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Characterization of the light harvesting proteins of the chromophytic alga, *Olisthodiscus luteus* (*Heterosigma carterae*)

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The light-harvesting complexes (LHC) of the unicellular marine chromophyte, *Olisthodiscus luteus* (*Heterosigma carterae*), were fractionated by sucrose-density gradient centrifugation following digitonin solubilization, and by non-denaturing SDS-PAGE. The sucrose gradient allowed for the isolation of a major light-harvesting complex fraction, containing approximately 53% of the total chlorophyll, the majority of the chlorophyll *c* and a single polypeptide of 19.5 kDa. Up to 12 different light-harvesting polypeptides were detected in thylakoids and in the lower Photosystem I (PS I) related fractions using polyclonal antibodies specific for barley light-harvesting complexes or a diatom fucoxanthin-Chl *a/c* complex. The non-denaturing gel system of Allen and Staehelin (Anal. Biochem. 194 (1991) 214–222) allowed the resolution of a number of large pigment-protein complexes, an improvement over previous electrophoretic separation methods applied to the chromophytic algae. Differences among the light-harvesting polypeptides in apparent molecular mass, immunological cross-reactivity and distribution among different pigment-protein complexes suggest that this protein family may be as complex as the family of chlorophyll *a/b* light-harvesting polypeptides in green algae and higher plants.

Introduction

The eukaryotic algae are generally divided into three major groups on the basis of a number of characteristics which include the pigment compositions of their light-harvesting antennas: the Chl *a/b* chlorophytes with antenna complexes similar to those of higher plants, the Chl *a/c* chromophytes and the rhodophytes which have neither Chl *b* nor Chl *c*, but have phycobilisomes resembling those of cyanobacteria. The chromophytic algae are a very diverse group characterized by being rich in xanthophylls, having Chl c_1 and/or Chl c_2, and usually having thylakoids arranged in groups of three [1].

Fucoxanthin is the primary accessory xanthophyll found in the antenna complexes of many chromophytic algae [2], including *Olisthodiscus* [3]. Fucoxanthin-chlorophyll-protein (FCP) complexes have been isolated from the Bacillariophyceae (diatoms) [4–9], the Chrysophyceae (golden-brown algae) [10], the Prymnesiophyceae [11] and the Phaeophyceae (brown algae) [12,13]. Chlorophyll *a/c* complexes lacking fucoxanthin have been reported in the Bacillariophyceae [6] and Phaeophyceae [13], but these studies use Triton X-100 and SDS, respectively, so the loss of fucoxanthin due to complex degradation can not be ruled out [2]. Typically, two or three light-harvesting polypeptides in the 15–20 kDa range have been described, as determined by SDS-PAGE, although a recent study detected 4–6 polypeptides that cross-reacted to a LHC specific antibody [14]. Recently, a novel pigment-protein complex with a single polypeptide of 31 kDa has been isolated from the chrysophyte, *Ochromonas danica* [15]. The light-harvesting complexes of other chromophytes that do not have fucoxanthin as a main accessory pigment, are discussed in a recent review [2].

In the diatom *Phaeodactylum tricornutum*, inhibitor studies have shown these light-harvesting polypeptides to be nuclear encoded and synthesized on cytoplasmic ribosomes [16]. Members of this fucoxanthin-Chl *a/c* antennal family are the only ones that have been cloned and sequenced [17] and found to have sequence similarity to the Chl *a/b* binding proteins of higher plants. An almost complete amino acid sequence has been obtained from the dinoflagellate, *Amphidinium*, and a partial sequence from the prymnesiophyte

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Abbreviations: PS I, Photosystem I; PS II, Photosystem II; P700, reaction center chlorophyll of PS I; LHC, light-harvesting complex; Chl, chlorophyll; FCP, fucoxanthin chlorophyll protein; CP1a, PS I plus its specific LHC in higher plants; CAB, chlorophyll *a/b*.

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Pavlova [18]; they are clearly related to the diatom proteins as well as to the Chl *a/b* (CAB) proteins [18,19].

The chromophyte alga previously known as *Olisthodiscus luteus* was the first alga shown to carry the small subunit of ribulose-1,5-bisphosphate carboxylase on its chloroplast genome [20], in contrast to its nuclear localization in higher plants and chlorophyte algae. The taxonomic position of this isolate, *Olisthodiscus luteus* Carter, has been uncertain for a number of years, but recent studies place it in the Raphidophyceae and suggest that it may belong to the genus *Heterosigma* [21]. If this is the case, it should be named *Heterosigma carterae* [22,23]. In spite of the large amount of work done on the chloroplast genome of this alga, no raphidophyte chlorophyll-protein complexes have been characterized. Our initial work was directed towards isolation and characterization of the various light-harvesting complexes (LHC's) and their pigment binding proteins, in order to determine the complexity of the LHC family and its relatedness to LHC's of other algae and higher plants.

