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# Isolation and characterization of Photosystems I and II from the red alga *Porphyridium cruentum*

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**Abstract**

Photosystem I and Photosystem II complexes were isolated from the red alga *Porphyridium cruentum* (*P. purpureum*) following solubilization of thylakoid membranes with dodecyl-β-D-maltoside. More than 90% of the total chlorophyll was recovered in two green bands on a sucrose gradient. PS I and PS II complexes, present in the lower green band, were separated by anion exchange chromatography. The purified PS II complex, with an absorption maximum at 674 nm and a fluorescence emission maximum at 692 nm (77 K), contained the reaction center polypeptide D2 and the core antenna proteins CP43 and CP47 as shown by immunoblot analysis. Two bands (< 14 kDa), presumably the cyt *b*-559 subunits were also observed after silver-staining SDS-PAGE gels. The photoactivity rate of 58 μmol DCPIP/mg Chl per h with DPC for PS II core complexes, and 112 μmol DCPIP/mg Chl per h for thylakoids (minus phycobilisomes), was sensitive to DCMU inhibition. The purified PS I holocomplex had approx. 130 chlorophyll per P700, an absorption maximum at 680 nm and a fluorescence emission maximum at 730 nm (at 77 K). Prominent silver-stained bands in SDS-PAGE gels at 62 and 57 kDa polypeptides were immunologically identified as the P700 apoproteins. Polypeptides of 23.5, 23, 22, 20, 19.5, 18, 16.5, 15.5, and 11 kDa and at least three low molecular mass polypeptides (< 11 kDa) were also observed. Of particular interest are the 18–23.5 kDa polypeptides in the PS I complex, since such polypeptides were previously unknown in phycobilisome-containing organisms. In fact, we have found that the PS I complex of *P. cruentum* is structurally similar to that of higher plants, especially since polypeptides of 18–23.5 kDa were shown to be immunologically related to LHC I of higher plants (Wolfe, G. et al. (1994) Nature 367, 566–568). These results clearly suggest that the thylakoid structure of this red alga is more complex than in cyanobacteria, which like rhodophytes, also have phycobilisomes as major antenna complexes.

*Keywords:* Rhodophyte; Photosystem I; Photosystem II; Light harvesting complex I; (*Porphyridium*)

## 1. Introduction

Red algae have been evolutionarily linked to cyanobacteria based upon the gross similarities of their photosynthetic apparatus [1,2]. All oxygen-evolving photosynthetic organisms, including the rhodophytes and cyanobacteria, have two photosystems which mediate the transduction of light energy to chemical energy and the transfer of electrons from water to $NADP^+$. Chlorophyll (Chl) *a*-binding proteins that comprise the photosystem cores are apparently ubiquitous throughout cyanobacteria and photosynthetic eukaryotes; however, extreme variability exists in the peripheral antenna complexes. Red algae and cyanobacteria are unique in that they contain phycobilisomes, extrinsic antenna complexes with bilin chromophores, on the stromal surface of thylakoids [3]. Higher plants, and

Abbreviations: β-DM, dodecyl-β-D-maltoside; Chl, chlorophyll; CP, chlorophyll–protein complex; CP43, Chl-protein of Photosystem II core and product of the *psbC* gene; CP47, Chl-binding protein of the Photosystem II core and product of the *psbB* gene; CP I, Photosystem I reaction center apoproteins and products of the *psaA* and *psaB* genes; cyt *f*, cytochrome *f*; D2, Photosystem II reaction center apoprotein and product of the *psbD* gene; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea); DCPIP, 2,6-dichlorophenolindophenol; DPC, 1,5-diphenylcarbazide; LHC, chlorophyll-binding light harvesting complex; LGB, lower green band; MES, (2[*N*-morpholino]ethane sulfonic acid); PS I, Photosystem I; PS II, Photosystem II; SDS-PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; UGB, upper green band.

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algae other than the rhodophytes, have Chl *a*/*b*-or *a*/*c*-binding light-harvesting complexes (LHC) which serve as antenna systems directing absorbed light energy towards the photosystem cores [4]. LHCs are integral membrane complexes composed of numerous nuclear encoded polypeptides with molecular masses typically of 20–30 kDa (for reviews see [5,6]). The occurrence of LHCs or LHC-like proteins has not previously been demonstrated in any phycobilisome-containing organism [6–9]. Phycobilisomes are thought to fulfill the role of primary light-gathering antenna and as being functionally equivalent to Chl *a*/*b* and Chl *a*/*c* complexes. Hence, it has been assumed that cyanobacteria and rhodophytes have no LHCs and that all of the Chl antenna pigments are associated with the polypeptides of the core complexes of the two photosystems.

Although the structure of phycobilisomes in red algae has been well studied (cf. [3,10]), there is a paucity of data on the composition of the photosystems from red algae. Redlinger and Gantt [8] examined the Chl-protein complexes (CP) in thylakoids of *Porphyridium cruentum* (also known as *P. purpureum*) by low temperature SDS-PAGE. Three separate CP (CP I, CP III and CP IV) were isolated and characterized: CP I contained the Photosystem I (PS I) reaction center apoproteins (approx. 68 kDa), and Photosystem II (PS II) components occurred in CP III (51 kDa) and CP IV (40, 48 and 52 kDa). The existence of other Chl-binding complexes or polypeptides could not be ascertained because much of the Chl migrated at the electrophoretic front.

Phycobilisome-PS II particles were also isolated from the red alga *P. cruentum* [11,12] but polypeptide components of the PS II core complex were difficult to identify in the presence of the much more abundant polypeptide subunits of the phycobilisomes. A more recent study reported the isolation of a PS I complex from detergent solubilized thylakoid membranes of *P. cruentum* and lists polypeptides of 68, 60, 54 and 13 kDa as constituents [7]. With this milder procedure much of the Chl was associated with protein. In the acidophilic red alga *Cyanidium caldarium* thylakoids were fully solubilized, and whereas typical high molecular mass polypeptides of the PS I reaction center were found, LHC-type polypeptides were not reported [9].

