Carotenoid Pigments in Goldfish (Carassius auratus L.) III. Metabolism of Ingested Cynthiaxanthin

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Carotenoid Pigments in Goldfish (*Carassius auratus* L.)
III. Metabolism of Ingested Cynthiaxanthin

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Summary

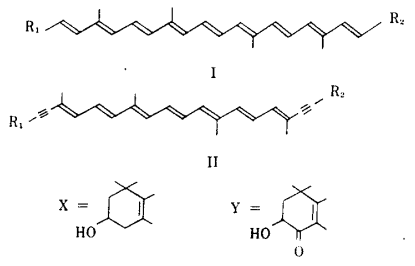
Goldfish (*Carassius auratus*) metabolized cynthiaxanthin to two new ketocarotenoids, 4-ketocynthiaxanthin and 4,4′-diketocynthiaxanthin. Large amounts of cynthiaxanthin were accumulated in the eggs.

These results indicate that cynthiaxanthin is well metabolized like zeaxanthin and astaxanthin would be biosynthesized from zeaxanthin.

The carotenoid composition of goldfish (*Carassius auratus*) was reported in the previous paper (1). The principal carotenoids in goldfish integument were astaxanthin and ketolutein esters, and the formation of these carotenoids was discussed on the basis of the analytical data.

Recently "astarin säure" was identified as the astaxanthin analogue 7,8 dehydroastaxanthin or 7,8,7′,8′-tetradehydroastaxanthin (2). This carotenoid containes a triple bond and has a characteristic absorption spectrum.

If zeanxanthin is metabolized to astaxanthin, cynthiaxanthin (7,8,7′,8′-tetradehydrozeaxanthin) (3) would be metabolized to the astaxanthin analogue, 4,4′-diketocynthiaxanthin. The metabolized carotenoids can be characterized by



Zeaxanthin I ($R_1=R_2=X$)
Adonixanthin I ($R_1=X$, $R_2=Y$)
Astaxanthin I ($R_1=R_2=Y$)
Cynthiaxanthin II ($R_1=R_2=X$)
4-Ketocynthiaxanthin II ($R_1=X$, $R_2=Y$)
4,4′-Diketocynthiaxanthin II ($R_1=R_2=Y$)

FIG. 1. The structure of carotenoids.

visible and infrared absorption spectroscopy. Acetylenic carotenoids are not normally observed in fresh water organisms. Therefore the conversion of cynthiaxanthin to 7,8,7′,8′-tetradehydroastaxanthin can be used to determine the ability of the goldfish to convert the 3-hydroxy-β-ring into a 3-hydroxy-4-keto-β-ring, as in the formation of astaxanthin from zeaxanthin. The ability of the animals to metabolize zeaxanthin to astaxanthin can be inferred.

Materials and Methods

Materials

Goldfish (*Carassius auratus*, Orandashishigashira) were cultured in an aquarium for two years. They were a pale orange colour. The mean body weight was 18.5 g and the mean body length was 6.3 cm.

Cynthiaxanthin

Cynthiaxanthin was prepared from *Halocynthia roretzi* (v. Drashe). The carotenoid was extracted with acetone, and purified by chromatographic methods, and crystallized from methanol-water as reddish plates, m.p. 191°C. The absorption maxima were (422), 450,479 nm in petroleum ether, (425), 453, 481 nm in ethanol, (436), 464, 494 nm in benzene and 481,513 nm in carbon disulphide.

Preparation of diet and feeding

The composition of the diet was as follows: cynthiaxanthin, 12 mg; Tween 80,120 mg; assorted feed for carp (Nisshin Jiryo Co., Tokyo), 20 g; gelatin, 3 g; water, 40 g. Cynthiaxanthin and Tween 80 were dissolved in a small quantity of chloroform, and the chloroform was evaporated under a stream of N_2. A small quantity of water was added and the mixture was stirred vigorously to disperse the carotenoid before addition of warm gelatine solution and the assorted feed. The prepared diet was kept in a refrigerator. About one g. diet per animal was fed daily for one month. This represents a total of 9 mg cynthiaxanthin per animal. The water temperature was maintained at about 25°C.

