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Light- and $MgCl_2$-dependent characteristics of four chlorophyll-protein complexes isolated from the marine dinoflagellate, *Glenodinium* sp.

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Chlorophyll-protein complexes previously isolated from low-light (80 $\mu E \cdot m^{-2} \cdot s^{-1}$) log cultures of the marine dinoflagellate, *Glenodinium* sp., were further characterized. SDS solubilization in combination with polyacrylamide gel electrophoresis in the presence of Deriphat 160-C resolved four discrete chlorophyll-protein bands. In order to elucidate the functional role of *Glenodinium* sp., room-temperature absorption and fluorescence spectra, protein composition, and pigment molar ratios were obtained for each complex. Results indicated that complex I was analogous to the green plant Photosystem I complex and was also associated with light-harvesting chlorophyll c_2. Complex II was highly enriched in chlorophyll c_2, devoid of peridinin, and demonstrated energy transfer from chlorophyll *c* to chlorophyll *a* within the complex, indicating the presence of a light-harvesting component. Based on peridinin:chlorophyll *a* ratios and fluorescence excitation spectra analyses for complexes III and IV, it was concluded that these complexes contained functional peridinin–chlorophyll *a*–protein complexes. Changing the ionic environment during isolation of the complexes, or altering the growth irradiance of *Glenodinium* sp. cultures, resulted in a significant alteration of distribution of chlorophyll *a* among the chlorophyll-protein complexes.

Introduction

In photosynthesis, light energy absorbed by light-harvesting pigments is transferred to the P-680 and P-700 reaction centers of Photosystems (PS) II and I, respectively. Structural components of the photochemical reaction centers are believed to be organized similarly within the thylakoid membranes of diverse plant groups, while the nature of light-harvesting pigment-protein components (LHC) differs among these groups. Experimental studies of detergent-solubilized membrane complexes representing PS I, PS II, and light-harvesting pigment-protein complexes have provided the bases for several conceptual models describing the organization of the photosynthetic apparatus in green plants [1–4].

Based upon a variety of biochemical and physiological data, photosystem arrangements modelled after those proposed for green plants have been suggested for Chl *c*-containing marine algae [3,5]. It is known that all Chl *c*-containing algae examined thus far contain spectrally similar P-700-Chl *a* reaction center I complexes[3,6,7]. However, it is the arrangement of the LHCs within the subgroups of Chl *a*–Chl *c*–carotenoid-containing algae that remains to be defined. From some

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Abbreviations: Chl, chlorophyll; LHC, light-harvesting component; PMSF, phenylmethylsulfonyl fluoride; PS I, Photosystem I; PS II, Photosystem II.

brown algae, diatoms, chrysophytes and dinoflagellates, large amounts of carotenoid-Chl complexes can be isolated and shown to serve major blue-green light-harvesting functions. Characterized carotenoproteins include the fucoxanthin–Chl *c*–Chl *a*–protein complexes of diatoms and brown macroalgae [6,7], and the uniquely water-soluble peridinin–Chl *a*–protein complex found only in dinoflagellates [8]. The dinoflagellates appear particularly well suited for studies of the organization of LHCs in marine plants as their peridinin and Chl c_2 appear distributed in distinct peripheral and integrally-bound membrane components respectively. A membrane-associated light-harvesting Chl *c*–Chl *a*–protein complex, highly enriched in accessory chlorophyll c_2, was first hypothesized [7] and then isolated and preliminarily characterized from marine dinoflagellates [9].

Initially, four Chl-protein complexes were resolved from low-light populations of *Glenodium* sp., using SDS solubilization and a Deriphat electrophoretic system [9]. This preliminary study suggested that complex I was a Photosystem I (PS I) component, while complexes II, III and IV appeared to be light-harvesting components containing significant amounts of Chl *c* and peridinin accessory pigmentation. To better define the roles that these complexes play in the photosynthetic apparatus of *Glenodinium* sp., a study was undertaken to identify the room-temperature fluorescence characteristics, chromophore ratios, and protein components of the four Chl-protein complexes. In addition, light levels during growth, and ionic environments during isolation and separation procedures, were also altered, so as to determine the effect that irradiance and ionic strength might have on the organization of pigmentation within the photosynthetic apparatus of dinoflagellates.

Methods and Materials

Algal culture and harvesting. Methods for the mass culturing and harvesting of *Glenodinium* sp. at low irradiances (80 $\mu E \cdot m^{-2} \cdot s^{-1}$) (L. Provasoli, M. Bernard strain; UCSB code No. 5M29) have been previously described [9]. High-light populations were grown in batch cultures in 2.8 l Fernbach flasks containing 1–2.5 l f/2 medium (22°C, 300 $\mu E \cdot m^{-2} \cdot s^{-1}$, General Electric Power-Groove fluorescent lamps) and were harvested by centrifugation (Sorvall RCB-2, 15°C, 20 min, $1000 \times g$) during late-exponential growth. Pelleted cells were routinely resuspended in 50 mM Tris-HCl/1 mM EDTA/1 mM PMSF (pH 8.0). However, when determining the effects of magnesium on Chl organization, harvested cells were resuspended in 50 mM Tris-HCl/4 mM $MgCl_2$/1 mM PMSF (pH 8.0). Cells were frozen rapidly with liquid N_2 and stored at −10°C.

