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Correlation of photosystem-II complexes with exoplasmatic freeze-fracture particles of thylakoids of the cyanobacterium *Synechococcus sp.**

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Abstract. The supramolecular structure of the exoplasmic freeze-fracture particles of thylakoids of the thermophilic cyanobacterium *Synechococcus sp.* is compared with that of isolated photosystem-II complexes. The in-situ EF particles are scattered on the thylakoids or organized in rows of variable length; the latter aligned particles measure 10 nm × 20 nm and are separated perpendicular to their long axis into two parts. We propose that they represent dimers composed of two monomeric 10-nm EF particles side by side. Isolated photosystem (PS)II particles correspond in size to the monomeric 10-nm EF particles as analysed by negative contrast and freeze-fracture electron microscopy. Dimeric PSII particles, very similar to the in-situ 10 nm × 20 nm EF particles, are obtained after incorporation of purified PSII complexes into liposomes made from phospholipid and cholesterol. Each monomeric complex consists of the reaction center, the water-splitting system, the chlorophyll antennae and phycobilisome-binding polypeptides. We propose that the dimeric complexes bind one hemidiscoidal phycobilisome at their domains exposed to the external side of the thylakoids. The implications of this arrangement of the PSII-phycobilisome complexes within the thylakoids upon excitation-energy distribution are discussed.

Key words: Cyanobacteria – Photosystem II – Phycobilisome – *Synechococcus* – Thylakoid (freeze-fracture).

Introduction

The phycobilisomes are the dominant light-harvesting pigment-protein complexes of cyanobacteria and red algae (for a review, see Mörschel and Rhiel 1987). They are bound to the external surface of the thylakoids and transfer the absorbed light energy mainly to photosystem (PS)II. As the efficiency of excitation-energy transfer approaches 95% of the initially absorbed light quanta (Ley and Butler 1977), it was postulated that phycobilisomes and the reaction centres of PSII should be closely spatially arranged. This relationship was recently established by freeze-fracture analysis. Cyanobacterial thylakoids showed a structural alignment of the hemi-discoidal phycobilisomes on top of the aligned EF freeze-fracture particles, which were supposed to be PSII complexes (Mörschel and Mühlethaler 1983). Furthermore, this relationship was recently corroborated by the isolation of allophycocyanin containing PSII (Schatz and Witt 1984) and of PSII-phycobilisome complexes (Clement-Metral and Gantt 1983). It was the aim of this study to examine the hypothesis that the 10-nm exoplasmic freeze-fracture particles correspond to PSII complexes. For this purpose we compared in-situ EF particles with oxygen-evolving PSII complexes which were isolated, reconstituted into liposomes and visualized by electron microscopy. The detailed information gained of the arrangement of PSII and the phycobilisomes shows the structural basis for effective energy transport.

Material and methods

Strains and culture conditions. The thermophilic cyanobacterium *Synechococcus spec.* was cultured in medium D of Castenholz (1970) supplied with trace elements and 0.5 g·l^{-1} $NaHCO_3$ at pH 8.2. Cultures were grown at 45° C either in glass culture tubes, gassed with a mixture of 2% CO_2 in air

* This study is dedicated to Professor W. Nultsch on the occasion of his 60th birthday

Abbreviations: EF = exoplasmic fracture face; LDS = lithium dodecyl sulfate; PAGE = polyacrylamide gel electrophoresis; PS = photosystem; SDS = sodium dodecyl sulfate; SPC-buffer = 0.5 M sucrose, 0.5 M K_2HPO_4/KH_2PO_4, 0.3 M Na-citrate, pH 7.0

or in 2 l Erlenmeyer flasks maintained without aeration. Light was provided by fluorescent lamps with an intensity of 3000 l×.

Isolation of PSII complexes. Oxygen-evolving PSII complexes were isolated according to the procedure developed by Schatz and Witt (1984). Spheroplasts of *Synechococcus sp.* were prepared by lysozyme treatment (0.1%) followed by passage through a French or Yeda press. Thylakoids were released from the spheroplasts by osmotic shock. The thylakoid fraction was then incubated with the detergent Sulfobetain 12 (0.35%; Serva, Heidelberg, FRG) in 20 mM 2-(N-morpholino)ethanesulfonic acid-(Mes)-NaOH, 10 mM $MgCl_2$, 2 mM K_2HPO_4, 500 mM mannitol, pH 6.0 (MMPM-buffer) and glycerol (80/20 v/v) buffer. The suspension was centrifuged at 140000·*g* to separate the solubilized PSII complexes from the membrane fraction. The supernatant was sedimented (240000·*g*, 14 h) and further purified by density gradient centrifugation (7–30% sucrose, 180000·*g*, 90 min TFT 65 rotor; Kontron, München, FRG) in 20 mM 4-(2-hydroxyethyl)-1-piperazine ethane sulfonic acid (Hepes)-NaOH, 10 mM $MgCl_2$, 2 mM K_2HPO_4, 500 mM mannitol, pH 7.8 (HMPM-buffer) or 0.5 M sucrose, 0.5 M K_2HPO_4/KH_2PO_4, 0.3 M Na-citrate, pH 7.0 (SPC buffer).

Oxygen evolution of PSII complexes and reconstituted liposomes was detected using a Clark-type electrode under the following conditions: MMPM-buffer (pH 6.0) with 1 mM $K_3Fe(CN)_6$ and 0.1 mM phenyl-*p*-benzoquinone as electron acceptors, 20° C, chlorophyll concentration 2–4 µM, illumination with saturating red light (RG 610 filter; Schott, Mainz, FRG).