We describe here another method devised for the isolation of active PS I and PS II complexes from thylakoid membranes of the unicellular, marine, red alga, *P. cruentum*. At least 90% of the Chl is recovered in Chl-binding protein fractions when thylakoids are solubilized by this procedure. We show that there is greater complexity in subunit composition of PS I from *P. cruentum* than was previously anticipated from comparisons with cyanobacteria. The PSI complex of *P. cruentum* contains at least 14 subunits including prominent polypeptides of approx. 18–23.5 kDa that had not been previously found in a photosystem complex isolated from phycobilisome-containing organisms. Preliminary findings of this work were presented at the IXth International Congress on Photosynthesis [13].

## 2. Materials and methods

### 2.1. Cell culture

*Porphyridium cruentum* (ATCC 50161) was grown in Jones' artificial seawater medium [14] at 18°C. One-liter batch cultures were bubbled with 5% carbon dioxide/95% air and shaken on a rotary shaker at 80 cycles/min with continuous illumination (Sylvania VHO, daylight, 115 W fluorescent lamps) filtered through cheesecloth to adjust light intensity. Cultures were maintained at an irradiance level of 15 $\mu$E $m^{-2} s^{-1}$ and were harvested at a cell density of 3–4 · $10^6$ cells/ml.

### 2.2. Isolation of thylakoid membranes

Thylakoid membranes were isolated by a method similar to that described by Cunningham et al. [15]. Cultures of *P. cruentum* were harvested in the exponential growth phase by centrifugation at $5900 \times g$ (6000 rpm in a Sorvall GSA rotor) for 6 min. Pelleted cells were washed in deionized water and then centrifuged at $16000 \times g$ (10000 rpm in a Sorvall GSA rotor) for 10 min. Pellets were resuspended in 2 ml of 50 mM Na phosphate buffer (pH 7.0) per gram of cells. All subsequent steps were carried out at 4°C or on ice. The cells were broken in a French pressure cell (AMINCO) at 15000–16000 psi and the pressate was centrifuged for 60 min at $305000 \times g$ (55000 rpm in a Beckman 60 Ti rotor). Pellets were homogenized in 0.5 M sucrose, 2.5 mM $Na_2$-EDTA, 10 mM Na phosphate buffer (pH 7.0) and 10 ml of the homogenate was layered onto sucrose step gradients composed of 8 ml of 1.6 M sucrose and 19 ml of 0.8 M sucrose each in 10 mM Na phosphate buffer (pH 7.0). Sucrose gradients were centrifuged for 3 h at $113000 \times g$ (25000 rpm in a Beckman SW-28.1 rotor). Thylakoid membranes were collected with a syringe from the 0.8 M/1.6 M sucrose interface and stored at −80°C for later use.

### 2.3. Isolation of β-DM extracted photosystem complexes

Thylakoid membrane fractionation was carried out at 4°C throughout the entire procedure. Membranes were suspended in cold 50 mM Na phosphate buffer (pH 7.0), 150 mM NaCl and centrifuged for 30 min at $45000 \times g$ (26000 rpm in a Beckman Type 65 rotor).

For further removal of extrinsic proteins the pellet was suspended in 1 M NaBr according to the procedure of Malkin [16] and after 30 min was centrifuged as above. The thylakoid membranes were prepared for solubilization in dodecyl-β-D-maltoside (β-DM) according to a procedure adapted from that described by Fotinou and Ghanotakis [17]. To remove residual NaBr, membranes were washed in 0.4 M sucrose, 50 mM (2[*N*-morpholino]ethane sulfonic acid) (MES) (pH 6.0), 10 mM NaCl, and were then resuspended in the same buffer to a final concentration of 350 μg Chl/ml. The resuspended thylakoids were mixed with an equal volume of 50 mM MES (pH 6.0), 1 M sucrose, 10 mM NaCl, 1.32% β-DM (w/v) and incubated for 30 min and after that were centrifuged for 2 h at 35000 × *g* (21000 rpm in a Beckman Type 65 rotor). The supernatant was diluted with an equal volume of 50 mM MES (pH 6.0) and 2.0 ml (350 μg Chl) layered onto 15–30% (w/v) linear sucrose gradients containing 50 mM MES (pH 6.0), 10 mM NaCl, 10% glycerol (v/v) and 0.04% β-DM (w/v). The gradients were centrifuged for 16 h at 252000 × *g* (50000 rpm in a Beckman 60 Ti rotor). Two distinct and well-separated green bands were resolved on the gradients.

