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Organization of the thylakoid membrane from the heterotrophic cyanobacterium, *Aphanocapsa* 6714

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The polypeptide composition of thylakoid membrane fractions from the heterotrophic cyanobacterium *Aphanocapsa* 6714 was examined by electrophoretic and immunoblotting procedures. We have identified thylakoid cytochromes *f*, b_6, *c*-550 and *c*-553 by tetramethylbenzidine staining of lithium dodecyl sulfate polyacrylamide gels; we also have identified the Rieske Fe-S center protein and subunit 4 of the cytochrome b_6/f complex. We have characterized phycobilisomes and active core preparations of PS I and PS II. PS I is comprised of five polypeptides (62 kDa, 14.5 kDa, 10 kDa, and two proteins of less than 10 kDa), and our PS II preparation is highly enriched for three chlorophyll-binding proteins of 48, 45 and 36 kDa. Furthermore, we have resolved the chlorophyll-binding complexes on non-denaturing gels and have determined the polypeptide composition of each chlorophyll-containing band. Three bands are associated with PS I (I, IIa and IIb) and three bands are PS II components (III′, IIIa and IIIb) as judged by low-temperature fluorescence emission spectra. Band III′ contains a 64 kDa antenna polypeptide, IIIa contains the 48 kDa and 45 kDa polypeptides, and IIIb is comprised solely of a 36 kDa protein. The IIIb apoprotein represents a novel PS II component; its possible role in photochemistry is discussed.

Introduction

Cyanobacteria are prokaryotes capable of oxygenic photosynthesis and thus provide a model system for understanding the organization and assembly of the photosynthetic apparatus of higher plant chloroplasts. These organisms are particularly useful for genetic studies of photosynthesis, and strain of the genus *Aphanocapsa* have proven to be ideal for applying such genetic techniques. *Aphanocapsa* sp. are unicellular cyanobacteria capable of photoheterotrophic growth, a property which permits the isolation of mutations defective in photosynthetic electron transport [1,2]. We are currently generating both transposon insertions and point mutations in *Aphanocapsa* 6714 with the goal of generating a bank of photosynthesis mutants. This strategy will help identify gene products that are involved in the photosynthetic mechanism and in directing the synthesis and assembly of thylakoid components.

In order to identify photosynthetic mutants at the structural level, the organization and polypeptide composition of the *Aphanocapsa* membrane must be well characterized. Recent work in this laboratory has concentrated on the organization of the *Anacystis nidulans* R2 thylakoid membrane; characterization of chlorophyll-protein

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Abbreviations: Chl, chlorophyll; CP, chlorophyll-protein; DCMU, 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea; DCIP, 2,6-dichlorophenolindophenol; DPC, diphenylcarbazide; Mes, 4-morpholinoethanesulfonic acid; Tricine, *N*-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine; PS, Photosystem; Cyt, cytochrome.

complexes [3], an oxygen-evolving particle [4] and thylakoid cytochromes [5] have been documented. We report here a similar approach to *Aphanocapsa* 6714 that has allowed a detailed characterization of its thylakoid components. We have identified important components of the five major complexes involved in photosynthesis: PS I and PS II core particles, phycobilisomes, ATP synthase, and the cytochrome b_6/f complex. The immunological relatedness of *Aphanocapsa* membrane proteins to well-characterized polypeptides of other species has enabled the identification of the analogous components in *Aphanocapsa*. Furthermore, we have resolved *Aphanocapsa* chlorophyll-binding proteins on non-denaturing gels and have assigned each protein as to its structural role in PS I, PS II, or the antennae complexes. This work has enabled us to identify an important PS II component, a 36 kDa chlorophyll-binding polypeptide which is also seen in PS II particles isolated from *A. nidulans* [6]. Hence, in addition to providing information for future genetic manipulations of the photosynthetic apparatus in *Aphanocapsa* 6714, this work will demonstrate the importance of specific proteins in cyanobacterial photosynthesis, especially with respect to PS II.

Materials and Methods

Strain and medium

Aphanocapsa 6714 *str*-5, a spontaneous mutant resistant to 25 μg/ml streptomycin, was employed throughout this study. Strain 6714 *str*-5 was grown in the medium described by Herdman et al. [7]. Large volumes of cells were grown with aeration at 25°C in 15-l carboys under constant illumination (0.5 mW/cm^2).

Standard methods

Thylakoid membrane isolation, lithium dodecyl sulfate polyacrylamide gel electropheresis and tetramethylbenzidine staining were performed according to Guikema and Sherman [5,8]. The polypeptide composition of the membrane and submembrane complexes were examined by gel electrophoresis, and aliquots of each sample were solubilized at either 0°C or 70°C prior to loading. Comparison of polypeptide patterns at the two solubilization temperatures allowed the identification of certain ligand binding species, since chlorophyll-binding proteins and cytochrome *f* have altered electrophoretic mobility after heating. Determination of chlorophyll concentration utilized the equations of Arnon [9]. Extracts for chlorophyll-protein (CP) gels were prepared by breaking *Aphanocapsa* cells (suspended in 50 mM Mes, pH 6.5, at 400 μg Chl/ml) in a chilled French pressure cell, and adding dodecyl-β-D-maltoside (Calbiochem) to a detergent/Chl ratio of 5 : 1. After centrifugation at 4°C at 34000 $\times$ *g* for 15 min, supernatant samples containing 10 μg Chl were electrophoresed according to Delepelaire and Chua [10], except that dithiothreitol and LDS were omitted from the samples. Low-temperature (77 K) fluorescence emission was measured by excising green bands from the CP gels, placing the gel slice into the wide end of a pasteur pipette, and aligning the gel slice in the excitation beam (excitation wavelength, 435 nm) within the sample chamber of an SLM 8000 spectrofluorimeter.