Reconstitution experiments. The PSII complexes were reconstituted into liposomes composed of IVS soybean phosphatidylcholine (Sigma, Deisenhofen, FRG). The PSII suspensions in HMPM or SPC buffer containing 0.35% Sulfobetain 12 were mixed with phospholipids (5 or 10 mg·ml^{-1}) suspended in the same buffer to give phospolipid to protein ratios between 5 and 20. The preparations were subsequently flushed with nitrogen and submitted to three cycles of freeze-thawing. Then they were dialysed against SPC buffer without detergent for 12 h in order to induce formation of proteoliposomes.

An alternative reconstitution of PSII complexes was performed by using liposomes made of soybean phosphatidylcholine plus cholesterol as follows: 9 g phosphatidylcholine and 3 g cholesterol were sonified (sonifier B 15, Branson, Danburg, Conn., USA) in 18 ml MMPM-buffer for 15 min, whereupon PSII suspensions were added at a protein-mass equivalent of 0.6 g and the mixture was sonified again for 1 min. This alternative method resulted in liposomes with a completely reconstituted capacity for oxygen evolution, i.e. specific rates as high as with PSII complexes alone. The resulting proteoliposomes were concentrated by centrifugation (100000·*g*, 30 min), resuspended in 35% glycerol and processed for freeze-fracture electron microscopy.

Electron microscopy. Cells and PSII preparations were concentrated and placed on gold or thin copper supports and frozen in liquid propane cooled by liquid nitrogen. Freeze-fracture was performed in a Balzers BAF 301 freeze-fracture unit (Balzers, Liechtenstein) at −109° C according to Moor and Mühlethaler (1963). The freeze-fracture apparatus was equipped with electron guns fitted with an automatic shutter device. Preparations were shadowed with 2.0-nm carbon-platinum at an angle of 45° C. Replicas were cleaned in 40% chromic acid followed by Na-hypochlorite and examined in a Philips 301 G electron microscope (Philips, Eindhoven, The Netherlands). Particle sizes were determined from micrographs enlarged to 200000×. Negative staining was as described by Mörschel et al. (1980). Photosystem-II proteoliposomes in 0.1 M phosphate buffer (pH 7.0) were sedimented by centrifugation (30 min, 100000·*g*, 4° C) to remove dissociated biliproteins. The proteoliposomes were resuspended in 0.1 M phosphate buffer containing 1% (w/v) Triton X-100 to disintegrate the liposome membranes.

Analytical procedures. Chlorophyll-protein complexes of PSII particles were separated by lithium dodecyl sulfate (LDS)-polyacrylamide gel electrophoresis (PAGE) as developed by Delepelaire and Chua (1979) in 7.5–15.5% acrylamide gradient slab gels. Samples were incubated at 4° C with LDS (chlorophyll: LDS = 1:20) and subsequently separated at the same temperature. Only the electrophoresis-buffer contained 0.1% LDS as detergent. For two-dimensional gel electrophoresis, strips were cut out, incubated in sodium dodecyl sulfate (SDS)-buffer and processed according to the procedure of Laemmli (1970). Fluorescence emission spectra at 77 K were recorded on a purpose-built photometer equipped with a Hamamatsu R1104 photomultiplier tube (Dr. Seitner, Herrsching, FRG). The band pass on the emission side was 1.0 nm.

Results

Spectroscopic analysis. Photosystem-II particles were prepared using the method described by Schatz and Witt (1984) and further purified by density gradient centrifugation in SPC buffer without any loss in PSII activity. The high degree of enrichment of PSII was indicated by chlorophyll (Chl)/PSII ratios between 70 to 90. Typical absorbance spectra of cells and of PSII complexes are shown in Fig. 1. The main pigments of PSII complexes were allophycocyanin and chlorophyll as shown by their absorption maxima at 652 and 680 nm, respectively. The low-temperature fluorescence emission spectra of whole cells and PSII preparations are given in Fig. 2A, B. Whole cells showed a strong emission peak at 730 nm and minor peaks at 660, 685 and 695 nm when excited at 445 nm (Fig. 2A). The 730-nm emission peak was attributed to PSI. Purified PSII preparations lacked this 730-nm emission maximum and conse-

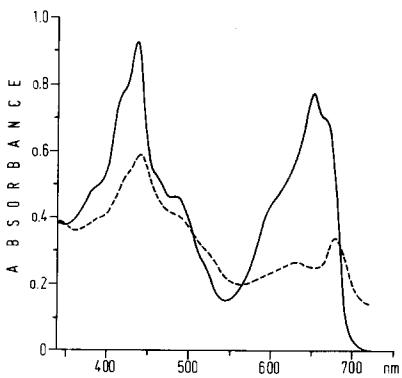


Fig. 1. Absorption spectrum of purified PSII complexes (——) compared with that from whole cells (-----) of *Synechococcus*

