Carotenoid Pigments in Rainbow Trout, Salmo gairdueri irideus

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Carotenoid Pigments in Rainbow Trout, *Salmo gairdneri irideus*

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Summary

The composition and the distribution of carotenoids in tissues were studied in rainbow trout cultured in different conditions.

The carotenoids of integument were of the ester form and the other tissues, muscle, liver, ovary and digestive organs were of the free form.

The tissue specificity on the carotenoid composition was observed distinctly. The main carotenoids were as follows; depigmented rainbow trout, lutein and zeaxanthin. pigmented rainbow trout, astaxanthin, lutein and zeaxanthin. The integument shows an affinity to xanthophylls and muscle to ketocarotenoids, especially to astaxanthin.

Salmon and trout are two of the most important food and game fish and they have been cultured for a long time in many countries. They are characterized by red muscle and their commerical values are usually determined by the degree of muscle pigmentation. Therefore pigmentation of muscle and red spoted integument of salmonoid have been attempted by many fish culturists for a long time without a sufficient knowledge of the nature of pigments (1, 2). At present, it is well known that crustaceans are very suitable foods for pigmentation.

The pigments of muscle of salmonoid have been studied by many investigators (3–7). These studies confirmed that the red muscle colour is based on the carotenoid pigments, especially astaxanthin. Detailed studies of carotenoids in salmonoid, however, have never been done.

This paper deals with the composition and the distribution of carotenoid pigments in the tissues of rainbow trout cultured in different conditions.

Experiments

Materials

Rainbow trout; Two groups of fishes cultured in different conditions were used. The one group was cultured in fresh water in the Toggata trout farm, Mt.

TABLE 1. *The Distribution of Carotenoids in Rainbow Trout*

Tissue	Carotenoid				Existence form
	Depigmented fish		Pigmented fish		
Integument	0.252 mg/fish	86.2%	0.701 mg/fish	55.2%	ester
Muscle	0.039	13.5	0.570	44.8	free
Digestive organ	trace	trace	trace	trace	free
Liver	trace	trace	trace	trace	free
Ovary	0.002	0.4	trace	trace	free

Zao, Miyagi prefecture. Five unmatured female fish, body length 28–31 cm, body weight 310–480 g, were used. They were fed assorted feeds only. They did not show pigmentation of muscle. The other group was cultured in sea water in the bay of Okachi, Miyagi prefecture. They ate shrimp, assorted feed and fish. They showed pigmentation of muscle. Only one unmatured female fish, body length 30 cm, body weight 536 g was used.

Analysis of carotenoids

Carotenoids were extracted with acetone and separated with silicic acid (silicic acid: Celite 545 2:1) column chromatography and thin layer chromatography (TLC). The identification of carotenoids was carried out by the following methods; (1) absorption spectra, (2) the colour of chromatogram on column and thin layer chromatography, (3) comparison with authentic standards on TCL. Thin layer plates used were as follows; silica gel (Wakogel B5), magnesia (MgO: silica gel 1:1), alumina (Merck, precoated), (4) reduction with sodium borohydride (8), (5) saponification, carried out in 1/2N KOH-EtOH in the dark at room temperature over night.

The amount of carotenoid was calculated from the E value of absorption maximum. The $E^{1\%}_{1cm}$ value was posturated as 2000.

Results

Distribution of carotenoids

The total amount of carotenoids in the tissues were calculated from the E value of absorption maximum in petroleum ether before saponification. The results are given in Table 1. The form of the carotenoids (free or esterified) was determined with silica gel TLC.

Composition of carotenoids

Integument; The carotenoids of the integument of depigmented rabinow trout were separated into five fractions with silicic acid column chromatography (Table 2). Astacene was expressed as astaxanthin in all tables. The carotenoids of pigmented rainbow trout were separated into four fractions with silicic acid

TABLE 2. *The carotenoids of the Integument of Depigmented Rainbow Trout*

Fraction	Eluent ethyl ether in PE(%)	Absorption maximum (nm)*		Percentage	Carotenoid
		PE	CS_2		
1	5	(417), 443. 472	(446), 474, 504	58. 2	Lutein ester
		(418), 448, 476	480, 509	15. 6	Zeaxanthin ester
2	10	453-461, (475)	497	3. 7	Unknown (F2a) ketoester
		400, 428, 454	427, 456, 485	2. 3	Unknown (F2b) ester
		398, 425, 450	423, 453, 481	1. 6	Unknown (F2c) ester
3	20	419, 446, 469	448, 476, 503	7. 4	Unknown (F3a) ester
		479**	508-519**	0. 5	Astaxanthin ester
4	30	417, 445, 472	—	3. 4	Unknown (F4a) ester
		448, 478	—	0. 6	Unknown (F4b) ester
5	50	467	504	2. 3	Canthaxanthin
		423, 449, 478	—	3. 0	Unknown (F5b)
		423, 448, 476	—	1. 2	Unknown (F5c)

