Prorocentrum micansにおけるペリジニン-クロロフィルa-たん白質複合体の存在

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Occurrence of Peridinin-Chlorophyll a-Protein Complex in Red tide Dinoflagellate *Prorocentrum micans*

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Peridinin-chlorophyll **a**-protein complex was isolated and partially purified from *P. micans*. The PCP had a molar ratio of peridinin: chlorophyll a=4: 1. MW was 37,000. pI values were 8.3, 7.9, 5.2. λ_{max} values were 440,480,670 nm.

It is well known that peridinin is a characteristic carotenoid of dinoflagellates.[1,2] Recently it was found that peridinin existed in the form of peridinin-chlorophyll **a**-protein complex (PCP) as an effective photosynthetic light harvesting pigment in some marine dinoflagellates.[3-10]

The major carotenoid of red ride dinoflagellate *P. micans* is peridinin.[11,12] However little is known about the occurrence of PCP in *P. micans*. This paper deals with the PCP in *P. micans*.

Experimental

Biological Material

P. micans was collected in the summer 1976 from the Kesennuma Bay, Miyagi and stored at −20°C until use.

Isolation of PCP

P. micans was sonicated with 5 vol (v/w) of 0.1 M Tris-HCl buffer, pH 8.5, and centrifuged at 3,000 g for 20 min. The supernatnat was applied to a Sephadex G-100 column (4.5×70 cm) and eluted with 0.01 M Tris-HCl buffer, pH 8.5, containing 0.2 M NaCl. All procedures were performed at 5°C. The eluted PCP was concentrated using Toyo ultrafilter, UP-20 (Toyoroshi, Co. Ltd., Tokyo).

Isolation and Identification of Chromophore of PCP

The pigments of PCP were extracted with acetone. The extract was transferred to petroleum ether, washed with water to remove acetone, and dried over dry sodium sulfate. The crude pigments were obtained by removing petroleum ether *in vacuo*, and then applied to a silicic acid column (silicic acid, Mallinckrodt AR100: Celite 545= 2: 1) or a cellulose column. Carotenoid was eluted from the silicic acid column with 30% acetone-petroleum ether and further purified by silica gel TLC (30% acetone-petroleum ether). Carotenoid was identified by the following methods; saponification with 1/2 N KOH-EtOH, silica gel (Silica gel 60, Merck), alumina (aluminium oxide 60, F254, Merck) and MgO (MgO: silica gel=1: 1) TLC, UV, acetylation,[13] HCl-MeOH test,[13] silylation,[14] reduction with $NaBH_4$, MS (Hitachi RMU-6M, 20 eV, 160–180°C), IR (film on KBr).

Chlorophyll was eluted from the cellulose column with petroleum ether containing small amounts of isopropanol. The eluted chlorophyll was converted to phaeophytin by oxalic acid in acetone[15] and compared with authentic standard phaeophytin.

Molecular Weight

Molecular weight of PCP was determined by Sephadex G-100 gel filtration (2.5×80 cm) using proteins of known molecular weights.

Electrophoresis

Polyacrylamide disc gel electrophoresis of PCP was performed using 7% polyacrylamide gel, pH 9.5, for 90 min at 3 mA/tube at 5°C.

Isoelectric focusing electrophoresis was performed using 7% polyacrylamide gel containing carrier ampholyte (Ampholine, pH 3.5–10, LKB) for 4 h at 250 V at 4°C. After electrophoresis, the gel was scanned with a dual wave length TLC scanner and sliced in 1 mm at intervals of 10 mm and measured for pH with a micro pH electrode.

Results and Discussion

The naturally grown *P. micans* was chocolate brown. The contents of pigments were chlorophyll 6.9 mg/g dry (chl. **a**: chl. **c**=10: 4) and

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carotenoids 5.1 mg/g dry.

The brick-red extract of *P. micans* was applied to a Sephadex G-100 column and eluted with the buffer. The elution profile showed a single peak of PCP. The PCP was not available in sufficient amounts for purification and further analyses. Therefore, this partially purified PCP was used for following experiments without further purification.

Composition of Chromophore of PCP

The chromophore of PCP was readily extracted with acetone. Two pigments, peridinin and chlorophyll **a**, were isolated by column chromatography.