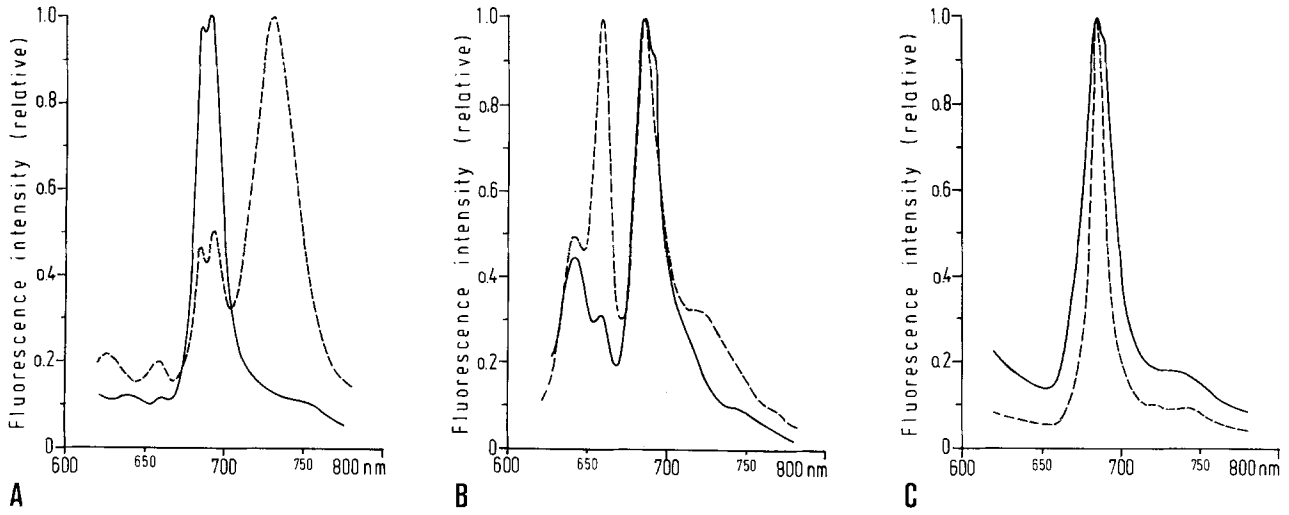


Fig. 2. A, B 77 K fluorescence emission spectra of PSII complexes (——) compared with those of whole cells (-----) of *Synechococcus*. Excitation wavelengths were at 445 nm (**A, C**) and 590 nm (**B**). **C** 77 K fluorescence emission spectra of chlorophyll protein (CP) IIa and CP IIb which are very similar (——) and CP IIc (-----). CP IIa, b have a shoulder at 692 nm which is absent in CP IIc

quently PSI. This corresponds well with the high Chl/PSI ratio of >1500 of the PSII preparations compared with ratios of 300–350 in unfractionated thylakoids (Schatz and Witt 1984). When excited at 445 nm the PSII preparations showed a minor maximum at 660 nm, characteristic for allophycocyanin, and two major maxima at 685 and 693 nm (Fig. 2A). The fluorescence at 685 nm was attributed to an interplay of allophycocyanin B, the large membrane-phycobilisome linker (L_{CM}) and a chlorophyll antenna, whilst the 693-nm fluorescence belonged to a chlorophyll antenna alone. The phycobilisome constituents that were not dissociated from the complex were tightly coupled to PSII, as was evident from excitation within the absorption range of phycocyanin at 590 nm (Fig. 2B). Most of the energy was transferred to both the final phycobilisome emitters and the PSII antennae, as shown from the emission peak at 685 nm and the shoulder at 692 nm. Only a minor part of the fluorescence was emitted by uncoupled phycocyanin and allophycocyanin at 640 and 660 nm, respectively. Purification of the PSII complexes in 0.1 M phosphate or 20 mM Mes/NaOH, 10 mM $MgCl_2$, 2 mM K_2HPO_4 (MMP) buffer resulted in a loss of phycocyanin and part of allophycocyanin; this is further evidence that phycobilisomes were bound to PSII complexes.

Electrophoretic analysis. The chlorophyll protein and polypeptide pattern of PSII complexes were determined by two-dimensional gel electrophoresis (Fig. 3). In the first dimension, run in the presence of LDS for optimal yield of intact chlorophyll complexes, three chlorophyll proteins termed CP II a, b, c as well as the biliproteins were resolved (Fig. 3A; C, first dimension). Apparent molecular weights of 110, 79 and 45 kDa were determined for the three chlorophyll proteins. In the second dimension, the polypeptide pattern after complete denaturation by SDS was determined (Fig. 3C). Prominent polypeptides could be attributed to the α- and β-biliprotein subunits of allophycocyanin (14, 16 kDa) and phycocyanin (15, 18 kDa) and at least four phycobilisome-linker polypeptides corresponding to 29, 31, 34 and 120 kDa. After SDS-PAGE, the chlorophyll complexes CP IIa and b were resolved as apoproteins of 47 kDa and CP IIc as one of 41 kDa. The chlorophyll complexes CP II b, c contained no other proteins. Complex CP IIa (Fig. 3E) contained an additional faint band of 80 kDa, which may be an apoprotein aggregate. After separation in the first dimension, the chlorophyll proteins were cut out of the gels and analysed by fluorescence emission spectroscopy at the temperature of liquid nitrogen (Fig. 2C). Complexes CP IIa and b were very similar in their spectroscopic properties and showed maxima at 685 nm and shoulders at 693 nm, whilst CP IIc exhibited only one peak at 686 nm, when excited at 445 nm. Thus the isolated chlorophyll complexes had emission properties similar to the in-situ PSII antennae.

Freeze-fracture analysis of thylakoids. The supramolecular organization of thylakoids was investigated by freeze-fracture analysis of whole cells. The protoplasmic fracture face contained broad fields

