector of *hMMH*. The purified protein revealed both glycosylase activity and AP-lyase activity on duplex DNA containing 8-OH-G/C. From the analysis of the cleaved product, it was concluded that the enzyme was likely to cleave the 8-OH-G-containing DNA at 8-OH-G-l-P-dN *via* β-elimination. The hMMH protein was able to rescue a spontaneous mutatior strain of *E. coli* lacking *mutM* and *mutY*. These recent work on human homologue of *mutM* will be discussed in detail.

RNA STRUCTURE OF TRINUCLETIDE REPEATS

Wlodzimierz J. Krzyzosiak, Marek Napierala, Maciej Drozdz

Laboratory of Cancer Genetics, Institute of Bioorganic Chemistry, Polish Academy of Sciences, Noskowskiego 12/14, 61-704 Poznan , Poland

The expansion of trinucleotide repeats has been shown to be the underlying cause of at least a dozen human neurological diseases (1-3), which include the Fragile X syndrome, Myotonic Dystrophy and Huntington Disease. DNA structure of the single-stranded trinucleotide repeats has been intensively studied (4) in order to understand the molecular basis of the repeat expansion which occurs during DNA replication. In contrast, a research on the structure and function of RNA bearing the repeated sequences is less advanced, and several questions regarding the significance of RNA effects in normal and pathological function of the repeats need to be answered.

Recently, we have analysed the structure of the CUG repeat region of human DMPK RNA which is implicated in pathogenesis of myotonic dystrophy. Using lead-induced cleavages and enzymatic digestions we have shown that the repeats form a novel type of hairpin structure which contains a quasistable stem (5). The stability of the hairpin increases with its length. We postulate that long hairpins, most likely complexed with the specific (CUG)n binding proteins, are important factors in the DM pathogenesis.

While analyzing human FMR-1 mRNA fragment containing CGG repeats and the AGG interruptions, we found its remarkable propensity to form different stable secondary structures. We developed two alternative protocols for RNA structure probing, in order to obtain cleavage patterns specific for the individual conformers and to determine their structures. The stable conformers were separated in nondenaturing polyacrylamide gel either before or after the probing of their structure. As shown by nuclease digestions and lead cleavages the analysed fragment of the FMR-1 mRNA forms both the single, distorted hairpin and the bifurcated Y-shaped structure.

References:

1. Richards R.I. and Sutherland G.R., Dynamic mutation: possible mechanisms and significance in human disease, Trends Biochem. Sci. 1997, 22:432 - 436.
2. Reddy P.S. and Housman D.E., The complex pathology of trinucleotide repeats, Curr. Opin. Cell Biol. 1997, 9:364-372.
3. Korade-Mirnics Z., Babitzke P. and Hoffman E., Myotonic dystrophy: molecular windows on a complex etiology, Nucleic Acids Res. 1998, 26:1363-1368.
4. Mitas M., Trinucleotide repeats associated with human disease, Nucleic Acids Res. 1997, 25:2245-2253.
5. Napierala M. and Krzyzosiak W.J., CUG repeats present in myotonin kinase RNA form metastable "slippery" hairpins, J. Biol. Chem. 1997, 272:31079-31085.

PATHWAYS FOR RNA FOLDING

Jerzy Ciesiołka

Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznań, Noskowskiego 12/14, Poland

In recent years enormous progress has been made in RNA research: the crystal structures of the hammerhead ribozyme and the P4-P6 domain of the *Tetrahymena* self-splicing intron have been solved, *in vitro* selection method has been continuing in providing new RNAs with unusual properties and unexpected structures. These achievements emphasize once again that our understanding of RNA structure and properties largely based on classic studies of tRNA molecules is far unsatisfactory. A simple model of tRNA folding pathway assumes that secondary structure elements form first and tertiary structure forms once the secondary structure elements are present. Recent studies of folding pathways of the *Tetrahymena* ribozyme, the ribozyme from RNase P and fragments of ribosomal and messenger RNAs show that the hierarchical model of tRNA folding pathway does not reflect all aspects of folding of other RNA molecules.
Recently our laboratory has been engaged in studies on structure and function of the HDV ribozymes. In particular, we are interested in RNA folding pathway by which these RNAs achieve their active conformations. We synthesized several progressively shortened 3'-truncates of the genomic and antigenomic ribozymes that may be considered as consecutive stages in the transcription of the ribozyme regions. We analyzed their structures with single- and double-strand specific RNA probes. A computer simulation of the ribozyme folding pathway was also performed based on a search for the minimal free energy of consecutive stages in the process of RNA growth. The results of experimental and computer simulation approaches were compared and discussed in terms of folding and folding pathways of the HDV ribozymes.

This work was supported by the grant No. 6 P04A 001 12 from the State Committee for Scientific Research.

ELECTRONIC NORTHERNS: GENE EXPRESSION INFORMATION FROM HIGH THROUGHPUT EST SEQUENCING

Antoni Rafalski, Mike Hanafey, Guo-Hua Miao, Ada Ching, Jian-Ming Lee, Maureen Dolan and Scott Tingey.

DuPont Agricultural Biotechnology - Genomics. Delaware Technology Park, Suite 200,
1 Innovation Way, PO Box 6104, Newark, DE 19714-6104. Email: J-Antoni.RAFALSKI@usa.dupont.com

DuPont's EST (Expressed Sequence Tag) program provides access to a large number of ESTs from major crop plants, including corn, soybean, rice, wheat. In addition to the homology of each EST to genes in Genbank, information about homology to all ESTs in the DuPont data base is available. This allows us to count the number of times each gene has been seen in different cDNA libraries, form different tissues, developmental stages or induction conditions. This quantitation of message levels is quite accurate for highly expressed messages and, unlike conventional northern blots, allows comparison of expression levels between different genes. Lists of most highly expresses genes in different libraries can be compiled. also, if EST data is available for cDNA libraries derived from different developmental stages, gene expression profiles across development can be assembled. We will present examples of such profiles for soybean seed development.

Gene expression data obtained from Electronic Northern analysis can be confirmed and extended beyond the realm of highly expressed genes by using high density DNA arrays. The ESTs identified as interesting can be arrayed on nylon or glass and probed with total labelled cDNA first strand from the tissue of interest. Two-color fluorescent labeling allows accurate mRNA ratio measurements. We are currently using the DNA array technology to study chemical induction of gene expression and the biosynthesis of oil in corn embryo.

INSPIRATIONS DERIVED FROM THE THYMIDYLATE SYNTHASE REACTION

Upendra K. Pandit

Laboratory of Organic Chemistry, University of Amsterdam,
Nieuwe Achtergracht 129, 1018 WS Amsterdam. The Netherlands.

The enzyme thymidylate synthase (TS, E.C. 2.1.1.45) catalyses the conversion of 2'-deoxyuridine-5'-monophosphate (dUMP) to 2'-deoxythymidine-5'-monophosphate (dTMP), an essential constituent for the biosynthesis of DNA. The enzyme utilizes the cofactor methylenetetrahydrofolate as the source of the *methyl group* for this transformation. The mechanistic basis of the latter reaction, which involves the sequential transfer of a *methylene* group and a *hydride* equivalent - from the cofactor to the dUMP substrate - is without precedent in organic chemistry.

As a part of a research program on models of folate cofactors, we have investigated the mechanistic and synthetic aspects of molecular systems that are capable of transferring *one carbon units* at different oxidation levels. This has led to the devlopment of a methylenetetrahydrofolate model that can transfer a *methyl group* to a suitably activated uracil derivative. This constitutes the first and only known example of a mimic of the TS reaction.

Recent mechanistic model studies of the TS reaction have inspired the development of potential enzyme inhibitors. The synthesis of these compounds and their preliminary biological activity data will be presented.

GENETIC RECOMBINATION IN (+) RNA VIRUSES

Marek Figlerowicz

Institute of Bioorganic Chemistry Polish Academy of Sciences, Noskowskiego 12/14, Poznań, Poland

Genetic recombination has been described for human, animal, plant and bacterial viruses. It can occur between genomic RNA of either the same virus, different viral strains or different viruses. Recombination between viral and host RNA was also observed. Beside an obvious role in the evolution of RNA viruses, recombination is also considered as an important factor eliminating errors arising during viral genom replication.

In spite of extensive studies conducted during last decade molecular mechanism of RNA recombination is still not well recognized. At present it is generally accepted that RNA viruses recombine according to a copy-choice mechanism. This assumes that recombinants are formed when viral replicase initiates RNA progeny synthesis from one template (RNA donor), then switches to another RNA molecule (RNA acceptor) and using it as a template resumes nascent strand elongation. On the bases of the nature of the donor and acceptor molecules (their structure and function) and sites of crossing over, RNA recombinants can be divided into three types: homologous, aberrant homologous and nonhomologous.

A great opportunity to study RNA recombination provides a unique experimental system developed in J. Bujarski's laboratory. Contrary to other systems it can efficiently induce all three types of recombinational crossovers. They occur between the genomic RNAs of brome mosaic virus (BMV). The BMV genom is composed of three RNA molecules: RNA1, RNA2 and RNA3. All three BMV RNAs share a highly structured 3' noncoding region where crossovers are targeted. Full-length cDNA clones from which infectious BMV RNAs can be synthesized are available. Recombination of the virus can be tested under different selection pressure (in systemic or local lesion host and in protoplasts).

Former studies on a mechanism of genetic recombination in BMV allowed to identify some RNA motifs involved in this process. It was demonstrated that nonhomologous recombination can be targeted by local complementarity while homologous by local homology between RNA molecules. Recently, we have shown that both BMV encoded proteins forming viral replicase are involved in recombination process. Especially interesting results have been obtained for BMV polymerase mutants which in different ways influence nonhomologous and homologous recombination. This reveals substantial differences between molecular mechanisms operating in both types of recombination. In addition some elements in RNA structure which can be responsible for site-specific nonhomologous recombination have been identified.

TOBACCO SMOKE-INDUCED DNA DAMAGE IN HUMAN TISSUES

K.Szyfter[1], Z.Szmeja[2], W.Szyfter[2], K.Hemminki[3], J.Banaszewski[2], R.Jaskuła-Sztul[1], M.Kujawski[1], P.Dąbrowski[2], A.Hirvonen[4]

[1] Inst. of Human Genetics, Polish Acad. of Sciences., Poznań, Poland
[2] Clinics of Otolaryngology, K.Marcinkowski Medical Univ., Poznań, Poland
[3] Center for Nutrition and Toxicology, Karolinska Inst., Huddinge, Sweden
[4] Institute of Occupational Health, Helsinki, Finland

Tobacco smoke contains an abundance of carcinogens capable to induce DNA lesions and chromosome aberrations in human cells. It is estimated that tobacco smoking is responsible at least for 1/3 of all types of cancer.

In 1992 we have undertaken the multilaboratory collaboration on genotoxic and clastogenic effects of tobacco smoke exposure in relation to laryngeal cancer.

In DNA extracted from tumour and non-tumour laryngeal cells there were detected aromatic and alkylated carcinogen:DNA adducts generated by polycyclic aromatic hydrocarbons and N-nitrosoamines, respectively. Formation and removal of both types of DNA adducts proceeds fairly independently that can be explained by different types of enzymes responsible for activation and detoxification of carcinogens as well as by various DNA repair mechanisms involved in DNA lesions removal.

The levels of DNA adducts appear to be strongly inter-individually varied. Thus, we turned our attention for factors affecting DNA adduct level. An extent of tobacco smoking was found to be the main cofounder modulating DNA adduct level followed by subjects gender, disease progression and age. A significance of genetic factor was proven in the course of studies on relationship between defects of genes coding detoxifying enzymes.

Fixation of DNA damage as mutations was demonstrated in tumour suppressor gene p53. Mutations in p53 gene together with loss of heterozygosity at p53 and p16 loci were found responsible for uncontrolled cell proliferation proven by expression of PCNA and Ki67 proteins known as markers of cell proliferation.

Studies on chromosomal levels provided demonstration of:

- increased chromosomal instability in peripheral blood lymphocytes of larynx cancer subjects shown by bleomycin test,
- frequent loss of Y chromosome in laryngeal tumour cells studied by fluorescent in situ hybridization (FISH),
- frequent losses of DNA copy number at the stage of lymph node metastases demonstrated by comparative genome hybridization (CGH).

The presented results support the model of multistage carcinogenesis induced by tobacco smoking in laryngeal subjects.

TETRACYCLINE-REPRESSOR ACTS AS A MOLECULAR SWITCH REGULATED BY TETRACYCLINE BINDING

W. Hinrichs, P. Orth, C. Kisker, J. Schnappinger, W. Hillen, W. Saenger
Freie Universität Berlin, Institut für Kristallographie, Takustr. 6, D-14195 Berlin and Universität Erlangen, Institut für Mikrobiologie, Staudtstr. 5, D-91058 Erlangen

Tetracyclines are a class of molecules that have antibiotic action against bacteria by binding to their ribosomal 30S subunit and inhibiting protein synthesis. Since the tetracyclines have been used and misused in the past 50 years, bacteria have developed resistance mechanisms of different types. These mechanisms are brought about by proteins encoded on moveable genetic elements like transposons or plasmids, which can be transmitted from one bacterium to the next so that a very efficient transfer of the genetic information occurs. The most common resistance mechanism in gram negative bacteria is due to a protein which is located in the bacterial membrane and exports tetracycline molecules as soon as they have entered the bacterial cell so that the tetracycline cannot reach the ribosomal 30S subunits. The biosynthesis of this membrane protein (TetA) is regulated by tetracycline repressor (TetR) which binds to a specific DNA operator sequence located in front of the gene for the TetA molecule and also in front the gene for TetR. In the absence of tetracycline, the homodimeric TetR binds tightly to the operator by virtue of two helix-turn-helix motifs, which are a frequently observed supersecondary structural element interacting with the major groove of DNA. If, however, tetracycline enters the bacterial cell, it binds very tightly to TetR, induces a conformational change that widens the distance between the helix-turn-helix motifs in the TetR homodimer so that operator DNA is no longer tightly bound and released. This permits expression of the genetic information and production of TetA protein. It is inserted into the bacterial membrane and can now export tetracycline that has entered the cell, thereby preventing binding of tetracycline to the ribosomal 30S subunit; the bacterium is now resistant against the antibiotic.

We have determined the crystal structures of TetR as such and in complexes with tetracyclines and with operator DNA. These studies show clearly how the antibiotic tetracycline is complexed with the TetR protein by a number of specific hydrogen bonds and less specific hydrophobic interactions. The geometry of the binding site explains why some tetracyclines, which are chemically modified, bind with very different association constants. The binding of tetracycline to TetR induces a conformational change by unwinding one turn of an α-helix which in turn triggers a larger movement of the helix-turn-helix motifs so that DNA operator is no longer bond to TetR. We have also determined the crystal structure of TetR complexed with operator DNA which clearly suggests why this specific DNA sequence is recognized and tightly bound by the helix-turn-helix motif of TetR.

In summary, the crystal structures of uncomplexed and complexed TetR suggest the mechanism by which the antibiotic tetracycline acts as a switch in regulating the expression of the protein TetA. It elucidates how nature makes use of small conformational changes to trigger larger movements of parts of a protein molecule that are further away and have a very profound influence on the binding strength of TetR to operator DNA. This system shows that we can learn much in the field of supramolecular chemistry if we have deeper insight into functional mechanisms that are used by biological systems.

1. W. Hinrichs, C. Kisker, M. Düvel, A. Müller, K. Tovar, W. Hillen, W. Saenger, Structure of the Tet-repressor-tetracycline complex and regulation of antibiotic resistance. Science 264: 418-420 (1994).
2. Kisker, W. Hinrichs, K. Tovar, W. Hillen, W. Saenger, The complex formed between Tet repressor and tetracycline-Mg^{2+} reveals mechanism of antibiotic resistance. J. Mol. Biol. 247: 260-280 (1995).

EFFECTS OF PROMOTER DNA BENDING ON TRANSCRIPTION INITIATION BY *E.COLI* RNA POLYMERASE

Kazimierz L.Wierzchowski

Institute of Biochemistry & Biophysics, Polish Academy of Sciences,
ul.Pawińskiego 5a, 02-106 Warszawa, Poland

Bending of DNA by repetitive A_n .T_n (n = 5,6) tracts located upstream the -35 promoter region is known to be an important factor involved in regulation of transcription in procaryotic systems. On the other hand, such bending sequences were found rather rarely within the promoter sequences of *E. coli* promoters. It seemed thus worth to attempt to use perturbation of the promoter DNA structure by these sequences as a structural probe in studies on molecular interactions involved in formation of the transcription initiation complexes with the cognate RNA polymerase (RNAP). For this purpose a set of consensus-like *E.coli* promoters carrying A_n .T_n (n = 4-6) sequences located in different orientation in one or more functional promoter regions (the -35,-10 and spacer domains) has been constructed and used in comparative *in vitro* investigations on: the gross-structure of the open complexes and the kinetics and thermodynamics of their formation and dissociation, by the PAGE gel-shift and steady-state kinetic methods, respectively. The results of these studies will be shortly summarized. They have indicated that the most pronounced effect on the promoter gross-structure (lowering of PAGE mobility) is exerted by T_4[-34...-37] and T_5[-34...-38] tracts located in the nontemplate DNA strand and overlapping partially the -35 consensus hexamer TTGACA. In connection with the available 3D low-resolution model of RNAP this effect can be attributed to a perturbation of the subtle specific interactions between (i) the -35 recognition hexamer and the hypothetical helix-turn-helix motif of the σ^{70} subunit and (ii) the upstream DNA helix with the α subunit of the enzyme. Bending sequences were also found to influence significantly both the kinetic and thermodynamic parameters of open complex formation, the magnitude of which depends on the number and localization of the bending tracts within the promoter sequence. However, the rate constants for dissociation of the open complex proved practically independent of the presence of these tracts. This indicates that once an open complex has formed, the promoter DNA therein adopts the same conformation irrespective of its bendability.

THE RNA HYDRATION. TWO EXAMPLES

Ryszard W. Adamiak

Institute of Bioorganic Chemistry, Polish Academy of Sciences,
Noskowskiego 12/14, 61-704 Poznań, Poland.

Till present, only four X-ray RNA structures have been solved at the resolution higher than 1.50 Å, which allows to analyse the pattern of RNA hydration precisely(1-4). In contrast to the DNA field (5), no NMR studies conducted to observe the phenomena of RNA hydration have been reported so far. Molecular dynamics sinulation in aqueous solution is a powerful tool to study RNA hydration. However, examples of the latter approach are still rare (6,7). The choice of appropriate force field and calculation protocols and enormous processor time necessary to search the conformational space are major barriers of this method.

Recently, our laboratory has got interested in the elucidation of specific RNA/water interactions using those approaches; stress is laid not only on the RNA structure but also on the behaviour of single water molecules within the structural shell. An analysis of interactions and dynamics of particular water molecules should add new information on factors governing the RNA folding.

Two examples of RNA hydration studies will be shown.

Example I. The architecture of the water net in the crystal structure of 2'-O-Me$(CGCGCG)_2$ - the modified RNA duplex at 1.3 Å resolution (3). Solution structure of this duplex was recently reported (8).

Example II. The dynamics of water sorrounding the bulge loop region of a RNA duplex analysed by simulation of molecular dynamics in aqueous solution (9).

References

1. Wahl,M.C., Rao,S.T. and Sundaralingam,M. (1996) *Nature Structural Biology*, **3**, 24-31.
2. Egli,M., Portman,S. and Usman,N. (1996), *Biochemistry*, **35**, 8489-8494.
3. Adamiak,D.A., Milecki,J., Popenda,M., Biała,E., Adamiak,R.W., Dauter,Z. and Rypniewski,W.R. (1997) *Nucleic Acids Res.*, 25 (22), 4599-4607.
4. Correll,C.C., Freeborn,B., Moore,P.B. and Steitz,T.A. (1997) *Cell* , 91, pp. 705-712,.
5. Wuthrich,K., Billeter,M., Guntert,P., Luginbuhl,P., Rick,R. and Wider,G. (1996) *Faraday Discuss*, 103, 245-253.
6. Auffinger,P., Louise-May,S. and Westhof,E. (1996) *J. Amer. Chem. Soc.*, 118, 1181-1189; and references therein.
7. Cheathan,T.E. and Kollman,P.A. (1966) *ibid*, 119, 4805-4825; and references therein.
8. Popenda,M., Biała,E., Milecki,J. and Adamiak,R.W (1997) *Nucleic Acids Res.*, 25 (22), 4589-4598
9. Bielecki,Ł and Adamiak,R.W. *unpublished*

STRUCTURE OF IRE STUDIED BY COBALT (III) HEXAMMINE BINDING, MOLECULAR MODELING AND NMR SPECTROSCOPY

[2]Zofia Gdaniec, [1]Hanna Sierzputowska-Gracz, [1]Elizabeth. C Theil

[1] Department of Biochemistry , North Carolina State University, Raleigh, NC 27695,
[2]Institute of Bioorganic Chemistry, Polish Academy of Science , Poznan. Poland

The IRE (iron responsive element) family of iso-elements is a well characterized control element in normal animal mRNAs encoding proteins of iron metabolism. All IREs recognize a family of RNA binding proteins, the IRPs (iron regulatory proteins); some IREs recognize other proteins as well, such as initiation factors. Single-copy IREs in the 5'-untranslated regions of mRNAs regulate ribosome binding while pentuple-copy IREs in the 3'-untranslated regions are part of a rapid turnover element regulating mRNA stability. The ferritin IRE is a highly conserved (96-99% in vertebrates) hairpin loop with a conserved C residue and terminal hexaloop (CAGUGU); The translation regulatory element is found in animal m-rna. Molecular modeling (using MC-SYM and DOCKING) and the heteronuclear multidimensional NMR spectroscopy were used to stydy the ferritin IRE both using unlabeled and $^{13}C/^{15}N$ labeled 30 mer ferritin IRE synthesized by T7 polymerase (1).

