An improved electron density map of photosystem I (PSI) calculated at 4-Å resolution yields a more detailed structural model of the stromal subunits PsaC, PsaD, and PsaE than previously reported. The NMR structure of the subunit PsaE of PSI from *Synechococcus* sp. PCC7002 (Falzone, C. J., Kao, Y.-H., Zhao, J., Bryant, D. A., and Lecomte, J. T. J. (1994) Biochemistry 33, 6052–6062) has been used as a model to interpret the region of the electron density map corresponding to this subunit. The spatial orientation with respect to other subunits is described as well as the possible interactions between the stromal subunits. A first model of PsaD consisting of a four-stranded β-sheet and an α-helix is suggested, indicating that this subunit partly shields PsaC from the stromal side. In addition to the improvements on the stromal subunits, the structural model of the membrane-integral region of PSI is also extended. The current electron density map allows the identification of the N and C termini of the subunits PsaA and PsaB. The 11-transmembrane α-helices of these subunits can now be assigned uniquely to the hydrophobic segments identified by hydrophobicity analyses.

In cyanobacteria, green algae, and higher plants, photosystem I (PSI) forms part of the photosynthetic apparatus of oxygenic photosynthesis. It is a multisubunit protein complex located in the thylakoid membrane. In cyanobacteria, PSI consists of 11 subunits, whose nomenclature (PsaA to PsaF and PsaM) has been derived from the encoding genes *psaA* to *psaF* and *psaI* to *psaM*. Most of the subunits are membrane-integral. The large subunits PsaA and PsaB coordinate the majority of cofactors both of the electron transfer system and of the antenna system. The remaining antenna chlorophylls are bound by the smaller membrane-integral subunits. Some of these subunits have additional specialized functions. Thus, for example, PsaL and PsaI are responsible for the formation of PSI-trimers, while PsaF and PsaJ stabilize PsaE in the PSI complex (1, 2).

PsaC, PsaD, and PsaE are non-membrane-integral, extrinsic subunits that may be removed from the membrane-integral core complex by chaotropic reagents (1). Combinations of electron microscopy studies (3, 4), cross-linking experiments (5, 6), and x-ray crystallographic investigations (7–9) have led to progressively more detailed models of the stromal ridge, showing these subunits to be in close neighborhood to each other.

Only in the case of PsaC could an x-ray structural model be suggested based on a previous electron density map (8–10). The model was derived from the x-ray structure of a 2Fe4S4-[ferredoxin from *Peptostreptococcus asaccharolyticus*](11). Due to the high degree of 2-fold rotational symmetry inherent to the core of PsaC, a 2-fold ambiguity regarding its orientation in the PSI complex remained (8–10). In contrast to PsaC, the characteristic structural features of PsaE could unexpectedly not be located in the previous electron density map. The NMR structure of PsaE had revealed a five-stranded β-barrel forming the core of this subunit (12).

No structural model has previously been proposed for PsaD. CD- and NMR-spectroscopical investigations indicate that a central portion of the PsaD sequence is folded into a β-sheet, whereas the N- and C-terminal regions are mobile (13). An assignment of the transmembrane α-helices of the x-ray structural model to the membrane-integral subunits of PSI has been suggested (8, 9). Regarding the connectivity of the subunits PsaA/PsaB, some unobserved loops prevented an unambiguous assignment of individual α-helices to the primary structure. This was especially true of the N-terminal, antenna-binding domains of PsaA and PsaB.

Here we confirm the NMR structure of PsaE and describe its position and orientation with respect to the PSI complex. A first structural model of PsaD is presented. It indicates that PsaD partly covers PsaC stromally in a clasplike manner. PsaD thereby approaches and interacts with PsaE. The orientation of the subunit PsaC is discussed in relation to recent biochemical and spectroscopic experiments. A unique assignment of the α-helices of PsaA/PsaB is discussed.

