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Stereochemical Investigation of Astaxanthin in the Ovaries of Chum Salmon *Oncorhynchus keta* during Spawning Migration

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Abstract

The carotenoids from the ovaries in chum salmon *Oncorhynchus keta* during spawning migration were investigated from the stereochemical point of view. Astaxanthin fraction was a dominant carotenoid in the ovaries. The composition of three astaxanthin isomers, (3R, 3'R)-astaxanthin (11-13%), (3R, 3'S; *meso*)-astaxanthin (0.6-0.7%) and (3S, 3'S)-astaxanthin (86-88%), was constant, although the total amounts of carotenoids in the ovaries increased greatly during spawning migration.

Introduction

The bright yellow, orange or red colors of the eggs of salmonid fish are due to the presence of carotenoids in the yolk. Fish, like all other animals, are unable to synthesize carotenoids *de novo* and must obtain them from their diet, depending ultimately on the plant kingdom for carotenoid synthesis. In salmonids, carotenoids are ingested and deposited mainly in the muscle (Matsuno *et al.*, 1980, 1984; Schiedt *et al.*, 1981, 1985; Kitahara, 1983, 1984a; Foss *et al.*, 1984; Storebakken *et al.*, 1985). With sexual maturation, however, the carotenoids are mobilized from the muscle and transported to the integument and ovaries (Kitahara, 1983; Ando *et al.*, 1985, 1986a, b, c, d). Little has been reported on the changes in carotenoid composition of the ovaries of salmonids during spawning migration (Kitahara, 1983), although there are a number of reports on the carotenoids in the ovaries of salmonids (Matsuno *et al.*, 1980, 1984; Miki *et al.*, 1982; Kitahara, 1984a, b; Ando *et al.*, 1986a, c).

High performance liquid chromatography (HPLC) on an optical resolution column has been recently attempted to analyze the carotenoid composition (Maoka *et al.*, 1985; Matsuno *et al.*, 1984, 1985a, b). Matsuno *et al.* (1984) have revealed that (3S, 3'S)-astaxanthin is a dominant carotenoid in the ovaries of chum salmon, although they have not dealt with the changes of carotenoid composition in the ovaries during spawning migration from the stereochemical point of view. This paper presents the results of stereochemical investigation of the carotenoids from the ovaries in chum salmon during spawning migration.

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Materials and Methods

Materials

Ovaries from chum salmon *Oncorhynchus keta* specimens in different physiological states were used as the materials (Table 1).

Extraction of carotenoids

Carotenoids were extracted from the ovaries with acetone. The total carotenoid content was calculated, assuming the $E^{1\%}_{1cm}$ value in acetone at 477 nm to be 2,200.

After concentration under reduced pressure, the carotenoids were transferred to diethyl ether by the addition of distilled water. The aqueous phase was extracted with ether several times. The combined ethereal layer was concentrated under reduced pressure. The residue was submitted to HPLC.

HPLC analysis of carotenoids

HPLC was carried out on a Shimadzu LC-6A instrument with a Shimadzu SPD-2A VIS spectrophotometer set at 470 nm. The column used was a 250×4 mm I.D. stainless steel column packed with 5 μm Sumipax OA-2000 (Sumitomo Chemical Co., Ltd.). Separation was achieved with a mobile phase of *n*-hexane-CH_2Cl_2-EtOH (50 : 20 : 0.5), flow-rate of 0.8 ml/min. Identification of each carotenoid was accomplished by co-thin layer chromatography and co-HPLC with authentic specimens. The authentic carotenoids used were as follows: Astaxanthin diester, astaxanthin monoester and astaxanthin were extracted and purified from the Antarctic krill *Euphausia superba* (Yamaguchi *et al.*, 1983; Maoka *et al.*, 1985). Zeaxanthin, diatoxanthin and cynthiaxanthin were extracted and isolated from *Spirulina maxima* (Miki *et al.*, 1986).

Table 1. Characteristics of chum salmon specimens

Stage	Sex	Age	Date and locality of collection	Fork length (cm)	Body weight (g)	Gonadosomatic index*	Hepatosomatic index**
Feeding migration	Female	03	June 27, 1985. Shizunai coast of Hokkaido	62	3170	4.59	2.49
Spawning migration	Female	03	Sept. 22, 1985. Shibetsu coast of Hokkaido	69	3800	13.06	2.73
Upstream migration	Female	03	Oct. 6, 1985. Lower reaches (2.4 km) of Shibetsu River, Hokkaido	66	3060	20.27	1.43

* (Gonad weight/Body weight)×100.

** (Liver weight/Body weight)×100.

Results and Discussion

Astaxanthin fraction was a dominant carotenoid, although the total amounts of carotenoids in the ovaries increased greatly during spawning migration (Table 2). A typical chromatogram on an optical resolution column (Sumipax OA-2000) of carotenoids from the ovaries is shown in Fig. 1. Similar chromatograms, consisting of seven main peaks, were observed irrespective of the migratory stage. Peaks 5, 6 and 7 were shown to be identical with (3R, 3'R)- astaxanthin, (3R, 3'S ; *meso*)-astaxanthin and (3S, 3'S)-astaxanthin, respectively. As shown in Fig. 1 and Table 3, (3S, 3'S)-astaxanthin was a dominant carotenoid in the ovaries during spawning migration. More noticeable is the constancy of the composition of three astaxanthin isomers, (3R, 3'R)-astaxanthin (11-13%), (3R, 3'S ; *meso*)-astaxanthin (0.6-0.7%) and (3S, 3'S)-astaxanthin (86-88%), during spawning migration (Table 3). This indicates that there was no preferential transport of the optical isomers of astaxanthin from the muscle to the ovaries.

