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Update on Photosynthetic Electron Transport

Photosystem I[1]

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Plants harvest light energy by oxygenic photosynthesis, which is undoubtedly one of the most important biological processes on the earth. By liberating oxygen, consuming carbon dioxide, and generating biological sources of energy and reducing power, oxygenic photosynthesis has made our planet hospitable for the survival and evolution of complex life forms. The light reactions of oxygenic photosynthesis, in which the energy of photons is converted into chemical energy, take place in the thylakoid membranes of chloroplasts and cyanobacteria. During the photosynthetic electron transport, three membrane-protein complexes, PSII, Cyt b_6f, and PSI, function in a coordinated fashion to yield a proton gradient across membrane and strong reductants on the stromal side. ATP synthase of thylakoids uses the proton motive force to synthesize ATP.

PSI is the light-driven generator of the reducing power in chloroplasts. It is a heteromultimeric pigment-protein complex that functions as a light-driven plastocyanin-Fd oxidoreductase (see Chitnis et al., 1995, for details and additional refs.). Reduced Fd is used by Fd-$NADP^+$ oxidoreductase to generate NADPH. Thus, PSI plays a vital role in chloroplast metabolism; it utilizes light to generate reducing power, NADPH, and reduced Fd. These reductants are used in diverse biochemical processes, including Calvin cycle, nitrite reduction to ammonia, fatty acid desaturation, pyruvate decarboxylation, glutamate synthesis, sulfite reduction, and enzyme regulation through reduced thioredoxin. PSI also participates in cyclic electron flow and contributes to the proton gradient that is used to generate ATP. In chloroplasts, PSI is localized in the nonappressed regions of thylakoids.

The PSI complex contains polypeptides and cofactors that are required for absorption and use of photons. PSI consists of at least 11 different polypeptides in cyanobacteria and 13 in chloroplasts (Table I). All PSI proteins are believed to be present as one copy per P700 reaction center. They vary considerably in their molecular weights, hydrophobicities, and locations with respect to the lipid bilayer. In addition to proteins, the PSI complex contains approximately 100 Chl *a* molecules, several β-carotenes, two phylloquinone molecules, and three [4Fe-4S] clusters. The cofactors of PSI are bound to the PsaA, PsaB, and PsaC proteins. The remaining subunits of PSI do not bind any redox centers. In plants and green algae, PSI associates with multiple membrane-embedded LHCIs, which serve as accessory antennas to harvest the light and to funnel its energy to the PSI reaction center (Preiss et al., 1993). LHCI contains hydrophobic apoproteins with a apparent molecular masses of 11 to 24 kD that bind Chl *a*, Chl *b*, violaxanthin, and lutein. Such separate, membrane-embedded, PSI-specific LHCs have not been found in cyanobacteria.

In the past 10 years, multidisciplinary research efforts have led to characterization of the components, the electron transfer reactions, and the structure of PSI. Biophysicists have elucidated the electron transfer pathway, biochemists have determined protein and cofactor composition of the purified PSI complexes, crystallographers have undertaken a formidable task of understanding the structure of this membrane-protein complex, and molecular geneticists have embarked on extensive mutational dissection of structure-function relations of PSI. Recent progress in our understanding of the function and structure of PSI is discussed in this paper.

ELECTRON TRANSFER REACTIONS

The PSI complex absorbs photons and uses their energy to catalyze the photooxidation of plastocyanin, a copper protein in the lumen of thylakoid membranes, and the photoreduction of Fd, a [2Fe-2S] protein in chloroplast stroma and cyanobacterial cytoplasm. PSI converts the energy of photons into chemical energy with a remarkable quantum efficiency of 39 to 44%. This estimate is especially impressive because plastocyanin and Fd, the products of the photochemical reaction catalyzed by PSI, are stable and diffusible chemicals.

Electron transfer reactions within the PSI complex are studied by various spectroscopic methods, including flash-induced optical and EPR spectroscopy (Lagoutte and Mathis, 1989). These studies have characterized discrete redox and spectroscopic properties of the primary reaction center P700 and subsequent electron transfer centers (Fig. 1). Chl

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Abbreviations: ATCC, American Type Culture Collection; Chl, chlorophyll; EPR, electron paramagnetic resonance; LHC, light-harvesting complex; $t_{1/2}$, half-time.

Table I. *Polypeptide subunits of PSI*

The subunit nomenclature used in this review is given in the body of the table; the alternative names in the literature are shown in parentheses. The molecular mass predicted from the deduced primary sequences of *Synechocystis* sp. PCC 6803 PSI is given; the apparent mass observed during PAGE is in parentheses. For PsaG, PsaH, and PsaN, molecular masses for the proteins from higher plants are given.