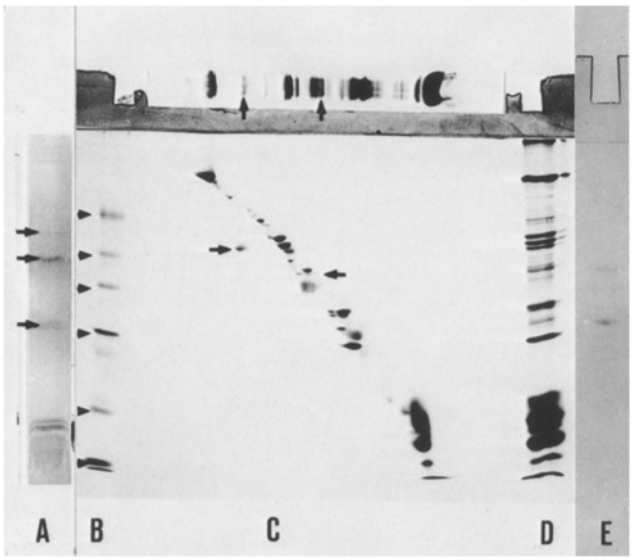


Fig. 3A–E. Polyacrylamide gel electrophoresis of PSII particles isolated from *Synechococcus*. **A** Photosystem-II complexes solubilized in LDS. The gel is not stained and shows (from the top) the chlorophyll proteins (CP) IIa, b, c (*arrows*) and the biliproteins. **C** Two-dimensional electrophoresis of PSII complexes. 1st dimension: Separation of chlorophyll-proteins after LDS-solubilization as in **A**. 2nd dimension: Separation in SDS after complete denaturation; the apoproteins of CP IIb, c are marked by **arrows**. **E** Separation of CP IIa after SDS-PAGE. **D** Denatured PSII complexes separated by SDS-PAGE. **B** Reference proteins, from the top (*arrowheads*): bovine serum albumin (67000), ovalbumin (45000), glyceraldehyde-3-phosphate dehydrogenase (36000), carbonic anhydrase (29000), trypsinogen (24000), α-lactalbumin (14200)

of densely packed particles with diameters around 7.0–9.0 nm (not shown). The EF particles were scattered on the thylakoids (Fig. 4e) or aligned in short or long rows often running parallel to each other as shown in Fig. 4a, b. In this case, the spacing of neighbouring rows was 45 to 75 nm, thus matching the known centre-to-centre distance of parallel phycobilisome rows at the outer surface of the thylakoids. The EF particles within one row were packed with little or no space between them; their centre-to-centre periodicity was 10–12 nm and thus corresponded to the centre-to-centre distance of phycobilisomes as reported by Mörschel and Mühlethaler (1983). The size of the EF particles was determined by measuring the axes running parallel and perpendicular to the direction of the rows; the latter axis could only be determined accurately when the particle rows were shadowed parallel to their direction (Fig. 4). The particles were 20 nm long and 10 nm wide and were aggregated linearly with their longitudinal faces. Many particles had a central band or furrow perpendicular to the long axis, dividing them into two side-by side domains of 10 nm × 10 nm each (Fig. 4). Some particles within the rows measured only 10 nm; they probably originated from the dissociation of the 20 nm × 10 nm particles. The aligned 10 nm × 20 nm particles showed an additional faint furrow parallel to their long axes (Fig. 4a, d, c). This furrow was also observed in the 10-nm EF particles which were not organized into rows (Fig. 4e).

Electron microscopy of PSII preparations. In order to test the correlation of the EF freeze-fracture particles with PSII complexes, the purified PSII complexes were incorporated into liposomes made from soybean phosphatidylcholine. Figure 5b shows a typical PSII-proteoliposome fraction after negative staining. The liposome fraction was nearly uniform and consisted mainly of unilamellar vesicles with diameters in the range of 30–300 nm; multilamellar and multivesicular liposomes were rarely observed. The incorporation of the PSII particles into the liposomes seemed to be almost complete. This was evident from the distribution of chlorophyll and of protein between pellet and supernatant. The degree of incorporation of PSII particles varied within the liposome population, some liposomes showing no incorporation of protein particles at all. The protein particles within the plane of the liposome membranes were clearly visible as electron-translucent dots. The diameter of the incorporated particles was about 10 nm. Dissociated biliprotein aggregates were observed as soluble discs or double discs in the suspension between the proteoliposomes (Fig. 5b).

Figure 5a shows a typical negatively stained fraction of PSII particles solubilized by Triton X-100 from proteoliposomes which were separated from free biliproteins. Two particle classes are distinguished: spherical-ellipsoidal and binary particles. The dominant structures are the binary particles, which are preferably deposited on the support by their broad faces. They measure 20–25 nm × 10–14 nm and are divided perpendicular to their long axis by a band into two spherical-ellipsoidal parts of about 10–13 nm. Aggregations of these binary particles to rows also occurred as shown in Fig. 4 (inset). Within these rows, particles are visible in top view and measure about 24 nm × 12 nm; the central division, separating the particles into two parts of about 12 nm size, is clearly visible. These binary particles are very similar in their appearance to the in-situ EF particles of Fig. 4. The second particle class is represented by spherical-ellipsoidal particles of about 10–12 nm × 14–16 nm; they are probably the building blocks of the the binary particles.