Spectral data were stored, corrected and plotted using software provided by the manufacturer. Protein blots of polyacrylamide gels were performed according to Towbin et al. [11]; antibody-antigen complexes were visualized by immunodecoration with peroxidase-conjugated goat anti-rabbit IgG followed by *o*-dianisidine treatment. Antibodies to *Chlamydomonas* thylakoid polypeptides 5 and 6 were a gift from Dr. N.-H. Chua; antibody against spinach cytochrome *b*-559 was a gift from Dr. W.A. Cramer; antibodies against the cytochrome b_6f complex components and antibodies against the light-harvesting chlorophyll protein complex (LHC-II) were gifts from Dr. W. Taylor. *Chlamydomonas* anti-β subunit of ATP synthase was provided by Dr. B. Selman. Phycobilisomes were isolated by the methods of Gantt et al. [12], and Triton X-114 phase partitioning was as described by Bricker and Sherman [13]. PS I activity (diaminodurene/ascorbate to methyl viologen) and PS II activity (DPC to DCIP) was measured essentially as described by Newman and Sherman [14].

Isolation of Aphanocapsa PS I

Frozen cells (concentrated from a 15-l culture) were thawed, washed and resuspended to 1 mg Chl/ml in 20 mM Tricine (pH 7.5). All steps were carried out at 4°C in the presence of protease

inhibitors (1 mM each of benzamidine, ϵ-amino caproic acid and phenylmethylsulfonyl fluoride). After passage through a chilled French pressure cell, the broken cells were diluted to 0.3 mg Chl/ml in 20 mM Tricine (pH 7.5). Dodecyl-β-D-maltoside was added to a detergent/Chl ratio of 10 : 1, and the mixture was immediately centrifuged (30 min at 34000 × *g*). The supernatant was layered onto an 8–25% sucrose density gradient containing 20 mM Tricine (pH 7.5) and 0.05% Triton X-100. Following centrifugation (20 h at 65000 × *g* in a Beckman SW27 rotor), a dark green band migrating below the phycobilisome material was collected, diluted in 10 mM sodium pyrophosphate/20 mM Tricine (pH 7.5) (to dissociate phycobilisomes) and pelleted. The pellet was washed once in the same pyrophosphate-containing solution, then suspended to a concentration of 0.12 mg Chl/ml. LDS was added to 0.5%, and the LDS-treated material was layered onto a 5–20% sucrose density gradient containing 20 mM Tricine (pH 7.5)/0.05% Triton X-100. After centrifugation (12 h at 154000 × *g* in a Beckman SW41 rotor), a light green band migrating just below a darker green band was collected and found to contain a highly purified PS I reaction center. PS I activity was assayed by monitoring oxygen uptake as a result of methyl viologen photoreduction, using diaminodurene/ascorbate as the electron donor. This preparation yielded rates of 350 μmol O_2 consumed per mg Chl per h.

Aphanocapsa PS II core preparation

Cells concentrated from a 15-l carboy were washed and resuspended to approx. 1 mg Chl per ml in 50 mM Mes (pH 6.5). All procedures were carried out at 4°C and protease inhibitors were included in all buffers. After cell breakage by two passages through a chilled French pressure cell, sodium pyrophosphate was added to dissociate phycobilisomes from the membranes; this material was centrifuged for 5 min at 3000 × *g* to remove unbroken cells and envelope membranes. The supernatant was spun in a Beckman Ty 60Ti rotor at 115000 × *g* for 1 h to collect thylakoid membranes. The membrane fraction was resuspended in 50 mM Mes (pH 6.5) to a concentration of 350 μg Chl per ml. Dodecyl-β-D-maltoside was added to yield a detergent/Chl ratio of 5 : 1, and the mixture was recentrifuged at 115000 × *g* for 1 h in the 60 Ti rotor. These extraction conditions yielded a PS I pellet and a supernatant enriched in PSII. The supernatant was layered onto a 8–25% sucrose gradient (containing 50 mM Mes, pH 6.5, and 10 mM sodium pyrophosphate) and centrifuged at 70000 × *g* in a Beckman SW27 rotor for 20 h. This gradient resolved two major bands and a pellet; the upper (blue-green) band contained PS II and was free of contaminating PS I. The upper band from each tube was pooled, diluted 4-fold in 50 mM Mes/10 mM sodium pyrophosphate (pH 6.5), and pelleted overnight (10–16 h) at 90000 × *g*. The pellet was resuspended in 2 ml buffer containing 50 mM Mes (pH 6.5)/10 mM sodium pyrophosphate/0.05% Triton X-100, and run over a Sephadex G-200 column (1.5 × 60 cm) equilibrated in the same buffer. The green PS II particles eluted in the void volume ahead of residual blue material. The green fractions were pooled and pelleted, and the material was then resuspended in 1 ml 50 mM Mes (pH 6.5). The preparation could mediate transfer of electrons from DPC to DCIP at a rate of 143 μmol DCIP reduced per mg Chl per h. The addition of 10 μM DCMU reduced the rate to 69 umoles DCIP reduced per mg Chl per h. PS II samples were stored at −70°, and aliquots were removed for electrophoretic analysis.