Subunit	Gene	Mass	Properties	Functions
PsaA (Ia, PSI-A)	*psaA*	83.0 (66)	Transmembrane proteins with 8–11 putative helices; together, they bind approximately 100 Chl *a*, P700 Chl *a* dimer, 12–16 β-carotenes, two phylloquinones, and one [4Fe-4S] cluster (F_X)	Light-harvesting; charge separation; photoprotection; charge stabilization
PsaB (Ib, PSI-B)	*psaB*	82.4 (66)		
PsaC (VII, PSI-C)	*psaC*	8.9 (8)	Peripheral on stromal (cytoplasmic) side; binds two terminal [4Fe-4S] centers (F_A, F_B)	Terminal acceptor; donate electrons to Fd
PsaD (II, PSI-D)	*psaD*	15.6 (17.7)	Peripheral on stromal (cytoplasmic) side	Fd docking; assembly of PsaC; normal EPR properties of F_A and F_B
PsaE (IV, PSI-E)	*psaE*	8.0 (8)	Peripheral on stromal (cytoplasmic) side	Facilitates interaction with Fd; essential for cyclic electron transport
PsaF (III, PSI-F)	*psaF*	15.7 (15.8)	One putative transmembrane helix; large exposure on luminal side; may bind Chl *a*	Plastocyanin docking for fast electron transfer
PsaG (PSI-G)	*psaG*	10–10.8	Two putative transmembrane helices; present only in chloroplasts	Interaction with LHCI
PsaH (VI, PSI-H)	*psaH*	10.2–11	Peripheral on stromal side; present only in chloroplasts	Interaction with LHCI
PsaI (IX, PSI-I)	*psaI*	4.3 (3.4)	One transmembrane helix	Normal organization of PsaL
PsaJ (VIII, PSI-J)	*psaJ*	4.4 (3.0)	One transmembrane helix	Normal organization of PsaF
PsaK (PSI-K)	*psaK*	8.5 (5.1)	Two transmembrane helices	Unknown
PsaL (V, PSI-L)	*psaL*	16.6 (14.3)	Two transmembrane helices	PSI trimerization
PsaM (PSI-M)	*psaM*	3.4 (2.8)	One transmembrane helix	Cyclic electron flow
PsaN (PSI-N)	*psaN*	9	Peripheral on *p* side	Unknown

molecules of LHCI and of the PSI antenna absorb photons, and the excitation energy is transferred to the primary electron donor P700, which is a dimer of distinct Chl *a* molecules. Excitation of P700 leads to charge separation. The primary photochemical charges are stabilized by spatial displacement of electrons through a series of redox centers. In a forward reaction, an electron from the excited P700 Chl is transferred to the primary acceptor A_0, a Chl *a* monomer, from which it is transferred to the intermediate acceptor A_1. PSI contains two phylloquinone molecules, one of which serves as A_1. The next intermediate acceptor is the [4Fe-4S] cluster F_X. The subsequent path of electrons through F_A/F_B remains an unresolved area in the electron transfer pathway in PSI (Jung et al., 1995; Mannan et al., 1996). The electrons may travel in a series: F_X to $F_{B/A}$ to $F_{A/B}$ to Fd. Alternatively, the electrons from F_X may be transferred to F_A or F_B and then one or both of these reduced clusters can donate electrons to Fd. The proposed locations of these redox centers in the x-ray crystallographic structure are at different distances from F_X, thus

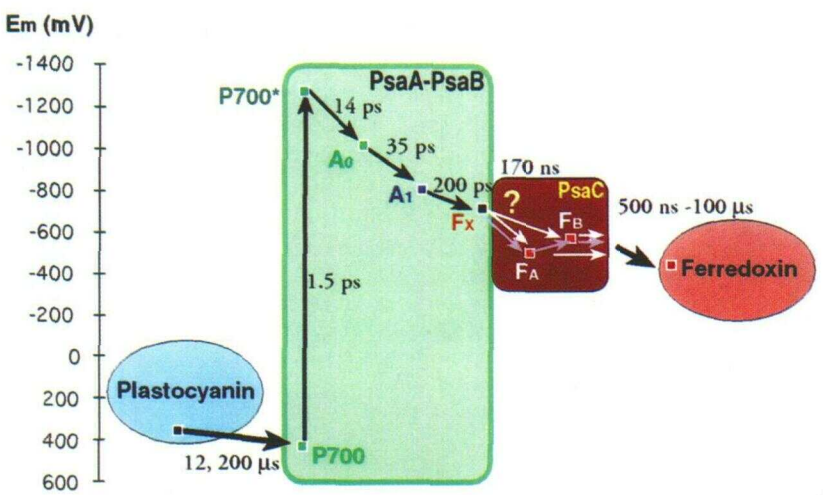

Figure 1. Electron transfer in PSI. The values of midpoint potentials (Em) and the rates ($t_{1/2}$) of forward reactions are representative; estimates from different laboratories vary depending on the technique and source of PSI. In PsaC, blue arrows show serial electron transfer from F_X to Fd, and white arrows show the alternative parallel mode of electron transfer.

implying a serial flow of electrons between F_B and F_A (Krauss et al., 1993). Selective inactivation of F_B with mercurials indicates that photoreduction of F_A is independent of F_B (Jung et al., 1995). In contrast, Fd-mediated electron transfer from PSI requires functional F_B (Jung et al., 1995). Therefore, F_B may be functionally and spatially more distant to F_X than F_A.

