Chlorophyll-Protein Complexes from the Red-Tide Dinoflagellate, *Gonyaulax polyedra* Stein1

**ISOLATION, CHARACTERIZATION, AND THE EFFECT OF GROWTH IRRADIANCE ON CHLOROPHYLL DISTRIBUTION**

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**ABSTRACT**

A comparison of high (330 microeinstans per meter squared per second) and low (80 microeinstans per meter squared per second) light grown *Gonyaulax polyedra* indicated a change in the distribution of chlorophyll a, chlorophyll c2, and peridinin among detergent-soluble chlorophyll-protein complexes. Thylakoid fractions were prepared by sonication and centrifugation. Chlorophyll-protein complexes were solubilized from the membranes with sodium dodecyl sulfate and resolved by Deriphat electrophoresis. Low light cells yielded five distinct chlorophyll-protein complexes (I to V), while only four (I′ to IV′) were evident in preparations of high light cells. Both high molecular weight complexes I and I′ were dominated by chlorophyll a absorption and associated with minor amounts of chlorophyll c. Both complexes II and II′ were chlorophyll a-chlorophyll c2-protein complexes devoid of peridinin and unique to dinoflagellates. The chlorophyll ac2 c2 molar ratio of both complexes was 1:3, indicating significant chlorophyll c enrichment over thylakoid membrane chlorophyll ac2 c2 ratios of 1.8 to 2.1. Low light complex III′ differed from all other high or low light complexes in that it possessed peridinin and had a chlorophyll ac2 c2 ratio of 1:1. Low light complexes IV and V and high light complexes III′ and IV′ were spectrally similar, had high chlorophyll ac2 c2 ratios (4:1), and were associated with peridinin. The effects of growth irradiance on the composition of chlorophyll-protein complexes in *Gonyaulax polyedra* differed from those described for other chlorophyll c-containing plant species.

An increasing number of investigators have become interested in the isolation of pigment protein complexes from the thylakoid membranes of the chromophytic algae. Chromophytic algae typically contain Chl c as their accessory Chl and may have accessory carotenoids, such as peridinin or fucoxanthin, that efficiently transfer absorbed light energy to photosynthetic reaction centers (10, 27). Particular attention has been given to Chl a-Chl c-Chl c2-fucoxanthin containing diatoms and brown macroalgae, where the P700-Chl a-protein complex of PSI reaction centers has been isolated (1, 18). Barrett and Anderson (3, 4) have also characterized two light-harvesting pigment-protein complexes (LHCc) from brown macroalgae, including a Chl a-Chl c-fucoxanthin complex and a Chl a-Chl c complex. Recently, Friedman and Alberte (8) and Owens and Wold (18) have isolated a LHC from the diatom, *Phaeodactylum tricornutum* which contained Chl a, Chl c, and fucoxanthin. Owens and Wold (18) also demonstrated the presence of a distinct Chl a-Chl c LHC in *P. tricornutum* which was devoid of fucoxanthin.

Photosynthetic dinoflagellates, a major group of primary producers in the marine environment, also possess the Chl a-Chl c-carotenoid pigment system characteristic of many groups of aquatic primary producers. Dinoflagellates, however, possess the carotenoid peridinin instead of fucoxanthin and have only one type of Chl c, i.e. Chl c2 (12). These light-harvesting pigments are associated with various Chl a-protein complexes within the thylakoid membranes. Complexes containing the reaction center of PSI have been isolated from two species of dinoflagellates (22). These P700-Chl a-protein complexes have spectral and kinetic properties similar to PSI complexes isolated from other plant species. In addition, a major light-harvesting complex of dinoflagellates has been characterized as a water-soluble peridinin-Chl a-protein complex (PCP). The PCP complex contains the majority of cellular peridinin, up to 20% of the cellular Chl a and is devoid of all other photosynthetic pigments found in the chloroplast (cf. 21). The species distribution, photophysiology, and molecular topology of PCP has been extensively characterized (19–26). Recently, a second major light-harvesting complex was isolated from three dinoflagellate species and characterized as a Chl a-Chl c2-protein complex devoid of peridinin but which transferred absorbed light energy from Chl c2 to Chl a (5–7). The organization of Chl-protein complexes within the thylakoid membranes of dinoflagellates has been modeled after that described for vascular plants (22, 28, 29).