Results

Our initial approach in this study was to employ techniques which allowed us to identify photosynthesis-associated components in whole thylakoid membranes. These methods included tetramethylbenzidine staining of membrane samples, protein blot analysis with heterologous antibody probes, and resolution of chlorophyll-binding proteins on non-denaturing gels. After having used these methods to identify components of PS I, PS II, and the cytochrome b_6/f complex, we next isolated PS I, PS II, and phycobilisome particles to examine more closely the structure and polypeptide composition of each complex. Thus, the composition of whole membranes will be described first, followed by the analyses of the submembrane fractions.

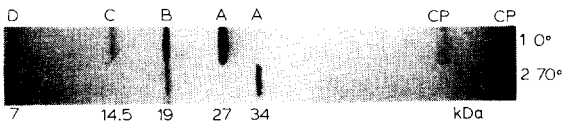


Fig. 1. *Aphanocapsa* 6714 cytochromes detected by tetramethylbenzidine staining. Membrane samples solubilized at 0°C (lane 1) and after heating to 70°C (lane 2) were electrophoresed and stained for heme-dependent peroxidase activity. The four heme-staining species are labeled A–D. The bands labeled CP are chlorophyll-protein (green) complexes present in the unheated samples.

Cytochrome identification by tetramethylbenzidine staining of membrane polypeptides

Aphanocapsa 6714 membranes were electrophoresed following solubilization at either 0°C or 70°C. After electrophoresis, the gels were stained for heme-dependent peroxidase activity with tetramethylbenzidine/H_2O_2 [5] to identify cytochromes. *Aphanocapsa* membranes yield four distinct tetramethylbenzidine staining species; samples solubilized at 0°C yielded bands corresponding to cytochromes having apparent molecular masses of 27, 19, 14.5 and 7 kDa (bands A–D), whereas heated samples showed cytochrome species of 34, 19 and 14.5 kDa (bands A–C of Fig. 1). Band A of Fig. 1 shifted to a higher apparent molecular mass after heating; this property is characteristic of cytochrome *f* [5,15]. Both bands A and B corresponded to intrinsic cytochromes as determined by Triton X-114 phase partitioning. Band B corresponded to a cytochrome of similar molecular mass as cytochrome b_6 isolated from various sources [15]. The identity of bands A and B as cytochromes *f* and b_6 was verified by Western blot analysis; antibodies raised against maize cytochromes *f* and b_6 crossreacted to the same protein bands (A and B, respectively) that stained with tetramethylbenzidine (data not shown). Band C corresponded to an extrinsic polypeptide which is likely cytochrome *c*-550, since *c*-550 from other cyanobacteria are soluble polypeptides of approx. 14 kDa [16]. Although band D was not analyzed further, it most likely corresponds to cytochrome *c*-553, since this cytochrome has been shown to be a soluble polypeptide of molecular mass less than 10 kDa [17]. Band D is not cyrochrome *b*-559, as this band did not crossreact to antibody raised against spinach Cyt *b*-559, and was not present in active *Aphanocapsa* PS II core preparations.

Chlorophyll-protein complexes of Aphanocapsa

Non-denaturing polyacrylamide gel electrophoresis of dodecyl-β-D-maltoside extracts of *Aphanocapsa* membranes resolved three sets of green bands, labeled I–III in Fig. 2A. Bands I, IIa and IIb corresponded to different oligomeric forms of PS I as judged by low-temperature fluorescence emission spectra [18]; bands excised from the gels emitted fluorescence at F_{max} of 716–720 nm (excitation wavelength, 435 nm). The broad peak at 675 nm was identical to the peak arising from free chlorophyll (Fig. 2B). The band III region, comprised of bands III′, IIIa and IIIb, was fluorescent when transilluminated with long-wavelength (365 nm) ultraviolet light; the isolated bands had fluorescence emission peaks at 684–686 nm when illuminated with 435 nm light at 77 K (Fig. 2C). These data are consistent with these chlorophyll-protein complexes being associated with PS II and its antennae [3]. Bands I, IIa and IIb were not fluorescent during ultraviolet transillumination, a fact that is likely due to the presence of the effective quencher of fluorescence, P-700 [19]. Note that bands III′, IIIa and IIIb are plainly visible in these gels. By comparison, PS II-associated CP bands of *A. nidulans* R2 are only clearly visible as green bands when the thylakoids are prepared from iron deficient cells [20]. These data suggest that *Aphanocapsa* PS II components bind chlorophyll more stably than the chlorophyll-binding proteins of the *A. nidulans* R2 PS II core.

