Three-dimensional Structure of Higher Plant Photosystem I Determined by Electron Crystallography

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We describe the three-dimensional structure of higher plant photosystem I (PSI) as obtained by electron microscopy of two-dimensional crystals formed at the grana margins of thylakoid membranes. The negatively stained crystalline areas displayed unit cell dimensions \( a = 26.6 \text{ nm}, b = 27.7 \text{ nm}, \text{ and } \gamma = 90^\circ \), and \( p22_12_1 \) plane group symmetry consisting of two monomers facing upward and two monomers facing downward with respect to the membrane plane. Higher plant PSI shows several structural similarities to the cyanobacterial PSI complex, with a prominent ridge on the stromal side of the complex. The stromal ridge is resolved into at least three separate domains that are interpreted as representing the three well-characterized stromal subunits, the \( \text{psa} C, D, \text{ and } E \) gene products. The luminal surface is relatively flat but exhibits a distinct central depression that may be the binding site for plastocyanin. Higher plant PSI is of dimensions \( 15-16 \times 11-12.5 \text{ nm} \), and thus leaves a larger footprint in the membrane than its cyanobacterial equivalent \( (13 \times 10.5 \text{ nm}) \). It is expected that additional membrane-bound polypeptides will be present in the higher plant PSI complex. Both higher plant and cyanobacterial complexes span about 8–9 nm in the direction orthogonal to the membrane. This report represents the first three-dimensional structure for the higher plant PSI complex.

Photosystem (PS)\(^1\) I is a multiprotein complex found in the photosynthetic membranes of plants and cyanobacteria. It carries out the second light-driven electron transfer step in the linear Z scheme of photosynthesis (1). In higher plants, a significant proportion of the PSI complexes is located in the stromal lamellae (2). Cyclic electron transfer may be carried out by this pool of PSI for at least part of the time. It is also thought that higher plant PSI may be located around the periphery of the grana stacks (the grana margins) where they interact with photosystem II (PSII) complexes, and probably take part in linear electron transfer (2). On the basis of spectroscopic data, it has been proposed that there is heterogeneity in the structure of higher plant PSI (3), with stromally located PSI complexes predicted to have a smaller photosynthetic unit size (i.e., a reduced number of light-harvesting pigments) than their granally located sisters. Until now, there has been little direct structural investigation of higher plant PSI. The paucity of structural data was largely due to the lack of two- or three-dimensional crystals of the higher plant complex. In the absence of crystals, the analysis of freeze-fractured or freeze-etched thylakoid membranes by shadowing and electron microscopy was carried out (e.g., see Dunahay and Staehelin (4)), but only the overall dimensions of the complexes were determined, and uncertainty over their identification as PSI was not eliminated. In a second approach, higher plant PSI complexes have been purified after detergent solubilization and then examined by electron microscopy after negative staining (5). These studies estimated the dimensions of the various PSI subcomplexes and described their general shape (approximately elliptical). Without correction for attached detergent, average dimensions for three types of monomeric PSI complexes were found to be approximately \( 15.3 \times 10.5 \text{ nm}, 16.2 \times 11.1 \text{ nm}, \text{ and } 20.0 \times 16.0 \text{ nm} \). Each of the three types of PSI particle were representative of different preparation methods yielding complexes with different polypeptide and pigment compositions.

Structural studies on two-dimensional crystals of higher plant PSI obtained in spinach thylakoid membranes were recently reported (6). These represented the first reported two-dimensional crystals of the higher plant complex. It has been demonstrated that the crystals are well ordered, and they have been used to generate a projection map of the higher plant PSI complex in negative stain at about 2.5-nm resolution. This revealed the overall shape of the complex. Further structural details, such as the high density domain (“ridge”) predicted to contain the extrinsic polypeptides on the stromal side of the complex, were suggested by the projection structure. These earlier studies were also consistent with the hypothesis that a population of PSI complexes exists around the periphery of the grana stacks in the thylakoids (6, 7).

