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Carotenoids in fish. 31. Occurrence of α-doradexanthin in fish in Poland

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Abstract — By means of adsorption column and thin layer chromatography the author investigated the occurrence of α-doradexanthin in individuals of 45 fish species living in Polish salt and fresh waters.

Key words: α-doradexanthin in fish, biosynthesis of astaxanthin in fish.

1. Introduction

During the first half of this century, when intensive investigations on the occurrence of carotenoids in fish were carried out, it was believed that fish contained mainly carotenoids such as β-carotene, lutein, zeaxanthin, and, above all, astaxanthin (Goodwin 1951, Fox 1957).

Investigations carried out in the second half of our century confirmed that the most frequent carotenoid, and occurring in great amounts, was astaxanthin; at that time a number of new carotenoids were also described. It was found that not only derivates of β-carotene can be transformed into astaxanthin, though it can also be formed from lutein as a derivate of α-carotene.

Hirao et al. (1957); who investigated the content of carotenoids in individuals of *Thunnus orientalis*, found the occurrence of a new carotene which was called tunaxanthin. Later investigations showed that tunaxanthin is a derivate of ε-carotene (3.3'-dihydroxy ε-carotene) and

occurs not only in fish but is specific of all crustaceae (Czeczuga 1975) and even amphibians (Czeczuga 1980a). Some years later when investigating the occurrence of carotenoids in individuals of *Carassius auratus* Hirao et al. (1963) found the presence of a carotenoid resembling astaxanthin but with somewhat different spectrometric data. Repeating Hirao's et al. (1963) investigations Katayana et al. (1970a) found that the absorption maximum of the new carotenoid which was called α-doradexanthin lay approximately at the same place as the maximum of astaxanthin; simultaneously its structure was determined as a derivate of α-carotene. Moreover it was established that α-doradexanthin was inherent in the process of transformation of lutein into astaxanthin. Recently several publications have appeared on the occurrence of α-doradexanthin in a number of salt water fish (Tanaka 1978).

Considering that the existing publications on the occurrence of α-doradexanthin in fish referred mainly to salt water fish species it has been found appropriate to investigate the occurrence of that carotenoid in fresh water fish species living in the Polish waters, as well as to give data on some salt water species in which this carotenoid was not previously recorded.

2. Material and methods

The investigations were carried out on individuals of 45 species of fish belonging to families such as *Clupeidae* (2 species), *Salmonoidae* (7), *Osmeridae* (1), *Thymallidae* (1), *Esocidae* (1), *Cyprinidae* (16), *Cobitidae* (3),*Siluridae* (1), *Anguillidae* (1), *Gadidae* (2), *Gasterosteidae* (2), *Percidae* (3), *Centrarchidae* (1), *Ammodytidae* (1), *Cyclopteridae* (1), and *Pleuronoctidae* (2) whose representants inhabit either fresh or salt waters teritorially belonging to Poland. Muscles together with skin and fins, without internal organs were subjected to analysis. The material was homogenized in a glass homogenizer for more than ten minutes.

A part of the homogenized material was transferred into dark glass bottles and immersed in 95% acetone, it was kept under nitrogen in a refrigerator in temperature — 4°C till the moment of chromatographic analysis. Separation of carotenoid pigments was carried out by means of adsorption column chromatography and thin layer chromatography. Prior to chromatography the material was subjected to hydrolysis in 10 per cent KOH in nitrogen atmosphere at room temperature for 24 hours. After hydrolysis the extract was passed on a column filled with Al_2O_3. The length of the columns (produced by Quickfit — England) varied from 15 to 25 cm. Particular fractions were eluted by use of various compositions of solvents (Czeczuga, Czerpak 1976). Subsequently

the eluent was evaporized and the remains were dissolved in a appropriate solvent in order to develop the absorption curve the maxima of which served, among other purposes, to identify patricular carotenoids.