Preparation and solubilization of thylakoid membranes. Thawed cells were ruptured by sonication and thylakoid membrane fractions were prepared and solubilized as previously described [9], except that 1 mM PMSF was added to all resuspension buffers. SDS-solubilized membrane extracts were recovered from the supernatant and loaded onto either polyacrylamide tube gels (0.5×8.0 cm) or a slab gel (2.5×115 mm). Both electrophoretic gel systems contained 5% acrylamide (Bio-Rad), 0.25% *N,N*-methylene bisacrylamide (Bio-Rad), 0.125% ammonium persulfate (Sigma), 0.1% (v/v) *N,N,N′,N′*-tetramethylethylenediamine (Eastman Kodak), 6.2 mM Tris, 48 mM glycine and 0.1% Deriphat 160-C (partial sodium salt of *N*-lauryl-*β*-iminodipropionic acid (gift from the Henckel Corporation). However, the quality of Deriphat 160-C varied, i.e., two of five lots were unsuitable for electrophoretic studies.

Electrophoresis was carried out at 75 V for 35–45 min at room temperature using an electrode buffer containing 12.4 mM Tris, 96 mM glycine, and 0.2% Deriphat 160-C. For thylakoid preparations prepared in the presence of magnesium, 2 mM $MgCl_2$ or 4 mM $MgCl_2$ was added to the gel buffer. In some cases an equivalent concentration of $MgCl_2$ was added to the electrophoresis buffer.

Relative Chl *a* distribution was determined in the gels as described previously [9]. Chl-containing bands were excised from tube gels and were analyzed immediately or stored at −76°C for future protein determinations. Chl-containing components were recovered from the gel in 0.3% SDS/50 mM Tris, (pH 8.0) as previously described [9].

Deriphat-containing slab gels were used to determine the relative mobility of solubilized Chl *a*-containing components in reference to standard

proteins. Marker proteins (Bio-Rad) included: myosin (200 kDa), β-galactosidase (130 kDa), phosphorylase *b* (92 kDa), bovine serum albumin (68 kDa), ovalbumin (43 kDa), carbonic anhydrase (31 kDa), trypsin inhibitor (21 kDa) and lysozyme (14 kDa). Marker proteins were denatured by boiling preparations for 3 min in 6.2 mM Tris/48 mM glycine/1% SDS/1% 2-mercaptoethanol/10% (v/v) glycerol. Slab gels were fixed overnight in 10% acetic acid and then stained for protein with 0.2% Coomassie Blue R in methanol: H_2O: acetic acid (5 : 5 : 1, v/v) and destained in methanol : H_2O : acetic acid (5 : 5 : 1, v/v).

Pigmentation. Chl *a*:Chl c_2 molar ratios were determined using acetone extraction procedures as previously detailed [8]. Furthermore, complex III and IV samples were eluted, extracted with 90% acetone, and then pigment components were transferred to diethyl ether, and samples were concentrated under nitrogen for quantitative TLC analyses of carotenoid and Chl components. One-dimensional TLC was carried out on cellulose plates according to the methods of Jeffrey [10]. Pigment molar ratios were calculated from previously presented extinction coefficients and molecular weights [9].

Protein analyses. Frozen gel slices of each Chl-containing band were thawed by soaking them for 15–20 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 electrophoresed in a Laemmli buffer system with a 12% resolving gel and a 3% stacking gel [11]. Protein bands were visualized using the silver-staining technique described by Morrissey [12] and gels were scanned with an ISCO Model 1310 gel scanner with an ISCO UA-5 absorbance/fluorescence monitor. Molecular weight standards used were identical to those listed for the slab gel system described above.

Absorption and fluorescence (emission and excitation) spectra. Absorption spectra of gel slices, eluted solutions and extracted pigments were recorded on an Aminco DW2 spectrophotometer. Room-temperature fluorescence emission and excitation spectra were recorded for gel slices suspended in 50 mM Tris (pH 8.0) or, for buffered solutions of eluted Chl-protein complexes, 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 quantum-corrected for wavelength variation in xenon lamp energy output. Emission spectra were corrected for instrument sensitivity. All fluorescence measurements were on samples not more than 0.1 absorbance unit at the Chl *a* red absorption maximum. Excitation slit widths were 2–8 nm; emission slit widths were 2–4 nm.

Results

A total of five major Chl *a*-containing protein complexes can be obtained from *Glenodinium* sp. cells grown under low-light (80 $\mu E \cdot m^{-2} \cdot s^{-1}$) conditions: the water-soluble peridinin–Chl *a*–protein complex, and four Chl *a*-containing protein complexes extractable with SDS from a brownish-green thylakoid membrane fraction. Fig. 1 is a photograph of a representative Deriphat-polyacrylamide tube gel, illustrating the resolution of the four integral Chl *a*-containing protein com-

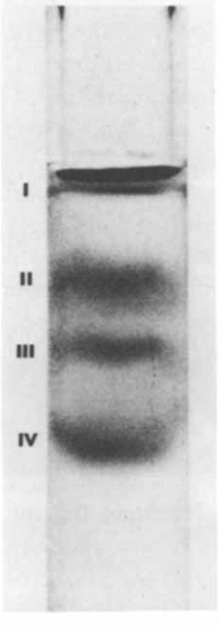

Fig. 1. Photograph of a representative Deriphat polyacrylamide gel showing the relative positions of chlorophyll-protein complexes I, II, III and IV from *Glenodinium* sp.

