

# Light Regulation of Peridinin-Chlorophyll *a*-Protein (PCP) Complexes in the Dinoflagellate, *Glenodinium* sp.<sup>1</sup>

USE OF ANTI-PCP ANTIBODIES TO DETECT PCP GENE PRODUCTS IN CELLS GROWN IN DIFFERENT LIGHT CONDITIONS

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## ABSTRACT

As a step toward developing the tools needed to study the molecular bases of light regulation of gene expression in dinoflagellates, light-harvesting peridinin-chlorophyll *a*-protein (PCP) complexes from *Glenodinium* sp. were purified and used to generate anti-PCP antibodies. Affinity purified anti-PCP antibodies were isolated from the crude anti-PCP antiserum resulting in improved specificity of immune reactions. The affinity purified anti-PCP antibodies were shown to react strongly and specifically with all major isoforms of PCP complexes in *Glenodinium* sp. cells, and were used to assess qualitative changes in the levels of PCP gene products in cells grown under different light conditions. Western blot analysis revealed a two- to three-fold increase in detectable PCP apoprotein in low light compared to high light grown cells. *In vitro* translation reactions supplied with total RNA from high and low light grown *Glenodinium* sp. cultures also showed an approximate twofold increase in translatable PCP mRNAs in low light grown cells as determined by immunoprecipitation of the primary translation products with affinity purified anti-PCP antibodies. In addition, PCP apoproteins appear to be encoded as larger pre-proteins, since the major immunoprecipitated products from *in vitro* translation are 23 and 22 kilodaltons, while mature PCP apoproteins are 15.5 kilodaltons. The parallel increases in PCP apoprotein and translatable PCP mRNAs indicate that light regulation of PCP complexes occurs at the level of PCP mRNA abundance.

Light-harvesting pigment-protein complexes are ubiquitous among photosynthetic organisms where they function to absorb photosynthetically available radiation and transduce excitation energy to the photochemical reaction centers of photosynthesis. Most interestingly from a regulatory standpoint, a wide variety of different light-harvesting complexes appear to be light inducible, *i.e.* intracellular levels of light-harvesting complexes increase under conditions of lower growth irradiance (1, 19, 20). The mechanisms by which an external environmental signal such as light regulates the levels of light-harvesting complexes are not well understood. Although some progress on light regulation of gene expression has been made with the light-harvesting Chl *a/b*-binding proteins of several higher plants (for reviews, see Refs. 1, 13, and 23) and the green alga *Chlamydomonas* (12), very

little is known about the light regulation phenomenon in the chromophytic (Chl *c*-containing) algae that account for a major fraction of ocean primary productivity. Dinoflagellates, as members of the chromophytic algae, provide a good model system for studying the mechanisms of light regulation of gene expression in these important algal groups.

The major light-harvesting component of dinoflagellates is a brick red, water-soluble PCP<sup>2</sup> complex whose molecular topology, photosynthetic function, and adaptive photophysiology have been well characterized (19). PCP complexes, located peripherally on thylakoid membranes in close association with other components of the photosynthetic apparatus, function to absorb blue-green light and transduce it with high efficiency to the photochemical reaction centers (19). PCP levels are known to increase in low light environments, presumably to increase light-harvesting efficiency in light-limited environments (19), but little is known about the molecular mechanism of this light induction. It is the variability of PCP content that determines dinoflagellate cell color, has a major effect on their light-harvesting capabilities, and influences the optical properties of the water columns in which they are found (20).

Knowledge of the molecular mechanism underlying light regulation of light-harvesting complexes in dinoflagellates awaits the development of appropriate molecular probes for the detection, characterization and quantification of identifiable products of transcription and translation in these cells. Such probes must include antibodies directed against specific protein components of light-harvesting complexes, as well as cloned genes that code for these components. The model system we have chosen for studying the molecular mechanism of light regulation is the light-harvesting pigment-protein complexes in the dinoflagellate, *Glenodinium* sp. As a major step toward generating the molecular probes necessary for such studies, we have purified the chromoprotein from *Glenodinium* sp. and generated highly specific anti-PCP antibodies. We report here the characterization of these anti-PCP antibodies and their use in preliminary experiments on light regulation of PCP.

## MATERIALS AND METHODS

**Purification of PCP Complexes.** All reagents used were of analytical grade. Procedures for mass culturing dinoflagellates have been previously described (21, 22). *Glenodinium* sp. strain used was that isolated by L. Provasoli and M. Bernard, UCSB

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<sup>2</sup> Abbreviations: PCP, peridinin-Chl *a*-protein; TBS, Tris-buffered saline; TBST, Tris-buffered saline with 0.1% Tween-20; IEF, isoelectric focusing; PVP-40, PVP, minimum mol wt 40,000; pI, isoelectric point.