In contrast to the higher plant situation, structural studies of the more robust cyanobacterial PSI complex are well advanced. Both two- and three-dimensional crystals of the cyanobacterial complex have been available for the last 10 years (8–14). There have been various electron microscopical investigations of two-dimensional crystals of the complex (8–11), as well as a major x-ray crystallography effort, which has now resolved cyanobacterial PSI structure to a level where the assignment of secondary structure, chlorophyll molecules, and electron transfer components has become possible (14, 15). The latest x-ray data shows that the cyanobacterial PSI complex contains at least 31 transmembrane \( \alpha \) helices and 86–100 chlorophyll (Chl) \( \alpha \) molecules (15).\(^2\) The cyanobacterial PSI complex contains 11 polypeptides, but the two largest polypeptides, of mass roughly 80 kDa each, bind the majority of the electron transfer compo-

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\(^1\) The abbreviations used are: PS, photosystem; LHC, light harvesting complex; Chl, chlorophyll; MES, 2-(morpholino)ethanesulfonic acid; r.m.s., root mean square.

\(^2\) N. Krauss, personal communication.
nents and pigment molecules. The central core of the PSI complex appears to be similar in construction to the basic purple bacterial reaction center, with 2\(3\)5 transmembrane helices related by an axis of 2-fold rotational pseudosymmetry. In the crystals, the PSI is found as a trimeric complex, which is readily isolated after detergent solubilization of cyanobacterial membranes (8). Although these trimeric complexes have been observed in reconstituted proteoliposomes at high protein:lipid ratios and under high salt conditions (8), they have so far not been convincingly detected in native cyanobacterial membranes, where only monomeric particles have been observed (11) to date. It therefore remains a strong possibility that, like the higher plant situation, cyanobacterial PSI is normally monomeric \textit{in vivo}, and that unlike the higher plant equivalent, it oligomerises \textit{in vitro} upon detergent solubilization of the membranes. A subunit present in both the cyanobacterial PSI and higher plant PSI (the \textit{psa L} gene product) has been implicated in the oligomerization of the cyanobacterial complex into trimers (16) and has therefore been assigned to a position at the center of the trimeric complex (14, 15, 17). The location of this protein in the higher plant PSI complex will thus be of particular interest since oligomerization of the higher plant complex does not appear to occur.

The light-harvesting system of higher plant PSI consists of an evolutionarily conserved, core light-harvesting system containing about 100 Chl \(\alpha\) molecules that can possibly be modeled on the cyanobacterial PSI complex. However in higher plants, but not in cyanobacteria, this core is surrounded by several evolutionarily divergent peripheral light-harvesting proteins (LHCl proteins) which bind an additional 100–150 Chl \(\alpha\) and \(\delta\) pigments. The size of this peripheral antenna is controlled by environmental factors, such as the intensity and spectral composition of the incident light energy and may also be modulated by the location of the PSI complex in the thylakoid membrane.
Photosystem I Three-dimensional Structure

FIG. 2. Projection maps of the higher plant PSI unit cell in untilted crystals and applying (a) p2 or (b) p22.2, symmetry. Unit cell dimensions are \( a = 26.6 \text{ nm}, b = 27.7 \text{ nm}, \gamma = 90^\circ \). The maps were contoured in evenly spaced steps beginning at \( 0.5 \times \text{r.m.s. deviation} \) above the mean density level. There are four PSI complexes in the unit cell, each complex measuring approximately 15–16 nm in length and 11–12 nm across. A central ridge of density runs across the complex, almost perpendicular to its longest axis.

In addition, it is thought that higher plant PSI can associate with phosphorylated LHCl polypeptides migrating from PSII in a reversible manner, in a process that is under redox control (2). At least four different LHCl polypeptides have been identified (22–25 kDa), present in about eight copies per PSI complex (17), thus forming a sizeable proportion (–50%) of the total mass of the higher plant PSI complex.

In addition to the LHCl polypeptides (which are completely absent in cyanobacteria), higher plant PSI contains other polypeptides not found in the cyanobacterial PSI preparations.