The photosynthetic adaptation of unicellular algae to various light intensities has been documented in a wide variety of algal groups (cf. 23). Cellular responses to a change in light intensity can include changes in pigment concentration and/or composition, photosynthetic unit density and optical cross-section, and photosynthetic carbon fixation parameters (cf. 20). Recent studies of the diatom, *P. tricornutum* documented changes in the major detergent-soluble light harvesting complex (18) and its apoprotein (9) in response to changes in light intensity. The present study characterizes the biochemical features of Chl a-protein complexes isolated from populations of the red-tide dinoflagellate, *Gonyaulax polyedra* grown under high and low light conditions.

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1 Abbreviations: LHC, light-harvesting complex; LL, low light; HL, high light; PCP, peridinin-chlorophyll a-protein; PMSF, phenylmethylsulfonylfluoride.
irradiances. Results indicate that growth irradiance does influence the distribution of pigments in the Chl α-protein complexes of G. polyedra.

MATERIALS AND METHODS

Growth Conditions. Low light (LL, 80 μE m⁻² s⁻¹) and high light (HL, 330 μE m⁻² s⁻¹) cultures of Gonioaulax polyedra Stein, clone 70A (University of California, Santa Barbara, UCSC Code No. 5M20) were grown in unialgal batch cultures in 2.8 L Fernbach flasks containing 1.5 L of I/2 medium (15). Cells were cultured in an alternating 12 h light, 12 h dark schedule at 22°C ± 1°C. Illumination was provided by General Electric cool-white fluorescence lamps. Doubling times were 4 to 5 d for both populations, which were harvested in mid-log phase (4–5 x 10³ cells/ml) by centrifugation (15°C, 20 min, 1000g). Pelleted cells were resuspended in 50 mM Tris/HisCl-1 mM EDTA-1 mM PMSF (pH 8.0).

Preparation and Solubilization of Thylakoid Membranes. Cell suspensions were sonicated at 0°C (Sonifier model W185) until 80 to 90% cell breakage was determined microscopically. Whole cells and large cellular debris were sedimented by centrifugation at 500g, 10 min, 4°C. Thylakoid membranes were pelleted by centrifugation (30,000g, 70 min, 4°C) and the pellet washed by recentrifugation following resuspension in 50 mM Tris/HisCl-1 mM EDTA-1 mM PMSF (pH 8.0).

Membranes were solubilized in 1.5% SDS at an SDS:Chl a ratio of 22:1 and centrifuged at 38,000g for 15 min at 4°C.

Table I. Electrophoretic and Pigmentation Properties of Chl α-Protein Complexes Isolated from LL (80 μE m⁻² s⁻¹) Grown G. polyedra. Cellular Chl αc2 was 2:1

<table>
<thead>
<tr>
<th>Band Position</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV &amp; V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mobility</td>
<td>0.04</td>
<td>0.32</td>
<td>0.51</td>
<td>0.76</td>
</tr>
<tr>
<td>Approximate mol wt (kD)</td>
<td>133</td>
<td>92</td>
<td>71</td>
<td>51</td>
</tr>
<tr>
<td>% Chl a ^a</td>
<td>48 (3.0)</td>
<td>7 (0.4)</td>
<td>12 (4)</td>
<td>34 (4.7)</td>
</tr>
<tr>
<td>% Chl c ^a</td>
<td>17</td>
<td>44</td>
<td>22</td>
<td>17</td>
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<tr>
<td>Chl a:c (molar ratio)</td>
<td>5.0</td>
<td>0.32 (0.1)</td>
<td>1.01 (0.5)</td>
<td>4.0 (0.5)</td>
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</table>

^a Percentage of total Chl a on the Deriphat gel. ^b Numbers in parentheses indicate standard deviations from the mean; n = 5 in all cases.