TABLE I

CHARACTERISTICS OF SDS-SOLUBILIZED CHROMOPROTEINS ISOLATED FROM LOW-LIGHT (80 $\mu E \cdot m^{-2} \cdot s^{-1}$) -GROWN *GLENODINIUM* SP. BY DERIPHAT GEL ELECTROPHORESIS

	Band			
	I	II	III	IV
Mobility	0.09	0.47	0.53	0.85
Chl *a* (%)	21 (2.0) *	30 (2.0)	24 (2.6)	24 (4.0)
Associated proteins (kDa)	69, 57, 43, 39	37, 24, 22, 19	49, 19	32–35, 19

* Numbers in parentheses represent 1 S.D. of the mean of triplicate measurements.

plexes. Apparent molecular weight equivalences of the complexes, determined by Deriphat slab-acrylamide gel electrophoresis, were: complex I, 115 000; complex II, 71 000; complex III, 61 000; and complex IV, 47 000). The apparent molecular weight equivalences of complexes II, III and IV were about 10–20 kDa higher than those originally reported using Deriphat tube gels [9]. Because of this discrepancy, the term 'unit equivalence' is used when comparing molecular weight estimates for Chl *a*-protein complexes to known molecular weights of standard marker proteins. The term should also indicate that the Chl *a*-containing complexes could not be prepared for electrophoresis identically as were marker proteins. Therefore, the apparent molecular weights are most useful as comparative indices of size differences between different complexes and between similar complexes isolated from different growth or preparation conditions.

Low-light populations

Complex I. The low-mobility complex I contained about 20% of the Chl *a* on the Deriphat gel and had an absorption spectrum dominated by Chl *a* peaks at 443 and 674 nm (Fig. 2). A shoulder at 485 nm reflected the presence of β-carotene, whose presence was verified by TLC (data not shown). When irradiated with a 438 nm excitation wavelength, the room temperature fluorescence emission spectrum displayed a major emission maximum at 678 nm and a minor Chl c_2 emission peak at 643 nm. Previously, Chl c_2 was not detectable by TLC chromophore analyses of complex I [9]. Room-temperature excitation spectra for Chl *a* emission at 678 nm resolved two excitation peaks (Fig. 2b); the one at 442 nm was attributable to Chl *a*, while the 416 nm peak had to be assigned to unknown secondary components.

When electrophoresed upon a Laemmli system, complex I consistently contained undissociated material stained by silver at the top of the gel that accounted for about 50% of all silver-stained material on the gel (Fig. 3a). Addition of SDS or β-mercaptoethanol did not improve solubilization, while further heating of the gel only increased the relative amount of undissociated material. Proteins of 69 kDa and 57 kDa constituted the major components resolved from complex I (Table I). Other peptides of 43 kDa and 39 kDa were occasionally present, perhaps representing additional

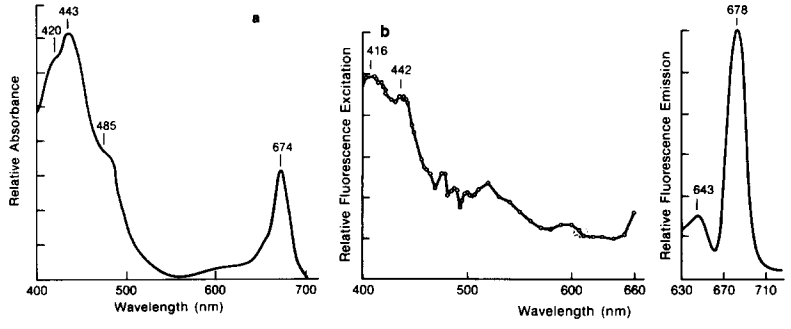

Fig. 2. Relative room-temperature (a) absorption and (b) fluorescence excitation (open circles) and emission (solid line) spectra of complex I gel slices isolated from low-light (80 $\mu E \cdot m^{-2} \cdot s^{-1}$) -grown *Glenodinium* sp. Excitation wavelength was 438 nm. Emission wavelength was 678 nm.

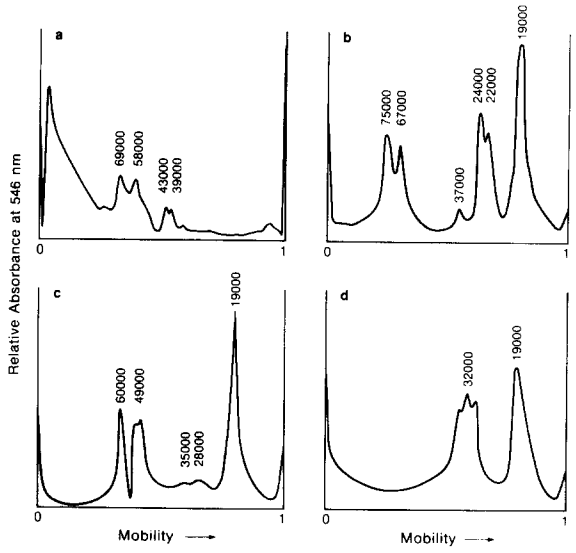

Fig. 3. Silver-stained protein patterns of chlorophyll *a*-protein complexes of *Glenodinium* sp. Deriphat gel slices of (a) complex I, (b) complex II, (c) complex III, and (d) complex IV were reelectrophoresed onto SDS-polyacrylamide gels and scanned at 546 nm.

proteins released from the undissociated sample.

