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Isolation and Characterization of Three Membrane-Bound Chlorophyll-Protein Complexes from Four Dinoflagellate Species

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Isolation and characterization of three membrane-bound chlorophyll–protein complexes from four dinoflagellate species

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SUMMARY

Employing discontinuous sucrose density gradient centrifugation of *n*-dodecyl β-d-maltoside-solubilized thylakoid membranes, three chlorophyll (Chl)–protein complexes containing Chl *a*, Chl c_2 and peridinin in different proportions, were isolated from the dinoflagellates *Symbiodinium microadriaticum*, *S. kawagutii*, *S. pilosum* and *Heterocapsa pygmaea*. In *S. microadriaticum*, the first complex, containing 13% of the total cellular Chl *a*, and minor quantities of Chl c_2 and peridinin, is associated with polypeptides with apparent molecular mass (M_r) of 8–9 kDa, and demonstrated inefficient energy transfer from the accessory pigments to Chl *a*. The second complex contains Chl *a*, Chl c_2 and peridinin in a molar ratio of 1:1:2, associated with two apoproteins of M_r 19–20 kDa, and comprises 45%, 75% and 70%, respectively, of the cellular Chl *a*, Chl c_2 and peridinin. The efficient energy transfer from Chl c_2 and peridinin to Chl *a* in this complex is supportive of a light-harvesting function. This Chl *a*–c_2–peridinin–protein complex represents the major light-harvesting complex in dinoflagellates. The third complex obtained contains 12% of the cellular Chl *a*, and appears to be the core of photosystem I, associated with a light-harvesting complex. This complex is spectroscopically similar to analogous preparations from different taxonomic groups, but demonstrates a unique apoprotein composition. Antibodies against the water-soluble peridinin–Chl *a*–protein (sPCP) light-harvesting complexes failed to cross-react with any of the thylakoid-associated complexes, as did antibodies against Chl *a*–*c*–fucoxanthin apoprotein (from diatoms). Antibodies against the P_{700} apoprotein of plants did not cross-react with the photosystem I complex. Similar results were observed in the other dinoflagellates.

1. INTRODUCTION

The photosynthetic unit of oxygen-evolving primary producers is functionally organized into two types of pigment–protein complexes; the antenna complexes which are responsible for photon capture and energy excitation transfer, and the reaction centres where primary photochemistry occurs. Although these components are ubiquitous among photosynthetic organisms, aquatic primary producers have evolved a variety of light-harvesting complexes. Green algae and plants possess antenna complexes containing Chl *a* and *b* (Thornber *et al.* 1987). Cyanobacteria, red algae and cryptomonads contain water-soluble phycobiliproteins as major light-harvesting complexes (Ingram & Hiller 1983). 'Chromophytes' possess carotenoids, in addition to Chl *a* and *c*, as major light-harvesting pigments (Larkum & Barrett 1983).

In contrast to cyanobacteria and plants in which the structure of the photosynthetic machinery has been extensively documented, relatively few attempts have been made to characterize the photosynthetic apparatus of 'chromophyte' algae in general (Barrett & Anderson 1980; Alberte *et al.* 1981; Freidman & Alberte 1984; Owens & Wold 1986; Katoh *et al.* 1989; Passaquet *et al.* 1991), and dinoflagellates in particular (Prézelin & Alberte 1978; Boczar *et al.* 1980; Boczar & Prézelin, 1986, 1987; Knoetzel & Rensing 1990), despite the significant role of this group in global primary production. Lack of appropriate fractionation procedures has hindered the characterization of the photosynthetic apparatus in this group. Techniques that have proved reliable for thylakoid fractionation in plants, such as non-denaturing sodium dodecyl sulphate (SDS)-Deriphat 160-polyacrylamide gel electrophoresis (PAGE) (Markwell *et al.* 1978, 1979), appear to be inadequate when applied to 'chromophytes'. The use of SDS in thylakoid solubilization appears to prevent the isolation of native 'chromophyte' Chl–protein complexes (Friedman & Alberte 1984), complicating any interpretation concerning their function. Regarding dinoflagellates in particular, all previously reported efforts of isolation of membrane-bound Chl–protein complexes, resulted in a loss of energy transfer from the accessory pigments to Chl *a* (Boczar *et al.* 1980; Boczar & Prézelin 1986, 1987).

In a previous study (Iglesias-Prieto *et al.* 1991), we characterized the water-soluble peridinin–Chl *a*–protein (sPCP) antenna complexes from three species of

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symbiotic dinoflagellates. Here we characterize the spectroscopic properties and apoprotein composition of three membrane-bound Chl–protein complexes isolated by *n*-dodecyl β-d-maltoside (DDM)-solubilization and sucrose density gradient centrifugation, from the symbiotic dinoflagellate *Symbiodinium microadriaticum* and show their similarity to complexes from three other dinoflagellate species. Our analyses indicate that sPCP is a minor light-harvesting complex, and instead, a Chl *a*–c_2–peridinin–protein complex represents the major light-harvesting component of the photosynthetic apparatus in these algae.

2. MATERIALS AND METHODS

(*a*) *Algal cultures*

Symbiodinium microadriaticum (originally isolated from the jellyfish *Cassiopeia xamachana*), *S. kawagutii* (from the stony coral *Montipora verrucosa*, and *S. pilosum* (from the zoanthid *Zoanthus sociatus*) (Trench & Blank 1987) were cultured axenically in 2.8 l Fernbach flasks containing 1.0 l of ASP-8A (Blank 1987). Cultures were maintained under a 14 h:10 h (light:dark) photoperiod at 26°C. Illumination was provided by cool white fluorescent lamps delivering 250 µmol quanta $m^{-2} s^{-1}$ photosynthetically active radiation (PAR). *Heterocapsa pygmaea* was grown as described by Nelson & Prézelin (1990).

(*b*) *Isolation of thylakoid membranes*

Cultures in late exponential or early stationary phase were harvested by centrifugation at 8000 *g* for 10 min at 10°C. The pellet was resuspended in 25 ml of ice-cold TB buffer (100 mM Tris-borate pH 8.0, 2 mM $MgCl_2$, 2 mM Na_2 EDTA, 1 mM phenylmethylsulphonyl fluoride) (Owens & Wold 1986), and broken by three passes through a French pressure cell at 8.3×10^7 Pa. The resulting lysate was centrifuged at 500 *g* for 10 min at 4°C to remove unbroken cells and cell debris. The crude homogenate was rendered 50% saturated with $(NH_4)_2SO_4$ at 4°C, and centrifuged for 10 min at 8000 *g* at 4°C. Water-soluble PCP (sPCP; Iglesias-Prieto *et al.* 1991) was recovered from the supernatant. The precipitated material was resuspended in 8 ml of ice-cold TB and centrifuged at 225 000 *g* for 2 h at 4°C to pellet the thylakoid membranes. This procedure was conducted within 2 h of the initial rupture of the cells.