Materials and Methods

Growth and cell fractionation

An axenic culture of *Olisthodiscus luteus* was maintained as previously described [24]. Late log phase cells were harvested at $400 \times g$ for 12 min, resuspended in 0.33 M sorbitol, 1 mM $MgCl_2$, 50 mM Hepes (pH 7.6) and proteinase inhibitors (1 mM phenylmethylsulfonyl fluoride, 5 mM ϵ-amino-*n*-caproic acid, 1 mM benzamidine-HCl, 1 mg/ml leupeptin). Cells were lysed under 4000 kPa (600 psi) nitrogen in a Yeda Press (Yeda Research and Development Co., Rehovot, Israel) to release the chloroplasts. The chloroplasts were separated by differential centrifugation in a swinging bucket rotor at $6500 \times g$ for 12 min at 4°C. Chloroplasts were washed three times in 0.1 M NaCl, 5 mM $MgCl_2$, 20 mM Tricine (pH 8.0) (including proteinase inhibitors) yielding a washed thylakoid fraction. Thylakoids to be used for non-denaturing gel electrophoresis were made up to 10% glycerol prior to quick freezing in liquid nitrogen and storage at −80°C [25]. Thylakoids used for sucrose gradient fractionation were used fresh and solubilized with digitonin (BDH) at a detergent to chlorophyll ratio of 100:1, on ice for 4 h. After centrifugation at $40\,000 \times g$ for 30 min, the supernatant was loaded onto a 0.3 M–1.2 M linear sucrose gradient on top of 1.3 M and 1.6 M sucrose cushions. Sucrose solutions were made up in 10 mM Tricine (pH 8.0) containing 0.05% (w/v) digitonin. Samples were centrifuged for 24 h at $250\,000 \times g$ in a swinging bucket rotor at 4°C. Fractions 2 and 3 (Fig. 1) were precipitated at $40\,000 \times g$ following dialysis at 4°C in 0.1 M $CaCl_2$, 10 mM $MgCl_2$, 10 mM Tricine (pH 8.0) including proteinase inhibitors (as above). Due to the large amount of detergent at the top of the gradient, Fraction 1 was pelleted at $100\,000 \times g$ following extended periods of dialysis with many changes of the dialysis buffer. Chlorophyll concentrations were determined according to Jeffrey and Humphrey [26].

Denaturing SDS-PAGE and Western blotting

For denaturing gel electrophoresis, samples were solubilized in 2% SDS, 65 mM Tris-HCl (pH 6.8), 50 mM dithiothreitol, 10% glycerol and heat-denatured for 1 min at 100°C. Gel slices from non-denaturing PAGE were incubated in 2 × sample buffer (4% SDS, 132 mM Tris-HCl (pH 6.8), 0.1 M dithiothreitol, 20% glycerol) at room temperature for 2 h, then heated to 80°C for 20 min. Polypeptides were separated on 12–16% or 7.5–15% SDS polyacrylamide gels (acrylamide:bisacrylamide 37.5 : 1) containing 0.05% SDS and 1.32 M Tris-HCl (pH 8.8), with a 2 cm stacking gel containing 5% acrylamide, 0.1 M Tris-HCl (pH 6.1)

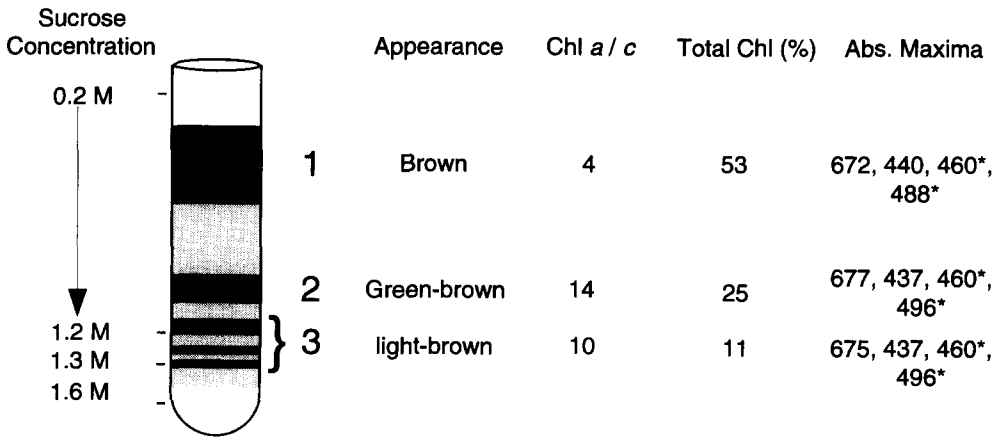


Fig. 1. Schematic representation of sucrose gradient fractionation of digitonin solubilized thylakoids, with Chl *a/c* ratios, percentage of total chlorophyll in each fraction and absorption maxima for the three major fractions. An asterisk in the absorption data indicates a shoulder.

and 0.1% SDS. Gels were run for 18 h at 4°C with the Laemmli buffer system [27]. Colored molecular mass standards (Amersham) were used to estimate molecular mass.

Proteins were electrotransferred to nitrocellulose in 50 mM sodium acetate (pH 7.0) overnight at 200 mA and 4°C. Western blotting was carried out as previously described [28]. Nitrocellulose was reblotted after stripping the membrane in 0.1 M glycine-HCl (pH 2.2), 20 mM magnesium acetate, 50 mM KCl [29] followed by reblocking in 3% Hipure liquid gelatin (Norland Products, New Brunswick, NJ) in phosphate-buffered saline (1.37 M NaCl, 27 mM KCl, 81 mM Na_2HPO_4, 15 mM KH_2PO_4, (pH 7.4)). Proteins were Coomassie stained using standard methods. Polyclonal antibodies used in this study were α-CP1a, specific for barley CP I plus LHC I [28] and α-FCP, specific for *Phaeodactylum tricornutum* fucoxanthin-chlorophyll *a/c* (FCP) protein complex [16]. Other antibodies include the α-PsaD antibody specific for a PS I associated subunit (also called PS I subunit No. 2) [30] and α-D1, specific for the PsbA polypeptide of PS II, provided by L. McIntosh.

