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Comparative biochemistry of Carotenoids in algae — IV.

Carotenoids in Cyanophyta, blue-green algae, *Spirulina platensis*

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Abstract

The carotenoids in spirulina were extracted, saponified, purified on the columns, characterized by absorption spectra, the behavior on the columns, absorption maxima of the reduction products and co-chromatography with authentic samples.

In spirulina besides β-carotene, echinenone, cryptoxanthin, zeaxanthin and lutein, the exisence of α-carotene, hydroxyechinenone and euglenanone was confirmed. β-Carotene was most abundant in this blue-green algae.

In crustacea, it was confirmed that β-carotene was a precursor of astaxanthin and converted to astaxanthin through the steps of cryptoxanthin, echinenone, canthaxanthin and phenicoxanthin. The color of zeaxanthin is red, spirulina would be a good additives for fish food to improve their red color.

It is generally accepted that fish, like all animals do not have the ability to synthesize carotenoids from acetic acid or pyruvic acid de novo, but they are capable of altering alimentary carotenoids and storing the resulting products.

The present investigation was undertaken to clarify the carotenoids in spirulina from the view of the standpoint of the precursor of the fish carotenoids.

The existence of β-carotene, lutein[1)], zeaxanthin[1)], echinenone and canthaxanthin were found in algae[1-13)]. Those β-carotene, lutein, zeaxanthin, echinenone and canthaxanthin could be easily converted to astaxanthin in fish or transferred those carotenoids to their body and stored them.

Goodwin et al.[16)] reported that the main carotenoids in *Cyanidium culdarium* were β-carotene and zeaxanthin and also Strain[2)] clarified the carotenes were primarily β-carotene and zeaxanthin in Cyanophyta.

On carotenoids in spirulina, Homma[17)] has clarified the existence of β-carotene, echinenone, cryptoxanthin, zeaxanthin and diolketone. By this invesitigation, besides those carotenoids listed above, α-carotene, hydroxyechinenone and euglenanone were found in Spirulina.

Methods and Materials

I. Methods of Cultivation

Cultures of Spirulina were grown in the medium shown in Table 1.

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Table 1. Defined medium for spirulina

$NaHCO_3$	16.8 g	$MnCl_2 \cdot 4H_2O$	1.81 〃
K_2HPO_4	0.5 〃	$ZuSO_4 \cdot 7H_2O$	0.22 〃
$MgSO_4 \cdot 7H_2O$	0.2 〃	$CuSO_4 \cdot 5H_2O$	0.08 〃
K_2SO_4	1.0 〃	Na_2MoO_4	0.021 〃
NaCl	1.0 〃	Water	1000 ml
$CaCl_2$	0.04 〃	Conc H_2SO_4	1.0 drop
$FeSO_4 \cdot 7H_2O$	0.01 〃	**B_6-Sol.	
EDTA	0.08 〃		
*A_5-Sol.	1.0 ml	NH_4VO_3	229.6 mg
**B_6-Sol.	1.0 〃	$CrK(SO_4)_4 \cdot 24H_2O$	960.2 〃
Urea	1.0 〃	$NiSO_4 \cdot 6H_2O$	447.8 〃
Water	1000 〃	$Co(NO_3)_2 \cdot 6H_2O$	493.8 〃
		$NaWO_4 \cdot 2H_2O$	179.4 〃
*A_5-Sol.		***Ti-Sol.	20 ml
H_3BO_3	2.86 g	$1/10NH_2SO_4$	1000 ml

*** 736.6 mg Titanium oxisalate were dissolved on dist. water and ammonium hydroxide solution was added in order to alkalify it. Some prepicitates were obtained, collected them and dissolved them in 20 ml of 1/10 NH_2SO_4 solution.

II. Separation and Identification of the carotenoids in Spirulina

The carotenoid pigments of spirulina were completely extracted with acetone in a Waring blender. The pigments were transferred to petroleum ether from acetone with water. The petroleum ether solution of the pigments was washed with water to remove acetone, dried over anhydrous sodium sulfate, and evaporated under reduced pressure. The pigments were saponified by dissolving them in 100 ml of absolute ethanol, adding 10 ml of 60 percent (w/v) aqueous potassium hydroxide solution, and leaving them over night[17]. The saponified pigments were then transferred to petroleum ether and water was added. The petroleum ether solution of pigment washed thoroughly with water, dried over anhydrous sodium sulfate, and evaporated to an oil under reduced pressure, The pigments were dissolved in a small volume of petroleum ether and chromatographed on a magnesium oxide column (magnesium oxide: hyflosupercel=1: 2) using petroleum ether as developing solvent. Three bands were obtained: Fr-I (lower band) Fr-II (middle band) and Fr-III (upper band)

The pigments of Fr-I (lower band) were rechromatographed on a magnesium oxide column (magnesium oxide: hyflosupercel=1: 2), using petroleum ether as developing solvent. Three bands were obtained: Fr-I-A (lower band), Fr-I-B (middle band), Fr-I-C (upper band).

α-carotene: The pigment of Fr-I-A was repurified on an aluminum oxide column by using 0.2 % 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 pure α-carotene. This pigment was confirmed to be α-carotene.