Table II. Electrophoretic and Pigmentation Properties of Chl α-Protein Complexes Isolated from HL (330 μE m⁻² s⁻¹) Grown G. polyedra. Cellular Chl αc2 Was 1:8:1

<table>
<thead>
<tr>
<th>Band Position</th>
<th>I'</th>
<th>II'</th>
<th>III'</th>
<th>IV'</th>
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<tr>
<td>Mobility</td>
<td>0.09</td>
<td>0.30</td>
<td>0.45</td>
<td>0.76</td>
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<tr>
<td>Approximate mol wt (kD)</td>
<td>125</td>
<td>95</td>
<td>78</td>
<td>51</td>
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<tr>
<td>% Chl a ^a</td>
<td>37 (1.5)</td>
<td>14 (0.8)</td>
<td>17 (1.1)</td>
<td>32 (0.5)</td>
</tr>
<tr>
<td>% Chl c ^a</td>
<td>12</td>
<td>66</td>
<td>10</td>
<td>12</td>
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<tr>
<td>Chl a:c (molar ratio)</td>
<td>4.5 (1.5)</td>
<td>0.33 (1.1)</td>
<td>2.5 (1.5)</td>
<td>4.0 (1.5)</td>
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^a Percentage of total Chl a on the Deriphat gel. ^b Numbers in parentheses indicate standard deviations from the mean; n = 5 in all cases.

solubilized membrane extracts were recovered from the supernatant and loaded onto either polyacrylamide tube gels (0.5 x 8.0 cm) or a slab gel (2.5 x 115 mm). Both electrophoretic gel systems contained 5% acrylamide (Bio-Rad), 0.25% N,N-methylen bis acrylamide (Bio-Rad), 0.125% ammonium persulfate (Sigma), 0.1% (v/v) N,N',N'-tetra methyl ethylene diamine (Eastman Kodak), 0.2 mM Tris, 48 mM glycine, and 0.1% Deriphat 160C (partial sodium salt of N-lauryl B-iminodipropionic acid, Gift from the Henkel Corporation). The quality of Deriphat 160C varied from lot to lot, with two of five lots tested found to be unsuitable for experimental work. Electrophoresis was carried out at 75 V for 35 to 45 min at room temperature, using an electrode buffer containing 12.4 mM Tris, 96 mM glycine, and 0.2% Deriphat 160C.

Following electrophoresis, protein bands were detected by fixing Deriphat tube gels overnight in 10% acetic acid, protein staining with 0.2% Coomassie blue R in methanol:H₂O:acetic acid (5:5:1 v/v/v) and destaining the gel background with methanol:H₂O:acetic acid (5:5:1 v/v/v). Relative Chl a distribution in the Deriphat gel was determined from gel scans measured at 670 nm with a Beckman Acta III spectrophotometer. Gel scan patterns were photographed and individual peaks were cut and weighed. Chl α-containing bands were then excised from tube gels and either used immediately, stored at 77°C for future protein analyses, or the Chl α-containing components were eluted from the gel with 0.3% SDS in 50 mM Tris (pH 8.0) by methods previously described (6). Deriphat-containing slab gels were used to determine the relative mobility of solubilized Chl α-containing components as compared to standard proteins. Marker proteins (Bio-Rad) included: myosin (200 kD); β-galactosidase (130 kD); phosphorylase b (92 kD); BSA (68 kD); ovalbumin (43 kD); carbonic anhydrase (31 kD); trypsin inhibitor (21 kD); and lysozyme (14 kD). All protein markers were denatured prior to electrophoresis by boiling solutions for 3 min in 6.2 mM Tris, 48 mM glycine, 1% SDS, 1% 2-mercaptoethanol, 10% (v/v) glycerol. Protein-stained slab gels, prepared in an identical manner as tube gels, were used to estimate mol wt of the native complexes.

