

# Supramolecular structure of the photosystem II complex from green plants and cyanobacteria

(transmission electron microscopy/image analysis/subunit positioning)

EGBERT J. BOEKEMA\*, BEN HANKAMER†, DIRK BALD‡, JOCHEN KRUIP‡, JON NIELD†, ARJEN F. BOONSTRA\*, JAMES BARBER†§, AND MATTHIAS RÖGNER‡

\*BIOSON Research Institute, Biophysical Chemistry, University of Groningen, Nijenborgh 4, NL-9747 AG Groningen, The Netherlands; †Biochemistry Department, Imperial College of Science, Technology and Medicine, London SW7 2AY, United Kingdom; and ‡Institute of Botany, University of Münster, Schlossgarten 3, D-48149 Münster, Germany

Communicated by George Porter, Imperial College, London, United Kingdom, September 12, 1994 (received for review June 23, 1994)

**ABSTRACT** Photosystem II (PSII) complexes, isolated from spinach and the thermophilic cyanobacterium *Synechococcus elongatus*, were characterized by electron microscopy and single-particle image-averaging analyses. Oxygen-evolving core complexes from spinach and *Synechococcus* having molecular masses of about 450 kDa and dimensions of  $\approx 17.2 \times 9.7$  nm showed twofold symmetry indicative of a dimeric organization. Confirmation of this came from image analysis of oxygen-evolving monomeric cores of PSII isolated from spinach and *Synechococcus* having a mass of  $\approx 240$  kDa. Washing with Tris at pH 8.0 and analysis of side-view projections indicated the possible position of the 33-kDa extrinsic manganese-stabilizing protein. A larger complex was isolated that contained the light-harvesting complex II (LHC-II) and other chlorophyll a/b-binding proteins, CP29, CP26, and CP24. This LHC-II-PSII complex had a mass of about 700 kDa, and electron microscopy revealed it also to be a dimer having dimensions of about 26.8 and 12.3 nm. From comparison with the dimeric core complex, it was deduced that the latter is located in the center of the larger particle, with additional peripheral regions accommodating the chlorophyll a/b-binding proteins. It is suggested that two LHC-II trimers are present in each dimeric LHC-II-PSII complex and that each trimer is linked to the reaction center core complex by CP24, CP26, and CP29. The results also suggest that PSII may exist as a dimer *in vivo*.

Photosynthetic organisms contain light-harvesting antenna systems to capture solar energy and transfer it to photochemically active reaction centers where energy conversion occurs. Photosystem II (PSII), which is present in cyanobacteria, red and green algae, and higher plants, contains a reaction center that brings about the splitting of water to molecular oxygen and reducing equivalents (1). The latter are transferred via electron-transport chain/proton carriers, including a second photosystem (PSI), ultimately to be utilized in the reduction of carbon dioxide to organic molecules. PSII possesses its own specific light-harvesting system, which is composed of two chlorophyll (Chl) a-binding proteins known as CP43 (PsbC) and CP47 (PsbB) and several chlorophyll a/b-binding proteins (encoded by *cab* genes and called Cab proteins) of which the most abundant is the light-harvesting complex II (LHC-II). Recently the structure of LHC-II has been determined to a resolution of 3.4 Å by electron crystallography (2). The other Cab proteins of PSII are CP29, CP26, and CP24 (3). In cyanobacteria the Cab proteins are not present and are replaced by phycobiliproteins, which also act as light-harvesting systems for PSII. In most other respects PSII is very similar in all types of oxygenic photosynthetic organisms (1)

and contains a reaction center that is likely to be similar to that of purple photosynthetic bacteria (4), where the structure has been revealed to atomic resolution by x-ray crystallography (5).

Electron microscopy (EM) of negatively stained specimens is a technique that can reveal low-resolution structures of protein complexes and has been applied to several types of PSII preparations, including ordered arrays from spinach (6–9). Single-particle averaging has also been performed on electron micrographs of PSII complexes isolated from the cyanobacterium *Synechococcus* sp (10, 11) and spinach (12). High-resolution EM is possible only with unstained, large, well-ordered two-dimensional crystals, but these are often difficult to grow and, despite the success with LHC-II (2), have not been obtained for PSII cores or reaction centers. For PSI with a mass comparable to that of PSII, it was shown that single-particle analysis is a worthwhile alternative to yield projection images of about 25-Å resolution (13). We have therefore applied single-particle averaging to the characterization of isolated PSII complexes differing in subunit composition. The results presented here give new and important information about the structure and organization of PSII.