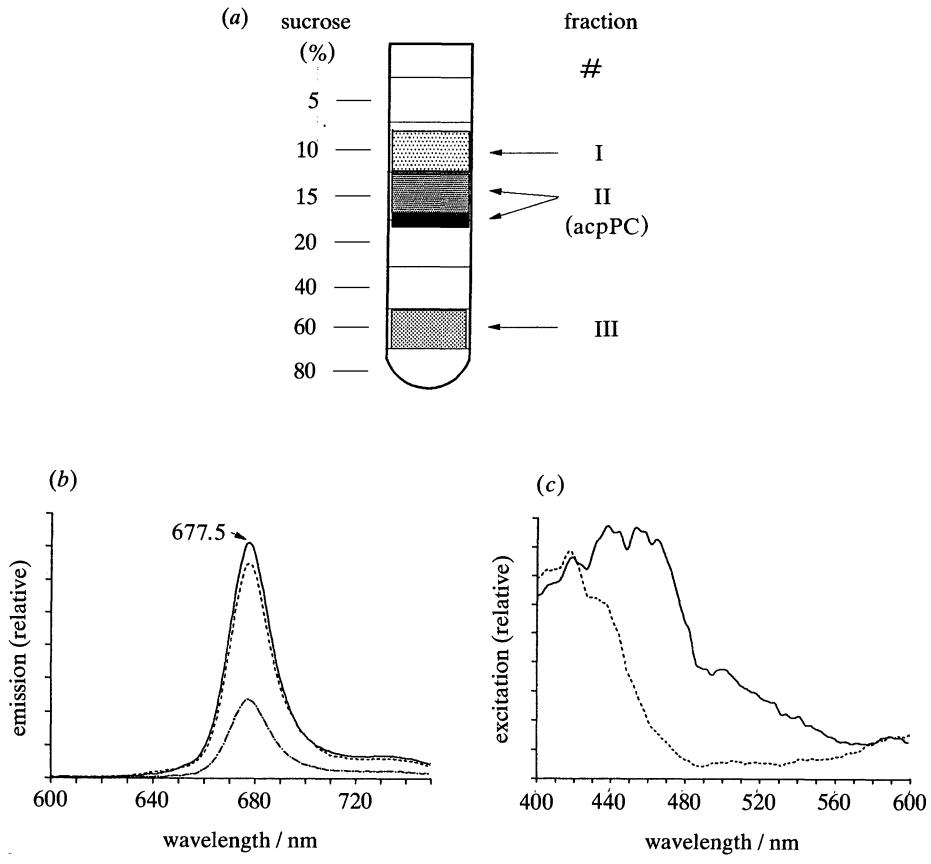

Figure 1. (*a*) *Symbiodinium microadriaticum*. Schematic representation of the result of a discontinuous sucrose density gradient analysis. One millilitre of 1% DDM-solubilized thylakoids was loaded on top of the gradient containing 0.1% DDM. (*b*) Uncorrected fluorescence emission spectra of purified thylakoid membranes. Excitation at 435 nm (solid line); at 465 nm (dashed line); and at 520 nm (dot-dashed line). (*c*) Fluorescence excitation spectra of purified thylakoids (solid line), and the same preparation after exposure to 0.1% SDS at room temperature for 40 min in the dark (dashed line).

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

(*c*) *Membrane solubilization and isolation of pigment–protein complexes*

Pellets containing the photosynthetic membranes were resuspended in ice-cold TB buffer at 250 μg Chl *a* ml^{-1}, and the sample was made 1% (w/v) with *n*-dodecyl β-d maltoside (DDM) (Sigma) at a surfactant:Chl *a* ratio of 70:1 (w/w). The membranes were solubilized by gentle stirring at 4°C for 2 h, and the resulting solution centrifuged for 10 min at 21 000 *g*. The soluble supernatant was loaded onto a discontinuous sucrose density gradient (5, 10, 15, 20, 40, 60 and 80% sucrose in 0.1% DDM in TB (w/v)). The gradients were centrifuged for 16 h at 225 000 *g* at 4°C. Portions of the gradient containing the pigment–protein complexes (figure 1*a*) were removed. All the isolation procedures were performed under dim light to minimize photo-destruction of the complexes.

(*d*) *Spectroscopic analyses*

Absorption spectra of the isolated pigment–protein complexes were obtained at room temperature (23°C) using a Varian Techtron Series 634 UV/VIS spectrophotometer. The spectra were digitized using the procedure previously described (Iglesias–Prieto *et al.* 1991). Each spectrum shown is the average of 15 individual spectra. The fourth derivative of the absorption spectra were obtained as described by Butler & Hopkins (1970).

Fluorescence excitation and emission spectra at room temperature (23°C)and 77 K were obtained using a Perkin-Elmer LS 50 spectrofluorometer equipped with a red-sensitive photo multiplier (Model R-928). The instrument was operated with emission and excitation slit widths of 2.5 and 4 nm, respectively. Samples were diluted in TB to a final concentration of 200 nM Chl *a* to minimize reabsorption. Excitation spectra were corrected to compensate for wavelength-dependent energy variations in the output of the excitation lamp. Emission spectra are presented without correction. Each spectrum shown is the result of averaging 25 individual spectra.

Pigments were extracted in 90% (v/v) acetone and Chl *a* and Chl c_2 concentrations were determined spectrophotometrically using the equations of Jeffrey & Humphrey (1975). Peridinin concentrations were determined after separation by thin layer chromotography (TLC) as described by Chang *et al.* (1983). Chl *a* concentration in the native pigment–protein complexes was estimated using a molar extinction coefficient of 60 $mM^{-1}\,cm^{-1}$ (Shiozawa *et al.* 1974). The concentration of P_{700} in crude lysates and in isolated complexes was determined with a Hewlett Packard 8252A diode array spectrophotometer, using light-induced photo-bleaching of P_{700} as described by Smith & Alberte (1991). As the measuring beam of the spectrophotometer produced photo-bleaching of P_{700} in the PS I-enriched fraction, a neutral density filter was used to attenuate the measuring beam by 90%. Owing to the detrimental effect of Triton X-100 on P_{700} activity in this preparation (see results), this detergent was substituted with 1% (w/v) DDM.

(*e*) *Electrophoretic analysis of pigment–protein complexes*

Samples of the different pigment–protein complexes, and samples of the photosynthetic membranes in various stages of purification, containing equal amounts of Chl *a* were separated using sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE, 15–20% acrylamide linear gradients). The proteins were visualized by staining the gels with silver (BioRad). Similar preparations were separated by SDS-PAGE (10–20% acrylamide linear gradients); the proteins were electroblotted onto nitrocellulose filters as described previously (Roman *et al.* 1988), and the blots probed with anti-ScPCP serum diluted 1:500 (Iglesias-Prieto *et al.* 1991), anti-Chl *a*–*c*–fucoxanthin protein serum diluted 1:1000, and with CC I serum (anti-P_{700} apoprotein (Verling & Alberte 1983)),

Table 1. *S. microadriaticum. Pigment distribution in photosynthetic membranes and DDM-solubilized fractions*

(The average values (± 1 standard deviation) for Chl *a* content are the result of six independent analyses. The molar ratios of chl *a*:P_{700} were derived from six independent analyses, with nine replicates each. Carotenoids in each fraction are represented as: Per, peridinin; DX, dinoxanthin; DD, diadinoxanthin; Car, β-carotene. n.d., not detected.)

fraction	Chl *a* %	Chl *a*:Chl c_2	Chl *a*:Per	Chl *a*:P_{700}	other carotenoids
cell lysate	100	1.73	0.77	1171 ± 109	DX, DD, Car
sPCP	5		0.25		
thylakoid membranes	90	1.70	0.88		DX, DD, Car
detergent-soluble fraction	71	1.65	0.75		DX, DD, Car
detergent insoluble fraction	19	2.00	1.90	n.d.	DX, DD, Car
sucrose density gradient of the detergent-soluble fraction[a]					
fraction I	12.8 ± 3.5	4.20	0.75	n.d.	DX, DD, Car
acpPC	44.6 ± 2.1	1.00	0.50	n.d.	DX, DD
fraction III	11.7 ± 1.4	> 10.00	5.40	112 ± 10	DX, DD, Car

[a] Approximately 3% of the Chl *a* loaded onto the sucrose density gradient remained at the interface between fraction I and acpPC.

diluted 1:1000 (Smith & Alberte 1991). Preimmune serum was used as control in all assays.

3. RESULTS

(*a*) *Chlorophyll–protein complexes of the thylakoids*

Approximately 90% of the total Chl *a* present in the cell lysate was recovered in the membrane fraction (table 1). In *S. microadriaticum*, sPCP represents approximately 5% of the total Chl *a* and 15% of the total peridinin; in *S. kawagutii*, *S. pilosum* and *H. pygmaea*, sPCP represents 5%, 1% and 12% respectively, of the cellular Chl *a*. In *S. microadriaticum*, the ratio of Chl *a*:Chl c_2 in the purified thylakoid membranes is very similar to that observed in the intact cells, but there is a reduction in the peridinin content of the membranes relative to the intact cells, which is accounted for by sPCP. The molar ratio of Chl *a*:P_{700} in the cell lysate was 1171 ± 109, which is similar to the ratio reported for the symbiotic dinoflagellates from *Stylophora pistillata* collected from low light environments (Falkowski & Dubinsky 1981).

The fluorescence emission spectrum (figure 1*b*) of purified thylakoids excited with light preferentially absorbed by Chl *a* (435 nm), shows a major emission maximum at 677.5 nm and a minor peak at approximately 735 nm. Excitation with light preferentially absorbed by Chl c_2 (465 nm), or peridinin (520 nm), also produced a Chl *a* emission maximum at 677.5 nm, with negligible Chl c_2 emission, indicating effective excitation transfer from the accessory pigments to Chl *a*. This observation is corroborated by the excitation spectrum of the purified thylakoids (figure 1*c*), which shows substantial contribution from Chl c_2 and carotenoids to the 677.5 Chl *a* emission.