The lower green band (LGB), which contained the photosystem complexes, was further fractionated on a Q Sepharose Fast Flow (Pharmacia) anion exchange column (10 mm i.d. × 3 cm) equilibrated with 5 mM $LiClO_4$ in 20 mM Bis-Tris (pH 6.0), 0.3 M sucrose, 10% glycerol, 0.04% β-DM. By this method Fotinou and Ghanotakis [17] had shown good recovery of PS II with β-DM-solubilized membranes and $LiClO_4$ elution. Following sample (200–300 μg Chl) loading, proteins were eluted from the column with a step gradient consisting of 5 mM, 75 mM, 125 mM and 200 mM $LiClO_4$ in 20 mM Bis-Tris (pH 6.0), 0.3 M sucrose, 10% (v/v) glycerol, 0.04% β-DM (w/v) at a flow rate of 1.0 ml/min, and 4 ml fractions were collected. Successive buffer steps were applied until the elution of protein was no longer detectable by absorbance at 280 nm. The non-binding fractions, those that eluted during sample loading and with 5 mM $LiClO_4$, were combined, dialyzed for 5 h against 20 mM Bis-Tris (pH 6.0), 10% glycerol (v/v), 0.04% β-DM to remove MES (an anionic buffer), then loaded onto a second Q Sepharose Fast Flow column equilibrated with 5 mM $LiClO_4$ in 20 mM Bis-Tris (pH 6.0), 10% glycerol, 0.04% β-DM without sucrose. Fractions were eluted with a linear gradient of 5–125 mM $LiClO_4$ in the buffer used for dialysis. Following the linear gradient, elution in 125 mM $LiClO_4$ was continued until protein in the eluate was no longer detectable at $A_{280}$. The column was subsequently washed with 200 mM $LiClO_4$. Relative amounts of protein and Chl per fraction were examined by measuring absorbance at $A_{280}$ (protein) and $A_{675}$ (Chl) for each fraction.

### 2.4. Quantitation of chlorophyll

Chlorophyll concentrations were determined in *N,N*-dimethylformamide from the absorbance at 664 nm according to the method of Moran [18]. The concentration of *N,N*-dimethylformamide after the addition of samples was never less than 95%.

### 2.5. Cytochrome analysis

Cytochrome *f* (cyt *f*) was assayed at 554 nm by examining ascorbate-reduced minus potassium ferricyanide-oxidized difference spectra [19].

### 2.6. Absorption and fluorescence spectroscopy

Absorption spectra were measured at room temperature using a Perkin Elmer Lambda 6 spectrophotometer. Fluorescence spectra were determined at 77 K with an Aminco-Bowman spectrophotofluorometer. Samples (2 parts by volume) were suspended in 1 part glycerol, and 4 parts potassium glycerophosphate. The mixture was transferred to NMR sample tubes (5 mm o.d.) and frozen in liquid nitrogen. This produced a clear 'glass', thereby minimizing scattering effects. Emission spectra were obtained using an excitation wavelength of 440 nm.

### 2.7. Polyacrylamide gel electrophoresis and gel staining

Prior to electrophoresis, samples were incubated in 50 mM Tris-HCl (pH 6.8), 10% glycerol (w/v), 2% sodium dodecyl sulfate (SDS), at room temperature for 30–45 min. SDS–polyacrylamide gel electrophoresis (SDS-PAGE) was carried out in 1.5 mm thick, 7–15% polyacrylamide slab gels according to Laemmli [20]. Protein bands were visualized by silver staining following the procedure of Oakley et al. [21].

### 2.8. Immunoblotting

Proteins separated on polyacrylamide gels were transferred to Immobilon-P® membranes (Millipore) according to the method of Peluso and Rosenberg [22]. Immunoprobing was carried out as described in Mustardy et al. [23].

Polyclonal rabbit antibodies to the PS I core components (CP I, presumed *psaA* and *psaB* gene products) and to CP47, the PS II core antenna polypeptide (presumed *psbB* gene product) and allophycocyanin (phycobiliprotein from the phycobilisome core) were elicited with antigens derived from *P. cruentum* [23], while the CP43 (presumed *psbC* gene product) antiserum was obtained using an antigen derived from *Nostoc* sp. thylakoids.

The antiserum to D2 (PS II reaction center apoprotein and *psbD* gene product) was a gift from Dr. B.A. Diner of the E.I. Du Pont de Nemours Co., Wilmington, DE. and was produced as described in Nixon et al. [24].

### 2.9. PS I reaction center determination

The concentration of P700 in PS I was determined from sodium ascorbate-reduced minus potassium ferricyanide-oxidized difference spectra as described by Kawamura et al. [25], and using an extinction coefficient of 77 $mM^{-1}cm^{-1}$ ($\Delta A_{700\ nm} - \Delta A_{750\ nm}$) based upon the difference spectrum as published for Triton X-100 solubilized thylakoids by Hiyama and Ke [26]. P700 determinations were performed on samples at a concentration of 4–10 $\mu$g Chl/ml in 20 mM Bis-Tris (pH 6.1), 10% glycerol (v/v), 0.04% $\beta$-DM. PS I preparations were first oxidized by the addition of potassium ferricyanide from a 100 mM stock solution to a final concentration of 1 mM [27,28]. Samples were then reduced by the addition of solid ascorbate.

### 2.10. Photosystem II activity

PS II activity was determined spectrophotometrically in a Perkin Elmer Lambda 6 spectrophotometer with actinic white light (Dolan Jenner Fiber-Lite model 170-D) provided at right angles to the reference beam (3400 $\mu E\ m^{-2}\ s^{-1}$ for 30 s). The reference cuvette was shielded by an opaque screen and the photomultiplier was protected by a hand-operated shutter. Samples with 50 $\mu$M DCPIP, 1 mM DPC, 0.5 M sucrose, 0.05 M $KPO_4$, and 0.04% $\beta$-DM at pH 7.5 were assayed at a concentration of 1 $\mu$g Chl per ml. Absorbance changes at 600 nm were measured and an extinction coefficient of 20.6 $mM^{-1}\ m^{-1}$ was used to calculate photoreduction of the electron acceptor 2,6-dichlorophenolindophenol (DCPIP) [29]. The reported values represent light minus dark rates. Both thylakoid preparations and PS II complexes were inhibited by 10 $\mu$M DCMU.

