Mem. Fac. Fish., Kagoshima Univ.
Vol. 24 pp. 127~131 (1975)

Comparative biochemistry of carotenoids in algae–V

Carotenoids in *Rhodomonas baltica* Karsten and *Nostoc commune* Vancher

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Abstract

From the standpoint of the precursor of the carotenoids in aquatic animals, the carotenoids in *Rhodomonas baltica* Karsten and *Nostoc commune* Vancher were separated and identified by using column chromatography. The existence of α-carotene, diatoxanthin, diadinoxanthin, fucoxanthin, and neofucoxanthin was confirmed in *Rhodomonas baltica* Karsten. β-carotene, echinenone, canthaxanthin, P-446 and zeaxanthin were found in *Nostoc commune* Vancher.

Carotenoids are reported to have a number of function in photosynthetic and phototactic organisms. By absorbing light in the region where absorption by chlorphyll is low and transferring this energy to chlorophyll, they increase the capacity of plants to gather light for photosynthesis.

Carotenoids also protect the cell from photodynamic destruction. It has also been suggested that carotenoids play a role in the transport of oxgen. It is also very interesting to note that the origin of the carotenoids in aquatic animals can be traced to the algae consumed by the aquatic animals, because aquatic animals, like all other animals do not have the ability to synthesize catotenoids from acetic acid or pyruvic acid de novo, but they are capable of altering alimentary carotenoids and storing the resulting products[1-4]. The carotenoids in Nostoc were already reported by Hager et al[5], and there have been few investigations of the carotenoids of dinoflagellates[6-9]

The present investigation was undertaken to clarify the carotenoids in *Rhodomonas baltica* Karsten and *Nostoc commune* Vancher from the standpoint of the precursor of the carotenoids in aquatic animals. The existence of α-carotene, diatoxanthin, diadinoxanthin, fucoxanthin and neofucoxanthin was clarified in *Rhodomonas baltica* Karsten. Betacarotene, echinenone, canthaxanthin, P–446 and zeaxanthin were found in *Nostoc commune* Vancher.

Methods and Materials

I. The carotenoids in *Rhodomonas baltic* Karsten.

a) Methods of cultivation.

Rhodomonas baltica Karsten were grown in the medium shown in Table 1[10].

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Table 1. Defined medium for *Rhodomonas baltica* Karsten.

NaCl	1.8 g	Na_2EDTA	3.0 mg
$MgSO_4 \cdot 7H_2O$	0.5 g	Fe (as Cl^-)	0.08 mg
KCl	0.06 g	Zn (〃)	15.0 μg
Ca (as Cl^-)	10 mg	Mn (〃)	0.12 mg
$NaNO_3$	5 mg	Co (〃)	3 μg
K_2HPO_4	0.5 mg	Cu (〃)	0.12 μg
$Na_2SiO_3 \cdot 9H_2O$	15 mg	B (as H_3BO_3)	0.6 mg
TRIS	0.1 g	H_2O	100 ml.
B_{12}	0.2 μg	pH	7.6–7.8
*Vitamin Mix S3	1 ml		

* 1 ml. of vitamin Mix S3 contains: Thiamine HCl, 0.05 mg; nicotinic acid, 0.01 mg; Ca pantothenate, 0.01 mg; p-aminobenzoic acid, 1.0 μg; biotin, 0.1 μg; inositol, 0.5 mg; folic acid, 0.2 μg; tyhmine,

b) Separation and identification of the carotenoids in *Rhodomonas baltica* Karsten.

The carotenoid pigments of *Rhodomonas baltiac* Karsten were extacted with acetone in a Waring blender. The pigments were transferred to petroleum ether from acetone by addition of water. The petroleum ether solution of the pigments was washed with water to remove the trace of acetone, dried over anhydrous sodium sulfate and evaporated under reduced pressure. The pigments were dissolved in a small volume of petroleum ether and chromatographed on a magneisum oxide column (MgO: Hyflosupercel=1: 2), using 5% acetone in petroleum ether as developing solvent[11]. Two bands were obtained Fr–I (lower band), and Fr–II (upper band).

α-carotene: The pigment of Fr–I 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 behavior on the column were all identical with pure α-carotene. The pigment was confirmed to be α-carotene.