**EXPERIMENTAL PROCEDURES**

**Protein Purification and Crystallization**—Photosystem I was isolated and purified from the thermophilic cyanobacterium *Synechococcus elongatus*; crystals were grown by microdialysis by lowering the salt con-
Photosystem I: The Stromal Subunits

X-ray data sets used for the calculation of the new electron density map

| Derivative | Resolution (maximum) | $R_{	ext{sym}}$ | Average redundancy | No. of reflections with $I > 3\sigma$ | Completeness |
|------------|----------------------|-----------------|-------------------|--------------------------------------|--------------|
| Native     | 3.5                  | 10.7            | 4.8               | 88.6                                 | 93.4         |
| Hg1        | 3.8                  | 10.2            | 3.8               | 77.1                                 | 83.4         |
| Hg2        | 3.8                  | 9.6             | 2.9               | 83.7                                 | 91.8         |
| Pt         | 3.6                  | 6.7             | 3.2               | 78.5                                 | 86.5         |
| U          | 4.1                  | 11.9            | 3.6               | 92.5                                 | 93.5         |
| Xe         | 4.6                  | 11.5            | 2.5               | 65.5                                 | 75.7         |

$a$ $R_{	ext{sym}} = \sum \frac{\sigma(I(h)) - \langle I(h) \rangle}{\sigma(I(h))}$, where $I(h)$ are individual intensities of any reflection and $\langle I(h) \rangle$ is the mean intensity of this reflection.

$b$ Obtained by co-crystallization of PSI with 1 mM sodium-2-ethylmercurithiosalicylate.

Electron density maps calculated at various levels of resolution and cross-phased isomorphous difference Fourier syntheses, were refined using the standard modus suggested for SHARP. The relatively low resolution of the available data prevents heavy atom temperature factors from being refined. The heavy atom model was improved by inspecting residual log likelihood gradient electron density maps (18). They permit correctly and incorrectly identified positions to be distinguished and indicate possible new sites. This procedure was repeated cyclically until convergence was achieved.

The resulting phases were further improved by the solvent flattening procedure as implemented in the program SOLOMON (17, 19). The phase information introduced in MLPHARE produced a better multiple isomorphous replacement electron density map than the current version of SHARP. The quantity of data, the isotropic temperature factor of all heavy atom sites could not be refined. This resulted from phasing with MLPHARE using heavy atom derivatives, is generally of poorer quality than the best map with phases obtained from SHARP using five heavy atom derivatives, and generally remained consistent with the newly optimized model. The earlier heavy atom sites nevertheless generally lead to the formation of multiple heavy atom sites, which is crucial in obtaining optimal phase angles by the SHARP method.

Previously, major heavy atom sites were identified from isomorphous difference Patterson maps. Additional minor sites were detected from cross-phased isomorphous difference Fourier maps. The occupancies and coordinates of these sites were optimized using vector space refinement techniques as implemented in VECREF (17). Due to the low resolution, temperature factors of the heavy atom sites could not be refined. This heavy atom model was incorporated into MLPHARE (21) for phase calculation. Further refinement of the heavy atom parameters could not be achieved, since occupancy optimization resulted in large parameter shifts without convergence. The calculated electron density maps were not of optimal quality.

Small improvements were achieved by origin correlation refinement along the c-axis between heavy atom derivatives using the program HEAVY (22, 23) and reintroducing the refined parameters into MLPHARE for phasing. (See Fig. 1 for the mean figures of merit (FOM) for acentric (black dots) and centric (white) reflections before (continuous lines) and after (dashed lines) refinement.) This procedure is seen to slightly increase the mean FOM in the case of the acentric reflections in the medium resolution shells and somewhat more in the low resolution shells. The latter results in an improved final electron density map after solvent flattening as low resolution terms strongly influence the correctness of the mask determined from the initial MIracis electron density map. The described procedures, VECREF/MLPHARE-DM and VECREF/HEAVY/MLPHARE-DM, produce electron density maps with strong radial quality variations, the highest quality coinciding with the center of the PSI monomer. This causes the difficulties encountered in interpreting the stromal and luminal sections of PSI as well as the peripheral membrane integral regions (9).

The most significant improvement in the quality of the electron density map was, however, achieved by introducing the programs SHARP (18) for refinement of heavy atom parameters and phase calculation and SOLOMON (19) for density modification including solvent flattening. Starting with the existing heavy atom model determined from isomorphous difference Patterson maps, the analysis of the residual log likelihood gradient maps increased the number of minor sites for each derivative used. The earlier heavy atom sites nevertheless generally remained consistent with the newly optimized models (see Table II). Due to the low resolution of the diffraction data, the isotropic temperature factor of all heavy atom sites was fixed at $B = 30 \AA^2$.