These include the products of the \( \text{psa} \, G \) and \( H \) genes as well as the \( \text{psa} \, N \) gene product. All three polypeptides are small, in the region of 9–11 kDa, and one of these (the \( \text{psa} \, H \) gene product) seems to be an extrinsic protein. Higher plants apparently lack only one of the cyanobacterial PSI proteins, the \( \text{psa} \, M \) product (–4 kDa). The significance of the differences in polypeptide composition between plants and cyanobacteria is not well understood; indeed the roles of most of the PSI subunits in either plants or cyanobacterial are still unknown (17). There may even be different roles for the same polypeptide. For example, the \( \text{psa} \, F \) gene product in plants appears to have a role in binding the soluble electron donor protein plastocyanin on the luminal side of the complex (31). In contrast, this function for \( \text{psa} \, F \) has not been found in cyanobacteria.

Given the wealth of structural detail available for the cyanobacterial PSI complex, a three-dimensional structural study of the higher plant version can be considered to be long overdue. The use of rapid in situ two-dimensional crystallization combined with electron crystallography has now allowed an alternative approach to be developed. It is now possible to judge the degree to which the cyanobacterial structure is applicable to the plant PSI complex and we can begin to explore the extent of the structural evolution of the higher plant complex that has given rise to its unique light-harvesting system and environment in the thylakoid membrane. In this article, we present the three-dimensional structure of the higher plant complex and discuss its relationship to the cyanobacterial PSI reaction center complex.

EXPERIMENTAL PROCEDURES

Spinach (Spinacia oleracea) PSII-enriched grana membranes (BBYs) were prepared as described previously (6). Chlorophyll concentrations were determined as described by Arnon (19). Crystal containing PSII-enriched grana membranes were prepared by a two-step incubation procedure. Thylakoid membranes were left to stack in the dark for 2 h; the chlorophyll concentration adjusted to 2 mg ml\(^{-1}\) and Triton X-100 added for a detergent to chlorophyll ratio of 13.75:1 (w/w) and incubated in the dark for 20 min at room temperature (20 °C). The sample was then centrifuged (4 °C) at 13,000 \( \times \) g and the pellet was resuspended in Buffer A (20 mM MES/NaOH (pH 6.3), 5 mM MgCl\(_2\), 15 mM NaCl) at 4 °C. Chlorophyll concentrations were then adjusted to 2 mg ml\(^{-1}\) for a second incubation step (20 °C) with a detergent to chlorophyll ratio of 5:1 (w/w) in the dark for 20 min. The sample was then centrifuged (4 °C) at 13,000 \( \times \) g for 5 min, and the pellet was resuspended in cold Buffer A (4 °C). An aliquot was taken and diluted to a suitable concentration (20–30 pg Chl ml\(^{-1}\)) for electron microscopic studies. Membrane preparations were assayed for PSI content by measuring the chlorophyll \( a:b \) ratio and the extent of P700 oxidation as described previously (6).

Samples were negatively stained as described before (6) and examined in a Phillips 400 or CM10 transmission electron microscope operated at an acceleration voltage of 100 keV (18). Electron micrographs were recorded at calibrated magnifications of \( \times 27,500 \) and \( \times 38,450 \) on Kodak electron microscope film (ESTAR Thick Base 4489) and digitized for a pixel size of 0.78 or 0.74 nm at the specimen level. The digitized images of tilted crystalline areas were selected and analyzed using the PC-based CRISP and TRIMERGE image-processing packages (20). For each Fourier transform, amplitudes and phases were extracted after refinement of the reciprocal lattice parameters. A similar procedure was applied to the image of the untilted crystal to allow the determination of the tilt axis relative to the crystallographic axes and to determine the tilt angle. A resolution cutoff of 2.5 nm was applied, before individual data sets were merged for calculation of the three-dimensional map. Because the crystals were relatively small, each set of structure factors was noisy, and there was the possibility of ambiguity in the discrimination of the correct phase origin from one located at \( (\pi, -\pi) \) (28). This problem was addressed by comparing average interimage phase residuals for the two possible origins using initially only reflections where \( (h + k) = \text{odd} \), which are reflections which will have different phases for the two alternative phase origins. The phase origin associated with the lowest mean interimage phase residuals was contoured in evenly spaced steps beginning at 0.5 \times \text{r.m.s. deviation} above the mean density level. There are four PSI complexes in the unit cell, each complex measuring approximately 15–16 nm in length and 11–12 nm across. A central ridge of density runs across the complex, almost perpendicular to its longest axis.