Non-denaturing gel system

Thylakoids were solubilized with a mixture of 0.9% octylglucoside, 0.9% decyl maltoside and 0.2% lithium dodecyl sulphate (in 2 mM Tris-maleate (pH 8.0), 10% glycerol and with proteinase inhibitors) and resolved on a non-denaturing gel system according to Allen and Staehelin [25] except that a 7% acrylamide gel was used with an acrylamide to bisacrylamide ratio of 150:1. A stacking gel was not used because it resulted in degradation of the pigment-protein complexes. Samples were solubilized on ice for 30 min at an anionic detergent to chlorophyll ratio of 30:1 with occassional mixing, then centrifuged in an microfuge for 20 min at 4°C. Samples were electrophoresed at 10 mA for 1.5 to 2.5 h at 4°C. Estimations of molecular mass were done using non-denatured, high molecular mass markers (Pharmacia). Gel bands were excised and electrophoresed on a denaturing gradient gel as described above. Samples to be used for fluorescence data were excised from the gel and quick frozen in liquid nitrogen prior to storage at −80°C.

Spectroscopy and fluorescence measurements

Absorption spectra were recorded on a Cary 210 Spectrophotometer at room temperature. P700 content was measured from the sucrose gradient fractions directly by monitoring the recovery of absorption at 700 nm after photo-oxidation by saturating red light with 1.7 mM ascorbate and 0.075 mM methylviologen present in the reaction mixture [31].

Fluorescence emission spectra were recorded with a Perkin Elmer LS50 fluorometer with the 77 K low-temperature attachment and red sensitive photomultiplier. Excitation wavelength was 440 nm and the excitation and emission slit widths were adjusted to 10 nm and 5 nm, respectively. A 530 nm cut off filter helped to remove Rayleigh scatter in the 620 nm range. Gel slices from the non-denaturing gel system were fitted in the cuvette with 60% glycerol and frozen in liquid nitrogen prior to measuring. Emission spectra were corrected for the drop in photomultiplier sensitivity in the 600–800 nm range using an averaged correction factor provided by Perkin Elmer.

Results

Fractionation of digitonin-solubilized membranes by sucrose gradient centrifugation

Thylakoid membranes solubilized with digitonin were resolved into three major fractions on a sucrose gradient (Fig. 1). The top dark brown fraction (fraction 1) was rich in fucoxanthin and chlorophyll *c* as demonstrated by an absorbance peak at 440 nm and a prominent shoulder at 460 nm, respectively. Fraction 1 also showed visible red fluorescence upon excitation with long-wavelength UV light, indicating the detachment of the light-harvesting complex from the reaction center. Both Fractions 2 and 3 were enriched in PS I with Chl *a*/P700 ratios of 340 and 420 compared to 1200 for Fraction 1.

SDS-PAGE (Fig. 2A) showed that there was a single polypeptide in Fraction 1. It cross-reacted with an antibody specific for the FCP from the diatom *Phaeo-*

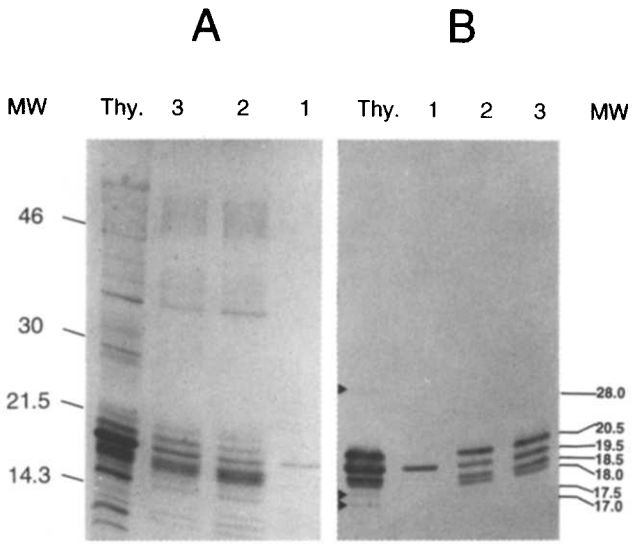
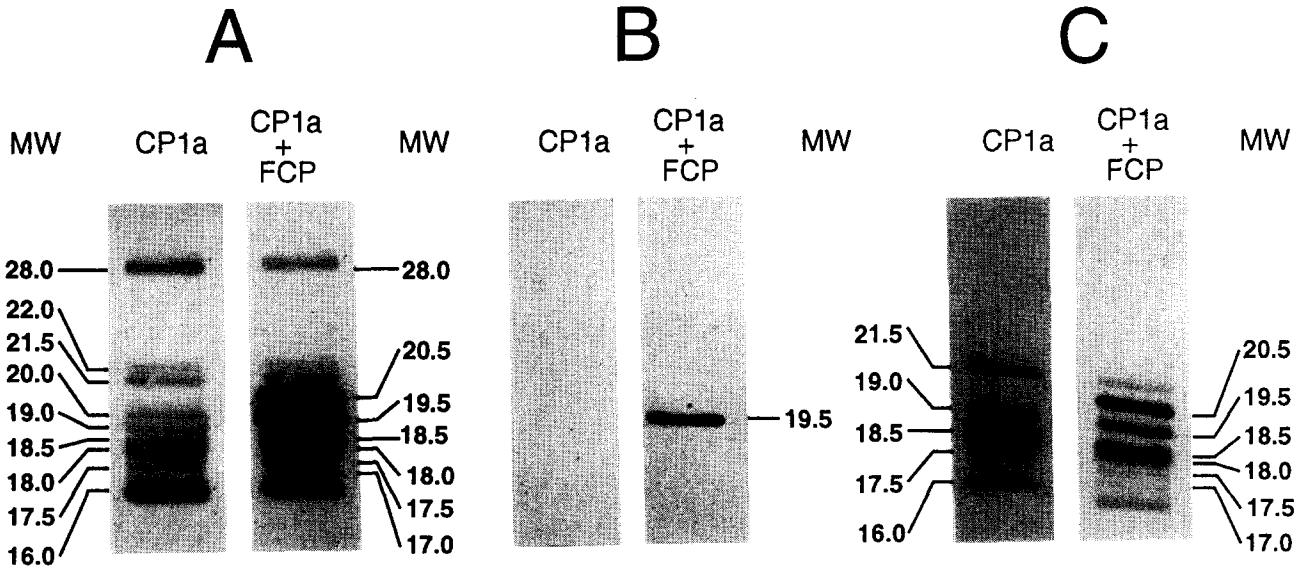