Complex II. The absorption spectrum of complex II was dominated by Chl c_2 absorption, accounting for the 453 and 636 nm peaks. Chl *a* absorption accounted for the 432 nm shoulder and the 672 nm red peak (Fig. 4a). The complex contained about 30% of the Chl *a* and more than 80% of the Chl c_2 on the gel, giving a Chl c_2 : Chl *a* molar ratio of 4.2. The emission spectrum of excised gel slices of complex II excited at 438 nm showed two peaks (Fig. 4b). A major peak at 675 nm was assignable to Chl *a* emission, while the secondary emission peak at 640 nm suggested uncoupled Chl c_2 fluorescence. Transfer of absorbed light energy from Chl c_2 to Chl *a* was indicated by the fluorescence excitation spectrum (Fig. 4b). Fluorescence emission by Chl *a* at 676 nm was attributable to both Chl *c* (452, 587, 640 nm) and Chl *a* (432, 442 nm) excitation peaks. The excitation spectrum for Chl c_2 emission at 640 nm (Fig. 4c) showed the major in vivo absorption peak for Chl c_2 to be a signature at 452 nm. Complex II had one major protein band at 19 kDa with minor bands at 24, 22 and 37 kDa (Table I) (Fig. 3b). Larger peptide components (greater than 50 kDa) appeared irregularly and represented comigrating proteins which may or may not be functionally associated with complex II.

Complexes III and IV. The fastest-moving Chl *a*-containing protein bands, complexes III and IV, had almost identical absorption and fluorescence emission/excitation spectra (Fig. 5). These orange complexes III and IV contained about 24% and 24%, respectively, of the gel Chl *a* (Table I). Neither contained detectable amounts of Chl c_2, while the peridinin 485 nm shoulder was appreciable in absorption spectra of excised gel slices of both bands. Complex III had a peridinin : Chl *a* molar ratio of 3.4, while that of complex IV was 3.2. Fluorescence excitation spectra for Chl *a* 672 nm emission in complexes III and IV showed a broad excitation band around 520 nm, i.e., the spectral region dominated by peridinin absorption

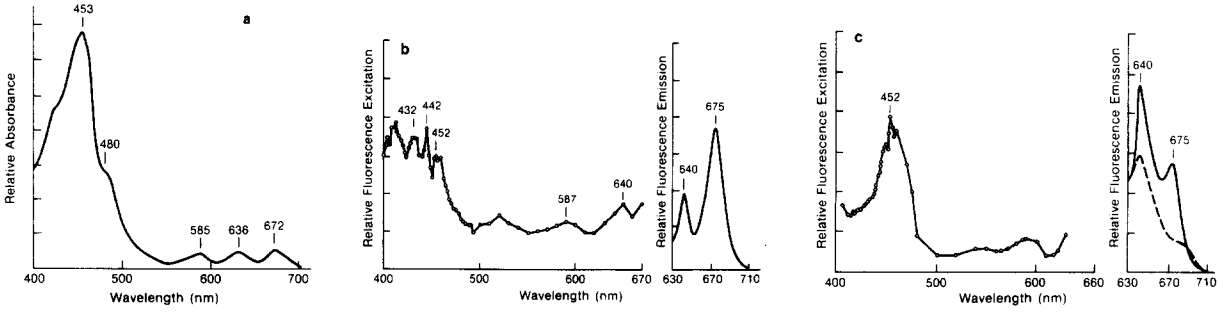

Fig. 4. (a) Room-temperature absorption spectrum of complex II gel slices from low-light (80 $\mu E \cdot m^{-2} \cdot s^{-1}$) -grown *Glenodinium* sp. (b) Room-temperature fluorescence excitation spectrum (open circles) measured at the 675 nm Chl *a* emission peak. Emission spectrum (solid line) was measured with an excitation wavleength of 438 nm. (c) Room-temperature fluorescence excitation spectrum (open circles) measured at the 640 nm Chl *c* emission peak. Emission spectra, measured with 452 nm excitation wavelength, were determined in gel slices (solid line) and following elution of the complex into Deriphat electrophoresis buffer (dashed line). Fluorescence excitation and emission units are relative.