Co(III)hexammine was added to the RNA in some experiments, as a model for hydrated Mg^{2+} ion-solvated metal ions, often important for the proper folding and function of RNA. A complete model of the IRE-30 mer was developed which shows a dynamic G-U base pair and G-C base pair, conserved in ferritin IREs that spans an internal loop/bulge in the middle of an A-helix. Based on effects of Co(III)hexammine on the ^{1}H-NMR spectrum and results of automatic docking into the IRE model, we concluded that the IRE bound Co(III)hexammine, at the pocket in the major groove of the IRE (2). Mg^{2+} may bind to the IRE at the same site, based on analogy to Co(III)hexammine and the Mg^{2+} inhibition of $Cu(phen)_2$ cleavage at the site. Distortion of the IRE helix by the internal loop/bulge near a conserved unpaired C required for IRP binding and adjacent to an IRP crosslinking site suggests a role for the pocket in ferritin IRE/IRP interactions.
Understanding the relation of IRE structure to function and protein binding provides a particularly attractive model for the other mRNA's and interactions between regulatory protein and mRNA.

1.Sierzputowska-Gracz, H., McKenzie, A., Theil, E.C. (1995) *Nucl. Acids Res. 23,* 146-153.

2. Gdaniec, Z., Sierzputowska-Gracz, H., Theil, E.C. (1998) *Biochemistry. 37,* 1505-1512.

ENGINEERING OF NOVEL PROTEOLYTIC SPECIFICITIES OF TRYPSIN

Jean-Marc Chobert and Thomas Haertlé

Groupe Protéines, LEIMA, Institut National de la Recherche Agronomique, BP 71627, 44316 Nantes Cedex 03, FRANCE.

The aspartyl 189 residue of trypsin (S_1) is crucial for substrate orientation and specific lysis of its Arg-X and Lys-X bonds (P_1). Sequence alignments of other serine proteases reveal that the vicinal amino acid residue 188 (S_2) is highly conserved and can be either a lysine or an arginine. Consequently, K188 was replaced with aromatic amino acids or histidine in order to change the interactions of the substrate binding site and, hence, modulate catalytic properties of this protease. The catalytic properties of mutated trypsins K188F, K188Y, K188W and K188H were determined with tetrapeptide synthetic substrates and natural substrate β-casein. Kinetic analysis show that all the mutants conserved the capacity to split peptide bonds involving arginyl and lysyl residues. The optimal pH of activity changed considerably according to the mutation. As shown by proteolysis of the natural substrate, all aromatic mutants acquired capacity to hydrolyse β-casein at C-termini of amidated amino acids (Q and N). The same lysyl residue 188 was replaced with histidine in order to build a metal chelation site in the substrate binding pocket of trypsin. K188H mutation does not affect catalytic efficiency at all, changing only slightly the specificity of the mutant protease. In the presence of Cu^{2+}, trypsin K188H exhibits a 30 to 100-fold increase of K_m, while k_{cat} is only slightly decreased. Hydrolytic activity of this mutant can be fully restored by addition of EDTA. Thus, in contrast to chelation of active site, different mode of metal-dependent inhibition of the activity of trypsin by building a co-ordination site in the substrate binding pocket of the protease was achieved.

SEQUENCE DEPENDENT STRUCTURAL AND BIOCHEMICAL PROPERTIES OF DNA

Kazimierz Grzeskowiak

Centre of Structural Biology and Bioinformatics, NIBH-AIST, Tsukuba , Japan
and
Molecular Biology Institute, University of California Los Angeles,
Los Angeles, CA 90095, USA*

Crystallization of DNA oligomers is a process involving recombination of complementary DNA strands and the nucleation of the DNA double helices into condensation state.

Porschke at all (D. Porschke, O. C. Uhlenbeck, and F. H. Martin, Biopolymers, 1973, 12, 1313-35.) results on the recombination and nucleation of RNA oligomers were implied to the crystallization of DNA oligomers. The kinetics of helix formation from their work indicate that the recombination rate of given chain length is faster when two GC pairs are at the end of a helix than when they are in the middle.

Structural analysis of the first set decamers, CGATCGATCG, CGATTAATCG, and CGATATATCG revealed the very tight packing and parallel helix to helix interactions caused by presence of CG pairs at the ends of helix. Three oligomers offered the opportunity to study different sequences in the same crystal environment. Structures of these decamers have strengthened the evidence supporting the large conformational flexibility of the TA step.

Structures of second set of oligomers, CTCTCGAGAG and CTCAGCTGAG revealed that when the only one GC pair is present in the middle of sequence the double stranded helices packed in crystals with the crossing angle between helices of 42° and 90° respectively. Additionally the effect of cations on the compactness of DNA oligomers has been observed in CTCTCGAGAG decamer and its external and internal morphology may be related to thermal stability of DNA.

Among of several DNA oligomers of 10 to 20 base pairs long the two of them, CTCAGCTGAG and its double repeat CTCAGCTGAGCTCAGCTGAG contain CAG triplet that are thought to cause Huntington's disease when this triple (CAG)n is expended on chromosomes up to more than 40 repeats. These two oligomers show different patterns of compactness in crystal form.

This work was possible to present thanks to support from Dr. Masashi Suzuki, NIBH-AIST and Dr.Richard E. Dickerson, UCLA.

* - Mailing Address

RAPID PROTEIN CRYSTAL STRUCTURE DETERMINATION USING 3RD GENERATION SYNCHROTRON SOURCES

M. A. Walsh, I. Dementieva, G. Evans, R. Sanishvili, and A. Joachimiak

Argonne National Laboratory, Structural Biology Center, Argonne, IL 60439 USA.

Recent developments at 3rd generation synchrotron sources bring a promise to revolutionize protein structure determination. These instruments provide well collimated, very bright, stable, and tunable x-ray sources. Synchrotron radiation from the Structural Biology Center's undulator beamline was used to collect a multiwavelength anomalous diffraction (MAD) data at 100K. The four wavelength experiment was completed to 2.25 Ĺ resolution in 23 min. on a single crystal of 16 kDa protein containing three SeMet residues. The SeMet-labeled protein from a thermophilic bacteria was produced in the *E. coli* over expression system that was supplemented with SeMet under conditions known to suppress *E. coli*"s methionine biosynthesis and resulted in a 93% incorporation of SeMet into protein. Protein was purified in two steps and it crystallized readily in the orthorhombic space group $C222_1$ with unit cell parameters a=62.6 Ĺ, b=64.8 Ĺ, c=74.3 Ĺ, a=b=g=90°. At each wavelength, in a single pass, sixty 2° oscillation images were collected in 345 sec using mosaic 3x3 CCD detector. Data were processed with HKL2000. Two of the three selenium sites were located automatically using the program RSPS in CCP4 suite. These sites were refined with the MLPHARE which give an overall FOM of 0.72 for data between 10-2.25 Ĺ. The overall map correlation coefficient of the solvent flattened map produced with the DM and the map calculated from coordinates of the refined model is 0.82. Further evidence of the high quality of the data were obtained with the program wARP which was used first to extend phases to 1.7 Ĺ, and then automatically trace a complete main chain of the protein with polyalanine/serine. This ultra rapid MAD data collection has recently been successfully executed at the SBC undulator beamline for two other larger protein structures. The high photon brilliance, very fast CCD detector, newly designed efficient data reduction software combined with multi-cpu computing environment allows for new approach to synchrotron data collection. The complete MAD experiment including data acquisition, processing, and initial phase determination may take less than a day. The approach provides a basis to revolutionize protein structure determination using x-ray crystallography at the synchrotron facilities.

MOLECULAR RECOGNITION MOTIFS IN NUCLEOSIDE SALTS

Mariusz Jaskolski

Center for Biocrystallographic Research, Institute of Bioorganic Chemistry, Pol. Acad. Sci. and Department of Crystallography, A.Mickiewicz Univ., Poznan, Poland

A subject of dividing controversy among structural scientists earlier, C-H...Y hydrogen bonds are now widely accepted and even believed to play decisive, scale-tipping roles in a number of processes that require precise molecular recognition. An excellent example of the use of C-H...Y interactions in molecular mimicry was provided by the crystal structures of adenosinium and ethenoadenosinium chlorides which are isostructural in spite of drastic differences in the cation structure [1]. Recently, the issue of C-H...Y interactions in nucleic acids has provoked several exciting studies, such as those by Hunter on C-H...Y bonds in base pairing [2] or by Seddon in his critique [3] of the communication by Kool [4] questioning the role of even canonical hydrogen bonds in nucleic acids structures. This paper will describe a characteristic motif, a supramolecular synthon, recurring in crystal structures of cytidinium salts with composite anions and will demonstrate that it can be realized by both conventional and C-H...Y hydrogen bonds. In its classic form, ($N3^+$-H..., N4-H41...)...Y_2X, this pattern, consisting of two hydrogen bonds (from the $N3^+$-H protonation site and from the *cis* N4-H41 group) accepted by one composite anion, XY_n, was first recognized by Sundaralingam [5] in the crystal structure of 2'-deoxycytidine 5'-monophosphate monohydrate. We have later confirmed its existence in a number of crystal structures of cytidinium and 2'-deoxycytidinium salts with anions as diverse as $H_2PO_4^-$, NO_3^-, SO_4^{2-}, SiF_6^{2-}, ClO_4^- and BF_4^-. Encouraged by the prevalence of this motif on the active, $N3^+$-H, side of the cytosinium cation, and by the recent demonstration by Desiraju [6] that favored supramolecular synthons will also prevail when classic hydrogen bonds are replaced by C-H...Y interactions, we have re-examined the (2'-deoxy)cytidinium salts focusing on the biologically inactive, C5-H, side of the cation. The results indicate that in many cases the (C5-H..., N4-H42...)...Y_2X motif is indeed formed, confirming the importance of C-H...Y hydrogen bonds in molecular recognition.

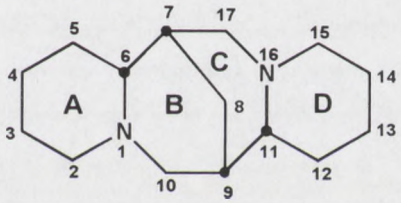
[1] M.Jaskolski, *J. Cryst. Spectr. Res.* **14**, 45-57 (1984).
[2] G.A.Leonard, K.McAuley-Hecht, T.Brown, W.N.Hunter, *Acta Cryst.* D**51**, 136-139 (1995).
[3] T.A.Evans, K.R.Seddon, *Chem. Commun.* 2023-2024 (1997).
[4] S.Moran, R.X-F.Ren, E.T.Kool, *Proc. Natl. Acad. Sci. USA,* **99**, 10506-10511 (1997).
[5] M.A.Viswamitra, B.S.Reddy, G.H.-Y.Lin, M.Sundaralingam, *J. Am. Chem. Soc.* **93**, 4565- 4573 (1971).
[6] K.Biradha, A.Nangia, G.R.Desiraju, C.J.Carell, H.L.Carell, *J. Mater. Chem.* **7**, 1111-1122 (1997).

BIS-QUINOLIZIDINE ALKALOIDS - NEW DERIVATIVES

Władysław Boczoń

Department of Stereochemistry, Faculty of Chemistry
A. Mickiewicz University, Poznań, Poland

Bis-quinolizidine alkaloids feature a complex spatial structure, which, depending on conditions, may undergo specific changes.



The basic bis-quinolizidine alkaloid: SPARTEINE

Most common of them is an inversion of nitrogen atom joining the C, D rings of the molecule. Its dynamic stereochemistry was of our special interest. This demanded determination of a degree at which even small structural changes may affect the stereoelectronic properties as well as basicity of the sparteine (bis-quinolizidine) system. For this reason, several new sparteine derivatives were obtained and studied.

LUPIN ALKALOIDS AT PRESENT

Waleria Wysocka

Adam Mickiewicz University, Faculty of Chemistry,
Grunwaldzka 6, 60-780 Poznan, Poland

Development of a wide variety of new spectroscopic methods, particularly the experimental NMR techniques is of crucial importance for the natural product chemistry. These methods allowed a complete elucidation of the structure of several quinolizidine alkaloids isolated from seeds of a new variety of *Lupinus albus*. These alkaloids contain either the skeleton of sparteine or cytisine with the γ-oxo-α,β-enamine or γ-pyridone systems in ring A of the molecules. The NMR techniques became also a powerful tool for determination of stereochemistry (conformational equilibria and geometry) of these alkaloids. The question of the conformation of alkaloids with a sparteine skeleton was qualitatively covered by Wiewiórowski et al. in '60s and '70s.

A method based on ^{1}H and ^{13}C NMR spectroscopy provided us with a possibility of a quantitative determination of conformational equilibria in 12 bis-quinolizidine alkaloids and some their salts. In view of the three criteria, i.e. ^{13}C chemical shifts for C12 and C14 atoms as well as the coupling constant H1-H17β, the results were consistent. Using the modified Karplus (Haasnoot) equation we also determined the geometry of tricyclic quinolizidine-piperidine alkaloids in solution which did not differ very much from that in the solid state and that obtained by MM calculations.

The modern spectroscopic methods allowed a full determination of stereochemistry of reduction, methylation and halogenation of multiflorine - an alkaloid containing a γ-oxo-α,β-enamine system in ring A.

The knowledge of the precise stereochemistry of alkaloids under investigation afforded a possibility to study their biological activity as well as that of some of their analogs (e.g. containing sulphur).

METALATION OF BIOGENIC AMINES INVESTIGATED IN SOLID STATE BY FT-IR AND X-RAY

Anna Perkowska, Hanna Małuszyńska,* Maria Danuta Bratek-Wiewiórowska and Maciej Wiewiórowski

Institute of Bioorganic Chemistry, Polish Academy of Sciences, Noskowskiego 12/14, 61-704 Poznań.
* Institute of Physics, A. Mickiewicz University, Umultowska 85, 61-614 Poznań.

For several years work in our laboratory has been directed toward the understanding of the structure of polyamines and nucleic acid. The initial impetus for this research was the discovery, that polyamines play a crucial role in various biological processes such as stabilizing the secondary and tertiary structures of nucleic acid. The specific function of polyamines is still obscure, but their ubiquitous distribution and their high concentration in cells have stimulated research on them.

In our first approach we studied the conformation of protonated polyamine with different counter anions in the crystal structures (1).

The next step was to perform a comparison between the structrure of perprotonated and metalated polyamines. So we obtained a series of polyamines complexes with the salts of bivalent metals and we undertook the crystallographic and spectroscopic studies of them (2, 3, 4).

In this paper we present crystal and molecular structures of spermine ^{343} complexes (Spm^{343} $=NH_2\text{-}(CH_2)_3NH(CH2)_4NH(CH_2)_3NH_2$) and their analogues: Spm^{323} , Spm^{333}, with $Cu(NO_3)_2$, $Cu(ClO_4)_2$, $CuSO_4$, $NiSO_4$ and we discuss the relation between protonation and metalation , the influence of the cations and anions and the length of polyamine chain on the structure of these complexes and the role of H-bonding and other weak interactions existing in these compounds.

Literature:

1. M.D. Bratek-Wiewiórowska, M. Alejska, M. Figlerowicz, J. Barciszewski, M. Wiewiórowski, M. Jaskólski, W. Zielenkiewicz, A. Zielenkiewicz, Pure Appl. Chem. 59, 1987, 407.
2. Małuszyńska, A. Perkowska, E. Skrzypczak-Jankun, J. of Chem. Cryst. 25, 1995, 19.
3. Perkowska, H. Małuszyńska, J. of Chem. Cryst. - submitted for publication 1998.
4. K. Surma, A. Perkowska, M.Gawron , Polish J. Chem. 68, 1994, 481.

THE DIFFERENT PATTERNS OF INTERACTIONS OF HYDROGENPHOSPHATE ANIONS WITH PROTONATED BIOGENIC DIAMINES PUTRESCINE AND ITS NOR-HOMOLOGUE

Marek Figlerowicz, Magdalena Alejska, Maria D. Bratek-Wiewiórowska, Maciej Wiewiórowski

Institute of Bioorganic Chemistry Polish Academy of Sciences, Noskowskiego 12/14, Poznañ Poland

Presented studies concern the hydrogenphosphate salts of two biogenic polyamines, i. e. putrescine (Put, 1,4-diaminobutane) and its desmethylene homologue called nor-putrescine (nPut, 1,3-diaminopropane), which exists beside or instead of Put in thermophilic organisms. We intended to find out the way in which the relatively small change in the structure of the diprotonated Put and nPut cations can influence the pattern of their interactions with the hydrogenphosphate dianion. We also tried to determine whether the fact that nPut can act as a substituent for Put in thermophilic organisms would have an effect on the structure and properties of the salts under formation. Our studies were based on the results obtained from x-ray analysis, infrared spectroscopy and thermogravimetric measurement.

Undertaken comparative structural analysis of nPut and Put salts reveals that there are some similarities only in the case of interactions between cationic and anionic parts. Both protonated diamines directly interact with phosphate dianions by means of analogous hydrogen bonds N-H...O-P. However in this case we also observe one significant distinction. The Put forms hydrogen bonds exclusively with the phosphate dianions, while nPut interacts not only with the anionic part but also with water molecules. Other interaction systems formed between particular components of studied salts are of clearly different character. In nPut phosphate, dianions are coupled by means of P-O-H...O-P hydrogen bonds forming infinite polyanionic chains, whereas in Put phosphate the direct interactions between anions are not observed. Water molecules present in the crystal network of Put salt interact with the anionic part and with each other, while in the crystals of nPut salt one-water bridges link cationic and anionic parts.

IR studies indicate that release of the water molecules from Put phosphate leads to crucial changes in its crystal network although even after complete dehydration the salt remains a crystalline compound. In nPut phosphate the first stage of the dehydration does not lead to the significant structural variation. However further removing of the crystal water molecules from the partially dehydrated substrate is connected with deep physical and chemical transformation of the salt.

THE INVOLVEMENT OF WATER MOLECULES IN INTERACTIONS BETWEEN PHOSPHATE DIANIONS AND EITHER BIOGENIC DIAMINE OR MAGNESIUM CATIONS

Marek Figlerowicz, Ewa Utzig[1] Magdalena Alejska, Maria D. Bratek-Wiewiórowska, Maciej Wiewiórowski

Institute of Bioorganic Chemistry Polish Academy of Sciences, Noskowskiego 12/14, Poznań Poland
[1] Institute of Physical Chemistry Polish Academy of Sciences, Kasprzaka 44/52, Warsaw, Poland

Phosphates play a very special role in all living organisms because most biologically active molecules are constantly or temporarily phosphates derivatives. This unusual commonness of phosphates indicates their non-specific character, while their participation in many biochemical processes requires very specific and often unique properties. Previously, we have demonstrated that negatively charged phosphates can acquire this specificity by interaction with different types of cations. In addition, it is generally accepted that the hydration system can also strongly affect the structure of biomolecules. That is why, we have decided to check whether the structure and properties of the cations can influence the behaviour of water molecules solvating phosphate anions. Our studies are of a model character and concern hydration systems existing in the hydrogenphosphate salts of magnesium and two biogenic polyamines i.e. putrescine (Put) and nor-putrescine (nPut). In each salt, the anionic part is the same, whereas the three dications which make up the cationic part are different in terms of their physical, chemical and biological properties. Our comparative studies of the hydration systems were based on x-ray data and IR, NMR and calorimetric measurements.

Using x-ray data we could determine some principal differences in the location of the water molecules in the crystal networks of the phosphates under study. However, only by application of the other methods were we able to detect the nature and size of these differences. We observed that water molecules are particularly strongly bound in nPut phosphate although each molecule is linked by three hydrogen bonds only. A hydration system existing in magnesium salt where each water molecule is built into the crystal network not only by means of three hydrogen bonds but also by a coordinate bond is visibly less stable. Especially an unstable hydration system is present in Put salt. In this case water molecules can be released from crystal even at room temperature if relative humidity of the atmosphere is reduced to about 5%. We also tested how Put, nPut and magnesium cations behave when they are forced to interact with phosphate dianion in the absence of water molecules. We observed that anhydrous Put hydrogenphosphate is crystalline up to 180 C when it starts to convert into pyrophosphate. Magnesium cations and phosphate dianions can form a well-ordered interaction system only in the presence of water molecules; hence anhydrous salt is amorphous. Unexpectedly, we found that pure nPut hydrogenphosphate does not exist as a completely anhydrous salt, although maximally dehydrated (in 70-80 %) is still a crystalline compound. Moreover we have discovered, that contrary to anhydrous magnesium phosphate, maximally dehydrated nPut and anhydrous Put salts can undergo rehydration when placed in an atmosphere of 86% relative humidity. The rehydration process takes place in the solid and leads to the reconstruction of the hydrated salts structure.

