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Communication

Unique carotenoid lactoside, P457, in *Symbiodinium* sp. of dinoflagellate*

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The dinoflagellates are a large group of unicellular alge in marine and fresh water. Some are an endosymbiont of marine animals. Photosynthetic dinoflagellates have peridinin, a light-harvesting carotenoid. In addition, a unique carotenoid, P457, was found from *Amphinidium*. The presence of P457 in *Symbiodinium* derived from marine animals has not been reported. We reconfirmed the molecular structure of P457, a neoxanthin-like carotenoid with an aldehyde group and a lactoside, from *Symbiodinium* sp. NBRC 104787 isolated from a sea anemone. In addition, we investigated the distribution of P457 and peridinin in various *Symbiodinium* and scleractinian coral species, and possible biosynthetic pathways of these carotenoids are proposed.

Key words: carotenoid, carotenoid glycoside, dinoflagellate, P457, peridinin, *Symbiodinium*

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INTRODUCTION

The pigment composition of plants and algae has been used as a chemotaxonomic marker. Chlorophylls (Chls) are Chl *a*, Chl *b* and Chl *c*. Carotenoid profiles in photosynthetic eukaryotes are much more complex, such as fucoxanthin, peridinin, diadinoxanthin. Takaichi (2011) has summarized the distribution of carotenoids in algae and land plants.

The division Dinophyta (dinoflagellates) is a large group of algae, and is characterized by the presence of peridinin, as the most dominating carotenoid, which has never been detected in any other algae (Britton *et al.*, 2004; Takaichi, 2011). Interestingly, however, some dinoflagellates contain fucoxanthin instead of peridinin (Jeffrey *et al.*, 1975).

A unique carotenoid lactoside, P457, was found from symbiotic dinoflagellates from corals and clams as a highly polar pink-orange pigment, and was a sole glycoside of carotenoid known in algae (Johansen *et al.*, 1974). The structure of P457 derived from free-living *Amphidinium carterae* was determined (Aakermann *et al.*, 1993; Englert *et al.*, 1995). Jeffrey *et al.* (1975) examined pigment profiles in dinoflagelates. P457 was detected in 19 dinoflagellates that contained also peridinin, whereas P457 and peridinin were not observed in three species that had fucoxanthin. Considering that dinoflagellates might have originated from a plastid from red algae or other algae such as diatoms *via* secondary endosymbiosis (Palmer, 2003), investigation of the distribution of P457 in dinoflagellates and other kinds of algae is valuable to pursue the evolutional relations of algae.

In this study, P457 was purified from a symbiotic *Symbiodinium* sp. NBRC 104786 isolated from a sea anemore. Its molecular structure was re-determined, and a possible biosynthetic pathway of P457 and also peridinin was proposed. The distribution of P457 and peridinin was screened using *Symbiodinium* spp. and various species of marine algae and reef-building corals, and was discussed as a taxonomic and phylogenetic marker.

MATERIALS AND METHODS

Several marine algae including *Symbiodinium* sp. NBRC 104787 and *Amphidinium carterae* NBRC 102919 were cultivated in Daigo IMK medium under shaking at 25ºC with a 16 h light/8 h dark cycle under daylight fluorescence lamp. Artificially cultivated reef-building corals were obtained from the Okiden Kaihatsu Inc. (Okinawa, Japan).

Pigments were extracted with methanol. They were analyzed by HPLC equipped with a μBondapak C_{18} column (RCM type; Waters, USA) and eluted with methanol/water (8:2, v/v) for the first 6 min, methanol/water (9:1, v/v) for the next 14 min, and then 100% methanol (1.8 ml/min). Pigments were separated using silica gel 60 TLC (Merck, Germany) developed with petroleum ether/acetone (7:3, v/v), and then collected from the HPLC. Very polar pigments on the TLC plates were purified with TLC developed with dichloromethane/ethyl acetate/acetone/methanol (2:4:2:1, by vol.), and one green and one orange bands were separated. The orange band was further purified by the HPLC.

We measured the absorption spectra of the pigments using an MCPD-3600 photodiode array detector (Otsuka Electronics, Japan) attached to the HPLC system. The relative molecular masses of the carotenoids were measured using an FD-MS; M-2500 double-focusing GC-MS equipped with a field-desorption apparatus (Hitachi, Japan). The circular dichroism spectrum was measured using a J-820 spectropolarimeter (JASCO, Japan) in diethyl ether/2-pentane/ethanol (5:5:2, by vol.) at room temperature. The ^{1}H-NMR (500 MHz) spectra of the carotenoid in CD_3OD at room temperature were measured using the UNITY *INOVA*-500 system (Varian, USA).

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Abbreviations: Chl, chlorophyll

RESULTS

Pigments in *Symbiodinium* sp. NBRC 104787

Some pigment peaks were detected by the HPLC for the pigments extracted from *Symbiodinium* sp. NBRC 104787. Two peaks were identified Chl c_1+c_2 and Chl *a* based on their absorption spectra. The non-polar carotenoid was identified as β-carotene based on the compatible absorption spectrum and retention time on HPLC with authentic β-carotene, and its relative molecular mass was 536. Similarly, diatoxanthin, diadinoxanthin, pyrrhoxanthin and peridinin were identified (Fig. 1).