β-carotene: The pigment of Fr-I-B was repurified on an aluminum oxide column

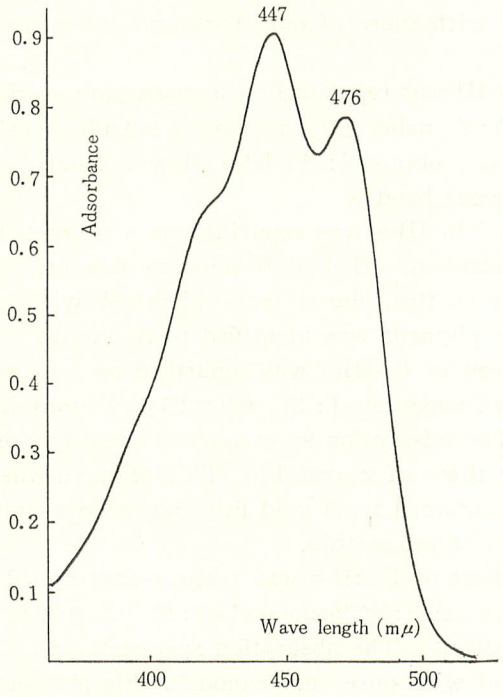

Fig. 1. The absorption spectrum of the crude carotenoids in Spirulina.

(grade II), using petroleum ether as developing solvent. The absorption spectra and the behavior on the column were in agreement with those of β-carotene. This pigment was co-chromatographed on an aluminum oxide column (grade II) with pure β-carotene obtained from prawn, using petroleum ether as developing solvent and made a unitary zone. This pigment was identified to be β-carotene.

echinenone and cryptoxanthin: The pigments of Fr-I-C were rechromatographed on an aluminum oxide column, using 3 % acetone in petroleum ether as developing solvent. Two bands were obtained; Fr-I-C-a (lower band) and Fr-I-C-b (upper band). The absorption spectrum of Fr-I-C-a and its behavior on the column were identical with pure echinenone. The pigment was reduced by the addition of a tiny amount of sodium borohydride in 95 % ethyl alcohol at 5°C. The reaction was allowed to proceed for 30 minutes. The absorption spectrum of the reduced pigment agreed with that of β-carotene. These results were all identical with those of echinenone. This pigment was confirmed to be echinenone.

The absorption spectrum of the pigment of Fr-I-C-b and its behavior on the column were in agreement with pure cryptoxanthin. This pigment was identified to be cryptoxanthin.

Hydroxyechinenone: The pigment of Fr-II was rechromatographed on an aluminum oxide column, using 3 % acetone in petroleum ether as developing solvent. Only one band was obtained. The absorption spectra showed to be 460 mμ in petroleum

ether. After reduction with sodium borohydride, λ_{max}=425,450, 477 mμ. These values were all identical with those of pure hydroxyechinenone which was found by Krinsky[6].

The pigments of Fr-III was repurified on a magnesium oxide column (magnesium oxide: hyflosupercel=1: 2), using 25 % acetone in petroleum ether as developing solvent. Three bands were obtained: Fr-III-a (lower band), and Fr-III-b (middle band), and Fr-III-c (upper band).

Lutein: The pigment of Fr-III-a was repurified on a magnesium oxide column, using 20 % acetone in petroleum ether as developing solvent. The absorption spectrum and the behavior on the column were identical with pure lutein obtained from gold fish.[17] This pigment was identified to be lutein.

Zeaxanthin: The pigment of Fr-III-b was repurified on a magnesium oxide column (magnesium oxide: hyflosupercel=1: 2), using 25 % acetone in petroleum ether as developing solvent. The adsorption spectrum and the behavior on the column were all in agreement with those of zeaxanthin. This pigment was co-chromatographed with pure zeaxanthin obtained from gold fish and made a unitary zone. This pigment was confirmed to be zeaxanthin.

Euglenanone: The pigment of Fr-III-c was rechromatographed on a magnesium oxide column (magnesium oxide: hyflosupercel=1: 2), using 30 % acetone in petroleum ether as developing solvent. The absorption spectrum and the behavior on the column are all identical with pure euglenanone[6], this pigment was identified to be euglenanone.

Results and Discussions

In spirulina besides β-carotene, echinenone, cryptoxanthin, zeaxanthin and lutein, the existence of α-carotene, hydroxyechinenone and euglenanone were confirmed. β-carotene was most abundant in this blue-green algae. The carotenoids in spirulina are listed in Table 2 in the order in which they were eluted from the columns. The relative amount of each pigment is given as a percentage of that total.

Table 2. Spectral characteristics and relative abundance of the carotenoids in Spirulina

Compound	%	λmax (mμ)
α-carotene	7.0	418, 443, 472
β-carotene	67.4	425, 447, 473
echinenone	6.8	453,
cryptoxanthin	6.0	422, 446, 472
pigment 425	2.5	405, 426. 452
hydroxy-echinenone	1.4	460
lutein	2.7	(420), 445, 468
zeaxanthin	5.8	425, 448, 473
euglenanone	0.01	452

In crustacea, it was confirmed that β-carotene was a precursor of astaxanthin and converted to astaxanthin through the steps of cryptoxanthin, echinenone, canthaxanthin and phenicoxanthin. The color of zeaxanthin is red, Spirulina would be a good additives for fish food to improve their red color.

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