## MATERIALS AND METHODS

**Purification of PSII Complexes.** PSII core complexes from *Synechococcus elongatus* have been isolated and purified as described (11). Spinach PSII core complexes were made as described by Hankamer (14) and involved the solubilization of oxygen-evolving core particles, similar to those of Ghanotakis *et al.* (15), with dodecyl maltoside prior to sucrose density-gradient centrifugation. Spinach particles containing the core complex plus LHC-II were obtained from membranes enriched in PSII (BBYs) (16) by incubation of chlorophyll at 0.5 mg/ml in 30 mM dodecyl maltoside and subsequent sucrose density-gradient centrifugation, which resulted in a separation into four bands, from which the lowest one was used. Washing core complexes with 1 M Tris (pH 8.0) (spinach) or 0.5 M Tris (pH 8.0) (*Synechococcus*) in the presence of 2 mM dodecyl maltoside resulted in the removal of the 33-kDa subunit.

**EM and Image Analysis.** For EM, material was prepared by the droplet method, with 1% uranyl acetate as the negative stain. EM was performed with a JEOL 1200-EX electron microscope at  $\times 60,000$  magnification. Micrographs were digitized with a Kodak Eikonix charge-coupled device camera with a step size of 25  $\mu\text{m}$ . Analysis was carried out on a Convex C220 minisupercomputer. For single-particle averaging, data sets from five different PSII complexes were collected. Image analysis was carried out as described (17, 18). First, projections were pretreated and then aligned by correlation methods.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: PSI and PSII, photosystems I and II; LHC II, light-harvesting complex II.

§To whom reprint requests should be addressed.

Aligned projections were treated with multivariate statistical analysis in combination with classification (17, 19). Finally, for sums of projections belonging to the various classes, the best 50–70% of the class members were taken, and the correlation coefficient in the alignment procedure was used to judge the quality of the analysis. The resolution of the various averaged sums ranged from 1.6 to 2.5 nm as judged from Fourier-ring correlation criteria (20).

## RESULTS

**Analysis of PSII Reaction Center Cores of *S. elongatus*.** PSII cores isolated from *Synechococcus* evolved oxygen and did not contain phycobilisomes (10, 11). SDS/PAGE revealed their composition to consist of chlorophyll-binding proteins CP43 and CP47 (*psbC* and *psbB* gene products, respectively), the 33-kDa manganese-stabilizing protein (*psbO* gene product), and the reaction center proteins—namely, D1 and D2 (*psbA* and *psbD* gene products, respectively) and the  $\alpha$  and  $\beta$  subunits of cytochrome *b559* (*psbE* and *psbF* gene products, respectively). Other small polypeptides are also probably contained in these particles, such as the *psbI* gene product. HPLC size-exclusion analyses (10, 11) indicated that the molecular mass of this particle is  $450 \pm 50$  kDa (corrected for its detergent micelle).

The cyanobacterial PSII core particle was subjected to EM (see Fig. 1A). Both top views and side views were selected and analyzed. For top views, the projections were fairly similar with the same type of handedness, which indicated a strong preference in attachment to the carbon support of one of its hydrophilic sides. The projections were analyzed without imposing symmetry, but no substantial deviation from a twofold symmetry was found. Therefore, the best 150 top views were averaged assuming twofold symmetry, and the resulting image is shown in Fig. 2A. As can be seen, this averaged projection clearly identified four areas of high density surrounding an area of low density in the center.

In contrast to the top-view analysis, classification of the side views of the cyanobacterial core particle resulted in two main types of projections. In one type, two separated protrusions were visible (see Fig. 2C), while in the other only one protrusion was observed in the center (Fig. 2D). These different projections indicate that the cyanobacterial particle can be attached in different ways to the carbon support, causing the protrusions to be separated or superimposed in the overlap view of Fig. 2D. The different positions also caused the projection of Fig. 2D to be slightly longer than that of Fig. 2C. The protrusions are most likely due to the 33-kDa subunit. To verify this, particles were washed with Tris at pH 8.0, a treatment that specifically removes the 33-kDa subunit. Unfortunately, the electron micrographs of these washed particles did not give a sufficient number of side views for a satisfactory analysis. However, the number of top views was sufficient to give the average projection shown in Fig. 2E.