STRUCTURAL STUDIES OF A MODEL POLYAMINE...PHOSPHATESYSTEM

E.Bartoszak-Adamska[1], M.Figlerowicz[2], M.Wiewiórowski[2], T.Gustafsson[3], I.Olovsson[3], M.Jaskólski[1,2]

[1]Department of Crystallography, A.Mickiewicz Univ., [2]Institute of Bioorganic Chemistry, Pol. Acad. Sci., Poznan, Poland, [3]Institute of Chemistry, Univ. of Uppsala, Sweden

Biogenic polyamines are simple organic compounds occuring in all living organisms. It is known that polyamine levels suddenly increase in rapidly growing cells which indicates that they can be engaged in cell maturation or differentiation processes. Aliphatic polyamines, such as spermine and spermidine, are able to influence the secondary and tertiary structure of nucleic acids. Being strong bases, they exist in physiological pH as polycations which can interact with the negatively charged sugar-phosphate backbones of nucleic acids. However, unlike metal cations, polyamines cannot be treated as point charges. Perprotonated polyamines possess two, three or four separated charges. Their spatial arrangement depends on the conformation of the carbon chain linking the ammonium groups. The molecules of these amines contain the -$N(CH_2)_4N$- unit alone [putrescine $NH_2(CH_2)_4NH_2$] or in combination with -$(CH_2)_3N$- (spermidine, spermine). This renders the molecules quite flexible and allows them to adopt many different conformations. The diprotonated desmethyl homologue of putrescine (Put), called nor-putrescine (nPut), has been studied crystallographically in a number of salts. Here we describe the structure as well as thermal stability and spectroscopic properties of the $nPutH_2^{2+}$ cation in its hydrated monohydrogenphosphate salt ($nPut.2H^+.HPO_4^{2-}.H_2O$). The crystal structure has been determined for the protonated as well as (O,N)-deuterated analogs at two different temperatures, 290 and 25 K. Exchange of the mobile hydrogen atoms for deuterium strongly affects the thermal stability of the salt. The cation exists in an extended all-*trans* conformation. It occupies a general position in the crystal but deviates only slightly from C_2 symmetry. The structure contains a 3D network of H bonds in which all available proton donors are engaged. On its N2 end, the cation forms three H bonds with three different anions. Those hydrogen bonds have relatively similar parameters and can be classified as medium strong. The H bonds formed by the -$N1H_3^+$ donor cover a somewhat wider range of N...O distances and include a bifurcated component. The hydroxyl group of the $HOPO_3^{2-}$ ion is a donor in a relatively strong H bond with another anion and does not show any acceptor activity. Each of the remaining phosphate O atoms accepts three H bonds but the character of the bonds accepted at each center is quite different. A characteristic motif in the crystal packing is an infinite chain of H-bonded anions running along **c**. All unit-cell lengths increase on deuteration. The relatively largest change is the increase in the *c* lattice constant. The shortest H bond is the phosphate...phosphate O-H...O bond (2.63Å). This corresponds to an asymmetric, double minimum type for which an expansion is expected on deuteration. As this H bond has its major component along **c**, the expansion of this bond on deuteration is the main factor responsible for the increase of the *c* lattice parameter. The unit-cell volume as well as the *a* and *b* parameters contract on cooling from 290 to 25 K, whereas the *c* parameter expands slightly. The unusual behavior of the *c* parameter is probably related to ionic repulsions. The ions stacked in the **c** direction are of the same polarity, whereas alternating cationic and anionic components populate the **a** and **b** directions. Thus, the increased electrostatic attraction accompanying the thermal contraction of *a* and *b* is possibly the dominating force, whereas the increased separation of the ions along **c** is a secondary effect, which partially relieves the coulombic repulsion.

STRUCTURE AND PROPERTIES OF CYTIDINIUM HYDRODIFLUORIDE

M. Gawron, M. D. Bratek-Wiewiórowska, J. Sarzyńska and M. Wiewiórowski

Institute of Bioorganic Chemistry, Polish Academy of Sciences,
Zygmunta Noskowskiego 12/14, 61-704 Poznań, Poland

This work is part of our spectroscopic and crystallographic studies on the influence of counter-anions on the conformation and properties of protonated nucleosides [1-4]. This paper is focused of the crystalline cytidinium hydrodifluoride salt ($CydH^+ HF_2^-$).

To obtain crystalline cytidinium mono-hydrodifluoride, an excess (3 to 6 times) of HF is needed. However, re-crystallization of cytidinium mono-hydrodifluoride usually leads to trans-crystallization procedure, producing cytidinium hemi-hydrodifluoride ($Cyd_2H^+ HF_2^-$) with some amount of Cyd.

To better characterize the release of the hydrogen fluoride molecule from the salts under discussion, additional temperature studies of mono- and hemi-hydrodifluoride salt were carried out and monitored by FTIR-PAS spectroscopy. Finally, the crystal and molecular structure of cytidinium hydrodifluoride has been determined by X-ray analysis.

The crystals are monoclinic, space group $P2_1$. X-ray diffraction data were collected on a KM4 diffractometer using CuK_α radiation. The structure was solved by direct methods and its model was refined to final R = 0.035. All hydrogen atoms were located from difference-Fourier maps and included in the refinement.

Protonation of the cytosine moiety at the N(3) site induces changes in the delocalization of the π-electrons in this region: the electron charge is increased between C(2) and O(2) and, simultaneously, decreased between C(2) and N(3). It means that the resonance structures with negative charge located on O(2) have negligible contribution to the resonance hybrid of the cytidinium cation. This situation is reflected by the distribution of bond lengths and angles in cytidinium hydrodifluoride and in cytidine [5].

The ribofuranose ring adopts a twist conformation with major C(2')-endo and minor C(3')-exo puckering ($^2T_3$): P = 166.0(8)° and τ_m = 42.9(6)°. This conformation is close to the ideal C(2')-endo conformation with P = 162°. Relative to the sugar moiety, the pyrimidine ring is in *-ac* conformation, the torsion angle O(4')-C(1')-N(1)-C(2) (χ) being -134.8(2)°. The conformation about the C(4')-C(5') bond is *+sc*, the torsion angle C(3')-C(4')-C(5')-O(5') (γ) being 54.5(2)°.

Electrostatic forces are the most important factors arranging the ions in the crystal, where the cations and anions form alternating layers. At the same time, the crystal lattice is built in a way that enables all proton donors to be involved in hydrogen bonding. The hydrodifluoride anion is cemented with a strong, formally symmetrical, hydrogen bond with F···F equal to 2.257(2) Å.

1. Wiewiórowski, M., Bratek-Wiewiórowska, M.D., Alejska, M., Perkowska, A., Krzyżosiak, W., Chemica Scripta **26**, 229-240, 1986
2. Bratek-Wiewiórowska, M.D., Alejska, M., Malinowska, N., Sarzyńska, J., Figlerowicz, M., Utzig, E., Zielenkiewicz, W., Wiewiórowski, M., Tetrahedron **48**, 4893-4904, 1992
3. Jaskólski, M., Wiewiórowski, M., Proceedings of the 8th International Symposium on Organic Crystal Chemistry, Poznań-Rydzyna, 203-217, 1992
4. Sarzyńska, J., Ph. D. Thesies, IBCh, Poznań, 1996
5. Ward D.L., *Acta Cryst.* (1993), **C49**, 1789-1792

POLYMORPHISM OF 2'-DEOXYCYTIDINE VERSUS MONOMORPHISM OF CYTIDINE

Magdalena Alejska, Maria D.Bratek-Wiewiórowska, Maciej Wiewiórowski.

Institute of Bioorganic Chemistry, Polish Academy of Sciences Noskowskiego 12/14, 61704 Poznań, Poland

We have no doubt that cytidine (Cyd) crystallizes only in one and the same monomorphic form. Molecules are arranged in clusters: one central molecule is surrounded by six analogous molecules of Cyd, which have an identical sugar conformation.
However it has been found that 2'-deoxycytidine (dCyd) crystallizes in three different forms, designated: dCydA [1], dCydB [2] and dCydC·H_2O [3].
The third form dCydC·H_2O, differs from dCydA and dCydB not only because of the presence of crystalline water molecules but also because it contains only one conformer in unit cell.
The three forms of dCyd are characterized by different conformation of the sugar residue and by significantly different networks of hydrogen bonds.
In the crystal lattice of dCydC·H_2O, each crystalline water molecule acts as a double proton donor and a single proton acceptor bridging directly three molecules of dCyd by hydrogen bonds. Apart from these three hydrogen bonds involving the participation of a crystalline water molecule, there are also three other hydrogen bonds linking the nucleosides directly.The hydrate of dCydC is easily transformed into a fully dehydrated form: dCydC-anhydro.
dCydC-anhydro can be rehydrated to crystalline dCydC·H_2O by placing it for only 2 minutes at RT over saturated aqueous KCl .
The de- and re-hydration processes have been monitored by FT-IR-PAS spectroscopy.
In the solid state, under suitable conditions even five forms of 2'-dCyd could be obtained: dCydA, dCydB, dCydC·H_2O, dCydC-anhydro and dCydD.
The conditions of transformation in the solid state of one form of dCyd into another have been precisely determined and a comparative analysis of all the forms has been carried out. The significant differences and similarities between all the forms of dCyd and Cyd can be visualized in the FT-IR-PAS spectra.

References:
[1]. D.W.Young, H.R. Wilson Acta Cryst., sect. B, 31, (1975), 961.
[2]. K.Surma, M.Alejska, M.Gawron, Polish J.Chem. 68, (1994), 2553
[3]. M.Gawron, M.D.Bratek-Wiewiórowska, Acta Cryst., in preparation
[4]. M.Wiewiórowski, M.Alejska, N.Malinowska, M.D.Bratek-Wiewiórowska, J.Mol. Struct. 436-437, (1997), 627-635

FORM *A* OF 2'-DEOXYCYTIDINIUM SULFATE. STRUCTURE AND BEHAVIOUR.

M. Gilski[1,2], M.D. Bratek-Wiewiórowska[1], J. Sarzyńska[1], M. Wiewiórowski[1]

[1]Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznań, Poland
[2]Department of Crystallography, Faculty of Chemistry, A. Mickiewicz Univ., Poznań, Poland

This work is a part of our general conformational studies of protonated nucleosides. Their electronic structure, association modes and conformational preferences forced by different counter anions, are of similar importance, but less widely recognized, than similar properties of nucleosides and nucleotides.

2'-deoxycytidinum sulfate $(dCydH^+)_2SO_4^{2-}$ the subject of the present communication, crystallizes in two forms *A* and *B*. Form *A* crystallizes from methanol or methanol/water mixture with methanol excess. Form *B* crystallizes from water solutions.

The X-ray analysis of form *A* has established that the crystals are solvated, probably with one methanol molecule per one $(dCydH^+)_2SO_4^{2-}$ formula unit. The crystals are monoclinic, *C2*, a=33.457(3), b=6.792(1), c=11.502(3)Å, β=105.35(3)°, V=2520.5(4) $\cdot Å^3$

The molecule of methanol in the crystal structure of form *A* is disordered with the OH group in two orientations. Also disordered are the side chains of the two independent $dCydH^+$ cations ($\mathbf{A}^+$ and $\mathbf{B}^+$). The SO_4^{2-} dianion and both $dCydH^+$ cations form ion clusters in which each cation is connected *via* $N(3)^+$-H and N(4) H(41) with two O acceptors of a sulfate anion. This pattern of hydrogen bonds seems to be a characteristic of cytosinium salts with composite anions.

The cations and the anion form ion clusters ($\mathbf{A}^+ \cdot SO_4^{2-} \mathbf{B}^+$) which are arranged into columns. Two adjacent and parallel columns are H-bonded through their sugars **B** and the methanol molecule. The hydroxyl groups from the sugars **B** form a channel (parallel to the column axis) in which the methanol molecules are trapped.

The sulfate salt of dCyd in form *A* undergoes rapid solid state transformation when mixed with sodium chloride or potassium bromide. This solid state reaction transforms 2'-deoxycytidinium sulfate into 2'-deoxycytidinium chloride. This solid state ion exchange has been documented by FTIR-PAS spectroscopy. This method surpasses the IR spectroscopic methods used earlier, since it eliminates other factors that could influence the ion exchange like: fluorolube, nujol, NaCl plate or KBr plate and others.

The rate of ion exchange of 2'-deoxycytidinium sulfate with inorganic halides has been compared with the analogue reactions of 2'-deoxycytidinium sulfate form *B* and cytidinium sulfate. Deutered derivatives of 2'-deoxycytidinium sulfate have been obtained, and are also characterized by FTIR-PAS spectroscopy which probably will help us to interpret characteristic absorptions of 2'-deoxycytidinium sulfate.

SUPRAMOLECULAR SYNTHONS IN CYTIDINIUM AND CYTOSINIUM SALTS WITH COMPOSITE ANIONS

M. Gilski, M. Jaskólski

Center for Biocrystallographic Research, Institute of Bioorganic Chemistry, Polish Academy of Sciences and Department of Crystallography, Faculty of Chemistry, A. Mickiewicz Univ., Poznañ, Poland

The term „synthon" was originally introduced to describe synthetic organic structural features, but it has been re-purposed by Desiraju [1] as a description of supramolecular subunits. As supramolecular chemistry develops, it is hoped that the synthons can be designed so that general synthetic patterns can be identified and applied to unknown structures.

This poster will describe a general motif of hydrogen bonding framework existing in crystal structures of cytidinium/cytosinium salts with composite anions. Our investigations are based on the structures extracted from the Cambridge Structural Database and on our own unpublished data. We have analyzed 25 different crystal structures that contain the cytosinium cation together with a composite anion. These two units form a characteristic *supramolecular synthon* that dominates the packing pattern. It is formed by H-bonded association between the cation and the anion involving a pair of $N^{+}(3)$-H···Anion and N(4)-H(41)···Anion interactions. Similarly, it can be realized on the „hydrophobic edge" of the cytosinium cation, where, in addition to N(4)-H(42)···Anion, an „unconventional" C(5)-H(5)···Anion hydrogen bond is formed.

Among the 25 structures there are as many as 18 cases where the basic motif of interaction on the $N^{+}(3)$-H side of the cytosinum cation is formed by two acceptor atoms of a single anion. In addition, in four structures these two H-bonds are accepted by just one atom of the anion. On the other side of the cytosinium cation the motif of hydrogen-bonded interactions is formed in 13 cases. In each of the 25 crystal structures analyzed here at least one of the above motifs is formed. The three structures in which the dominating $N^{+}(3)$-H motif is not present have been analyzed individually. In all those cases there always exists a structural reasons (e.g. base pairing) explaining the absence of this motif.

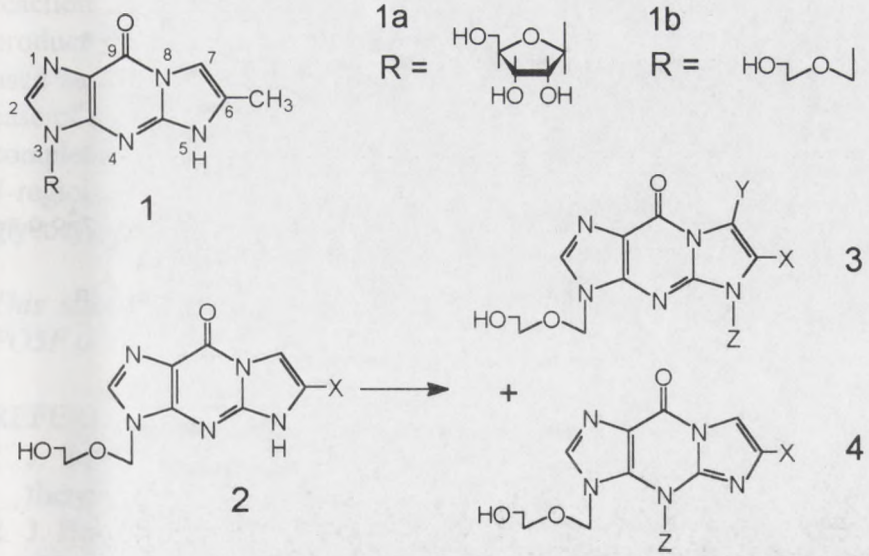
[1] G.R.Desiraju, Angew.Chem., Int.Ed.Engl. 1995, 34, 2311.

INFLUENCE OF SUBSTITUENTS IN THE 6 POSITION ON THE REACTIVITY OF 3,9-DIHYDRO-3-[(2-HYDROXYETHOXY)METHYL]--9-OXO-5*H*-IMIDAZO[1,2-*a*]PURINE. PART 2. BENZYLATION.

Tomasz Ostrowski, Tomasz Gośliński, Joanna Zeidler and Bożenna Golankiewicz

Institute of Bioorganic Chemistry, Polish Academy of Sciences, Noskowskiego 12/14, 61-704 Poznań, Poland

Tricyclic system of 3,9-dihydro-6-methyl-9-oxo-3-(β-D-ribofuranosyl)-5*H*-imidazo-[1,2-*a*]purine **1a** is a key intermediate for the synthesis of wyosine. Transformation of **1a** into wyosine as well as its 3-[(2-hydroxyethoxy)methyl] congener **1b** into acyclo-wyosine (i.e. N-4-methylation) can be accomplished by means of diazomethane / zinc iodide. Alkylation of **1a** and **1b** with methyl iodide or benzyl bromide leads to the formation of N-1-alkyl products or N-5-alkyl products (under alkaline conditions).
We now present a method for introducing the benzyl group in N-4 position of this system. Replacing 6-methyl substituent in **1b** with aryl groups had an impact on the site of benzylation. After reacting **2a** (X = phenyl), **2b** (X = 4'-methoxyphenyl) and **2c** (X = 2',5'-dimethoxyphenyl) with benzyl bromide / K_2CO_3, N-5-benzyl derivative **3** (Y = H, Z = Bn) was the major product. In addition however, 7,N-5-dibenzyl **3** (Y = Bn, Z = Bn), N-4-benzyl **4** (Z = Bn; yield 2-18 %) and small amounts of other derivatives were isolated. Using **2d** (X = t-butyl) allowed to increase the efficiency of N-4-benzylation to 24 %. Thus obtained compound **4** and its ribofuranose analogue may be substrates for the preparation of 3-benzylacyclovir and 3-benzylguanosine.



Work supported by KBN Grant Nr 4PO5F03008.

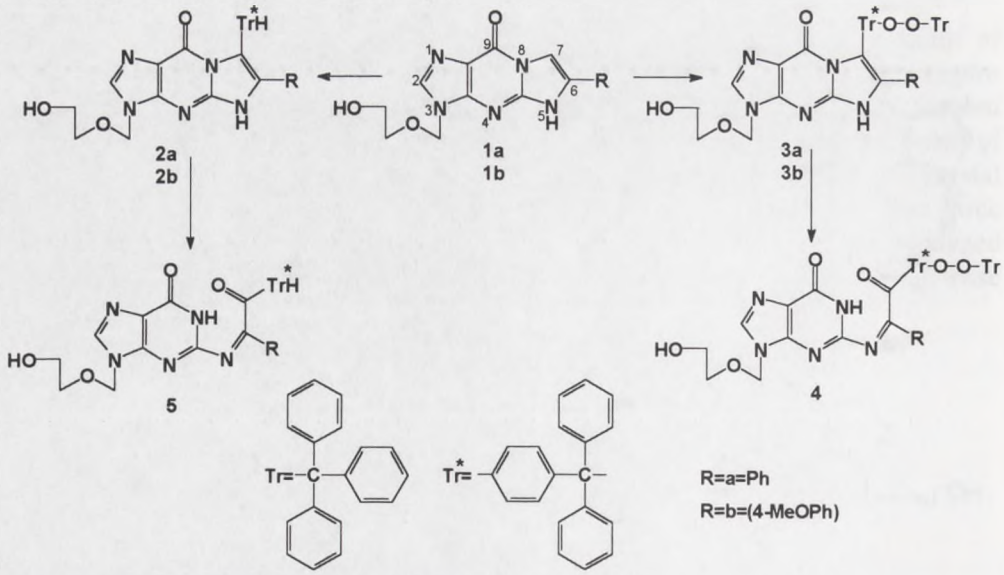
INFLUENCE OF SUBSTITUENTS IN THE 6 POSITION ON THE REACTIVITY OF 3,9-DIHYDRO-3-[(2-HYDROXYETHOXY)METHYL]--9-OXO-5*H*-IMIDAZO[1,2-*a*]PURINE. PART 1. TRITYLATION.

Tomasz Gośliński, Joanna Zeidler and Bożenna Golankiewicz

Institute of Bioorganic Chemistry, Polish Academy of Sciences, Noskowskiego 12/14, 61-704 Poznań, Poland

We have recently reported that 3,9-dihydro-3-[(2-hydroxyethoxy)methyl]-6-methyl--9-oxo-5*H*-imidazo[1,2-*a*]purine, tricyclic antiviral derived from acyclovir, when tritylated under conditions suitable for regioselective N-5 alkylation undergoes instead C-substitution to give 7-trityl (major) and 7-(4-benzhydrylphenyl) (minor) derivatives. We present now the results of replacement of the 6-methyl substituent with aryl ones. When 6-phenyl, **1a** and 6-(4-methoxyphenyl), **1b** derivatives were subjected to tritylation, the general course of the reaction, C-7 substitution, was the same. 7-(4-Benzhydrylphenyl) compounds, **2a** and **2b**, however, became the major products and the minor products, **3a** and **3b**, incorporated in the 7 position 4-benzhydrylphenyl linked by its central carbon to triphenylmethyl peroxy group. 6-(4-Methoxyphenyl) derivatives, **2b** and **3b** turned out to be unstable and transformed upon work-up into the bicyclic, highly fluorescent compounds **4** and **5**.

The structures of the new compounds were deduced from the analysis of UV, ^{1}H and ^{13}C NMR and mass spectra.



Work supported by KBN Grant Nr 4PO5F03008.