Fig. 2. Polypeptides of digitonin sucrose gradient fractions separated by denaturing 12–16% gradient SDS-PAGE. (A) Stained with Coomassie blue. Thy.: Whole thylakoids; 1: Fraction 1; 2: Fraction 2; 3: Fraction 3. (B) Western blot immunoprobed with the α-FCP antibody. Lanes labelled as in panel A. MW, molecular mass standards in kDa. Arrowheads on left of Panel B denote faint bands at 28.0, 17.0 and 17.5 kDa in whole thylakoids (Thy.) only.

dactylum tricornutum (Fig. 2B). A number of tryptic peptides from this polypeptide have been sequenced: three of these peptide sequences could be aligned with the sequences of the diatom FCP's and higher plant CABs in the region of the first transmembrane helix ([32], Durnford, unpublished data), indicating that the antibody results were due to sequence conservation.

Both lower fractions showed a similar polypeptide pattern with a number of bands of 16–22 kDa, a sharp band at 37 kDa and diffuse bands in the 49–55 kDa range (Fig. 2A). The four polypeptides estimated as 20.5, 19.5, 18.5 and 18.0 kDa cross-reacted strongly with the α-FCP antibody (Fig. 2B). Two minor polypeptides at about 17.5 kDa and 16.5 kDa were faintly immunostained (lower arrowheads). The FCP antibody also detected a polypeptide of apparent molecular mass 28 kDa, found only in the thylakoid fraction (upper arrowhead, see also Fig. 3A). Note that in this figure and subsequent figures, the apparent molecular masses determined by SDS-PAGE are used as labels to identify distinguishable polypeptides, and are not meant to imply accurate molecular mass determinations.

Using an antibody specific for barley CP1a (PS I core complex plus its corresponding light-harvesting polypeptides [28]), a different subset of cross-reacting polypeptides was found (Fig. 3). The α-CP1a antibody detected five major bands at approx. 16, 17.5, 18.5, 19 and 21.5 kDa in fractions 2 (Fig. 3C, left) and 3, while an additional four bands of about 18, 20, 22, 28 kDa could be resolved in thylakoids (Fig. 3A, left). To identify which of the polypeptides reacting with the α-CP1a antiserum were also immunostained with the α-FCP antiserum, the immunoblots shown in the left panels of Fig. 3A–C were stripped and reblotted with the α-FCP (shown in Fig. 3A–C, right panels). The bands of 20.5, 19.5, 18.5, and 18.0 that were prominent in thylakoids immunostained only with α-FCP (Fig. 2) are heavily stained in Fig. 3A (right panel) and are clearly distinguished from the bands labeled 21.5 and



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	Molecular Weight (kDa)											
Antiserum	28.0	22.0	21.5	20.5	20.0	19.5	19.0	18.5	18.0	17.5	17.0	16.0
CP1a	+	+	+	-	+	-	+	+*	+*	+	-	+
Fac	+	-	-	+	-	+	-	+	+	+	+	-

*An apparent single band at 18-18.5 kDa may be a doublet

Fig. 3. Western blot of sucrose gradient fractions immunoprobed with α-CP1a (left panels) then stripped, blocked and immunoprobed with α-FCP (right panels) on the same blot. (A) Whole thylakoids (B) Fraction 1 from sucrose gradient (C) Fraction 2 from sucrose gradient. (D) Table indicating the cross-reactivity of the polypeptides detected on the western blot. Approximate molecular masses (kDa) are used as labels to distinguish individual bands.

22.0 above them and the 16.0 band below which only cross-reacts with the α-CP1a antiserum. Similar results were obtained with fraction 2 when it was reblotted with α-FCP (Fig. 3C, right panel). Note that the major light-harvesting polypeptide in fraction 1, which was immunostained with the α-FCP, was not detected using the α-CP1a (Fig. 3B). Only four polypeptides, at 28, 18.5, 18 and 17.5 kDa, appeared to react with both antisera. Results of the immunoblotting with the two antisera are summarized in Fig. 3D. These results show that there are up to 12 polypeptides in the FCP/CAB family in *Olisthodiscus*, a larger number than previously reported for any chromophytic alga.

Fractionation by non-denaturing PAGE

Pigment-protein complexes were isolated from *Olisthodiscus* thylakoids solubilized in 0.9% octylglucoside, 0.9% decyl maltoside and 0.2% lithium dodecyl sulphate, and separated by means of the non-denaturing gel system of Allen and Staehelin [25] (Fig. 4). With this system, eleven pigment-protein complexes were resolved (Fig. 4A). Bands 1 and 10 were resolved into two complexes (1b, 10b) with longer periods of electrophoresis (Fig. 4B), but this resulted in some degradation of the central pigment-protein complexes. The first ten pigment-protein complexes were green and lacked noticeable fucoxanthin while band 11 was a brown fraction making up approx. 40% of the total protein.