The electron from F_A/F_B is used to reduce Fd, and the electron lost by P700 is gained by oxidizing plastocyanin. Thus, the PSI complex interacts with soluble electron carrier proteins on both sides of the thylakoid membrane. The interprotein electron transfer on the reducing side of PSI is a complex process. It involves several different PSI-Fd complexes and three different first-order components with $t_{1/2}$ of approximately 500 ns, 13–20 μs, and 100–123 μs (Setif and Bottin, 1995). The 500-ns phase corresponds to electron transfer from F_A/F_B to Fd. When grown under iron-deficient conditions, cyanobacteria contain flavodoxin as an additional electron acceptor of PSI. The structural requirements for efficient electron transfer in *Anabaena* Fd and flavodoxin are highly dependent on the reaction partner (Navarro et al., 1995). On the oxidizing side of PSI, the interaction between plastocyanin and PSI involves a fast phase with a $t_{1/2}$ of 12 to 14 μs and a second slower phase with a lifetime $t_{1/2}$ of 200 μs (Haehnel et al., 1994). The fast rate can be attributed to the association of plastocyanin close to P700, whereas the slower phase may represent a distant plastocyanin population. In cyanobacteria and green algae, Cyt c_6, a second electron donor, is synthesized during growth in copper-depleted medium. Recently, interaction of PSI with plastocyanin or Cyt c_6 proteins from diverse organisms were studied by laser flash absorption spectroscopy (Hervas et al., 1995). PSI reduction by plastocyanin or Cyt shows different kinetics depending on the organism from which the photosystem and metalloproteins are isolated. These findings suggest that PSI was able to first optimize its interaction with positively charged Cyt and that the evolutionary replacement of the ancestral Cyt by plastocyanin, as well as the appearance of the fast kinetic phase in the plastocyanin/PSI system of higher plants, would involve structural modifications in both the donor protein and PSI (Hervas et al., 1995).

POLYPEPTIDE SUBUNITS OF PSI

The primary structures of individual PSI proteins and the overall mechanism of PSI function are remarkably conserved among cyanobacteria, green algae, and plants. The PsaA and PsaB proteins form the photoreactive core of PSI. Three peripheral proteins (PsaC, PsaD, and PsaE) and five integral membrane proteins (PsaL, PsaK, PsaF, PsaI, and PsaJ) are present in the PSI complexes from the cyanobacteria, green algae, and plants. PsaM has been detected in the cyanobacterial PSI but has yet to be shown in the PSI preparations from eukaryotes. In contrast, PsaG, PsaH, and PsaN have been shown only in the PSI preparations from green algae and plants. Genes for all known subunits of PSI have been cloned from several plants, algae, and cyanobacteria. These genes are designated as *psaX*, where X is assigned in the order of discovery. The protein subunits of eukaryotic PSI are encoded in both chloroplast and nuclear genomes; the *psaA, psaB, psaC, psaI,* and *psaJ* genes are localized on the ctDNA, and the others are present in the nucleus. It is interesting that *psaM* is present in the chloroplast genome of liverwort but not in that of rice or tobacco. The nuclear-encoded PSI proteins are synthesized as precursors with amino-terminal transit peptides and are imported posttranslationally into chloroplasts. The precursors of PsaF and PsaN have bipartite transit sequences that target them to thylakoid lumen.

Analysis of the deduced protein sequences, along with biochemical data and phenotypes of PSI mutants, can be used to propose a model for the organization of PSI proteins (Fig. 2A). Two large subunits (PsaA and PsaB) and some low-molecular-weight polypeptides (PsaF, PsaL, PsaK, PsaG, PsaI, PsaJ, and PsaM) are integral membrane proteins with highly hydrophobic transmembrane domains. In contrast, PsaC, PsaD, PsaE, and PsaH are peripheral proteins on the reducing side of PSI, whereas PsaN associates with the luminal side. Functions of some PSI proteins have been studied by a biochemical resolution-reconstitution approach. In recent years, many researchers have also used molecular genetic strategies to decipher structure-function relations in PSI. The cyanobacteria (*Synechocystis* sp. Pasteur Culture Collection (PCC) 6803, *Synechococcus* sp. PCC 7002, and *Anabaena variabilis* ATCC 29413) and the green alga *Chlamydomonas reinhardtii* have been the most popular and versatile model systems to generate PSI mutants. The elegant combination of molecular genetics, protein biochemistry, and spectroscopy has revealed functions of most PSI proteins (Table I).