TRANSGLYCOSYLATION REACTIONS OF 6-THIOXO AND 6-THIOMETHYL ANALOGUES OF ACYCLOVIR

Andrzej Manikowski, Jerzy Boryski

Institute of Bioorganic Chemistry, Polish Academy of Sciences,
ul. Noskowskiego 12/14, Pl-61704 Poznań, Poland

The fully protected derivatives of 6-oxopurine nucleosides *e.g.* inosine, guanosine and their acyclic analogues, like diacetylacyclovir (9-(2-acetoxy-ethoxy)methyl-N^2-acetylguanine), have been so far the only known series of compounds which readily undergo a fully reversible 7 $\rightleftarrows$ 9 isomerization.[1] In order to establish whether that unique transglycosylation process takes place in a closely related series of derivatives, we have synthesized 9-(2-acetoxy-ethoxy)methyl-N^2-acetyl-6-thioguanine - a model compound, in which oxygen in the position 6 has been replaced by sulfur. The latter compound undergoes two different glycosyl migration reactions under transglycosylation conditions (220°C, without solvents, 7 min; or refluxing in chlorobenzene in the presence of 6% mol of *p*-toluenesulfonic acid, 2 h): i) the 7 $\rightleftarrows$ 9 isomerization, which gives the respective 7-regioisomer as the major product (yield 50% in chlorobenzene); ii) the 9 $\rightleftarrows$ S^6 glycosyl migration, which leads to a 9,S^6-di-substituted product (7%, respectively), and N^2-acetyl-6-thioguanine. The formation of the 7-isomer has been somehow surprising, since a similar reaction of tetraacetyl-6-thioguanosine yields only the 9,S^6-disubstituted product.[2] A counter reaction, in which the 7-isomer of 6-thioacyclovir was used as a substrate, gave an almost identical mixture of compounds as in the case of the 9-isomer. S^6-Methylation of 6-thioacyclovir (CH_3I, K_2CO_3, MeOH) completely stopped reversibility of transglycosylation. Thus, the respective 7-regioisomer of 6-thiomethyl derivative underwent a quantitative trans-glycosylation to the thermodynamically more stable 9-regioisomer.

This study was supported by the Komitet Badań Naukowych, Project No 4 PO5F 007 12.

REFERENCES

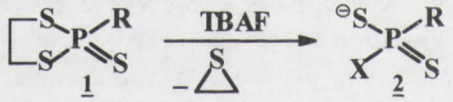
1. J. Boryski, *Nucleosides Nucleotides*, **15**, 771 (1996); and references cited therein.
2. J. Boryski, A. Manikowski, *Collect. Czech. Chem. Commun., Special Issue* **61**, S38 (1996).

NEW STRATEGIES FOR CHEMICAL SYNTHESIS OF PHOSPHORODITHIOATES DERIVED FROM 3'- or 5'-THIONUCLEOSIDES

W. Dąbkowski and I. Tworowska

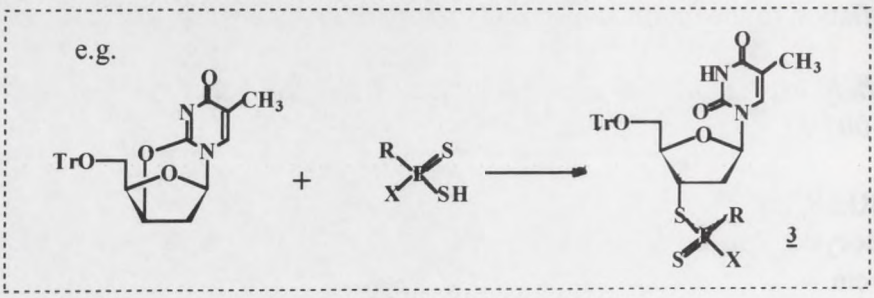
Centre of Molecular and Macromolecular Studies,
Polish Academy of Sciences,
Sienkiewicza 112, 90-363 Łódź, Poland
(E- mail: wdabkow@bilbo.cbmm.lodz.pl)

A variety of phosphorus dithioacids **2** are available *via* dithiaphospholane approach based on reaction of compound **1** with tetra-n-butylammonium fluoride (TBAF).



X= F	**R**= H_3C-, NC-, L-proline-N-,$(CH_3)_3CO$- $NC(CH_2)_2O$-, $(CF_3)_2CHO$-, nucleoside cholesterol, citronellol
X= nucleoside	**R**= H_3C-, NC-, L-proline-N-, $(CH_3)_3CO$-, $(CF_3)_2CHO$- ,cholesterol, citronellol

We discovered that anhydronucleosides react readily with phosphorus dithioacids RR'P(S)SH **2** in almost quantitative yield.[1] This general and simple procedure allows efficient synthesis of phosphosphorodithioates derived from 3'- or 5'-thionucleosides **3**.



1. W. Dąbkowski, M. Michalska, I. Tworowska, *J. Chem. Soc., Chem. Commun.*, **1998**, 427.

SYNTHESIS OF 2'-O-RIBOSYLADENOSINE 5"-PHOSPHATE, A MINOR NUCLEOSIDE OF EUCARYOTIC INITIATOR tRNAs

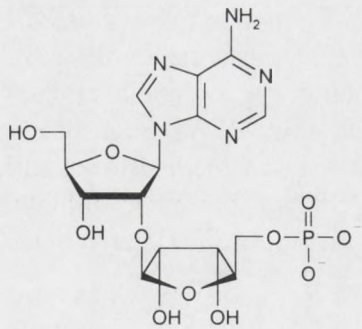
Marcin Chmielewski and Wojciech T. Markiewicz

Institute of Bioorganic Chemistry, Polish Academy of Sciences, Noskowskiego 12, PL-61704 Poznań, Poland

Two novel modified nucleotides were found in eukaryotic cytoplasmic initiator methionine tRNAs in position 64. These nucleotides were identified as β-anomers of *O*-ribosyl(1"→2')-5"-phosphates of adenosine (*Ar(p)*) and guanosine (*Gr(p)*) respectively[1,2].

We have reported the synthesis of these nucleosides as well as their pyrimidine analogues, however, non-phosphorylated at 5" position[3,4]. These nucleosides were obtained from *N*-protected 3',5'-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)ribonucleosides and 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose in the presence of tin tetrachloride in 1,2-dichloroethane. Recently we described structural studies of these nucleosides using X-ray and NMR analysis[4]. The influence of a 2'-O-ribosylation on a structure of oligoribonucleotides was also studied by NMR spectroscopy using ribofuranosyl(1"→2')adenylyl(3'→5')guanosine (*ArpG*) as a model compound[4].

In this communication the synthesis of *O*-ribosyl(1"→2')-adenosine-5"-phosphate (*Ar(p)*) will be presented.



Ar(p)

References

1. Glasser, A.-L.; Desgres, J.; Heitzler, J.; Gehrke, C. W.; Keith, G. (1991) *Nucleic Acids Research,* **19**, 5199-5203.
2. Keith, G.; Glasser, A.-L.; Desgres, J.; Kuo, K. C.; Gehrke, C. W. (1990) *Nucleic Acids Research,* **18**, 5989.
3. Niewczyk, A.; Krzyżaniak, A.; Barciszewski, J.; Markiewicz, W. T. (1991) *Nucleosides and Nucleotides,* **10**, 635-638.
4. Markiewicz, W. T., Niewczyk, A., Gdaniec, Z., Adamiak, D. A., Dauter, Z., Rypniewski, W., Chmielewski, M. (1998) *Nucleosides and Nucleotides,* **17**, 411-424.

REACTIVITY OF 5'-O-DMT-THYMIDINE 3'-O-(1,3,2-OXATHIAPHOSPHOLANE) TOWARDS NUCLEOPHILES IN THE PRESENCE OF DBU

Konrad Misiura and Wojciech J. Stec

Department of Bioorganic Chemistry, Centre of Molecular and Macromolecular Studies, Polish Academy of Sciences, Sienkiewicza 112, 90-363 Łódź, Poland

Diastereomers of 5'-O-DMT-nucleoside 3'-O-(1,3,2-oxathiaphospholane)s are used as monomers for stereocontrolled synthesis of oligo(nucleoside phosphorothioate)s (1). Oxathia(dithia)phospholane method was also used for the synthesis of other types of oligonucleotide analogues eg. phosphorodithioates (2), phosphoroselenoates (3), and mixed backbone phosphate/phosphorothioates (4). It is assumed that in syntheses mentioned above 5'-hydroxyl group of nucleoside acts as a nucleophile activated by the DBU. The only other types of nucleophiles used in oxathiaphospholane chemistry were diphosphate (5) and fluoride anions (6). To get better understanding of mechanism of coupling and to explore synthetic applicability of oxathiaphospholane method we performed studies on reactivity of 5'-O-DMT-thymidine 3'-O-(1,3,2-oxathiaphospholane) towards other nucleophiles including water, alcohols, phenols, mercaptans, amines, carboanions, halogen- and pseudohalogen anions. We have found that nucleophilic ring-opening condensation reactions proceeded only in the presence of DBU, however, only reactions with alcohols, phenols, mercaptans, and amines gave desired products in good yields. After DMT deprotection appropriate analogues of thymidine 3'-O-phosphorothioate were isolated by DEAE-Sephadex chromatography. Oxathiaphospholane chemistry offers a new method for synthesis of nucleoside O-alkyl(aryl) phosphorothioates, S-alkyl phosphorodithioates, and N-alkyl phosphoroamidothioates. The mechanism of DBU activation will be discussed.

(1) Stec, W.J., Grajkowski, A., Karwowski, B., Kobylańska, A., Koziołkiewicz, M., Misiura, K., Okruszek, A., Wilk, A., Guga, P., Boczkowska, M. (1995) *J.Am.Chem.Soc.*, **117**, 12019-12029.

(2) Okruszek, A., Sierzchała, A., Fearon, K.L., Stec, W.J. (1995) *J.Org.Chem.*, **60**, 6998-7005.

(3) Misiura, K., Pietrasiak, D., Stec, W.J. (1995) *J.Chem.Soc., Chem.Commun.*, 613-614.

(4) Stec, W.J., Karwowski, B., Guga, P., Koziołkiewicz, M., Boczkowska, M., Wieczorek M.W., Błaszczyk, J. (1998) *J.Am.Chem.Soc.* - accepted for publication.

(5) Okruszek, A., Olesiak, M. (1994) *J.Med.Chem.*, **37**, 3850-3854.

(6) Misiura, K., Szymanowicz, D., Stec, W.J. (1998) *Chem.Commun.*, 515-516.

THE HYDRATION OF ACHIRAL 5'-METHYLENEPHOSPHONATE DINUCLEOSIDE ANALOGUE IN WATER. MD COMPUTER SIMULATIONS AND FT-IR SPECTROSCOPY

K. Kulińska [1], T. Kuliński [1], A. Laaksonen [2] and J. Stawiński [3]

[1] Institute of Bioorganic Chemistry, Polish Academy of Sciences, Noskowskiego 12/14, 61-704 Poznan, Poland
[2] Division of Physical Chemistry, Arrhenius Laboratory, Stockholm University, S-106 91 Stockholm, Sweden
[3] Department of Organic Chemistry, Arrhenius Laboratory, Stockholm University, S-106 91 Stockholm, Sweden

The replacement of phosphodiester internucleotide linkage in natural nucleic acids by the methylphosphonate function leads to nuclease-resistant phosphate diester's analogues of significant potential for genome targeting therapy *via* the antisense or the antigen approach. The methylphosphonate analogs, by virtue of chirality at the phosphorus centre, exist in the form of two diastereomers (R_P and S_P) and are electrically neutral.

The hydration pattern of thymidyl(3'–>5')thymidine and those of R_P and S_P diastereomers of the corresponding methylphosphonate analogue, have been studied using Molecular Dynamics (MD) computer simulation and FTIR spectroscopy [1, 2]. It was found that the methylphosphonate modification leads to significant changes in the coordination of water molecules around the internucleotidic linkage and these, in turn, affect the hydration pattern of other parts of the molecule. The most notable differences between R_P and S_P diastereomers were found to occur at the deoxyribose moieties of the nucleosid-5'-yl units.

The problem of chirality at the phosphorus centre in nucleoside C-phosphonate diesters may be circumvented by placing the P-C bond into the bridging position of the phosphonate group to produce the corresponding achiral, isosteric, and ionic 3'-methylenephosphonate or 5-methylenephosphonate analogues. According to the ^{1}H NMR spectroscopy data 5-methylenephosphonates adopt preferentially (-) *gauche/trans)* conformation around the C4'-C5' bond [3].

In this comunication we will present studies on the conformation and the hydration pattern of the achiral 5'-methylenephosphonate dinucleoside analogues in water by Molecular Dynamics computer simulations and FT-IR spectroscopy.

References

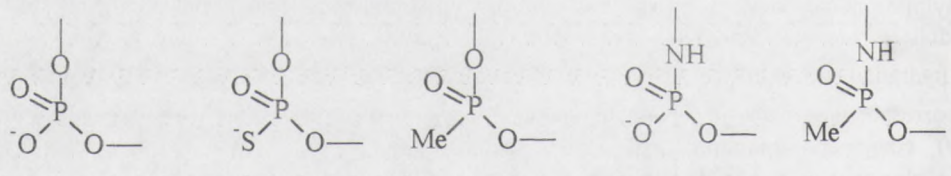
1. Kulińska K., Sarzyńska J., Szabó T. and Stawiński J., J. Biomol. Struct. Dynam. **15** (1), 119-128 (1997)
2. Kulińska K., Kuliński T., Stawiński J and Laaksonen A., J. Biomol. Struct. Dynam. (1998), in press.
3. Szabó T. and Stawiński J., Tetrahedron, **51**, 4145-4160 (1995)

NOVEL INTERNUCLEOTIDE 3'-NH-P(CH_3)(O)-O-5' LINKAGE. OLIGO(DEOXYRIBONUCLEOSIDE METHANEPHOSPHONAMIDATE)S; SYNTHESIS, STRUCTURE AND HYBRIDIZATION PROPERTIES.

Barbara Nawrot, Małgorzata Boczkowska, Marzena Wójcik, Marek Sochacki, Sławomir Kaźmierski and Wojciech J. Stec

Department of Bioorganic Chemistry, Centre of Molecular and Macromolecular Studies, Polish Academy of Sciences, Sienkiewicza 112, 90-363 Łódź, Poland

High avidity of oligonucleotide analogue towards complementary DNA or RNA strand, its stability against endo- and exonucleases and low affinity towards proteins promotes oligonucleotide as a good candidate for antisense strategy (1, 2). Taking into account the advantageous properties of [R_p]-methanephosphonates (3) and N3'-P5' phosphoramidates (4) it was tempting to prepare new oligonucleotides with incorporated structural motifs related to both aforementioned classes of oligomers, namely methanephosphonamidates.



Diastereomeric dithymidine methanephosphonamidates (TnpmT) were synthesized by reaction of 3`-amino-3`-deoxythymidine with 3`-*O*-acetylthymidin-5-yl-methanephosphono chloridate. Separated dinucleotide TnpmT-fast and TnpmT-slow diastereomers were used as building blocks to prepare chimeric oligothymidylates (12mers and 20mers), possessing various number of TnpmT units, by means of phosphoramidite automated solid phase synthesis. As expected, methanephosphonamidate internucleotide linkage is resistant toward nuclease P1, svPDE and 3'-exonuclease from human plasma. Degradation of oligothymidy lates possessing modified internucleotide linkages in alternate positions proved some "hopping" properties of 3`-exonuclease. In thermal denaturation experiments all the oligomers show lower binding ability for duplex formation, with either DNA and RNA targets. Oligomers originated from TnpmT-fast dimeric unit decrease the Tm's to lower extend than their diastereomeric TnpmT-slow counterparts. Oligo(deoxyribonucleoside methanephosphonamidate)s originated of TnpmT-fast unit exhibit higher ability for triplex formation with d($A_{21}C_4T_{21}$) hairpin oligomer than parent T_{20} oligomer. Thus, nuclease resistance and some hybridization properties make oligo(deoxyribonucleoside methanephos phonamidate)s attractive objects for further investigations as both antisense and antigene constructs.

(1) Uhlmann, E. and Peyman, A. (1990) *Chem. Rev.* **90**, 544-584. (2) Crooke, S. T. and Lebleu, B. (1993), *Antisense Research and Applications*, CRC Press, Boca Raton, Florida. (3) Leśnikowski Z. et al. (1988) *Nucleic Acids Res.* **16**, 11675-11689, Agrawal, S., et al. (1995) *Clin. Pharmacokinet.* **28**, 7-16, Reynolds, M. A., et al. (1996) *Nucleic Acids Res.* **24**, 4584-4591, (4) Gryaznov, S. M. et al. (1994) *J. Am. Chem. Soc.* **116**, 3143-3144.

NEW COST-EFFECTIVE STEREOSELECTIVE SYNTHESIS OF [R_P]-DINUCLEOSIDE (3',5')-METHANEPHOSPHONATES

Lucyna A. Woźniak, Arkadiusz Chworoś and Wojciech J. Stec

Department of Bioorganic Chemistry, Centre of Molecular and Macromolecular Studies, Polish Academy of Sciences, Sienkiewicza 112, 90-363 Łódź, Poland

In spite of numerous papers reporting the synthesis and assignment of absolute configuration in nucleoside 3'-O-(S-alkyl/aryl methanephosphorothioates) (**1**) (1) the reports on their use in the stereocontrolled synthesis of oligonucleotides bearing dinucleoside (3',5')-methanephosphonates (**2**) of predetermined configuration at phosphorus were published only from this laboratory (2). Since [R_P]-**2** have been demonstrated useful synthons for the synthesis of chimeric oligonucleotides tested as potential antisense therapeutics (3) we extended our efforts towards diastereoselective synthesis of [R_P]-**2**.

In this communication we present an improved procedure for the stereocontrolled synthesis of [R_P]-**1**. The step requiring the synthesis of nucleoside 3'-O-methanephosphoranilidothioates has been omitted (4). Instead, 5'-O-DMT-base protected (except thymidine) nucleosides react with $MeP(S)Cl_2$ and β-cyanoethanol in pyridine to provide diastereomeric mixture of 5'-O-DMT-protected nucleoside (except thymidine) 3'-O(O-β-cyanoethyl methanephosphonothioate) (**3**) which undergoes separation on silica gel. FAST-eluted diastereomer **3** is, after decyanoethylation with ammonia, alkylated with benzyl bromide and resulting **1** is used for the DBU/LiCl-assisted condensation with 5'-OH function of 3'-O-protected nucleoside providing, after deprotection, [R_P]-$N_{PMe}N'$ (**2**). SLOW-migrating **3**, after phosphonothioate deprotection, is converted into 5'-O-DMT-N-protected nucleoside (except thymidine) 3'-O-(O-2,4,6-trimethylbenzoyl methanephosphonothioate) (**4**) which then is hydrolysed in the presence of DBU; this process occurs with full chemoselectivity (P attack proved by ^{18}O-labelling studies) and with full inversion of configuration at phosphorus atom. Resulting nucleoside 3'-O-(methanephosphonothioate) after S-alkylation provides the substrate for synthesis of [R_P]-**2**.

(1) Brill, W., Caruthers, M.H. (1987) *Tetrahedron Lett.*, **28**, 3205; (1988) *ibid*, **29**, 1227; (1989) *ibid*, *Nucleosides & Nucleotides*, **8**, 1011; Brill, W.K., Caruthers, M.H., Haltiwanger, R.C. (1989) *Acta Cryst.*, **C45**, 1639; Lebedev, A.V. *et al.*, (1990) *Tetrahedron Lett.*, **31**, 855; Brill, W.K. (1995) *Tetrahedron Lett.*, **36**, 703.
(2) Woźniak, L.A. *et al.* (1994) *J.Org.Chem.*, **59**, 5843.
(3) Reynolds, M.A. *et al.* (1996) *Nucleic Acids Res.*, **24**, 4584.
(4) Stec, W.J. *et al.* (1997) *Antisense & Nucleic Acid Drug & Dev.*, **7**, 383.

SYNTHESIS AND SOME APPLICATIONS OF NUCLEOSIDE ARYL H-PHOSPHONATES

Jacek Cieślak[1], Adam Kraszewski[1], Jacek Stawiński[2]

[1]Polish Academy of Sciences, Institute of Bioorganic Chemistry, Noskowskiego 12/14, 61-704 Poznań, Poland.
[2]Stockholm University, Department of Organic Chemistry, Arrhenius Laboratory, S-106 91 Stockholm, Sweden.

It was found that the most important factors affecting the formation of nucleoside aryl-H-phosphonates in the reaction of nucleoside H-phosphonate monoester and phenols were: (i) the nature of a coupling agent used for the condensation, (ii) acidity of a phenol, and (iii) basicity of the reaction medium. Searching for optimal reaction conditions for nucleoside aryl H-phosphonates synthesis, we found that diphenyl chlorophosphate in methylene dichloride containing 10 % (v/v) of pyridine, was the most efficient coupling system for majority of the investigated phenols, securing rapid and clean formation of the desired product.
Various synthetic applications of thus produced nucleoside aryl H-phosphonates, will also be presented.

APPLICATIONS OF 9-FLUORENEMETHYL-H-PHOSPHONOTHIOATE IN THE SYNTHESIS OF NUCLEOSIDE H-PHOSPHONOTHIOATES, PHOSPHOROTHIOATES AND PHOSPHORODITHIOATES

Jadwiga Jankowska[1], Adam Kraszewski[1], Jacek Stawiński[2]
[1]Polish Academy of Sciences, Institute of Bioorganic Chemistry, Noskowskiego 12/14, Poznań, Poland.
[2]Stockholm University, Department of Organic Chemistry, Arrhenius Laboratory, S-106 91 Stockholm, Sweden.