Independently of column chromatography the obtained acetone extract was divided into individual strains by use of thin layer chromatography. For this purpose glass plates 15 × 40 cm in size were used. These were covered with silica gel (produced by Merck) and subsequently acetone extract was pipetted with a special micropipette onto the starting line using simultaneously various compositions of solvents (Czeczuga, Czerpak 1976). Subsequently the value R_f was determined according to generally adopted principles. Absorption maxima were determined by use of a Spectromom- 203 spectrophotometer and a Specol spectrocalorimeter.

The identification of α-doradexanthin in the investigated material was carried out in a similar way as it was done by Lenel et al. (1978) upon the basis of the absorption maxima in various solvents (hexan, ethanol, benzen) of α-doradexanthin itself and lutein, which is the reduced form of that carotenoid (through an addition of $NaBH_4$). Moreover, the epi- and hypophase coefficient was determined according to Petracek, Zechmaister (1956).

The percentage content of α-doradexanthin and the total amount of carotenoids was determined according to Davies' method (1976).

3. Results

The fraction isolated as α-doradexanthin (fig. 1) showed maximum absorption in hexan within the 451 and 470 nm range, in ethanol 458 nm and 473 nm, and in bezen 461 and 482 nm. After an addition of $NaBH_4$ the same fraction showed maximum absorption typical of lutein (in hexan — 420, 445, 475 nm, in ethanol 420, 445, 475 nm). The coefficient of epi- and hypophase varied within the 11—15: 89—85 range.

The total content of carotenoids (in μg/g fresh weight) and the percentage content of α-doradexanthin in individuals of the investigated fish species was given in Table I.

As to α-doradexanthin, the percentage content of that carotenoid in the investigated fish species varied from 0.3 *(Cobitis taenia)* to 25.8 per cent of the total content of carotenoids *(Rutilus rutilus)*.

4. Discussion

Since the occurrence of α-doradexanthin in fish was described for the first time (Hirao et al. 1963), a number of publications reporting the presence of that carotenoid in other fish species have appeared.