Binding to Redox Centers and Cofactors

In the late 1980s, Golbeck and colleagues developed resolution-reconstitution assays to identify the PSI proteins that bind redox centers. Chaotropes were used to prepare a PsaA–PsaB core that lacked peripheral subunits, including PsaC (Golbeck et al., 1988b). Such core complexes contain all pigments and are capable of charge separation and electron transfer to F_X. F_A and F_B can be reconstituted in purified PsaC and subsequently assembled on a PsaA-PsaB core (Golbeck et al., 1988a). These analyses demonstrated that PsaA, PsaB, and PsaC bind all electron-transfer centers of PSI. Recently, a powerful combination of in vitro reconstitution and molecular genetics has been used to determine the role of the conserved cysteinyl residues in PsaC. PsaD, PsaE, and PsaC of *Synechococcus* sp. PCC 7002 have been produced in *Escherichia coli* for in vitro reconstitution with a biochemical preparation of a PSI core. These experiments showed that Cys 21, 48, 51, and 54 in PsaC provide ligands to the F_A cluster, whereas Cys 11, 14, 17, and 58 are ligated to the F_B cluster. Low-temperature EPR analysis of the free and rebound mutant PsaC proteins showed that replacement of Cys 14 by Asp results in the formation of a [3Fe-4S] cluster in the F_B site of the free protein; there is a mixed-ligand [4Fe-4S] cluster in the F_B site of the rebound protein that is fully competent at electron transfer (Yu et al., 1995c). The [3Fe-4S] cluster