code No. 5M29. Detailed methods for the purification of PCP complexes from *Glenodinium* sp. have also been described (21) and were followed except for minor modifications (see Table I). Briefly, frozen packed *Glenodinium* sp. cells were resuspended in extraction buffer and broken by vortexing with glass beads (0.476 mm in diameter, B. Braun Melsungen, FRG). After  $(\text{NH}_4)_2\text{SO}_4$  fractionation, the crude preparation was chromatographed successively on Sephadex G-100 (Pharmacia), DEAE-Cellulose (Sigma), HPLC Superose-12 (Pharmacia) and HPLC Mono-Q (Pharmacia). Column fractions during chromatography were monitored for absorbance at 280 nm (protein) and 478 nm (peridinin), and pools were made of the fractions yielding the highest  $A_{478}:A_{280}$  ratios (11, 21). Pooled fractions were routinely concentrated by Amicon pressure concentration or dialysis against PVP-40 (Sigma). Protein concentrations in samples during purification were estimated by absorbance at 280 nm and 260 nm using the formula:  $1.54(A_{280}) - 0.76(A_{260})$ . Mono-Q purified PCP was estimated to be about 95% pure in silver/Coomassie doubly stained SDS gels (14, 17), and was further purified by preparative SDS gel electrophoresis as follows. Mono-Q purified PCP was electrophoresed through preparative 10 to 20% linear gradient acrylamide SDS gels (3 mm thick), lightly stained with Coomassie (0.1% Coomassie, 5% MeOH, 10% acetic acid for 10 min) and destained for 3 h with MeOH/acetic acid. The single band tracking at 15.5 kD was cut out and electroeluted into dialysis bags, dialyzed overnight against 5 mM Tris (pH 7.4), lyophilized, and resuspended in 20 mM sodium phosphate buffer (pH 7.2). This preparation was judged >99.9% pure by visualization in 10 to 20% SDS gels doubly stained with silver and Coomassie.

**Immunological Methods.** The approximately 95% pure Mono-Q PCP preparation was used to immunize a mature New Zealand White rabbit and antiserum was recovered by standard procedures (2).

**Affinity Purified Anti-PCP Antibodies.** Antibodies were obtained by the construction of a PCP affinity column through which crude anti-PCP antiserum was chromatographed. Covalent linkage of 0.35 mg of gel-purified PCP apoprotein to 0.5 mL (packed volume) CNBr-activated Sepharose 4B (Sigma) was done according to March *et al.* (15). One mL aliquots of crude PCP antiserum were passed five times through the column, nonspecifically bound material washed off the column with 20 column volumes of wash buffer (20 mM sodium phosphate buffer, 0.5 M NaCl, 0.1% Tween-20, pH 7.2) and anti-PCP antibodies eluted in 0.5 M  $\text{NH}_4\text{OH}$ , 3 M KSCN, and immediately dialyzed against 20 mM sodium phosphate buffer, 0.5 M NaCl (pH 7.2). A single column run on 1 mL of crude antiserum typically yielded 25  $\mu\text{g}$  anti-PCP antibodies. Dialyzed antibodies were precipitated by 35% saturation with  $(\text{NH}_4)_2\text{SO}_4$  and stored as precipitates. Pooled precipitates were recovered by centrifugation and resuspended in 20 mM sodium phosphate buffer, 0.5 M NaCl (pH 7.2) to a final concentration of 1 mg/mL.

**Western Blot Analyses.** Analyses using anti-PCP antibodies were performed according to the methods of Towbin *et al.* (24) with modifications as noted below. *Glenodinium* sp. protein preparations were electrophoresed on discontinuous 10 to 20% linear gradient acrylamide SDS gels and electroblotted to nitrocellulose sheets. The blots were air dried, fixed in 10% acetic acid/25% isopropyl alcohol for 15 min, rinsed several times with water, then incubated for 5 min at 25°C in TBS (50 mM Tris, 0.2 M NaCl, pH 7.4). Nitrocellulose blots were blocked, washed, incubated with first and second antibodies, and developed by standard methods, except that 20% fetal calf serum was used as blocking agent instead of gelatin or BSA. Antibody dilutions were done in TBST (TBS plus 0.1% Tween 20)—see specific figure legends for more details. Visualization of antigen/antibody

complexes was by horse raddish peroxidase conjugated second antibody and the 4-chloro-1-naphthol color development reaction.

**IEF Gels.** Gels (pre-poured LKB Ampholine PAGplates, pH 3.5–9.5) were run and stained according to the manufacturer's directions. IEF gels were sometimes used for Western blot analysis, in which case the gels were removed from their supporting matrix to Whatman 3MM paper and preequilibrated in Western transfer buffer (3.0 g Tris-HCl, 14.4 g glycine, 250 mL MeOH per liter) for 20 min prior to electrophoretic transfer to nitrocellulose sheets. Crude preparations of isoelectric conformers of PCP were obtained by first size fractionating crude *Glenodinium* sp. proteins on Sephadex G-100, pooling fractions with high absorbance at 478 nm, and then chromatographing this material on DEAE-Sephacel (21). Elution of PCP conformers from DEAE-Sephacel was accomplished by a 500 mL linear decreasing pH gradient starting with 50 mM Tris (pH 8.0) and ending with 50 mM Hepes (pH 6.6). This resulted in four major regions of 478 nm absorbing material, called DEAE-Sephacel 1–4 in the order of their elution from the column. A final peak of 478 nm absorbing material was eluted with 50 mM Hepes (pH 6.6) 0.4 M NaCl (DEAE-Sephacel 5). These preparations were concentrated by dialysis against PVP-40. Aliquots of from 1 to 20  $\mu\text{L}$  (0.02 to 0.77 OD<sub>478</sub> units) of the concentrated DEAE-Sephacel peaks and the G-100 crude material used to generate them were loaded onto LKB PAGplate gels for IEF analysis.

