Mem. Fac. Fish., Kagoshima Univ.
Vol. 25, No. 1, pp. 27~32 (1976)

Comparative biochemistry of carotenoids in algae—VI.

Carotenoids in *Phylloderma sacrum*, *Lyngbya* sp., and *Spirogyra* sp.

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Abstract

The carotenoids in *Phylloderma sacrum*, *Lyngbya* sp., and *Spirogyra* sp. were extracted, purified on column chromatography and identified by the absorption spectra, the behaviors on the columns, special chemical tests and co-chromatography with authentic carotenoids.

In *Phylloderma sacrum*, the existence of β-carotene, β-zeacarotene, echinenone, lutein and zeaxanthin was confirmed. In *Lyngbia* sp., β-carotene, β-zeacarotene, echinenone, α-cryptoxanthin, cryptoxanthin and zeaxanthin were found. α-Carotene, β-carotene, lutein and zeaxanthin were confirmed in *Spirogyra* sp.

It is commonly considered that fish, like all other animals, do not possess any ability to synthesize carotenoids de novo from mevalonic acid, but they can alter alimentary carotenoids by oxidation or can deposite them without modification.

It was clarified that *Crustacea* can oxidize the 3 and 3′ positions of β-ionone rings, and also can oxidize the 4 and 4′ positions of β-ionone rings of carotenoids[1,2].

It was elucidated that fresh water red fish can oxidize the 4 and 4′ positions of the ionone rings of carotenoids but not the 3 and 3′ positions of the ionone rings of the carotenoids[3,4].

The present investigation was undertaken to clarify the carotenoids in *Phylloderma sacrum*, *Lyngbya* sp., and *Spirogyra* sp. from the point of the view of the precursors of the aquatic animal carotenoids.

The existence of β-carotene, β-zeacarotene, echinenone, lutein and zexanthin was confirmed in *Phylloderma sacrum*. In *Lyngbya* sp., β-carotene, β-zeacarotene, echinenone, α-cryptoxanthin, cryptoxanthin and zeaxanthin were found. The existence of α-carotene, β-carotene, lutein and zeaxanthin was elucidated in *Spirogyra* sp.

From the point of the view of the "Biosynthesis of Astaxanthin" in aquatic animals, it was elucidated that *Phylloderma sacrum* and *Lyngbya* can be used to supplement the diet for the prawn to improve their pigmentation.

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Materials and Methods

I. The carotenoids in *Phylloderma sacrum*:

Alga, *Phylloderma sacrum*, cultured at Fukuoka, were obtained. The carotenoid pigments were completely extracted with acetone in a Waring blender until the residues became colorless. The pigments of acetone solutions were transferred to petroleum ether by the addition of water. The petroleum ether solution of the pigments was washed with water to remove traces of acetone. The light petroleum ether solution of the pigments was dried over anhydrous sodium sulphate and concentrated under vacuum to an oil. The pigments were saponified by dissolving them in about 100 ml of absolute ethanol, adding 10 ml of 60 per cent (W/V) aqueous potassium hydroxide solution and leaving them in the dark at room temperature. The saponified pigments were then transferred to petroleum ether by the addition of water. The petroleum ether solution of the pigments was washed with water, dried over anhydrous sodium sulphate and concentrated under vacuum to an oil. The adsorbents for column chromatography were chosen according to the types of the carotenoids to be separated[5]. The pigments were first chromatographed on a magnesium oxide column (MgO: Hyflosupercel=1 : 2), using petroleum ether as developing solvent. Two bands were obtained: Fr–I (lower band) and Fr–II (upper band).

β-Carotene: The pigment of Fr–I (lower band) was repurified on a magnesium oxide column (MgO: Hyflosupercel=1 : 2), using petroleum ether as developing solvent. Only one band was obtained. The absorption spectra and the behaviors on the column were all identical with pure β-carotene. This pigment was confirmed to be β-carotene.

The pigments of Fr–II (upper band) were rechromatographed on a magnesium oxide column, using 4% acetone in petroleum ether as developing solvent. Three bands were obtained. Fr–II–a (lower band), Fr–II–b (middle band) and Fr–II–c (upper band). The pigments of Fr–II–a were repurified on an alumina column (activity grade II), using 0.5% acetone in petroleum ether. Two bands were obtained: Fr–II–a–1 (lower band) and Fr–II–a–2 (upper band).

β-Zeacarotene: The pigment of Fr–II–a–1 (lower band) was eluted from the column with acetone and transferred to petroleum ether by the addition of water. The absorption spectra and the behavior on the column were in agreement of pure β-zeacarotene. This pigment was confirmed to be β-zeacarotene.