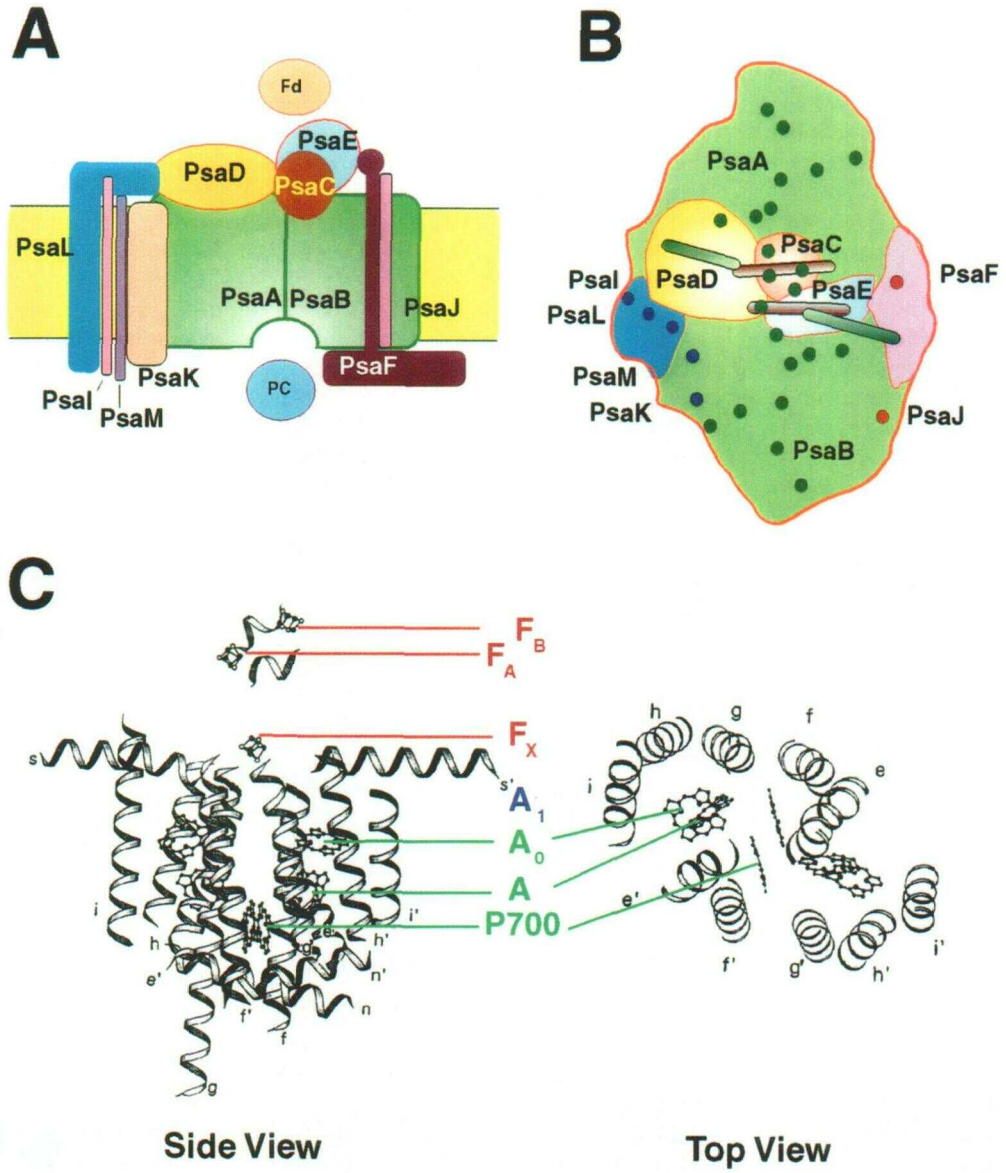

Figure 2. A model for the organization of PSI proteins. A, *A side view of the cyanobacterial PSI complex.* A detailed discussion of the supporting evidence can be found in Chitnis et al. (1995). The PsaH subunit of plant PSI is a stromal, peripheral protein. PsaG is an intrinsic membrane protein in plant PSI. It interacts with PsaA–PsaB and with LHCI. PsaN is present on the luminal side; its interactions with other subunits are not known. B, A top view of the cyanobacterial PSI complex. The overall shape is based on electron density and electron microscopy data. Approximate locations of the transmembrane helices on the stromal side are shown with filled circles. The green helices are arranged with a 2-fold axis of symmetry and have tentatively been assigned to PsaA and PsaB. The red and blue helices belong to other integral membrane proteins of PSI. The parallel helices are shown as cylinders; the green helices are on the top (reducing) side and the brown helices are on the luminal side. The assignment of helices to individual subunits and locations of different PSI proteins are tentative and are based on topological and genetic evidence. C, The inner core of PSI. The structure is based on x-ray diffraction analysis of PSI crystals at a 4.5-Å resolution. The helices are shown as spirals. There are two possibilities for the accessory Chl A and the electron acceptor A_0. These cofactors are arbitrarily labeled in the figure; the molecules present at symmetric positions are equally possible alternatives. A_1 could not be identified at a 4.5-Å resolution. The iron-sulfur clusters and the helices that are outside the lipid bilayer are omitted from the top view. The original figure was kindly provided by Professor H.T. Witt.

cannot be found in the mutant PsaC that is rebound to the PSI core.

Biochemical evidence for the function of PsaA, PsaB, and PsaC has been corroborated by the phenotypes of cyanobacterial and algal mutants. Inactivation of *psaA* or *psaB* results in the absence of PSI in the mutant thylakoids, showing that these subunits cannot form homodimers and the presence of the PsaA–PsaB heterodimer is essential for the assembly of the complex (Smart et al., 1991). With the availability of subunit-deficient mutants, vigorous site-directed mutagenesis of PsaA–PsaB is now focused on identifying the residues that bind different cofactors. An obvious choice for such an approach was mutations in the conserved cysteinyl residues that have been proposed to function as F_X ligands. The replacement of these residues affects F_X and drastically reduces accumulation of PSI in the membranes (Smart et al., 1993). Therefore, the conserved cysteinyl residues in PsaA and PsaB coordinate F_X, which in turn is essential for stable assembly of the PSI core. The cyanobacterial mutant strain that contains inactive *psaC* lacks EPR signals that can be attributed to F_A/F_B and does not contain PsaD and PsaE in the membranes (Yu et al., 1995b). The absence of PsaC has different effects on the assembly of the core complex in different organisms. The PSI core is unstable in the PsaC-less mutant of *C. reinhardtii* (Takahashi et al., 1991) but is assembled and functional in charge separation in the cyanobacterial mutants (Mannan et al., 1991; Yu et al., 1995b). The mutant strains of *A. variabilis* ATCC 29413, in which cysteinyl ligands for the iron-sulfur clusters were mutated to aspartate, can grow under photoautotrophic conditions (Mannan et al., 1996).

Interaction with Soluble Electron Acceptors

On the reducing side, PSI-Fd complex formation precedes electron transfer and the rate constants for complex formation depend on ionic concentration, suggesting electrostatic interactions between Fd and PSI. Fd accepts electrons from F_A-F_B clusters of PsaC, implying that these proteins should contact each other. The major obstacle in the association between PsaC and Fd is the unfavorable electrostatic interactions; both proteins have strong electronegative surfaces at the physiological pH. Therefore, docking proteins are required to facilitate the interaction by providing amino acid clusters of opposite charges. Numerous recent studies involving biochemical reconstitutions, chemical cross-linking, and subunit-deficient mutants have revealed crucial roles of PsaD and PsaE in the docking of Fd (Chitnis et al., 1995). Thus, PsaC, PsaD, and PsaE form the reducing site of PSI, on which Fd can dock and accept electrons. On the oxidizing side, PsaF may form a docking site for plastocyanin (Farah et al., 1995). In addition to the linear electron transfer that results in $NADP^+$ photoreduction, PSI also participates in cyclic electron flow. Characterization of cyanobacterial mutant strains has shown that PsaE and PsaM may be involved in cyclic electron flow around PSI (Yu et al., 1993).

Protection of the Electron Transfer Centers

The electron transfer centers are extremely electronegative (≤ -530 mV). Protection of these centers is an essential condition for the PSI complex to function in an oxygenic photosynthetic environment. Some accessory subunits of PSI may provide physical isolation of the electron transfer centers, protect them from wasteful oxidation, and allow only regulated oxidation to occur (Chitnis et al., 1995). The PsaD–PsaE-less mutant strain of *Synechocystis* sp. PCC 6803 dies under high light intensity or temperature. PsaD and PsaE may have protective roles on the reducing side of PSI.