Pigmentation. Chl αc2 molar ratios were determined using the acetone extraction procedures detailed in Prezelin and Haxo (24). In addition, all resolvable complexes were eluted from the
Chlorophyll-Protein Complexes from *Gonyaulax Polyedra* Stein

**Wavelength (nm)**

<table>
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<td>400</td>
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<td>600</td>
<td>700</td>
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Fig. 2. A comparison of room temperature absorption spectra for (a) LL (80 μE m⁻² s⁻¹) complex I, (b) HL (330 μE m⁻² s⁻¹) complex I', (c) LL complex II, and (d) HL complex II' isolated from LL and HL grown populations of *G. polyedra*.

Absorption and Fluorescence (Emission and Excitation) Spectra. Absorption spectra were recorded using an Aminco DW2 spectrophotometer. Room temperature fluorescence emission and excitation spectra of gel slices suspended in 50 mM Tris (pH 8), or of solutions of eluted Chl α-protein complexes, were recorded on a Perkin-Elmer MPF-2A spectrofluorimeter. Fluorescence data were relayed to a Bascom-Turner microprocessor at 2 nm intervals and subsequently displayed on the X-Y plotter of an Aminco DW2 spectrophotometer. Excitation spectra were quanta corrected for wavelength variation in xenon lamp energy output. Emission spectra were corrected for instrument sensitiv-

Gel and pigments extracted with 90% (v/v) acetone. The clarified supernatant was transferred to diethyl ether, and concentrated under N₂ for further chromatographic analyses of carotenoid and Chl components. One-dimensional TLC was carried out on cellulose plates according to the methods of Jeffrey (13).

**Protein Analysis.** Gel slices of each Chl α-containing band were either used immediately for protein analyses or frozen for subsequent use. In either case, gel slices were equilibrated for 15 to 20 min in a solution of 50 mM Tris, 3% SDS, and 1% 2-mercaptoethanol. The gel slices were then heated for 2 min at 70°C and reelectrophoresed, using a Laemmli buffer system with a 12% resolving gel and a 3% stacking gel (14). Protein bands were resolved using the silver-staining technique described by Morrissey (17) and gels were scanned with an ISCO model 1310 gel scanner with an ISCO UA-5 absorbance/fluorescence monitor. Mol wt protein standards were the same as those used in the slab gel system.
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FIG. 3. A comparison of room temperature fluorescence emission spectra of LL (80 µE m⁻² s⁻¹, ---) complex I gel slices and HL (330 µE m⁻² s⁻¹, ---) complex I' gel slices isolated from SDS/Deriphat preparations of LL and HL grown populations of G. polyedra. Excitation wavelength was 435 nm.

RESULTS

Low and high light grown Gonyaulax polyedra populations had similar whole cell Chl a:c molar ratios, i.e. 2:1 and 1.8:1, respectively (Tables I and II). In LL cultures, the Chl content of log phase cells was about twice as concentrated as those measured for cells growing under HL intensities. The average Chl a and c content of LL cells was about 40 ± 4 and 20 ± 2 µM cell⁻¹ (10⁶) (n=3), respectively. Previous studies (26) also indicated that the peridinin content of LL G. polyedra is up to 2-fold greater than that of HL cells. When thylakoid membranes isolated from either LL and HL cultures were solubilized with 1.5% SDS, more than 85% of the total Chl a in the detergent homogenate was recovered in the SDS supernatant. Analyzed just prior to Deriphat electrophoresis, Chl a:c molar ratios of solubilized thylakoid membrane fractions were 1.9:1 and 1.6:1, respectively, for LL and HL preparations.