**RNA Isolation, *In Vitro* Translation and Immunoprecipitation.** RNA was isolated from freshly harvested *Glenodinium* sp. cells grown in low light (80  $\mu\text{E m}^{-2}\text{s}^{-1}$ ) and high light (300  $\mu\text{E m}^{-2}\text{s}^{-1}$ ) batch cultures (22). Cultures in late exponential phase of growth were harvested by centrifugation (6000 g, 10 min) and the pelleted cells washed once with HDHB (60 mM Hepes (pH 7.4), 1.2 mM EDTA, 0.75 M sorbitol). RNA was isolated by a slight modification of the method of Cathala *et al.* (4).

The methods for immunoprecipitation of PCP from *in vitro* translation reactions were based on those of Oelmüller *et al.* (18). From 0 to 10  $\mu\text{g}$  RNA were translated in a noncommercial rabbit reticulocyte lysate (gift from S. Benson) in the presence of [<sup>35</sup>S]methionine (NEN, translation grade, 1000 Ci/mmol, 10–20  $\mu\text{Ci}$ /reaction). A 1  $\mu\text{L}$  aliquot was taken from each reaction and analyzed by TCA precipitation and scintillation counting to determine total [<sup>35</sup>S]methionine incorporation. A 4  $\mu\text{L}$  aliquot from each reaction was saved for direct analysis by SDS-PAGE, and the remainder was diluted 10-fold with TBST, divided into two equal volumes, incubated with either affinity purified anti-PCP antibody (at a final concentration of 5  $\mu\text{g}/\text{mL}$ ) or rabbit preimmune serum (at a final dilution of 1:200 for whole serum) and precipitated with Staph A cells (Calbiochem). Whole translation reaction samples and solubilized immunoprecipitates were electrophoresed through SDS-polyacrylamide (10–20%) gels and labeled proteins were visualized by autoradiography.

## RESULTS

**Purification of Peridinin-Chl *a*-Protein Complexes.** When *Glenodinium* sp. cells are homogenized and the cell debris removed, the soluble fraction is a deep brick red color (21). The solubility of PCP was investigated by stepwise addition of  $(\text{NH}_4)_2\text{SO}_4$  and monitoring of the absorbance at 478 nm (peridinin) and 280 nm (protein) of the resuspended protein precipitates at each increment of  $(\text{NH}_4)_2\text{SO}_4$ . The ratio of peridinin to protein absorbance ( $A_{478}:A_{280}$ ) is an indication of the purity of PCP complexes (11), and the fraction with the highest optical ratio remained soluble above 70% saturation with  $(\text{NH}_4)_2\text{SO}_4$  (data not shown). This approach provided a convenient initial fractionation, and we routinely brought our crude homogenates to 70% saturation before any further purification steps.

Table I summarizes the steps required to purify PCP to a single polypeptide species as visualized on SDS gels. Ion-exchange

Table I. Summary of Purification of Peridinin-Chl *a*-Protein Complexes

Purification began with 37.7 g of frozen whole *Glennodinium* sp. cells. Changes in chromophore and protein absorbance, as well as protein yield are shown for the stepwise purification of complexes used for the generation of antibodies.

Preparation	Ratio 478:280	Protein Conc <sup>a</sup> mg/ml	Total Protein mg	PCP Purity <sup>b</sup> %
Crude	0.22	9.53	4765	5.6
Ammonium sulfate 70%	0.96	43.1	733	24
G-100 No. 1	1.34	0.63	22.6	
G-100 No. 2	1.63	1.07	32.1	40.8
DEAE-Cell No. 1	2.06	0.26	7.93	51.5
DEAE-Cell No. 2	0.38	0.22	9.11	
DEAE-Cell No. 3	0.12	6.05	10.21	
DEAE-Cell No. 4	0.14	0.27	2.11	
Superose-12	2.62	0.229	2.36	65.5
Mono-Q No. 1	3.74	0.38	0.35	
Mono-Q No. 2	4.31	0.156	1.58	~95 <sup>c</sup>
Preparative SDS gel	— <sup>d</sup>	0.71 <sup>d</sup>	0.71	>99.9 <sup>c</sup>

<sup>a</sup> Protein concentrations determined by the formula  $1.54(A_{280}) - 0.76(A_{260})$  unless otherwise noted. <sup>b</sup> PCP purity estimated as  $[(A_{478}/A_{280})/4.0] \times 100$ , unless otherwise noted. <sup>c</sup> Purity estimated by silver and Coomassie double staining in SDS gels (see "Materials and Methods" for details). <sup>d</sup> Protein concentration determined by Bradford protein assay (3).

chromatography resolves PCP complexes into several species that differ in isoelectric points (21). This was observed both with NaCl gradient elution from DEAE-cellulose columns, as during PCP purification (data not shown), or with pH gradient elution from DEAE-Sephacel, as during later analysis of PCP isomers (see below). For the purposes of purification, DEAE-Cellulose peak 1 was used since it yielded the highest  $A_{478}:A_{280}$  ratio, and the other minor peaks from DEAE-cellulose were not further pursued. DEAE-cellulose peaks are numbered according to their order of elution during the increasing NaCl step gradient. Prior results indicated that PCP complexes approach purity when the  $A_{478}:A_{280}$  ratio exceeds 4.0 (11, 21), and therefore the Mono-Q 2 preparation (see Table I) was used for immunization of a rabbit. Upon further analysis of SDS gels, however, this preparation was found to contain several minor contaminating species (data not shown), and thus it was necessary to purify the PCP apoprotein from preparative SDS gels. Figure 1 shows gel purified PCP apoprotein treated two different ways before electrophoresis. The material in lane 1 was boiled for 3 min in 1X electrophoresis buffer prior to loading and shows an apparent aggregate at about 40 kD. The material in lane 2 was not boiled, but instead brought to 2% SDS and incubated 30 min at room temperature prior to loading. This sample is devoid of the 40 kD band, and so protein samples were routinely prepared in this way for electrophoresis to avoid the apparent aggregation associated with boiling.