Role in PSI Organization and Its Interaction with LHCI

Some PSI proteins, such as PsaL, are essential components of the trimeric quaternary structure of PSI, which has been demonstrated in the photosynthetic membranes of cyanobacteria (Boekema et al., 1994). Trimers of PSI cannot be obtained from cyanobacterial mutants that lack PsaL (Chitnis et al., 1995). PSI contains the hydrophobic small polypeptides PsaJ, PsaI, and PsaM (Table I). They have one putative transmembrane helix flanked by short hydrophilic, charged domains. Recent characterization of PsaI- and PsaJ-less mutants of *Synechocystis* sp. PCC 6803 indicates that these polypeptides assist in the correct organization of PsaL and PsaF, respectively, presumably by stabilizing their transmembrane domains in the lipid bilayer (Xu et al., 1995).

Primary sequences of two PSI proteins, PsaK and PsaG, show some similarity. These proteins can be chemically cross-linked to LHCI apoproteins. Therefore, they may function in connecting the PSI reaction center with LHCI. Incidentally, PsaG is not found in cyanobacteria. It may play a role in regulating the transfer of excitation energy from the light-harvesting Chl to the reaction center Chl of the eukaryotic PSI.

STRUCTURE OF PSI

During the past few years, we have witnessed major advances in our understanding of PSI structure. Electron microscopy studies have provided valuable information concerning the global shape and size of PSI complexes (Boekema et al., 1994), x-ray crystallography has permitted a more detailed knowledge of secondary and tertiary structural elements in PSI (Krauss et al., 1993; Schubert et al., 1995), and biochemical studies have revealed some details of PSI topography and subunit interactions. On the basis of the electron density maps, a structural model for trimeric PSI from *Synechococcus elongatus* was initially proposed at a 6-Å resolution (Krauss et al., 1993). From new data, many aspects of this model were later refined to a 4.5-Å resolution (Schubert et al., 1995). Although the PSI structure at atomic resolution may be forthcoming from the finer x-ray diffraction analyses of better PSI crystals, topological explorations and electron microscopy of the PSI complexes have aided and complemented interpretation of the currently available x-ray crystallographic data. These data can be used to propose a model for the organization of PSI proteins (Fig. 2). It needs to be emphasized that many

facets of this model are still tentative because of the low resolution of the currently available information.

Overall Organization

The PSI complex has an elongated shape, with a local pseudo-2-fold symmetry. When viewed from the top, the electron density map and electron microscopy images show a similar shape (Fig. 2B). The crystal analysis of PSI has indicated the location of the [4Fe-4S] clusters F_X, F_A, and F_B, 64 Chl *a* molecules, 29 transmembrane α helices, and 4 α helices that are parallel to the membrane plane. Most transmembrane helices are tilted to various extents from the membrane normal. A monomer of PSI consists of a "catalytic domain" and a smaller "connecting domain" that links monomers to form a trimer. The connecting domain does not significantly extend beyond the lipid bilayer and contains three transmembrane helices (Fig. 2B). The absence of trimers in the PsaL-less and PsaI-less mutants of *Synechocystis* sp. PCC 6803 indicates that the three transmembrane helices in the connecting domain may belong to PsaL and PsaI (Chitnis et al., 1995). The remaining helices belong to the PSI core and other subunits in the catalytic domain, which also contains all cofactors. This domain protrudes 15 and 35 Å on the luminal and stromal sides, respectively.

Electron Transfer Chain and Chl Molecules

In the 4.5-Å model, electron density in the central region of PSI can be fitted to identify several Chl molecules and iron-sulfur centers (Schubert et al., 1995). This model includes some striking features that were not previously detected by spectroscopy. Analogous to the purple bacterial reaction center, PSI has been proposed to contain two alternative symmetrical electron transfer paths between P700 and F_X. Another unexpected aspect of the proposed pathway is the presence of accessory Chl molecules, designated as A and A′ in Figure 2, between P700 and A_0.

Because of the distinctly high electron density, the iron-sulfur centers of PSI are clearly identified in the crystal structure of PSI. F_X is located at the edge of the membrane plane (Fig. 2C). From the top view of PSI, F_X is at the center of the PSI complex. The terminal iron-sulfur clusters F_A and F_B are located 15.4 and 22.2 Å from F_X, respectively. The F_A–F_B axis is tilted 54° from the membrane perpendicular axis (Fig. 2). Two α helices are present close to F_A and F_B, thus resembling two iron-sulfur centers of the soluble Fd of *Peptococcus aerogenes*. The distance between centers of *Peptococcus* Fd (12 Å) is similar to that between F_A and F_B (12.5 Å). Although biochemical evidence suggests that the distal cluster is F_B (Jung et al., 1995), an unambiguous assignment of F_A and F_B to the respective clusters is not yet possible.