9-Fluorenemethyl H-phosphonothioate, a stable and easy to prepare reagent, was found to be exceptionally effective in transferring an H-thiophosphonyl group to nucleosides. Since the produced nucleoside H-phosphonothioate diesters can be converted under oxidative conditions in nearly quantitative yields to P(V) derivatives, this approach can be considered as a versatile method for the preparation of variety of biologically important phosphate analogues, *e.g.* nucleoside phosphorothioates, phosphorodithioates, phosphoramidothioates, *etc.*

THE CHEMICAL SYNTHESIS AND PURIFICATION OF HIV-1 29-MER TAR RNAs MODIFIED WITH 5-FLUORO-URIDINE

Mikołaj Olejniczak, Artur Fischer and Ryszard W. Adamiak

Institute of Bioorganic Chemistry, Polish Academy of Sciences,
Noskowskiego 12/14, 61-704 Poznań, Poland

Fluorine chemical shifts are extremely sensitive to the nucleus environment thus making fluorine an ideal NMR spin label in the study of nucleic acids interactions with proteins. The small radius of fluorine atom implying little steric hindrance, 100% natural abundance of ^{19}F nucleus, high sensitivity of detection (83,3% of that of ^{1}H) and large chemical shift dispersion are also advantages of using ^{19}F NMR in the study of RNA-protein interactions (1).

The interaction between TAR RNA element, present at the 5'-end of all HIV-1 mRNAs, and Tat-1 protein is a crucial step in the HIV-1 replication cycle (2). We have decided to synthesize TAR RNA elements labelled with fluorine atoms at specific positions aiming at the use of ^{19}F NMR in the study of the interactions between TAR RNA element of HIV-1 and recombinant Tat-1 protein (3).

5-Fluoro-uridines have been regioselectively incorporated into 29 nucleotides long TAR RNA HIV-1 fragments by means of phosphoramidate chemical synthesis on solid support (4). Fluorine labels have been introduced into functionally important bulge and loop regions of TAR RNAs. *U* (in italic) stands for 5-fluoro-uridine residue

```
                 UCU       CU
5'-GGCAGA        GAGC       G
3'-CCGUCU____CUCG           G
                          AG

                 UCU       CU
5'-GGCAGA        GAGC       G
3'-CCGUCU____CUCG           G
                          AG
```

After synthesis and deprotection with fluoride anion the correct length oligomer has been isolated from the reaction mixture using a set of procedures including polyacrylamide gel electrophoresis in denaturing conditions, electroelution of RNA from gel slices and desalting on Sephadex G-25 columns. Total yields 3-5%.

References

1. W. J. Metzler, K. Arndt, E. Tecza, J. Wasilewski, P. Lu, *Biochemistry*, 1985, **24**, 1418-1424
2. A. D. Frankel, *Current Opinion in Genetics and Development*, 1992, **2**, 293-298
3. E. Wyszko et al., *unpublished*
4. A. Fischer, Z. Gdaniec, E. Biała, M. Łożyński, J. Milecki & R. W. Adamiak, *Nucleosides & Nucleotides*, 1996, **15**, (1-3), 477-488

^{13}C-NMR SPECTRA SUPPORT UNEXPECTED SIMILARITIES BETWEEN r(CGCGCG)$_2$ AND 2′-O-Me(CGCGCG)$_2$ DUPLEX STRUCTURES

Mariusz Popenda and Ryszard W. Adamiak

Institute of Bioorganic Chemistry, Polish Academy of Sciences
Noskowskiego 12/14, 61-704 Poznań, Poland

Recently we have determined the structures of r(CGCGCG)$_2$ and 2′-O-Me(CGCGCG)$_2$ by NMR spectroscopy under low salt conditions (1). Both duplexes form half-turn, right-handed helices with several conformational features which deviate significantly from a canonical A-RNA structure. Duplexes are characterised as having C3′-endo sugar pucker, very low base-pair rise and high helical twist and inclination angles. Helices are overwound with less than 10 base pairs per turn. Within CG steps of both duplexes, the planes of the inter-strand cytosines are not parallel while guanines are almost parallel. For the GC steps this pattern is reversed. Surprisingly, the solution structures of both duplexes are similar; the effect of 2′-O-methylation on the parent RNA structure is small. The X-ray crystal structure of 2′-O-Me(CGCGCG)$_2$ at 1.30 Å resolution, the highest one to be reported so far in the RNA field, was also solved (2). Similarities between the solution and crystal structures of 2′-O-Me(CGCGCG)$_2$ were also observed. Although our knowledge on the effects of 2′-O-methylation of a RNA structure is far from being complete, it is a common presumption that 2′-O-methylation has a considerable impact on the RNA structure, particulary in respect to sugar puckering.

Here we are presenting ^{13}C-NMR spectra of r(CGCGCG)$_2$ and 2′-O-[^{13}C]Me(CGCGCG)$_2$. The spectra of both duplexes, especially within the region of aromatic carbons, are very similar and confirm a structural unification within both duplexes as far as stacking interactions are concerned. Also other data clearly support an earlier suggestion (1,2) that intrinsic properties imposed by alternating CG base pairs govern the overall conformation of both duplexes. The impact of 2′-O-methylation on the r(CGCGCG)$_2$ structure is small. That is certainly not true for the hydration pattern of both duplexes, which is a key factor for A-RNA to Z-RNA transformation (3,4). From them both, only the r(CGCGCG)$_2$ undergoes helicity reversal to form a Z-RNA structure (5,6).

References

1. Popenda,M., Biała,E., Milecki,J. and Adamiak,R.W (1997) *Nucleic Acids Res.*, 25 (22), 4589-4598.
2. Adamiak,D.A., Milecki,J., Popenda,M., Biała,E., Adamiak,R.W., Dauter,Z. and Rypniewski,W.R. (1997) *Nucleic Acids Res.*, 25 (22), 4599-4607.
3. Hall,K., Cruz,P., Tinoco,I. Jr., Jovin,T.M.and Van de Sande,J.H. (1984) *Nature*, **311**, 584-586.
4. Adamiak,R.W., Gałat,A. and Skalski,B. (1985) *Biochim. Biophys Acta,* **825**, 345-352.
5. Davis,P.W., Adamiak,R.W.and Tinoco,I. Jr. (1990) *Biopolymers,* **29,** 109-122.
6. Krzyżaniak,A., Sałański,P., Adamiak,R.W., Jurczak,J. and Barciszewski,J. (1996) In Hayashi,R. and Balny,C. (eds.), *High Pressure Bioscience and Biotechnology.* Elsevier Science, Amsterdam, pp. 189-194.

DYNAMICS OF RNA BULGE LOOPS. MD SIMULATION OF 2-AMINOPURINE LABELLED ADENOSINE LOOPS IN AQUEOUS SOLUTION

Łukasz Bielecki and Ryszard W. Adamiak

Institute of Bioorganic Chemistry, Polish Academy of Sciences,
Noskowskiego 12/14, Poznań, Poland

The 2-aminopurine riboside appeared to be the nucleosidic fluorophore of choice for labelling adenosine containing bulge loops in RNA duplexes (1). The aim of this study was the evaluation of the conformation of the bulged region in respect to spectrofluorimetry results (2,3); see also accompanying poster by T. Kuliński *et al.*

A short RNA duplex containing a trinucleotide bulge modified with 2-aminopurine:

```
          A(2AP)A
5'-GUCG        GCUG
3'-CAGC_______CGAC
```

was analysed by the molecular dynamics simulation in aqueous solution. The orientation of the bulge towards the stem region was taken under special consideration.

Using the MSI/Biosym software (InsightII and Discover programs with the AMBER 4.0 force field implemented), the duplex was built and placed in a periodic box filled with water molecules and sodium counterions were located near the phosphorus atoms. After the initial energy minimization, the molecular dynamics simulation was performed for 250 ps in 300 K (cutoff 16Å).

During the entire MD run, the stem regions retained their conformational stability. Molecule is bend. The hydrogen bonds and stacking interactions within them were preserved and the sugar puckering remained typical for A-RNA. On the other hand, the bulged nucleotides showed a tendency to „bulge out" to the exterior which resulted in destabilization of their closest neighborhood, which was clearly observed by the conformational parameters of the stem/bulge contact region . On the other hand, the stacking interactions within the bulge are well retained during the prevalent parts of the dynamics simulation.

References

1. I. Zagórowska & R.W. Adamiak „2-Aminopurine labelled RNA bulge loops. Synthesis and thermodynamics", *Biochimie* (1996), 78, 123-130.
2. T. Kuliński, Ł. Bielecki, I. Zagórowska & R. W. Adamiak: „Introductory data on dynamics of RNA bulge duplexes. 2-Aminopurine labelled adenosine loops", *Collect. Czech Chem. Commun.* (Vol 61) Special Issue 1996, pp. 265-267.
3. T. Kuliński, Ł. Bielecki, I. Zagórowska & R. W. Adamiak: „Dynamics of RNA bulge duplexes: 2-Aminopurine labelled adenosine loops", in preparation to *Biophys. J.*

DYNAMICS OF RNA BULGE LOOPS. SPECTROFLUORIMETRY OF 2-AMINOPURINE LABELLED ADENOSINE LOOPS

T. Kuliński[1] , I. Zagórowska[1] , R. Rigler[2] and R. W. Adamiak[1]

[1] Institute of Bioorganic Chemistry, Polish Academy of Sciences, Pl-61704 Poznan, Poland.
[2] Department of Medical Biochemistry and Biophysics, Karolinska Institutet, S-171 77 Stockholm, Sweden

RNA bulge loops are considered as important elements in terms of the RNA structure formation and the specificity of RNA-protein binding, intron splicing and other biological processes [1]. We present experimental data on dynamics of adenosine bulges which prevail in ribosomal RNAs. Duplexes resulted from annealing of oligoribonucleotides: GUCG**P**GCUG, GUCG**P**AGCUG, GUCGA**P**GCUG and GUCGA**P**AGCUG with CAGCCGAC, where 2-aminopurine (**P**) has been used as fluorescent conformational probe have been studied by time-resolved spectrofluorimetry. Free energy increments for bulged loops [2] suggest that the structural properties of 2-aminopurine in such RNA bulge loops are very similar to that of isomeric adenine and 2-aminopurine may be applied as the fluorescent probe with non-disruptive character to the structure of interest [3].

The fluorescence intensity decay and anisotropy decay have been measured in a premelting temperature range from 4^{o} C to 30 ^{o} C on the picosecond laser spectrofluorimeter with time-correlated single photon counting technique [4]. The multiexponential fluorescence decay and its temperature dependence suggest that the 2AP base exists in oligomers in different conformational states. Four exponential components in the fluorescence decay were found in all oligomeric structures, with the lifetimes ranging around 7 ns, 1.5 ns, 0.3 ns and 0.05 ns. The temperature dependence is mostly pronounced for the longest decay component, which becomes shorter by 50% when temperature increases to 30 C, approaching melting temperature of oligomers.

Fluorescence anisotropy decay show two distinct components, long one ranging from 4 ns to 1 ns, and short one from 0.5 ns to 100 ps for low to high temperature, respectively. The longer rotational correlation time found in the analysis reflects overall rotation of the oligomers: the values of those correlation times correspond to theoretically predicted rotational correlation times of the molecules of that dimension, as well as its temperature dependence, following temperature change of the viscosity of water in the premelting region of temperatures. The short correlation times reflect internal motions of the 2AP in respect to the whole molecule, and may involve only its nearest neighbors. The extent of the mobility is different in investigated oligomers as reflected by different initial partial anisotropy values r^{o}_{i} .

The fluorescence intensity decays are sequence dependent, where neighboring G acts as a fluorescence quencher.

References

1. Wyatt J.R., Tinoco Jr. I. in: *The RNA World*, (R.F. Gesteland and J.F. Atkins, Eds), p.465, Cold Spring Harbor Laboratory Press (1993).
2. Zagórowska I., Adamiak R.W.: *Biochimie*, 78 (2), 1 (1996)
3. T. Kuliński, L. Bielecki, I. Zagórowska and R.W. Adamiak. *Collection of Czechoslovak Chemical Communications*, 61, 265-267(1996).
4. Rigler R, Claesens F, Kristensen O *Analytical Instrumentation* 14, 525-546(1985).

THE INFLUENCE OF THE MODIFICATIONS LOCATED AT THE 3'- DANGLING ENDS OF SELF-COMPLEMENTARY RNA DUPLEXES ON THEIR THERMODYNAMIC PARAMETERS

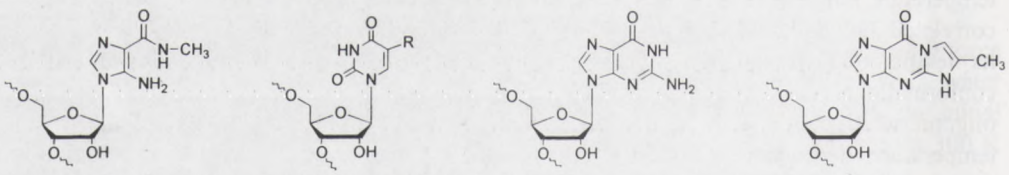
Krzysztof Ziomek, Ryszard Kierzek

Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznań

The main forces stabilizing the RNA duplex structure are stacking interactions and Watson-Crick hydrogen bonding. In case of paired nucleotides located in complementary strands the competition between these two forces can be observed. When the nucleotide is located at 3' dangling end of RNA duplex, its heterocyclic base does not form Watson-Crick hydrogen bonds and as a consequence it is not forced to adopt any arrangement which is not preferred by stacking interactions. The base can freely move and locate in position at which stacking interactions are the highest. The observations concerning the thermodynamic stability of the oligoribonucleotides containing nucleoside at the 3' dangling end indicate, that the influence of all purine bases is similar. The same situation can be observed in case of pirimidine bases. The set of experiments, characterizing the influence of some modifications (Fig.2) located at the 3'-dangling ends of self-complementary RNA duplexes (Fig.1) on their thermodynamic parameters, has been conducted.

UCUAGAU^{R}
^{R}UAGAUCU

Fig. 1



Where R: F, Cl, Br, I, H, Me, Et, nPr

Fig. 2

Results of our studies demonstrated that not the substituents but the π-electron structure of the heterocyclic base is mainly (or at least in higher degree) responsible for the stacking interactions. The halogen atoms introduce only small change in stability of the duplex (ΔT_m vary between 0.0 and $+0.6^0$C per modification, $\Delta\Delta G^0_{37}$ not more than about 0.1 kcal/mol per modification), when the bases having different π-electron systems change the parameters of the duplex in much bigger range (ΔT_m from -3.5^0C to $+5.0^0$C per modification with $\Delta\Delta G^0_{37}$ per modification from 0.5 kcal/mol to 1.0 kcal/mol respectively). We observed that the thermodynamic stability of the duplexes containing modified nucleotides at 3' dangling ends rises in the following direction : 3'-imidazole derivative < 3'-uridine derivatives < 3'-guanosine < 3' -desmethylwyosine. This testifies that the magnitude of the dipole moment, responsible for the stacking interactions, is a derivative of the quantity of π-electrons in the heterocyclic system. Introduction of electron donating and electron withdrawing groups in C5 position of uridine changes thermodynamic parameters much less than in case of the systems having different quantity of the π-electrons in the heterocyclic base. Considering these data it can be concluded that the substituents in C5 position of uridine do not affect the stacking interactions in a very big extend.

THE INFLUENCE OF MODIFIED INTERNAL BASE PAIRS ON THERMODYNAMIC STABILITY OF RNA DUPLEXES

Krzysztof Ziomek, Ryszard Kierzek

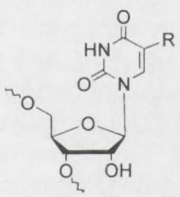
Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznań

Modified nucleosides occur in all types of ribonucleic acids (RNA). Especially often they can be found in transfer ribonucleic acids (tRNA), where they constitute 15% of whole nucleoside composition. Altogether in all types of RNA examined, 95 different kinds of modified nucleosides have been found. There are many suspicions about the significance of modifications, but the functions of most of them are not really known. The main forces stabilizing the RNA duplex structure are stacking interactions and Watson-Crick hydrogen bonding. In case of paired nucleotides, located in complementary strands, the competition between these two forces can be observed. As a consequence arrangement of heterocyclic bases in double helix is an 'average' between optimum values for hydrogen bonding and stacking interactions. Substituents at the C5 position of the pyrimidine bases are known to affect the base pairing properties with complementary purines, by modifying the acceptor strength of carbonyls situated at positions C2 and C4.

In our research we focused on the influence of some modifications introduced instead of H5 of uridine and located in nucleotides being the internal base pairs. The set of modified duplexes (Fig.1) has been prepared. Hydrogen atom (H5) of uridine has been substituted by one of seven groups (Fig.2). The substituents have been chosen to have changeable electron withdrawing and electron donating properties and different sizes and shapes.

AUCU^{R}AGAU
UAGA^{R}UCUA

Fig. 1



where R: F, Cl, Br, I, H, Me, Et, nPr

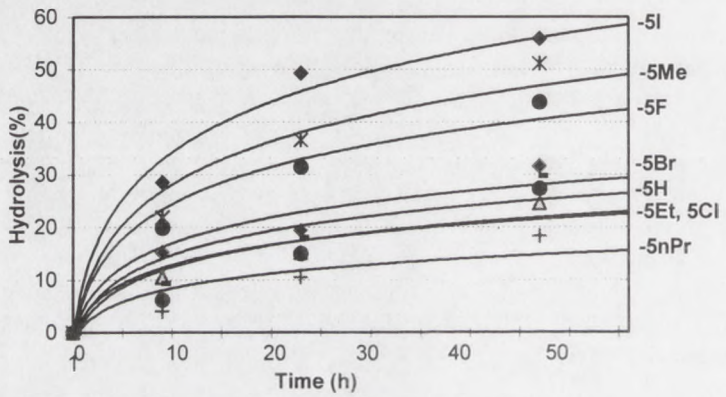
Fig. 2

The aim of presented research was to establish any 'rule' that could help to predict some properties of modified RNA duplexes. Although any simple 'rule' cannot be established, it can be reported that the three modified nucleotides containing halogen atoms (Cl, Br, I) in position C5 of heterocyclic base increase the thermal stability of corresponding RNA duplexes (ΔT_m from $+2.0^0$C to $+3.0^0$C per modification with $\Delta\Delta G^0_{37}$ from 0.2 kcal/mol to 0.5 kcal/mol per modification). Moreover it can be also noticed that chlorine and bromine atoms, having similar sizes and electronegativities, have very comparable effects. Smaller increase of stability, observed in case of iodine substituent, can be due to its bigger radius. Such a large atom can create some steric hindrance and through this may weaken the effect of electronegative substituent. The interesting exception is fluorine atom, which in spite of the smallest size and the biggest electronegativity decreases the stability of the corresponding duplex (ΔT_m -0.6^0C with $\Delta\Delta G^0_{37}$ of 0.2 kcal/mol per modification). The observations concerning alkyl groups seem to be even more clear. The methyl group slightly increases the stability of the modified duplex, while the ethyl group introduces unfavorable steric hindrance which causes substantial decrease of duplex stability (ΔT_m -3.1^0C with $\Delta\Delta G^0_{37}$ of 0.54 kcal/mol per modification). In case of more bulky n-propyl group this effect, according to expectations, is comparable (ΔT_m -2.5^0C with $\Delta\Delta G^0_{37}$ of 0.47 kcal/mol per modification). The duplex destabilization can be also explained by hydrophobic effect, i.e. by the reduction of the cation solvation.

THE INVOLVMENT OF URIDINE AND ADENOSINE RESIDUES IN SPECIFIC NONENZYMATIC RNA HYDROLYSIS

A. Bibiłło, M. Figlerowicz, R. Kierzek
Institute of Bioorganic Chemistry PAS, Noskowskiego 12/14, Poznań, Poland

Instability of the tested samples is the most common problem encountered during different studies involving RNA. However, there are several lines of evidence suggesting that RNA degradation cannot be considered as a random process. This phenomenon seems to be closely related to specific nonenzymatic RNA hydrolysis studied in our laboratory. We observe that in the absence of enzymes which could catalyze RNA cleavage (nucleases or ribosimes) some single-stranded RNA fragments are hydrolyzed in specific manner. Undertaken studies demonstrated that two types of phosphodiester bonds are especially susceptible to nonenzymatic hydrolysis: pyrimidine-A and pyrimidine-C. Their hydrolizability depends on the length of the single-stranded RNA fragment and the cleaved bond vicinity. Collected data allowed us to identify the most sensitive sequences. From among those RNA motifs, we chose one, namely UCGU↓AAC displaying especially high rate of specific nonenzymatic hydrolysis (an unstable bond between U and A is pointed with an arrow). This oligomer was applied in our further studies on the mechanism of RNA hydrolysis. Since we observed a distinct relationship between the rate of hydrolysis and RNA primary structure we intended to determine which elements in nucleoside structures decide that UpA phosphodiester bond is the most sensitive to hydrolysis. Our former studies indicated that the base stacking and nucleotide conformation around N-glycoside bond are two the most important factors which potentially can influence the rate of specific nonenzymatic hydrolysis. To answer this question three series of modified oligoribonucleotides UCGU↓AAC were obtained. In the first and the second series, forming sensitive phosphodiester bond uridine was replaced with its 5 substituted derivatives. In the first series the substitution consisted in exchange of the H5 for one of the halogen either F, Br, Cl or I. We assumed that electronegativity of these atoms should influence aromatic character of the heterocyclic bases and this way affect their stacking. In the second series H5 was exchanged for methyl, ethyl or n-propyl group. In this case we expected that steric hindrance of the introduced alkyl groups would disrupt base stacking without significant affecting of the electron distribution within heterocyclic base. The third series comprise of two derivatives only 6-methyluridine and 8-bromoadenosine. In both cases introduced substituents change nucleoside conformation from anti- to syn-. The influence of particular modifications on the rate of specific nonenzymatic hydrolysis of UCGUAAC oligoribonucleotide is presented bellow.