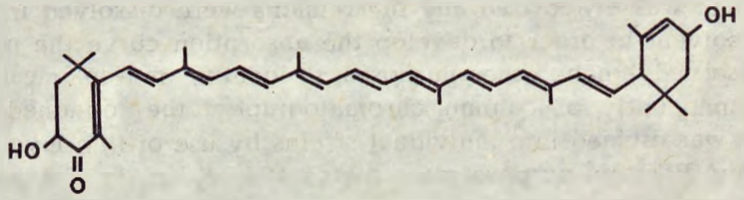

Fig. 1. α-doradexanthin structure

Table I. The content of α - doradexanthin in the investigated species of fish

Family	Species	Total content of carotenoids (in μg/g of fresh weight)	α- doradexanthin in %
Clupeidae:	Clupea harengus Linne	1.235	4.5
	Sprattus sprattus (Linne)	1.815	7.3
Salmonidae:	Salmo salar Linne	9.105	12.4
	Salmo trutta morpha trutta Linne	7.125	8.2
	Salmo trutta morpha fario Linne	8.311	4.9
	Hucho hucho (Linne)	1.954	2.8
	Coregonus albula (Linne)	2.119	12.5
	Coregonus lavaretus (Linne)	1.973	5.5
	Coregonus peled (Gmel.)	0.547	3.1
Osmeridae:	Osmerus eperlanus (Linne)	0.596	1.8
Thymallidae:	Thymallus thymallus (Linne)	1.324	4.6
Esocidae:	Esox lucius Linne	1.312	7.3
Cyprinidae:	Tinca tinca (Linne)	1.542	2.9
	Carassius carassius (Linne)	1.353	1.8
	Carassius auratus gibelio (Bloch)	2.976	2.1
	Cyprinus carpio Linne	0.287	3.6
	Abramis brama (Linne)	0.567	9.1
	Blicca björkna (Linne)	2.158	3.6
	Leuciscus idus (Linne)	1.980	4.2
	Phoxinus phoxinus (Linne)	5.873	5.7
	Chondrostoma nasus (Linne)	0.099	11.3
	Gobio gobio (Linne)	1.902	0.8
	Leucaspius delineatus (Heckel)	8.117	0.5
	Alburnus alburnus (Linne)	29.779	8.6
	Rutilus rutilus (Linne)	1.812	25.8
	Scardinius erythrophthalmus (Linne)	0.658	1.6
	Ctenopharyngodon idella Val.	0.775	1.3
	Hypophthalmichthys molitrix Val.	1.593	12.4
Cobitidae:	Misgurnus fossilis (Linne)	1.262	7.6
	Nemachilus barbatulus (Linne)	1.102	1.8
	Cobitis taenia Linne	16.513	0.3
Siluridae:	Silurus glanis Linne	0.722	1.7
Anguillidae:	Anguilla anguilla (Linne)	2.501	10.8
Gadidae:	Gadus callarias Linne	0.786	11.3
	Lota lota (Linne)	0.932	1.5
Gasterosteidae:	Gasterosteus aculeatus Linne	2.314	4.2
	Pungitius pungitius (Linne)	11.730	3.0
Percidae:	Lucioperca lucioperca (Linne)	0.512	17.8
	Acerina cernua (Linne)	3.753	10.4
	Perca fluviatilis Linne	0.692	8.2
Centrarchidae:	Micropterus salmoides (Lacepede)	0.456	9.1
Ammodytidae:	Ammodytes tobianus Linne	6.052	1.8
Cyclopteridae:	Cyclopterus lumpus Linne	0.034	9.3
Pleuronectidae:	Limanda limanda (Linne)	12.840	3.8
	Platichthys flesus (Linne)	45.767	12.1

These are mostly data concerning salt water fish, though there are also reports on the occurrence of that carotenoid in some fresh water species (T a n a k a 1978).

α-doradexanthin is a derivate of α-carotene (3.3'-dihydroxy-4'-keto-α--carotene) and is formed from lutein being a 4-ketolutein. As it is known

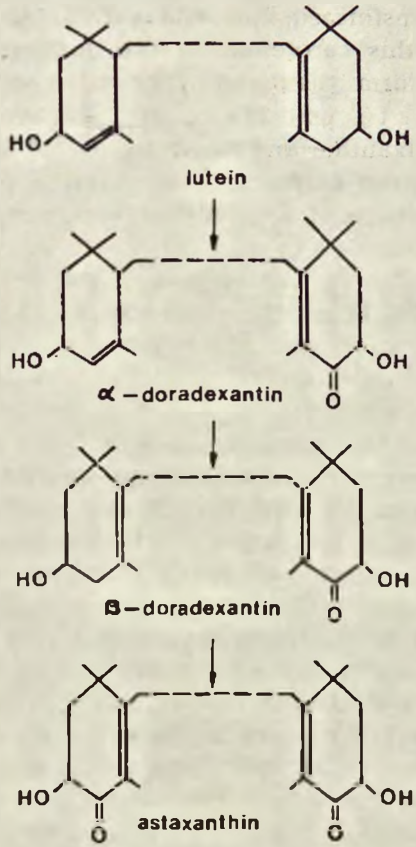

Fig. 2. Biosynthesis of astaxanthin from lutein

lutein is a carotenoid most common among plants and animals feeding on plants. K a t a y a m a et al. (1970a) were the first to forward the view that astaxanthin in fish can be formed in the process of lutein transformation through α- and β-doradexanthin (fig. 2). Investigating the occurrence of α-doradexanthin in individuals of *Carassius auratus* variety B e n i b u n a, K a t a y a m a et al. (1970b) concluded, on the basis of the carotenoid they had found, that astaxanthin in these individuals was formed also in the course of transformation of lutein ester through, α-doradexanthin ester and β-doradexanthin ester. This course of transformation of lutein into astaxanthin was confirmed by K a t a y a m a et al. (1972) on the example of individuals of *Cyprinus carpio* var. red carp, applying hydrogen titrated β-carbonate. At the same time, applying zeaxanthin and carbon titrated lutein H a t a and H a t a (1972a, 1972b) showed that in individuals of golden fish, astaxanthin is formed mainly on the way of transformation of zeaxanthin into β-doradexanthin, the