**Analysis of PSII Cores from Spinach.** Two forms of oxygen-evolving core complexes, having similar subunit composition to the *Synechococcus* cores, were isolated from spinach. These higher plant particles did not contain the Cab proteins or the 18- and 23-kDa subunits of the water-splitting complex. HPLC size-exclusion analysis showed them to have molecular masses of about  $240 \pm 25$  kDa and  $450 \pm 50$  kDa.

EM revealed the 450-kDa particle to be preferentially oriented on the carbon support in a way similar to that observed with the cyanobacterial preparation (see Fig. 1B). Analyses of 300 top-view projections gave the image presented in Fig. 2B, which clearly shows twofold symmetry and is very much like the top-view image obtained for the *Synechococcus* particle. The spinach particle was also subjected to washing with Tris at pH 8.0 to remove the 33-kDa protein. The top-view analysis of the resulting electron micrograph is shown in Fig. 2F.

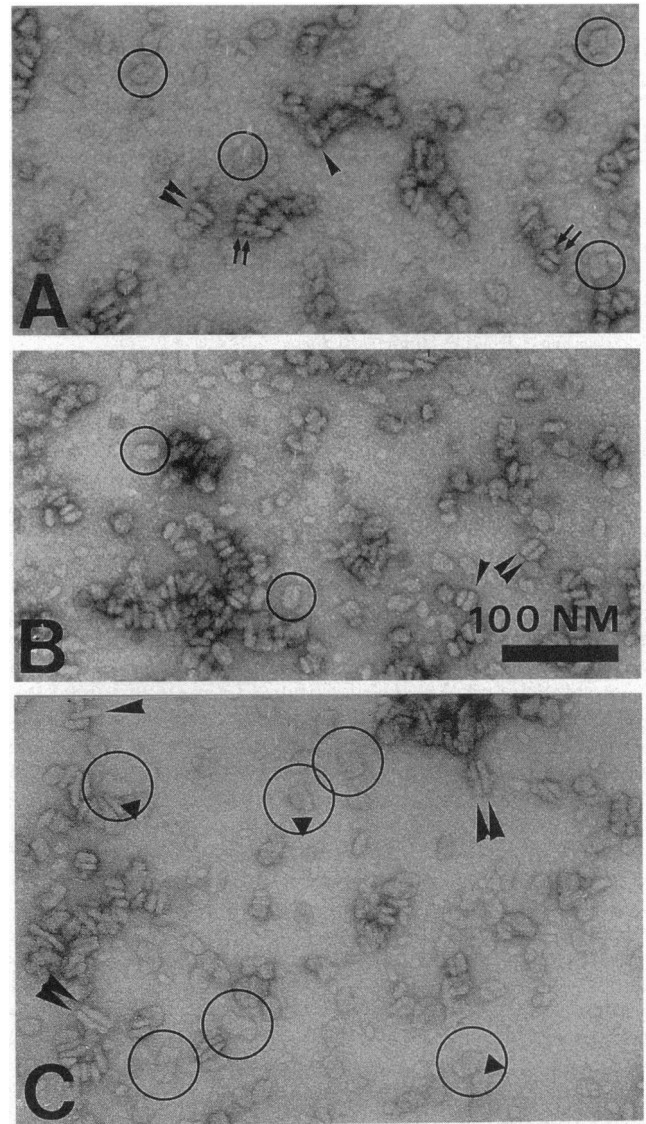


FIG. 1. EM of PSII complexes. (A) The core complex dimer from *Synechococcus*. Top views are indicated by circles. In side-view position (arrowheads), often two protrusions are visible (small arrows). (B) The core complex dimer from spinach. (C) The LHC-II-PSII complex dimer. Top views have been indicated by large circles. In side-view position (arrowheads), dimers often aggregate into pairs. Sometimes a specific part of a large dimer was dissected (marked by a triangle).

Both the cyanobacterial and higher plant particles having molecular masses of about 450 kDa seem to be composed of two monomers as indicated by the twofold symmetry in projection and the existence of two 33-kDa proteins per particle. This was confirmed by the analysis of electron micrographs obtained with the PSII core from spinach having a molecular mass of about 240 kDa. The sum of 100 top views of this 240-kDa particle is given in Fig. 2H, where it is clearly seen to be equal to one half of the larger core particle (e.g., Fig. 2B). A similar monomeric core has also been isolated recently from *Synechococcus* (unpublished data).