By visual judgment, the electron density map, calculated with phases obtained from SHARP using five heavy atom derivatives, is generally of poorer quality than the best map resulting from phasing with MLPHARE using heavy atom models for 10 derivatives. The difference in the number of derivatives used is based on program-inherent parameter limitations in the current version of SHARP. The quantity of information introduced in MLPHARE produced a better multiple isomorphous replacement electron density map than the more accurately defined heavy atom parameters of the limited selection of derivatives used in the SHARP refinement (see Fig. 1 and Table II). The nominal mean FOM-values for both centric and acentric reflections reported by SHARP are significantly smaller than the ones obtained using MLPHARE.
lower than those from MLPHARE, although this may be due to small differences in FOM definitions. Furthermore, SHARP determines similar average FOM values for both centric and acentric reflections, whereas centric reflections have significantly better values as reported by MLPHARE. On the other hand, comparing phasing statistics for individual derivatives indicates an overall improvement for each derivative (higher phasing power and lower $R_{\text{cullis}}$ values) during refinement by SHARP, except, that is, for the platinum derivative. This confirms the improvement of the heavy atom models by using SHARP, whereas the lower quality of the MIRAS phases calculated with SHARP seems simply to be due to the smaller number of derivatives used.

None of the MIRAS electron density maps allow PsaE or the second phylloquinone molecule (two previously undetected structural motifs) to be identified (Fig. 2). Comparing the SHARP and MLPHARE electron density maps, the $\alpha$-helices in the former appear more detailed but less continuous, whereas the MLPHARE maps reveal more connected electron density stretches.

The situation is dramatically reversed following solvent flattening by SOLOMON (19). The electron density map obtained by combination of MLPHARE and SOLOMON is of higher quality than that obtained with MLPHARE and DM (data not shown). Consequently, SOLOMON was used for all further solvent flattening procedures. Fig. 1, b and c, depicts the FOM and mean phase differences following solvent flattening for three procedures combining VECREF/HEAVY/MLPHARE-DM, VECREF/HEAVY/MLPHARE-SOLOMON, and SHARP-SOLOMON.

Clearly, the combination SHARP-SOLOMON provided the best phases, followed by VECREF/HEAVY/MLPHARE-SOLOMON. Presumably, the more elaborately defined phase probability distributions of SHARP allow for optimal combination of phase information during solvent flattening procedures, leading finally to more accurately defined phases.

Furthermore, little or no additional information is made available by phase extension to between 4.0 and 3.5 Å. The limit of resolution was therefore taken to be 4 Å. To avoid being misled by statistics, the subjective visual impression (compactness and continuity) of each electron density map was taken as the final quality criterion. An example of the variations in the quality of the electron density maps is given in Fig. 2, which shows identical sections of differently calculated electron density maps corresponding to the subunit PsaE.

**Location of the Three Stromal Subunits of Photosystem I—**PSI possesses three small stromal subunits, PsaC, PsaD, and PsaE of molecular masses 8.7, 15.2, and 8.3 kDa in *S. elongatus*, respectively (24). Together, they form a stromal ridge that extends ~30 Å beyond the membrane-integral regions (Fig. 3a), as first revealed by electron microscopy studies (3). PsaC is positioned such that the 2-fold pseudorotation axis $C_2(C)$, which relates PsaA and PsaB, intersects the local 2-fold axis $C_2(A)$ of PsaC at an angle of 62° (cf. Fig. 6). PsaE is located adjacent to PsaC, facing away from the trimeric axis $C_3$, whereas PsaD faces toward this axis (9). In projection onto the a,b-plane, connecting vectors between the approximate “centers of mass” (COM) of PsaD, PsaC, and PsaE from an obtuse angle of ~160° (Fig. 3a); the angle between vectors $C_2(C)$ and $C_2(A)$ of PsaD, $C_2(A)$ and PsaE are approximately 45° (155°). In this projection, PsaD, PsaC, and PsaE are located ~22 and ~26 Å from PsaC, Together with some loops from membrane-integral subunits, the three stromal subunits form a wide cavity, the docking site for ferredoxin and flavodoxin, as

