Peridinin-Chlorophyll a Proteins of Dinoflagellate AlgaeHarold W. Siegelman^{*}, J. Helen Kycia^{*}, and Francis T. Haxo[†]

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INTRODUCTION

The pigments which function as the energy collection systems for photosynthesis are either tetrapyrroles or carotenoids. In the oceans, which cover over 70% of the surface of the earth, carotenoids may be more important than chlorophylls for photosynthetic energy capture by phytoplankton. A characteristic feature of the principal marine phytoplankton is the possession of a high carotenoid content. In general each major group of phytoplankton has a specific carotenoid which accounts for a major portion of the total carotenoid content. The accessory pigment function of fucoxanthin and peridinin have long been known⁶, and the same function was recently demonstrated for 19'-hexanoyloxyfucoxanthin with Coccolithus huxleyi clone BT-6 (F. T. Haxo, unpublished). The distribution of these photosynthetically-active carotenoids are as follows:

<u>Algal class</u>	<u>Predominant carotenoid</u>
Dinophyceae (dinoflagellates)	Peridinin ¹⁵
Bacillariophyceae (diatoms)	Fucoxanthin ⁴
Coccolithophoraceae (coccolithophorids)	
Coccolithus huxleyi (clone BT-6)	19'-Hexanoyloxyfucoxanthin ¹

The occurrence of 19'-hexanoyloxyfucoxanthin in other coccolithophorids has not yet been established, and fucoxanthin is generally considered the dominant carotenoid of the coccolithophorids. In consequence of their carotenoid pigment content, the marine phytoplankton are yellow-brown to yellow-green in color.

These organisms all contain chlorophylls a and c.

The spectral composition of the light which penetrates the water column changes with increasing depth. The red portion of the spectrum is attenuated by the red absorbance bands of water. The blue portion of the visible spectrum is diminished by Rayleigh scattering and absorption by yellow pigments. The blue-green to green portion of the visible spectrum (480-570 nm) has maximal transmission in the water column, and is the region of maximal absorbance of many carotenoids.

Schutt¹² first isolated peridinin and provided substantial evidence for the presence of soluble carotenochlorophyll proteins in dinoflagellate algae in 1890. Bode² and Bode and Hastings³ found that aqueous extracts of Gonyaulax polyedra were red and that the color was due to peridinin bound to protein. Haidak et al.⁵ reported that the red protein from G. polyedra has a molecular weight of about 38,000 and contained both peridinin and chlorophyll.

ALGAL CULTURES

Detailed studies on the peridinin-chlorophyll a-proteins were initiated at Brookhaven National Laboratory with Amphidinium carterae (Plymouth 450). Dr. Luigi Provasoli suggested the use of A. carterae for these studies and generously provided the culture and advice on growing the alga. Our initial efforts were concerned with the development of large-scale culture techniques to provide a continuous and dependable supply of algae⁷. Culture methods previously used for large scale culture of other microalgae proves satisfactory¹³. The alga was readily cultivated on a large scale, 160 liters, in the ASP₇ artificial marine media of Provasoli¹¹ supplemented with a soil extract. A pure culture of the alga was obtained by the use of agar plating and antibiotics.

Large scale axenic cultures were maintained by using autoclaved or pasteurized media and antibiotics. The cultures were stirred and aerated with sterile air. Recently, we found that aeration with 0.1% CO₂ improved the growth of A. carterae and several other dinoflagellate algae. The algal cells were harvested during late log phase at cell densities of about 2.7×10^5 cells ml⁻¹. Harvesting large volumes of cells was simplified by making the culture media to 1 mM KAl(SO₄)₂ which caused the algae to aggregate and settle out to the bottom of the culture vessel. The bulk of the culture media was removed by pumping, and the thick slurry of cells remaining were collected by centrifugation. The KAl(SO₄)₂ treatment has no effect on the extraction or properties of the peridinin-chlorophyll a proteins.

PURIFICATION

The extraction of the peridinin-chlorophyll a proteins from A. carterae and other dinoflagellate algae was most conveniently achieved by repeated freeze-thawing and grinding. In a typical extraction, the KAl(SO₄)₂-harvested cells from 300 liters of culture were ground with 0.1 M Tris-HCl buffer, pH 8.4 at liquid nitrogen temperature in a large mortar. After thawing, the cells were removed by centrifugation and reextracted until the extracts were colorless. The combined extracts were concentrated by ultrafiltration and then applied to a large Sephadex G-100 column (9 x 90 cm). The principal orange component which eluted from the column was then preparatively separated into several homogenous components by repeated DEAE-cellulose chromatography.