The similarity in the size and shape of the spinach and cyanobacterial complexes is consistent with their protein compositions and PSII function. The overall dimensions of the four sets of core complex dimers, as measured from the top views, did not vary very much, with an average length (measured along the long axis) of 20.6 nm and width of 13.1 nm. However, these values need to be corrected for attached detergent.

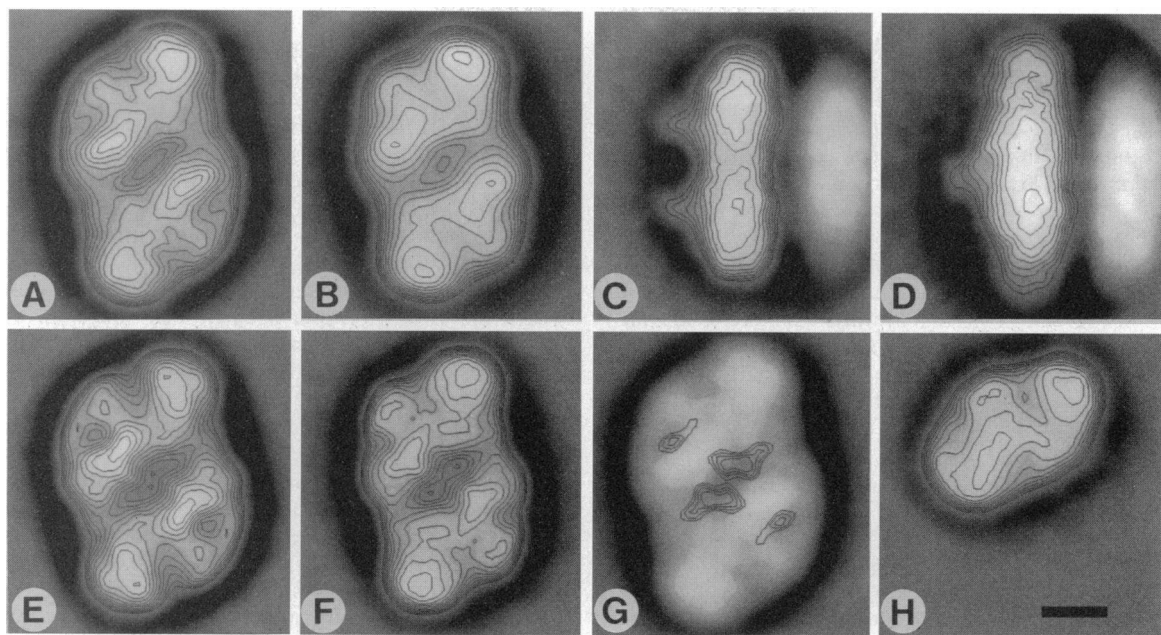


FIG. 2. Averaged projections of PSII core complexes. (A) A sum of 150 top views from *Synechococcus* core dimers. (B) Sum of 300 top views of spinach core complex dimers. (C and D) Sums of 85 and 30 projections from a classification of side views of *Synechococcus* core dimers. (E) Sum of 70 projections of Tris-washed *Synechococcus* core dimers. (F) Sum of 160 projections of Tris-washed spinach core dimers. (G) Difference images of (A + B) - (E + F). The areas showing the largest difference have been contoured, and the lines of these regions are imposed on the image of B. (H) Sum of 100 top views of spinach core complex monomers. Note that twofold symmetry was imposed on images A, B, E, and G. (Bar = 5 nm.)

When a detergent layer of 1.7 nm is assumed (11), then the size of the dimer is about  $17.2 \times 9.7$  nm. The height of the PSII complex measured from the image in Fig. 2C is 9.1 nm, including the protrusion and about 6.0 nm at the edge.

A difference image of the nonwashed (Fig. 2A and B) and the Tris-washed projections (Fig. 2E and F) was generated to identify the possible position of the 33-kDa protein. As Fig. 2G shows, two areas with differences in electron density are clearly visible in each monomer.