Solubilization of purified thylakoids with the glycosidic surfactant DDM released 71% of the cellular Chl *a*. The detergent-insoluble component contained 19% of the cellular Chl *a*. In *S. microadriaticum*, the detergent-solubilized material is enriched in Chl c_2 and peridinin, relative to the purified thylakoids. Discontinuous sucrose density gradient centrifugation of solubilized thylakoid membranes resulted in the formation of three discrete pigmented zones (table 1 and figure 1*a*). They all contain Chl *a*, Chl c_2 and peridinin in different proportions. Essentially identical patterns were obtained from the other three species of dinoflagellates analysed.

In *S. microadriaticum*, a green fraction containing 13% of the cellular Chl *a* was recovered from the 10% sucrose region of the gradient. This fraction (hereafter referred as fraction I), was depleted in Chl c_2 and peridinin, compared with the starting material, and showed no P_{700} activity. β-carotene and the xanthophylls dinoxanthin and diadinoxanthin, were detected by TLC.

A second Chl–protein complex (fraction II) was isolated from the 15% sucrose region of the gradient and the interface between the 15 and 20% sucrose regions. Most (80%) of this material was recovered from a narrow brown band at the 15–20% sucrose interface; the remaining 20% was recovered from the bulk 15% sucrose region (figure 1*a*). As both fractions posses identical spectroscopic characteristics and SDS-PAGE profiles, they were pooled (table 1). This complex (hereafter referred to as Chl *a*–Chl c_2–peridinin–protein complex (acpPC)), contains 45% of the Chl *a* and most (75%) of Chl c_2, and (70%) of the peridinin present in intact cells. Analysis of this complex by TLC shows the presence of dinoxanthin and diadinoxanthin, but not β-carotene. P_{700} activity was not detected in the acpPC fraction.

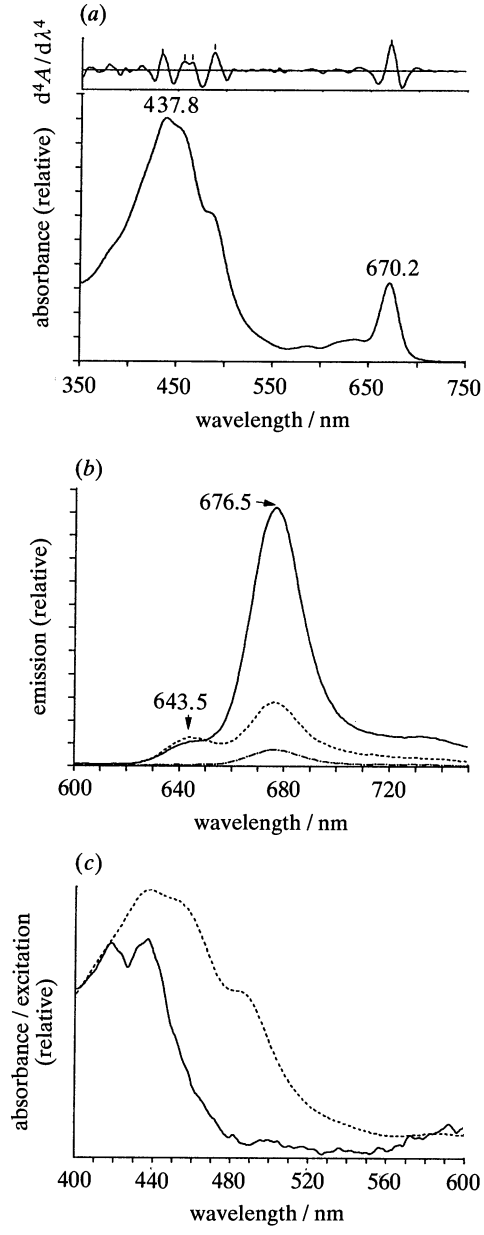

Figure 2. Fraction I from sucrose density gradient centrifugation of solubilized membranes from *S. microadriaticum*. (*a*) Room temperature absorption spectrum and (upper frame) the fourth derivative of the spectrum. (*b*) Uncorrected fluorescence emission spectra. The excitation wavelengths were as in figure 1*b*. (*c*) Corrected fluorescence excitation spectrum (solid line) detected at 676.5 nm. The absorption spectrum of the same samples is presented for comparison (dashed line).

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

A green band (fraction III), containing 12% of the cellular Chl *a* was recovered from the 60% sucrose region. This fraction contains small amounts of both Chl c_2 and peridinin. Analysis by TLC revealed the presence of dinoxanthin, diadinoxanthin and β-carotene. The P_{700} content of this fraction showed a 10 fold increase relative to the cell lysates.

(*b*) *Spectroscopic characteristics of the isolated Chl–protein complexes*

The absorption spectrum of fraction I shows a Chl *a* absorption maximum at 437.8 nm in the Soret region, and a second maximum at 670.2 nm in the red portion of the spectrum. The fourth derivative revealed the presence of components absorbing at 456.0, 464.8 and 487.4 nm, consistent with the presence of Chl c_2 and carotenoids in this complex (figure 2*a*). When excited at 435 nm, the emission spectrum of fraction I shows the presence of a shoulder at 643.5 nm and a peak at 676.5 nm (figure 2*b*). When the same sample was excited at 465 nm, the emission spectrum showed two distinct maxima at 643.5 nm and 675 nm, the first one resulting from Chl c_2, indicating uncoupled excitation energy transfer to Chl *a*. The fluorescence excitation spectrum of the 676.5 nm emission shows a very small contribution from both Chl c_2 and peridinin (figure 2*c*), indicating poor energy transfer between the accessory pigments and Chl *a*.

The absorption spectrum of acpPC (fraction II) shows two maxima in the Soret region at 441.0 and 456 nm, due to Chl *a* and Chl c_2, respectively (figure 3*a*); Chl *a* shows a peak at 672.8 nm. The fourth derivative of this spectrum shows the presence of components absorbing at 488.0 nm due predominantly to peridinin, and 633.0 and 646.4 nm, due to the Chl *a*, and Chl c_2, respectively. The emission spectrum shows a major peak at 676.5 nm and a minor one near 735 nm. When the complex was excited at 465 or 520 nm, the fluorescence emission spectra were very similar to that observed with excitation at 435 nm. Negligible Chl c_2 emission was observed (figure 3*b*). The excitation spectrum for the 676.5 nm emission shows substantial contributions from Chl c_2 and carotenoids, indicating efficient energy transfer between the accessory pigments and Chl *a* (figure 3*c*). When the fluorescence excitation spectrum is compared with the absorption spectrum, it is evident that some carotenoids are not associated with excitation transfer. These carotenoids may play a photo-protective role.

The absorption spectrum of the photosystem I (PS I)-enriched fraction III shows (figure 4*a*) two Chl *a* maxima at 435.8 and 676.0 nm. The fourth derivative of the absorption spectrum revealed the presence of a Chl *a* maximum at 414 nm, a Chl c_2 component at 463 nm, and a carotenoid component at 490 nm, and also demonstrated heterogeneity of Chl *a*, as indicated by the presence of two peaks at 669.0 nm and 681.8 nm. The fluorescence emission quantum yield of fraction III was very low (figure 4*b*). Simultaneous analysis of the excitation and absorption spectra, revealed a larger Chl c_2 and peridinin contribution to Chl *a* emission than that predicted from their relative abundance. This observation is consistent with the low fluorescence emission quantum yield characteristic of PS I-enriched preparations (figure 4*c*).

The fluorescence emission spectra of the three isolated Chl–protein complexes at 77 K is presented in figure 5. The major emission peaks in all complexes are red-shifted compared with their emission maxima at room temperature. The emission spectrum of the PS I-enriched fraction III shows a broad maximum at 684 nm and a characteristic shoulder at 709.5 nm.