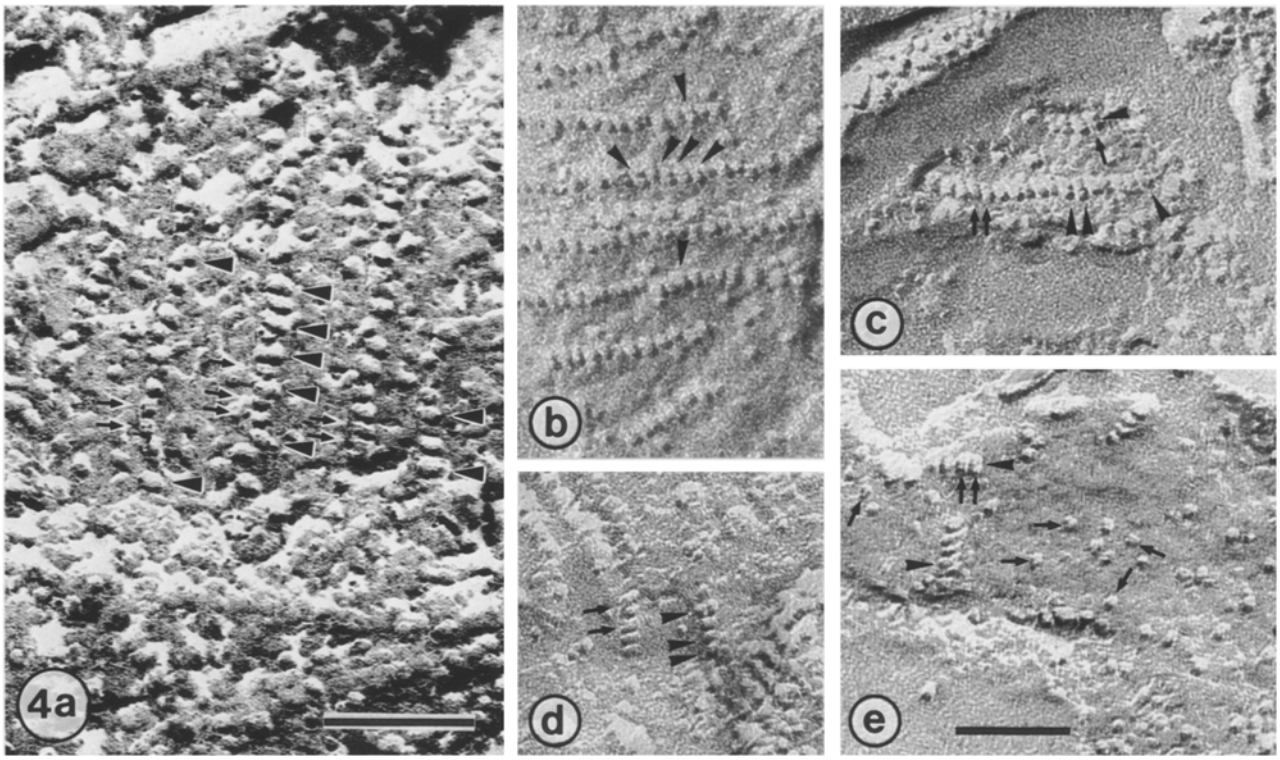


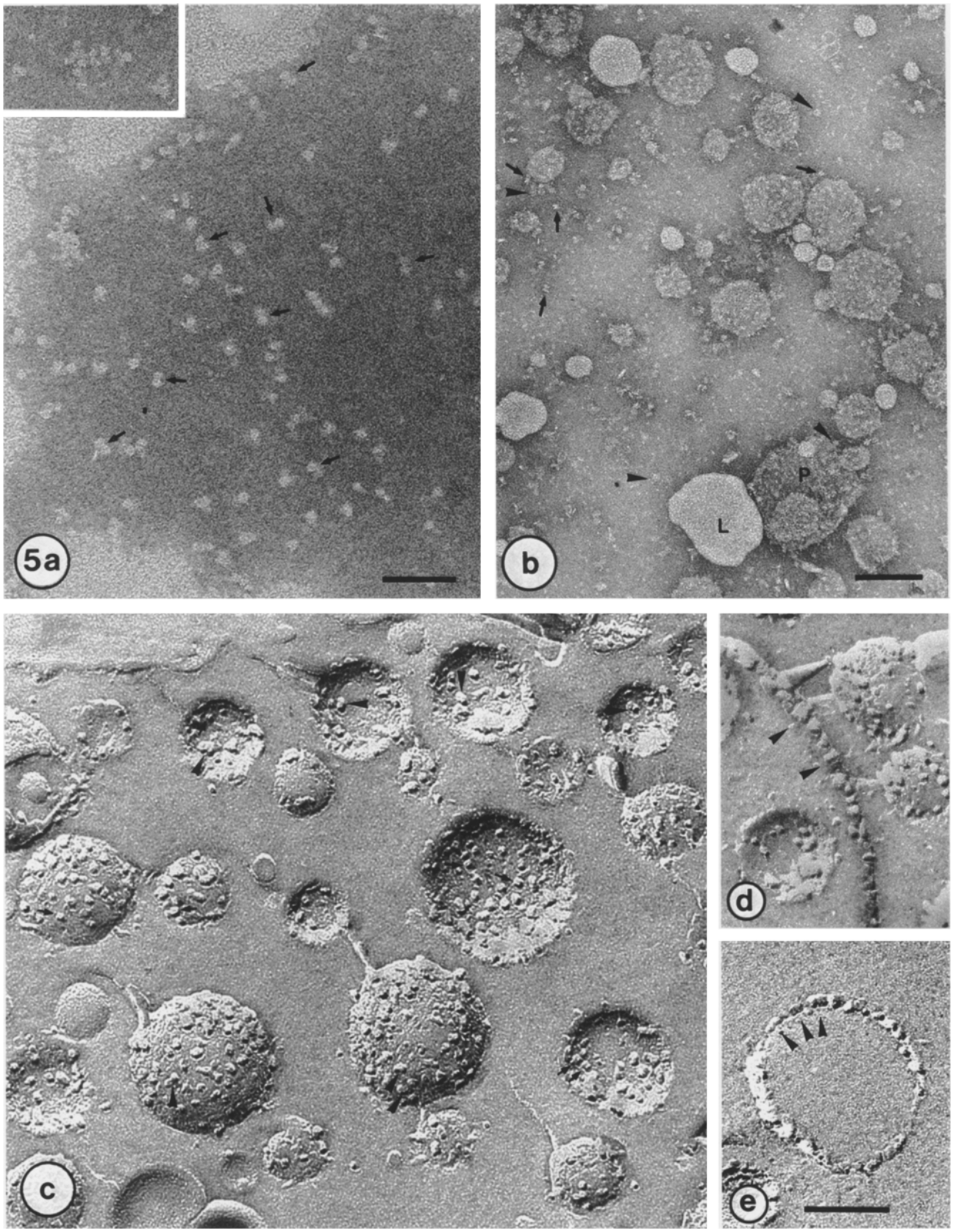
Fig. 4a–e. Freeze-fracture through *Synechococcus* thylakoids exposing EF fracture faces. **a, b, c, e** show an extensive alignment of EF particles; in **e**, aligned and scattered EF particles are visible. Many aligned EF particles are 10 nm × 20 nm and are divided into two parts of 10 nm × 10 nm by a central band perpendicular to their long axis (*arrowheads*). A further furrow (marked by *arrows*) divides these particles parallel to their long axis (**a–e**) and also the scattered 10-nm EF particles (**e**). **a** ×200000; **b–e** ×150000; bars = 100 nm