## 3. Results

### 3.1. Fractionation of P. cruentum thylakoid membranes

Peripheral proteins, including phycobiliproteins, were removed from thylakoid membranes by the buffer washes and treatment with 1.0 M NaBr prior to solubilization. Solubilization of thylakoid membranes with the nonionic detergent $\beta$-DM resulted in high recovery of Chl–protein complexes with minimal free pigment. A summary encompassing the procedural steps is presented in Fig. 1. Approximately 90% of the Chl in the thylakoids was solubilized by this method. Two Chl-containing bands were obtained on a sucrose gradient after centrifugation of the solubilized membranes. Approximately 16% of the Chl was in the upper green band (UGB) with 74% occurring in the lower green band (LGB) and 10% left in the pellet after thylakoid membrane solubilization. It is significant that free Chl was not detectable on the gradient nor were any other colored bands, since phycobiliproteins were removed in previous rinses.

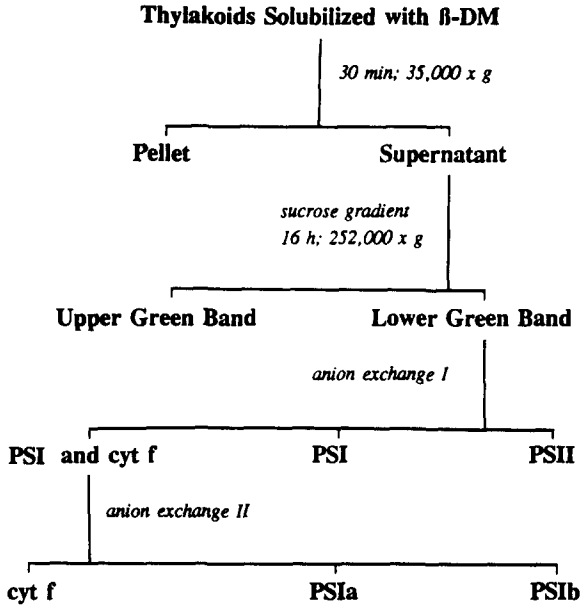


Fig. 1. Flow diagram for the isolation of PS I and PS II complexes from $\beta$-DM solubilized thylakoids of *P. cruentum*. (See Sections 2 and 3 for details.)

Spectral differences of the Chl-binding UGB and LGB bands recovered from the sucrose gradient were significant. The UGB had an absorbance maximum at 672 nm (Fig. 2), and a fluorescence maximum at 683 nm at 77 K (not shown), and considerable absorption

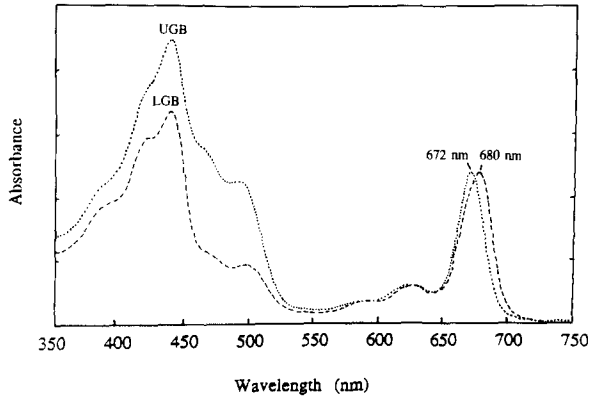


Fig. 2. Room temperature absorption spectra of the upper green band (UGB) and lower green band (LGB) obtained after sucrose gradient centrifugation of $\beta$-DM-solubilized (0.66%) *P. cruentum* thylakoids.

in the carotenoid region (460–520 nm). The LGB, by comparison, had an absorption maximum at approx. 680 nm with a lower absorption in the carotenoid region.

The UGB contained many protein bands some of which were also distinct in unfractionated thylakoids as seen by silver staining of SDS-PAGE (Fig. 3, lanes 1 and 2). P700 and the PS I reaction center apoproteins (57 and 62 kDa region) were lacking in the UGB as confirmed by P700 analysis and the absence of a reaction by immunoblot analysis with anti-PS I. Immunoblot analysis also showed that this green band did not contain PS II core complexes (Fig. 3, lanes 3–6). Whereas CP43, the peripheral PS II core antenna protein was present, other PS II core polypeptides including CP47 and D2 were not detected. Particularly prominent were polypeptides of approx. 18–23.5 kDa. We presume that most of the Chl and carotenoid in this fraction is bound to polypeptides of this molecular mass region and that they belong largely to PS I. This assumption is based on results from analysis of the PS I holocomplex where it was shown that polypeptides of such molecular mass can be stripped from the PS I holocomplex and recovered along with the Chl [30]. Since PS I and PS II reaction centers were lacking from the UGB we did not analyze it further at this time.

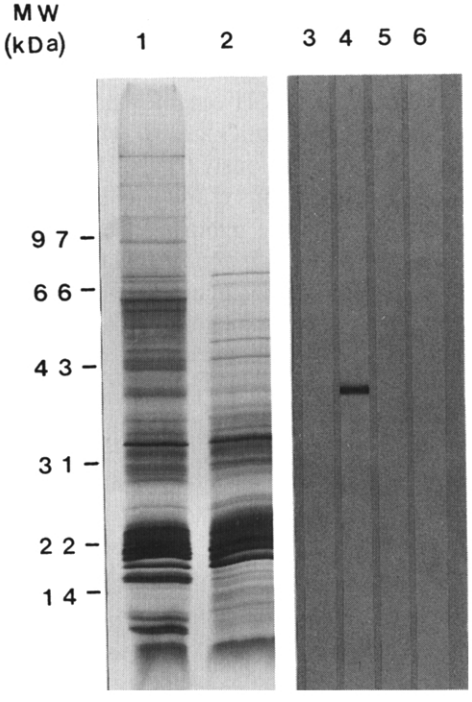


Fig. 3. SDS-PAGE of thylakoids (lane 1) and of the upper green band (lane 2) after silver staining. Immunoblots of the upper green band probed with anti-CP47 (lane 3), anti-CP43 (lane 4), anti-D2 (lane 5) and anti-CP I (lane 6) demonstrate that the upper green band is deficient in PS I and PS II reaction center core polypeptides.