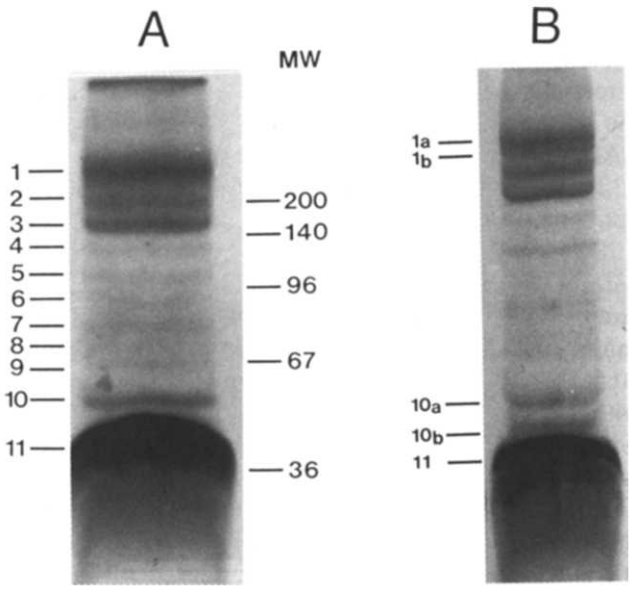

Fig. 4. Unstained 7% polyacrylamide gel separating pigment-protein complexes of thylakoids solubilized with 0.9% octylglucoside, 0.9% decyl maltoside, 0.2% lithium dodecyl sulfate [25]. (A) Electrophoresed for 1.5 h at 10 mA at 4°C. (B) Similar gel electrophoresed for 2.5 h. Apparent molecular masses are given in kDa.

Denaturing SDS-PAGE of complexes 1–3 (Fig. 5A)

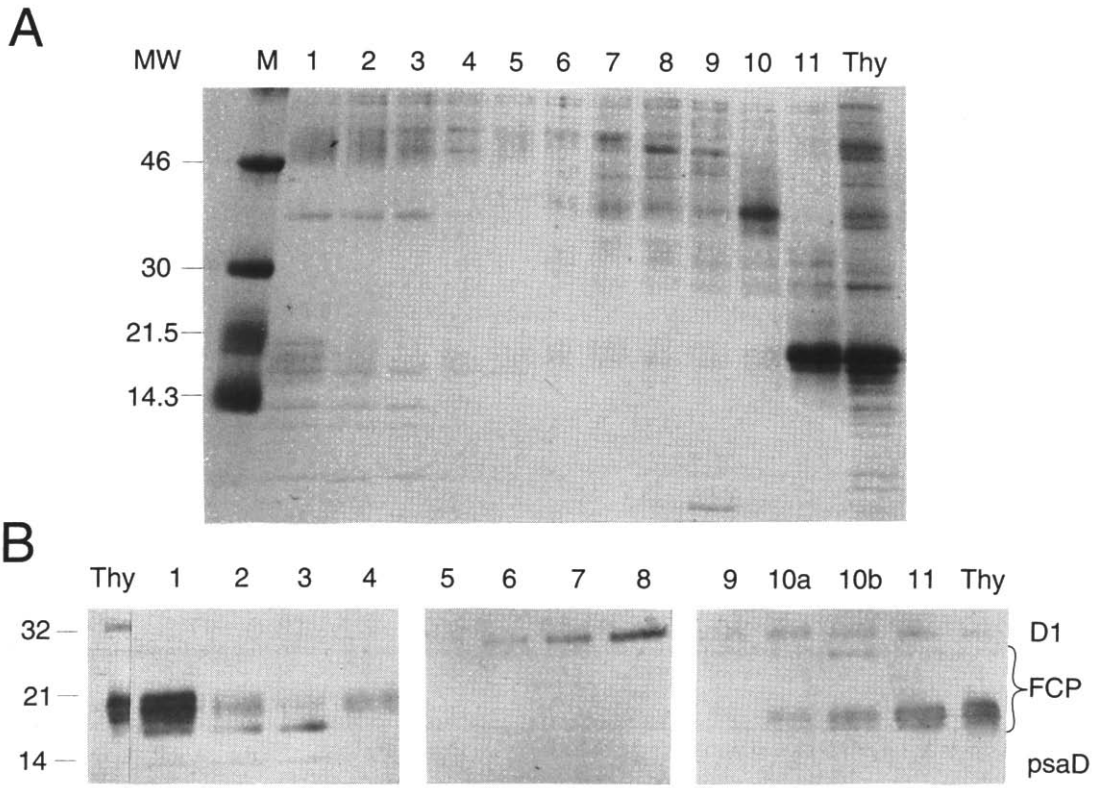

Fig. 5. Analysis of isolated pigment-protein complexes. (A) 7.5–15% denaturing SDS-PAGE of pigment protein complexes 1–11 separated from non-denaturing PAGE. (B) Western blot analysis of the same pigment protein complexes. Antibody used in sequential immunoprobing indicated on the right; Molecular masses in kDa on the left.

showed a broad stained band of about 53 kDa, a 37 kDa band, and a number of sharper bands in the molecular mass range (10–21 kDa) typical of the non-pigmented subunits of PS I [33]. A immunoblot of samples from a similar gel was sequentially probed with several antibodies to determine the composition of the various bands in Fig. 4. Immunodetection of the PS I-D (*psaD* gene product, Fig. 5B), and CP I polypeptides (data not shown) indicated that pigment-protein complexes 1–3 were PS I complexes. PS I core polypeptides were not detected in the other fractions. When blots were probed with the α-FCP antibody, there appeared to be a number of antennal polypeptides in the 17–20.5 kDa range associated with the PS I core complex (Fig. 5B). Complex 1 had the long-wavelength absorption maximum at 677 nm typical of PS I (Fig. 6A) and a long-wavelength chlorophyll *a* fluorescence emission maximum at 717 nm (Fig. 6B), similar to that attributed to the PS I specific light-harvesting complex of higher plants [34,35]. It also had a second fluorescence emission maximum at 676 nm which may indicate uncoupled chlorophyll resulting from the detergent treatment (Fig. 6B).