**Characterization of Anti-PCP Antibodies.** Western blot analyses were performed to determine the reactivity and specificity of the crude antiserum generated by immunization with Mono-Q prepared PCP. Figure 2, lane 1, shows such a blot. The specificity of the crude antiserum is insufficient, since several *Glennodinium* sp. proteins are recognized by this antibody preparation. To improve the specificity of the antibodies, a PCP affinity column was constructed (see "Materials and Methods"), allowing for the purification of highly specific affinity purified anti-PCP antibodies from the crude serum. A comparative Western blot using affinity purified antibodies is shown in Figure 2, lane 2, and demonstrates an improvement in antibody specificity.

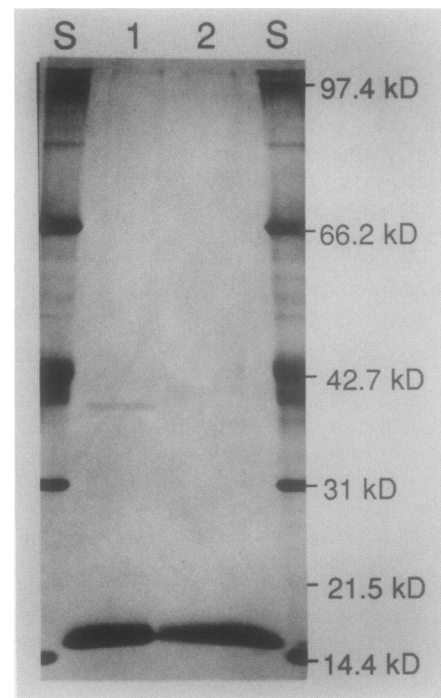


FIG. 1. SDS-PAGE analysis of gel purified PCP apoprotein. Each lane has 15  $\mu$ g gel-purified PCP. Lane 1 material was boiled 5 min in 1X electrophoresis buffer immediately prior to electrophoresis through the 10 to 20% linear acrylamide gradient gel. Lane 2 material was brought to 2% SDS in 1X electrophoresis buffer and left to incubate 30 min at room temperature prior to electrophoresis. "S" denotes SDS-PAGE low mol wt standards. Visualization of protein in the gel was by double staining with silver and Coomassie.

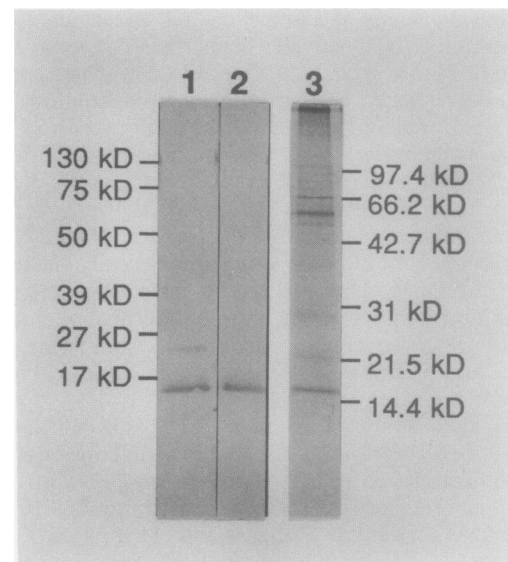


FIG. 2. Western blot analysis of crude and affinity purified anti-PCP antibodies. Lanes 1 and 2: 3  $\mu$ g of crude *Glennodinium* sp. protein per well was electrophoresed through an SDS-polyacrylamide gel (10–20%), electroblotted to nitrocellulose and probed with either crude anti-PCP antiserum (lane 1) at a 1:1000 dilution, or affinity purified anti-PCP antibodies (lane 2) at a dilution of 1:500, equal to 2  $\mu$ g/mL IgG. Lane 3: 10  $\mu$ g of crude *Glennodinium* sp. protein stained with silver and Coomassie.

The affinity purified antibodies detect a single band at  $M_r$  of 15.5 kD in SDS gels on crude protein extracts of *Glenodinium* sp.

Since PCP complexes are resolved into several pI isomers during purification, it was important to determine which species of PCP were recognized by the affinity purified antibodies. Elution of PCP complexes from DEAE-Sephacel by a decreasing linear pH gradient resolved the complexes into four distinct 478 nm absorbing peaks (DEAE-Sephacel 1-4). A final peak of 478 nm absorbing material (DEAE-Sephacel 5) eluted from the column only after the addition of 0.4 M NaCl to the acidic end of the pH gradient. These fractions were concentrated and analyzed by IEF gel electrophoresis, as shown in Figure 3A. No staining of the gel in Figure 3A was done—the bands appear by virtue of their native orange color due to the peridinin in the complexes. To assess the reactivity of the major isoforms of PCP with the affinity purified antibodies, a Western blot was performed on a similar focusing gel, except that one twentieth of the material was loaded in each lane (compared to the loading in Fig. 3A), electrophoresed, then blotted to nitrocellulose for reaction with antibodies. Figure 3B is a Western blot of such a gel, and shows that the affinity anti-PCP antibodies recognize all of the major pI isomers of PCP complexes found in *Glenodinium* sp.