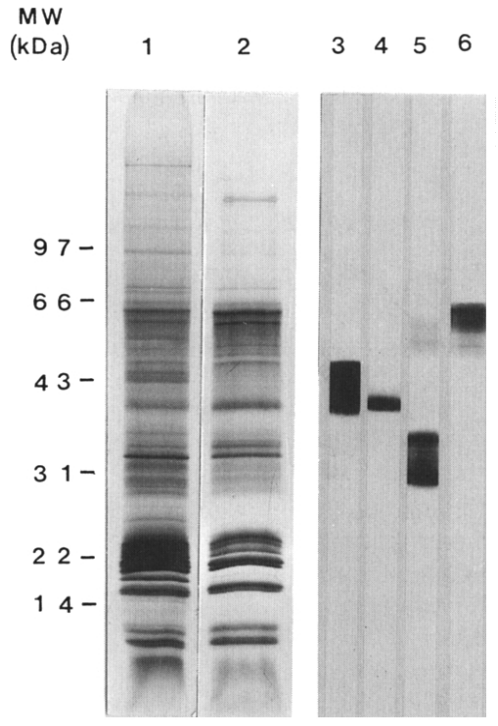


Fig. 4. SDS-PAGE of thylakoids (lane 1) and of the lower green band (lane 2) after silver staining. Immunoblots of the lower green band probed with anti-CP47 (lane 3), anti-CP43 (lane 4), anti-D2 (lane 5) and anti-CP I (lane 6) show that the PS I and PS II reaction center polypeptides are present.

### 3.2. Analysis of the lower green band

Enrichment of PS I and PS II apoproteins in the LGB is indicated by silver-stained SDS-PAGE (Fig. 4, lane 2) and is confirmed by immunoblots. Both the PS I and PS II core polypeptides were present in the LGB as shown by immunoblot analysis (Fig. 4, lanes 3–6). By SDS-PAGE, two prominent bands in the 62–57 kDa region were present in unfractionated thylakoids (lane 1) and in the LGB (lane 2). These were identified as the PS I reaction center apoproteins (lane 6) by immunoblotting with anti-CP I. Presence of the PS II core components was verified by positive reactions with anti-D2 (lane 5), anti-CP43 (lane 4) and anti-CP47 (lane 3).

Separation of PS I and PS II complexes was accomplished with column chromatography. Components of the LGB were fractionated by two consecutive anion exchange columns. Chl monitored at $A_{675}$ and protein at $A_{280}$ were found to co-elute, indicating that Chl maintained its association with protein. The presence of PS I and PS II was ascertained by immunoprobing of the eluting fractions (not shown), while cyt $f$ was followed by a spectroscopic assay. PS II was largely separated from other components of the LGB on the first Q-Sepharose Fast Flow anion exchange column (Fig. 5A). Under the conditions employed, the majority

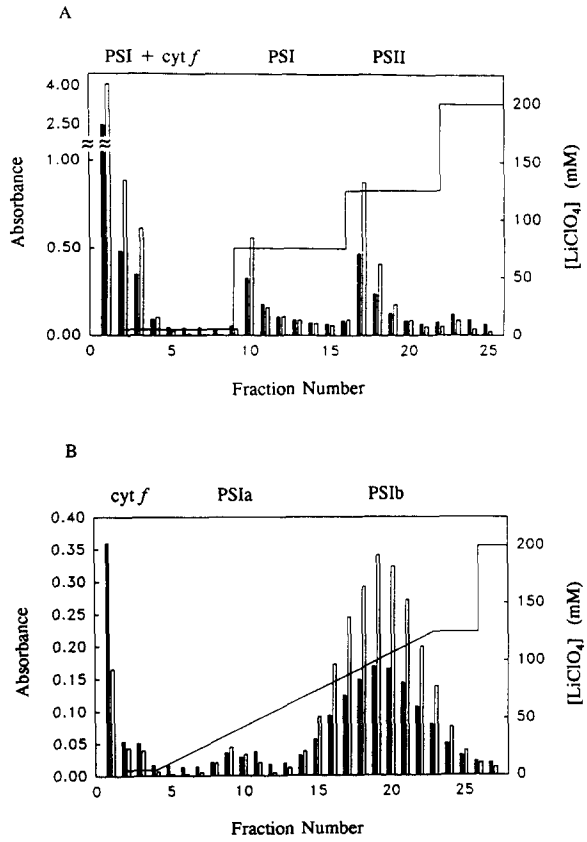


Fig. 5. Elution profiles on Q-Sepharose Fast Flow anion exchange columns of (A) the constituents of the lower green band, and (B) the components of the nonbinding fraction from the first column, namely, cyt *f*, PS Ia and PS Ib. Absorbance of protein was measured at 280 nm (solid bars) and of chlorophyll at 675 nm (open bars). Thylakoid components eluting in 4 ml fractions of various $LiClO_4$ concentrations (solid line) are indicated at the top of each figure.

of PS I and 48% of the total Chl did not bind to the column and was recovered in fractions 1–5. These fractions also contained cyt *f*. All of the PS II bound to the column as did a small amount of PS I. The bound PS I was eluted with 75 mM $LiClO_4$ (fractions 10–16) and represents approximately 8% of the total Chl. About 14% of the total Chl was present in fractions enriched in PS II and eluted from the column with 125 mM $LiClO_4$ (fractions 16–21).