Echinenone: The pigment of Fr–II–a–2 was rechromatographed on a magnesium oxide column, using 4% acetone in petroleum ether as developing solvent. Only one band was obtained. The absorption spectra and the behavior on the column were all identical with those of pure echinenone. The pigment was reduced by adding a tiny crystal of sodium borohydride to a solution of the pigment in 95% ethanol. The solution was then left overnight in a refrigerator. The absorption spectra of the re-

duced pigment were identical with those of isocryptoxanthin. The pigment was co-chromatographed with pure echinenone and only one band was obtained. Those results show that this pigment was confirmed to be echinenone.

The pigments of Fr–II–b were repurified on a magnesium oxide column, using 8% acetone in petroleum ether as developing solvent. Two bands were obtained: F–II–b–1 (lower band) and Fr–II–b–2 (upper band).

α-Cryptoxanthin: The absorption spectra of the pigment of Fr–II–b–1 (lower band) and the behavior on the column were all identical with those of α-cryptoxanthin. This pigment was confirmed to be α-cryptoxanthin.

Lutein: The pigment of Fr–II–b–2 (upper band) was rechromatographed on a magnesium oxide column, using 15% acetone in petroleum ether as developing solvent. The absorption spectra and the behavior on the column were all in agreement with pure lutein. This pigment was confirmed to be lutein.

Zeaxanthin: The pigment of Fr–II–c was repurified on a magnesium oxide column, using 25% acetone in petroleum ether as developing solvent. Only one band was obtained. The absorption spectra and the behavior on the column were all identical with those of pure zeaxanthin. This pigment was confirmed to be zeaxanthin.

II. The carotenoids in *Lyngbya* sp.:

Alga, *Lyngbya* sp. were collected at the Ota river in Kagoshima city. The pigments in *Lyngbya* sp. were extracted with acetone in a Waring blender until no further pigments were obtained. The acetone solutions of the pigments were transferred to petroleum ether by the addition of water and washed repeatedly with water to remove traces of acetone. The petroleum ether solution of the pigments was dried over anhydrous sodium sulphate, concentrated under vacuum to an oil and saponified as already mentioned. The saponified pigments were dissolved in about 10 ml of petroleum ether for chromatography.

The pigments were first chromatographed on a magnesium oxide column, using petroleum ether as developing solvent. Two bands were obtained: Fr–I (lower band) and Fr–II (upper band).

β-Carotene: The pigment of Fr–I (lower band) was repurified on a magnesium oxide column, using petroleum ether as developing solvent. Only one band was obtained. The absorption spectra and the behavior on the column were all in agreement with those of pure β-carotene. This pigment was confirmed to be β-carotene.

The pigments of Fr–II were rechromatograped on a magnesium oxide column, using 4% acetone in petroleum ether as developing solvent. Two bands were obtained: Fr–II–a (lower band) and Fr–II–b (upper band). The pigments of Fr–II–a were repurified on an alumina column (activity grade-II), using 0.5% acetone in petroleum ether as developing solvent and two bands were obtained: Fr–II–a–1 (lower band) and Fr–II–a–2 (upper band).

β-Zeacarotene: The absorption spectra and the behavior on the column of

Fr–II–a–1 (lower band) were all identical with those of pure β-zeacarotene. This pigment was confirmed to be β-zeacarotene.

Echinenone: The pigment of Fr–II–a–2 was repurified on a magnesium oxide column, using 5% acetone in petroleum ether as developing solvent. Only one band was obtained. The absorption spectra, the behavior on the column, the absorption spectra of the reduced product and the results of thin-layer co-chromatography with pure echinenone are all in agreement with pure echinenone. Thus this pigment was confirmed to be echinenone.

The pigments of Fr–II–b were rechromatographed on a Microcel-C column, using 10% acetone in petroleum ether as developing solvent. Three bands were obtained: Fr–II–b–1 (lower band), Fr–II–b–2 (middle band) and Fr–II–b–3 (upper band).

α-Cryptoxanthin: The pigment of Fr–II–b–1 (lower band) was repurified on a magnesium oxide column, using 6% acetone in petroleum ether as developing solvent. Only one band was obtained. The absorption spectra and the behavior on the column were all identical with those of pure α-cryptoxanthin.

Cryptoxanthin: The pigment of Fr–II–b–2 was repurified on a magnesium oxide column, using 8% acetone in petroleum ether as developing solvent. Only one band was obtained. The absorption spectra and the behavior on the column were all in agreement with those of pure cryptoxanthin.