**Regulation of PCP Complexes.** Affinity purified anti-PCP antibodies were used in two different experiments to assess qualitative changes in the amounts of PCP gene products in *Glenodinium* sp. when cells were grown under different light conditions. Following harvesting of high and low light cultures, parallel protein and RNA preparations were made (see "Materials and Methods"). In the first experiment, crude protein extracts from high light and low light grown cells were normalized for protein concentration and electrophoresed on 10 to 20% SDS polyacrylamide gels, blotted to nitrocellulose and probed with affinity purified anti-PCP antibodies. Results of such a blot are shown in Figure 4, lanes 1 and 2, and demonstrate a two- to threefold increase of PCP apoprotein in low light grown cells relative to high light grown cells. This low light increase of PCP apoprotein observed in Western blots is also seen in the silver/Coomassie stained gel of total *Glenodinium* sp. proteins (Fig. 4, lanes 3 and 4). These results correlate with the quantities of total peridinin as determined by organic extraction and HPLC pigment analysis for high light and low light grown *Glenodinium* sp. (N Nelson, unpublished data).

The second experiment looking at PCP gene products in cells grown in different light conditions was based on *in vitro* translation of total RNA extracted from the high light and low light cultures. In this experiment, equivalent amounts of total RNA from high light and low light grown cells were translated *in vitro* in the presence of [ $^{35}$ S]methionine, and the products subjected to immunoprecipitation with affinity purified anti-PCP antibodies, followed by gel electrophoresis and autoradiography. The results of this experiment appear in Figure 5, A and B. Both the autoradiogram in Figure 5A and the densitometric scan in Figure 5B show an approximate twofold increase in precipitated PCP apoprotein from the translation mix supplied with RNA from low light grown cells (lane 2) relative to that from high light grown cells (lane 5). This increase in precipitable products from the *in vitro* translation reactions must arise from an increase in translatable PCP mRNAs in the total RNA population in low light grown cells and roughly correlates with the increase in PCP apoprotein observed in protein extracts.

Figure 5A also reveals that more than one species of primary translation product are specifically precipitated by the affinity purified anti-PCP antibodies, and that these products are larger than native PCP apoprotein. There are two major species (one at 23 kD and the other at 24 kD) and two very minor species (one at 22 kD and one at about 16 kD) specifically precipitated from these reactions (see Fig. 5A, lanes 2 and 5). These results

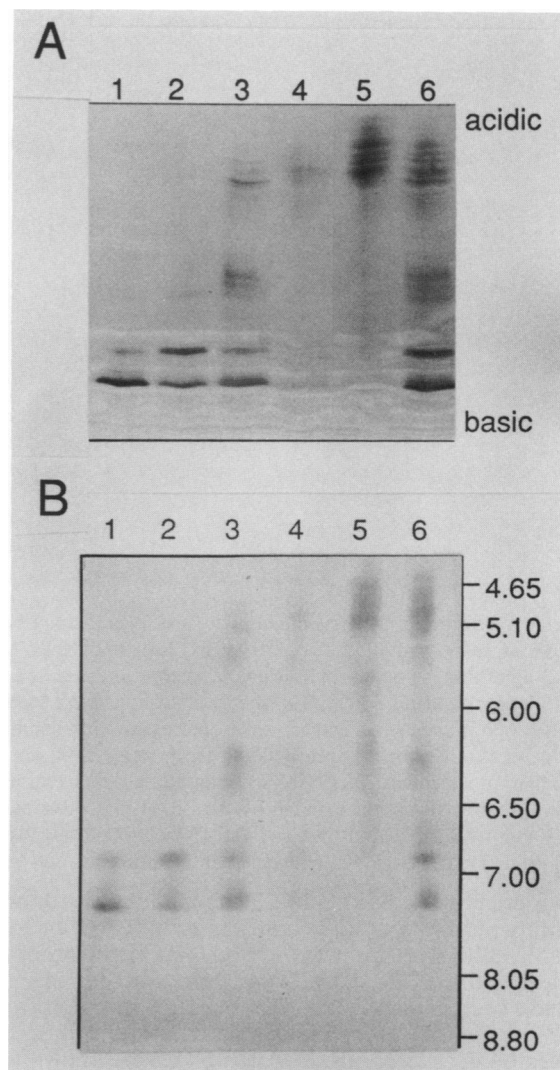


FIG. 3. IEF gel electrophoresis of PCP isomers. A, 20  $\mu$ L each (equal to 0.4 to 0.8  $OD_{478}$  units) of the DEAE-Sephacel pools I to V (lanes 1 through 5) and a Sephadex G-100 pool (lane 6) were applied to loading strips directly on the surface of the LKB Ampholine PAGplate gel (pH 3.5–9.5) and electrophoresed for 2 h according to the manufacturer's directions. No staining was employed for visualization of protein. The bands that appear were dark orange colored due to their peridinin content. B, 1  $\mu$ L each of the same PCP samples (equal to from 0.02 to 0.04  $OD_{478}$  units) was loaded directly onto the LKB Ampholine PAGplate gel (without loading strips) and similarly electrophoresed. Protein was then electroblotted to nitrocellulose and probed with affinity purified anti-PCP antibodies (diluted 1:200; equal to 5  $\mu$ g/mL IgG) in a standard Western blot assay.