### 3.3. Photosystem I

The non-binding fractions from the first anion exchange column (fractions 1–5) were dialyzed and then further fractionated on a second Q-Sepharose Fast Flow anion exchange column eluted with a linear gradient of 5 to 125 mM $LiClO_4$ (Fig. 5B). Under these conditions, approximately 5% of the total Chl and all of the cyt *f* eluted during sample loading in 5 mM $LiClO_4$ (fractions 1–4). Fractions enriched in PS I complexes were eluted subsequently. A minor PS I component, designated PS Ia, eluted in 30–40 mM

Table 1
Characteristics of isolated Photosystem I and Photosystem II complexes from *Porphyridium cruentum*

| | PS I Holocomplex | PS I Core | PS II Core |
|---|---|---|---|
| Chl/P700 [a] | 130±12 | 98±4 | – |
| Absorbance max. nm | 680 | 679 | 674 |
| Emission max. nm [b] | 730, (680) | 720, (680) | 692 |
| DCP > DCPIP | – | – | 58 [c] |

[a] From Ref. [30].
[b] Samples at 77 K, minor peaks in parentheses.
[c] $\mu$mol DCPIP (mg Chl)$^{-1}$ h$^{-1}$ (3400 $\mu$E m$^{-2}$ s$^{-1}$).

$LiClO_4$ and contained about 3% of the total Chl (fractions 7–12). A major component designated as PS Ib, with 41% of the total Chl, eluted in approximately 100 mM $LiClO_4$ (fractions 13–26). This was the predominant PS I population and contained the PS I holocomplex. Such a complex was shown to have approx. 130 Chl per P700 by oxidized minus reduced difference spectra (Table 1).

Room temperature absorption spectra of PS I and PS II are shown in Fig. 6. Absorbance maxima in the red region of PS I and PS II complexes were at 680 nm and 674 nm, respectively. Both spectra lacked significant absorbance between 545 and 650 nm, indicating that phycobiliproteins were not associated with the photosystems in these preparations. This was also confirmed by immunoblotting (not shown). Fluorescence emission spectra (77 K, excitation at 440 nm) showed that PS I has a major peak at 730 nm and a lesser emission peak at 680 nm (Fig. 7A) (Table 1). A 730–735 nm emission peak is characteristic of PS I holocomplexes (cf. [31]). The PS II complex had a single emission maximum at 692 nm (Fig. 7B) which is consistent with data reported previously (cf. [7,31,32]).

Most of the PS I in this study was isolated as PS Ib, and we consider this the major, and perhaps the only, PS I holocomplex in *P. cruentum* thylakoids. The PS Ib polypeptide composition included those of apparent

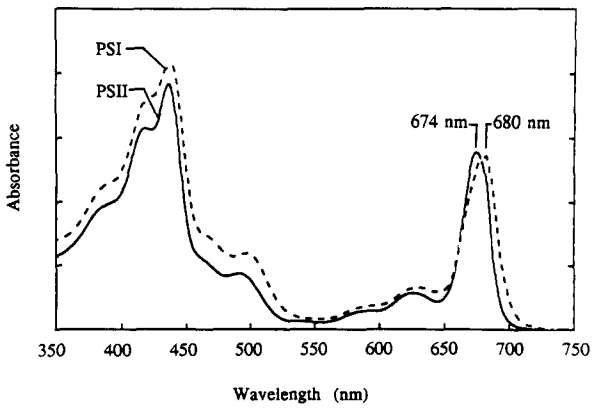


Fig. 6. Room temperature absorption spectra of isolated complexes of PS I (dashed line) and PS II (solid line). Absorbance maxima in the red region are indicated.

molecular masses of 62, 57, 23.5, 23, 22, 20 19.5, 18, 16.5, 15.5, 11 kDa and at least three low molecular mass polypeptides of < 11 kDa (Fig 8, lane 2). The two high molecular mass proteins, the P700 apoproteins, crossreacted with anti-CP1 (Fig. 8, lanes 3 and 4), but PS II reaction center polypeptides were not seen by silver staining and indeed no reaction was observed after probing the same blots with anti-D2. PS Ia was deficient in polypeptides of approx. 22, 19.5 and 18 kDa (Fig. 8, lane 1). Whereas heterogeneity among PS I has been reported in both cyanobacteria [33] and higher plants [34,35], and may exist here, a more conservative explanation is that the PS Ia purified in this study reflects a loss of subunits during the isolation procedure.

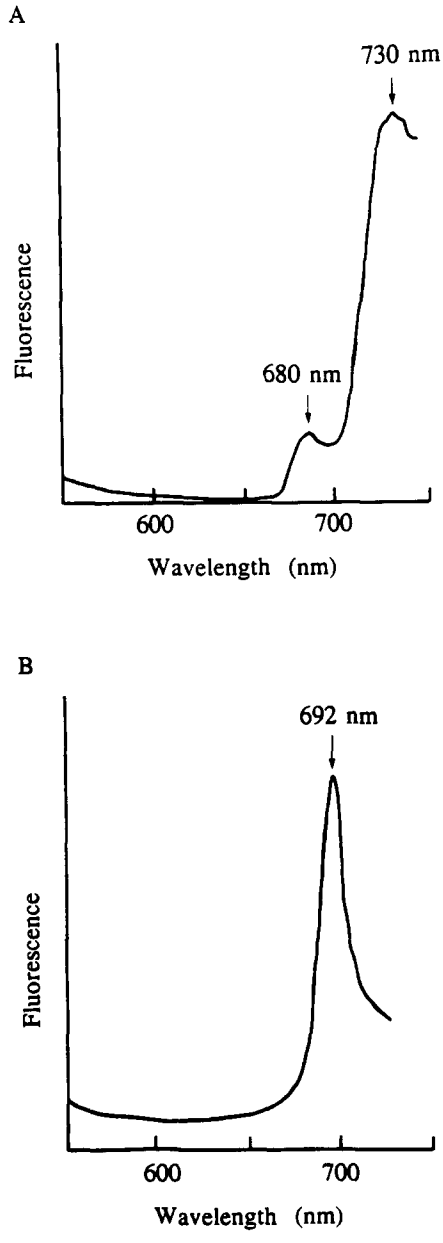


Fig. 7. 77 K fluorescence emission spectra of isolated complexes of PS I (A) and PS II (B) with fluorescence maxima are indicated. Excitation at 440 nm.