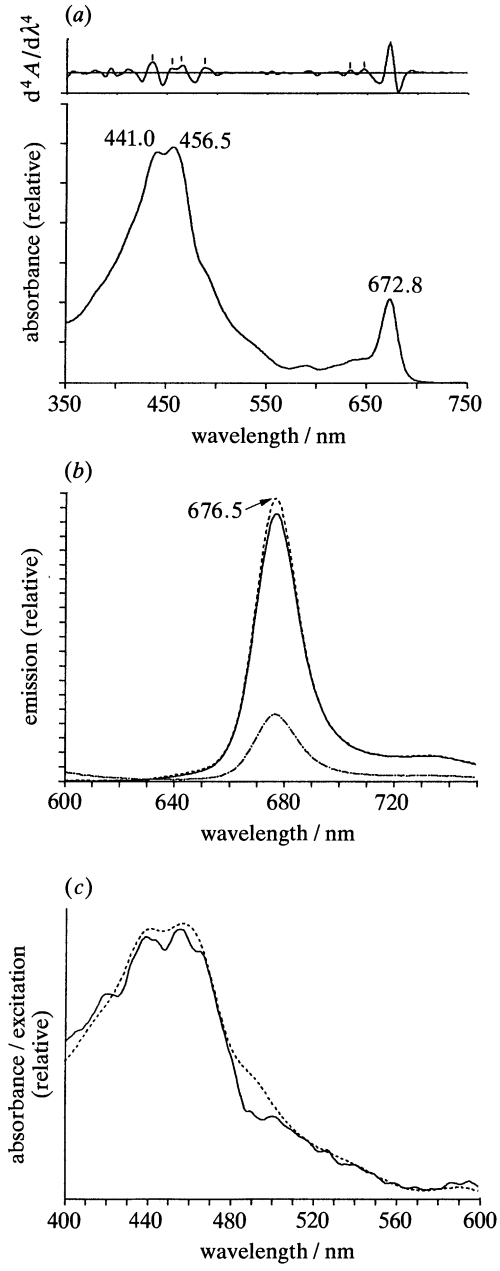

Figure 3. Fraction II, acpPC, from sucrose density gradient centrifugation of solubilized membranes from *S. microadriaticum*. (*c*) Room temperature absorption spectrum and (upper frame) the fourth derivative of the spectrum. (*b*) Uncorrected fluorescence emission spectra. The excitation conditions are as in figure 1*b*. (*c*) Corrected fluorescence excitation spectrum detected at 676.5 nm (solid line). The absorption spectrum of this fraction (dashed line) is shown for comparison.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

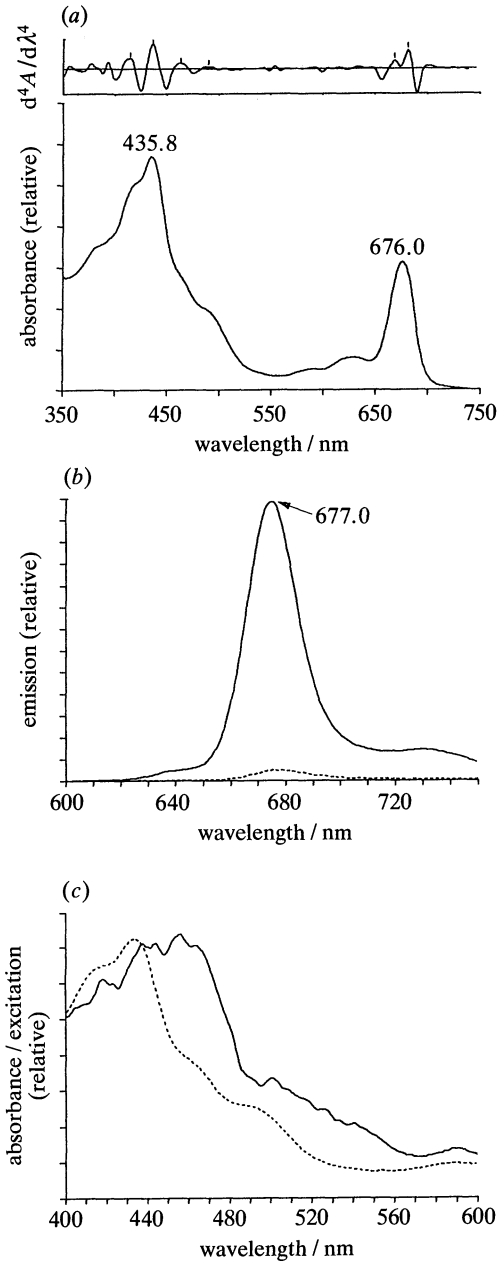

Figure 4. Fraction III from sucrose density gradient centrifugation of solubilized membranes from *S. microadriaticum*. (*a*) Room temperature absorption spectrum and (upper frame) the fourth derivative of the spectrum. (*b*) Uncorrected fluorescence emission spectra (excitation at 435 nm), before (dashed line), and after (solid line) treatment with 0.05% Triton X-100. (*c*) Corrected fluorescence excitation spectrum detected at 677 nm (solid line). The absorption spectrum of this fraction (dashed line) is presented for comparison.

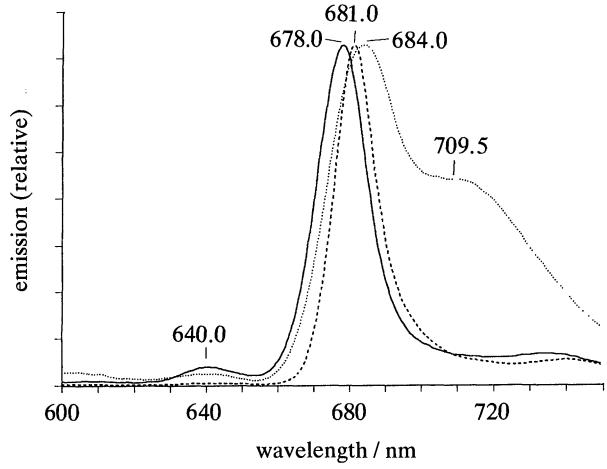

Figure 5. Low temperature (77 K), uncorrected fluorescence emission spectra of the three pigment–protein complexes isolated by density gradient centrifugation from *S. microadriaticum*. Fraction I (solid line); fraction II (acpPC) (dashed line); fraction III (dashed line).

The room temperature absorption spectra (612–730 nm) of the PS I-enriched fraction III show (figure 6*a*) that the absorption maximum of the complex shifted from 676 to 669.6 nm upon addition of 0.05% (v/v) Triton X-100. The fourth derivative of the intact fraction III demonstrates the presence of at least four spectral forms of Chl *a* with maxima at 662, 670, 678 and 684 nm. The fourth derivative of the Triton X-100-treated fraction III is dominated by two maxima at 670 and 676 nm. The fluorescence emission quantum yield increased 24-fold immediately after addition of the detergent (figure 4*b*). Along with the spectroscopic changes described, P_{700} activity of the complex was also reduced, and was not detectable after 10 min of Triton X-100 treatment.

The fluorescence emission spectra of purified thylakoids treated with 0.1% SDS indicate a time-dependent loss of excitation transfer from Chl c_2 to Chl *a* (figure 6*b*). The fluorescence excitation spectrum of the Chl *a* emission after 40 min of SDS treatment indicates very limited contribution from Chl c_2 and carotenoids (figure 1*c*). Similar effects were observed in the isolated Chl–protein complexes treated with SDS. The various fractions isolated from the other three dinoflagellate species exhibited spectroscopic properties essentially identical to those described for *S. microadriaticum*.

(*c*) *Polypeptide composition*

The polypeptide composition of the cell lysate, purified thylakoid membranes, and the isolated Chl-protein complexes from *S. microadriaticum* are shown in figure 7. The electrophoretic profile of the purified thylakoids (figure 7, lane B) shows a decrease in the number of polypeptides relative to the crude cell lysate (lane A). The $(NH_4)_2SO_4$ treatment successfully removed both the dimeric and monomeric sPCP apoproteins. The polypeptide composition of the detergent-solubilized membranes (lane C) is very similar to that of the purified thylakoids. Fraction I appears to be composed of two polypeptides with M_r 8 and 9 kDa, respectively (lane D). The polypeptide composition of acpPC (fraction II) is dominated by a doublet with M_r of 19–20 kDa in all four dinoflagellate species (figure 8). Based on comparison of the intensity of the silver staining in lane D with lane A (figure 7), these polypeptides represent a large fraction of the total protein present in these organisms.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

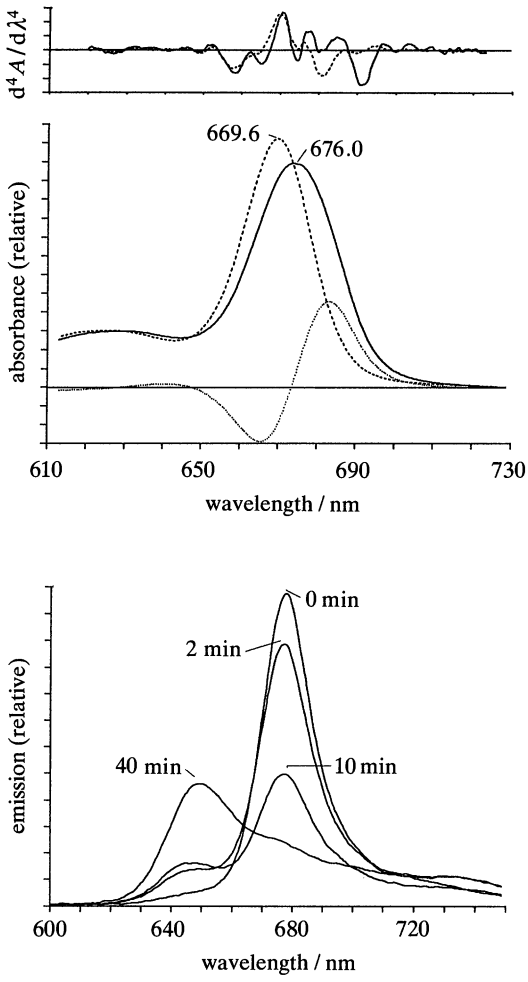

Figure 6. Analyses of fraction III from *S. microadriaticum*. (*a*) Room temperature absorption spectra before (solid line), and after treatment (dashed line) with 0.05% Triton X-100 (3 min in the dark at room temperature). Fourth derivative (upper frame) and the difference spectrum (dotted line). (*b*) Temporal effect of 0.1% SDS on the fluorescence emission spectrum of purified thylakoid membranes.