In addition, it is thought that higher plant PSI can associate with phosphorylated LHCl polypeptides migrating from PSII in a reversible manner, in a process that is under redox control (2). At least four different LHCl polypeptides have been identified (22–25 kDa), present in about eight copies per PSI complex (17), thus forming a sizeable proportion (–50%) of the total mass of the higher plant PSI complex.

In addition to the LHCl polypeptides (which are completely absent in cyanobacteria), higher plant PSI contains other polypeptides not found in the cyanobacterial PSI preparations.
Discrimination between the two alternative phase origins was particularly important for low tilt angle data, where the phases approximate to 0 or π. For all lattice lines, smooth curves fitted to the data are sampled at 1/16 nm⁻¹ in order to extract amplitude and phase for reciprocal lattice positions along z*.

RESULTS

Fig. 1a shows a typical crystalline area attached to the periphery of a grana membrane, and in Fig. 1b, the same area is shown tilted at an angle of 40°. In preparations, such crystals could be observed at the edges of most of the grana membranes and were found to be of variable size. The crystals shown in the figure are of average dimensions, and the largest crystals observed were about twice the area of the larger crystal shown.

The effect of differential negative staining of the p22 121 complexes in the unit cell have a higher maximum density and are much better stained than the other two.

Fig. 3 shows data for phase and amplitude along two lattice lines for (h, k) (2, –2) and (2, −5). The scatter of phases around the line of best fit is reasonable for both lattice lines, although the scatter is somewhat worse for the higher resolution reflection. It can be seen that sufficient data has been collected for both reflections to allow a reasonably good extrapolation of the phases to about 4-nm resolution along z*. In contrast, the variation of amplitudes along the same lattice lines is quite large. The effect of less well defined amplitudes compared with phases on the final calculated three-dimensional structure is to make the positions of domains well defined, while the density of the domains is much less accurately defined. Thus in interpreting the three-dimensional map, the relative positions of domains may be relied upon, but any discussion of their relative sizes (when contoured similarly) will be less reliable.

Three-dimensional representations of higher plant PSI and the PSI unit cell are shown in Figs. 4 and 5. In Fig. 4, sections through the three-dimensional map are shown that are parallel to the crystal plane, starting at the lower surface of the crystal (Fig. 4a) and moving in 1.3-nm equidistant steps to the upper face of the crystal (Fig. 4f). Each section is contoured to indicate areas of increasing protein density, with the first contour at 0.5 times r.m.s. deviation above the mean density level. The lower sections (Fig. 4, a–c) are more deeply stain-embedded and hence the higher contrast gives higher apparent density. In these sections, two complexes in the unit cell show a stromal face, while the other two complexes show the lumenal face. The stromal face is characterized by a ridge that is roughly L-shaped (section a), being composed of two elliptical domains at right angles to each other, and a third domain positioned at the apex of the “L.” Only weak density can be found for the lumenal face in section a, suggesting that the stromal face of the complex extends much further than the lumenal face (see also Fig. 5). In subsequent sections (b, c), the stromal side is characterized by a very strong central domain with a density maxima directly underneath and between the two larger elliptical domains of the L-shaped ridge. In these sections, density for the lumenal face becomes apparent, formed by two domains separated by a shallow central pit. In the central section (d), the double-lobed shape of this domain begins to be replaced by a
single off-center domain, while the strong central domain of the stromal side has become a relatively featureless density. Moving toward the opposite side of the crystal, one would expect to see the reappearance of the double-lobed feature and the central ridge, and these features should be apparent in the oppositely oriented molecules (i.e. the ones flipped with respect to the membrane plane. Thus, where a stromal ridge was apparent, there should now be a double-lobed shape, and vice versa.