Polypeptide composition of chlorophyll-protein bands

Excised bands from non-denaturing gels were placed in the wells of 10–20% denaturing LDS polyacrylamide gels and electrophoresed to determine the protein composition of each chlorophyll-containing band. Bands I, IIa and IIb were comprised of PS I polypeptides, as the 62 kDa PS I apoprotein (see Fig. 6A) was the predominant species in these samples (data not shown). Band IIIa contained several polypeptides of 40–45 kDa and IIIb was comprised solely of a 36 kDa protein (Fig. 3, lanes 3 and 4). Band III′ always contained a 64 kDa protein Fig. 3, lane 2); polypeptides of lower molecular mass were often present, but since

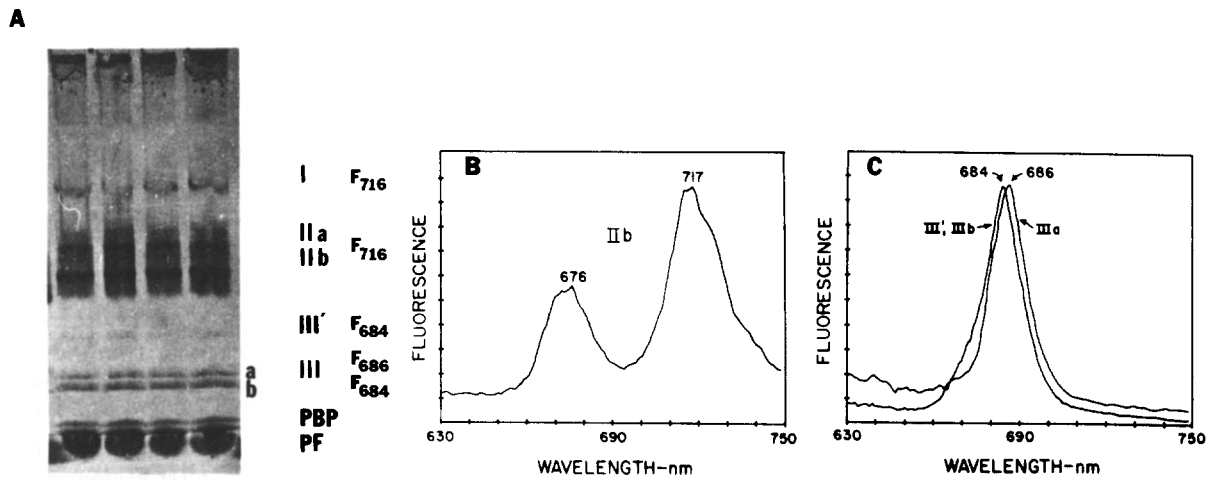


Fig. 2. (A) Chlorophyll-protein complexes of *Aphanocapsa* 6714 resolved by electrophoresis on non-denaturing, gradient (5–15%) acrylamide gels. The bands are numbered from I to III in order of decreasing apparent molecular mass. The associated fluorescence emission peak at 77 K is also indicated for each band. PF, pigment front; PBP, phycobiliproteins. (B) Low-temperature (77 K) fluorescence emission spectrum of CP IIb (excitation at 435 nm). (C) Low-temperature (77 K) fluorescence emission spectra of bands III′, IIIa, and IIIb (excitation at 435 nm).

the 64 kDa protein was the only species seen in all III′ samples so far examined, we believe this protein was the Chl-binding component. The 64 kDa polypeptide crossreacted to antibody raised against the maize light-harvesting chlorophyll protein, LHC-II, and will be discussed in a future communication (Bullerjahn, G.S. and Sherman, L.A., unpublished results).

Protein blot analyses of thylakoids and chlorophyll-proteins

Whole membrane samples and submembrane fractions were electrophoresed and electroblotted onto nitrocellulose in order to detect polypeptides which were crossreactive to antibodies prepared against the chlorophyll-binding, PS II core polypeptides 5 and 6 from *Chlamydomonas* (Ref. 21; Fig. 4). Such blots revealed that the *Aphanocapsa* species immunologically related to component 5 was a 48 kDa polypeptide upon solubilization at 0°C, which shifted to 40 kDa when solubilization was carried out at 70°C (Fig. 4, lanes 10–13). Anti-component 6 crossreacted to various species of 38–45 kDa after 0°C solubilization and to a 45 kDa polypeptide which appeared after the mixture was heated to 70°C before loading (Fig. 4, lanes 6 and 7). Using these antibodies to probe protein blots of membranes from a related strain, *Aphanocapsa* 6803, revealed a similar pattern except that anti-6 crossreacted to a 47 kDa polypeptide after heating the sample to 70°C (lanes 8, 9, 14 and 15, Fig. 4). When these antibodies were used to probe blots of the CP III region polypeptides, the band IIIa proteins crossreacted to both anti-5 and anti-6. Lastly, the 36 kDa polypeptide of CP IIIb did not crossreact to either of these polyclonal antibodies (data not shown).