The pigments of Fr–II (middle vand) were repurified on a magnesium oxide column (MgO: Hyflosupercel=1: 2), using 10% acetone in petroleum ether as developing solvent. Two bands were obtained Fr–II–a (lower band) and Fr–II–b (upper band)

Diatoxanthin and diadinoxanthin[8]: The pigments of Fr–II–a (lower band) were rechromatographed on a silica gel column by suing 50% ethylether in petroleum ether as developing solvent. Two bands were obtained Fr–II–a–1 (lower band) and Fr–II–a–2 (upper band). The pigment of Fr–II–a–1 (lower band) was eluted out from the column with acetone. The absorption spectra and the behavior on the column were all identical with pure diatoxanthin. The pigment of Fr–II–a–1 was confirmed to be diatoxantin. The pigment of Fr–II–a–2 (upper band) was eluted from the column with acetone. The absorption spectra and the behavior on the column were all in agreement with pure diadinoxanthin. This pigment was confirmed to be diadinoxanthin.

Fucoxanthin and neofucoxanthin[8)12-15]: The pigments of Fr–II–b were

rechromatographed on a silica gel column by using 50% dichloromethane in petroleum ether as developing solvent. Two band were obtained, Fr–II–b–1 (lower band) and Fr–II–b–2 (upper band). The pigment of Fr–II–b–1 (lower band) was eluted from the column with acetone. The pigment in acetone was transferred to petroleum ether. The absorption spectra and the behavior on the column were identical with those of pure fucoxanthin. The pigment was confirmed to be fucoxanthin.

The pigment of Fr—I–b–2 was eluted from the column with acetone. The absorption spectra and the behavior on the column were all in agreement of pure neofucoxanthin. The pigment was confirmed to be neofucoxanthin.

II. The carotenoids in *Nostoc commune* Vancher.

Algae, *Nostoc commune* Vancher were collected at Shirahama, Wakayama. The carotenoid pigments of Nostoc were completely extracted with acetone in a Waring blender untile the residue became colorless. The carotenoid pigments were first chromatographed on a magnesium oxide column, using petoleum ether as developing solvent. Three bands were obtained. Fr–I (lower band), Fr–II (middel band), Fr–III (upper band).

β-carotene: The pigment of Fr–I (lower band) was repurified on a magnesium oxide column[11], using petroleum ether as developing solvent. The absorption spectra and the behavior on the column were identical with pure β-carotene. This pigment was confirmed to be β-carotene.

Echinenone: The carotenoid of Fr–II (middle band) was rechromatographed on a magnesium oxide column, using 4% acetone in petroleum ether as developing solvent. The absorption spectra and the behavior on the column were identical with those of pure echinenone. This pigment was confirmed to be echinenone.

The pigments of Fr–III were rechromatographed on a magnesium oxide column, using 10% acetone in petroleum ether as developing solvent. Three bands were obtained, Fr–III–a (lower band), Fr–III–b (middle band), Fr–III–c (upper band).

Canthaxanthin: The pigment of Fr–III–a (lower band) was eluted from the column with acetone, and transferred to petroleum ether solution. The absorption spectra and the behavior on the column were all identical with those of pure canthaxanthin. This pigment was confirmed to be canthaxanthin.

P–446: The pigment of Fr–III–b (middle band) was eluted from the column. The absorption spectra and the behavior on the column were not identical with those of the known carotenoids.

Zeaxanthin: The pigment of Fr–III–c (upper band) was eluted from the column. The absoption spectra and the behavior on the column were in agreement of pure zeaxanthin. This pigment was confirmed to be zeaxanthin.

Results and Discussions

In *Rhodononas baltica* Karsten, α-carotene, diatoxanthin, diadinoxanthin, fucoxan-

Table 2. Spectral characteristics and relative abun dance of the carotenoids in *Rhodomonas baltica.*

Compound	%	λ max (mμ) in *P. E.
α-carotene	2.5	419, 442, 472
Diatoxanthin	36.4	428, 449, 477
Diadinoxanthin	0.8	430, 449, 476
Fucoxanthin	50.4	430, 450, 475
Neofucoxanthin	9.8	428, 449, 476

*P. E.: Petroleum ether.

Table 3. Spectral characteristics and relative abundance of the carotenoids in *Nostoc commune.*

Compound	%	λ max (mμ) in *P. E.
β-carotene	20.4	426, 451, 478
Echinenone	43.4	458, 469
Canthaxanthin	27.3	468
P-446	2.6	421, 446, 473
Zeaxanthin	6.2	429, 453, 481

*P. E.: Petroleum ether.

thin, and neofucoxanthin were found to be present. Fucoxanthin was most abundant in this alga. In *Nostoc commune* Vancher, echinenone was most abundant. The carotenoids in *Rhodomonas baltica* are listed in Table 2 in the order in which they were eluted from the column and the relative amount of each pigment is given as a percentage of that total. The carotenoids in *Nostoc commune* are listed in Table 3 in the order in which they were eluted from the column.

In crustacea, it was confirmed that β-carotene, echinenone and canthaxanthin were precursors of astaxanthin[2)4)]. Nostoc would be a good additive for crustacea to improve their red color.

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