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**TABLE II**

| No. of sites | No. of common sites | $R_{\text{cullis}}$ | Phasing power | $R_{\text{cullis}}$ |
|-------------|---------------------|---------------------|---------------|---------------------|
|            |                     | $\%$                | $\%$          | $\%$                |
| Hg$^{1+}$  | 5 (NA)              | 12.7                | 1.22 (NA)     | 0.83 (NA)           |
| Hg$^{2+}$  | 5 (6)               | 9 21.8              | 0.92 (0.90)   | 0.93 (0.87)         |
| Pt         | 12 (9)              | 14.5                | 0.92 (0.79)   | 0.89 (0.97)         |
| U          | 12 (6)              | 5 5.6               | 1.08 (0.69)   | 0.91 (0.97)         |
| Xe         | 14 (8)              | 8                   | 0.92 (0.87)   | 0.89 (0.97)         |

* The current number of sites is shown; number of sites according to previous model (9) is shown in parentheses.
* $R_{\text{cullis}} = \sum_{h|F(h)}|F(h)^{\text{der}} - F(h)^{\text{nat}}|/\sum_{h|F(h)}|F(h)^{\text{der}}|$, where $F(h)^{\text{der}}$ and $F(h)^{\text{nat}}$ are derivative and native structure factor amplitudes.
* Previous values are shown in parentheses. Phasing power was determined by the programs SHARP (18) (current values) and MLPHARE (21) (previous values).
* Anomalous scattering data included in the calculation of phases.
* NA, not applicable.

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**FIG. 1. Distribution of FOM.** a. MIRAS statistics. Distribution of mean FOM versus resolution for the procedures described in determining the heavy atom model. b. Distribution of mean FOM. c. Estimated errors in the phase angle versus resolution ($\Delta$ Phi represents mean phase angle difference between MIRAS phases and phases obtained after density modification).
first suggested by Fromme et al. (25) and later confirmed by combined cross-linking and electron microscopy studies (26, 27) (Fig. 3c).

Orientation of PsaE in the PSI Complex—In all previously calculated electron density maps of PSI, the precise positioning of the subunit PsaE proved elusive. The region presumed to be occupied by PsaE had been located, but an interpretation as in the case of PsaC was not possible. The current electron density map (see Figs. 2d and 5a) clearly reveals the five-stranded β-barrel of the PsaE core as well as an extended loop region directed toward the PSI core.

Initially, a representative NMR model of PsaE (12) was visually fitted into the electron density map. The structural model of PsaE from Synechococcus sp. strain PCC7002 was then modified to reflect the sequence of S. elongatus (see Fig. 4). Following Falzone et al. (12), the β-strands are referred to as βA to βE and the connecting loops as AB to DE, Fig. 5b. An additional proline was introduced into the loop BC and a glutamine into loop DE, slightly elongating these loops. Except for the C-terminal end of βE (Val 68 has been substituted by an alanine), all remaining substitutions are not located in the β-barrel, nor do they disturb the length of the loops. The C-terminus of PsaE in S. elongatus and the loops BC and DE are longer than in Synechococcus sp. PCC7002; they have been modified as indicated by the electron density map (Fig. 5a).

Superimposing the NMR and x-ray model structures of the subunit PsaE reveals both to be almost identical in the core region (Fig. 5b). The long loop CD, as described in the NMR study (12), is observed to adopt a twisted conformation in the x-ray structural model directed away from the PsaE core in a finger-like manner. Although changes in the main conformation of the PsaE model are anticipated to be minimal, a shift in the amino acid positions by one or two is possible, although this remains uncertain, since individual amino acid side chains are not visible in the electron density map.

The position and orientation of PsaE within the PSI complex may be described as follows. The N- and C termini face the stroma and are directed away from the C3-axis toward the outer rim of the PSI trimer (Figs. 3 and 5). The loop AB is located close to the loop j′-k′ (−6.5 Å based on Cα positions), which links the α-helices j′ and k′ of one of the core subunits PsaA or PsaB (Fig. 3). It is similarly also near (−5.5 Å) the extension of a membrane-integral α-helix y1, not previously described (see below and Figs. 3b and 8), as well as being nearest PsaD (−6.5 Å, Fig. 3b). The loop BC stromally forms a large part of the surface of PsaE and is involved in contacts (−4.5 Å) to the central loop of PsaC, which links the two Fe4S4 cluster binding domains in this subunit (Ref. 1; see Figs. 3c and 6).