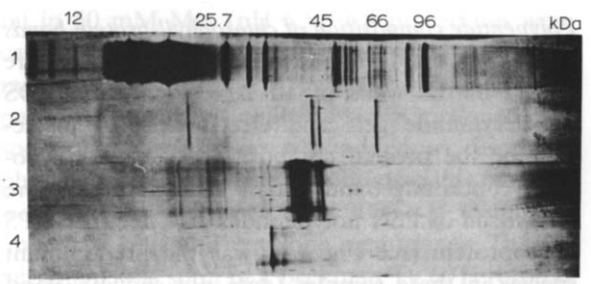


Fig. 3. Gel electrophoresis of chlorophyll-protein bands (10–20% LDS-polyacrylamide gel electrophoresis) excised from non-denaturing gels. All samples were solubilized at 70°C, and the gel was silver stained. Lane 1, purified phycobilisomes (PBS); lane 2, band III′ material; lane 3, band IIIa material; lane 4, band IIIb material.

Protein blots probed with cytochrome b_6/f complex antibodies from maize revealed that the

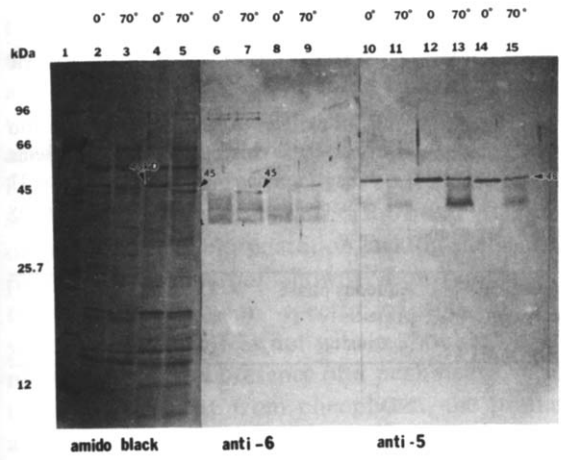


Fig. 4. Protein blots of *Aphanocapsa* 6714 and 6803 membranes run on 10–20% gel and probed with antibodies raised against the PS II Chl-binding polypeptides 5 and 6 from *Chlamydomonas* (21). Lanes 1–5 reveal total membrane polypeptides transferred to nitrocellulose as detected by amido black staining. Lane 1, molecular weight markers; lane 2, 6803 membranes solubilized at 0°C; lane 3, 6803 membranes solubilized at 70°C, lane 4, 6714 membranes solubilized at 0°C, lane 5, 6714 membranes solubilized at 70°C, lanes 6–9 represent protein blots probed with antibodies raised against *Chlamydomonas* polypeptide 6; lanes 6 and 7, immunostaining of anti-6 against 6714 membranes solubilized at 0°C (lane 6) and at 70°C (lane 7); lanes 8 and 9, immunostaining of anti-6 cross-reacting to 6803 membranes solubilized at 0°C (lane 8) and at 70°C (lane 9); lanes 10–15 represent blots probed with antibodies raised against *Chlamydomonas* polypeptide 5; lanes 10 and 11, 6714 membranes unheated (lane 10) and heated (lane 11); lanes 12 and 13, same as 10 and 11 respectively, except that 3-fold more protein was applied to the gel; lanes 14 and 15, 6803 membranes unheated (lane 14) and heated (lane 15). Note that the material cross-reacting with anti-6 forms a smear from 45 to 38 kDa in the unheated lanes, but upon heating, a higher molecular mass species is generated (47 kDa in 6803, 45 kDa in 6714). Blots probed with anti-5 yield one 48 kDa band in unheated samples; upon heating, most of the cross-reacting material is converted to a 40 kDa species. Both the 48 kDa polypeptide cross-reacting to anti-5 and the 45–47 kDa polypeptide cross-reacting to anti-6 are visible stained bands in whole membrane preparations.