The polypeptide composition of the PS I-enriched fraction III is more complex than that of either fraction I or acpPC. Seven discrete polypeptides with M_r ranging from 14.5 to 29 kDa, and a minor one with M_r of 55 kDa (figures 7 and 8) were resolved.

Monospecific, polyclonal antibodies against sPCP failed to cross-react with the membrane-bound Chl–protein complexes. Positive controls containing purified sPCP, and cell lysates, show the presence of both monomeric and dimeric forms of sPCP (figure 9*a*). Polyclonal antibodies against the Chl *a*–*c*–fucoxanthin apoprotein from the diatom *Phaodactylum tricornotum* did not cross-react with preparations of the thylakoids nor with electrophoretically separated acpPC apoprotein. Under conditions of high stringency no cross-reactivity was observed when the cell lysate, intact thylakoids, or the three detergent-solubilized thylakoid fractions, were probed with CC I antibodies. In contrast, these antibodies reacted with components of a preparation of solubilized spinach thylakoids, showing (figure 9*b*) the presence of the two conformational

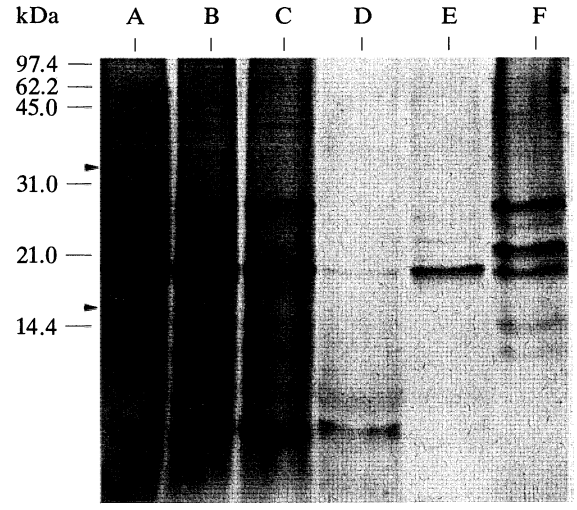

Figure 7. SDS-PAGE analysis of the polypeptides associated with the photosynthetic apparatus in *S. microadriaticum*. Lane (A), cell lysate, arrows identify the 35 and 15 kDa sPCP apoproteins, respectively; lane (B), purified thylakoids; lane (C), DDM-solubilized membrane fraction; lane (D), fraction I; lane (E), acpPC (fraction II); and lane (F), fraction III.

variants of the 60 kDa polypeptide characteristic of PS I of plants (Verling & Alberte 1983). However, under conditions of low stringency, a very faint reaction with the CC I antibodies was observed. We do not interpret this observation as indicating a positive reaction. In no instance did control blots probed with preimmune sera show any cross-reactivity.

4. DISCUSSION

The use of the glycosidic surfactant DDM, in combination with sucrose density gradient centrifugation, allowed us to isolate the majority of the water-insoluble Chl–protein complexes in *S. microadriaticum* and three other species of dinoflagellates. In contrast

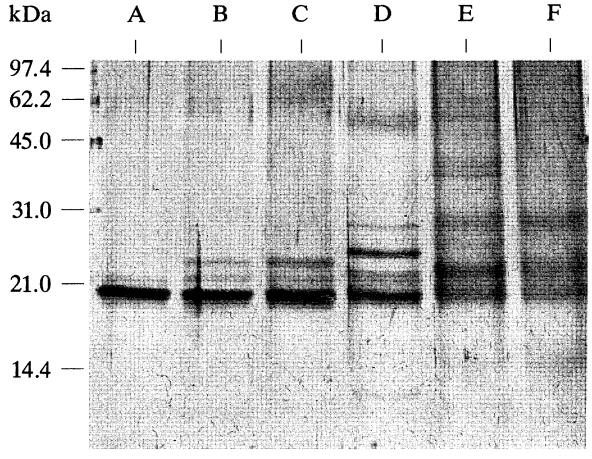

Figure 8. SDS-PAGE analysis of the polypeptides associated with acpPC (lanes A–C) and fraction III (Lanes D–F). Lanes A and D, *H. pygmaea*; lanes B and E, *S. kawagutii*; lanes C and F, *S. pilosum*.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

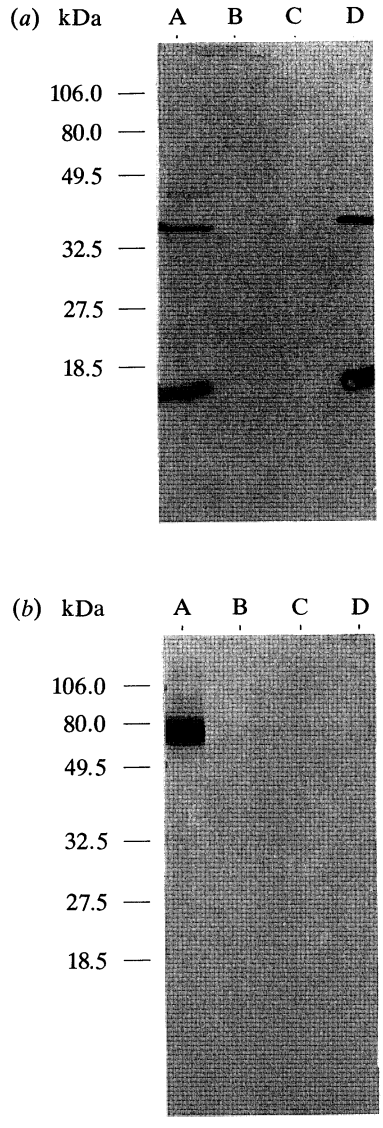

Figure 9. Immunoblot (Western) analyses of components of the photosynthetic apparatus of *S. microadriaticum*. (*a*) Immunoblot probed with anti-Sc-PCP serum. Lane (A), cell lysate; lane (B), purified thylakoids; lane (C), apcPC (Fraction II) (1 μg Chl *a* each); lane (D), sPCP (0.1 μg Chl *a*). (*b*) Immunoblot probed with anti-CCI serum. Lane (A), spinach thylakoids; lane (B) cell lysate; lane (C), purified membranes; lane (D), DDM-solubilized membranes, lane (E) fraction III (2.5 μg Chl *a* each). Note that fraction III exhibits at least three times more P_{700} activity than the spinach thylakoids.

to the Chl–protein complexes isolated by non-denaturing SDS-Deriphat electrophoresis (Boczar *et al.* 1980; Boczar & Prézelin 1986; 1987), thylakoid complexes isolated in the present study retain efficient energy transfer from peridinin and Chl c_2 to Chl *a*.

Compared to sPCP, which has been extensively studied, only a limited number of studies have attempted to characterize the membrane-bound components of dinoflagellate thylakoids. The first membrane-bound component characterized in this group was the P_{700}–Chl *a*–protein (Prézelin & Alberte 1978). This complex was isolated in its active form from *Heterocapsa pygmaea* and *Gonyaulax polyedra* by Triton X-100 solubilization, followed by hydroxyapatite chromatography. Using non-denaturing Deriphat-PAGE of SDS-solubilized thylakoids, several Chl–protein complexes have been characterized in *H. pygmaea*, *G. polyedra* and *Exuviella* sp. (Boczar *et al.* 1980, 1990; Boczar & Prézelin 1986; 1987; Knoetzel & Rensing 1990). These Chl–protein complexes include one with spectroscopic characteristics very similar to those observed in the P_{700}–Chl *a*–protein complex isolated by Prézelin & Alberte (1978), another devoid of peridinin, which bound most of the Chl c_2, several complexes with spectral characteristics similar to sPCP, and various complexes in which both chlorophylls and peridinin were present. We detected neither Chl *a*–*c* complexes, nor complexes exclusively composed of Chl *a* and peridinin, in *S. microadriaticum*, *S. kawagutii*, *S. pilosum* or *H. pygmaea*.