**Analysis of LHC-II-PSII Complex from Spinach.** PSII-enriched membranes known as BBYs (16) were treated with dodecyl maltoside and subjected to sucrose density-gradient centrifugation. The lowest of four bands was taken for EM. According to SDS/PAGE, this band contained, in addition to the PSII core proteins, the *cab* gene products LHC-II, CP29, CP26, and CP24, and HPLC size-exclusion analysis showed it

to be dominated by a particle having a molecular mass of  $700 \pm 70$  kDa (without detergent). Fig. 3A shows the average of 200 top views of this LHC-II-PSII supercore complex analyzed without imposing symmetry. Nevertheless, the particle clearly possessed twofold symmetry as indicated by the Fourier-ring correlation criterion (20), which found the two halves of the unsymmetrized dimer to be identical above 2.5-nm resolution. The dimensions of the LHC-II-PSII complex are  $30.2 \times 15.7$  nm, which decreases to  $\approx 26.8 \times 12.3$  nm after correction for the detergent layer. Although the top view was the preferred orientation of the particle, side-view projections were also found (see Fig. 1C). The side views often consisted of the aggregation of two particles (Fig. 3B), as was also found for the smaller dimeric core particles (see Fig. 2C and D). In both cases it seems that the aggregation is due to an interaction between the two stromal exposed surfaces, since the nonin-

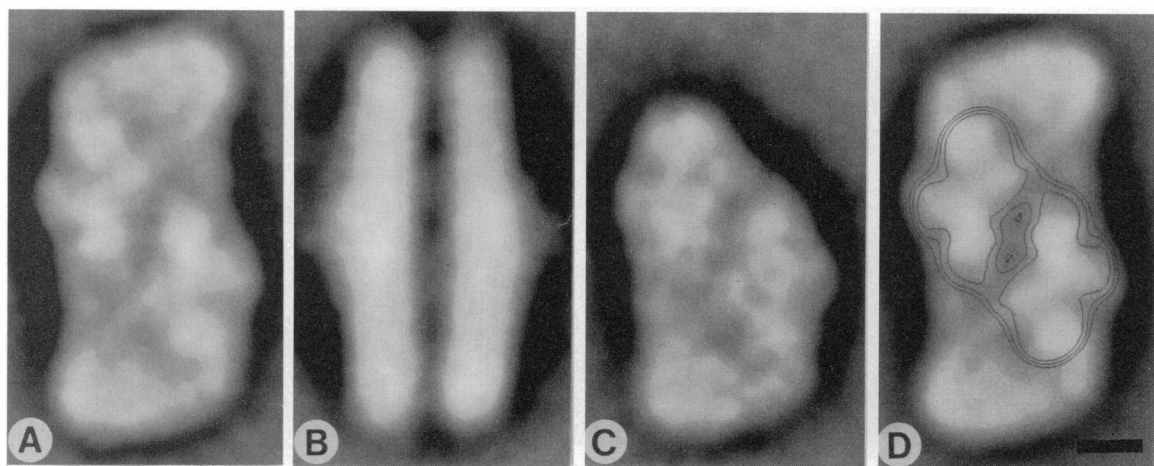


FIG. 3. Averaged projections of the LHC-II-PSII complex. (A) Sum of 200 top views without symmetry imposed. (B) Sum of 80 side views. (C) Sum of 75 projections of complexes missing a flap on the upper side. (D) Complex in top-view projection with the small core complex outline, including its detergent shell, superimposed. (Bar = 5 nm.)

teracting surfaces bind the 33-kDa protein. The analysis of 80 side views given in Fig. 3B shows a broad single protrusion, which presumably is due to the partial alignment of two 33-kDa proteins. The image also indicates the thickness of the particle to be about 6.0 nm at the tip and 9.0 nm at the center. In addition to the top-view projection shown in Fig. 3A, some particles had modified top views (marked with triangle in Fig. 1C), and the analysis of 75 such particles yielded the image shown in Fig. 3C. The meaning of this modified image is revealed when the projections of the spinach core dimer complex are superimposed on the image of the LHC-II-PSII complex, as in Fig. 3D. From this overlay it is quite clear that the LHC-II and other Cab proteins are located in the "flap" regions surrounding the central core. Thus, the particle shown in Fig. 3C has apparently lost half of its complement of Cab proteins.

## DISCUSSION

**Location of Chlorophyll-Binding Proteins.** We have shown that the core complex of PSII can be isolated from spinach and *Synechococcus* either as a monomer or dimer. These complexes have similar composition and structural features. Since cyanobacteria do not contain Cab proteins, it can be concluded that LHC-II, CP29, CP26, and CP24 are also absent from spinach cores. This is consistent with SDS/PAGE analysis. The four main protein densities observed in the top projection of the core dimer are similar to those seen in the projection of PSII dimers in two-dimensional crystals (7), and we have adopted the same notation used in this work. The position of the four densities is shown in Fig. 4, which gives the projection of the LHC-II-PSII complex. These densities must be due to the components that make up the PSII core—namely, CP47, CP43, D1 and D2 proteins, and  $\alpha$  and  $\beta$  subunits of cytochrome *b559*. Comparisons with data obtained from two-dimensional crystals (6) and labeling studies with D1 antibod-