To a certain degree, this expectation is confirmed in the upper sections (e) and (f); however, the stain on this side of the crystal is considerably shallower, and thus the level of noise in these low contrast sections may well be too high for a detailed analysis of the structure. It is therefore opportune that both sides of the PSI complex are exposed on the lower, better stained side of the crystal.

In Fig. 5, the three-dimensional structure of the PSI complex is depicted using surface rendering and simulated illumination to give the impression of a solid object. In panels a and b, only the nearest part the complex is viewed from the better stained side of the crystal, and for clarity, the rest of the crystal (more distant from the observer in this case) is removed from the representation. The L-shaped stromal ridge and the double-lobed lumenal surface are apparent. Note that the handedness of these features is reversed compared with Fig. 4, because the viewing direction is now from the opposite side of the crystal.

When rotated through 90° and viewed from a direction parallel to the membrane plane (panel c), the complex shows a thickness of about 8 nm, with the stromal ridge comprising about 2.5 nm of this distance. In comparison, the lumenal side of the complex seems to project to only about half the height of the stromal ridge, explaining why the lumenal surface is almost absent in the lowermost section of the third-dimensional map in Fig. 4 (section a). The parallel white lines in c represent the expected limits of a 4.5-nm thick lipid bilayer.

**DISCUSSION**

The higher plant PSI complex has been the subject of much biochemical and genetic investigation, and much is now known
about its polypeptide subunits and electron transfer components (1, 17, 29). In contrast, relatively little has been learned about the three-dimensional structure of higher plant PSI and how these subunits come together in the complex. In the absence of such information, it has been necessary to rely on comparisons with the cyanobacterial PSI complex, for which excellent structural data are available (14, 15). Such comparisons, though, have inevitably been of limited application since, as discussed earlier, the higher plant complex contains several additional features that make it larger and more complicated than the cyanobacterial version. Fortunately, it seems very likely that the conserved reaction center core of the complex will show few structural variations; there is a high (≥80%) amino acid sequence homology between the reaction center subunits Psa-A and Psa-B from plants and cyanobacteria (21).

Therefore, as an initial exercise in modeling the higher plant PSI complex, we have attempted to correlate the cyanobacterial reaction center structure with the molecular envelope of the higher plant PSI complex in negative stain. Such an exercise may highlight significant differences between the two systems and point to possible structural explanations for the differing behavior of the cyanobacterial and plant complexes. In Fig. 6, schematic drawings of the smaller cyanobacterial PSI complex (right-hand side) and the larger higher plant PSI complex (left) are displayed in top and side views as well as a space-filling representations. The approximate predicted positions of some of the PSI subunits are highlighted using the letters that are used for their nomenclature.

The overall shapes of the two complexes are similar as shown in the lower panel in Fig. 6, although the higher plant complex is slightly larger (except in the dimension orthogonal to the membrane). The dimensions for the higher plant complex (15 × 12.5 × 8 nm) shown in Fig. 5 may be an underestimate, as it is feasible that subunits that protrude only to a minor extent from the lipid bilayer may have escaped staining. It is known that several PSI subunits, including the LHCl polypeptides, fall into this category (1, 17). Further electron crystallography studies on unstained crystals will be needed in order to resolve this question.

The extrinsically bound stromal subunits in PSI (Psa-C, -D, and -E) appear to be arranged close together on the stromal surface of the large Psa-A and -B subunits. By comparison with the cyanobacterial arrangement, we predict that the Psa-C subunit will be located in the central domain of the ridge, while the Psa-D and -E subunits will flank it. There is no evidence from the structural data that these subunits stack on top of each other. Electron transfer from the iron-sulfur centers of subunit Psa-C to the soluble electron acceptor protein, ferredoxin, must involve the binding of ferredoxin in very close proximity to Psa-C, although it is known that this binding appears to be mediated by the Psa-D and -E subunits (22, 23). The structural basis of this binding is not yet clear, although some studies of ferredoxin binding have been carried out using electron microscopy of the cyanobacterial complex (24).