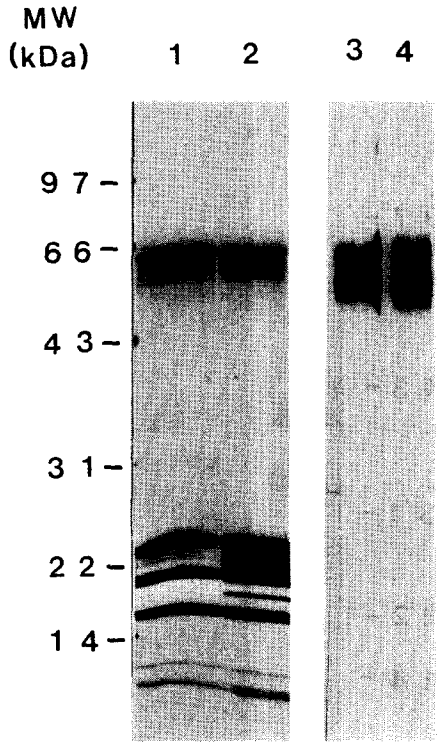


Fig. 8. SDS-PAGE of PS Ia (lane 1) and PS Ib (lane 2) silver-stained. Immunoblots of PS Ia (lane 3) and PS Ib (lane 4) were probed with both anti-CP I and anti-D2, confirming PS II absence.

### 3.4. Photosystem II

Analysis of the PS II core complex of *P. cruentum* suggests that it contains polypeptides that are also typical of cyanobacteria and higher plants. As seen in Fig. 9 it contains prominent polypeptide regions at approx. 40–47 (diffuse), 39–40 and 32–34 (diffuse) kDa, and at least two additional lower molecular mass polypeptides (< 14 kDa) of lesser intensity. Immunoblot analysis with anti-D2 showed that the 32–34 kDa polypeptide region reacted, as did a broad band of around 55 kDa. This higher molecular mass band probably represents aggregates of core components. Similar immunostaining patterns have been described previously for both cyanobacterial [33,36] and higher plant [37] PS II preparations. The polypeptide at 39–40 kDa reacted with anti-CP43, and the broad, diffuse band at 40–47 kDa crossreacted with anti-CP47. A polypeptide of approx. 16.5 kDa was not identified, and we presume that the apoproteins below 14 kDa to be the $\alpha$ and $\beta$ subunits of cyt *b*-559. Our PS II preparation was free of PS I as verified by lack of a 730 nm fluorescence emission and by immunoblotting (Fig. 9, lane 5). Also lacking was evidence of antennae components

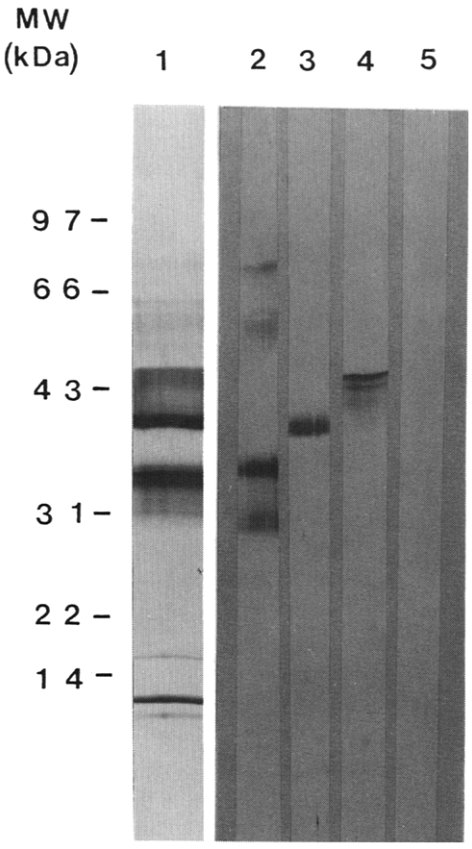


Fig. 9. SDS-PAGE of PS II silver-stained (lane 1) and immunoblots of PS II probed with anti-D2 (lane 2), anti-CP43 (lane 3), anti-CP47 (lane 4), and anti-CP I (lane 5), showing PS I absence.

including the 92 kDa thylakoid-membrane polypeptide of phycobilisomes [38] (immunoprobing data not shown).

Thylakoids no longer evolved oxygen after removal of phycobilisomes and other extrinsic proteins; thus the photochemical activity of the PS II core complex was compared with that of thylakoids by assaying the reduction of DCPIP in the presence of DPC [39]. At a light intensity of 3400 $\mu$E $m^{-2} s^{-1}$ the activity of thylakoids was approx. 112 $\mu$mol DCPIP (mg Chl)$^{-1}$ $h^{-1}$. The PS II complexes under the same conditions had an activity of 58 $\mu$mol DCPIP (mg Chl)$^{-1}$ $h^{-1}$ (Table 1).