Complexes 6–10a appeared to be PS II-related, as they had a number of polypeptides migrating as somewhat diffuse bands in the 30–50 kDa range [36]. An antibody specific to D1 (PS II reaction center polypeptide encoded by *psbA* gene) (Fig. 5B) showed that it was found in all these fractions but was absent from complexes 1–5. Complexes 5–7 had long-wavelength maxima at 674 and complexes 8–10b at 671 nm (data not shown). Complex 10a had a fluorescence emission maximum at 686 nm (Fig. 6) which is typical of PS II core complexes [8,35]. Variability in the resolution of pigment-protein complexes 4–9 was observed between different preparations of thylakoids, possibly the result of different cell culture densities which could have altered light conditions.

Complex 10b, detectable after longer periods of electrophoresis, was enriched in the 28 kDa polypeptide that cross-reacted with the α-FCP (Fig. 5). It had absorbance peaks typically associated with Chl *a*, Chl *c*, and fucoxanthin, but since it also contained some of the lower molecular mass light-harvesting polypeptides, we cannot say whether the 28 kDa polypeptide binds these pigments or not. This polypeptide may be analogous to the 31 kDa pigment-protein complex containing only Chl *a* characterized from another chromophyte, *Ochromonas* [15].

Complex 11 contained the majority of the fucoxanthin and had a low Chl *a*/Chl *c* ratio as estimated from the peaks at 440 nm and 460 nm, respectively (Fig. 6A). It appeared to be similar to fraction 1 from the sucrose gradient, but was not as pure. Rather than a single polypeptide, it had the three major light-harvesting polypeptides at 20.5, 19.5 and 18.5 kDa that cross-reacted with the diatom α-FCP antibody. One-third the amount of Complex 11 was loaded onto the gel in Fig. 5 in order to prevent overloading. Complex 11 had a fluorescence emission maximum at 681 nm at

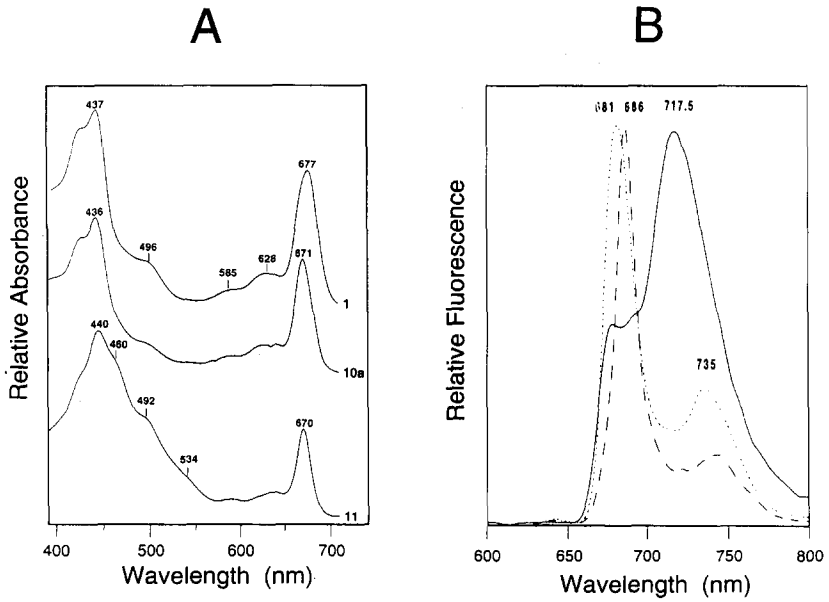

Fig. 6. Spectral characteristics of pigment-protein complexes 1, 10a, and 11 in gel slices from the non-denaturing SDS-PAGE. (A) Room temperature absorption spectrum. Spectra are offset for clarity. (B) Fluroescence emission spectra at 77 K of pigment-protein complexes. Spectra are Complex 1 (———); Complex 10a (- - - -); Complex 11 (· · · · ·).

77 K (Fig. 6) which is comparable to the LHC II of higher plants [35]. Although some D1 is immunodetected in complexes 10b and 11, it would be premature to conclude that the 28 kDa polypeptide or any of the FCP's are preferentially associated with PS II, as it is impossible to rule out comigration of individual polypeptides in this region of the gel.

An orange free pigment zone migrated just ahead of complex 11. Absorbance spectrum indicated that it contained carotenoids and a small amount of chlorophyll (data not shown). No polypeptides were detected following Coomassie staining.

Discussion

Sucrose gradient separation following digitonin solubilization has been successfully used in the isolation of light-harvesting complexes from a number of algae [2,37,38]. This fractionation technique usually results in a light-harvesting antennal fraction at the top of the gradient and a few additional denser pigment-protein complexes. We have found that the Raphidophycean alga, *Olisthodiscus*, like other chromophytic algae, has a predominant fucoxanthin-chlorophyll *a/c* pigment-protein complex released by digitonin solubilization. This complex has a single polypeptide with an apparent molecular mass of 19.5 kDa and spectral characteristics comparable to the predominant LHC from other chromophytes [2]. Since it is easily dissociated from the core complexes, this suggests it may be peripherally located and possibly analogous to higher plant LHC II. In contrast, the FCP fraction obtained by non-denaturing SDS-PAGE appeared to contain all the major FCP polypeptides.