Identification of carotenodis

The carotenoid pigments were extracted with acetone, separated on a silicic acid column (silicic acid (Mallinckrodt, AR–100): celite 545 3:1) and identified by the previous reported methods (1). The infrared absorption spectra were determined with an IR-S infrared spectrometer (Japan Spectroscopic Co., Ltd). Crystalline samples were prepared by the melting method (4). Other smaples were prepared as thin films on KBr disks.

Results

Colour change

A slight colouration of the integument after 7 days and a distinct colouration after 10 days was observed. After 30 days, the fish were coloured as distinctly as normal fish, but the colour was more pinkish and clear than that of normal fish.

The total carotenoid extracted was 1.9% of the total administered. ($E^{1\%}_{1cm}$ taken as 2,000 at 477 nm (max) in petroleum ether.)

Integument

Fraction 1. Cynthiaxanthin ester.

This fraction was eluted with 5% (v/v) ethyl ether-petroleum ether. The absorption maxima were (422), 451, 480nm in petroleum ether. The absorption spectrum showed no change after saponification.

Fraction 2. 4-ketocynthiaxanthin ester

This fraction was eluted with 10% (v/v) ethyl ether-petroleum ether. The absorption maxima were (447), 470, (500) nm in petroleum ether, 504, (586) nm in carbon disulphied before saponification, and (450), 473 nm in petroleum ether, (500), 505 nm in carbon disulphiede after saponification. The absorption spectra are shown in Fig. 2. This fraction was reduced with sodium borohydride before and after saponification, and the saponified product reduced to give a compound with absorption maxima at (422), 451, 480 nm in ethanol. These results indicate the presence of a carbonyl group in the original compound, and the production of a second carbonyl group during saponification. The Rf value of the reduction product on silica gel TLC was intermediate between those of cynthiaxanthin and tetrahydroxy-β-carotene indicating the presence of three hydroxyl groups in

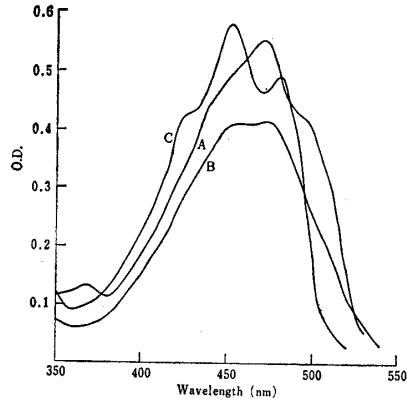


FIG. 2

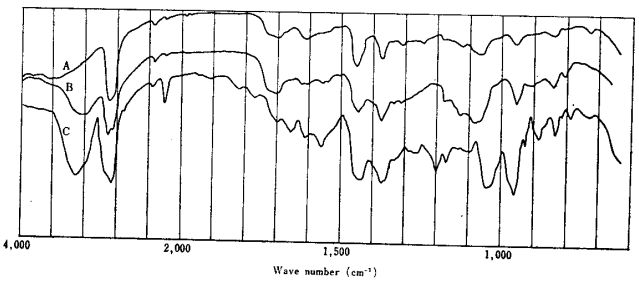


FIG. 3

FIG. 2. The absorption spectra of fraction 2.
A: before saponification (PE) B: after saponification (PE) C: reduction (EtOH)

FIG. 3. Infrared absorption spectra
A: fraction 3 (after saponification, film), B: fraction 2 (after saponification, film), C: cynthiaxanthin (melt)

the reduction product. The acetylation reaction was positive. The infrared spectrum indicates the presence of C≡C bonds (2,200 cm^{-1}) and OH groups (3,250 cm^{-1}) (Fig. 3). This fraction was therefore identified as 4-ketocynthiaxanthin ester. This carotenoid is changed to 3-dehydro-4-ketocynthiaxanthin by saponification. This carotenoid is a new carotenoid, an analogue of adonixanthin (3,3′-dihydroxy-4-keto-β-carotene).