The photoactivity rates for *P. cruentum* obtained here are highly similar to those for spinach obtained by Vernon and Shaw under similar conditions [39]. Previously, Chereskin et al. [11] measured the activity of PS II-phycobilisome (PS II-PBS) particles and found an activity of 261 $\mu$mol DCPIP (mg Chl)$^{-1}$ $h^{-1}$, and a DCMU inhibition of 92% at 1000 $\mu$E $m^{-2} s^{-1}$. Marquardt and Ried [7] found a lesser activity in Deriphat-treated thylakoid membranes (minus phycobilisomes) (13–17 $\mu$mol DCPIP (mg Chl)$^{-1}$ $h^{-1}$) and no detectable PS II activity in green bands from fractionated thylakoids. The large difference between the relatively low activity of the PS II complexes purified here and the high activity of PS II-PBS particles [11] is attributable to partial inactivation upon removal of phycobilisomes and isolation of PS II complexes.

## 4. Discussion

### 4.1. Thylakoid solubilization

The fractionation procedure developed here for thylakoids of *Porphyridium cruentum* resulted in recovery of approx. 90% of the total Chl in protein-containing fractions. This is in contrast to previous results where solubilization using SDS resulted in considerable loss of free Chl [8], and where lauryl-$\beta$-iminodipropionate yielded incomplete solubilization with 20–40% of the Chl recovered in protein complexes [7].

Most of the Chl in *P. cruentum* is associated with PS I as evidenced by the 52% recovery in PS I fractions of the LGB (Fig. 5). It is likely that much of the Chl (16% of total) in the UGB is bound to LHC I-type apoproteins. Whereas, this assumption requires further confirmation, it is based on the presence of significant amounts of the 18–23.5 kDa polypeptides in the UGB that are comparable in molecular mass to polypeptides in the PS I complexes from the LGB (Fig. 3, lane 2; Fig. 8, lanes 1–2), and our recent demonstration that such polypeptides bind Chl and are immunologically related to LHCs [30]. A small amount of the Chl in the UGB is no doubt part of CP-43, but it is unlikely that it accounts for much of the Chl since polypeptides of the LHC I region are the most prevalent (Fig. 3 lane 2).

Presently there is no evidence that the PS II core in red algae exists in association with LHC II-polypeptides. However, such a possibility cannot be ruled out until the UGB is more fully analyzed. If LHC II polypeptides exist in red algae, we would expect them also to be in the 18–23.5 kDa range and to be present in much lower concentration than LHC I polypeptides.

### 4.2. The thylakoid structure of Porphyridium cruentum is more complex than that of cyanobacteria

Characteristics of the photosystems of *P. cruentum* are summarized in Table 1, which also includes data from a more extended analysis of the PS I holocomplex [30]. Chl *a* was the only type of Chl present in the PS I holocomplex and in intact thylakoids. Upon detergent solubilization (1% $\beta$-DM) of the energetically functional holocomplex, a PS I core fraction and LHC I fraction were obtained. Chl declined to approx. 100 Chl/P700 in the PS I core as did polypeptides of 19.5–23.5 kDa, but the P700 apoprotein region was greatly enhanced. The 19.5–23.5 kDa polypeptides were

recovered in the green LHC I fraction. Immunoblot analysis showed that at least five of the red algal PS I polypeptides (19.5, 20, 22, 23 and 23.5 kDa) were immunodecorated with an antiserum raised to barley PS I complex (CP Ia). However, immunoblotting of thylakoids of a cyanobacterium (*Nostoc* sp.) with the same antiserum showed no reactive LHC I-type polypeptides [30]. Thus, by subunit composition and immunorelatedness the PS I holocomplex of *P. cruentum* resembles that of other eukaryotes rather than those of cyanobacteria.

The finding of the LHC I-associated polypeptides in a red alga is contrary to the common view that red algal thylakoid structure is like that of cyanobacteria. In fact, the lack of LHCs in cyanobacteria [33,40,41] in comparison to green algae [42,43] and higher plants [44–46] has been repeatedly documented, and whereas the PS I holocomplex from cyanobacteria resembles the PS I core complex of higher plants and green algae [47], the polypeptides at approx. 18–23.5 kDa are lacking.

Our results on the occurrence of LHC I in the PS I complex in red algae differ from the studies made by two other laboratories on *P. cruentum* [7] and *Cyanidium caldarium* [9]. Both studies concluded that the red algal PS I lacked LHC polypeptides and were therefore similar to the cyanobacterial type PS I. In characterizing PS I prepared with lauryl-β-iminodipropionate Marquardt and Reid [7] noted significant detergent effects that tended to shift fluorescence emission peaks. A detergent effect may explain the apparent disparity in emission from our PS I preparation of 730 nm vs. the 718 nm emission from their preparation. In higher plants it is known that the removal of the LHC I from the PS I holocomplex results in a shift in the fluorescence maximum at 730 nm from the holocomplex, to an emission at 717–722 nm from the PS I core complex ([43], cf. [30]). Of interest is the PS I prepared from Triton-X100 solubilized thylakoids of *C. caldarium* [9], because this red alga also appears to lack LHC-type polypeptides. A comparison of red algal thylakoids solubilized by β-DM may resolve the present discrepancies, because by immunoblotting we found that thylakoids of the red alga *Aglaothamnion neglectum* also have LHC I-type polypeptides [30]. This leads us to expect that LHC-type polypeptides will be found in many other red algal species.

It has been proposed that chloroplasts of higher plants share a common lineage with cyanobacteria [2]; however the origin of the LHCs in higher plants remains a point of contention. We therefore find it quite intriguing that the subunit composition of the PS I holocomplex isolated from *P. cruentum* bears closer resemblance to the subunit composition of other eukaryotic organisms than it does to the PS I complex from phycobilisome-containing prokaryotes. We believe that our results provide the first indication for the existence of LHCs in a phycobilisome-containing organism. Currently we are involved in the characterization of the polypeptides of 18–23.5 kDa in order to determine how closely these subunits are related to the LHC I of other eukaryotes. Such studies may provide insight into the phylogenetic relationships between cyanobacteria and photosynthetic eukaryotes.

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