In the cyanobacterial PSI structure (15), the location of the Psa-L subunit has been predicted to lie close to the 3-fold axis of the trimers that are found in the crystals (Fig. 5). These predictions are based on the observation that mutants lacking this subunit have fully functional PSI, but no longer form trimeric complexes after detergent solubilization (25). However, higher plant PSI complexes contain the Psa-L subunit, but do not form trimeric complexes, which suggests that
Psa-L may have a function that is unrelated to the native oligomeric form of the complex. Indeed, it has been proposed that the trimeric cyanobacterial complex may be a product of detergent solubilization (8, 11), and structural evidence for the existence of the trimer in native cyanobacterial membranes is scant (11, 27, 28) while the observation of the monomeric form has been reported in cyanobacterial thylakoids and in reconstituted membranes (8, 11, 27). In the higher plant PSI structure, the overall shape of the complex is rather similar to the cyanobacterial form, and both show the apical domain that is predicted to be the Psa-L location. There is no obvious structural feature in the higher plant complex that would prevent trimerization around this apical domain, and the two sides of the complex that straddle the apical domain subtend an angle of about 120° (Fig. 6), which would allow packing into a trimeric complex.

The luminal side of PSI in cyanobacteria has been shown to project only about 1 nm into the aqueous phase, and in some reports, the presence of a small central depression has been observed (8, 9). This feature is also present in the higher plant PSI structure, where it is found to be approximately 3 nm in diameter and about 0.5–1.0 nm in depth. The depression is partly flanked by two domains that may be formed from luminally exposed loops of the integral membrane proteins Psa-A and -B. In order to transfer its electron to the oxidized primary donor of PSI (P700⁺), plastocyanin must bind somewhere very close to the pseudo-2-fold axis of rotational symmetry on the luminal face of the PSI complex. This binding location would therefore be close to the center of the luminal depression. In higher plants the subunit Psa-F has been reported to be involved in mediating the binding of the soluble electron donor protein plastocyanin to PSI (29, 31), although there is no supporting evidence for this role in cyanobacterial studies (32). There is no obvious domain in the three-dimensional map that could immediately be assigned to Psa-F, and further studies are required to address this.

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REFERENCES

1. Bryant, D. A. (1991) in Current Topics in Photosynthesis (Barber, J., ed) Vol. 11, pp 1–27, Elsevier, Amsterdam.
2. Anderson, B., and Anderson, J. M. (1980) Biochim. Biophys. Acta 692, 427–440.
3. Anderson, E., and Albertsson, P.-A. (1993) Biochim. Biophys. Acta 1141, 175–182.
4. Dunahay, Y., and Staehelin, L. A. (1985) Plant Physiol. 78, 606–613.
5. Boekema, E. J., Wynn, R. M., and Malkin, R. (1990) Biochim. Biophys. Acta 1017, 49–56.
6. Kitmitto, A., Holzenburg, A., and Ford, R. C. (1997) J. Biol. Chem. 272, 19497–19501.
7. Wollenberger, P. A., Stefansson, H., Yu, S. G., and Albertsson, P.-A. (1994) Biochim. Biophys. Acta 1184, 93–102.
8. Ford, R. C., Hefi, A., and Engel, A. (1990) EMBO J. 9, 3067–3075.
9. Böttcher, B., Graber, P., and Boekema, E. J. (1992) Biochim. Biophys. Acta 1100, 125–136.
10. Karrasch, S., Tölke, D., Walz, T., Miller, M., Tsotis, G., and Engel, A. (1996) J. Mol. Biol. 262, 336–348.
11. Hefi, A., Ford, R. C., Miller, M., Cox, R. P., and Engel, A. (1992) FEBS Lett. 296, 29–32.
12. Ford, R. C., Picot, D., and Garavito, R. M. (1987) J. Mol. Biol. 194, 427–440.
13. Witt, I., Witt, H. T., Di Fiore, D., Roegner, M., Hinrichs, W., Saenger, W., and Engel, A. (1996) Biochim. Biophys. Acta 1100, 125–136.
14. Krauss, N., Schubert, W. D., Klukas, O., Fromme, P., Witt, H. T., and Saenger, W. (1996) J. Mol. Biol. 262, 336–348.
15. Witt, I., Witt, H. T., Di Fiore, D., Roegner, M., Hinrichs, W., Saenger, W., and Engel, A. (1996) FEBS Lett. 296, 29–32.
16. Ford, R. C., Picot, D., and Garavito, R. M. (1987) J. Mol. Biol. 194, 427–440.
17. Witt, I., Witt, H. T., Di Fiore, D., Roegner, M., Hinrichs, W., Saenger, W., and Engel, A. (1996) Biochim. Biophys. Acta 1100, 125–136.
18. Karrasch, S., Tölke, D., Walz, T., Miller, M., Tsotis, G., and Engel, A. (1996) J. Mol. Biol. 262, 336–348.
19. Arnon, D. I. (1949) Plant Physiol. 24, 1–15.