ies (unpublished data) indicate that area 4 may be tentatively attributed to CP43 and area 2 to the D1/D2 heterodimer. It is also clear that Cab proteins must be contained in the peripheral "flaps" on each side of the core dimer. The LHC-II-PSII complex has a height of 6 nm at its outer tip, which is consistent with the height of LHC-II as determined from high-resolution electron crystallography (2). Further support for the location of LHC-II at the periphery of the larger complex comes from superimposition of the projected structure of the trimer (21) of this chlorophyll-binding protein onto the electron micrograph as shown in Fig. 4. As can be seen, the LHC-II trimer seems to fit snugly in the region labeled 9. As a consequence, the remaining four densities marked 5–8 are suggested to be due to CP24, CP26, and CP29, which have been proposed as linker proteins between LHC-II and the PSII core complex (22, 23). These assignments are tentative and will need to be supported by further studies using procedures such as immunolabeling.

**Location of the 33-kDa Subunit.** The two predominant side views of the dimeric core complexes showed protrusions that were either in an overlap or nonoverlap position (Fig. 2C and D). In part, this protrusion can be attributed to the 33-kDa extrinsic protein. In the case of the LHC-II-PSII complex, only one type of side view was identified with a single central protrusion (Fig. 3B). It seems likely that the elongated shape of the LHC-II-PSII complex, compared with that of the core dimer, gives rise to a favored orientation with the two protrusions overlapping. The difference image of top views resulting from the comparison of Tris-washed and nonwashed core dimers (Fig. 2G) identified two regions of change in density—namely, in the central region, where the two monomers join, and in two rotational symmetrically related regions in the monomers themselves. Taking into account the side-view projections, it seems likely that the latter density changes can be attributed to the 33-kDa protein. This proposed location of the 33-kDa protein is within density 2 in Fig. 4 and

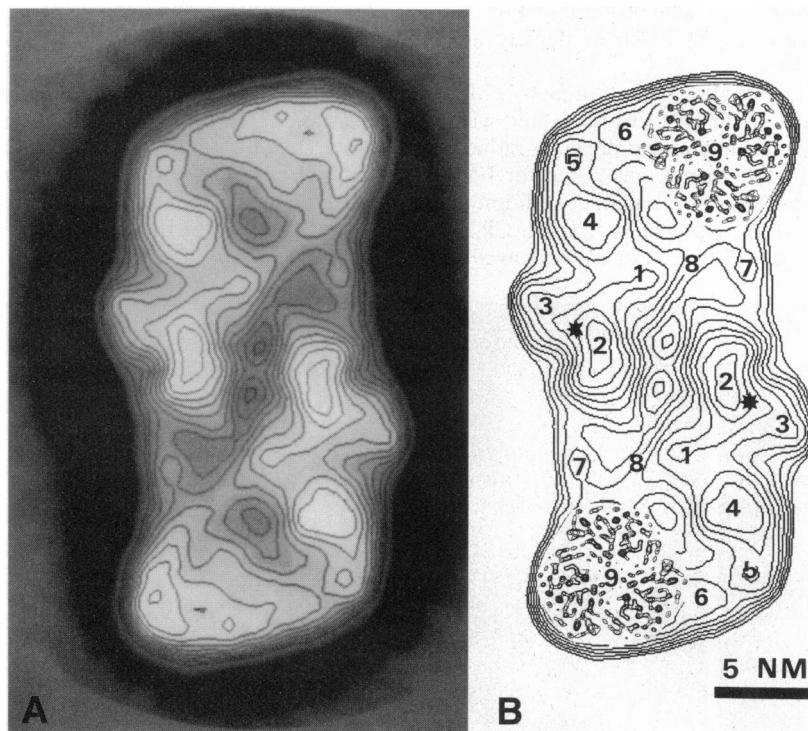


FIG. 4. The projected structure of the LHC-II-PSII complex (A) accompanied by a scheme for its structural organization (B). In the central part, occupied by the core complex, four densities can be seen, which are also visible in the images given in ref. 7. The mass around position 9 is most compatible to LHC-II trimer, and the projected density of LHC-II (21) has been reproduced in this position. Numbers 5–8 mark additional densities connecting the LHC-II trimer to the core complex. The proposed position of the 33-kDa subunit is indicated by an asterisk.



is marked with an asterisk. The reason for the density change at the center of the core dimer after removal of the 33 kDa protein is unknown but could be due to a conformational change induced by the removal of the extrinsic protein or by the Tris washing itself.

