The Biosynthesis of Astaxanthin—VIII. The Conversion of Labelled β -Carotene-15, 15'- $^3\text{H}_2$ into Astaxanthin in Prawn, *Penaeus japonicus* Bate

Teruhisa KATAYAMA, Tadashi KAMATA, Makoto SHIMAYA*,
Osamu DESHIMARU**, and C. O. CHICHESTER***

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To clarify the metabolic pathway from β -carotene to astaxanthin which was proposed in this thesis series, the present investigation was undertaken.

Astaxanthin is the dominant pigment in prawn, *Penaeus japonicus* Bate. Prawn were cultured in the laboratory for 21 days by feeding artificial food containing labelled β -carotene-15, 15'- $^3\text{H}_2$ dissolved in plant oil. In prawn, β -carotene-15, 15'- $^3\text{H}_2$ was converted to labelled astaxanthin via echinenone, canthaxanthin and phoenicoxanthin. The metabolic pathway from β -carotene to astaxanthin in prawn is as follows:

CHICHESTER *et al.*^{1,2)} recently found that in a Californian strain of *Artemia salina*, the two step conversion of β -carotene into canthaxanthin was the apparent pathway as follows: This was achieved by feeding [^{14}C] labelled β -carotene.

On the other hand, the existence of astaxanthin, β -carotene, canthaxanthin, echinenone, phoenicoxanthin, 3,3'-dihydroxy- ϵ -carotene in the order of abundance of the carotenoids and the traces of dihydroxy-piradixanthin, lutein and zeaxanthin were confirmed in the internal organs of prawn³⁾. In the carapaces of prawn⁴⁾, the existence of astaxanthin, phoenicoxanthin, dihydroxy-piradixanthin, and 3,3'-dihydroxy- ϵ -carotene and the traces of echinenone, canthaxanthin, lutein and zeaxanthin were confirmed. The metabolic pathway from β -carotene to astaxanthin was proposed³⁻⁴⁾.

It was confirmed that in prawn, astaxanthin was labelled when β -carotene-15,15'- $^3\text{H}_2$ was given together with the artificial diet.

* Lab. of Marine Biochemistry, Faculty of Fisheries, University of Kagoshima, Kagoshima, Japan.
(片山輝久・釜田 忠・島谷 周: 鹿児島大学水産学部)

** Kagoshima Prefectural Fisheries Experimental Station, Kagoshima, Japan. (弟子丸修: 鹿児島県水産試験所)

*** Department of Food and Resource Chemistry, University of Rhode Island, Kingston, Rhode Island, U.S.A. (C. O. チャイチェスター: ロードアイランド大学食糧資源化学科)

The carotenoids in gold fish, Benibuna, red carp and fancy red carp have intensively been studied and reported in the series of this thesis⁵⁻¹⁰⁾. The metabolic pathways of the carotenoids in those fish have also been proposed. As references, gold fish, red carp, and fancy red carp were cultured in a laboratory for the same number of days, and fed the same artificial diet containing β -carotene-15,15'- $^3\text{H}_2$. It was confirmed that in those fish, β -carotene-15,15'- $^3\text{H}_2$ was not incorporated into their astaxanthin ester. Thus in gold fish, red carp, and fancy red carp, it was clarified that β -carotene was not the major precursor of astaxanthin ester.

Materials and Methods

Alive and healthy prawn (about 5 cm length) were obtained at a local fish hatchery. At the bottom of the tank, sea sands were placed for about 5 cm depth in order to let them lie hidden into sand during daytime. The culture was started by inoculating them into 40 liters of natural sea water. The sea water was kept at 25°C, and aerated at 400 ml per minute.

Thirty-five prawn each were cultured for five days, and fed the standard artificial diet (Table 1). Then 10 gr of the artificial diet containing radioactive β -carotene (β -carotene-15,15'- $^3\text{H}_2$) was fed to them every day for 21 days.

By using the same method as already reported³⁻⁴⁾, the pigments were extracted and separated into three fractions: β -carotene fraction, echinenone and canthaxanthin fraction, and phoenicoxanthin and astaxanthin fraction. Each fraction of the pigments was saponified and purified on the columns. Pure β -carotene, echinenone, canthaxanthin, phoeniconone (3-keto-canthaxanthin), and astacin were obtained.