The PSII proteoliposomes were analyzed by freeze-fracture analysis. In general the size of the proteoliposomes was more homogeneous with this technique than with negative staining; their diameters were between 80–170 nm. The greater homogeneity is due to the fact that disruption and drying effects did not occur. Figure 5c gives an overview of the proteoliposome fraction. The freeze-fracture particles were randomly distributed and their diameter was about 10.3 nm on the concave and convex fracture faces as analyzed by the size-distribution measurements (Fig. 8a). Some particles showed a central furrow; side-by-side aggregations of two particles were observed too.

Proteoliposomes prepared with the addition of cholesterol looked very similar to those without cholesterol, the major difference being, however, that the capability for oxygen evolution (460 μmol $O_2 \cdot mg\,Chl^{-1} \cdot h^{-1}$) was completely preserved when cholesterol was present. Cholesterol is known to suppress lipid phase transitions and acts as a stabilizing factor in native membranes (Harrison and Lunt 1980). Parallel to the high oxygen evolution, significantly more (30–50%) of the PSII particles occurred in dimeric configurations (Fig. 6a–b). Sometimes, parts of phycobilisomes still bound to the incorporated PSII particles were observed on the outer surface of the proteoliposomes (Fig. 6c).

Cross-fractures running perpendicular to the plane of the liposome membrane showed that the particles were spanning the membrane (Fig. 5d, e). With a height of 13–16 nm they were considerably larger than the lipid bilayer and thus were exposed to the hydrophilic environment on both sides of the membrane. The width of the particles was about 10 nm; also, putative dimeric particles were visible in these micrographs, dependent on the exposure of their longitudinal face parallel to the fractured surface.

Freeze-fracture analysis of thylakoid fragments. Freeze-fracture replicas of membrane fragments depleted of PSII and containing nearly all of the PSI activity are shown in Fig. 7a, b. In this additional control experiment it was possible to distinguish between the different particle sizes of the exoplasmic – and protoplasmic thylakoid fracture faces. These fractions contained tightly appressed multilamellar thylakoid vesicles, as shown in fractures within the planes of the membranes (Fig. 7a)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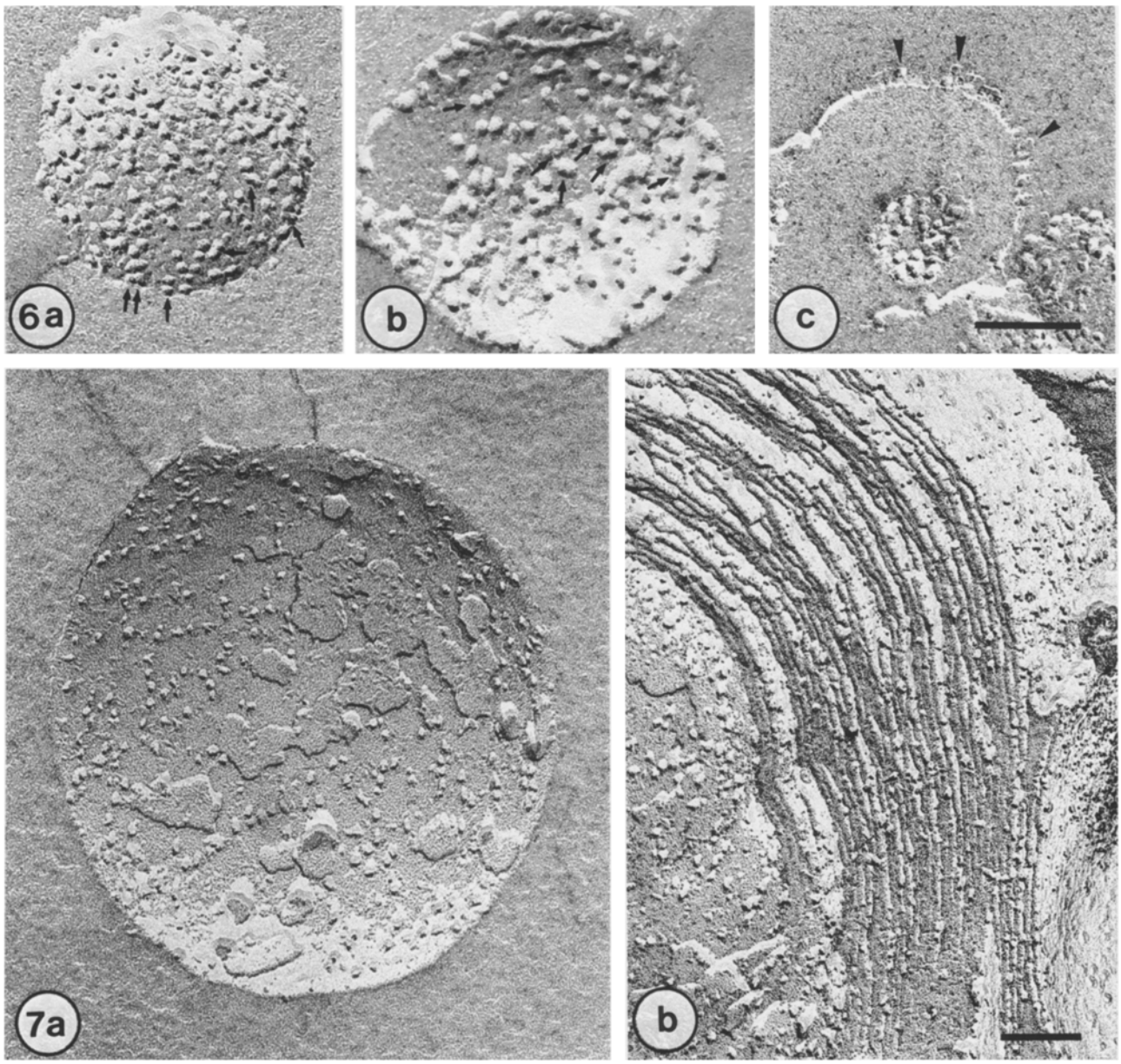
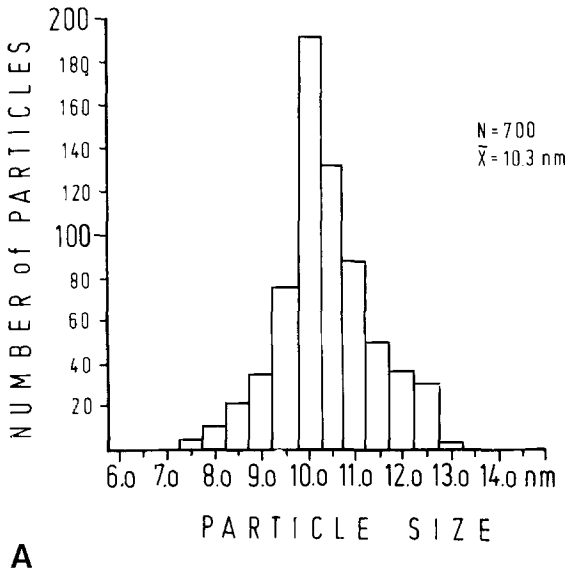