The presence of β-carotene and the low concentration of Chl c_2 in fraction I, suggests that this complex is associated with the core antenna of one of the reaction centres. Peripheral antenna complexes possess only trace amounts of β-carotene, whereas this pigment is very common in the core antennae and in the reaction centres (Siefermann-Harms 1985; Peter & Thornber 1991). In preliminary experiments, we found that using higher surfactant:Chl *a* ratios (150:1 w/w) during thylakoid solubilization, resulted in an increase in the amount of Chl *a* recovered in fraction I, without any appreciable change in the yield of either acpPC or fraction III. This result suggests that fraction I is not a dissociation product of the PS I-enriched fraction III. Peter & Thornber (1991) have reported the presence of a 9 kDa apoprotein in the core of PS II in barley. Therefore, we have tentatively identified fraction I as part of the core antenna of PS II. It is important to note that PS II has not been isolated from dinoflagellates. Although we did not analyse the DDM-insoluble material in detail, this was the only fraction in which pheophytin was detected by TLC. These observations suggest that it may be possible to isolate PS II using this fraction as starting material.

As most of the photosynthetic pigments in the dinoflagellates studied are associated with acpPC (45% Chl *a*, 75% Chl c_2 and 70% peridinin) in *S. microadriaticum* (even in *H. pygmaea*, acpPC contains twice as much peridinin as sPCP), we conclude that this complex represents the major light-harvesting complex in dinoflagellates. The presence of Chl *a*–Chl *c*–xanthophyll chromoproteins, with doublet apoproteins of M_r 17–22 kDa, as the major light harvesting complex, appears to be ubiquitous among 'chromophyte' algae. These complexes have been isolated in functional form from a variety of algal sources, and their apoproteins have been characterized (Friedman & Alberte 1984; Owens & Wold 1986; Passaquet *et al.* 1991). Our results are consistent with this pattern. Although the molecular mass of the Chl *a*–*c*–xanthophyll apoproteins is similar in different 'chromophytes', these complexes appear to be less conserved in terms of antigenic determinants (Friedman & Alberte 1987; Owens 1988; Passaquet *et al.* 1991), and chromophoric ratios (Barrett & Anderson 1980; Friedman

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

& Alberte 1984, Owens & Wold 1986; Kato *et al.* 1989; Passaquet *et al.* 1991). The Chl *a*:Chl *c* ratios of these complexes range from 3.3 in the brown alga *Dictyota dichotoma* (Katoh *et al.* 1989; Passaquet *et al.* 1991) to 1.0 in *S. microadriaticum*. Chl *a*:xanthophyll ratios range from 1.1 in the brown alga *Phylaiella litorallis* (Passaquet *et al.* 1991) to 0.5 in the diatom *P. tricornutum* (Friedman & Alberte 1984) and *S. microadriaticum* (this study).

In *S. microadriaticum*, sPCP accounts for only 12.6% of the cellular peridinin. This is in contrast to other dinoflagellates whose thylakoids membranes have been characterized, wherein it is reported that sPCP accounts for 40–90% of the total peridinin (Haxo *et al.* 1976; Prézelin & Haxo 1976). The yield of sPCP varies in different species grown under the same conditions of illumination. Prézelin (1987) suggested that the differences are related to the ease with which the different cells are broken, and implied the existence of a water-insoluble PCP (iPCP). Water-insoluble peridinin–Chl *a* proteins, devoid of Chl c_2, have been reported from *H. pygmaea* (Boczar *et al.* 1980; Boczar & Prézelin, 1986), and from *Exuviella* sp. (Boczar *et al.* 1990). Apparent support for the existence of iPCP was presented by Govind *et al.* (1990). Using affinity purified anti-sPCP antibodies, these authors were able to detect the presence immunoreactive polypeptides with molecular masses identical to the dimeric and the monomeric sPCP apoproteins in the SDS-solubilized membranes of *Prorocentrum concavum*, in which the yield of sPCP is negligible. We have observed that if the 50% $(NH_4)_2SO_4$ precipitation step is omitted from the isolation procedure, and instead, the thylakoid membranes are exhaustively washed with TB buffer until sPCP is no longer detectable, immunoblot analyses show the presence of components that react with anti-ScPCP antibodies. These polypeptides were effectively removed from the thylakoids by the $(NH_4)_2SO_4$ treatment (figure 8), suggesting that in the presence of buffers with low salt concentration, a portion of sPCP remains attached to the thylakoids.

Boczar & Prézelin (1986) suggested that the peridinin–chl *a* fractions isolated by non-denaturing Deriphat-PAGE may represent a dissociation product of a larger complex which includes the Chl *a*–Chl c_2 protein. This putative complex would show characteristics similar to the Chl *a*–Chl *c*–fucoxanthin–protein described by Friedman & Alberte (1984) in *P. tricornotum*. This interpretation was based on the observation that the dominant polypeptides associated with both the Chl *a*–c_2 and the Chl *a*–peridinin moieties have a molecular mass of approximately 19 kDa. In the four species that we analysed, most of the Chl c_2 and peridinin present in the thylakoid membranes are associated with a discrete complex with protein moieties in the 19–20 kDa range. The intact complex demonstrates efficient energy transfer from Chl c_2 and peridinin to Chl *a*. The results of immunoblot analyses presented here demonstrate that the acpPC complexes and sPCP do not possess common antigenic determinants. Indeed, our results indicate that iPCP does not exist in any of the dinoflagellates that we studied.

Energy transfer from Chl c_2 to Chl *a* approaches 100% efficiency (Govindjee *et al.* 1979; Owens 1988). This transfer appears to take place via the Föster dipole-dipole resonance interaction, which would proceed efficiently over 20–60 Å† distances (Friedman & Alberte 1984). Although the mechanism of energy transfer between carotenoids and Chl *a* is largely unknown (Siefermann-Harms 1985), in dinoflagellates the efficiency of this transfer is close to 100% (Prézelin & Haxo 1976). A topological configuration consisting of two peridinin dimers in close proximity to a Chl *a* monomer has been proposed for sPCP (Song *et al.* 1976; Koka & Song 1977; Iglesias-Prieto *et al.* 1991). In the context of acpPC it is reasonable to assume that the peridinin molecules are located in a topological configuration similar to that found in sPCP. This would imply the existence of two spectroscopic domains in the functional complex, one consisting of Chl *a* and Chl c_2, and a second one composed of peridinin and Chl *a*. Spectroscopic evidence supporting this internal heterogeneity has been provided for the Chl *a*–*c*–fucoxanthin–protein complex isolated from the brown alga *D. dichotoma* (Mimuro *et al.* 1990*b*). Furthermore, fractionation of 'chromophyte' SDS-solubilized thylakoids consistently produce Chl *a*–*c*–protein complexes devoid of xanthophylls, and Chl *a*-xanthophyll–protein complexes devoid of Chl *c* (Boczar *et al.* 1980, 1990; Alberte *et al.* 1981; Boczar & Prézelin 1986, 1987). These observations suggest that the spectroscopic domains may also reflect some degree of physical segregation in the holoprotein. Further research is required to clarify the molecular topology of the chromophores in the complex.

Fraction III possesses similar spectroscopic properties as the P_{700}–Chl *a*–protein isolated from plants and bacteria (Shiosawa *et al.* 1974; Verling & Alberte 1983), and from several 'chromophyte' algae (Prézelin & Alberte 1978; Barrett & Anderson 1980; Boczar *et al.* 1980, 1990; Boczar & Prézelin 1986, 1987; Owens & Wold, 1986; Knoetzel & Rensing 1990). The 77 K emission spectrum of this fraction shows the presence of a component emitting at 709 nm, similar to the one observed in PS I from several eukaryotic algae and *Prochloron* (Barrett & Anderson 1980; Schuster *et al.* 1985; Gibbs & Biggins 1991). Based on these similarities, the observed tenfold enrichment in P_{700} activity (when compared with the cell lysates), and the characteristic low fluorescence quantum yield at room temperature, we conclude that fraction III represents the core of PS I, perhaps associated with a minor light harvesting complex. Furthermore, the Chl *a*:P_{700} ratio and the relative yield of Chl *a* in this fraction, suggest that using the fractionation protocol described in the present communication, fraction III contains 100% of the PS I in the cells.