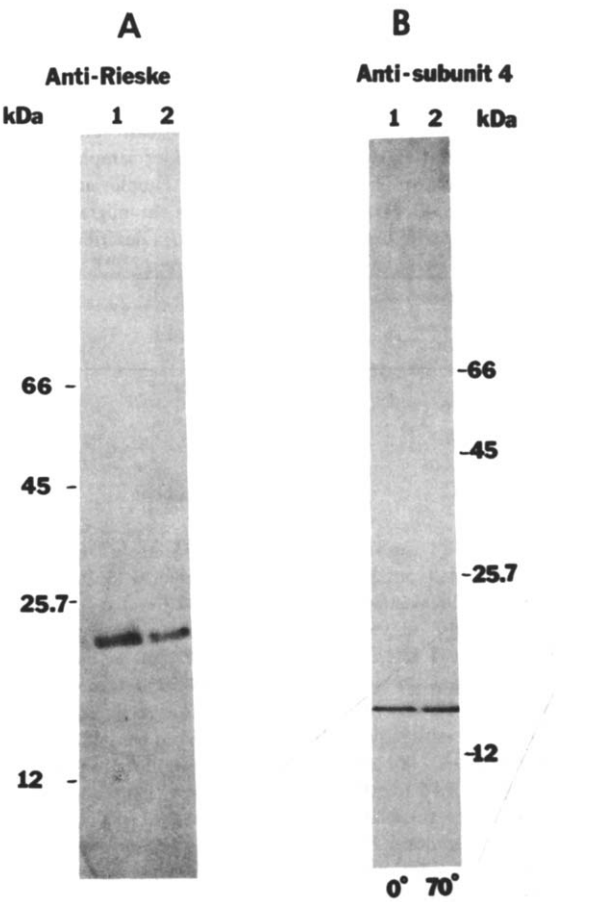


Fig. 5. Protein blots of cyanobacterial membrane samples probed with polyclonal antibodies to the maize Rieske Fe-S center and subunit 4 polypeptides of the cytochrome b_6/f complex. (A) Lane 1, *Aphanocapsa* 6714 membranes, and lane 2, *A. nidulans* R2 membranes probes with the Rieske antibody. In both lanes, the samples were solubilized at 70°C. (B) 6714 membranes solubilized at 0°C (lane 1) and at 70°C (lane 2) and probed with the subunit 4 antibody.

Rieske Fe-S polypeptide is a 21 kDa protein in both *A. nidulans* R2 and *Aphanocapsa* 6714; antibody to subunit 4 of the complex crossreacted to a 14 kDa protein (Fig. 5). Similar experiments were performed with anti-spinach cytochrome *b*-559 and anti-β subunit of ATP synthase from *Chlamydomonas*. These blots showed that two polypeptides of 8–10 kDa crossreacted to anti-*b*-559, whereas the β subunit antibody identified a 54 kDa protein (data not shown). A summary of the immunological findings is given in Table I.

Phycobilisome, PS I and PS II components

The polypeptide composition of *Aphanocapsa* 6714 phycobilisomes resembles that of other cyanobacteria, so we have been able to identify the phycobilisome proteins analogous to components of the well-characterized phycobilisomes from *Synechocystis* 6803 and *Synechococcus* 6301 [22,23]. These proteins include the 95 kDa anchor poly-

TABLE I

SUMMARY OF THE PHOTOSYNTHESIS-ASSOCIATED POLYPEPTIDES IDENTIFIED IN THIS PAPER, AND THEIR PHASE PARTITIONING PROPERTIES IN TRITON X-114.

Those polypeptides showing immunological crossreactivity to antibodies raised against analogous proteins from other species are also listed. Molecular masses were determined after sample solubilization at 70°C, since cytochrome *f* and chlorophyll-binding proteins show altered electrophoretic mobility when samples are prepared at 0°C; e.g., at 0°C, Cyt *f* has an apparent molecular mass of 27 kDa, and the 40 kDa PS II core component migrates at 48 kDa. The immunological crossreactivity has been determined by crossreactivity with heterologous antibodies as described in Results. ND, not determined.

Molecular mass (kDa)	Protein function	Immunological crossreactivity	Aqueous phase proteins (extrinsic)	Detergent phase proteins (intrinsic)
95	Phycobilisome anchor		+	+
64	III′ apoprotein	+	ND	ND
62	PS I apoprotein		−	+ +
54	β subunit, ATPase	+	ND	ND
52	Phycobilisome component		+ +	−
45	PS II core (IIIa)	+	−	+ +
40	PS II core (IIIa) (70°C)	+	−	+ +
36	PS II core (IIIb)		−	+ +
36	Phycobilisome linker		+ +	−
34	Cyt *f* (70°C)	+	+/−	+ +
32	Phycobilisome linker		+ +	−
28	Phycobilisome linker		+ +	−
21	Rieske Fe-S center	+	ND	ND
19	Cyt b_6	+	−	+ +
15–19.5	Phycobiliproteins (α and β subunits)		+ +	−
14.5	PS I component		−	+ +
14.5	Cyt *c*-550		+ +	−
14	Subunit 4 of Cyt b_6/f	+	ND	ND
10	PS I component		−	+ +
10	Cyt *b*-559	+	−	+ +
< 10	Cyt *c*-553		ND	ND
< 10	PS I component		−	+ +
< 10	PS I component		−	+ +

peptide and the three internal linker proteins of 28–36 kDa. Other components include the phycobiliproteins (α and β subunits of phycocyanin and allophycocyanin) of 15–19.5 kDa and two small polypeptides of less than 10 kDa (lane 3, Fig. 6A).