The loop CD is the longest and most flexible in PsaE (12). Its tip is directed toward Fx, the shortest distance between the two being −11 Å. It is furthermore located near α-helices e and f of PsaA or PsaB, (−6 Å) as well as to the loop n-o, which connects the “horizontal” α-helix n and the C-terminal helix o (−5 Å) (see Fig. 3).

The β-strands βB and βC form part of the ferredoxin binding site (Fig. 3c). It is therefore likely that some amino acids of these strands interact with the water-soluble electron acceptors. The mutation of Arg68 to Gln (R39Q) at the C terminus of the strand βC is known to partly inhibit ferredoxin reduction (28), an observation that is corroborated by the structural model. The shortest distance between PsaE and the loop w-x and α-helix u (PsaF, PsaJ, and PsaM; see below) are −12 and −20 Å, respectively (Fig. 3).

The orientation of PsaE in the PSI complex described here is not entirely compatible with that previously suggested by
Rousseau et al. (29), who on the basis of fluorescein isothiocyanate-labeled thylakoid membranes and mutational arginine replacement analyses in *Synechocystis* PCC6803, had concluded that both N and C termini are buried within the PSI complex. Interestingly, only two PsaE polypeptides with triple mutations (K7N,K11E,R12A and R4A,R9N,R12A) and another one with a stop codon at the position of Leu65 were not recovered in the PSI complex, while single mutations affected neither binding nor ferredoxin reduction behavior (29). Clearly, only drastic changes among the first 12 N-terminal amino acid residues, possibly affecting the overall structure of PsaE, suffice to prevent its binding to the PSI complex. Fluorescein isothiocyanate labeling of accessible amino groups previously performed on membranes at pH 9.3 failed to probe the N-terminal Ala1 and the C-terminal Lys73. When the same reaction is conducted at pH 9.8, a pH where amino groups with an alkaline-shifted pK are more likely to react, the N-terminal Ala1 and all ε-amino groups of lysines become labeled, except that of Lys11, suggesting that Lys11 could be interacting with the PSI core.2 This observation is in agreement with our structural model, since Lys11 of PsaE from *Synechocystis* PCC6803 corresponds to the C-terminal residue of the loop AB, which is located close to the loop j’-k’ of PsaA or PsaB. Recent biochemical studies on PSI complexes from *Synechocystis* sp. PCC6802 using the endoproteinase Glu-C reveal the residues Glu63 and Glu67 near the C terminus of PsaE as the most likely cleavage sites for this protease (30). These data would also confirm our structural findings of a solvent-exposed C terminus.

A strongly conserved aromatic region in the loop AB of PsaE,

PsaD and PsaE are indicated by white dots; for PsaC, it coincides with the symbol of the local 2-fold axis C2(AB). I, terminal extension of PsaD. It functions as a clasp for PsaC and is in close contact with PsaE. II, crystal contact surface of PsaE with the next trimer in the crystal lattice. III, loop CD of PsaE below PsaC. IV, binding site for ferredoxin/flavodoxin. b, the equivalent view as in a showing the observed secondary structure elements of PSI. The α-helices of the membrane-integral parts are drawn as cylinders and shaded gray. The stromal subunits PsaC, PsaD, and PsaE are depicted as coil models showing their secondary structure elements. In addition, a large loop region of PsaD involved in the contact between this subunit and the core of PSI is marked. c, model of the stromal ridge of PSI showing the secondary structure elements. The view is into the ferredoxin/flavodoxin binding pocket with the trimeric axis on the right. The stromal subunits PsaC, -D, and -E as well as the iron-sulfur cluster F430 are rendered in black. The protein backbone of the membrane-integral subunits (α-helices depicted as cylinders) are colored in gray. a was produced using Grasp (48); b and c were produced using BobScript (47).

![Diagram of Photosystem I: The Stromal Subunits](image-url)

**FIG. 3.** The stromal ridge of PSI shown from the stromal side onto the membrane plane. a, simulated surface of the stromal ridge. PsaC is depicted in yellow, PsaD in red, PsaE in blue, and membrane-integral subunits in white. The approximate centers of mass of subunits

**FIG. 4.** Sequence alignment of subunit PsaE from *Synechococcus* sp. Lines 1, PCC7002; lines 2, S. elongatus. The secondary structure element nomenclature (β-strands βA to βE) is taken from Ref. 12. Amino acids not conserved in both sequences are indicated by rectangular boxes.

