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Communication

α-Carotene and its derivatives have a sole chirality in phototrophic organisms?*

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Carotenoids in eukaryotic phototrophic organisms can be classified into two groups; β-carotene and its derivatives, and α-carotene and its derivatives. We re-examined distribution of α-carotene and its derivatives among various taxa of aquatic algae (17 classes) and land plants. α-carotene and its derivatives were found from Rhodophyceae (macrophytic type), Cryptophyceae, Euglenophyceae, Chlorarachniophyceae, Prasinophyceae, Chlorophyceae, Ulvophyceae, Charophyceae, and land plants, while they could not be detected from Glaucophyceae, Rhodophyceae (unicellular type), Chryosophyceae, Raphidophyceae, Bacillariophyceae, Phaeophyceae, Xanthophyceae, Eustigmatophyceae, Haptophyceae, and Dinophyceae. We also analyzed the chirality of α-carotene and/or its derivatives, such as lutein and siphonaxanthin, and found all of them had only (6'*R*)-type, not (6'*S*)-type.

Key words: algae, α-carotene, β-carotene, carotenoid, chirality, red algae

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INTRODUCTION

Eukaryotic phototrophic organisms produce not only chlorophylls but also some carotenoids, which can be classified into two groups; β-carotene and its derivatives (zeaxanthin, violaxanthin, neoxanthin, fucoxanthin, diadinoxanthin, etc.), and α-carotene and its derivatives (lutein, loroxanthin, siphonaxanthin, prasinoxanthin, etc.) (Fig. 1). All of eukaryotic phototrophic organisms necessarily contain β-carotene and its derivatives. On the other hand, distribution of α-carotene and its derivatives is reported to be limited in some taxonomic groups of phototrophic organisms. In addition, only (6'*R*)-type of α-carotene and its derivatives have been reported from algae and land plants, although C-6' in α-carotene between ε-end group and conjugated double bonds is chiral, (6'*R*)- and (6'*S*)-types (Fig. 2) (Bjørnland & Liaaen-Jensen, 1989; Rowan, 1989; Britton *et al*., 2004; Takaichi, 2011).

In this study, to confirm the reliability of chirality, we re-examined distribution of α-carotene and its derivatives among algae, and analyzed their C-6' chirality using circular dichroism (CD) or nuclear magnetic resonance spectra after purification of the carotenoids.

MATERIALS AND METHODS

Most of microalgae were cultured in the laboratory. Macrophytic seaweeds (Rhodophyceae, Phaeophyceae, Ulvophyceae) were collected at rocky seashore in Awaji-island, Japan. Nearly 40 species were analyzed.

Pigments were extracted with acetone/methanol (7:2, v/v). They were analyzed by HPLC equipped with a μBondapak C_{18} column (RCM type; Waters, USA) and eluted with methanol/water (9:1, v/v) for the first 20 min, and then 100% methanol (2.0 ml/min) (Iwai *et al*., 2008), and carotenes were analyzed with a Novapak C_{18} column (RCM type; Waters) and eluted with acetonitrile/methanol/tetrahydrofuran (58:35:7, by vol., 2.0 ml/min) (Takaichi, 2000). The absorption spectra of the pigments were measured using an MCPD-3600 photodiode array detector (Otsuka Electronics, Japan) attached to the HPLC (Takaichi & Shimada, 1992).

α-Carotene and/or its derivatives have typical absorption spectra (Takaichi & Shimada, 1992; Takaichi, 2000). They were purified with the combination of silica gel TLC plates (Merck, Germany), KC18 TLC plates (Whatman, USA), DEAE-Toyopearl 650M column chromatography (Tosoh, Japan), and C_{18}-HPLC described above.

After purification, the relative molecular masses of the carotenoids were measured using an FD-MS; M-2500 double-focusing gas chromatograph-mass spectrometer equipped with a field-desorption apparatus (Hitachi, Japan). The CD spectra were measured using a J-820 spectropolarimeter (JASCO, Japan) in diethyl ether/2-pentane/ethanol (5:5:2, by vol.) at room temperature (Buchecker & Noack, 1995).

RESULTS

Distribution of α-carotene and its derivatives

We could not detect α-carotene and/or its derivatives from Glaucophyceae (*Cyanophora paradoxa*, etc.), Chryosophyceae (*Ochromonas danica*, etc.), Raphidophyceae (*Heterosigma akashiwa*, etc.), Bacillariophyceae (*Phaeodactylum tricornutum*, etc.), Phaeophyceae (*Undaria pinnatifida*, etc.), Xanthophyceae (*Vaucheria terrestiris*,

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Abbreviations: CD, circular dichroism.