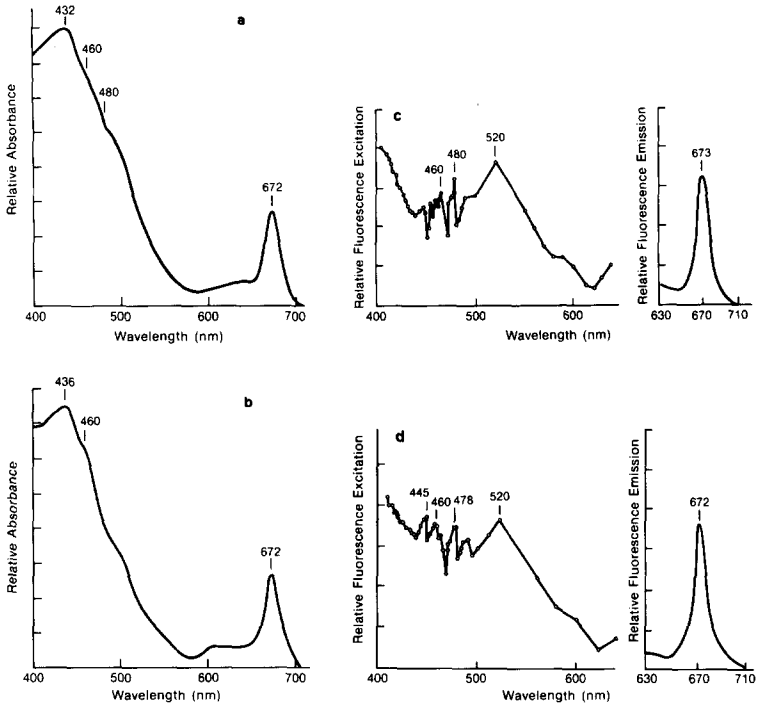

Fig. 5. (a) Room-temperature absorption spectrum of complex III gel slices from low-light (80 $\mu E \cdot m^{-2} \cdot s^{-1}$) -grown *Glenodinium* sp. (b) Room-temperature absorption spectrum of complex IV gel slices from low-light (80 $\mu E \cdot m^{-2} \cdot s^{-1}$) -grown *Glenodinium* sp. (c) Room temperature fluorescence excitation spectrum (open circles) measured at the 673 nm Chl *a* emission peak was determined in complex III gel slices, with an excitation wavelength of 438 nm. (d) Room-temperature fluorescence excitation spectrum (open circles) measured at 672 nm was determined in complex IV gel slices. Emission spectrum was measured at an excitation wavelength of 438 nm. Fluorescence excitation and emission units are relative.

in both photosynthetic fluorescence and oxygen action spectra [14]. Additional excitation maxima were detected at 480 nm and 444 nm. The room-temperature Chl *a* emission peak was at 672 nm, i.e., identical to that measured for purified *Glenodinium* sp. peridinin–Chl *a*–protein [8]. When eluted complex III was re-electrophoresed, major protein bands were resolved at 19 and 49 kDa, with secondary bands at 60 and 53 kDa (Fig. 3c). Complex IV also contained a major peptide at 19 kDa, a mix of peptides in the range 30–35 kDa, and occasional peptides at 40 kDa and 55 kDa (Fig. 3d).

Effect of magnesium on Chl-protein distribution

To determine whether cations could influence chlorophyll organization in *Glenodinium* sp., magnesium chloride was added to the preparation system in concentrations similar to those used to study the regulation of thylakoid rearrangements in higher plants [15]. When 2 mM $MgCl_2$ was added to the solubilization, the electrophoretic, and the polyacrylamide gel buffers, the distribution of Chl *a* in the complexes of low-light-grown *Glenodinium* sp. changed significantly (Fig. 6). Comparison of gel scans run in the presence and absence of $MgCl_2$ indicated that the presence of the ion increased the mobility of complex I, decreased that of complex II, and did not affect that of complex III. Most interestingly, complex IV was no longer evident. The presence of $MgCl_2$ markedly increased the Chl *a* content of complex III (57%) (Table II), presumably due to the incor-

TABLE II

CHARACTERISTICS OF SDS-SOLUBILIZED CHROMOPROTEINS ISOLATED FROM LOW-LIGHT (80 $\mu E \cdot m^{-2} \cdot s^{-1}$) -GROWN *GLENODINIUM* sp. BY DERIPHAT GEL ELECTROPHORESIS CONTAINING 2 mM $MgCl_2$ IN ALL SEPARATION BUFFERS

	Band		
	I	II	III
Mobility	0.21	0.30	0.54
Chl *a* (%)	18 (1.0) *	27 (1.5)	57 (3.0)
Associated proteins (kDa)	74–58, 39, 19	38, 19, 16	35, 19

* Numbers in parentheses represent 1 S.D. of the mean of triplicate measurements.

poration of complex IV with this band. The absorption spectra of all three complexes were little affected by the presence of magnesium. If magnesium content in the gel and electrophoretic running buffers was increased further, then only one complex was resolved, which had a mobility similar to that of the complex II found in a $MgCl_2$-free system.

The electrophoresis of magnesium-containing complex I on a Laemmli gel showed proteins in common with complex I from preparations without magnesium, containing a region of protein staining between 58 and 74 kDa (Table II). Complex I also contained proteins at 39 and 19 kDa. Complex II again contained a major 19 kDa protein, with secondary 38 ad 16 kDa proteins. Similarly, complex III also contained a major 19 kDa protein and a secondary band at 35 kDa.