One carotenoid was unusually very polar as eluted very early on C_{18} HPLC and strongly absorbed on silica gel TLC as described above. It had broad absorption spectrum, indicating the presence of a conjugated carbonyl group. After purification, its circular dichroism and ^{1}H-NMR spectra were compatible with those of P457 from *Amphidinium carterae* (Englert, 1995). Its relative molecular mass was 940. From these data, the polar carotenoid was identified as P457, (3*S*,5*R*,6*R*,3'*S*,5'*R*,6'*S*)-13'-*cis*-5,6-epoxy-3',5'-dihydroxy-3-(β-D-galactosyl-(1→4)-β-D-glucosyl)oxy-6',7'-didehydro-5,6,7,8,5',6'-hexahydro-β,β-caroten-20-al (Fig. 1).

The composition of carotenoids was β-carotene (5%, mol% of total), diatoxanthin (1%), diadinoxanthin (32%), P457 (5%), peridinin (54%) and pyrrhoxanthin (3%).

Distribution of P457 and peridinin in dinoflagellstes and related classes

Both P457 and peridinin were found in some dinoflagellates including *Symbiodinium* sp. NBRC 104787 and *Amphidinium carterae* NBRC 102919, and some reef-building corals including *Acropora pulchra* and *Porites cylindrical*. Both carotenoids were not found in several species belonging to Rhodophyta, Cryptophyta, Euglenophyta, Heterokontophyta and Haptophyta (Takaichi, 2011).

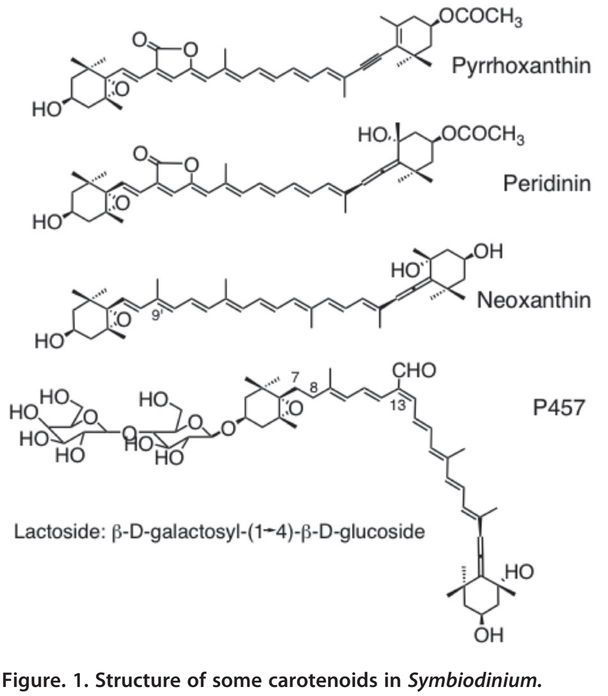

Figure. 1. Structure of some carotenoids in *Symbiodinium*.

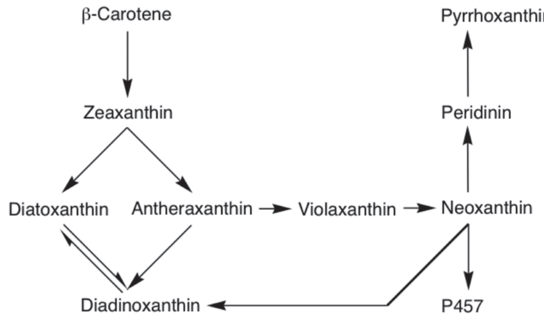

Figure. 2. Putative biosynthetic pathways of carotenoids in *Symbiodinium*.

DISCUSSION

The structure of P457 isolated from *Amphidinium carterae* was identified (Aakermann *et al.*, 1993; Englert *et al.*, 1995). In this paper, we reconfirmed the structure of P457 from *Symbiodinium* sp. NBRC 104787. P457 is the first carotenoid diglycoside found in nature. Carotenoid di-glycosides and monoglycosides are found in bacteria, but P457 is the first carotenoid glycoside found in algae (Britton *et al.*, 2004).

The identification of the carotenoids in this study has facilitated the elucidation of the carotenogenesis pathways, although little is known for the carotenogenesis pathways among algae (Takaichi, 2011). In Fig. 2, we propose biosynthetic pathways of P457 and peridinin in *Symbiodinium*.

Lycopene is cyclized to β-carotene, and then it is hydroxylated to zeaxanthin. Epoxy groups are introduced to form violaxanthin. Its one end group is changed to an allene group of neoxanthin. In the cell-free preparation of *Amphidinium carterae*, ^{14}C-labelled zeaxanthin was incorporated into neoxanthin, and then into diadinoxanthin and peridinin (Fig. 2) (Swift & Milborow, 1981; Swift *et al.*, 1982). In organic chemistry, the C-7,8 double bond of zeaxanthin can be oxidized to acetylenic (triple bond) carotenoid of diatoxanthin (Britton, 1998). The carotenoid moiety of P457 was 7,8-dihydroneoxanthin-20-al, which might be derived from neoxanthin.

Peridinin is the major carotenoid in most dinoflagellates, and always accompanied with P457, as shown in this study and Jeffrey *et al.* (1975). On the aspects of biosynthesis, both carotenoids are different final products of the different pathways (Fig. 2). The most dinoflagellates might be produced by symbiosis of red algae (Palmer, 2003), while red algae have not both carotenoids (Takaichi, 2011). Thus, the occurrence of both carotenoids is interesting phenomena during evolution.

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