2 B. Lagoutte, personal communication.
which contains a Tyr-Trp-Tyr triple, is thought to be involved in binding PsaC (12). This is supported by our structural model, where this loop faces PsaC, the protein backbones being ~7 Å apart. Replacement of the tyrosines by phenylalanine or of the tryptophan by alanine was not reported to affect the binding behavior of PsaE in the PSI complex (29). The x-ray structural model places the Tyr-Trp-Tyr triple close to j'-k' (core subunits), thereby indicating its possible importance in binding PsaE to the PSI core.

Apart from a suggested role in cyclic electron transfer (31, 32), PsaE has been implicated in stabilizing the stromal ridge of PSI (33) and is known to influence fast electron transfer between PsaC and ferredoxin (29). PsaD-less mutants show that PsaE binds to the PSI core in the absence of PsaD and that it appears to influence neither the binding of PsaC nor the photoreduction of the terminal iron-sulfur clusters F_A and F_B (45). In fact, however, the x-ray structural model reveals the loop CD of PsaE to be sandwiched between PsaC and the PSI core. Removing PsaE would therefore introduce an opening between PsaC and PsaA/PsaB, which could destabilize the correct binding of PsaC to the core. Three aliphatic residues (Val_43, Ala_49, and Leu_67/Val_68) in Synechococcus sp. PCC7002/S. elongatus in the loop CD of PsaE, which are not in contact with other amino acids within this subunit, probably interact with a hydrophobic surface area of PsaA/PsaB. Similarly, Tyr_45 and Tyr_48 (Phe_48 in Synechococcus sp. PCC7002), located in the loop CD of PsaE facing the PSI core, are possibly involved in binding PsaE to the core subunits. As noted previously, they are conserved, but no specific functional assignment could be made (12). Since the loop CD had to be remodeled in order to fit our electron density map, it is uncertain whether the orientations of these residues are similar to those in the original NMR structural model of PsaE. Consequently, the structural function of Tyr_45 and Tyr_48 presently remain open.

The Subunit PsaC—Following the identification of F_1 and F_2 in the electron density map (7) as well as additional structural elements belonging to the pseudosymmetrical core of PsaC, the orientation of PsaC within PSI was established, except that is, for a 2-fold ambiguity caused by the inherent pseudosymmetry of the PsaC core (8, 10). EPR experiments independently arrived at the same conclusion (34). The debate about the two orientations has been raging since, initial experiments favoring the orientation in which the N and C termini face the stroma and F_3 is the terminal electron acceptor (35). The first electron density map at 4 Å appeared to confirm this view, with a closed extended loop between the two symmetry-equivalent halves of PsaC being modeled near the interface between PsaC and the core subunits and an extended C terminus covering the subunit stromally (9). Recently, however, the opposite orientation (N and C termini on the interface between PsaC and the core subunits and F_B as the terminal cluster) has been gaining ground (36, 37).

Examining the improved electron density map indicates that the previous model cannot unreservedly be upheld. The electron density structure located close to the stromal end of the PsaC core, which was previously interpreted as the C-terminal extension of this subunit, now appears more realistically to belong to PsaD (see below and Fig. 6). Furthermore, electron density on the luminaly directed side of PsaC, which previously remained unidentified, is connected to the pseudosymmetrical core of PsaC in the improved electron density map and is seen to be at least compatible with a linear extension of the C terminus by 13–16 amino acid residues beyond the PsaC core. This would be in agreement with the C-terminal extension of PsaC by 14 residues as compared with the 2Fe_4S_4-ferredoxin from P. asaccharolyticus. Thus, the orientation in which the N and C termini face the membrane-integral subunits is now seen to be at least feasible. The only drawback to the interpretation of this orientation is that electron density that would accommodate the extended central loop insertion is connected to the PsaC core by weak electron density only, preventing a complete modeling of this part of PsaC. Overall, an orientation in which F_A is the proximal and F_B the distal iron-sulfur center would now appear most likely.