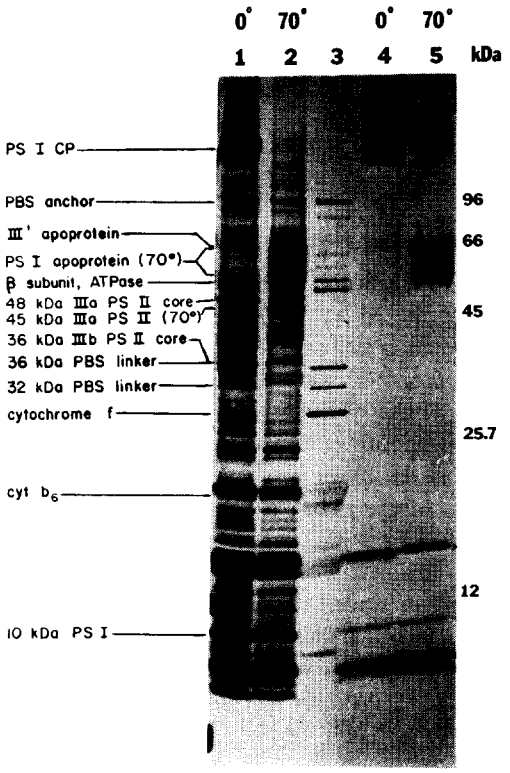
PS I core complexes were prepared as described in Materials and Methods, and were shown to be capable of methyl viologen photoreduction (350 μmol O_2 consumed per mg Chl per h). Analysis of these particles by electrophoresis showed that four polypeptides of low molecular mass (one of 14.5 kDa, another one of 10 kDa, and two of less than 10 kDa) were associated with the 62 kDa PS I chlorophyll-binding apoprotein (lanes 4 and 5, Fig. 6A). Low-temperature fluorescence emission spectra obtained from this preparation yielded a single peak at 718 nm (data not shown). This preparation is of comparable complexity to PS I from higher plant and other cyanobacterial sources [24,25].

The polypeptide composition of an active PS II core preparation included the 64 kDa III′ apoprotein, the 48 kDa polypeptide which was antigenically related to *Chlamydomonas* component 5 of PS II, the 45 kDa protein analogous to *Chlamydomonas* component 6, the 36 kDa protein and the species which cross-reacted to the cytochrome *b*-559 antibody (Fig. 6B). This particle contained the chlorophyll-binding components of CP bands III′, IIIa and IIIb. Furthermore, the DCMU sensitivity of the preparation (52% inhibi-

tion of activity after addition of 10 μM DCMU) shows that the non-Coomassie stainable Q_B apoprotein [26,27] was present in this fraction. We were able to deplete this preparation of the III' apoprotein by filtration on Bio-Gel A-0.5m without loss of activity, suggesting that the III' component serves an antenna function. However, we never obtained an active preparation lacking the 36 kDa polypeptide (data not shown). Low-temperature fluorescence emission spectra of this material yielded a peak at 693 nm with a shoulder at 687 nm (Fig. 6B). The presence of a peak near 696 nm is thought to arise from pheophytin, the primary acceptor of PS II [28]. Both the 77 K fluorescence spectra and the polypeptide patterns showed that PS I and phycobilisome components are absent in this preparation; however, this fraction contained the β subunit of ATP synthase and components of the cytochrome b_6/f complex (Fig. 6B).

Phase partitioning of Aphanocapsa membranes

Aphanocapsa thylakoids and PS I particles were phase fractionated with the non-ionic detergent



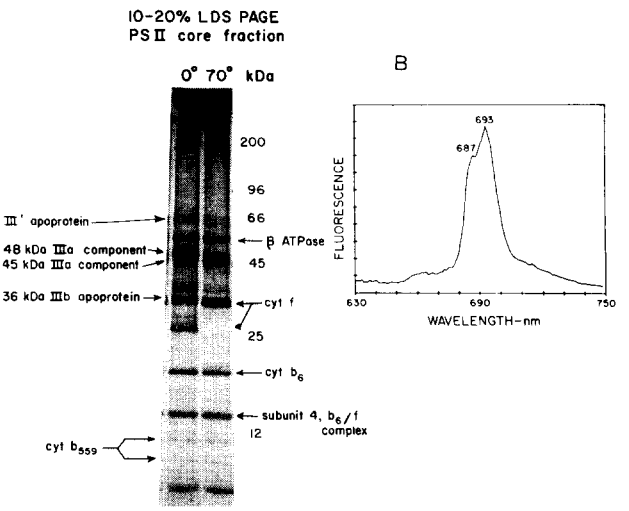


Fig. 6. Gel electrophoresis of phycobilisome, PS I and PS II complexes of *Aphanocapsa* 6714. (A) Lane 1, *Aphanocapsa* thylakoids solubilized at 0°C; lane 2, *Aphanocapsa* thylakoids solubilized at 70°C; lane 3, *Aphanocapsa* phycobilisomes solubilized at 70°; lane 4, *Aphanocapsa* PS I core complex solubilized at 0°C; lane 5, *Aphanocapsa* PS I solubilized at 70°C. The phycobilisome proteins include the 95 kDa anchor polypeptide, the three internal linker polypeptides (28–36 kDa) and the phycobiliproteins (15–19.5 kDa). PS I is comprised of five polypeptides; four small proteins (one of 14.5, another one of 10, and two of less than 10 kDa) in addition to the 62 kDa chlorophyll-binding PS I apoprotein. PS I solubilized at 0°C yields a high molecular mass green band (PS I CP) corresponding to the 62 kDa polypeptide complexed to chlorophyll. Some of the major proteins visible in whole membrane samples are identified on the left of the figure. (B) The PS II core preparation solubilized at 0°C and 70°C. The 48, 45 and 36 kDa proteins are highly enriched in this fraction. The contaminants identified in this preparation are predominately components of the cytochrome b_6/f complex and ATP synthase. Inset: 77 K fluorescence emission spectrum of the PS II core fraction (excitation at 435 nm). PAGE, polyacrylamide gel electrophoresis.