Craik (1985) has recently reviewed the relationship between egg quality and egg pigment in salmonid fish. He has pointed out that carotenoids act as a source of pigment for the chromatophores of the alevin. The physiological roles, however, of the optical isomers of carotenoids in the ovaries are obscure. In any event, it was

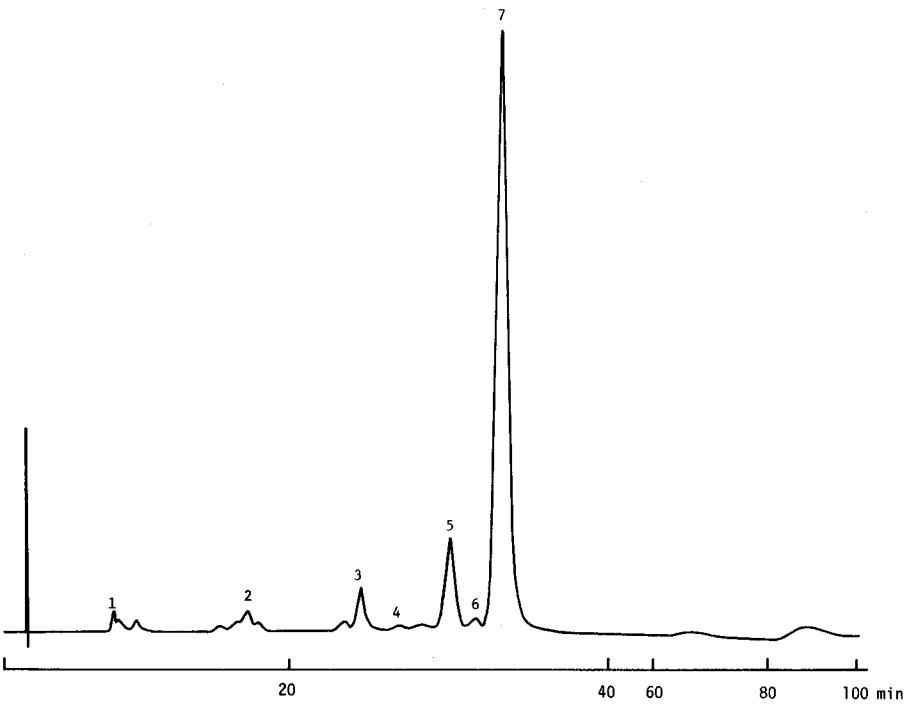

Fig. 1. HPLC separation of carotenoids extracted from the chum salmon ovaries at the upstream migration stage. Column, Sumipax OA-2000 ; Mobile phase, *n*-hexane-CH_2Cl_2-EtOH (50 : 20 : 0.5) ; Flow-rate, 0.8 ml/min ; Detection, 470 nm ; Peaks, 1 = canthaxanthin ; 2 = zeaxanthin ; 3 = 4-keto-zeaxanthin ; 4 = diatoxanthin + cynthiaxanthin ; 5 = (3R, 3'R)-astaxanthin ; 6 = (3R, 3'S ; *meso*)-astaxanthin ; 7 = (3S, 3'S)-astaxanthin.

Table 2. Content and percentage composition of carotenoids in the chum salmon ovaries during spawning migration

	Feeding migration stage	Spawning migration stage	Upstream migration stage
Total carotenoids (mg/whole ovary)	2.510 (100)[b]	2.580 (100)	4.863 (100)
Canthaxanthin(1)[a]	0.043 (1.73)	0.016 (0.61)	0.020 (0.41)
Zeaxanthin(2)	0.056 (2.24)	0.137 (5.31)	0.070 (1.44)
4-Keto-zeaxanthin(3)	0.049 (1.94)	0.164 (6.34)	0.193 (3.97)
Diatoxanthin (4) + Cynthiaxanthin (4)	0.011 (0.43)	0.034 (1.30)	0.046 (0.95)
Astaxanthin fraction(5, 6, 7)	2.181 (86.90)	2.109 (81.75)	4.276 (87.92)
Unidentified	0.170 (6.76)	0.121 (4.69)	0.258 (5.31)

[a] Peak numbers on chromatogram.
[b] Values in parentheses represent percentages, with total carotenoids being 100%.

Table 3. The percentage composition of three stereoisomers of astaxanthin in the chum salmon ovaries during spawning migration

	Feeding migration stage	Spawning migration stage	Upstream migration stage
Astaxanthin fraction			
(3R, 3'R)-Astaxanthin(5)*	13.4	11.9	11.1
(3R, 3'S; *meso*)-Astaxanthin(6)	0.6	0.7	0.7
(3S, 3'S)-Astaxanthin(7)	86.0	87.4	88.2

* Peak numbers on chromatogram.

proved that (3S, 3'S)-astaxanthin was a dominant carotenoid in the ovaries of chum salmon during spawning migration.

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