Five Chl a-protein complexes were resolved from LL G. polyedra preparations (Fig. 1a), although the resolution of complex V varied. Approximately half of the gel Chl a was associated with complex I (48%) and an additional third (34%) with complexes IV and V (Table I). The remainder of the Chl a was divided between complexes II (7%) and III (12%). Chl c was associated with all complexes, but was concentrated in complex II (44%), while peridinin was associated with complexes III, IV, and V. By comparison, HL grown populations of G. polyedra had four resolvable Chl a-protein complexes (Fig. 1b). Like LL cells, the majority of total Chl a in HL cells was associated with complexes I' and I' (Table II). However, there was a significantly greater gel Chl a (14%) and Chl c (66%) associated with complex II' in HL cells than observed in the comparable complex II of LL cells. By comparison, there was no detectable change in the percent gel Chl a associated with HL complex III' over that seen in LL complex III, but the percent gel Chl c' in complex III' was only half that of complex III. All pigmented bands on the Deriphat gels contained protein components which stained with Coomassie brilliant blue R.

FIG. 4. Laemmli gel silver stain patterns (scanned at 546 nm) of polypeptides isolated from G. polyedra (a) LL (80 µE m⁻² s⁻¹) complexes I and II, and (b) HL (330 µE m⁻² s⁻¹) complexes I' and II'. Determined mol wt shown for each polypeptide band is expressed as kD.
a major high mol wt polypeptide component (106 kDa) was resolved which could not be further dissociated by additional heating or by increasing SDS or 2-mercaptoethanol concentrations (Fig. 4a). Such a high mol wt polypeptide was not evident in similar preparations of complex I' (Fig. 4b). Two major polypeptides of 61 and 55 kDa were also associated with complex I while the major polypeptide associated with high light complex I' appeared to be 70 kDa. Four additional complex I' polypeptides were resolved at 74, 59, 23, and 16 kDa, but not evident in similar preparations of complex I.

**Complexes II and II'**. While Chl c appeared to be present in all low light pigment-protein complexes isolated by SDS solubilization and Deriphat electrophoresis, the largest percentage (44%) of Chl c was concentrated in the apple-green colored complex II (Table I). The absorption spectrum of complex II was dominated by a blue peak at 452 nm and absorption peaks at 672 and 635 nm (Fig. 2c). The Chl a:c molar ratio was 0.32:1. TLC analyses verified the absence of peridinin and the presence of yellow xanthophylls. Major polypeptide components occurred at 49 and 38 kDa on Laemmli gels, with others detected at 32, 21, and 16 kDa (Fig. 4a). The excitation spectrum of LL complex II indicated some coupling of light absorbed by Chl c (440-460) to Chl a fluorescence emission at 675 nm (Fig. 5). However, the fluorescence emission spectrum indicated that some fraction of Chl c was associated with complex II was energetically uncoupled from Chl a, as evidenced by the Chl c emission peak at 640 nm.

The electrophoretic mobility, chromophore ratio, approximate mol wt, and spectral characteristics of complex II were identical in HL cells. However, the percentage of total gel Chl a and Chl c associated with HL complex II' was 100 and 50%, respectively, greater than that of complex II. There were also major differences in polypeptide band patterns of complexes II and II' on Laemmli gels. Five polypeptides with mol wt less than 50 kDa were resolved in preparations of complex II (Fig. 4a). By comparison, three major HL complex II' polypeptides were resolved at 72, 60, and 51 kDa, with two additional minor polypeptides were detectable at 41 and 38 kDa (Fig. 4b).

**Complexes III and III'**. LL complex III was spectrally distinct from any detergent-solubilized complex previously isolated from a dinoflagellate (5). It had an equal proportion of both Chls, giving complex III a Chl a:c molar ratio of 1:1, and accounted for 22% of the total gel Chl c found on a typical Deriphat gel (Table I). The absorption spectrum was dominated by a major peak associated with Chl c, as well as a Chl a red peak at 672 nm (Fig. 6a). In contrast to the higher mol wt complexes I and II described above, TLC analyses indicated the presence of peridinin in complex III. Peridinin presumably accounted for the orange color of the complex, and the 485 nm shoulder in the absorption spectrum. While HL complex III' had a mobility and a mol wt approximating that of LL complex III, it had distinctly different spectral characteristics. The Chl a red peak remained at 672 nm but there were two peaks at 432 and 450 nm which dominated the blue region of the absorption spectrum (Fig. 6b). TLC analyses confirmed the presence of Chl c in complex III', which had a high a:c molar ratio of 2.5:1. Peridinin was also present, contributing to the orange color of the complex and the absorption shoulder centered around 485 nm.