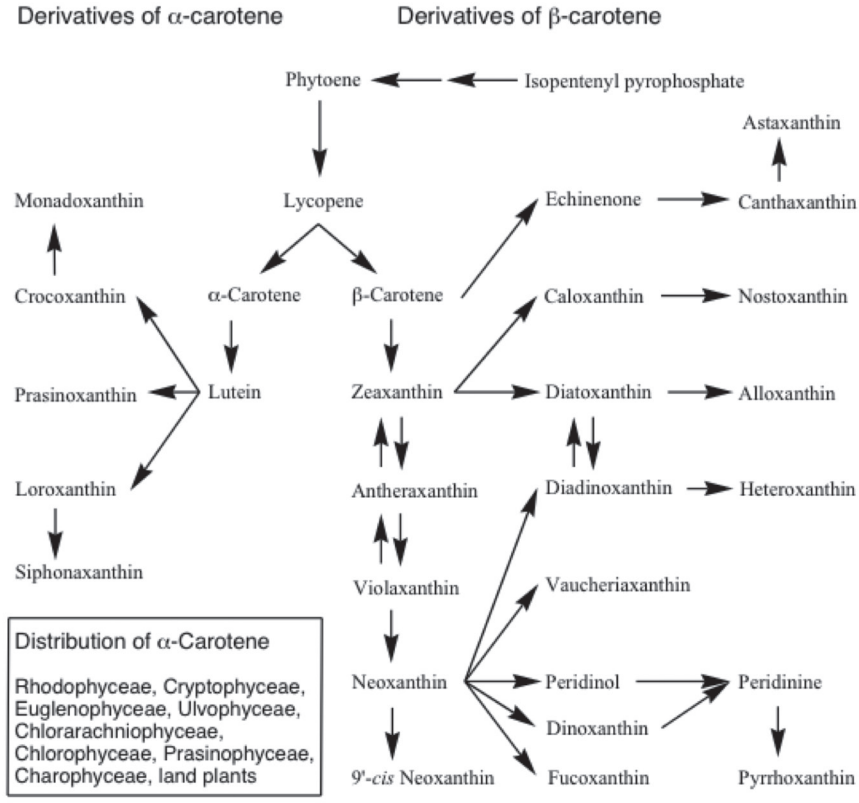

Figure. 1. α-Carotene, β-carotene and their derivatives, and carotenogenesis pathways in algae and land plants.

etc.), Eustigmatophyceae (*Nannochloropsis oculata*, etc.), Haptophyceae (*Emiliania hyxleyi*, etc.), and Dinophyceae (*Alexandorium tamarense*, etc.). Some species were reanalyzed from the HPLC data of Takaichi & Mimuro (1998). We found α-carotene and/or its derivatives from Cryptophyceae (*Cryptomonas ovata*, etc.), Euglenophyceae (*Euglena viridis*, etc.), Chlorarachniophyceae (*Chlorarachnion* sp., etc.), Prasinophyceae (*Pterosperuma cristatum*, etc.), Chlorophyceae (*Chlamydomonas reinharditt*, etc.), Ulvophyceae (*Ulvapertusa*, etc.), Charophyceae (*Spirogyra* sp.), and land plants (*Spinacia oleracea*, *Oryza sativa*, etc.). α-Carotene derivatives of loroxanthin and siphonaxanthin, which are synthesized from lutein (Fig. 1), were found from Euglenophyceae, Chlorarachniophyceae, Prasinophyceae, Chlorophyceae, and Ulvophyceae.

α-Carotene and lutein in Rhodophyceae

We found α-carotene and lutein from macrophytic type of Rhodophyceae; *Porphyra yezoensis*, *Grateloupia lanceolata*, *Chondrus giganteus*, *Antithamnion plumula*, etc., while we could not detect them from unicellular type of Rhodophyceae; *Cyanidioschzon merolae*, *Cyanidium caldarium*, etc.

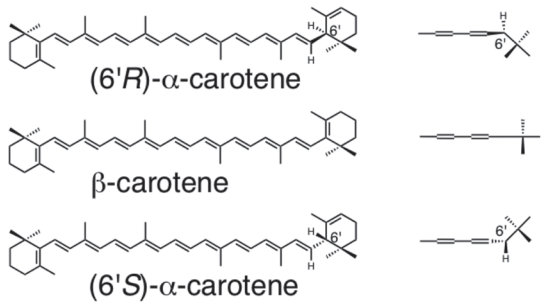

Figure. 2. Chirality of α-carotene.

Chirality of α-carotene and/or its derivatives

We analyzed chirality of α-carotene and/or its derivatives from more than 30 species described above including our previous papers (Yoshii *et al.* 2002; Kusaba *et al.*, 2009), and we obtained their chirality from around 20 species from reliable references. All of them had only (6'*R*)-type (Fig. 2).

DISCUSSION

In this study, we found that α-carotene and/or its derivatives (Fig. 1) were presented in the phylogenetically limited groups. One exception was Rhodophyceae (red algae), in which macrophytic type contained these carotenoids but unicellular type did not. All of α-carotene and its derivatives examined were (6'*R*)-type, and (6'*S*)-type was not found (Fig. 2).

In biosynthesis of α-carotene in land plants, both lycopene β-cyclase and lycopene ε-cyclase are needed to produce α-carotene from lycopene. They have high homology with each other, and therefore lycopene ε-cyclase gene might be produced by duplication of lycopene β-cyclase gene (Cunningham *et al.*, 1996). In enzymatic reaction of cyclization, the mechanisms of lycopene β-cyclase, lycopene (6'*R*)-ε-cyclase, and lycopene (6'*S*)-ε-cyclase are almost the same; the products are depending on the carbon number to eliminate H^+ and on the direction of elimination. Therefore, both lycopene ε-cyclases could be exist, but only lycopene

(6'*R*)-ε-cyclase was found based on the presence of only (6'*R*)-type. Since the stereochemistry of (6'*R*)- and (6'*S*)-α-carotenes are different for the direction of ε-end groups (Fig. 2), the binding site on the protein should not be identical. Consequently, the protein moiety might restrict to one chirality of α-carotene, (6'*R*)-α-carotene.

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