If $MgCl_2$ was omitted from gel or electrophoretic buffers, but 4 mM $MgCl_2$ was kept in all isolation steps prior to electrophoresis, then all four Chl *a*-containing complexes were resolved. Complexes I through IV had respective mobilities of 0.15, 0.30, 0.50 and 0.75, while their respective Chl *a* contents were 18%, 35%, 39% and 8%. Compared to $MgCl_2$-free preparations, the mobility of complex I was increased, the percentage of Chl *a* in complex III increased, and the percentage of Chl *a* in complex IV decreased. If $MgCl_2$ was added only to the electrophoresis buffer and was reduced from 4 to 2 mM $MgCl_2$, then no differences in mobility or Chl *a* distribution were noted as compared to low-light $MgCl_2$-free preparations.

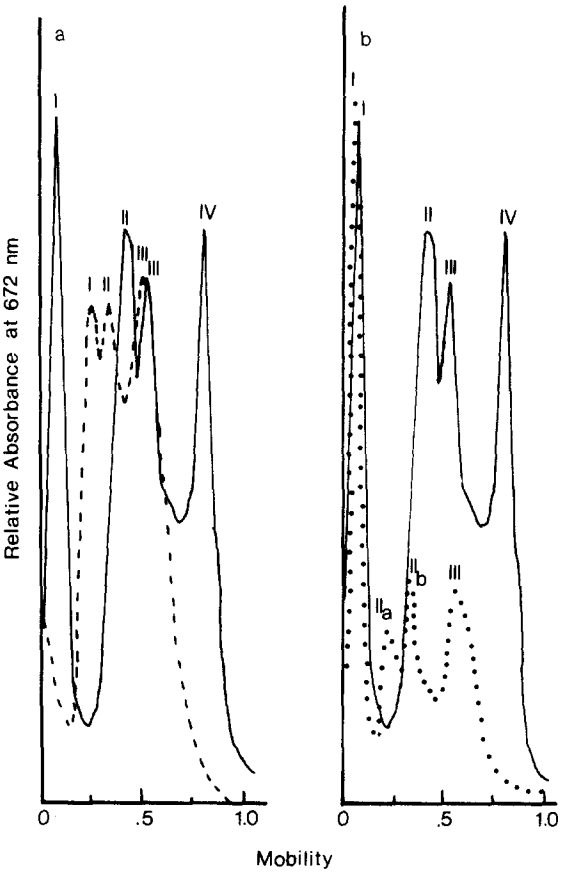

Fig. 6. Deriphat-polyacrylamide gel patterns of *Glenodinium* sp. SDS-solubilized chlorophyll-protein complexes scanned at 672 nm. (a) Comparison of gel scans of low-light (80 $\mu E \cdot m^{-2} \cdot s^{-1}$) -grown cells fractionated in the absence (solid line) and presence (dashed line) of 2 mM $MgCl_2$. (b) Comparison of gel scans of low-light (80 $\mu E \cdot m^{-2} \cdot s^{-1}$) (solid line) and high-light (300 $\mu E \cdot m^{-2} \cdot s^{-1}$) -grown cells. Peak heights are only relative.

Effects of growth irradiance on Chl-protein distribution

Glenodinium sp. cells were grown at a high light (300 $\mu E \cdot m^{-2} \cdot s^{-1}$), fractionated in the absence of $MgCl_2$, and electrophoresed on the Deriphat gel system. The resulting 672 nm gel scan was

TABLE III

CHARACTERISTICS OF SDS-SOLUBILIZED CHROMOPROTEINS ISOLATED FROM HIGH-LIGHT (300 $\mu E \cdot m^{-2} \cdot s^{-1}$) -GROWN *GLENODINIUM* sp. BY DERIPHAT GEL ELECTROPHORESIS

	Band			
	I	IIa	IIb	III
Mobility	0.09	0.25	0.32	0.60
Chl *a* (%)	40 (3.5) *	15 (3.0)	17 (0.5)	28 (4.0)
Associated proteins (kDa)	71, 61, 36, 17	19, 18	44, 18, 16	21, 18

* Numbers in parentheses represent 1 S.D. of the mean of triplicate measurements.

superficially similar to low-light (80 $\mu E \cdot m^{-2} \cdot s^{-1}$) gel patterns in that four Chl-protein complexes were resolved (Fig. 6). However, spectral analyses indicated significant changes in the Chl distribution between Chl *a*-containing protein complexes. The absorption spectra of high-light complexes I and III were similar to low light complexes I and III, respectively. However, high-light complex I contained 40% of Chl *a* compared to only 21% in low-light complex I. High-light complexes IIa and IIb were spectrally identical to each other and to low-light complex II. Taken together, high-light IIa and IIb contained a similar percentage of Chl *a* as low-light complex II (i.e., 32 vs. 30%, respectively), while the mobility of high-light IIa and IIb complexes respectively approached that of low-light II complex (compare Tables I and III). High-light complex III had a mobility intermediate to those of low-light complexes III and IV.

High-light Chl complexes electrophoresed on Laemmli gels showed no major changes compared to peptide patterns seen previously (Table III). Complex I showed the usual undissociated area at the top of the gel, and distinct proteins of 71 and 61 kDa. Proteins of 36 and 17 kDa wre also associated with this complex. Complexes IIa, IIb and III contained a major peptide of 18–21 kDa. In addition, complexes IIb and III contained low-molecular-weight components of 16–18 kDa. Complex IIb also contained a protein of 44 kDa.