SYNTHESIS OF POLYAMINOOLIGONUCLEOTIDE COMBINATORIAL LIBRARIES

Przemysław Godzina, Maria Markiewicz, Anna Astriab and Wojciech T. Markiewicz

Institute of Bioorganic Chemistry, Polish Academy of Sciences, Noskowskiego 12, PL-61704 Poznań, Poland

Studies on oligonucleotides carrying polyamine residues of different structure at various positions of oligonucleotide chains were recently described[1-6] as modifications that ease forming stable duplex and triplex structure with natural DNA. Thus, both 5'- and 3'-end modifications with natural polyamines were prepared and their analysis confirmed that formation of a triplex with double stranded DNA was easier than with unmodified oligodeoxynucleotides. Polyamine moieties were attached either *via* an appropriate linker to a 5', 3' or 2'-hydroxyl function[5,6] or to a sugar moiety or directly to a heterocyclic base residue[3,4].

We undertook a synthesis of phosphoramidites of deoxynucleosides carrying a protected polyamine moiety at pyrimidine and purine residues. Recently we described a new method of synthesis of phosphoramidite of 2'-deoxycytydine carrying a protected spermine moiety at *N*-4 position[7]. We reported also a synthesis of a polyaminooligonucleotide combinatorial library[7]. The analysis of the above library clearly showed that the presence of spermine moieties in oligodeoxyribonucleotides increases stability of their duplexes[7].

In this communication further studies on polyaminooligonucleotides and their combinatorial libraries will be presented.

References

1. Manoharan, M., Tivel, K. L., Andrade, L. K., Mohan,V., Condon, T. P., Bennett, C. F. and Cook, P. D. (1995) *Nucleosides and Nucleotides*, **14**, 969-973.
2. Olivas, W. M. and Maher, L. J. (1995) *Biochemistry*, **34**, 278-284.
3. Schmid, N. and Behr, J. P. (1995) *Tetrahedron Lett.* **36**, 1447-1450.
4. Prakash, T. P., Barawkar, D. A., Kumar, V. and Ganesh, K. N. (1994) *Bioorg. Med. Chem. Letters*, **4**, 1733-1738.
5. Tung, C.-H., Breslauer, K. J. and Stein,S. (1993) *Nucleic Acids Research*, **21**, 5489-5494.
6. Sund, C., Puri, N. and Chattopadhyaya, J .B. (1996) *Tetrahedron*, **52**, 12275-12290.
7. Markiewicz, W. T., Godzina, P., Markiewicz, M. and Astriab, A. *Nucleosides and Nucleotides,* in press.

SEMISYNTHETIC COMBINATORIAL LIBRARIES OF MURINE FAB

Piotr Kwaśnikowski and Wojciech T. Markiewicz

Institute of Bioorganic Chemistry, Polish Academy of Sciences, Noskowskiego 12, PL-61704 Poznań, Poland

The time consuming process of immunisation is being replaced by the *in vitro* generation of combinatorial libraries[1]. The success of this method strongly depends on the extent to which nature's mechanisms can be copied. Very large molecular diversity is considered to be essential starting feature for combinatorial antibody libraries that mimic immunisation *in vitro*. Also, unlike the hybrydoma method, combinatorial libraries can provide antibodies that are very hard, or even impossible to gain using the *in vivo* techniques[2].

Method utilises filamentous phages[1] being expressed in *E. coli*, as the carriers of antibody molecules exposed towards the environment. Since a single phage has only one sort of antibody molecule on its surface, monoclonal antibodies can be obtained from the library after selection procedure. Selected phage carries DNA sequence encoding antibody in its genome, thus evaluation of antibody sequence is assured. An expansion of immunological repertoire by the semisynthesis of antibody genes has been described[3].

In this communication the construction of two sublibraries of Fab fragment antibody chains randomised in the third complementarity-determining regions (CDR3s) will presented. These CDR3s are said to contribute most in terms of antigen binding contacts and diversity. As a „template" antibody murine anti-glicophorin N monoclonal antibody 425 has been chosen[4]. The original sequences of both light and heavy chain CDR3 (LCDR3 and HCDR 3, respectively) were substituted by the synthetic sequence within the PCR primer during PCR amplification of the 3' regions of antibody chains. Parallel, a 5' regions PCR amplification of both chains was carried out. Complete sequences of randomised antibody chains genes were obtained in the final PCR reaction of an overlap extension type, using previously amplified chain regions. The semilibrary of the light chain genes was successfully cloned in the pC3HSS vector of Barbas.

References

1. Smith, G. P. (1985) *Science*, **228**, 1315
2. Winter, G., Grffiths, A. D., Hawkins, R. E., Hoogenboom, H. R. (1994) *Ann. Rev.* Immunol., 12, 433.
3. Barbas III, C. F., Bain, J. D., Hoekstra, D. M., Lerner, R. A. (1992) *PNAS USA*, **89**, 4457.
4. Czerwiński, M., Siegel, D. L., Moore, J. S., Spitalnik, P. F., Spitalnik, S. L. (1995) *Transfusion*, **35**, 137.

APPLICATION OF SYNTHETIC OLIGONUCLEOTIDE COMBINATORIAL LIBRARIES IN TRIPLEX DNA STUDIES

Anna Astriab, Maria Markiewicz and Wojciech T. Markiewicz

Institute of Bioorganic Chemistry, Polish Academy of Sciences, Noskowskiego 12, PL-61704 Poznań, Poland

The possibility of using of a *Synthetic Oligonucleotide Combinatorial Libraries* (SOCL) to study triplex DNA formation was chosen for testing the library approach and as a first application. A binary system was chosen to study the formation of complex of nucleic acids able to form a triplex structure. However, the interaction studied consisted of three chains of nucleic acids. In the same time a selection consisted of one step procedure. Two chains forming a duplex part of selection complex belonged to the same molecule designed in such way that it formed a „hairpin" structure. This hairpin structure contained the appropriate labelling moiety. The label was either fluorescent dye (fluorescein or rhodamine) or biotin residue[1,2].
Recently the application of deoxyguanosine and deoxythymidine containing oligonucleotides (GT oligodeoxynucleotides, GT oligo) in the formation of a triplex DNA structure with the promoters of human epidermal growth factor receptor gene (HER2) was described[3].
Thus, the hairpin oligonucleotide labelled with rhodamine or fluorescein containing in a duplex region a sequence of the promoter of human epidermal growth factor receptor gene was used to screen the SOCL containing a 15-mer G/T region[4].
Results of the SOCL analysis will be presented.

References

1. Markiewicz, W. T., Gröger, G., Rösch, R., Zebrowska, A. and Seliger, H. (1992) *Nucleosides, Nucleotides*, **11**, 1703-1711.
2. Markiewicz, W. T., Adrych-Rożek, K., Markiewicz, M., Żebrowska, A. and Rożek, M. (1993) *Collection Czechoslov. Chem. Commun. Spec. issue*, **58**, 214-218.
3. Milligan, J. F., Krawczyk, S. H., Wadwani, S. and Matteucci, M. D. (1993) *Nucleic Acids Research*, **21**, 327-333.
4. Markiewicz, M., Astriab, A. and Markiewicz, W. T. (1996) *Collection Czechoslov. Chem. Commun. Spec. issue*, **61**, 120-121.

THERMODYNAMIC CYCLE BETWEEN DNA AND RNA CONSTITUENTS FOR CONFORMATION OF THE SUGAR RING

Mieczysław Remin

Department of Biophysics, University of Warsaw,
02-089 Warszawa, Poland

The effect a structural change of ribose to deoxyribose, by replacement of 2'-OH by 2'-H, on the conformational equilibrium of the sugar ring was described in terms of one thermodynamic cycle.* The method is based on the observation that conformational correlations of the sugar ring - side chain ensemble in DNA and RNA components show one general pattern, reflecting an intrinsic physical property of this ensemble. The pattern determines a choice of model systems to study. The systems consist of pairs of DNA and RNA components, nucleosides and nucleotides in aqueous solution, where all conformational factors are fully controlled.

This approach allowed us to describe the thermodynamic cycle and measure its fundamental parameters, equilibrium constants and free energy differences, $\Delta\Delta G$, from a nuclear magnetic resconance study. The $\Delta\Delta G$ values as detrmined for pairs of ribo- and deoxyribo- nucleosides in classes of syn-constrained and anti-preferred models, are comparable and lie in a narrow range, $\Delta\Delta G = 1.7 \pm 0.1$ [kJ/mol]. For pairs of ribo- and deoxyribo- nucleotides, the $\Delta\Delta G$ values also lie in narrow ranges, $\Delta\Delta G = 1.7 \pm 0.1$[kJ/mol] for 5' -phosphate nucleotides and $\Delta\Delta G = 1.9 \pm 0.1$ [kJ/mol] for 3' -phosphate nucleotides, i. e. similar to those observed for nucleosides.

The measured quantity, $\Delta\Delta G$, is generally observed in a relatively narrow range, $\Delta\Delta G = 1.75 \pm 0.15$ [kJ/mol], irrespective of the model system. This quantity represents a "pure" constant contribution, per one sugar moiety, as a "driving force" for the N--> S shift in the sugar ring conformational equilibrium, when one compares RNA and DNA. Ultimately this importrant thermodynamic quantity, $\Delta\Delta G$, is revealed in the tendency to adopt S(C2'endo) sugar puckering domain by the majority of DNA structures, whereas RNA generally adopt an N(C3'endo) puckering domain. A possible biological significance of the quantity $\Delta\Delta G$ may include evolutionary aspects of nucleic acids.

*M. Remin, J. Biomolecular Structure & Dynamics 15(2), 251-264 (1997).

MOLECULAR DYNAMICS SIMULATION OF 5S rRNA FRAGMENTS

J. Sarzyńska[1,2], L. Nilsson[2], T. Kuliński[1]

[1]Institute of Bioorganic Chemistry, Polish Academy of Sciences, Pl-61704 Poznań, Poland.
[2]Center for Structural Biochemistry, Karolinska Institutet, S-171 77 Stockholm, Sweden

5S rRNA is an essential component of the ribosome in both prokaryotic and eukaryotic organisms and has been the object of extensive functional and structural studies. Up to now, techniques, which give a direct insight into the molecular structure, have only contributed information about selected fragments of the 5S rRNA molecules. Significant contributions toward understanding 5S rRNA structure in solution has been obtained by biochemical methods such as enzymatic digestion, oligonucleotide binding, or metal ions hydrolysis.
Microcalorimetric and hydrolytic data point to the existence of stable regions extending standard helices by nonstandard interaction within loops regions, which is thought to explain the larger experimental enthalpy of the structure compared to those calculated theoretically [1,2].
The loop *c* itself exhibited unexpected stability and resistance against metal ion hydrolysis. On the other hand bases of the loop *c* should be involved in the tertiary interaction between two arms, as in the proposed model for eucariotic 5S rRNA. The evaluation of the structure and its dynamics should give the answer about the possible engagement in tertiary interactions and resistance against hydrolysis.
We analyze the structural details of the fragment containing loop *c* of 5S rRNA from Lupin seeds and their conformational equibria at the atomic resolution in different ionic conditions in aqueous solution by molecular modelling and by molecular dynamics simulation. In particular the formation of water mediated hydrogen bonds and its participation in the stabilization of structural domains and stacking interaction is examined.

1. Kuliński,T.,Bratek-Wiewiórowska, M.D.,Wiewiórowski,M., Zielenkiewicz, A., Żółkiewski,M. and Zielenkiewicz, W., (1991) Nucleic Acid Research 19, 2449
2. Kuliński,T.,Bratek-Wiewiórowska, Zielenkiewicz, A., Zielenkiewicz, W., (1997) J. Biomolecular Structure and Dynamics 14,495-507.

Lagrangian and Quaternion Molecular Dynamics of Nucleic Acids.

Witold R. Rudnicki,[1] Grzegorz Bakalarski,[1,2] Maciej Maciejczyk[1] and Bogdan Lesyng[1]

[1] Interdisiplinary Centre for Mathematical and Computational Modelling, Warsaw University, Pawińskiego 5A, 02-106 Warsaw, Poland.
[2] Department of Biophysics, Agricultural University –SGGW, Rakowiecka 26, 02-528, Warsaw, Poland.

We present an application of a general model for mezoscopic molecular dynamics applied to simulations of nucleic acids. LQMD model can be applied to simulations with objects of different scale and properties e.e. atoms, pseudoatoms, rigid and pseudoelastic bodies, described by the Cartesian coordinates and internal degrees of freedom. Pseudoelastic body is defined as a body with inertial properties dependent on the internal degrees of freedom. The Lagrangian approach was used to derive equations of motion. The quaternion representation is used for the description of dynamics of rigid and pseudoelastic bodies, replacing the conventional Euler-type equations of motion, the latter resulting from the Lagrangian approach. The quaternion formalism allows for fast and numerically stable algorithms.

The potential energy function for the model was based on the results of the all atom simulations, and was obtained as the PMF parameterized with respect to the phases of pseudorotation.

One nanosecond LQMD simulations were performed for the DNA decamer. Pseudoatomic model was compared with the mixed pseudoatomic and rigid body model. Stability of the algorithms for various time-steps was tested. It was found that the mixed model allowed for significantly longer time-steps. The total energy, momentum and angular momentum were conserved for time steps up to 20 fs in the case of the mixed model. Simulations for the test system with the LQMD code were three orders of magnitude faster than conventional all atom simulations for this system.

The *LQMD* model is not limited to nucleic acids. In principle, it can be used for modeling polymers, liquids and solid state systems. One should note, however, that simulations with this model are not straightforward. For each system, one has to select internal degrees of freedom, write the Lagrangian as a function of those degrees of freedom, and derive equations of motion. Then the appropriate algorithms for solving numerically particular equations of motion have to be applied. One of the directions of further research is to simplify or even automatize this process, using symbolic, artificial intelligence packages.

References

1. Rudnicki, W., Lesyng, B., and Harvey, S.(1994) Biopolymers 34, 383-392.
2. Rudnicki, W.R. (1997), *PhD Thesis*.
3. Rudnicki, W., and Lesyng, B., submitted to Molecular Simulation.
4. Maciejczyk, M., Rudnicki, W., and Lesyng, B. *in preparation*.

MOLECULAR BASIS OF DNA RECOGNITION BY bZIP TRANSCRIPTIONAL ACTIVATORS: MODELING STUDIES

Krzysztof Ginalski [1,2], Maria Miller [3], Maciej Geller [1,2]

[1] *Interdisciplinary Centre for Mathematical and Computational Modelling, Warsaw University, Pawińskiego 5a, 02-106 Warsaw, Poland; e-mail: kginal@icm.edu.pl;*
[2] *Department of Biophysics, Institute of Experimental Physics, Warsaw University, Żwirki i Wigury 93, 02-089 Warsaw, Poland;*
[3] *Macromolecular Structure Laboratory, NCI-Frederick Cancer Research Facility and Development Center, Frederick, MD 21702, USA*

Basic region-leucine zipper (bZIP) transcriptional activators can be classified into five distinct subfamilies based on their DNA sequence binding specificity. In order to understand the molecular basis of this specific DNA sequence recognition, we built 3D models of the bZIP peptides bound to its consensus DNA site for three activators (TEF, CEBA end EMP1), each of them belonging to a different bZIP subfamily, for which no structural data are available. These models were based on the 2.2 Å crystal structure of the complex of GCN4 DNA binding domain with 18 bp DNA oligomer containing the ATF/CRE site [1], PDB code: 2DGC. The modeling was done using the HOMOLOGY program from the INSIGHT II program package, version 95.5. All energy optimizations were performed using the DISCOVER program in the AMBER forcefield with distance dependent dielectric constant of 4r. Our modeling studies allowed to understand the basis for the bZIP-DNA interactions and the mechanism of the specific DNA sequence recognition which rely on the complicated network of interactions among the side chains of the recognition helix and the sugar-phosphate backbone.

Literature:

1. Keller, W., Konig, P. & Richmond, T. J. (1995) Crystal structure of a bZIP/DNA complex at 2.2 Å: determinants of DNA specific recognition. *J. Mol. Biol.* **254**, 675-667.

The studies were supported in part by the National Cancer Institute, DHHS, under contract with ABL, and the Polish State Committee for Scientific Research.

INHIBITION OF PAI-1 RELEASE IN EA.HY 926 CELL CULTURES BY 5'-END LIPOPHILIC MODIFICATION OF OLIGONUCLEOTIDES

Anna Kobylańska,[1] Elżbieta Pluskota,[2] Zofia Pawłowska,[2] Maria Koziołkiewicz,[1] Andrzej Okruszek,[1] Czesław Cierniewski[2] and Wojciech J. Stec[1]

[1]*Department of Bioorganic Chemistry, Centre of Molecular and Macromolecular Studies, Polish Academy of Sciences, Sienkiewicza 112, 90-363 Łódź, Poland*
[2]*Medical University in Łódź, Department of Biophysics, Lindleya 3, 90-131 Łódź, Poland*

Type-1 Plasminogen Activator Inhibitor (PAI-1) plays an important regulatory role in fibrinolysis and thrombus formation, and its effective inhibition could be an alternative approach to a thrombolytic therapy. In our previous reports (1-3) the results were described on specific inhibition of PAI-1 biosynthesis in HUVEC cell cultures by phosphorothioate oligonucleotides complementary to various regions of PAI-1 mRNA.

In this communication we present the data on antisense inhibition of PAI-1 in cultured EA.hy 926 hybrid endothelial cells by oligonucleotide 5'-conjugates of general structure RO-P(S)(O^-)-(O^-)-[X]-$^{5'}$O-OLIGO, where RO are the residues of lipophilic alcohols (borneol, cholesterol, heptadecanol, menthol) covalently attached to the 5'-end of hexadecadeoxyribonucleotide (phosphodiester or phosphorothioate) complementary to signal peptide region of PAI-1 mRNA. The RO residues are connected to the oligonucleotide (*via* a phosphorothioate linkage) either directly or through a linker comprising three tetraethylene glycol units combined *via* phosphate or phosphorothioate linkages. Appropriate oligonucleotides and conjugates were used as controls.
The highest inhibition of PAI-1 release (up to 65% at 1.25 μM concentration) was observed for conjugates of menthol and heptadecanol.
The conjugates of phosphodiester oligonucleotide were found to be as active as those of corresponding phosphorothioate oligonucleotide. The presence of a linker had no major influence on inhibitory activity.

(1) Cierniewski, Cz.S., Babińska, A., Świątkowska, M., Wilczyńska, M., Okruszek, A., Stec, W.J. (1995) *European J.Biochem.*, **227**, 494-499.
(2) Stec, W.J., Cierniewski, Cz.S., Okruszek, A., Kobylańska, A., Pawłowska, Z., Koziołkiewicz, M., Pluskota, E., Maciaszek, A., Rębowska, B., Stasiak, M. (1997) *Antisense and Nucleic Acid Drug Development*, **7**, 567-573.
(3) Buczko, Wł., Cierniewski, Cz., Kobylańska, A., Koziołkiewicz, M., Okruszek, A., Pawłowska, Z., Pluskota, E., Stec, W.J. (1997) *Pharmacology & Therapeutics*, **76**, 161-175.

OLIGONUCLEOTIDE LIBRARIES AND RNase H IN STRUCTURE PROBING OF THE HDV RIBOZYMES

Jan Wrzesinski, Marcin Matysiak, Barbara Smólska and Jerzy Ciesiołka

Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznań, Noskowskiego 12/14, Poland

Escherichia coli ribonuclease H (RNase H) is an endonucleolytic ribonuclease which digests DNA-RNA duplexes. Oligodeoxynucleotides complementary to an RNA target sequence can be employed in determination RNA regions accessible for hybridization. Only exposed single-stranded regions form stable DNA-RNA duplexes recognized by RNase H and cleaved with high efficiency. That approach requires, however, at least preliminary information on secondary structure of RNA targets and synthesis and testing several oligonucleotides. Recently, a modification of the methods has been proposed that involves random oligonucleotide libraries instead of oligonucleotides of defined sequences (1, 2).

We have used oligodeoxynucleotide libraries to structure probing of RNAs derived from the genomic and antigenomic HDV ribozymes. Libraries composed of oligomers as short as 6-mers formed stable DNA-RNA hybrids recognized and cleaved by RNase H. The antigenomic ribozyme was more accessible to hybridization and digestion, particularly in the J1/2 and P4 regions. The J1/4 region was cleaved by RNase H with comparable efficiency in the both ribozymes. Almost identical digestion patterns were obtained for the full length ribozymes and their corresponding 73-nucleotide long 3'-truncates. We confirmed the accessibility of the J1/2 and J1/4 regions of the antigenomic ribozyme to hybridization using DNA oligomers of defined sequences. These oligomers are potentially useful in the antisense strategy applied to the antigenomic ribozyme region of the viral RNA.

1. Ho et al., (1996) *Nucleic Acids Res.* **24**, 1901-1907
2. Eckstein et al., (1997) *RNA* **3**, 429-437

This work was supported by the grant No. 6 P04A 001 12 from the State Committee for Scientific Research.