Triton X-114 [13] in order to determine which polypeptides are intrinsic membrane proteins. Table I summarizes the results for the major photosynthesis-associated proteins; those polypeptides fractionating into the detergent phase were considered to be hydrophobic, intrinsic proteins, whereas the polypeptides in the aqueous phase were considered extrinsic components. The 62 kDa PS I apoprotein, the 48 kDa protein (which has an apparent molecular mass of 40 kDa after heating–see Fig 4), the 45 kDa, the 36 kDa pro-

tein, cytochrome b_6 and cytochrome b-559 were the predominant intrinsic species; the aqueous phase was composed mostly of phycobilisome components.

Discussion

The thylakoid membrane of *Aphanocapsa* is comprised of approx. 80 polypeptides as judged by the number of silver-stained bands resolved by 1-dimensional gel electrophoresis (lanes 1 and 2, Fig. 6A). Table I lists 24 photosynthesis-associated polypeptides which we have identified in *Aphanocapsa* 6714, and Fig. 6A notes some of the polypeptides which are clearly visible in gels of whole membranes samples. The complexity of the polypeptide pattern may, in part, be due to a single protein yielding several bands on gels; for example, the antibody crossreacting to the 45 kDa protein also recognizes several other bands in this region. Currently we can attribute a role to approx. one-third of the abundant polypeptides of the thylakoid membrane; these proteins are components of PS I, PS II, phycobilisomes, the cytochrome b_6/f complex, and ATP synthase.

We have identified two novel PS II components, indicating that PS II and its antenna contains at least three chlorophyll-binding proteins in *Aphanocapsa*. These chlorophyll proteins comprise bands III′, IIIa and IIIb in non-denaturing gels. Band III′ contains the 64 kDa polypeptide, IIIa contains both the 48 kDa and 45 kDa polypeptides (analogous to *Chlamydomonas* components 5 and 6), and band IIIb is comprised solely of a 36 kDa protein (Figs. 2A and 3). The CP III region bands yield 77 K fluorescence emission peaks at 684–686 nm. We have been unable to obtain a CP species yielding fluorescence at 693–696 nm, indicating that the CP bands have probably been altered during electrophoresis. This is in agreement with the observation that F_{695} arising from *A. nidulans* CP band VI-2 (containing the 52 kDa PS II core polypeptide) is very labile [6]; if spectra are not obtained from gel pieces immediately after electrophoresis, the fluorescence emission peak shifts to 685 nm.

The CP IIIb 36 kDa protein is immunologically distinct from the proteins of CP IIIa showing that the IIIb apoprotein is probably not a proteolytic breakdown product of either the 48 or 45 kDa polypeptides. Although this 36 kDa polypeptide has not been seen in higher plants or algae, this protein is a PS II component in the cyanobacterium *A. nidulans*. It is this polypeptide which becomes the major chlorophyll binding species in *A. nidulans* R2 during growth in iron depleted medium [6].

Our current PS II preparation is contaminated with several proteins which we have been able to identify by immunoblotting and tetramethylbenzidine staining (Fig. 6B). The remaining polypeptides are all thought to be intrinsic components of the PS II core. Analysis of the polypeptide composition of *Aphanocapsa* PS II core particles reveals that the 48 kDa, 45 kDa, and 36 kDa proteins are abundant species (Fig. 6B). Since the 36 kDa polypeptide is highly enriched in our PS II preparation, and all active complexes contained this component, a possible role of the 36 kDa protein in primary photochemistry cannot be ruled out. There is some disagreement as to which proteins comprise the PS II reaction center. Work by de Vitry et. al. [29] showed that PS II activity depends on the presence of *Chlamydomonas* components 5 and 6 (analogous to the IIIa proteins of *Aphanocapsa*) in 1 : 1 stoichiometry, but more recent data from Diner B. (personal communication) show that PS II activity occurs in the absence of component 6. Both de Vitry's and Diner's PS II particles contain component 5, and an intrinsic 32 kDa polypeptide called D2; however, in the former preparation, D2 was not present in stoichiometric amounts [21,30]. Since the gene sequences encoding D2 and the M subunit of the photosynthetic bacterial reaction center share homology, D2 may be a reaction center component in *Chlamydomonas* PS II [30]. The possibility that the cyanobacterial 36 kDa polypeptide may be analogous to D2 warrants further investigation.

Analyses of the polypeptide composition of PS II particles from both *Aphanocapsa* and *A. nidulans* points toward a role of the 36 kDa protein as an essential component of the PS II core. Although the 36 kDa component has not been described previously, it is important to note that cyanobacterial oxygen-evolving preparations reported elsewhere all contain Coomassie-staining polypeptide(s) in the 36 kDa region [4,31,32]. The

O_2-evolving preparation of Satoh et al. [32], isolated from a thermophilic strain, deserves special mention because it is highly enriched in a 35 kDa species. The authors suggest that this protein is involved in water oxidation; however, the possibility remains that this component is analogous to the 36 kDa PS II protein described here. A detailed analysis of *Aphanocapsa* mutants defective in PS II electron transport will help determine whether the 36 kDa polypeptide is required for primary photochemistry.

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