Discussion

In the original study of detergent-solubilized thylakoid fractions of *Glenodinium* sp. [9], complex I was designated as being representative of Photosystem I because of its high mobility on Deriphat gels, its long-wavelength Chl *a* peak (674 nm), and its apparent lack of Chl c_2. Further analyses indicate that this complex had a long-wavelength Chl *a* fluorescence peak at 678 nm, and apoproteins in the 67 kDa range, which are characteristics shared with the complex I isolated by SDS-solubilization techniques from green plants [16]. The idea that Chl c_2 may be a part of the light-harvesting complement for PS I is in line with the recent investigation of PS I Chl *b*-containing light-harvesting component, where Chl *b* is an integral component of Photosystem I [17,18]. When magnesium is added to the separation system, the mobility of complex I increases. This new position of complex I is similar to the position occupied by the P-700–Chl *a*–protein when thylakoid membrane fractions are run under the conditions detailed by Prézelin and Alberte [3], who included magnesium in their protocol. Since the absorption properties of complex I changed little with the change in mobility, it is possible that an oligomeric-to-monomeric change occurred. Similar changes from oligomeric complex Ia to monomeric complex I have been documented when magnesium is added to green plant systems [18]. It is also possible that increased mobility resulted from the dissociation of lipids or other membrane components from the complex.

Because they contain photosynthetic pigments other than Chl *a*, complexes II, III and IV can be designated as possible light-harvesting components of *Glenodinium* sp. The longest fluorescence emission wavelength of these complexes is found at 675 nm in complex II, the Chl *a*/Chl c_2 complex. This Chl c_2-enriched chromoprotein had a similar unit equivalence (71 000), and similar apoproteins of 24 and 25 kDa, to the Chl c_2 light-harvesting complex extracted from *Chroomonas* sp. by Ingraham and Hiller [19]. Discrepancies similar to those found here were also noted by these investigators in the molecular weights of native Chl-protein complexes when different types of electrophoretic systems were used [19].

Since energy transfer from Chl c_2 to Chl *a* is apparent in complex II, its role in the thylakoid membrane can be designated as light-harvesting. Because a fraction of the energy transfer is uncoupled, it was impossible to determine whether or not all of the Chl c_2 associated with complex II was functional. The lability of Chl c_2 energy transfer efficiency has been noted in other attempts to isolate a functional Chl c_2-containing LHC [6,19]. SDS in particular has been shown to destroy energy transfer between Chl *c* and Chl *a* [20,21]. It is also possible that a single Chl c_2-protein, similar to that found in *Acrocapia* by Barrett and Thorne [22], may exist in *Glenodinium* sp., thus accounting for the lability of Chl c_2-to-Chl *a* energy transfer. Recently, Friedman and Alberte

[20] have isolated a major light-harvesting component from a diatom that contained chlorophylls *a* and *c* and fucoxanthin in a complex that showed efficient energy transfer from fucoxanthin and Chl *c* to Chl *a*. It is possible to speculate that the peridinin–Chl *a*–protein found in these studies to be closely associated with the membranes might be also closely associated with a Chl c_2 protein, and that SDS used as a solubilization agent destroys this association.

The presence of peridinin, and absence of Chl c_2, argues that complexes III and IV represent a form of water-soluble peridinin–Chl *a*–protein that is closely associated with the thylakoid membrane. The peridinin/Chl *a* ratios of complexes III and IV (3.2–3.4), are very similar to the chromophore ratio of 4.0 determined for the water-soluble peridinin–Chl *a*–protein [8]. The reduced peridinin/Chl *a* ratios may have indicated either a selective loss of peridinin during chromophore analysis or the presence of additional Chl *a*-protein components complexed with peridinin–Chl *a*–protein. The Chl fluorescence emission peaks of these complexes, at 672–673 nm, also coincide closely with that of peridinin–Chl *a*–protein [14]. Their fluorescence excitation patterns are similar to peridinin–Chl *a*–protein, with peaks at 440 nm and 486 nm, although the 520 nm shoulder is exaggerated in the gel slices in a manner comparable to the 520 nm in vivo shoulder assignable to peridinin–Chl *a*–protein in low-temperature absorption spectra [14] and in low-temperature absorption spectra of complex III [13]. Lower excitation spectra than expected in the 400–500 nm region can be attributable to carotenoids exterior to the photosynthetic machinery acting as filters of incident light. Such blue-light screening is known to occur in a number of photosynthetic systems [14,23–25]. In previous studies of peridinin–Chl *a*–protein in *Glenodinium* sp., the molecular weight of its apoprotein was determined to be 16.5 kDa using gel chromatography [8]. The major low-molecular-weight peptides associated with complexes III and IV are in the 19 kDa range. This discrepancy is, however, within experimental error. Addition of magnesium appears to cause some aggregation of these peridinin–Chl *a*–protein-containing bands. Preliminary data suggest that aggregation is due to magnesium effects during solubilization, since extracts isolated with magnesium, but electrophoresed without magnesium, showed the beginnings of redistribution of Chl *a* between complexes III and IV. These observations contrast to reports on soluble peridinin–Chl *a*–protein, where removal of salts induces aggregation states (Prezelin, B., unpublished data).