FOLDING OF THE HDV RIBOZYMES: STRUCTURAL ANALYSIS OF THEIR 3'-TRUNCATES

Marcin Matysiak, Jan Wrzesinski and Jerzy Ciesiołka

Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznań, Noskowskiego 12/14, Poland

A very similar secondary structure model of the pseudoknot type has been proposed for both the genomic and antigenomic HDV ribozymes. Experimental data show, however, that there are remarkable differences in their structures. In order to gain insight into the overall folding of both ribozymes we analyzed the structure of their progressively shortened 3'-truncates. We synthesized the following RNA oligomers: 84-mer corresponding to the 3'-product of the genomic ribozyme and two its 3'-truncates: 73-mer and 43-mer as well as 84-mer corresponding to the 3'-product of the antigenomic ribozyme and its 3'-truncate: 74-mer. The oligomers were subjected to structural probing with Pb2+ ions as well as single- and double-strand specific nucleases. The results for the genomic HDV ribozyme are consistent with its pseudoknot model. The P1 and P4 helical segments persist in the ribozyme in the final forms adopted already in the truncates. The region corresponding to the P3/L3 domain forms an ordered hairpin loop structure in the truncates. That hairpin undergoes major rearrangement upon the formation of a pseudoknot in the full length ribozyme. Free energy of the pre-formed RNA hairpin and stabilization energy of the pseudoknot are compared and a hypothetical model for that rearrangement is proposed. On the other hand, the P3/L3 domain in the antigenomic ribozyme and in its 74-nucleotide long 3'-truncate is much less ordered. The results of structural probing suggest that this region shows high conformational flexibility or tendency to form alternative conformers. The results will be discussed in terms of folding and folding pathways of the HDV ribozymes.

This work was supported by the grant No. 6 P04A 001 12 from the State Committee for Scientific Research.

HAMMERHEAD RIBOZYMES AS TOOLS FOR STUCTURAL STUDIES OF RNA

Maciej Szymański[1], Jens-Peter Fürste[2], Mirosława Z. Barciszewska[1], Volker A. Erdmann[2] and Jan Barciszewski[1]

[1]Institute of Bioorganic Chemistry, Polish Academy of Sciences, Noskowskiego 12/14, 61-704 Poznań, Poland;
[2]Institut für Biochemie, Freie Universität Berlin, Thielallee 63, 14195 Berlin, Germany

We synthesized and used hammerhead ribozymes designed for the GUC and AUC target sequences located in the terminal loop C of animal and plant 5S rRNAs to verify its involvement in a higher order structure formation of ribosomal RNA. The activity of both GUC and AUC specific ribozymes were prooved to be active against synthetic analogs of the stem III and loop C in the two types of 5S rRNAs. In contrast we did not observe any ribozyme-catalysed hydrolysis within the loop C of the native 5S rRNAs. We considered two possible explanations of the results: an involvement of the loop C in long range interactions and lack of a structural flexibility to form proper tertiary structure of the complex with the ribozyme. From our data it seems that the single stranded nucleotides within the loop C can not form correct RNA duplex structure required for ribozyme activity. It is clear that hammerhead ribozymes can be used for inhibition of different steps of expression of genetic information only if the target sequence occurs in appropriate context, that allows to form correct secondary and tertiary structure of the ribozyme-substrate complex.

INTERACTION OF HIV Tat MODEL-PEPTIDES WITH tRNA AND 5S RNA

Małgorzata Giel-Pietraszuk[1], Iwona Buśkiewicz[1)], Piotr Mucha[2], Piotr Rekowski[2], Gotfryd Kupryszewski[2] and Mirosława Z. Barciszewska[1)]

[1]) Institute of Bioorganic Chemistry, Polish Academy of Sciences, Noskowskiego 12/14, 61-704 Poznań
[2]) Faculty of Chemistry, University of Gdańsk, Sobieskiego 18, 80-952 Gdańsk

We present a new data on the interaction of arginine-rich peptides of human immunodeficiency virus (HIV-Tat) with native RNA molecules: $tRNA^{Phe}$ of *Saccharomyces cerevisiae* and 5S rRNA from *Lupinus luteus*. Both RNA species form complexes with the Tat1 peptide (GRKKRRQRRRA) and Tat2 peptide (GRKKRRQRRRAPQDSQTHQASLSKQPA). This was shown by RNase footprints assay and CD-spectra measurements. The nucleotide sequence UGGG located in the dihydrouridine loop of $tRNA^{Phe}$ as well as in the loop D of 5S rRNA is specifically protected against RNases. Our data identified direct interactions of guanine of RNA moieties with arginine residues. They are similar to those observed in DNA - protein complexes, but different from those previously observed in the TAR RNA-Tat complexes.

FIRST TRANSCRIPTION FACTOR IIIA FROM HIGHER PLANTS. PURIFICATION AND CHARACTERIZATION.

Eliza Wyszko and Mirosława Barciszewska

Institute of Bioorganic Chemistry, Polish Academy of Sciences, Noskowskiego 12/14, PL-61704 Poznań, Poland,

Transcription factor IIIA (TF IIIA) is known to bind and specifically activate transcription of eukaryotic 5S rRNA genes. It also forms of 7S RNP complex with mature 5S rRNA. In this paper we show a purification and properties of TF IIIA from higher plants. The purified protein from tulip (*Tulipa whittalii*) has a molecular mass of about 40 kDa and binds 5S rRNA and 5S rRNA genes as well. It also facilitates transcription of a 5S rRNA gene in a HeLa cell extract. To our knowledge this is the first report on a TF IIIA type protein from higher plants (pTF IIIA).

STRUCTURAL STUDIES OF THE BROME MOSAIC VIRUS RNA POLYMERASE; THE KEY PROTEIN IN GENETIC RNA RECOMBINATION. 1. EXPRESSION AND PURIFICATION OF THE POLYMERASE N-TERMINAL DOMAIN

Magdalena Alejska, Nelli Malinowska, Marek Figlerowicz

Institute of Bioorganic Chemistry Polish Academy of Sciences, Noskowskiego 12/14, Poznań, Poland

The genome of brome mosaic virus (BMV) is composed of three separately encapsided RNA molecules (RNA1, RNA2 and RNA3). RNA1 and RNA2 encode viral replicase proteins 1a and 2a respectively, while RNA3 encodes movement and coat proteins. Former biochemical studies demonstrated that 1a and 2a proteins are indispensable for virus RNA replication. The proteins bind each other and together with some still not well defined host factors form the viral replication complex (its organization is schematically presented in Fig. 1).

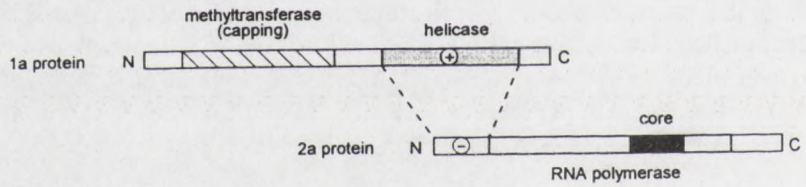


Fig.1. The domain organization and interaction between BMV 1a and 2a proteins within the viral replication complex. In the 1a protein two separate domains can be distinguished: methyltransferase and helicase. In the 2a central part of the protein constitutes RNA dependent RNA polymerase while its N-terminal portion is involved in 1a-2a interactions.

Previously it was found out that BMV can support the formation of all three types of RNA recombinants: homologous, aberrant homologous and nonhomologous. In addition, our studies revealed that BMV 2a protein is involved in the RNA recombination process. We observed that mutation of the viral polymerase can influence frequency, location and precision of recombinational crossovers. The structural studies of 2a protein have been undertaken to understand this phenomenon. In their initial stage we have focused on the 2a N-terminal domain. It has been expressed as a separate recombinant protein in a bacterial system. Then a suitable method for the 2a N-terminus purification has been elaborated.

CRYSTAL STRUCTURE OF A COMPLEX BETWEEN DNA METHYLTRANSFERASE FROM *THERMUS AQUATICUS* AND S-ADENOSYLHOMOCYSTEINE

Maciej Kozak[1] Gerd Schluckebier[2] and Wolfram Saenger[2]

[1]Deptment of Crystallography, A. Mickiewicz University, Poznań, Poland
[2]Institut für Kristallographie, Freie Universität Berlin, Germany

DNA methylation is found in all organisms ranging from bacteria to plants and mammals. This reaction is catalysed by methyltransferases (Mtases), which use S-adenosyl-L-methionine (AdoMet) as methyl group donor to yield the methylated DNA, the reaction product and S-adenosyl-L-homocysteine (AdoHcy). If accumulated to higher concentration, AdoHcy becomes an inhibitor of the methylation process.

The bacterium Thermus aquaticus possesses at least two resriction-modification systems. The TaqI system recognizes double stranded DNA at the palindromic sequence TCGA. The TaqI methyltransferase (MTaqI) modifies the adenine residue at the N6 position on each strand of the recognition site. The X-ray structure of a binary complex between MTaqI and the cofactor AdoMet substrate has been described (Labahn et al, 1994).

We have crystallized and determined the crystal structure of a binary complex between M.TaqI and AdoHcy. Crystals of the complex were grown using the vapour diffusion method. Drops containing 0.65 uM M.TaqI, 1mM AdoHcy ,1uM DNA 13-mer (with the 5'-TCGA-3' recognition motif) and 200 mM NaCl in 20 mM Tris/HCl buffer (pH 7.3) were equilibrated against solution containing 4% PEG 6000, 50 mM NaCl in 50 mM Tris/HCl buffer (pH 7.3). X-ray diffraction data were collected at room temperature using MarResearch image plate scanner and CuKa radiation. The crystals are isomorphous to those of the MTaqI-AdoMet complex, which could be used as a model. The electron density map clearly shows well defined difference electron density in the cofactor binding site. The inhibitor moiety was bulit into electron density. The structure of the complex, together with 69 water molecules, was refined using X-PLOR (Brunger, 1992). At the final stage of the refinement the crystallographic R-factor and R-free are 0.21 and 0.28, respectively and rms deviations from ideal geometry are 0.008 A (bonds) and 1.57 deg (angles).

The binding mode of AdoMet is identical in the adenosine fragment to that observed in the MTaqI-AdoMet complex. The amino acid fragment (homocysteine) is bound to the conserved 105-NPPY-108 motif IV, whereas is in the MTaqI-AdoMet complex the aminoacid moiety is located in the binding cleft.

- Labahn, J., Granzin, J., Schluckebier, G., Robinson, D.P., Jack, W.E., Schildkraut, I., Saenger, W. (1994), Proc. Natl. Acad. Sci. USA 91, 10957-10961.
- Brunger, A.T. X-Plor Version 3.1. (1992), New Haven and London, Yale Univ. Press.

ASPARAGINASES FROM GENOME SEQUENCING PROJECTS

Dominika Borek[1], Jan Podkowiński[2], David T. Bonthron[3] and Mariusz Jaskólski[1,2]

[1]Department of Crystallography, A. Mickiewicz University, Poznań, Poland
[2]Institute of Bioorganic Chemistry, Poznań, Poland
[3]Human Genetics Unit, University of Edinburgh, UK.

L-asparaginases hydrolyze L-asparagine to L-aspartate and ammonia. Enzymes with L-asparaginase activity are found in many organisms ranging from bacteria to mammals. Sequence analysis of these enzymes has revealed that there are at least two quite different families, which can be further divided into several subgroups.

We have searched the available genomic databases for sequences classified as L-asparaginases or having high similarity to the known L-asparaginases. Comparisons and alignments of 30 deduced amino-acid sequences from different organisms segregate them into the two L-asparaginase families. One family includes two E.coli enzymes, each of which defines a different subgroup. Enzymes in the subgroup typified by EcAII (E.coli isozyme II) are found in the periplasm of some bacteria. Because of their potent anti-leukemic activity, they have been thoroughly studied by X-ray crystallography. This subgroup also has eukaryotic members, from S. cerevisiae (ASP1, ASP3) and S. pombe. Many bacteria also carry a gene encoding a separate, type I asparaginase. Type I bacterial asparaginases are cytoplasmic and show noticable sequence differences from EcAII. Nevertheless, these two kinds of isozymes probably share the same principle of the enzymatic mechanism because of the conservation of the catalytic residues.

The second family of L-asparaginases comprises a new (third) E.coli gene, which is similar to plant L-asparaginases. Within this family, the plant asparaginases and two bacterial sequences (Synechocystis and E.coli) form a separate subgroup. A second subgroup comprises aspartyloglucosylaminidases (AGA), well known enzymes involved in the catabolism of N-glycosylated glycoproteins. They cleave asparagine from the residual N-acetylglucosamines in the final steps in lysosomal breakdown of glycoproteins. In humans, deficiency of an aspartyloglucosylaminidase causes a lysosomal disease known as aspartylglycosaminuria. The enzymes in the AGA subgroup also have L-asparaginase activity.

Data from genome sequencing projects open new possibilities for finding sequences with high homology to known target sequences, such as L-asparaginases, and for studing evolutionary relations among them.

PURIFICATION AND PROPERTIES OF LUPIN LDC

Maciej Astriab

Institute of Bioorganic Chemistry PAS,
Noskowskiego 12/14 St,
Pl-61-704 Poznań.
astriabm@ibch.poznan.pl

The distribution of lysine decarboxylase (LDC), the first enzyme of biosynthetic pathway of quinolizidine alcaloids, was studied in the various organs of *Lupinus albus*. Lysine decarboxylase activity is observed in all plant parts, but the highest total activity of this enzyme has been localised in roots. Conditions for optimum activity of the enzyme and its purification were described. We established the molecular characteristics of this enzyme, including N-terminal sequencing of LDC. Researching the mechanism of its activity and particularly the regulatory function of LDC we found the presence of a specific inhibitor. We think that this inhibitor is responsible for the regulatory mechanism of LDC activity.

REGULATORY MECHANISM OF PLANT FERRITIN BIOSYNTHESIS.

Joanna Smól

Institute of Bioorganic Chemistry PAS
Noskowskiego st 12/14
61 - 704 Poznań, Poland
jsmol@ibch poznan.pl

Ferritin is an iron storage protein. Plant ferritin biosynthesis is regulated at the transcriptional and translational level. We suggest an autoregulatory mechanism of ferritin biosynthesis (mechanism of autorepression and derepression of mRNA specific for ferritin) at the translational level.
For the purified protein we determined physico - chemical characteristic including N - terminal sequence of both subunits. We found that both subunits have the same N-terminal sequences. Following our findings the subunits are not programmed by different mRNAs but most probably they are a result of posttranslational modification. The smaller subunit (with lower molecular weight) is probably shortened by about 20 amino acids from the C-terminal end. Sequence data showed the identity of ferritins of different origin in the range 60-92,5%. This high sequence similarity was confirmed by the analysis of genomic DNA from maize and soybean. Both DNAs share 72% nucleotide sequence identity.

IRON PROTEINS IN LUPINE (*LIPINUS LUTEUS*) TISSUES

Paweł M. Stróżycki and Andrzej B. Legocki

Institute of Bioorganic Chemistry Polish Academy of Sciences, Noskowskiego 12/14, Poznań, POLAND

Biological nitrogen fixation is the process by which atmospheric nitrogen is reduced to ammonia in the nodule bacteroids of legume plants. Bacterial enzyme responsible for this reaction - nitrogenase, contains iron in its active center. Iron is involved in many key pathways and reactions including DNA and hormone synthesis. Nitrogen reduction requires a large energy and reducing power supply, which come ultimately from the oxidation of plant photosynthates. Strong reducing conditions, in the nodule central region, lead to the formation of activated oxygen. Iron can act catalytically to generate hydroxyl radicals, the most potent oxidizing agents, which can damage all cellular components, leading to a loss of integrity and cell death. The most important elements of the iron management in nitrogen fixing systems are leghemoglobins and ferritins.

Leghemoglobin, the most abundant plant protein in the nodule is a metalloprotein (iron in hem moiety), which protects nitrogenase from the excess of oxygen and supplies oxygen to the respiratory chain. Ferritin is a multimeric protein which stores iron in the nontoxic form . This ability makes ferritin a substantial source of iron and protection agent against iron-induced toxicities.

Lupine lb gene codes for the longest leghemoglobin polypeptide found. The only longer hemoglobins are those of nonsymbiotic type or from nonlegume plants. In spite of the absence of typical leghemoglobin elements, lupine lbI promoter directs an organ specific expression of the controlled gene.

Also the expression of the iron-storage protein gene - ferritin reveals several distinct features. A new class of ferritin RNA appears during the nodule formation. The nature of this appearance is currently under the investigation. Phylogenetic analysis of *L. luteus* leghemoglobin and ferritin sequences seems to confirm the opinion that lupine is one of the oldest members of the legume family.

This work was supported by grant from the State Committee for Scientific Research No. 6 P04B OO1 13.

Stróżycki PM and Legocki AB (1995) Leghemoglobins from an evolutionarily old legume, Lupinus luteus. Plant Sci. 110: 83-93

Stróżycki PM and Karłowski WM (1996) Nitrogen fixing systems and evolution of plant hemoglobins. In Biological Fixation of nitrogen for ecology and sustainable agriculture.: 325-328 A.B.Legocki, H.Bothe, A. Pühler eds, Springer-Verlag.

Stróżycki PM and Karłowski WM and Legocki AB. (1998) Yellow lupine gene coding for leghemoglobin I (Accession No.U50083) (PGR98-017). Plant Physiol. 116: 867

EXPRESSION OF YELLOW LUPINE GENES CODING FOR MITOTIC CYCLINS

Joanna Jeleńska, Andrzej B. Legocki

Institute of Bioorganic Chemistry, Polish Academy of Sciences,
Poznań, Noskowskiego 12/14, Poland

Cyclins, with adequate CDK kinases, regulate the eukaryotic cell division. They appeare only at specific phases of the cell cycle: at G1/S (start) and G2/M (mitosis) transition. Higher plants, as other Eukaryota, possess a family of numerous cyclins, playing different roles during the cell division.

We have characterized the expression of four *Lupinus luteus* cyclins: *Cyc*1, *Cyc*2, *Cyc*3 and *Cyc*4. Northern analysis with *Cyc*1 and *Cyc*2 cDNA probes revealed that cyclin genes were highly expressed in root and shoot meristem, less intense in lateral roots and undetectable in seeds, hypocotyl, root elongation zone and leaf. Since fourth day after inoculation with *Bradyrhizobium sp. (Lupinus)*, cyclin level was higher in nodulating root fragments that in uninfected ones, with maximum between 12^{th} and 16^{th} day.

Since cyclins are expressed only in few cells during short periods of time, a more sensitive method, RT-PCR, was used to recognize the role of each cyclin during plant development, including nodule formation, and in the presence of fitohormones. RT-PCR showed that four analyzed cyclins were expressed in different organs at various level and more than one cyclin was present in particular tissues. Transcription of all of them was stimulated in nodules: *Cyc*3 and *Cyc*4 at initial steps of nodule formation, *Cyc*1 and *Cyc*2 - later. Cyclin 3 may be considered as nodule specific. Auxin increased the expression of all analyzed genes, cytokinin affected the expression of *Cyc*1 and *Cyc*4, while abscsic acid had no influence.

In situ hybridization of nodules with *Cyc*4 probe showed that the transcript was localized in the meristem region and was the most abundant in very young, forming nodule.

YELLOW LUPINE PROTEIN OF PR10 CLASS REVEALS THE RIBONUCLEASE ACTIVITY *IN VITRO*

M.M. Sikorski, J. Wrzesiński, J. Ciesiołka and A.B. Legocki

Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznań, Poland

Intracellular Pathogenesis-Related proteins of PR10 class have been shown to be ubiquitous in the plant kingdom. They are structurally related to tree-pollen allergens and to major food allergens from celery and apple. The precise cellular localisation of PR10 proteins has not been determined but the absence of apparent signal peptides classify them as cytosolic. The PR10 proteins are composed of 154-161 amino acid residues with pI between 4.7-6.0. They are resistant to protease treatment. It was reported by several laboratories that PR10 proteins accumulate around the sites of pathogen invasion or wounding. There are also suggestions that PR10 proteins play an important function in the plant development. They have been identified in seeds, developing roots, senescent leaves and senescent nodules, stems and different parts of flower. It has recently been shown that birch pollen allergen *Betv*1 belonging to the PR10 protein class revealed RNAse activity *in vitro*. The biochemical and physiological function of intracellular pathogenesis-related proteins of PR10 class and their contribution to the defence mechanism still remains unknown. The structural similarity to ginseng ribonucleases may classify them as ribonuclease-like PR proteins.

Recently, the X-ray and NMR structure of birch pollen allergen *Betv*1 was determined. The presence of a P-loop with the conserved sequence GXGGXGXXK (positions 46-54 in lupine and ginseng PR10 proteins, 47-55 in birch allergen) may indicate a phosphate binding site of cleaved RNA.

We have identified two homologous proteins of PR10 class in yellow lupine. Both proteins, *Ll*PR10.1A and *Ll*PR10.1B are constitutively expressed in root and stem of non infected plant, while in petiole and leaf the *Ll*PR10.1B is only detected. The *Ll*PR10.1A protein was accumulated in leaves upon the biotic stress after infiltration with pathogenic bacteria, *P. syringae* pv. *pisi*, but not in response to wounding.

In order to analyse the structure and function of lupine PR10 proteins, the cDNA coding sequences were introduced into the pET-3a expression vector and proteins were overexpressed in *E.coli* cells. The recombinant proteins were purified to the homogeneity and used for either crystallisation or functional studies.