latter one is then transformed into astaxanthin. As regards lutein, in that species of fish, this carotenoid is transformed only into α-doradexanthin and in this form it is found in the organisms. The same results were obtained by Hata and Hata (1975) when they investigated transformations of zeaxanthin and lutein by use of labelled carotenoids in individuals of *Cyprinus carpio* var. fancy red carp.

As far as the presence of α-doradexanthin in representants of salt water fish is concerned, Matsuno et al. (1973) recorded the occurrence of α-doradexanthin in five species of salt water tadpoles. Katayama et al. (1973) found the presence of α-doradexanthin in sea bream *(Chrysophrys major)* and Matsuno et al. (1974a) reported it in individuals of *Spheroides niphobles* and *Diodon holacanthus*. Matsuno and Tatsuyama (1976a, 1976b) investigated the occurrence of carotenoids in six Leuciscinaeous species and nineteen species belonging to *Percichthyes;* they found that α-doradexanthin is specific to individuals of the majority of the investigated species. In some it was a dominant carotenoid, as it was the case in males of *Tribolodon hakonensis*, in which is constituted 63 per cent of the total number of the carotenoids.

As regards other fish species, it should be stated that trace amounts of α-doradexanthin were found in individuals of *Channa argus* (Matsuno et al. 1974a, 1974b) and in individuals of *Exocoetus volitans, Cololabis saira, Oryzias laiptes,* and in *Pungitius pungitius* (Matsuno, Katsuyama 1976a, 1976b, 1976c). The occurrence of that carotenoid in *Pungitius pungitius* living in Polish waters was reported by Czeczuga (1980b). Moreover, Matsuno and Katsuyama (1979) reported occurrence of α-doradexanthin in individuals of such fish species as *Serrasalmo nattereri, Lepomis macrochirus, Cichlasoma citrinellum, C. elisalium,* and *Channa maculata.*

Rodriguez et al. (1973) analyzing β-carotene transformation into astaxanthin recorded the presence of α-doradexanthin in individuals of golden fish. Spectral characteristics of α-doradexanthin in individual of golden fish were investigated by Tanaka et al. (1976) among others, absorption maxima of that carotenoid in various solvents and the coefficient of epi- and hypophase were also given.

Taking into consideration publications concerning occurrence of α-doradexanthin in fish and finding this carotenoid in a major number of fish specimens living in Polish waters it should be supposed that it a carotenoid widely spread in fish; recent investigations have reported even that α-doradexanthin is peculiar to species of various types of invertebrates. Its occurrence was found in numerous species both of salt water *Crustaceae* (Lenel et al. 1978) and fresh water *Crustaceae* (Czeczuga 1980c); its presence was also found in aquaeous and terrestrian molluscs (Czeczuga 1980d). According to the investigations

with carbon titrated carotenoids it should be assumed that α-doradexanthin is transformed in fish into astaxanthin; this may possibly not refer to all fish species, nevertheless, it is incontestably one of the possible ways of biosynthesis of astaxanthin, a carotenoid belonging to the group most common, not only among fish, but also among aquatic animals in general.

5. Polish summary

Karotenoidy u ryb. 31. Występowanie α-doradeksantyny u ryb w wodach Polski

Stosując adsorpcyjną chromatografię kolumnową i cienkowarstwową wykazano obecność α-doradexanthin u osobników 45 gatunków ryb zamieszkujących słodkie i słone wody polskie.

Badania wykazały, że zawartość α-doradexanthin wahała się od 0,3 *(Cobitis taenia)* do 25,8% ogólnej zawartości karotenoidów *(Rutilus rutilus)*.

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