indicate that translatable PCP mRNAs code for larger pre-protein forms of PCP apoproteins that are not processed to their native sizes (15.5 kD) in the reticulocyte translation system. It is possible that some of the less prevalent forms arise either from proteolysis or aberrant synthesis in a heterologous system (reticulocyte lysate) and are not necessarily biologically significant, but at least one (and probably both) of the larger, more predominant species must be reflective of mRNA coding sequences in the total RNA population.

## DISCUSSION

The crude anti-PCP antiserum generated here showed cross-reactivity with several *Glenodinium* sp. proteins, presumably due

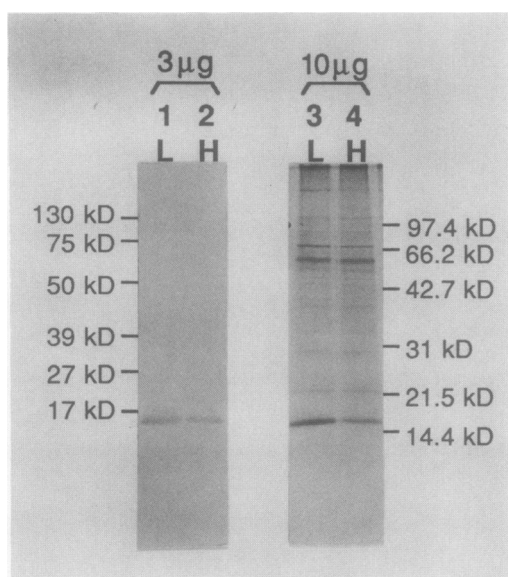


FIG. 4. Western blot analysis of *Glenodinium* sp. proteins from high light ( $300 \mu\text{E m}^{-2}\text{s}^{-1}$ ) and low light ( $80 \mu\text{E m}^{-2}\text{s}^{-1}$ ) grown cells. Lanes 1 and 2:  $3 \mu\text{g}$  of total soluble protein from *Glenodinium* sp. grown in low light (L, lane 1) and high light (H, lane 2) conditions were loaded per lane, electrophoresed as in Figure 2, electroblotted to nitrocellulose and probed with affinity purified anti-PCP antibodies at a 1:1000 dilution ( $1 \mu\text{g}/\text{mL}$  IgG) in a standard Western blot assay. Protein concentrations in the soluble extracts were determined by the Bradford protein assay (3). Lanes 3 and 4:  $10 \mu\text{g}$  each of the low light (L, lane 3) and high light (H, lane 4) protein extracts stained with silver and Coomassie.

to a lack of purity in the initial PCP preparations used as antigen. By affinity purification of the crude antiserum, we obtained the subset of antibodies from the serum that react strongly and specifically with PCP, and not with other protein species that may have been contaminating the original antigen preparation. Figure 2 demonstrates the improvement in antibody specificity achieved by these methods. PCP complexes are known to exist in *Glenodinium* sp. cells in several isoelectric forms (21 and Fig. 3A), so it was also necessary to determine whether or not the affinity purified anti-PCP antibodies would recognize all or only some of these forms. It was expected that these affinity purified anti-PCP antibodies would be cross-reactive with most, if not all, of the major isoforms of PCP, since these forms are presumed to have some epitopes in common. Figure 3, A and B, demonstrates that all major isoforms of PCP are recognized by the affinity purified anti-PCP antibodies.

Since the affinity purified anti-PCP antibodies recognize all major forms of PCP, they should be useful in experiments to determine the level(s) at which PCP antigen in *Glenodinium* sp. cells is regulated by light. The two experiments reported here show qualitatively that not only do the levels of PCP apoprotein rise in low light (relative to high light) grown cells, but there is also a concomitant rise in translatable PCP mRNAs. The rise in translational products—PCP antigen—is paralleled by a rise in total peridinin in low light grown cells (N Nelson, unpublished data), and so a post-translational assembly of a pool of stored PCP apoprotein with new pigment seems unlikely, although more quantitative determinations of PCP apoprotein in cells are now underway to investigate further this possibility.

The rise in translatable PCP mRNAs observed in low light grown cells, viewed in light of the concomitant increases in PCP apoprotein and chromophore pigmentation (*i.e.*, Chl *a* and peridinin), indicates that light regulation of PCP is at the level of PCP mRNA abundance. It is not yet known whether low light