Fig. 6. a, b Selected *Synechococcus* PSII proteoliposomes prepared with the addition of cholesterol showing PSII particles with binary structures (*arrows*). **c** Cross fracture through a PSII proteoliposome with putative triangular phycobilisome centres (*arrows*). × 160000; bar = 100 nm

Fig. 7a, b. Fractures through PSI-containing *Synechococcus* thylakoid fragments depleted in PSII. Less than 10% of the particles are in the size range of EF particles. **a** Fracture through the planes of the membranes. **b** Cross-fracture showing the tightly appressed membranes. × 120000; bar = 100 nm

Fig. 5. a Photosystem-II complexes isolated from *Synechococcus* and negatively stained with 1% phosphotungstic acid (pH 7). Many complexes have a binary structure (*arrows*). *Inset*: Binary complexes assembled to a short row. × 130000. **b** Overview of a PSII proteoliposome fraction. Soluble biliproteins are visible as disks in face view (*arrowheads*) or in profile (*arrows*). Liposome (*L*), proteoliposome (*P*). × 120000. **c** Freeze-fractured PSII proteoliposomes without addition of cholesterol. Particles with a central furrow are marked by *arrows*, binary particles by *arrowheads*. × 151000. **d, e** Cross-fracture perpendicular to the plane of the proteoliposome membrane showing the transmembrane organization of the PSII complexes; putative dimers are visible (*arrowheads*). Preparations without (**d**) and with cholesterol (**e**). × 151000. Bars = 100 nm



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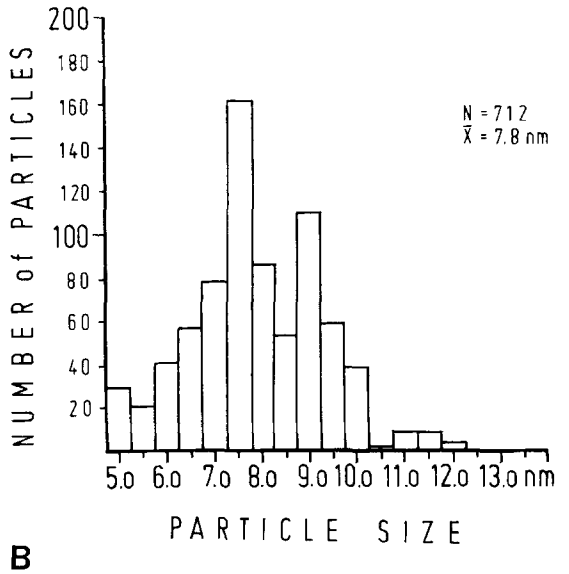

B

Fig. 8A, B. Histograms summarizing the particle-size distributions of **A** PSII particles incorporated into liposomes made from phospholipids and **B** particles in thylakoid fragments depleted in PSII

and also in cross-fractures (Fig. 7b). The size distribution of the thylakoid particles is given in Fig. 8b. With major particle classes of 7.5 and 9.0 nm, these membranes match the particle-size distribution pattern of the protoplasmic but not that of the exoplasmic thylakoid fracture faces.