Zeaxanthin: The pigment of Fr–II–b–3 was repurified on a magnesium oxide column, using 25% acetone in petroleum ether as developing solvent. The absorption spectra, the behavior on the column and co-chromatography with pure zeaxanthin obtained from Chinese lantern are all identical with those of pure zeaxanthin. This pigment was confirmed to be zeaxanthin.

III. The carotenoids in *Spirogyra* sp.:

Alga, *Spirogyra* were collected at the stream in Kagoshima city. The carotenoids were extracted with acetone in a Waring blender until the extracts became colorless. The acetone soltutions of the pigments were transferred to petroleum ether by the addition of water. Thus extracted carotenoids were saponified as already stated. The saponified pigments were first chromatographed on a magnesium oxide column, using petroleum ether as developing solvent. Three bands were obtained: Fr–I (lower band), Fr–II (middle band) and Fr–III (upper band).

α-Carotene: The pigment of Fr–I (lower band) was rechromatographed on an alumina column (activity grade-II), using petroleum ether as developing solvent. Only one band was obtained. The absorption spectra and the behavior on the column were all identical with those of pure α-carotene. This pigment was confirmed to be α-carotene.

β-Carotene: The pigment of Fr–II was repurified on a magnesium oxide column, using petroleum ether as developing solvent. Only one band was obtained. The absorption spectra and the behavior on the column were all in agreement with those of pure β-carotene. This pigment was confirmed to be β-carotene.

The pigments of Fr–III were rechromatographed on a magnesium oxide column, using 20% acetone in petroleum ether as developing solvent. Two bands were obtained: Fr–III–a (lower band) and Fr–III–b (upper band).

Lutein: The pigment of Fr–III–a (lower band) was repurified on a magnesium oxide column, using 15% acetone in petroleum ether as developing solvent. Only one band was obtained. The absorption spectra and the behavior on the column were all identical with those of pure lutein. This pigment was confirmed to be lutein.

Zeaxanthin: The pigment of Fr–III–b was rechromatographed on a magnesium oxide column, using 25% acetone in petroleum ether as developing solvent. Only one band was obtained. The absorption spectra and the behavior on the column were all in agreement with pure zeaxanthin. This pigment was confirmed to be zeaxanthin.

Results and Discussions

The carotenoids in *Phylloderma sacrum* are listed in **Table 1** in the order in which they were eluted from the column and the relative amounts of each pigment is given as a percentage of the total. The carotenoids in *Lyngbya* sp. and *Spirogyra* sp, are listed separately in **Tables 2** and **3**. As can be seen in **Table 1**, the major carotenoids in *Phylloderma sacrum* are β-carotene and zeaxanthin. In *Lyngbya* sp., the most a-

Table 1. Spectral characteristics and relative abundances of the carotenoids in *Phylloderma sacrum*.

Carotenoids	Relative abundances mg%	%	Spectral characteristics λ max in petroleum ether
β-Carotene	5.84	60.4	425, 449, 476
β-Zeacarotene	0.34	3.5	406, 427, 452
Echinenone	0.58	6.0	453
α-Cryptoxanthin	0.41	4.2	424, 445, 472
Lutein	0.41	4.2	425, 446, 473
Zeaxanthin	1.95	20.1	425, 449, 475
Unknown	0.16	1.6	—

Table 2. Spectral characteristics and relative abundances of the carotenoids in *Lyngbya* sp.

Cartenoids	Relative abundances mg%	%	Spectral characteristics λ max in petroleum ether
β-Carotene	7.49	94.5	425, 449, 476
β-Zeacarotene	0.003	trace	406, 427, 452
Echinenone	0.05	0.7	453
α-Cryptoxanthin	0.15	1.9	426, 446, 473
Cryptoxanthin	0.20	2.5	426, 450, 474
Zeaxanthin	0.03	0.4	425, 449, 476

Table 3. Spectral characteristics and relative abundances of the carotenoids in *Spirogyra* sp.

Carotenoids	Relative abundance mg%	Relative abundance %	Spectral characteristics λ max in petroleum ether
α-Carotene	0.30	4.7	421, 444, 475
β-Carotene	2.55	39.5	425, 448, 475
Lutein	3.33	51.6	420, 444, 472
Zeaxanthin	0.37	4.2	425, 449, 476

bundant pigment was β-carotene as shown in **Table 2.** The major carotenoids in *Spirogyra* sp. are lutein and β-carotene.

Those algae, which were investigated, can be used to supplement the food for *Crustacea* as the precursor of the astaxanthin, because those animals can oxidize the 3 and 3′ positions, and also the 4 and 4′ positions of the ionone rings of carotenoids as reported in the previous papers[1,2].

References

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