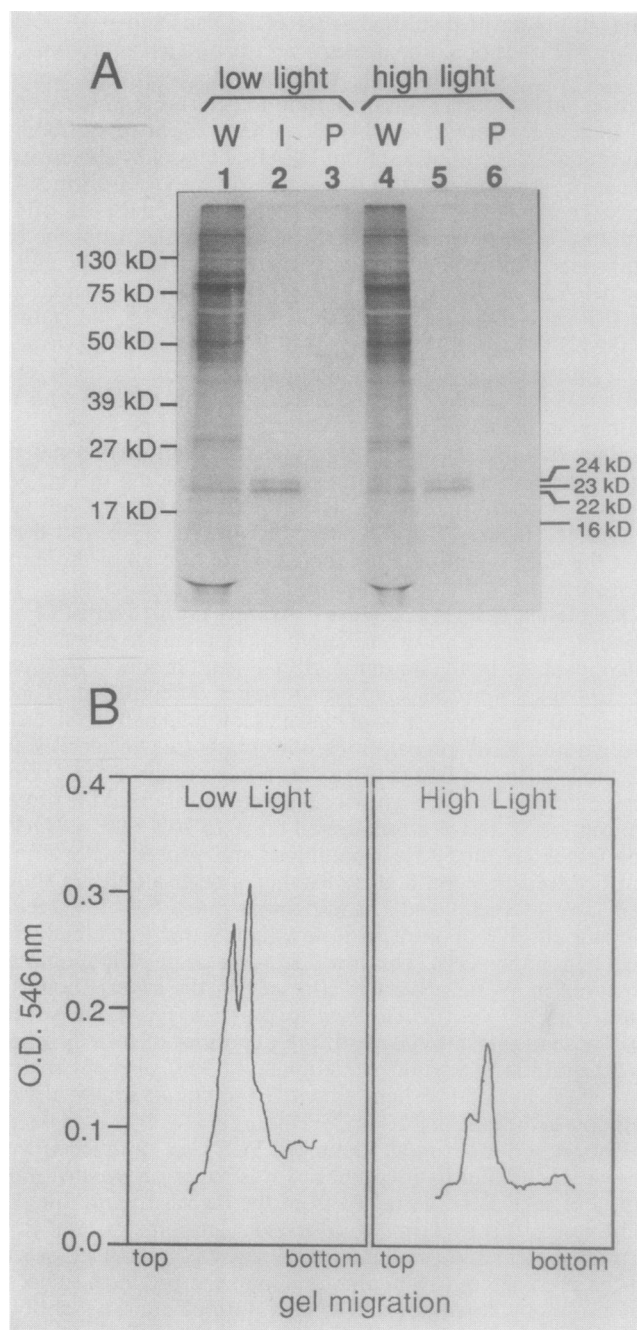


FIG. 5. Immunoprecipitation of PCP apoprotein from *in vitro* translation reactions supplied with total RNA from high light and low light grown *Glenodinium* sp. cells. A,  $10 \mu\text{g}$  total RNA from low light and high light grown cells was translated in  $40 \mu\text{L}$  translation reactions, immunoprecipitated with affinity purified anti-PCP antibodies, and the products analyzed on an SDS-polyacrylamide (10–20%) gel followed by autoradiography. Lanes 1 to 3 represent the reaction products from low light RNAs, and lanes 4 to 6 represent the reaction products from high light RNAs. W represents products from whole translation reactions; I denotes products immunoprecipitated with affinity purified anti-PCP antibodies; P denotes products immunoprecipitated with control serum. B, Densitometric scans of lanes 2 and 5 of the autoradiogram in A in the regions of the immunoprecipitated bands. The scanning rate was  $30 \text{ cm}/\text{h}$  and the chart speed was  $60 \text{ cm}/\text{h}$ , resulting in a twofold expansion of the horizontal axis.



growth conditions cause a rise in *de novo* transcription from PCP genes or, alternatively, the activation, stabilization or post-transcriptional processing of a pool of preexisting mRNA precursors. Most data on light regulated genes in higher plants implicate control at the level of transcription, but light effects on post-transcriptional events, such as mRNA degradation, translation and protein turnover have also been reported (for reviews, see Refs. 9 and 13). Resolution of these questions awaits the refinement of present techniques and the development of probes for PCP transcripts.

The observation of larger immunoprecipitated products from *in vitro* translation reactions suggests that PCP mRNAs code for precursor proteins that are normally processed. PCP apoproteins appear to be encoded by nuclear genes, since immunoprecipitable products of *in vitro* translation are observed in reactions supplied with polyadenylated RNAs, but are absent in reactions lacking polyadenylated RNAs (data not shown). This suggests that the function of the putative processing events may be in the transport and localization of cytoplasmically synthesized PCP pre-products into the chloroplast. Precedence for this includes the nuclear encoded Chl *a/b*-binding proteins (8) and the small subunit of ribulose biphosphate carboxylase (16) of higher plants that are synthesized as precursors and are processed in this fashion during transport into the chloroplasts.

Several other interesting questions are raised by these studies. For example, the significance of the various isoforms of PCP complexes in *Glenodinium* sp. cells is not known, but the phenomenon is clearly not restricted to *Glenodinium* sp. since multiple isoforms of PCP complexes are a common feature of all PCP-containing dinoflagellates (5, 19, 21). The exact nature of the differences between isoforms is not known, nor is there much information on when or how these differences arise. It is possible that in *Glenodinium* sp. only one (or perhaps several) major species is (are) light regulated and the other forms represent constitutive species in the cell. This type of differential regulation by light has been observed in the multigene families encoding ribulose 1,5-bisphosphate carboxylase (7, 10) and the light-harvesting Chl *a/b*-binding proteins in higher plants (1), as well as the light-harvesting phycobiliprotein system of cyanobacteria (6). Further experiments are underway to determine the degree of differential regulation of the various PCP isoforms in *Glenodinium* sp.. In addition, there is virtually no information on the kinetics of PCP regulation and PCP turnover in *Glenodinium* sp. cells. Most of these questions await the refinement of present techniques and the generation of additional molecular probes, but our results indicate that light regulation of PCP complexes in *Glenodinium* sp. is occurring mainly at the level of PCP mRNA abundance.