The PsaC Binding Surface of the PsaA/PsaB Heterodimer—Both PsaA and PsaB contain a strictly conserved F_X-binding motif CDGPGRRGGTCP, the two cysteines of which have been proposed to coordinate F_X (38). The intercysteine loops of this motif have been suggested to play a leading role in binding the extrinsic subunit PsaC (39, 40). In particular, one of the central arginines of the F_X-binding motifs (boldface R in the sequence motif) was postulated to form a salt bridge with an aspartate of PsaC (38). The intercysteine loops of this motif have been suggested to play a leading role in binding the extrinsic subunit PsaC (38). The intercysteine loops of this motif have been suggested to play a leading role in binding the extrinsic subunit PsaC (38). The intercysteine loops of this motif have been suggested to play a leading role in binding the extrinsic subunit PsaC (38).
remaining model, individual side chains cannot be identified directly in the electron density map and have consequently not been modeled.

Nevertheless, the structural model of the loop \( j-k \) indicates that (i) the central part of the conserved intercysteine \( F_X \)-binding loops of PsaA/PsaB containing the arginines, do indeed appear to interact with PsaC, while (ii) the aspartate residue next to the first cysteine (whose mutation to an arginine has a strong influence on the assembly of the PSI core (40)) could feasibly also be involved in interactions with PsaC. On the other hand, the intercysteine loops are rather closely associated with the membrane-intrinsic regions of PSI. On their own, they therefore represent a much smaller binding surface for PsaC than implied by the hypothetical model proposed previously (39–41). However, in addition to the intercysteine loops, the parts of the loops \( j-k \) (VIII–IX) N-terminal to the conserved \( F_X \)-binding motif additionally approach PsaC to within ~7 Å (as compared with the ~6 Å of the intercysteine loops), complementing the binding surface for PsaC (Fig. 3c).

**Fig. 6.** a, stereo view of the structural model of PsaC and its associated electron density map. Structural motifs of PsaE and PsaD in close contact to PsaC are also shown. The clasp of PsaD has been shortened to merely include the part covering PsaC. b, Structural model of PsaC. The view is onto the \( C_2(AB) \)-axis. This figure was produced using BobScript (47).

**Fig. 7.** Region of the electron density map showing the structural model of the conserved \( F_X \)-binding loops. The loops obey the pseudo-2-fold symmetry observed for the other features near \( C_2(AB) \). This figure was produced using Setor (49).

**Structural Model of PsaD**—Now that essentially all electron density belonging to both PsaE and PsaC has been identified, a first structural model of PsaD may be attempted. The model of PsaD presently consists of 125 Cα positions (as compared with the 138 amino acids determined from the corresponding gene psaD (24)). Hence, the present backbone represents a preliminary model only. This is especially true of the loops connecting the secondary structure elements. The ambiguity regarding their length presently prevents the protein sequence being assigned to the structure.

PsaD was previously reported to contain a single short \( \alpha \)-helix, \( D_a \), adjacent to PsaC (9). The current improved electron density map shows that this \( \alpha \)-helix is surrounded by a \( \beta \)-sheet (Fig. 3), consisting of at least three relatively long \( \beta \)-strands, which is in agreement with the observation that the structure of PsaD in solution contains a small amount of \( \beta \)-sheet (13). The electron density of the PsaD core is clearly connected to a stromal strand of electron density previously assigned to PsaC, indicating that this strand is actually part of PsaD. This means
that one terminus of PsaD partly covers PsaC, wrapping around PsaC in a clasplike manner (Figs. 3, a and c, and 6z). The distal end of the clasp is located above the transmembrane α-helices h', j', and k', thereby partly shielding PsaE, as well as the α-helix n (Fig. 3, b and c). The closest distance between the backbones of PsaE and PsaD is ~6.5 Å, supplying the structural explanation for the observed cross-linking between PsaD and PsaE (5). The other terminus of PsaD similarly appears to be located on the stromal surface. It is, however, not in contact with any other subunit. The current model thereby reveals the structural basis for the stabilizing role of PsaD for PsaC suggested previously for both cyanobacterial and higher plant PsaD (42), although this finding was later disputed (43). Since higher plant PsaD has an extended N terminus, possibly involved in the binding of other stromal subunits specific for higher plants, this may explain the more important role of PsaD in stabilizing the stromal ridge in barley (44). In cyanobacterial PSI complexes, functionally bound PsaC is retained under mild extraction procedures (43), whereas the stabilizing role of PsaD is increasingly noticeable when more aggressive detergents are used (45).