* after saponification ** astacene

TABLE 3. *The carotenoids of the Integument of Pigmented Rainbow Trout*

Fraction	Eluent ethyl ether in PE (%)	Absorption maximum*(nm)		Percentage	Carotenoid
		PE	CS_2		
1	5	418, 444, 473	(444), 464, 504	24. 7	Lutein ester
		(418), 446, 474	468, 504	12. 3	Zeaxanthin ester
		(422), 450, 477	472, 510	14. 1	Cynthiaxanthin ester
2	10-30	(401), 427, 453	—	10. 2	Unknown ester
3	30	397, 421, 448, 474	419, 448, 476, (504)	6. 2	Unknown ester
		472**	508**	23. 6	Astaxanthin diester
		474***	510***		
4	50	472***	506***	8. 9	Astaxanthin monoester

* after saponification ** astacene, extracted with alkaline ethyl ether
*** astacene, extracted with acidic ethyl ether

column chromatography (Table 3). The acetylenic bond of cynthiaxanthin was not confirmed distinctly on the infra red absorption spectrum because of the small amounts of the sample.

Muscle; The carotenoids of depigmented rainbow trout were separated into two fractions, alkaline ethyl ether extract and acidic ethyl ether extract, after saponification. The alkaline ethyl ether extract was separated into lutein and zeaxanthin with MgO column chromatography. The acidic ethyl ether extract was identified as astacene. The carotenoids of pigmented rainbow trout were separated into the above mentioned two fractions after saponification. The alkaline ethyl ether extract was separated into five fractions with silica gel TLC after removing the sterols with digitonin. They are lutein, zeaxanthin, cynthiaxanthin, canthaxanthin and an unknown carotenoid. The carotenoids of the muscle of rainbow trout are given in Table 4.

TABLE 4 *The Carotenoids of Muscle of Rainbow Trout*

Carotenoid	Relative abandance (%)	
	Depigmented fish	Pigmented fish
Lutein	73.9	27.7
Zeaxanthin	26.1	18.9
Cynthiaxanthin	—	4.5
Unknown	—	3.4
Canthaxanthin	—	3.1
Astaxann	trace	42.3

Discussion

The tissue specificity of the carotenoids was observed to determine form and composition. The ester form existed only in the integument, and most of the carotenoids in the integument were of the ester form. The other tissues contained the free form only. These results are the same as the previous reports (9–11).

The tissue specificity of accumulation of carotenoids was observed in ketocarotenoids, astaxanthin and canthaxanthin, and xanthophylls. Astaxanthin and canthaxanthin were observed in the muscle and integument. It was reported that canthaxanthin accumulated well in the muscle and integument as did astaxanthin (12–14). Canthaxanthin, however, did not accumulate in the integument in goldfish (15) and sea-bream (16) in which accumulate astaxanthin. It seems that the accumulation of astaxanthin and canthaxanthin is a specific characteristic of salmonoid because the existence of astaxanthin and canthaxanthin in the muscle is not observed in other fish (3, 4). The mechanism of specific accumulation of free form astaxanthin and canthaxanthin and their physiological function in the salmonoid muscle are unknown now. They are very interesting problems to study in comparative biochemistry and physiology.

Xanthophyll such as lutein and zeaxanthin accumulated well in the integument as in other fish. This result agrees with the previous results of the ^{14}C-labeled carotenoids feeding experiments (17). Cynthiaxanthin was observed only in the pigmented rainbow trout cultured in sea water. Cynthiaxanthin is a carotenoid of zeaxanthin analogue with acetylenic bonds in the isoprenoid chain and exists in diatom and marine organisms (18). It seems that the cynthiaxanthin observed in the pigmented rainbow trout cultured in sea water is derived from the ingested marine organisms.

Kanemitsu and Aoe (6) reported the carotenoid of the muscle of five North Pacific salmon was all astaxanthin. They did not observe any other xanthophylls. The results of Table 1 and 4, however, show a relatively high accumulation of xanthophylls in the muscle. It is well known that salmon eat fish, crustaceans, squid, amphipod and pteropod. (19). These organisms have many kinds of

carotenoids. Pacific salmons, however, have only astaxanthin. Therefore, the difference of accumulation of xanthophylls between salmon and rainbow trout may be due to the biochemical specificity rather than the food.

Astaxanthin, the main pigment of the muscle of salmonoid, has been assumed to be not biosynthesized by these fish from the results of *Oncorhynchus gorbuscha* (14), *O. keta* (20). *O. nerka kennerlyi* (21), *O. nerka* (22), *Salmo gairdneri* (14, 23), *S. irideus* (13,24–26), *S. clarkii clarkii* (14), *S. fario* and *S. umbra* (27), *S. trutta* (9. 24, 25, 28), *S. salar* (29), *Salvelinus fontinalis* (25, 30). Recently it was confirmed that rainbow trout could not convert β-carotene, zeaxanthin and lutein into astaxanthin (17). This result suggests that salmonoid can not convert β-carotene, zeaxanthin and lutein into astaxanthin and that the accumulated astaxanthin in the muscle of salmonoid is from those ingested. Therefore, astaxanthin itself must be added to the food for the pigmentation of salmonoid unlike goldfish which convert zeaxanthin into astaxanthin (15, 31).

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