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## LITERATURE CITED

- ANDERSON JM 1986 Photoregulation of the composition, function, and structure of thylakoid membranes. *Annu Rev Plant Physiol* 37: 93–136
- BENSON SB, EL TRIPLETT 1974 The synthesis and activity of tyrosinase during development of the frog *Rana pipiens*. *Dev Biol* 40: 270–282
- BRADFORD MM 1976 A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72: 248–254
- CATHALA G, J-F SAVOURET, B MENDEZ, BL WEST, M KARIN, JA MARTIAL, JD BAXTER 1983 A method for isolation of intact, translationally active ribonucleic acid. *DNA* 2: 329–335
- CHANG SS, RK TRENCH 1984 The isoelectric forms, quaternary structure and amino acid composition of peridinin-chlorophyll *a*-proteins from the symbiotic dinoflagellate *Symbiodinium microadriaticum* Freudenthal. *Proc R Soc Lond B* 222: 259–271
- CONLEY PB, PG LEMAUX, TL LOMAX, AR GROSSMAN 1986 Genes encoding major light-harvesting polypeptides are clustered in the genome of the cyanobacterium *Fremyella diplosiphon*. *Proc Natl Acad Sci USA* 83: 3924–3928
- DEAN C, P VAN DEN ELZEN, S TAMAKI, P DUNSMUIR, J BEDBROOK 1985 Differential expression of the petunia ribulose biphosphate carboxylase small subunit multigene family. *EMBO J* 4: 3055–3061
- DUNSMUIR P 1985 The petunia chlorophyll *a/b* binding protein genes: a comparison of *Cab* genes from different gene families. *Nucleic Acids Res* 13: 2503–2518
- ELLIS RJ 1986 Photoregulation of gene expression. *Biosci Rep* 6: 127–136
- FLUHR R, P MOSES, G MORELLI, G CORUZZI, N-H CHUA 1986 Expression dynamics of the pea *rbcS* multigene family and organ distribution of the transcripts. *EMBO J* 5: 2063–2071
- HAXO FT, JH KYCIA, GF SOMERS, A BENNETT, HW SIEGELMAN 1976 Peridinin-chlorophyll *a*-proteins of the dinoflagellate, *Amphidinium carterae*. *Plant Physiol* 57: 297–303
- KINDLE KL 1987 Expression of a gene for a light-harvesting chlorophyll *a/b*-binding protein in *Chlamydomonas reinhardtii*: effects of light and acetate. *Plant Mol Biol* 9: 547–563
- KUHLEMEIER C, PJ GREEN, N-H CHUA 1987 Regulation of gene expression in higher plants. *Annu Rev Plant Physiol* 38: 221–257
- LAEMMLI UK 1971 Cleavage of structural proteins during the assembly of the head of bacteriophage T<sub>4</sub>. *Nature* 227: 680–685
- MARCH SC, I PARIKH, P CUATRECASAS 1974 A simplified method for cyanogen bromide activation of agarose for affinity chromatography. *Anal Biochem* 60: 149–152
- MATSUOKA M, Y KAHO-MURAKAMI, Y TANAKA, Y OZAKI, N YAMAMOTO 1987 Nucleotide sequence of cDNA encoding the small subunit of ribulose 1,5-bisphosphate carboxylase from maize. *J Biochem* 102: 673–676
- MERRILL CR, D GOLDMAN, SA SEDMAN, MH EBERT 1981 Ultrasensitive stain for proteins in acrylamide gels shows regional variation in cerebrospinal fluid proteins. *Science* 211: 1437–1439
- OELMÜLLER R, I LEVITAN, R BERGFELD, VK RAJASEKHAR, H MOHR 1986 Expression of nuclear genes affected by treatments acting on plastids. *Planta* 168: 482–492
- PRÉZELIN BB 1987 Photosynthetic physiology of dinoflagellates. In M Taylor, ed. *The Biology of Dinoflagellates*. Blackwell, London, pp 174–223
- PRÉZELIN BB, BA BOCZAR 1986 Molecular bases of cell absorption and fluorescence in phytoplankton: potential applications to studies in optical oceanography. In FE Round, DJ Chapman, eds. *Progress in Phycological Research*, Vol. 4. Biopress, Bristol, pp 349–364
- PRÉZELIN BB, FT HAXO 1976 Purification and characterization of the peridinin-chlorophyll *a*-proteins from the marine dinoflagellates *Glenodinium* sp. and *Gonyaulax polyedra*. *Planta* 128: 133–141
- PRÉZELIN BB, HA MATLICK 1980 Time-course of photoadaptation in the photosynthesis-irradiance relationship of a dinoflagellate exhibiting photosynthetic periodicity. *Mar Biol* 58: 85–96
- TOBIN EM, J SILVERTHORNE 1985 Light regulation of gene expression in higher plants. *Annu Rev Plant Physiol* 36: 569–593
- TOWBIN H, T STAHELIN, J GORDON 1979 Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc Natl Acad Sci USA* 76: 4350–4354