アスタキサンチンの生合成IX

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The Biosynthesis of Astaxanthin—IX. The Transformation of Labelled Astaxanthin from the Diet of Sea Bream, *Chrysophrys major Temminck* and *Schlegel*, to their Body Astaxanthin

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The carotenoids in sea bream have been studied intensively and reported in the series of this thesis.

For two weeks, sea bream were cultured in the laboratory and fed radioactive β-carotene-15,15′-$^3H_2$ (group 1) and labelled astaxanthin (group 2). It was confirmed that radioactive β-carotene-15,15′-$^3H_2$ was not converted into astaxanthin in sea bream, but labelled astaxanthin was transferred to body astaxanthin.

In the integuments of sea bream, the existence of α-carofene, 3,3′-dihydroxy-ε-carotene, lutein, zeaxanthin, α-doradexanthin ester trace and astaxanthin was confirmed.[1,2] In the internal organs, β-carotene, echinenone, 3,3′-dihydroxy-ε-carotene, lutein, zeaxanthin and astaxanthin were found.[1,2]

In the series of this thesis,[1,2] it was clarified that the faded color of cultured sea bream was caused by the extremely small amount of astaxanthin contained in them. The contents of the stomach of natural sea bream were examined, and half digested *Squilla oratoria* and other Crustacea were found in them. Also, the carotenoids found in sea bream were similar to those in Crustacea, prawn *Penaeus japonicus* BATE,[3,4] and *Matapenaeopsis barbata*.[3] It was assumed that most astaxanthin was brought about from their food intake.[1,2]

The present investigation was undertaken to confirm the metabolic pathway from plant carotenoids to astaxanthin in sea bream. It was clarified that radioactive β-carotene-15,15′-$^3H_2$ was not converted into astaxanthin in sea bream, but they could transfer astaxanthin from their food to their body astaxanthin.

Materials and Methods

Alive and healthy sea bream (about 20 cm in length) were purchased at a local fish hatchery. Two aquarium tanks, 80×120 cm, were prepared. At the bottom of each

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Table 1. Composition of the modified artificial diet.

Defatted soybean powder	300.0	g
Dry pulp yeast	60.0	〃
Mineral mixture*	22.5	〃
DL-Methionine	1.5	〃
Green algae powder	6.0	〃
Unpurified soybean oil	26.0	〃
Cholesterol	0.3	〃
Vitamin A (natural)	6000.0	IU
〃 D (natural tocopherol)	24.0	mg
〃 C	1500.0	〃
〃 K_3	375.0	〃
〃 B_1	3.75	〃
〃 B_2	37.5	〃
〃 B_6 (pyridoxine)	22.3	〃
Nicotinic acid	225.0	〃
Ca-Pantothenate	375.0	〃
Choline-Cl	1500.0	〃
Folic acid	3.0	〃
Biotin	0.75	〃
P-Aminobenzoic acid	75.0	〃
Inositol	150.0	〃
Attractant**	300.0	ml
* Mineral mixture:		
Salt 1		
K_2HPO_4	2.31	g
KCl	0.724	〃
$MgSO_4$	1.14	〃
$FeSO_4 \cdot 7H_2O$	0.180	〃
$Ca_3(PO_4)_2$	2.11	〃
$MnSO_4 \cdot 7H_2O$	0.0154	〃
$CaCO_3$	1.29	〃
$ZnSO_4$	0.0154	〃
$CuSO_4$	0.0154	〃
Salt 2		
$CaCO_3$	1.0	g
$Ca_3(PO_4)_2$	2.0	〃
Salt 1 and salt 2 were mixed: Salt 1: salt 2=2: 1.		
** Attractant:		
Fresh cabbage	500.0	g
Mysis	75	〃
Water	200.0	ml

The mixtures of the attractant were boiled, homogenized, and filtered. 300 ml of this attractant were added.

3.5 mg of β-carotene-15,15′-$^3H_2$ (activity: 0.162 mC/mg) were dissolved in 26 g of soybean oil and added to the above mentioned artificial diet. The artificial diet was mixed, stuffed into the vinyliden chloride film like sausage, and steamed for twenty minutes. Thus obtained artificial diets were kept in a refrigerator until use.

aquarium tank, about 10 cm of sea sands were placed. The culture was started by inoculating each of four sea bream. During culture, the medium was circulated, aerated at

400 ml per minute, and kept at 25°C. One group of sea bream was fed the modified standard diet[5] containing β-carotene-15,15′-$^3H_2$. The other group of sea bream was fed cultured prawn. (For two weeks the prawn had been fed β-carotene-15,15′-$^3H_2$.)