PROPERTIES OF THE PERIDININ-CHLOROPHYLL A PROTEINS

The peridinin-chlorophyll a proteins eluted from a Sephadex G-100 column as a single symmetrical component with a molecular weight of about 39,000 (Fig. 1). When the Sephadex G-100 eluate was subjected to isoelectric focusing on polyacrylamide gel, it separated into six distinct components (Fig. 2). The gel was scanned at 476 and 669 nm and each separated component had absorbance which can be attributed to peridinin and chlorophyll a. The principal component has a pI of 7.5 and accounted for about 90% of the total chromoprotein. Preparative separation of three peridinin-chlorophyll a proteins (pI 7.6, 7.5, and 6.4) was achieved with DEAE-cellulose chromatography, and they were obtained in homogeneous form. Examination of the Sephadex G-100 eluate of the peridinin-chlorophyll a proteins or the three purified components by SDS-polyacrylamide gel electrophoresis revealed only a single polypeptide with a molecular weight of about 32,000. Treatment of the peridinin-chlorophyll a proteins with SDS released both chromophores from the proteins.

The absorption spectrum of the peridinin-chlorophyll a protein, pI 7.5 is shown in Fig. 3. The absorption maxima at 476 and at 435 and 669 nm are due to peridinin and chlorophyll a, respectively. The absorbance spectra of the Sephadex G-100 eluate and the three purified proteins were all identical. Spectral and chromatographic analyses of pigments of the peridinin-chlorophyll a proteins revealed only the two pigments. Fluorescence excitation and emission spectra of the peridinin-chlorophyll a proteins pI 7.5 are shown in Fig. 4. Recent studies indicated that energy transfer from peridinin to chlorophyll a in the peridinin-chlorophyll a protein from Glenodinium sp. was with 100% efficiency¹⁴.

Amino acid analysis of the A. carterae peridinin-chlorophyll a protein pI 7.5 are shown in Table I. The amino acid composition of the pI 7.6 and 6.4 proteins and the Sephadex G-100 eluate are essentially identical. Spectrophotometric analyses of the chromophore content of the pI 7.5 protein gave integral ratios of peridinin, chlorophyll a, and protein of 9:2:1.

The multiplicity of the peridinin-chlorophyll a proteins of A. carterae was anomalous. The increased sensitivity of newer protein separation methods, such as isoelectric focusing, has revealed several examples of multiple forms of proteins. Monomeric protein multiplicity is usually associated with progressive deamidation resulting in more acidic forms of the protein. Treatment of the peridinin-chlorophyll a protein, pI 7.5 under alkaline conditions (Fig. 5) converted the protein into more acidic forms implying that the multiplicity observed is due to partial deamidation of the glutamine and asparagine residues. The constancy of the isoelectric focusing behavior of the peridinin-chlorophyll a proteins on repeated isolation from fresh material suggests that the observed multiplicity is probably associated with enzymic deamidation. If cells of A. carterae are stored at -15° C for 7 days after harvest, on extraction, the peridinin-chlorophyll a proteins had more acidic pI values following isoelectric focusing. Extraction of the alga in the presence of several proteolytic enzyme inhibitors did not alter the isoelectric focusing patterns. We believe that the intrinsic multiplicity of the peridinin-chlorophyll a proteins represents the native state of these proteins in the cell.

The exact location of the peridinin-chlorophyll a proteins is not yet established. Localization in the chloroplast lamellae can be inferred from the oxygen photosynthetic action spectra of dinoflagellates known to contain appreciable amounts of peridinin-chlorophyll a protein such as Gonyaulax polyedra^{6,10}

and Glenodinium sp.¹⁰. The amount of peridinin-chlorophyll a protein increased markedly under low light growth conditions^{8,10}.

Peridinin-chlorophyll a proteins have been obtained from several species of dinoflagellates. At LaJolla they were isolated and purified by Prezelin and Haxo⁸ from Gonyaulax polyedra and Glenodinium sp. At Brookhaven, the peridinin-chlorophyll a proteins have also been purified from Cachonina niei, Gonyaulax tamarensis, and Gonyaulax polyedra. A summary of the properties of these proteins is given in Table II. Three species of dinoflagellates examined contained peridinin but did not yield peridinin-chlorophyll a proteins on extraction.