When low-light *Glenodinium* cells are shifted to high light, peridinin–Chl *a*–protein-associated Chl and peridinin decrease, and whole cell Chl *a*-to-Chl c_2 molar ratios decrease to less than one [27]. In the present study, high-light-induced pigment changes were reflected in Chl-protein redistribution. The percent Chl found in peridinin-containing complexes was drastically reduced, and, in fact, complex IV was lost. Unlike low-light populations, the highest percentage of Chl *a* was found in complex I, and the Chl a/c_2 component is split into two complexes. Complex I showed no increase in absorbance at 450 or 635 nm, which would have indicated an increase in Chl c_2 content. Calculations of the estimated Chl a/Chl c_2 ratio of complexes IIa and IIb from the amount of Chl added to the gel ($a/c_2 = 1.0$), showed that in order for these complexes to have peak heights, and thus Chl ratios, close to those seen in complex II of low-light cells, all of the Chl c_2 would have to be concentrated in complexes IIa and IIb. It would be difficult to determine at this time which complex might represent PS II. Because of the disappearance of complex IV in this system under high-light conditions, it would be unlikely that the reaction center would be found here; it would be more likely to be found associated with complexes II and III. On the basis of the fluorescence emission information presented here, and fluorescence assignments made by Govindjee et al. [27], it would be tempting to assign PS II to complex II. However, without low-temperature fluorescence and photosystem activity data, it is impossible to do so with any confidence.

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References

1 Thornber, J.P. (1975) Annu. Rev. Plant Physiol. 26, 127–158
2 Anderson, J.M. and Andersson, B. (1981) in Proceedings of the 5th International Congress on Photosynthesis (Akoyunoglou, G., ed.), Vol. III, pp. 23–31, Balaban International Science Services, Philadelphia, PA
3 Prézelin, B.B. and Alberte, R.S. (1978) Proc. Natl. Acad. Sci. USA 75, 1801–1804
4 Boardman, N.N., Anderson, J.M. and Goodchild, D.J. (1978) Curr. Top Bioenerg. 8, 35–109
5 Anderson, J.M., Barrett, J. and Thorne, S.W. (1981) in Proceedings of the 5th International Congress on Photosynthesis (Akoyunoglou, G., ed.), Vol. III, p. 301–317, Balaban International Science Services, Philadelphia, PA
6 Alberte, R.S., Friedman, A.L., Gustafson, D.L., Rudnick, M.S. and Lyman, H.L. (1981) Biochim. Biophys. Acta 635, 304–316
7 Barrett, J. and Anderson, J.M. (1980) Biochim. Biophys. Acta 590, 309–332
8 Prézelin, B.B. and Haxo, F.T. (1976) Planta 130, 251–256
9 Boczar, B.B., Prezelin, B.B., Markwell, J.P. and Thornber, J.P. (1980) FEBS Lett. 120, 243–247
10 Jeffrey, S.W. (1981) Limnol. Oceanogr. 26(1), 191–197
11 Laemmli, U.K. (1970) Nature 227, 680–685
12 Morrissey, J.H. (1981) Anal. Biochem. 117, 307–312
13 Prézelin, B.B. and Boczar, B.A. (1981) in Proceedings of the 5th International Congress on Photosynthesis (Akoyunoglou, G., ed.), Vol. III, pp. 417–427, Balaban International Science Services, Philadelphia, PA
14 Prézelin, B.B., Ley, A.C. and Haxo, F.T. (1976) Planta 130, 251–256
15 Argyroudi-Akoyunoglou, J.H. (1980) Photobiochem. Photobiophys. 1, 279–287
16 Anderson, J.M. (1980) FEBS Lett. 117, 327–331
17 Argyroudi-Akoyunoglou, J.H. and Thomou, H. (1981) FEBS Lett. 135, 177–181
18 Anderson, J.M., Brown, J.S., Lam, E. and Malkin, R. (1983) Photochem. Photobiol. 38(2), 205–210
19 Ingraham, K. and Hiller, R.G. (1983) Biochim. Biophys. Acta 722, 310–319
20 Friedman, A.L. and Alberte, R.L. (1984) Plant Physiol. 76, 483–489
21 Boczar, B. (1985) Ph.D. Thesis, University of California, Santa Barbara
22 Barrett, J. and Thorne, S.W. (1981) in Proceedings of the 5th International Congress on Photosynthesis (Akoyunoglou, G., ed.), Vol. III, pp. 347–357, Balaban International Science Services, Philadelphia, PA
23 Emerson, R. and Lewis, C.M. (1942) J. Gen. Physiol. 25, 579–595
24 Halldal, P. (ed.) (1970) Photobiology of microorganisms, Wiley-Interscience, London
25 Goedheer, J.C. (1972) Annu. Rev. Physiol. 23, 87–112
26 Prézelin, B.B. (1976) Planta 130, 225–233
27 Govindjee, Wong, D., Prezelin, B.B. and Sweeney, B.M. (1979) Photochem. Photobiol. 30, 405–411