Although fraction III exhibits kinetic and spectroscopic properties that closely resemble PS I preparations from plants, algae and cyanobacteria, PS I in *S. microadriaticum* shows several differences. The electrophoretic profile of fraction III shows that the major polypeptides associated with this complex have mole-

† 1 Å = 10^{-10} m = 10^{-1} nm.

cular masses in the 14–25 kDa range. This is in contrast to plant PS I preparations, in which the major polypeptides have M_r in the 60 kDa range, with several minor polypeptides in the 8–25 kDa range (Golbeck 1988; Andersen *et al.* 1992). These differences are not related to proteolytic degradation of fraction III polypeptides, since the samples showed normal P_{700} signals before electrophoresis. The results suggest that PS I from the four dinoflagellate species studied has a unique apoprotein composition. Smith & Alberte (1991) detected a major 25 kDa polypeptide that cross-reacted with CC I antibodies in several species of chlorophytes. Our results suggest that the apoprotein composition of PS I may be less conserved, in terms of its polypeptide composition and antigenic determinants, than previously recognized. P_{700} activity in *S. microadriaticum* was drastically reduced after addition of Triton X-100. This detergent is commonly employed during assays of P_{700} activity. In addition to its unique composition, fraction III polypeptides did not cross-react with CC I antibodies, suggesting that the P_{700}–Chl *a* proteins of are different from those of dinoflagellates.

In contrast to other functional P_{700}–Chl *a*–protein complexes isolated from 'chromophytes' (Prézelin & Alberte 1978; Barrett & Anderson 1980; Owens & Wold 1986), which showed Chl *a*:P_{700} ratios between 40–55, and were devoid of Chl *c*, fraction III in *S. microadriaticum* shows traces of both Chl c_2 and peridinin, and a Chl *a*:P_{700} ratio which is twice as high. Similar chromophore compositions were reported in Chl *a*–P_{700} proteins isolated by SDS–Deriphat from several species of dinoflagellates (Boczar & Prézelin 1987; Boczar *et al.* 1990; Knoetzel & Rensing 1990), although P_{700} activity was not assayed. The presence of pigments associated with the light-harvesting complexes in the PS I enriched fraction in *S. microadriaticum* may indicate the existence of a peripheral light harvesting complex specifically associated with PS I, analogous to LHCI in plants. Alternatively, since the fluorescence excitation spectrum of this fraction at room temperature shows contributions from both the Chl c_2 and peridinin that are very similar to the ones observed in acpPC (figures 3*c* and 4*c*), the small amounts of Chl c_2 and peridinin present in fraction III may represent a portion of acpPC that remained attached to the core of PS I.

Based on the kinetics of fluorescence induction curves at both room temperature and 77 K, Govindjee *et al.* (1979), suggested that in dinoflagellates the spectral differences between PS I and PS II may be minimal, leading to a balanced excitation of both photosystems. Using changes in fluorescence transients and modulated O_2 evolution in the diatom *P. tricornutum*, Owens (1986) suggested that the Chl *a*–*c*–fucoxanthin complex also distributes excitation equally to both photosystems. The simultaneous use of pulse amplitude modulated fluorescence and O_2 evolution potentially will clarify the role of acpPC in energy distribution between photosystems in dinoflagellates.

By virtue of the presence of Chl *c* in their photosynthetic apparatus, dinoflagellates have traditionally been grouped among the 'chromophyta' (Christensen 1989). Two independent lines of evidence render such affiliation questionable. First, phylogenetic reconstruction based on analysis of SSU rDNA sequences shows that the dinoflagellates, the ciliates and the Apicomplexa share a common ancestry (Johnson *et al.* 1990; Gajadhar *et al.* 1991; Barta *et al.* 1991; Sadler *et al.* 1992), and that the dinoflagellates are distantly related to the 'chromophytes' (Ariztia *et al.* 1991). Secondly, this study indicates that, although the acpPC in dinoflagellates is very similar in structure and composition to light-harvesting pigment–protein complexes in 'chromophytes', consistent with the observations of Friedman & Alberte (1987) and Hiller *et al.* (1988), the apoproteins associated with the complexes in the two groups are immunologically different. This is in contrast to the report of Manadori & Grossman (1990). Given the information currently available to us, it seems probable that the dinoflagellate chloroplast and those of the 'chromophytes' had a common ancestry, but subsequently diverged. A possible, but unlikely alternative, is that the dinoflagellate chloroplast is of independent evolutionary origin, and the acpPC demonstrates evolutionary convergence.

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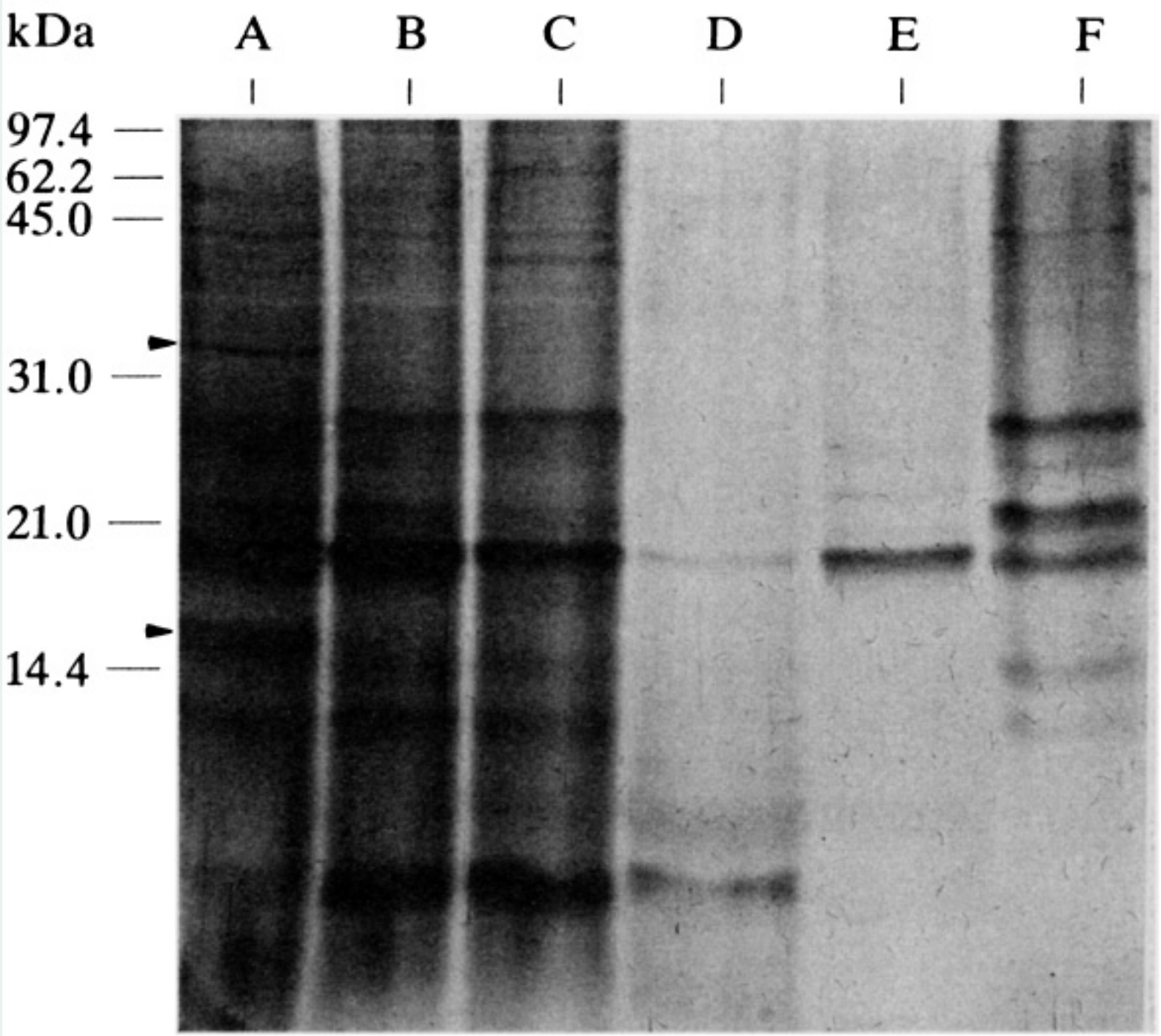

gure 7. SDS-PAGE analysis of the polypeptides associated th the photosynthetic apparatus in *S. microadriaticum*. Lane), cell lysate, arrows identify the 35 and 15 kDa sPCP oproteins, respectively; lane (B), purified thylakoids; ıe (C), DDM-solubilized membrane fraction; lane (D), ction I; lane (E), acpPC (fraction II); and lane (F), ction III.