An RNA oligomer, fragment of the genomic HDV ribozyme with well defined secondary structure was a substrate in the ribonuclease assay. So far only a preparation of *Ll*PR10.1B protein showed ribonuclease activity with pH optimum for hydrolysis in the range 6-8. Interestingly, single-stranded regions were preferentially cleaved with no apparent base specificity. The digestion pattern was, however, different from that generated by nuclease S1, another known a single strand specific nuclease. Control reactions showed that the ribonuclease activity of protein *Ll*PR10.1B was abolished by heat denaturation and proteinase K treatment and substantially decreased in the presence of urea.

Acknowledgement

This work was supported by grant from the State Committee for Scientific Research No.6 P04B 011 10.

Phylogenetic analysis indicates on a possibility of lateral transfer of the nodulation genes in lupine *Bradyrhizobium*

T. Stępkowski, A. Świderska, J. Biesiadka, K Miedzińska, M. Czaplińska, and A. B. Legocki.
Institute of Bioorganic Chemistry, Polish Academy of Sciences, 61-704 Poznań, ul. Noskowskiego 12, Poland

Bradyrhizobium sp.WM9 was isolated from yellow lupine (*Lupinus luteus*) plant grown in Roztocze region (eastern Poland). Among plant hosts which are effectively nodulated by WM9 are related with lupines broom (*Sarothamnus*) and laburnum (*Cytisus*), as well as unrelated with tribe Genisteae, seradella (*Ornithopus*) and bird's foot trefoil (*Lotus corniculatus*). The genetic analysis of the symbiotic loci indicates on similarity of the *nod* cluster in WM9 to the *nod* regions in other *Bradyrhizobium* spp. However, the nucleotide sequences of the whole *nod* cluster of WM9 shows an average 70% DNA homology to other bradyrhizobial *nod* genes, which is much lower than those reported for the *nod* gene homologies among *Bradyrhizobium* spp. It is noteworthy, that WM9 is the first intensively studied *Bradyrhizobium* isolate from Europe, and contrary to all other described *Bradyrhizobium* strains, infects plants, which evolved in temperate climate. On the other hand, comparison of the variable, 300 bp fragment of the SSU rRNA gene as well as a sequence of *dnaK* gene indicates on phylogenetic relatedness of WM9 to *Bradyrhizobium japonicum*. Similar observation concerning phylogeny based on SSU rRNA gene was reported for bradyrhizobia isolated from native for Mexico lupine species. This apparent discrepancy may indicate on a possibility of the lateral transfer of the symbiotic loci in *Bradyrhizobium*. Alternatively, the *nod* genes may evolve much faster than other functions. That difference may be particularly visible in comparison with the evolution rate of the SSU rRNA gene, which unlike in genera *Rhizobium* and *Mesorhizobium*, in *Bradyrhizobiums* exists as a single operon.

Acknowledgements. This work was supported by the grant No. 6 P04C 070 11 from the Polish State Committee for Scientific Research.

TRANSGENIC PLANTS AS A SOURCE OF VACCINES AGAINST HUMAN AND ANIMAL DISEASES

J. Kapusta[1], A. Modelska[2a], M. Figlerowicz[2b],J. Podkowiński[1], T. Pniewski,[1]O. Lisowa[1], P. Berbezy[1], D. Helias[1], J. Biesiadka[1], I. Femiak[1], M. Letellier[1], V. Yusibov[4], A. Płucienniczak[3], H. Koprowski[4] A.B. Legocki[1]

[1]/Institute of Bioorganic Chemistry, Noskowskiego 12/14, 61-704 Poznań, Poland,

[2a]/University of Medical Sciences, Department of Endocrinology, Przybyszewskiego 49, 60-355 Poznań, Poland,

[2b]/ University of Medical Sciences, Department of Pediatric, Szpitalna 27/33, 60-572 Poznań, Poland,

[3]/Institute of Biotechnology & Antibiotics, Sarościańska 5, Warsaw, Poland,

[4]/Thomas Jefferson University, 1020 Locust Street, Philadelphia, PA 19107, USA

Although achievements of preventive medicine in controlling some devastating infectious diseases is unquestionable, global control some of dangerous pathogenic microorganism still remains far from realization. Development of molecular biology and genetic transformation technology of plants has resulted in obtaining new genotypes with valuable superior traits. One of the most fascinating area of plants biotechnology research is designing plants able to produce the different types of compounds for pharmaceutical purposes including proteins. It was proven that antigen proteins expressed in plants can preserve their biological activity and immunogenicity. From applied point of view plant as producer of subunit vaccine i.e. vaccine based on single or mixture of immunogenic antigen protein in place of whole killed or attenuated pathogenic microbe, can be considered as safe, very cheap and efficient production system. We concentrated our interests on two pathogenic viruses: hepatitis B virus (HBV) and Classic Swine Fever Virus (CSFV). Hepatitis B is etiologic agent of one of the most harmful human disease. During acute illness phase HBV is able to cause a long-lasting and severe liver disorder and damage. CSFV is responsible for a death of millions of animals a year in all of the world, since effective vaccine and veterinary treatment is not available yet. We summarize here our approaches, focusing on plants as a target to express HBV and Classic Swine Fever Virus (CSFV) antigens. The coding sequences of HBV surface antigen protein gene (HBsAg) were cloned into *Agrobacterium tumefaciens* binary vector under the control of plant expression regulatory promoter. *Agrobacterium* was than used to transform tobacco, lettuce and lupin. For construction of plant transformation vector carrying CSFV antigen three derivative proteins E0, E1 and E2 were taken. They were processed via PCR and cloned into plant transformation vector containing plant expression signals. Tobacco and lettuce were transformed with E0 and E1 viral antigen. Putative transformed plants were analyzed with PCR to detect specific sequences. Presence of HBV viral antigen in protein extracts of transgenic tobacco and lupin callus tissue were detected with Abbott Kit based on monoclonal antibody directed to one of the epitope of HBsAg. Immunogenic properties of lupin transgenic calluses were evaluated with BALB/c mice. Animals were fed with transgenic lupin tissue harboring HBV surface antigen. Using standard ELISA analyses we have shown for the first time elicitation specific antibodies against HBsAg in mice orally immunized.

*/ Research partially granted by the State Committee for Scientific Research. Research project No 6 P04B 010 09

ROLE OF SECONDARY METABOLITES IN PLANT DEFENCE RESPONSES. QUINOLIZIDINE ALKALOIDS AND PHENOLIC COMPOUNDS IN LUPINS.

Paweł Bednarek, Mariola Piślewska, Maciej Stobiecki, Przemysław Wojtaszek, Krzysztof Gulewicz

Phytochemical Laboratory, Institute of Bioorganic Chemistry, Polish Academy of Sciences, Noskowskiego 12/14, 61-704 Poznań

The distinctive characteristics of plant species from tribe *Genisteae*, and particularly genus *Lupinus*, is the presence of quinolizidine alkaloids. Lupins are also characterised by the high content of isoflavone aglycones and glycosides. In order to establish the role of these groups of secondary metabolites in lupin defence responses, changes in the profiles of isoflavonoids and quinolizidine alkaloids of white lupin (*Lupinus albus* L. cv. Bac) plants and suspension-cultured cells, evoked by treatments with biotic (yeast cell wall extract) and abiotic ($CuCl_2$) elicitors were studied.

Phytoalexins are secondary metabolites with antimicrobial activity that are synthesised *de novo* in plants to inhibit the growth and development of invading pathogenic fungi or bacteria. In plant species of *Leguminosae* family, isoflavonoids with a pterocarpan-type structure are the predominant phytoalexins. However, in lupin shoots and leaves treated with both kinds of elicitors, only significant increases in the amounts of 2'-hydroxygenistein and prenylated isoflavonoids were observed. High levels of free isoflavonoid aglycones were detected in control lupin cell cultures, but in cells treated with either kind of elicitor increased amounts of prenylated derivatives were also noted. The observed increased activity of constitutively present isoflavone glucoside glucosidase indicated the possibility that release of aglycones from the pre-formed stores might be the putative mechanism of lupin defence response.

Quinolizidine alkaloids are thought to play a protective role in lupins and their increased levels in response to wounding have been observed previously. However, no data are available on their role in plant defence responses. In elicitor-treated lupin plants, relative quantities of quinolizidine alkaloids changed slightly in comparison to the control. The observed changes included: lower amounts of lupanine, and increased levels of 13-hydroxylupanine esters, mainly 13-tigloiloxylupanine.

Our results seem to indicate that the defence strategy of lupins is rather based on constitutive synthesis of numerous secondary metabolites, with end products of high antimicrobial activity. This is especially true for prenylated derivatives (luteone and wighteone) of two major lupin isoflavones: genistein and 2'-hydroxygenistein. Similarly, the antifungal activity of quinolizidine alkaloids has been observed in laboratory tests.

VARIOUS ASPECTS OF *LUPINUS* BIOLOGY

Krzysztof Gulewicz, Maciej Stobiecki, Przemysław Wojtaszek, Wojciech Wysocki, Paweł Bednarek, Mariola Piślewska, Danuta Ciesiołka, Małgorzata Zielińska

Phytochemical Laboratory, Institute of Bioorganic Chemistry, Polish Academy of Sciences, Noskowskiego 12/14, 61-704 Poznań, Poland

Three species of the genus *Lupinus* (tribe *Genisteae*, family *Leguminosae*): white lupin (*L. albus* L.), yellow lupin (*L. luteus* L.), and narrow-leafed lupin (*L. angustifolius* L.) are among the major legumes cultivated around the world. This is a very old genus (12-14 mln years old) and many aspects of its biology are different from those commonly found within leguminous species.

In our Laboratory, the biology of lupin species is studied using multidirectional approach. Both basic and applied research is carried out and it is concentrated on two major aspects: 1) the identification and biological activity of lupin natural products, and their use for ecological agriculture, and 2) the mechanisms of lupin responses to microorganisms, both symbiotic and pathogenic.

Lupin seeds are an exceptionally rich source of nutritional substances, due to high protein and oil content. However, their use is limited, mainly because of the presence of quinolizidine alkaloids. The method of the lupin seeds debittering (=alkaloid removal) has been elaborated, and both seeds and the extract have been analysed towards their potential economical use. Debittered seeds could be used for animal nutrition, while the extract could be used directly as e.g. yield-promoting agent or serve as a source of lupin natural products such as quinolizidine alkaloids or oligosaccharides of raffinose family. The former might be used as plant protection agents while the latter could have a profound health effect due to their role in human diet.

Plants belonging to genus *Lupinus* are among the few well documented examples of „non-mycorrhizal" plants. Also the formation of symbiotic nodules with rizobia differs in some aspects from two types of symbiotic interactions found in legumes. Research in our Laboratory indicated that similarly some lupin defence responses to pathogenic infections might be different. Firstly, only simple or prenylated isoflavones, but not pterocarpans, are synthesised in infected plants, serving as putative phytoalexins. Secondly, the presence of high levels of quinolizidine alkaloids could influence the course of infection. Thirdly, the presence of some defence-related proteins in cell walls could have the same effect on plant pathogens. It is suggested that in lupins the strategy of constitutive expression prevails over the activation of inducible defence mechanisms.

THERMOPHYSICAL PROPERTIES OF HYDRATED MONOHYDROSPHATES OF BIOGENIC APOLIAMINES

Maria D. Bratek-Wiewiórowska[1], Ewa Utzig[2], Wojciech Zielenkiewicz[2] and Maciej Wiewiórowski[1]

[1] Institute of Bioorganic Chemistry, Polish Academy of Sciences, Noskowskiego 12/14, 61-704 Poznań
[2] Institute of Physical Chemistry, Polish Academy of Sciences, Kasprzaka 44/52, 01-224 Warszawa

The biogenic polyamines: putrescine, spermidine and spermine have been intensively studied during the last 25 years particularly because of their biological functions and their applications in medicine and agriculture. These compounds are commonly present in cells of all living processes, for example biosynthesis of proteins, growth and cell differentiation. We consider the hydrated phosphates of polyamines as the very simple model compounds to study the polyamine - nucleic acid interactions. In this paper the results of calorimetric and thermogravimetric measurements of thermophysical properties of hydrated monohydrogenphosphates of polyamines: putrescine, spermidine and spermine are presented.

THE CONFORMATIONAL CHANGES OF 5SrRNA FROM LUPIN SEEDS AND $tRNA^{Phe}$ IN PRESENCE OF Ca^{2+}, Mn^{2+} CATIONS BY ADIABATIC SCANNING DIFFERENTIAL CALORIMETRY

Maciej Wiewiórowski[1], Anna Zielenkiewicz[2] and Wojciech Zielenkiewicz[2]

[1] Institute of Bioorganic Chemistry, Polish Academy of Sciences, Noskowskiego 12/14, 61-704 Poznań
[2] Institute of Physical Chemistry, Polish Academy of Sciences, Kasprzaka 44/52, 01-224 Warszawa

The studies of dynamic conformation of 5SrRNA in presence of different ions in conditions of temperature changes and at various ionic strengths of solution may lead to better understanding of structural property encoded in their sequences. At is well know, scanning adiabatic differential calorimetry DASM is convenient method for this kind of study. So far, DASM was applied by us for experimental investigations of conformational changes of 5SrRNA solutions from lupin seeds and wheat germs without and with addition of different amount of various anions: PO_4^{3-}, NO_3^-, ClO_4^-, Cl^-, Br^-, BF_4^-, COO^- and cations: Spm^{4+} (spermine), Spd^{3+} (spermidine), Mg^{2+}, Na^+, K^+, Cu^{2+}, Pb^{2+}. Structural interpretation of thermal unfolding patterns for lupin seeds and wheat germs was proposed.

In this paper of calorimetric studies of 5SrRNA from *Lupinus luteus* and of $tRNA^{Phe}$ both in the absence and in the presence of different concentration of cations Ca^{2+}, Mn^{2+} were reported. The temperature and the enthalpy of melting were determined. Using the deconvolution method the elementary transitions were distinguished and discussed.

CURRENT STATUS OF STUDIES ON SPECIFIC ENZYMES CATALYZING DEGRADATION OF UNCOMMON MONO- AND DI-NUCLEOSIDE POLYPHOSPHATES

Andrzej Guranowski

Katedra Biochemii i Biotechnologii, Akademia Rolnicza, ul. Wołyńska 35, 60-637 Poznań, Poland.

There occur in the cells at least the following uncommon mono- and di-nucleoside polyphosphates: adenosine 5'-tetraphosphate (p_4A), adenosine 5'-pentaphosphate (p_5A) and diadenosine 5',5'''-P^{α},P^{ω}-tri-, tetra-, penta- and hexa-phosphates (Ap_3A, Ap_4A, Ap_5A and Ap_6A). Some organisms (*Artemia*) accumulate mostly diguanosine tri- and tetra-phosphates (Gp_3G and Gp_4G). The adenylylated compounds are synthesized by some aminoacyl-tRNA synthetases (EC 6.1.1.x). LysRS, PheRS, ProRS and AlaRS are the most effective. They transfer ~AMP from aminoacyl~AMP onto pyrophosphate-moiety-containing acceptors: P_3, P_4, ADP, ATP, p_4A, and p_5A, respectively. Gp_4G and Gp_3G are produced by the GTP:GTP guanylyltransferase (EC 2.7.7.45) which transfers ~GMP *via* Enz~GMP onto GTP and GDP, respectively. Some acyl-CoA synthetases (EC 6.2.1.x) and the firefly luciferase (EC 1.13.12.7) can also synthesize p_4A, p_5A and various Ap_nN-s. Finally, ADP:ATP adenylyltransferase (EC 2.7.7.53) can produce certain Ap_nNs, too. Levels of those uncommon mono- and dinucleotides in the cells can be controled by various nonspecific and specific catabolic enzymes. Among the nonspecific ones which act on the dinucleotides are: nucleotide pyrophosphatase (EC 3.6.1.19), phosphodiesterase type I (EC 3.1.4.1) and the adenosine-phosphate deaminase (EC 3.5.4.17). The specific enzymes comprise: p_4A hydrolase (EC 3.6.1.14), Ap_3A hydrolase (EC 3.6.1.29), (*asymmetrical*) Ap_4A hydrolase (EC 3.6.1.17), (*symmetrical*) Ap_4A hydrolase (EC 3.6.1.41) and Ap_4A phosphorylase (EC 2.7.7.53). Interestingly, in yeast the p_4A and p_5A are dephosphorylated to ATP by the exopolyphosphatase (EC 3.6.1.11). At least one of each of the specific catabolic enzymes have been purified to homogeneity and sequenced. For most of them also the genes have been sequenced and in some cases these genes localized on eukaryotic chromosomes. Human *Fhit* protein, a member of the histidine triad (HIT) superfamily, is a typical Ap_3A hydrolase and its gene *FHIT* is localized to 3p14.2. The (*asymmetrical*) Ap_4A hydrolases from humans and plants are members of the MutT or 'nudix' family. Gene of the human enzyme *APAH1* is localized to 9p13. The related enzyme from *S. pombe* is also a HIT family member and two isozymes of the *S. cerevisiae* Ap_4A phosphorylase, *apa1* and *apa2*, have two regions of similarity with the latter dinucleoside polyphosphate hydrolase from *S. pombe*. Gene for the *S. cerevisiae* Ap_4A phosphorylase, *APA1*, is on the chromosome III. Very recently, the ORF YOR 163w protein of baker's yeast appeared to be a new hydrolase which prefers Ap_5A and Ap_6A as substrates. Details and up-to-date information concerning those catabolic enzymes will be presented in the poster.

WHY HAS NATURE CHOSEN PENTOFURANOSE NOT HEXOPYRANOSE IN THE CONSTRUCTION OF DNA AND RNA?

J. Chattopadhyaya
Department of Bioorganic Chemistry, Box 581, Biomedical Centre, Uppsala University, S-751 23 Uppsala, Sweden, E-mail: jyoti@bioorgchem.uu.se

We have earlier shown that the interplay of stereoelectronic anomeric and gauche effects are energetically important to drive the sugar-phosphate backbone in nucleosides and nucleotides, and their strengths are dependent upon the chiralities of each constituent diastereomeric carbon. The energy transmission from aglycone to the pentose sugar takes place through the orbital mixing of the non-bonded lone pair of O4' of the sugar to the σ^* orbital of the glycosyl bond [the anomeric effect: $[O4'(n) \rightarrow \sigma^*_{(C1'-N)}]$. The exact free-energy of the conformational drive of the sugar driving the phosphate backbone conformation is determined by the interplay of tunable anomeric and the inherent gauche $[\sigma_{(2'/3'/5'C-H)} \rightarrow \sigma^*_{(C4'-O4')}]$ effects of the sugar substituents. In this interplay, the anomeric effect of the pentose sugar in DNA and RNA acts an energy relay station to steer the sugar-phosphate backbone conformation (the energy pump). The *energy barrier* of the interconversion of the sugar conformation in hexopyranose-*vis-a-vis* pentofuranose-based polynucleotide dictates whether the free-energy change resulted from the alteration of the electronic character of a specific aglycone owing to the microenvironmental changes (such as pH or pK_a or any ligand complexation changing the protonation $\rightleftarrows$ deprotonation equlibrium of a specific aglycone depending upon its electronic character, or specific ligand association $\rightleftarrows$ dissociation equlibrium) in a folded polynucleotide chain can indeed drive the constituent sugar-phosphate backbone. The maximum transmission of free-energy from aglycone to the pentofuranose, and to the phosphate through the anomeric glycosyl bond is worth 1.5-5 kJ/mol, depending upon the exact electronic nature of the aglycone. Clearly, a precondition for the Aglycone-Sugar-Phosphate to act as a *wire* is that the free-energy generated as a result of the alteration of the electronic character of the aglycone as a response of the change of the microenvironment is really more than the activation energy barrier between the sugar conformers in order to make the free-energy transmittable from one end to the other to steer the sugar-phosphate backbone conformation. This is easily achieved for the pentose sugar with a potential energy barrier of ~0.2 kJ/mol among its various "infinite" number of pseudorotational forms, whereas the interconversion of conformational transitions of one conformer to the other in hexopyranoses requires an activation energy of about 8-40 kJ/mol, depending upon the nature, location and the configuration of the substituent. This means that the equilibration of various conformers is much more rapid in the pentofuranoses of DNA/RNA, and can very easily be prompted by a slight change in the electronic character of the constituent aglycone as a response to the environmental changes, which is difficult to achieve with the hexopyranose-based nucleotides. There lies the uniqueness and the importance of the pentofuranose-sugar based genetic machinery, *i.e*, its inherent ability to respond promptly by changing its local structure sequence-specifically as a result of a change in the environment.

Some Key References from this Lab on the Above Theme:

J. Chattopadhyaya *et al, Can. J. Chem.* **65**, 2089 (1987); *Tetrahedron* **43**, 2289 (1987). *J. Am. Chem. Soc.* **114**, 9934 (1992); *J. Chem. Soc. Chem. Comm.* 781 (1994); *J. Chem. Soc. Chem. Comm.* 537 (1994). *J. Am. Chem. Soc.* **116**, 4038 (1994); *J. Am. Chem. Soc.* **116**, 6558 (1994). *J. Am. Chem. Soc.* **116**, 8033 (1994). *Tetrahedron* **51**, 11775 (1995); *J. Org. Chem.* **61**, 266 (1996); *Pure and Applied Chemistry* **68**, 2137 (1996); *Tetrahedron* **53**, 6433 (1997). *Tetrahedron* **53**, 6903 (1997). *J. Org. Chem.* **62**, 8800 (1997); *Tetrahedron* **53**, 14043 (1997).

INDEX OF AUTHORS