1. Feeding radioactive β-carotene-15,15′-$^3H_2$ to sea bream: Every day for two weeks, each of four sea bream was fed 7 g of the modified standard diet[5] containing β-carotene-15,15′-$^3H_2$ (**Table 1**: increased amounts of mysis were added as an attractant to sea bream, which is three times the amount of mysis used as attractant in the standard diet[5]).

a. Extraction and purification of the carotenoids from sea bream: The dosal and ventral sections of the cuticle and all the epidermis of the cuticle were collected, and extracted with acetone until no further pigments could be obtained. The pigments were separated into four fractions using the same method[1,2] as already reported: α-carotene fraction, 3,3′-dihydroxy-ε-carotene fraction, lutein fraction and astaxanthin fraction. Each fraction of the pigments was saponified and purified on the columns, using the same method reported in the series of this thesis.[1,2] The carotenoids of the internal organs of the sea bream were extracted, saponified and purified on the columns.[1,2] Pure β-carotene, 3,3′-dihydroxy-ε-carotene and astacin were obtained.

b. Determination of radioactivity: Each purified pigment was assayed for $^3H_2$ activity, using a Beckman Scintillation Spectrometer. The method used was the same as the one reported in the series of this thesis.[5,6] Each purified pigment, obtained from the sea bream, to be assayed was transferred to a counting vial. After the removal of the solvent, it was dissolved in toluene (5 ml) and bleached with U.V. light. The vials were left in the dark overnight to allow the U. V.-induced phosphoresence to decay. After the addition of 5 ml of scintillation solution containing 20.5 g 2,5-diphenyloxazole (PPO) per 500 ml of toluene, the contents of the vials were then assayed. The following results were obtained (**Table 2**). It was confirmed that β-carotene could not be converted into astaxanthin in sea bream.

Table 2. Incorporation of β-carotene-15,15′-$^3H_2$ (purity: 95%) into astaxanthin in sea bream.

Pigments in sea bream	Radioactivity cpm/mg pigment
α-Carotene	140
3,3′-Dihydroxy-ε-carotene	220
Lutein	180
Astacin	850
β-carotene (in the internal organs)	320.000

2. Feeding radioactive astaxanthin to sea bream: The other group of four sea bream was fed labelled astaxanthin. It was confirmed and reported in the previous paper[5] that when prawn had been culutred and fed radioactive β-carotene-15,15′-$^3H_2$ for two weeks, the astaxanthin in prawn was labelled.

a. Preparation of labelled astaxanthin: The prawn (about 5 cm in length) were obtained from a local fish hatchery and fed for two weeks the standard diet containing β-carotene-15,15′-$^3H_2$ (3, 6 mg of β-carotene-15,15′-$^3H_2$ was added to 300 g of the standard diet;[6] $^3H_2$ activity: 0.162 mC/mg). The same aquarium tank was used as was reported in the previous paper.[5] It was confirmed that astaxanthin in prawn was labelled (**Table 3**).

b. Feeding labelled astaxanthin to sea bream: Each of twenty prawn, which had been fed β-carotene-15,15′-$^3H_2$ for two weeks, were then fed to each of four sea bream every day for two weeks. The dosal and ventral sections of the cuticle and all of the epidermis of cuticle were collected and extracted exhaustively with acetone in a Waring blender until no further pigments could be obtained. Each carotenoid pigment was purified, using the same method reported in the previous papers.[1,2] Thus purified, colored sample was added to counting vials and was assayed, using the same method reported in the previous paper.[6] The following results were obtained (**Table 3**). These results show that sea bream could transfer astaxanthin from their food to their body astaxanthin.

Table 3. Transformation of labelled astaxanthin from their food to the body astaxanthin of sea bream.

Pigments in sea bream	Radioactivity cpm/mg pigment
α-Carotene	20
3,3′-Dihydroxy-ε-carotene	30
Lutein	20
Astacin	110.000
Astacin in the prawn that had been fed β-carotene-15,15′-$^3H_2$ for two weeks, and was then fed to sea bream.	210.000

Results and Discussions

Labelled β-carotene-15,15′-$^3H_2$ was fed to sea bream every day for two weeks, but astaxanthin in sea bream was not labelled. This result shows that sea bream could not convert β-carotene into astaxanthin. After it had been confirmed that astaxanthin in prawn was labelled, prawn were fed labelled β-carotene-15,15′-$^3H_2$ every day for two weeks. Those prawn were then fed to sea bream for two weeks and it was found that astaxanthin in sea bream was labelled. This result clarifies that sea bream can transfer astaxanthin from their food to their body astaxanthin.

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