The denaturing SDS-PAGE system used allowed for the resolution of 12 separate cross-reacting LHC related polypeptides in *Olisthodiscus*. They were in the same size range (15–22 kDa) as those reported from other chromophytes [2]. Most published work has reported one to four light-harvesting polypeptides [2], although as many as six polypeptides from four chromophytic species have been reported to cross-react with an antibody raised to *Chlamydomonas* (Chlorophyceae) LHC [14]. Variations in the number of polypeptides detected may be due to different electrophoretic systems and to different sets of epitopes recognized by different antisera. In order to rule out the possibility that some of the immuno-reactive bands were the result of proteolytic cleavage of larger polypeptides, *Olisthodiscus* thylakoids were isolated and incubated at 37°C in the presence or absence of proteinase inhibitors. There were no differences in the number of LHC bands detected. Whole cells solubilized directly in 2 × SDS sample buffer also showed the same pattern as thylakoids (data not shown). The FCP antenna family in *Olisthodiscus* may therefore be as complex as the CAB antenna family in higher plants [32,39].

It is also interesting to note that these light-harvesting poplypeptides were detected using LHC specific antibodies raised against FCP's from a different class in the Chromophyta as well as with antibodies raised against higher plant CAB's. Other studies using antibodies specific for higher plant and chlorophyte LHC's showed cross-reactivity with various members of the Chromophyta [14,40,41]; others showed cross-reactivity within the Chromophyta [11]. These results indicate the presence of commonly conserved antigenic determinants associated with all light-harvesting polypeptides, suggestive of a common evolutionary origin. This structural similarity is confirmed by sequences of the FCP genes from the diatom, *Phaeodactylum tricornutum* [17]. Protein sequences of tryptic fragments from a Cryptomonad [42], the dinoflagellate, *Amphidinium* [18] and from *Olisthodiscus* (Ref. 32; Durnford, unpublished data) also demonstrate the apparent structural similarity between the Chl *a/b* proteins and the Chl *a/c* proteins. However, the observation that some *Olisthodiscus* polypeptides react only with one antiserum and not the other, suggests that there are significant structural differences between the FCP family members, as there are among members of the CAB family [19,39].

Solubilized chromophyte thylakoids have previously been fractionated by non-denaturing PAGE, especially in sodium deoxycholate [7,8] or Deriphat 160 gel systems [9,43–45]. We were unable to obtain satisfactory results with either of these gel systems or with the non-denaturing gel systems used successfully with higher plants [46,47]. However, a modification of the non-denaturing gel system devised by Allen and Staehelin [25] proved to be successful in the separation of *Olisthodiscus* pigment-protein complexes, allowing the preservation of a number of large pigment-protein complexes with apparent molecular masses of over 200 kDa. This system represents an improvement over other electrophoretic separation techniques for chromophytic algal pigment-protein complexes, being able to separate several PS I fractions, an LHC fraction and a number of PS II fractions.

An important feature of the non-denaturing electrophoretic separation technique is the ability to isolate light-harvesting antennal proteins still associated with the core complexes. Complexes 1–3 were PS I related and appeared identical except that the slowest migrating appeared to have larger amounts of associated light-harvesting polypeptides. Complexes 2 and 3 lacked the majority of the LHC and tended to retain high levels of the lower cross-reacting polypeptide (17 kDa) suggesting it may be closely associated with the PS I core complex. Previous studies on isolated PS I complexes of chromophyte algae found a 715–720 nm

fluorescence emission peak which is usually assumed to be due to PS I reaction center in association with its light-harvesting antenna [8,38]. It appears that the presence of a light-harvesting complex associated with PS I is a common feature of the Chromophyta [38], as is the case with green algae and higher plants where a number of unique antennal proteins in the size range of 21–24 kDa are specifically associated with PS I [34,48,49]. However, we can not yet assign any LHC polypeptides exclusively to PS I in *Olisthodiscus*.

The pattern of PS I and PS II complexes resolved from Olisthodiscus thylakoids is similar to that obtained with the green alga, *Chlamydomonas*, and higher plants [25]. However, the pattern is not dominated by multiple oligomeric forms of the major LHC, as it is in the chlorophytes [25]. At the present time we are unable to draw any conclusions about the organization of pigment-protein complexes in the thylakoid or whether the pigment-protein complexes separated on the non-denaturing gel system might represent different environments within the thylakoid membrane. Immunocytochemical localization of FCP and PS I complexes within the thylakoids of several members of the Chromophyta [50–52] showed that PS I and PS II were not as highly segregated within appressed and non-appressed regions as they are in higher plants, suggesting that there may be significant differences in macromolecular organization between chromophyte and chlorophyte thylakoids.