The fluorescence excitation spectrum (Fig. 7a) for the Chl a emission peak of complex III (Fig. 7b) indicated that both Chl c (440-460) and peridinin (460-520) transferred absorbed light energy to Chl a. The secondary 640 nm peak in the fluorescence emission spectrum (Fig. 7b) indicated that a fraction of the total Chl c present was energetically uncoupled. On Laemmli gels, a 45 kDa polypeptide appeared to be a major component in the complex III, with associated polypeptides at 33 and 24 kDa (Fig. 8a). Complex III' polypeptides also included 45 kDa and 32 to 35 kDa bands, as well as a distinct 57 kDa polypetide not seen in LL complex III (Fig. 8b).

**Complexes IV, V, and IV'**. Taken together, the brick-red LL complexes IV and V contained about 17% of the gel Chl c and their combined Chl a:c molar ratio approached 4:1 (Table I). These spectrally similar complexes (Fig. 6, c and d) contained peridinin, accounting for their color and spectral absorption between 480 and 520 nm. HL complex IV' had a mobility, an approximante mol wt, and a Chl a:c molar ratio similar to that of LL complex IV + V. While TLC analyses indicated that peridinin was present in complex IV', the markedly reduced spectral absorption in the carotenoid region between 480 to 520 nm suggested the relative amounts of peridinin were reduced in HL complex IV' over analogous LL complexes. LL complexes IV and V both contained polypeptide components in the 24 to 37 kDa range, as well as two lower mol wt polypeptides at 17 and 19 kDa (Fig. 8a). HL complex IV' had a polypeptide band pattern similar to LL complex V, with four polypeptide bands being resolved at 33, 23, 18, and 16 kDa (Fig. 8b).

**DISCUSSION**

Few investigators have attempted to isolate and characterize the membrane-associated Chl-protein complexes of dinoflagellates. Using the detergent Triton X-100 and column chromatography, Prezlin and Alberte (22) were able to isolate P700-Chl a-protein complexes from two marine dinoflagellates, *Glenodinium* sp. and *Gonyaulax polyedra*. However, they were unable to characterize any membrane-associated light-harvesting components by this method or by SDS electrophoresis. By using the SDS-solubilization/Deriphat electrophoresis method of Markwell et al. (16), Boczar and coworkers (6, 7) were able to separate the majority of *Glenodinium* sp. thylakoid Chl a into four distinct Chl a-protein complexes. Identical methods were used in the present study to separate Chl a-protein complexes from the red tide dinoflagellate, *Gonyaulax polyedra*. Results indicated that both the number and composition of Chl a-protein complexes in *G. polyedra* were influenced by growth irradiance, while the biochemical and spectral characters of these Chl a-protein complexes differ in detail from those described for cells of *Glenodinium* sp. cultured under similar light intensities (6).

Low light grown *G. polyedra* cells contained five SDS/Deriphat Chl a-protein complexes (I–V), while only four (I'–IV') were evident in identical preparations of high light cells. Both high mol wt Deriphat complexes I and I' were spectrally similar, but not identical, to the P700-Chl a complex routinely isolated from the PSI reaction center of many plant groups by SDS electrophoresis (28–30). But unlike the P700-Chl a-protein complex previ-
FIG. 6. A comparison of room temperature absorption spectra for (a) LL (80 μE m⁻² s⁻¹) complex III, (b) HL (330 μE m⁻² s⁻¹) complex III', (c) LL complex IV, (d) LL complex V, and (e) HL complex IV' isolated from LL and HL grown populations of G. polyedra.
of cellular Chl c (40–80%) and had consistently low Chl a:c molar ratios (0.26–0.33). Devoid of peridinin, but containing some yellow xanthophylls, it is clear these Chl a-Chl c-protein complexes contribute greatly to the blue light-absorbing capabilities of dinoflagellates. By comparison, Chl c-Chl c-protein complexes from the other algae had higher Chl a:c molar ratios, ranging from 0.5 to 4.0 (1, 3, 11, 18). The dinoflagellate Chl a-Chl c complexes were first postulated by Prezelin and Alberte to be LHC situated presumably between more peripheral peridinin-Chl a-protein complexes and photosystem II imbedded within the thylakoid membranes (22). While fluorescence excitation data presented here supports the functional role of complex II as an important light-harvesting Chl a-Chl c-protein complex, its placement within the photosynthetic apparatus of dinoflagellates remains to be defined.