**Relevance for *in Vivo* Organization.** The results presented in this communication not only give information about the relative position of the PSII antenna Cab proteins to the reaction center core complex but also indicate that PSII may exist as a dimer *in vivo*. We come to this conclusion because particles containing a monomeric core complex with bound LHC-II were never observed in our micrographs. Indeed, we found that the dimeric core is maintained even when the population of Cab proteins is removed from one side of the supercore complex (Fig. 3C). It is also worthy of note that the mild detergent conditions used to obtain the dimeric supercore yielded predominantly complexes of this type. The possibility that PSII exists as a dimer *in vivo* stems back to earlier work with *Synechococcus* (10, 11, 24) and more recent work with higher plants (7, 9, 25). In contrast, Holzenberg *et al.* (8) have argued to the contrary. Although their electron micrographs of PSII-ordered arrays in detergent-treated spinach BBYs revealed pseudodimeric features, they concluded that the twofold symmetry was not maintained across the complex going from the luminal to the stromal surfaces. Consequently, they argued that the PSII complex existed as a monomer *in vivo*. In fact, the structure they observed is similar in size and shape to the dimeric core particles analyzed in this communication. A comparison of projections in the membrane plane shows that the size of the pseudodimer reported by Holzenberg *et al.* (8) is  $18 \times 10$  nm, which is comparable to  $17 \times 10$  nm measured for the isolated core dimer of spinach and *Synechococcus*. It is possible, therefore, that the PSII particle characterized by Holzenberg *et al.* (8) was indeed dimeric and that the deviation from twofold symmetry on the stromal surface was due to its rather flat and presumably featureless characteristics as compared with the luminal surface (see Figs. 2C and D and 3B). Furthermore, Holzenberg *et al.* (8) also reported that the PSII complex had a central hole or cavity, a feature clearly seen in the dimeric (Fig. 2A and B) but not the monomeric (Fig. 2H) isolated core complexes. The structural information gained from the top and side views of the PSII core and LHC-II-PSII complexes also indicates that a previous EM analysis of isolated PSII (12) attributed to core particles must in part have been averaged from LHC-II-PSII complexes.

**Stoichiometries.** The monomeric PSII core isolated from spinach was estimated to have a molecular mass of about 240 kDa, which is roughly predicted if it contains one copy each of its protein components, although at this stage the possible presence of low molecular weight proteins has not yet been clarified. The dimer is about twice this molecular mass as expected. The LHC-II-PSII complex had a molecular mass in the region of 700 kDa of which 450 kDa is attributed to the core dimer. The remaining 250 kDa must be due to the Cab proteins. If there were two sets of LHC-II trimers (as suggested in Fig. 4), this would account for about 150 kDa. Given the errors, the remaining molecular mass probably accounts for two copies each of CP29, CP26, and CP24. Thus, the number of proteins within the isolated dimeric LHC-II-PSII complex could be  $2 \times$  LHC-II trimers,  $2 \times$  CP29,  $2 \times$  CP26,  $2 \times$  CP24,  $2 \times$  CP47,  $2 \times$  CP43,  $2 \times$  D1 protein,  $2 \times$  D2 protein, and  $2 \times$   $\alpha$ - and  $2 \times$   $\beta$ -subunits of cytochrome *b559*. The likely presence, however, of low molecular weight proteins should also be taken into account. The level of chlorophyll bound to the LHC-II-PSII complex has been roughly estimated to be about 200 (14) molecules or 100 chlorophylls per reaction center. The actual level of chlorophyll per PSII *in vivo* varies with growth conditions but is often assumed to be about 250

molecules per reaction center. Presumably in the natural membrane, the LHC-II-PSII complex we have isolated is surrounded by a more loosely bound pool of LHC-II, which has been postulated previously (26).

In conclusion, our data give firm experimental support to recent tentative models that suggest that the LHC-II complexes are attached only to certain regions of the core complex and that the other Cab proteins form linkers to facilitate this attachment (22, 23). We also concur with others who have suggested that PSII can exist as a dimeric complex *in vivo* (7, 9–11, 22–25, 27).

