Carotenoid Metabolism in Fancy Red Carp, *Cyprinus carpio*—III Accumulation of Lutein in the Integument

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The administration of lutein to fancy red carp resulted in a yellowish pigmentation of the white part of the integument. The yellow pigment of the white part was identified as lutein in its free form. Lutein was accumulated in the red part in ester form and in the white part in free form. This is the first reported example of mosaic accumulation of lutein in the integument.

Fancy red carp converts luttein to 4-ketolutein (α -doradexanthin) and accumulates these carotenoids in the integument in ester form.^{1,2)} In this experiment, we found that the white part of the integument of fancy red carp which fed lutein for a long time turned yellow. The pigment of this yellowish white part was extracted with acetone and identified as lutein in free form.

Experiments

Fish.

Fancy red carp (Koohaku in Japanese, two years old) were used.

Administration of lutein.

Crystalline lutein was administered until the white part of the integument turned yellow by the methods of our previous paper.¹⁾ The temperature of aquarium was kept at 25°C.

Extraction and identification of pigment.

The yellowish white part of the integument was collected carefully and the pigment was repeatedly extracted with acetone. The pigment was identified by the following methods: (a) visible light absorption spectrum in various organic solvents, (b) thin layer chromatography (silica gel, silica gel-magnesia (1:1)), (c) the change of chemical properties by saponification.

Results and Discussion

The yellow pigment was easily extracted with acetone. The thin layer chromatogram of the pigment extracted are shown in Fig. 1. The pigment of the red part, consisting of carotenoids in ester form, moved from the origin. The pigment of the white part, however, did not move from the origin (Fig. 1a). This pigment took R_f value as same as standard lutein before and after saponifica-

tion (Fig. 1b,c). The color of this pigment on the thin layer chromatogram was as yellow as lutein. The absorption spectrum and the absorption characteristics are given in Fig. 2 and Table 1. The spectrum properties of this pigment agreed with those of lutein.

In this experiment, it was confirmed that lutein in free form was accumulated in the white part of the integument of fancy red carp. The carotenoids of the red part were ester form and existed only in the xanthophore. However, the pigment cell in the white part was not observed by a microscope. These results suggest that lutein is esterified only in the xanthophore. The form of carotenoids in the integument of various fishes is ester form except cat fish.³⁾ This is the first report about the mosaic accumulation of lutein, free and ester, in the integument.

Table 1. The absorption characteristics of the yellow pigment in the white part

Pigment	λ max. (nm)
Yellow pigment	
(before saponification)	(417), 444, 472 (petroleum ether)
(after saponification)	(415), 443, 472 (petroleum ether)
	(418), 446, 475 (ethanol)
	(443), 477, 507 (carbon disulfide)
	(428), 456, 586 (chloroform)
Lutein	(420), 446, 475 (ethanol)
Zeaxanthin	(424), 453, 481 (ethanol)

Usually fancy red carp do not show yellowish

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Fig. 1. Thin layer chromatogram of the yellow pigment extracted with acetone.

- a) The pigments of red and white parts (before saponification, silica gel, petroleum ether: ethyl ether 9: 1), 1) Yellow, 2) Red, Astaxanthin ester, 3) Orange, *cis* Astaxanthin, artifact, 4) Orange, 4-Ketodihydroxycarotene esters, 5) Yellow, Xanthophyll esters, 6) Triglycerides
- b) The pigment in the white part (before saponification, silica gel-magnesia, petroleum ether: acetone 1: 1)
- c) The pigment in the white part (after saponification, silica gel-magnesia, petroleum ether: acetone 1: 1)

Fig. 2. The absorption spectrum of the yellow pigment in the white part (petroleum ether).

pigmentation on the white part because they have no chance of feeding a large amounts of lutein. Therefore, the metabolism of lutein in fancy fed carp is summarized as follows; lutein absorbed in the intestines is transferred to the integument in free form, converted to 4-ketolutein in the xanthophore, and accumulated in ester form. Lutein absorbed beyond the accumulating capacity of xanthophore is transferred to the nonpigment cells in the white part and accumulated in free form.

The equal amounts of zeaxanthin did not show yellowish pigmentation of the white part. It seems that the irregular accumulation of zeaxanthin does not occur.

The excessive administration of chlorella, green algae, resulted in yellowish pigmentation of the white part.^{4,5)} High content of lutein in chlorella must be responsible for this pigmentation. The fish culturists dislike this pigmentation of the white part as dirt, "shiro-ji no yogore" in Japanese. The administration of feed which contain a large amounts of lutein to fancy carp should be done carefully.

References

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