In addition to the iron-sulfur centers, the P700 Chl pair can also be assigned in the structure of PSI (Fig. 2C). The location of P700 close to the 2-fold rotation axis and near the luminal surface of PSI places it in an appropriate position to accept excitation energy from accessory Chl molecules and electrons from plastocyanin or Cyt c_6. The dihydroporphyrin rings of P700 Chl molecules are perpendicular to the membrane plane and are parallel to each other with a center-to-center distance of 9 Å. This orientation conforms to the existing spectroscopic evidence. The transmembrane helices f and f′ come close to the P700 Chl rings and may contain residues that provide ligands for the binding to P700. The amino acid residues that provide ligands to P700 have not been identified. It is highly likely that histidyl residues provide the ligands to the P700 Chl molecules. However, Gln residues and other amino acids have been found as ligands to Chls in LHCs (Kuhlbrandt et al., 1994). There are two possible electron transfer pathways between P700 and F_X. Two Chl molecules, A and A′, are located approximately 12 Å from P700 Chl molecules. They may serve as accessory Chl molecules that bridge P700 and A_0. A and A′ are not detected by spectroscopy, and therefore their role in electron transfer has not been examined. Approximately 9 Å from A and A′, two Chl molecules have symmetrical locations; one of these may serve as A_0. The phylloquinone molecule that serves as A_1 could not be identified at a 4.5-Å resolution. It is also not known whether only one or both sides of a possible electron transfer pathway are used during PSI function.

From biochemical estimates, a PSI complex contains approximately 100 Chl *a* molecules. Based on the crystal structure at a 4.5-Å resolution, locations of 64 Chl *a* molecules have been postulated. The proposed arrangement is different from that seen in the bacterial LHC or in LHCII of higher plants (Schubert et al., 1995). Clearly, an independent principle for light harvesting evolved in the intrinsic PSI antenna. Located 8 to 15 Å from each other, Chl *a* molecules are randomly positioned along the wall of an oval bowl with the bottom oriented toward the luminal side. This wall may function as a storage device for rapid delocalization of an excited state.

Structure and Topography of the PSI Core

Twenty-two transmembrane and 4 membrane-parallel helices in the catalytic domain are arranged in an approximate symmetry. Therefore, in agreement with the hydropathy analysis, the 2 homologous PSI core proteins, PsaA and PsaB, are proposed to contain 11 transmembrane helices each. The helix g is considerably longer than the rest of the transmembrane helices and therefore may correspond to a noticeably long helix in the hydropathy plot of PsaA.

A striking feature of the proposed PSI structure is the arrangement of helices around the electron transfer chain (Fig. 2C). The framework of the inner core of PSI consists of 10 helices of PsaA and PsaB that resemble similar helices of the L and M subunits of the bacterial reaction center. These tilted transmembrane helices, along with four parallel helices, make a cage for coordination and protection of the electron transfer chain. The luminal parallel helices are slightly inclined, whereas the helices on the reducing side lie parallel to the membrane plane.

The crystal structure at a 4.5-Å resolution is not sufficient for assigning the observed helices to the predicted helical regions in the primary sequence of PsaA and PsaB. Biochemical investigations have revealed some as-

pects of PSI topography. The N-terminal domains of PsaA and PsaB are more accessible to proteases, and the C-terminal domains are involved in dimerization and interaction with other subunits (Xu and Chitnis, 1995). The N termini of the core proteins may be located on the stromal side of the thylakoids (Vallon and Bogorad, 1993). Site-directed mutagenesis is also used to probe protein interactions in PSI. Both PsaA and PsaB have a conserved CDGPGRGGTC motif that contains the F_X-binding cysteinyl residues. Site-directed changes in the F_X domain of PsaB destabilize the subunit interaction in *C. reinhardtii*, thus indicating that the F_X domain in PSI may interact with PsaC (Rodday et al., 1995). A more detailed x-ray crystallographic structure of PSI or chemical cross-linking experiments may provide direct evidence for the intimate association between the F_X domain and PsaC. Further studies are also necessary to identify accurately surface topology of the PSI core.

Structural Organization of the Reducing and Oxidizing Sides

Based on the electron microscopy images and electron density maps, the PSI reaction center has a ridge of 35 Å height projecting from one side of the membrane (Krauss et al., 1993; Boekema et al., 1994). This ridge has been proposed to be composed of the PsaC, PsaD, and PsaE subunits (Fig. 2B). The solution conformation of *Synechococcus* sp. PCC 7002 PsaE, proposed from NMR analysis, has an antiparallel five-stranded β-sheet structure (Falzone et al., 1994). The Glu 63, Glu 67, and Lys 74 residues in PsaE of *Synechocystis* sp. PCC 6803 are exposed on the surface of PSI. In addition, a Lys-containing region of PsaC and the C-terminal region of PsaD are exposed on the surface of cyanobacterial PSI. Some of these surface-exposed domains of PsaC, PsaD, and PsaE may be involved in interaction with Fd. Computer modeling of Fd docking has predicted that the docking site may be on the outside of the ridge, tilted toward the membrane plane (Fromme et al., 1994). Recent electron microscopy studies on PSI complexes that have been cross-lined with flavodoxin have shown that flavodoxin docks in the ridge formed by the peripheral subunits, from the outside of a PSI trimer, and 7 nm from the center (Mühlenhoff et al., 1996).