We thank Dr. W. Keegstra for help with image analysis and W. Lamkemeyer for excellent technical assistance. This study was supported by The Netherlands Organization for Chemical Research with financial aid from the Netherlands Organization for Scientific Research (to E.J.B. and A.F.B.) and the Deutsche Forschungsgemeinschaft (to D.B., J.K., and M.R.). J.B. and M.R. are grateful for support from the Research Institute of Innovative Technology for the Earth. The Biotechnology and Biological Sciences Research Council supports J.B. and also gave studentships to B.H. and J.N. The British Oxygen Company kindly sponsored B.H.

1. Barber, J. (1989) in *Oxford Surveys of Plant Molecular and Cell Biology*, ed. Mifflin, B. J. (Oxford Univ. Press, Oxford), Vol. 6, pp. 115–162.
2. Kühlbrandt, W., Wang, D. N. & Fujiyoshi, Y. (1994) *Nature (London)* **367**, 614–621.
3. Jansson, S., Pichersky, E., Bassi, R., Green, B. R., Ikeuchi, M., Melis, A., Simpson, D. J., Spangfort, M., Staehelin, L. A. & Thornber, J. P. (1992) *Plant Mol. Biol. Rep.* **10**, 242–253.
4. Michel, H. & Deisenhofer, J. (1988) *Biochemistry* **27**, 1–7.
5. Deisenhofer, J., Epp, O., Miki, K., Huber, R. & Michel, H. (1985) *Nature (London)* **318**, 618–624.
6. Dekker, J. P., Betts, S. D., Yocum, C. F. & Boekema, E. J. (1990) *Biochemistry*, **29**, 3220–3225.
7. Lyon, M. K., Marr, K. M. & Furcinitty, P. S. (1993) *J. Struct. Biol.* **110**, 133–140.
8. Holzenberg, A., Bewley, M. C., Wilson, F. H., Nicholson, W. V. & Ford, R. C. (1993) *Nature (London)* **363**, 470–473.
9. Santini, C., Tidu, V., Tognon, G., Ghiretti Magaldi, A. & Bassi, R. (1994) *Eur. J. Biochem.* **221**, 307–315.
10. Rögner, M., Dekker, J. P., Boekema, E. J. & Witt, H. T. (1987) *FEBS Lett.* **219**, 207–211.
11. Dekker, J. P., Boekema, E. J., Witt, H. T. & Rögner, M. (1988) *Biochim. Biophys. Acta* **936**, 307–318.
12. Haag, E., Irrgang, K. D., Boekema, E. J. & Renger, G. (1990) *Eur. J. Biochem.* **189**, 47–53.
13. Boekema, E. J., Boonstra, A. F., Dekker, J. P. & Rögner, M. (1994) *J. Bioenerg. Biomembr.* **26**, 17–29.
14. Hankamer, B. (1994) Ph.D. thesis (University of London, London).
15. Ghanotakis, D. F., Demetriou, D. M. & Yocum, C. F. (1987) *Biochim. Biophys. Acta* **891**, 15–21.
16. Berthold, D. A., Babcock, G. T. & Yocum, C. F. (1981) *Biochim. Biophys. Acta* **764**, 179–183.
17. Harauz, G., Boekema, E. J. & Van Heel, M. (1988) *Methods Enzymol.* **164**, 35–49.
18. Boekema, E. J. & Böttcher, B. (1992) *Biochim. Biophys. Acta* **1098**, 131–143.
19. Van Heel, M. & Frank, J. (1981) *Ultramicroscopy* **6**, 187–194.
20. Saxton, W. O. & Baumeister, W. (1982) *J. Microscopy* **127**, 127–138.
21. Wang, D. N. & Kühlbrandt, W. (1991) *J. Mol. Biol.* **217**, 691–699.
22. Bassi, R. & Dainese, P. (1992) *Eur. J. Biochem.* **204**, 317–326.
23. Peter, G. F. & Thornber, J. P. (1991) *J. Biol. Chem.* **266**, 16745–16754.
24. Mörschel, E. & Schatz, G. H. (1987) *Planta* **172**, 145–154.
25. Peter, G. F. & Thornber, J. P. (1991) *Plant Cell Physiol.* **32**, 1237–1250.
26. Spangfort, M. & Andersson, B. (1989) *Biochim. Biophys. Acta* **977**, 163–170.
27. Seibert, M., De Witt, M. & Staehelin, A. L. (1987) *J. Cell Biol.* **105**, 2257–2265.