Discussion

The present study demonstrates that isolated PSII complexes from *Synechococcus sp.* belong to the 10-nm size family of freeze-fracture particles. Particles of this group are the dominant species of exoplasmic fracture faces of cyanobacterial thylakoids (Giddings et al. 1983; Golecki and Drews 1982; Lefort-Tran et al. 1973; Lichtlé and Thomas 1976; Mörschel and Mühlethaler 1983; Neushul 1970). Therefore we conclude that the 10-nm EF particles correspond to PSII complexes. This conclusion is supported by several lines of evidence: (i) A correlation between PSII activity and the number of 10-nm EF particles was observed in the wild-type and a phycobilisome-deficient mutant of the red alga *Cyanidium caldarium* (Wollman 1979). (ii) Thylakoids of heterocysts exhibit no PSII activity and concomitantly lack the 10-nm EF-particle class (Giddings and Staehelin 1979). (iii) Thylakoids from which PSII was extracted resemble the particle pattern of protoplasmic fracture (PF) faces (this study). The 10-nm particles contain at least the reaction-centre complex, the water-splitting system, the chlorophyll antennae with apoproteins of 41 and 47 kDa and a phycobilisome-binding polypeptide (L_{CM}). A functional PSII unit has a molecular weight of about 400000 kDa, as estimated from the minimum requirement for oxygen evolution (Ohno et al. 1986), including the phycobilisome linker L_{CM}. This value corresponds well to the weight of 418000 kDa for a spheric particle of 10.3 nm diameter calculated with a partial specific volume of 0.73 $ml \cdot g^{-1}$ for protein (Zipper et al. 1971).

In thylakoids of *Synechococcus*, a large population of EF particles is aggregated side by side yielding binary structures of 10 nm × 20 nm, which are attached at their longitudinal faces to form rows of variable length. We propose that these binary particles are transmembrane dimeric units made up of two individual 10-nm PSII complexes. This is confirmed by the following observations: (i) the central band dividing the particles perpendicular to their long axes; (ii) occasional dissociations releasing individual 10-nm particles, and the occurrence of 10- to 12-nm particles in negative staining and freeze-fracture preparations; (iii) a large population of dimeric PSII particles observed in proteoliposomes made of phospholipids and cholesterol in which oxygen evolution was almost completely preserved. Deep-etch studies by Giddings and coworkers (1983) showed that the EF particles protrude from the thylakoids with two domains. The formation of PSII dimers in vivo and their organization into rows appears to be a unique feature of cyanobacteria and red algae. Possible explanations are that either the PSII complexes might have developed specific binding sites to form these aggregations, or that the phycobilisomes may induce

this organization. The PSII complexes serve as binding sites for the light-harvesting system, the hemi-discoidal phycobilisomes. This structural relationship is shown by the tightly bound biliproteins and the phycobilisome linker polypeptides that could not be removed by density gradient centrifugation in SPC buffer conserving the phycobilisome structure. Isolation experiments, as well as energy-transfer and structural studies, support the concept of a supramolecular relationship between phycobilisomes and PSII. About 95% of the light energy initially absorbed by phycobilisomes is transferred to PSII (Khanna et al. 1983; Ley and Butler 1977, 1980). Gantt and coworkers (Chereskin et al. 1985); Clement-Metral and Gantt 1983) isolated water-splitting phycobilisome-PSII particles from the red alga *Porphyridium cruentum* and water-splitting PSII particles with bound biliproteins were isolated from *Synechococcus* (Schatz and Witt 1984) and *Anacystis nidulans* (Pakrasi and Sherman 1984). Structural studies showed that the aligned EF particles are associated with phycobilisomes in a 1 to 1 ratio (Mörschel and Mühlethaler 1983). The idea that the 10 nm × 20 nm EF particles are built up of two PSII complexes is supported by phycobilisome morphology, because the two basal phycobilisome core cylinders fit directly in size to the two PSII monomers. It is presumed that each of the basal core cylinders contains two terminal energy emitters, namely α-allophycocyanin B and the core-membrane linker L_{CM} (Glazer 1984; Redlinger and Gantt 1982, 1983; Zilinskas 1982), feeding their energy into underlying 10-nm PSII units (Mörschel and Rhiel 1987). The bigger hemi-ellipsoidal phycobilisomes of *Porphyridium cruentum* and *Griffithsia pacifica* are probably linked to a series of two to three neighboring EF particles and hence four to six PSII units. Photosystem II/phycobilisome ratios of up to 4 were determined by physiological measurements (Kursar and Alberte 1983; Ley 1984; Ohki and Fujita 1987; Stevens and Myers 1976).

Thus we propose that cyanobacteria contain phycobilisome-PSII supercomplexes composed of two PSII complexes and one hemi-discoidal phycobilisome, as shown in the model illustrated in Fig. 9. The aligned phycobilisome-PSII complexes may act as energy-conducting tracks. If this is true, energy is not only funneled within the complex to the PSII reaction centres, but also transferred to neighbouring PSII complexes and phycobilisomes. Energy transferred to PSII complexes may be distributed within the dimer and transferred to neighbouring complexes within the same row via the chlorophyll a antennae or via the phycobilisome core after reverse transfer. The resulting energy-conducting fibre system would allow for an efficient energy distribution along the plane of the thylakoid by connecting many PSII reaction centres.

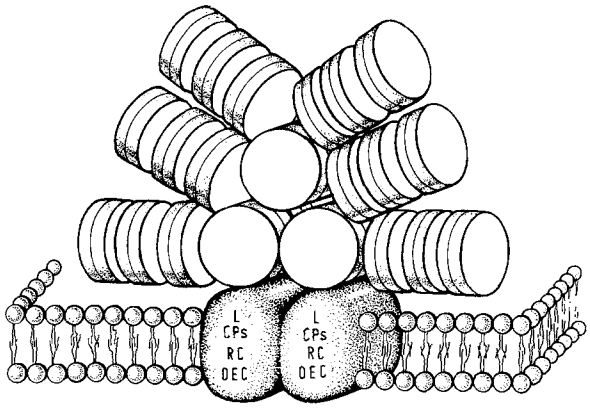

Fig. 9. Schematic model of the supramolecular structure of PSII-phycobilisome complexes in cyanobacteria. *L*, Linker polypeptide (120 kDa); *CPs*, chlorophyll-binding proteins (apoproteins 41 and 47 kDa); *RC*, reaction centre; *OEC*, oxygen-evolving complex. For composition of the reaction centre and oxygen-evolving complex see Ohno et al. (1986)

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