FIG. 6. Schematic representations of the structures of higher plant (left) and cyanobacterial (right) PSI, as determined by electron crystallography and x-ray crystallography, respectively. For the cyanobacterial complex, the representations are based on data described in Schubert et al. (15). The locations for various PSI subunits are also shown, as proposed in Schubert et al. (15), where the letters denote the subunit. In the upper panel, the schematic views are from a direction above the stromal side of the complex, while in the middle panel, the viewing direction is from along the center of the lipid bilayer along the longest axis of the complex. For the higher plant PSI complex (left-hand side), the outlines show the molecular envelope for the strongly stained part of the unit cell, and in the upper panel, the relative locations of the three densities that form the L-shaped stromal ridge are displayed using a line at about 2 × r.m.s. deviation above the mean density. The dashed line in the upper and middle panels denotes the expected position of the transmembrane region, which is known to be poorly stained, and hence may be under-represented in the structures presented in this study. In the lower panel, drawings representing space-filling models of the plant and cyanobacterial complexes are shown from a stromal viewpoint above the membrane plane. The stromal ridge appears to be extended in the higher plant complex by two additional domains around the putative location of subunit Psa-D.
20. Hovmoller, S. (1992) *Ultramicroscopy* 41, 121–135
21. Fish, L., Kuck, U., and Bogorad, L. (1985) *J. Biol. Chem.* 260, 1413–1421
22. Lagoutte, B., and Vallon, O. (1992) *Eur. J. Biochem.* 205, 1175–1185
23. Andersson, B., Koch, B., and Scheller, H. V. (1992) *Physiol. Plant.* 84, 154–161
24. Lelong, C., Boekema, E. J., Krupa, J., Bottin, H., Rogner, M., and Setif, P. (1996) *EMBO J.* 15, 2160–2168
25. Chitnis, V. P., and Chitnis, P. R. (1993) *FEBS Lett.* 336, 330–334
26. Deleted in proof
27. Ford, R. C., and Holzenburg, A. (1988) *EMBO J.* 7, 2287–2293
28. Hladik, J., and Sofrova, D. (1991) *Photosynth. Res.* 29, 171–175
29. Zilber, A., and Malkin, R. (1992) *Plant Physiol.* 99, 901–911
30. CCP4: Collaborative Computational Project No. 4 (1994) *Acta Crystallog. Sec. D* 50, 760–763
31. Max-Wynn, R., and Malkin, R. (1988) *Biochemistry* 27, 5863–5869
32. Fromme, P., Schubert, W. D., and Krauss, N. (1994) *Biochim. Biophys. Acta* 1141, 175–182