Each purified, colored sample was added to the counting vials. After removal of the solvent, it was dissolved in toluene (5 ml) and bleached with U.V. light. The vials were left in the dark over night to allow the U.V.-induced phosphorescence to decay and were assayed after 5 ml of scintillation solution containing 28.5 gr 2,5-diphenyl-oxazole (PPO) per 500 ml of toluene was added, each purified, bleached sample was then assayed for $^3\text{H}_2$ activity, using a Beckman Scintillation Spectrometer by the same method reported in the series of this thesis¹¹⁾. The results obtained are shown in Table 2. These results show that in prawn β -carotene was incorporated into their astaxanthin through echinenone, canthaxanthin and phoenicoxanthin (3-hydroxy-canthaxanthin).

As references, gold fish, red carp, and fancy red carp were cultured at the same time for the same number of days by feeding them every day 25 gr of the same artificial diet containing radioactive β -carotene. Carotenoids were extracted and purified on the columns by using the same method reported in the series of this thesis⁸⁻⁹⁾. Each purified, bleached astacin was assayed for $^3\text{H}_2$ activity using a Beckman Scintillation Spectrometer.

Table 1. Composition of the artificial diet to prawn

Defatted soybean powder	200.0 g
Dry pulp yeasts	40.0 "
Mineral mixture*	15.0 "
DL-Methionine	1.0 "
Green algae powder	4.0 "
Unpurified soybean oil	24.0 "
Cholesterol	0.2 "
Vitamin A (natural)	4000.0 IU
" B ₁ (thiamin-HCl)	2.5 mg
" B ₂	25.0 "
" B ₆ (pyridoxine)	15.0 "
" C	1000.0 "
" D (synthesized calciferol)	800.0 IU
" E (natural tocopherol)	16.0 mg
" K ₃	250.0 "
Nicotinic acid	150.0 "
Ca-Pantothenate	250.0 "
Choline-HCl	1000.0 "
Biotine	0.5 "
Folic acid	2.0 "
Inositol	100.0 "
P-Aminobenzoic acid	50.0 "
Attractant**	100.0 ml
* Mineral mixture:	
Salt 1	
K ₂ HPO ₄	2.31 g
KCl	0.724 "
MgSO ₄	1.14 "
FeSO ₄ ·H ₂ O	0.108 "
Ca ₃ (PO ₄) ₂	2.11 "
MnSO ₄ ·7H ₂ O	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 g
Mysis	25 "
Water	200 ml

The mixture of the attractant was boiled, homogenized, and filtered. 100 ml of attractant were added.

3.6 mg of β -carotene-15, 15'-³H₂ were dissolved in 24 g of soybean oil and added to the above mentioned artificial diet (activity of labelled β -carotene: 0.162 mC/mg).

Thus obtained artificial diet was mixed, stuffed into the vinyliden chloride film like sausage, and than boiled for twenty minutes. The artificial diets were kept in a refrigerator until use.

Table 2. Incorporation of β -carotene-15, 15'- $^3\text{H}_2$ into astaxanthin in prawn

Pigments in prawn	Radioactivity cpm/mg pigment
β -Carotene	76.000
Echinenone	25.000
Canthaxanthin	38.000
Phoeniconone	520.000
Astacin	310.000

Table 3. Incorporation of β -carotene-15, 15'- $^3\text{H}_2$ into astaxanthin in gold fish, red carp and fancy red carp.

Astacin in fish	Radioactivity cpm/mg pigment
Red carp	830
Gold fish	720
Fancy red carp	770

The following results were obtained (**Table 3**). These results clarified that β -carotene was not incorporated into astaxanthin ester in gold fish, red carp and fancy red carp.

Results and Discussions

In previous papers³⁻⁴), the existence of β -carotene, echinenone, canthaxanthin, phoenicoxanthin, astaxanthin, lutein, zeaxanthin, 3,3'-dihydroxy- ϵ -carotene and dihydroxy-piradixanthin was confirmed in prawn and the following metabolic pathway from β -carotene to astaxanthin was proposed.

Using radioactive β -carotene (β -carotene-15,15'- $^3\text{H}_2$), this investigation confirms the metabolic pathway from β -carotene to astaxanthin through the steps of echinenone, canthaxanthin and phoenicoxanthin. But in gold fish, red carp and fancy red carp, it is shown that β -carotene is not incorporated into astaxanthin ester, in other words, β -carotene is not the major precursor of astaxanthin ester.

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