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References

1 Staehelin, A. (1986) in Photosynthesis III: Photosynthetic membranes and light-harvesting systems (Staehelin, L.A. and Arntzen, C.A., eds.), Vol. 19, pp. 1–84, Springer, New York.
2 Hiller, R.G., Anderson, J.M. and Larkum, A.W.D. (1991) in Chlorophylls (Scheer, H., ed.), pp. 529–547, CRC Press, Boca Raton.
3 Riley, J.P. and Wilson, T.R.S. (1967) J. Mar. Biol. Ass. U.K. 47, 351–362.
4 Friedman, A.L. and Alberte, R.S. (1984) Plant Physiol. 76, 483–489.
5 Gugliemelli, L.A.(1984) Biochim. Biophys. Acta 766, 45–50.
6 Owens, T.G. and Wold, E.R. (1986) Plant Physiol. 80, 732–738.
7 Caron, L. and Brown, J. (1987) Plant Cell Physiol. 28, 775–785.
8 Brown, J.S.(1988) J. Phycol. 24, 96–102.
9 Boczar, B.A. and Prezelin, B.B. (1989) Plant Cell Physiol. 30, 1047–1056.
10 Wiedemann, I., Wilhelm, C. and Wild, A. (1983) Photosyn. Res. 4, 317–329.
11 Fawley, M.W., Morton, S.J., Stewart, K.D. and Mattox, K.R. (1987) J. Phycol. 23, 377–381.
12 Barrett, J. and Anderson, J.M. (1980) Biochim. Biophys. Acta 590, 309–323.
13 Alberte, R.S., Friedman, A.L., Gustafson, D.L., Rudnick, M.S. and Lyman, H. (1981) Biochim. Biophys. Acta 635, 304–316.
14 Plumley, F.G., Martinson, T.A., Herrin, D.L., M. Ikeuchi and Schmidt, G.W. (1993) Photochem. Photobiol. 57, 143–151.
15 Gibbs, P.B. and Biggins, J. (1991) Plant Physiol. 97, 381–387.
16 Fawley, M.W. and Grossman, A.R. (1986) Plant Physiol. 81, 149–155.
17 Grossman, A., Manodori, A. and Snyder, D. (1990) Mol. Gen. Genet. 224, 91–100.
18 Hiller, R.G., Wrench, P.M., Gooley, A.P., G. Shoebridge and Breton, J. (1993) Photochem. Photobiol. 57, 125–131.
19 Green, B.R. and Pichersky, E. (1993) Photosyn. Res., in press.
20 Reith, M. and Cattolico, R.A. (1986) Proc. Natl. Acad. Sci. USA 83, 8599–8603.
21 Hara, Y. and Chihara, M. (1987) Bot. Mag. Tokyo 100, 151–163.
22 Hulburt,, E.M.(1965) J. Phycol. 1, 87–94.
23 Taylor,, F.J.R.(1992) Gior. Botan. Ital. 126, 209–219.
24 Cattolico, R.A., Boothroyd, J.C. and Gibbs, S.P. (1976) Plant Physiol. 57, 497–503.
25 Allen, K.D. and Staehelin, L.A. (1991) Anal. Biochem. 194, 214–222.
26 Jeffrey, S.W. and Humphrey, G.F. (1975) Biochem. Physiol. Pflanzen 167, 191–194.
27 Laemmli, U.K. (1970) Nature 227, 680–685.
28 White, M.J. and Green, B.R. (1987) Eur. J. Biochem. 163, 545–551.
29 Legocki, R.P. and Verma, D.P.S. (1981) Anal. Biochem. 111, 385–392.
30 Bengis, C. and Nelson, N. (1975) J. Biol. Chem. 250, 2783–2788.
31 Marsho, T.V. and Kok, B. (1972) Methods Enzymol. 23, 515–522.
32 Green, B.R., Durnford, D.G., Aebersold, R. and Pichersky, E. (1992) in Research in Photosynthesis (Murata, N., ed.), Vol. I, pp. 195–201, Kluwer, Dordrecht.
33 Golbeck, J.H. and Bryant, D.A. (1991) in Current Topics in Bioenergetics (Lee, C.P., ed.), Vol. 16, pp. 83–177, Academic Press, San Diego.
34 Haworth, P., Watson, J.L. and Arntzen, C.J. (1983) Biochim. Biophys. Acta 724, 151–158.
35 Murata, N. and Satoh, K. (1986) in Light-emission by plants and bacteria (Govindjee, Amesz, J. and Fork, D.C., eds.), pp. 137–159, Academic Press, San Diego.
36 Green, B.R. (1988) Photosyn. Res. 15, 3–32.
37 Arsalane, W., Rousseau, B. and Thomas, J.C. (1992) J. Phycol. 28, 32–36.
38 Berkaloff, C., Caron, L. and Rousseau, B. (1990) Photosyn. Res. 23, 181–193.
39 Green, B.R., Pichersky, E. and Kloppstech, K. (1991) Trends Biochem. Sci. 16, 181–186.
40 Caron, L., Remy, R. and Berkaloff, C. (1988) FEBS Lett. 229, 11–15.
41 Passaquet, C., Thomas, J.C., Caron, L., Hauswirth, N., Puel, F. and Berkaloff, C. (1991) FEBS Lett. 280, 21–26.
42 Sidler, W., Wehrmeyer, W. and Zuber, H. (1988) Experientia 44, A60.
43 Boczar, B.A., Prezelin, B.B., Markwell, J.P. and Thornber, J.P. (1980) FEBS Lett. 120, 243–247.
44 Knoetzel, J. and Rensing, L. (1990) J. Plant Physiol. 136, 271–279.
45 Peyriere, M., Caron, L. and Jupin, H. (1984) Photosynthetica 18, 184–191.
46 Camm, E.L. and Green, B.R. (1989) Biochim. Biophys. Acta 974, 180–184.

47 Thornber, J.P., Morishige, D.T., Anandan, S. and Peter, G.F. (1991) in Chlorophylls (Scheer, H., ed.), Vol. pp. 549–586, CRC Press, Boca Raton.
48 Mullet, J.E., Burke, J.J. and Arntzen, C.J. (1980) Plant Physiol. 65, 814–822.
49 Lam, E., Ortiz, W., Mayfield, S. and Malkin, R. (1984) Plant Physiol. 74, 650–655.
50 Lichtlé, C., Spilar, A. and Gibbs, S.P. (1992) Protoplasma 166, 99–106.
51 Lichtlé, C., McKay, R.M.L. and Gibbs, S.P. (1992) Biol. Cell 74, 187–194.
52 Pyszniak, A.M. and Gibbs, S.P. (1992) Protoplasma 166, 208–217.