All peridinin-chlorophyll proteins isolated to date have similar molecular weights ranging from 34,500 to 39,000 (Tables I and II). The molecular weights of the polypeptide portion of the proteins, from amino acid analyses and SDS-polyacrylamide gel electrophoresis, have a smaller variability and a range of 30,000 to 32,000. All proteins exist in at least six pI forms and those of Cachonina niei were found to have twelve. The proteins from a particular species all have the same subunit number, and both monomeric and dimeric proteins were isolated. Comparison of the amino acid composition of the monomeric and dimeric peridinin-chlorophyll proteins in Table I reveal close similarities in content of several amino acids but significant differences also exist. Insufficient data on chromophore-protein ratios precludes detailed comparison of chromophore content. The proteins from A. carterae have a peridinin-chlorophyll a-protein ratio of 9:2:1⁷ while those of Glenodinium sp. have a ratio of 4:1:1⁹. In the latter case, a molecular arrangement of chlorophyll a and peridinin in the chromoprotein has been proposed¹⁴.

The peridinin-chlorophyll a proteins from each dinoflagellate examined exist in a unique and distinctive pattern as shown by isoelectric focusing on polyacrylamide gel. These patterns may be of value for species identification and provide a biochemical marker for a particular species. To date, no similar proteins have been isolated from other phytoplankton groups.

The peridinin-chlorophyll a proteins represent an important class of light-harvesting pigments for photosynthesis in the oceans and in some lakes. They contribute significantly to cellular absorption and may account for the order of one half of the total peridinin content. Their absorption coincides with the portion of the visible spectrum which has maximum penetration in the water column.

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Table I

Amino Acid Composition of Purified Peridinin-chlorophyll a Proteins from
Two Dinoflagellate Algae. Average Integral Ratios mol⁻¹

	<u>A. carterae</u> ⁽⁷⁾ pI 7.5	<u>Glenodinium sp.</u> ⁽⁹⁾ pI 7.3
Trp	2	2
Lys	27	30
His	4	6
Arg	3	0
Cys	0	0
Asp	35	34
Thr	10	9
Ser	22	25
Glu	21	17
Pro	15	7
Gly	22	24
Ala	55	54
Val	30	19
Met	10	13
Ile	14	13
Leu	19	16
Tyr	9	11
Phe	8	11
Total Residues per mole	306	291
Molecular Weight without chromophores	31,800	30,600

Table II
Peridinin-chlorophyll a Proteins of Several Dinoflagellates

Specie	Molecular weight including chromophores	pI forms	Subunits mol ⁻¹
<u>Amphidinium carterae</u> (Plymouth 450)	39,000	6	1
<u>Gonyaulux polyedra</u> ⁹	34,500	6	1
<u>Gonyaulux tamarensis</u>	37,000	6	1
<u>Cachonina niei</u>	36,000	12	2
<u>Glenodinium sp.</u> ⁹	35,500	6	2
<u>Peridinium cinctum</u>	-	-	-
<u>Amphidinium carterae</u> ⁹	-	-	-
<u>Dissodinium lunula</u>	-	-	-

FIGURE LEGENDS

FIGURE 1. Gel filtration of an extract of Amphidinium carterae on a Sephadex G-100 column (9 x 90 cm). Absorbance at 476 nm (●), 280 nm (○), and 415 nm (Δ).

FIGURE 2. Isoelectric focusing of the peridinin-chlorophyll a proteins on polyacrylamide gel. The pI values for each peak are indicated. Absorbance at 476 nm (—), 669 nm (-----) and pH (● — ●).

FIGURE 3. Absorption spectrum of the peridinin-chlorophyll a protein pI 7.5 at 20°C in 1 mM Tris-HCl buffer, pH 8.4.

FIGURE 4. Fluorescence excitation and emission spectra of peridinin-chlorophyll a protein pI 7.5. Quantum corrected excitation spectrum is shown from 260 to 600 nm. Emission spectra are uncorrected and are shown for 479 nm excitation (upper) and 330 nm excitation (lower).

FIGURE 5. Isoelectric focusing on polyacrylamide gel of peridinin-chlorophyll a protein pI 7.5 held for 8 days in universal buffer, pH 11.5 at 4°C. Absorbance at 476 nm (—) and pH (● — ●).

FIGURE 1

FIGURE 2

FIGURE 3

FIGURE 4

FIGURE 5