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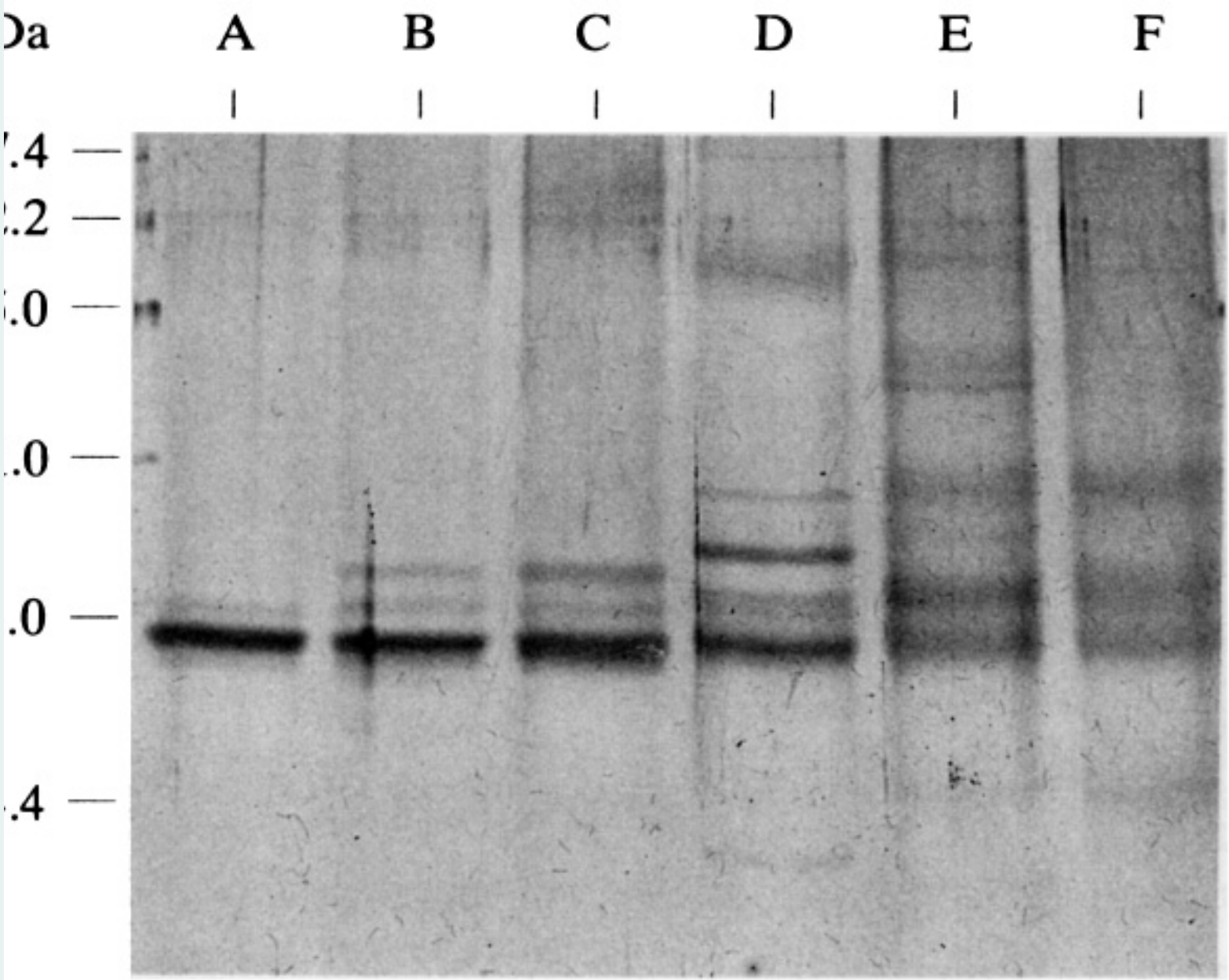
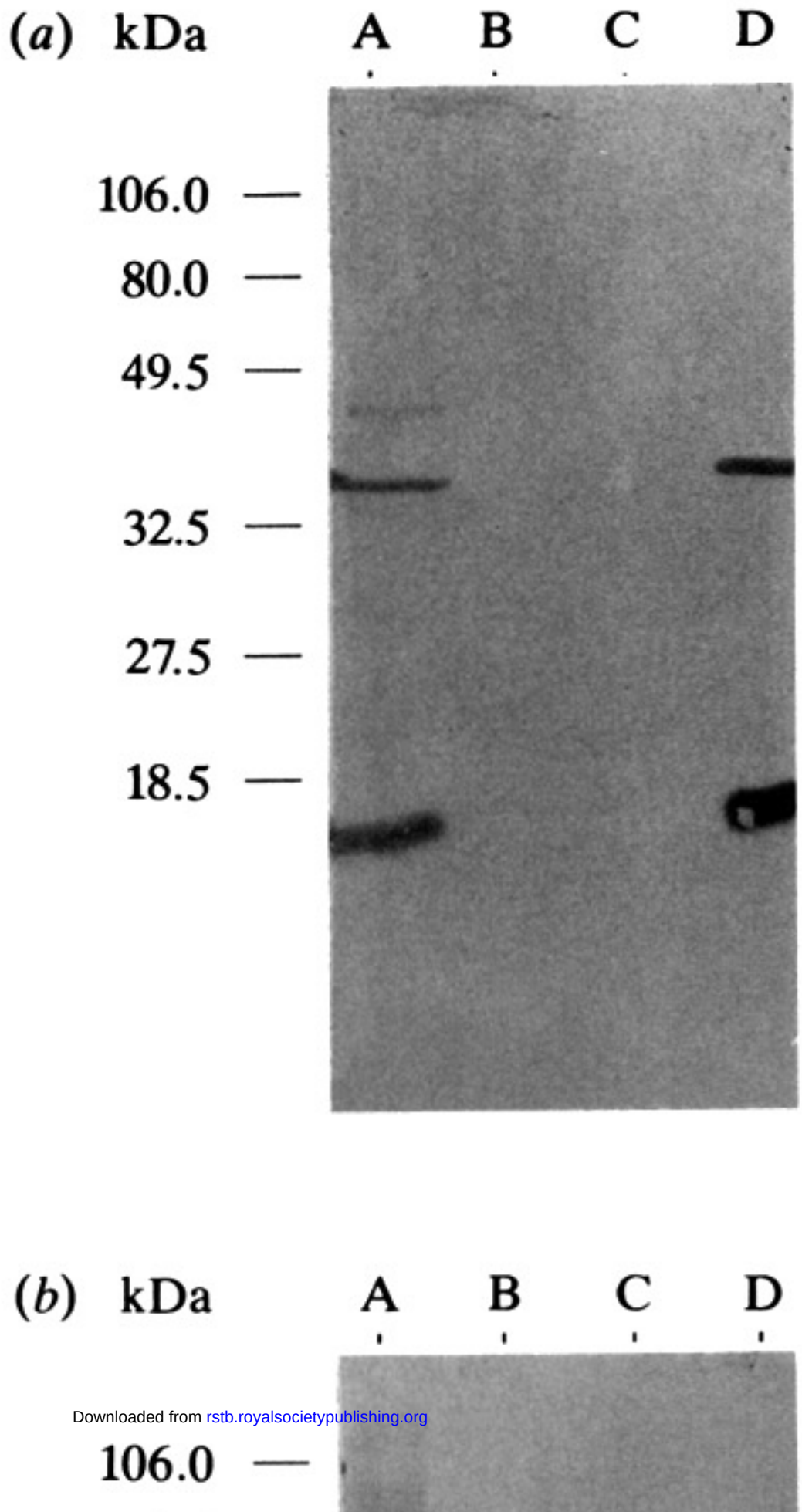


gure 8. SDS-PAGE analysis of the polypeptides associated th acpPC (lanes A–C) and fraction III (Lanes D–F). nes A and D, *H. pygmaea*; lanes B and E, *S. kawagutii*; lanes and F, *S. pilosum*.



igure 9. Immunoblot (Western) analyses of components the photosynthetic apparatus of *S. microadriaticum*. (*a*) nmunoblot probed with anti-Sc-PCP serum. Lane (A), cell sate; lane (B), purified thylakoids; lane (C), apcPC raction II) (1 µg Chl *a* each); lane (D), sPCP (0.1 µg Chl . (*b*) Immunoblot probed with anti-CCI serum. Lane (A), inach thylakoids; lane (B) cell lysate; lane (C), purified embranes; lane (D), DDM-solubilized membranes, lane) fraction III (2.5 µg Chl *a* each). Note that fraction III hibits at least three times more P_{700} activity than the inach thylakoids.