_Gonyaulax polyedra_ SDS/Deriphat complexes III, III’, IV, IV’, and V all represented Chl a-Chl c-peridinin complexes and might be considered analogous to Chl a-Chl c-fucoxanthin complexes found in diatoms and brown macroalgae. Of these dinoflagellate complexes, the LL _G. polyedra_ complex III had the lowest Chl a:c ratio (1:1). All other Chl a-Chl c-peridinin complexes from this dinoflagellate, as well as _Glenodinium_ sp., had Chl a:c ratios between 2.5 and 4.0. It is not known if all Chl c2 associated with peridinin in _G. polyedra_ represents distinct Chl a-Chl c2-protein complexes and/or distinct Chl c2-protein (PCP) complexes which associate with the water-soluble peridinin-Chl a-protein complexes, and/or if Chl c2 is associated with Chl a and peridinin in a single complex distinct from and not including PCP complexes. Fucoxanthin-containing algae are known to contain complexes representative of all of these chromophore arrangements (1–4, 8, 9, 18).

It is known that the initial fractionation procedure used here removes only a portion of the total cellular PCP prior to SDS solubilization. Since no free pigment is generated on the Deriphat gels, intact PCP is presumably associated with at least one of the peridinin-containing complexes. Also, all peridinin-containing complexes from _G. polyedra_ also contain protein components in the mol wt range (32 kD) of the apoprotein of _G. polyedra_ PCP. In addition, these complexes have spectral characteristics reminiscent of purified PCP, which accounts for at least 70% of the total peridinin in this dinoflagellate (19, 24, 25). However, immunological studies would be required to conclude that the PCP apoprotein is part of these peridinin-containing complexes.

While few studies in chromophytic algae have followed alterations of membrane-bound Chl a-protein complexes in response to light intensity, Owens and Wold did document changes in the
Chl a distribution among Chl a-protein complexes of P. tricornutum grown under three different light intensities (18). The photoadaptive responses of Glenodinium sp. and G. polyedra have been well characterized (19, 26). Increased pigmentation in LL grown Glenodinium sp. occurs primarily through increased cellular levels of PCP, while photoadaptation of G. polyedra to low growth irradiance involves cellular accumulation of both PCP and unidentified Chl a-Chl c-containing components. In the present study, growth irradiance was observed to affect the pigment composition of specific Chl a-protein complexes. Compared to high light cells, the percentage distribution of Chl c2 among the complexes of low light cells changed noticeably. The percent gel Chl c2 associated with complex II (II') was greater in HL grown cells, while Chl c2 associated with the remaining complexes was reduced. These changes were different than those documented in another dinoflagellate in response to HL. In Glenodinium sp., Chl c2 percentages did not change among complexes, while the percent of Chl a associated with peridinin decreased (6). Other changes in the number of resolvable Chl-protein complexes and Chl distribution among them also occurred in response to changes in G. polyedra growth irradiance. Most notable was the appearance in LL cells of a fifth complex, the enrichment of Chl c in LL complex III, and the relative reduction of total Chl c associated with all peridinin-containing complexes in HL cells. While the present study provides some molecular insights into the photoadaptive responses of G. polyedra, future studies are needed to identify the molecular topology, photochemical function, and supramolecular organization of these Chl a-protein complexes within the photosynthetic apparatus of dinoflagellates.

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