The purified carotenoid was rapidly decolorized in the presence of alkaline. UV: λ max. nm: 430,457,485 (petroleum ether), 475–481 (EtOH), 485,517 (CS_2). Reduction gave more polar product, UV; λ max. nm: 333,349,370 (MeOH). Acetylation gave two products. Fully acetylated product was silylated. HCl-MeOH test was positive and hypsochromic shift was observed after 20 min. IR (cm^{-1}): 3450 s, 2940 w, 1935 w, 1760–1740 s, 1530 m, 1470 m, 1370 m, 1250 m, 1180 w, 1160 w, 1125 w, 1030 m, 990 w, 950–940 w, 915–890 w. MS (20 eV, m/e): 630 (M^+). From these results, this carotenoid was identified as peridinin.[1,16-19]

Chlorophyll was eluted from the cellulose column before peridinin. The eluted chlorophyll was converted to phaeophytin by oxalic acid in acetone. Chlorophyll had UV: λ max. nm: 410sh, 430, 615, 660 (acetone). Phaeophytin had UV; λ max. mn: 410, 510, 538, 610, 663 (acetone). From these results, this chlorophyll was identified as chlorophyll **a**.

The composition of chromophore was peridinin: chlorophyll **a**=4: 1 in molar ratio. The extinction value used for peridinin was E_{466}=134 *l*/gcm in acetone[6] and chlorophyll a was E_{663}=88.15 *l*/gcm in acetone.[20] The Solet band correction was made by use following equation; peridinin= E_{466}–0.06E_{663}. This ratio is the same as those of *Glenodinium* sp.,[5] *Gonyaulax polyedra*,[3,5] and *Amphydinium rhyncocephaleum*.[21]

The absorption maxima of PCP were 440,480, 670 nm as shown in Fig. 1. The absorption maxima at 440 and 670 nm are attributed to the Soret and α bands of chlorophyll **a**. The broad absorption with a maximum at 480 nm is due to peridinin. The absorption maxima and shape of spectrum are the same as those of previous reports.[3-5]

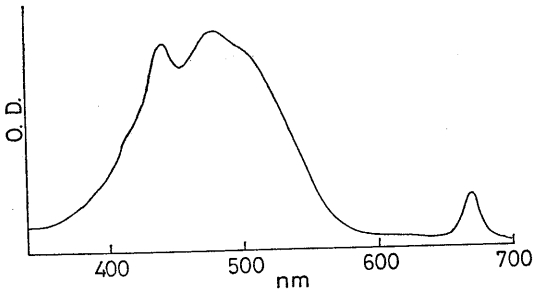

Fig. 1. Absorption spectrum of peridinin-chlorophyll a-protein in 0.01 M Tris-HCl buffer, pH 6.5.

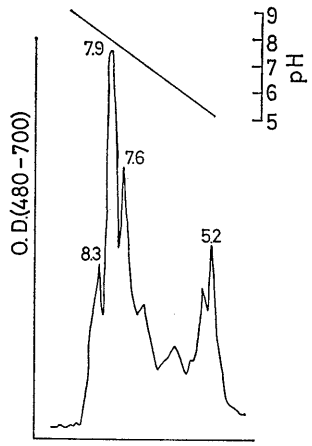

Fig. 2. Isoelectric focusing of peridinin-chlorophyll a-protein.

The molecular weight was estimated as 37,000 by gel filtration. This value is similar to those of known PCPs.[3-5,8]

Polyacrylamide disc gel electrophoresis showed a brick-red broad band, *Rf* 0.25-0.60. Isoelectric focusing showed seven multiple pI form of proteins (Fig. 2). The pIs of principal components were 8.3, 7.9, 7.6, 5.2. The multiple mobility of PCP on electrophoresis is due to the multiple pI forms of protein.

Recently the steric arrangement of peridinin and chlorophyll **a** on the apoprotein was proposed.[12,22] Peridinin forms two dimeric pairs and locates symmetrically to chlorophyll a molecule. However the mechanism of solar energy transfer to photosynthetic apparatus and the comformation of apoprotein are still unknown.[9,10]

Pyrrohoxanthin, peridinin like carotenoid, ex-

ists in free form in *P. micans*.* Occurrence of pyrrohoxanthin suggests that the binding of peridinin to apoprotein is very specific.

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