The loops of PsaD connecting the individual strands of the β-sheet appear largely directed toward the core subunits, thereby establishing the interface between the core and PsaD (Fig. 3c). In the PSI-complex, they are therefore buried and not accessible to attack by proteases. An extended loop forms a compact domain without recognizable secondary structure (Fig. 3c). It is located within a cavity formed by the central subunits PsaA/PsaB and is involved in the shortest contacts to the latter subunits.

Relative to PsaA/PsaB, the main volume of PsaD partly covers the interhelical loops n'-o', j-k, and i-h as well as the stromal ends of the α-helices i and j. The backbone-to-backbone distance between PsaD and other structural elements are currently modeled to be as follows: ~15 Å to e', ~7.5 Å to n, and ~7.5 Å to j-k. Note that the shortest distance of ~10 Å between PsaD and PsaL involves the surface α-helix p of PsaL. This newly introduced stromal surface α-helix is connected to the transmembrane α-helix p and located on the monomer-monomer interface (see Fig. 3, b and c). The distance between PsaD and α-helix r, probably part of PsaI, is ~15 Å. The current structural model indicates that PsaC and PsaD share multiple contacts to each other as well as each being in intimate contact with the core subunits.

The Subunits PsaF, PsaJ, and PsaM—The changes in the present model of PSI do not exclusively concern the stromal subunits. Note in particular that the poorly defined α-helix y previously tentatively identified with PsaM (9) is no longer discernable and has now been removed from the structural model of PSI. Thus, PsaM would not possess the trimer-stabilizing role proposed (9). Similarly, the luminal surface α-helix v has not been included in the present structural model of PSI, since the corresponding electron density could also be interpreted as belonging to an elongated loop structure.

On the other hand, the current electron density map allows a previously unreported stromal α-helical region distal to the C2-axis near the α-helices w and x to be included in the model. This α-helical structure has an unusual overall conformation (Fig. 8). After a short stromal loop region near PsaE (~5.5 Å), the backbone forms a small α-helix (y1), inclined by about 40–50° to the membrane plane, which, after reaching a third of the total membrane depth, bends back toward the stromal surface as a second α-helix (y2), ending just short of the electron density assigned to the stromal loop connecting α-helices w and x.

The exact assignment of the secondary structure elements in this region is not entirely clear; because w and x appear to be linked stromally (this is still the case in the present electron density map), these were assigned to PsaF (9), the only subunit long enough to possess two transmembrane α-helices. As a result, u was assigned to PsaJ. If this assignment is maintained, y1 and y2 would belong to PsaM, supporting the notion that PsaM is involved in cyclic electron transfer.

However, this assignment contradicts the positioning of PsaJ identified by electron microscopy, which indicates PsaJ to be located in the region of w and x (4). Possibly, therefore, y1 and y2 belong to PsaJ and u to PsaM. In this constellation, PsaM is distal to both PsaJ and PsaF, accounting for the lack of crosslinking products between PsaM and any other small PSI subunit (5). The position of PsaJ would match the electron microscopic conclusions, although PsaJ would not be transmembrane as generally assumed.

A third assignment is possible if the loop w-x is assumed to be an artifact. Then y1 and y2 could represent the C terminus of PsaF, while one of w and x would belong to PsaJ and the other to PsaF. This constellation would best account for the finding that PsaF does not contain two significantly hydrophobic stretches, although it is not in perfect agreement with the apparent stromal connection between w and x.

PsaA and PsaB—Except for the luminal connection k-l-m (Fig. 9), all other luminal interhelical loop regions have not to date been located in their entirety. This is due both to their length and to the lower effective resolution of these luminal regions of the electron density. By contrast, the short stromal connections have been identified to a much larger extent (9). Nevertheless, the previous model could not differentiate between a or d as the N-terminal transmembrane α-helix due to the ambiguity in the stromal (N-terminal) connection of α-hel-
Fig. 9. A schematic correlation of the secondary structures of the X-ray structural model to the amino acid sequence for the central subunits PsaA and PsaB. The N terminus is at α-helix f, the C terminus at α-helix o. Binding sites for the phylloquinone cofactors (Q_a) and the iron sulfur cluster F_{X} are indicated. An assignment of primed or unprimed α-helices to PsaA or PsaB is still not possible on purely structural grounds (however, see Ref. 46).

**antenna binding domain**

**reaction centre domain**
Photosystem I: The Stromal Subunits

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