Fraction 3 4,4′-Diketocynthiaxanthin

This fraction was eluted with 20–30% (v/v) ethyl ether-petroleum ether. The absorption maxima were (446), 474, 504 nm in petroleum ether and (478), 507, 540 nm in carbon disulphide. After saponification, this carotenoid could, like astaxanthin, be extracted with ethyl ether acidified acetic acid, and produced a compound with absorption maxima (450), 473 nm in petroleum ether and 500, (505) nm in carbon disulphide. The absorption spectra are shown in Fig. 4. The shapes of the absorption spectra are similar to those of asterin säure (2). The absorption maxima of the reduction product were (420), 449, 475 nm in ethanol. On silica gel TLC, this reduction product remained at the origin with 30% (v/v) acetone-petroleum ether as a developing solvent. This suggests the presence of four hydroxyl groups. The acetylation reaction was negative. The infrared spectrum was similar to that of fraction 2, indicating the presence of C≡C groups. The presence of no free hydroxyl group suggests that this carotenoid does not exist in the enol form.

The carotenoid composition of the integument is given in Table 1.

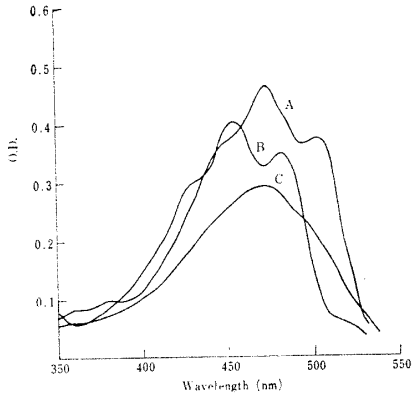


FIG. 4. The absorption spectra of fraction 3.
A: before saponification (PE) B: reduction (EtOH) C: after saponification (PE)

Egg

Egg carotenoids were not esterified. These carotenoids were fractionated on silcia gel TLC and identified after saponification. The carotenoid composition of the eggs is given in Table 2.

TABLE 1. *The Carotenoid Composition in Integument of Goldfish Fed Cynthiaxanthin.*

Carotenoid	%
Cynthiaxanthin ester	22.6
4-ketocynthiaxanthin ester	39.0
4,4′-Diketocynthiaxanthin ester	33.0
Others	5.4

TABLE 2. *The Carotenoid Composition in Egg of Goldfish Fed Cynthiaxanthin*

Carotenoid	%
Cynthiaxanthin	66.0
4,4′-Diketocynthiaxanthin	17.0
Others	16.6

Hepatopancreas

The carotenoids were not investigated fully because of the small amounts of smaples. The absorption spectrum suggests that the main carotenoid is cynthiaxanthin or β-carotene.

Discussion

In these experiments, it was observed that goldfish oxidized cynthiaxanthin to ketoderivatives of cynthiaxanthin. These carotenoids are analogues of adonixanthin and astaxanthin. These results suggest that goldfish are also able to metabolize zeaxanthin to astaxanthin.

In the eggs, a large amount (about 66%) of cynthiaxanthin was observed. This result is similar to the case of zeaxanthin in the normal goldfish. This indicates that cynthiaxanthin is accumulated in a similar way to zeaxanthin. The reason why large amounts of xanthophylls are accumulated in the eggs is not known.

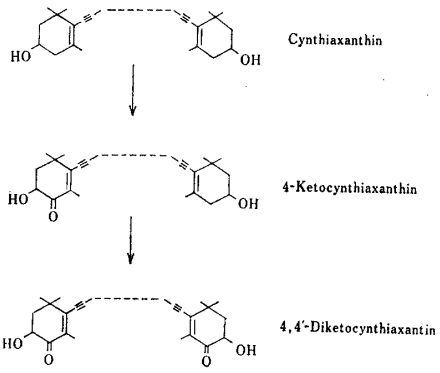


FIG. 5. The metabolic pathways of ingested cynthiaxanthin in goldfish.

The probable metabolic pathways of cynthiaxanthin are shown in Fig. 5. Crustanceans cannot metabolize xanthophylls to astaxanthin or ketocarotenoids. They can only metabolize β-carotene to ketocarotenoids such as echinenone, canthaxanthin and astaxanthin (5, 6, 7, 8, 9, 10, 11).

The difference in metabolic ability between crustaceans and goldfish is very interesting from the viewpoint of comparative biochemistry.

Acknowledgement

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