Electron microscopy and crystallographic studies show that the luminal side is flat with a 10-Å protrusion beyond the membrane boundary. There is a 3-nm-deep indentation in the center, perhaps caused by a partial separation between PsaA and PsaB, where plastocyanin can dock during electron transfer (Krauss et al., 1993; Boekema et al., 1994). A substantial part of PsaF is localized on the luminal side; however, it is believed to be appressed against the PsaA–PsaB core. Two α helices that lie parallel to the membrane plane are analogous to similar helices seen in the purple bacterial reaction center. These helices may provide a hydrophobic docking region that is postulated to be involved in interaction with the hydrophobic surface of plastocyanin (Haehnel et al., 1994).

IMPLICATIONS, QUESTIONS, AND FUTURE DIRECTIONS

Elegant use of diverse approaches and techniques, from genetic engineering of cyanobacteria to crystallography of complete PSI complexes, has led to a better understanding of the structure and function of PSI. Yet, we are far from completely understanding how this pigment-protein complex is built for efficient conversion of light energy. Further experimentation needs to use clues from current knowledge of PSI and related photosynthetic reaction centers. PSI, the P840 photosynthetic reaction center of green sulfur bacteria, and the P800 reaction center of Heliobacteria share structural and functional similarities, indicating a common evolutionary origin (Golbeck, 1993). The reaction centers of *Chlorobium* and *Heliobacterium* are homodimers of the 80-kD proteins that share significant homology to PsaA and PsaB of plant and cyanobacterial PSI. The 22-kD PscB protein of *Chlorobium* shows significant functional and some structural homology to PsaC of PSI. The reaction centers of these anoxygenic bacteria do not seem to have as many polypeptides as in the PSI complex. The presence of these proteins in cyanobacteria and chloroplasts may be a recent addition to meet the protective and interactive demands of the oxygenic photosynthesis. The tentative arrangement of the PSI electron transfer chain, especially in the P700-A_1 region, is analogous to the locations of redox centers in the purple bacterial reaction center, which has a common evolutionary origin with PSII. Therefore, despite variations in the details, all photosynthetic reaction centers seem to share a common structural and functional theme.

As discussed before, a collaborative use of molecular genetics and biochemistry has led to characterization of the roles of many PSI proteins. Yet, functions of some PSI proteins are still controversial or unidentified. The function of PsaK is not known. Similarly, PsaL has been shown to mediate trimer formation in cyanobacteria, but its role in plants and algae is yet to be demonstrated. In higher plants, an oligomeric structure of the PSI entity has been shown to exist in vitro (Preiss et al., 1993), but the existence of PSI trimers in membranes has not been confirmed. In chloroplasts, PsaF is an essential docking protein for plastocyanin. However, it is not needed for electron transfer to P700 in the cyanobacterial PSI. PsaG and PsaN are found only in chloroplast PSI; their functions are unknown. Now we know the identities, spectral properties, and some aspects of the arrangement of electron transfer centers in PSI. However, the role of the protein scaffold in defining attributes and function of the redox centers is just beginning to unfold. For example, detailed studies of site-directed alterations in Cys ligands of iron-sulfur clusters have demonstrated the impact of the protein environment on quantum efficiency and redox properties (Vassiliev et al., 1995; Yu et al., 1995a). Extensive site-directed mutagenesis and chemical modifications should lead to the identification of the ligands to photoactive Chl and phylloquinone molecules. It should also provide an attractive approach to find which of the two possible paths between P700 and F_X is used for the electron transfer.

Although the 4.5-Å structure has provided some tentative assignments regarding organization of the PSI complex, a more accurate assignment of the different protein subunits within the complex and their relation to the thylakoid membrane could not be accomplished because of the low resolution of this analysis. Use of complimentary approaches are needed for rapid progress in determining details of the PSI structure. These include protein topography analysis, chemical cross-linking of interacting proteins, and generation of simpler systems for structural analysis. Several subunit-deficient cyanobacterial mutants are now being used in electron microscopy studies to determine approximate locations of PSI subunits in the overall architecture of PSI. For example, comparison of the electron microscopy images of the wild type and PsaF–PsaJ-less PSI trimers from *Synechocystis* sp. PCC 6803 shows that the lack of PsaF and PsaJ causes a change in a contiguous area on the opposite side from the trimer-forming domain (Kruip et al., 1996). A promising approach for rapid progress in x-ray diffraction studies may involve use of subunit-deficient PSI preparations for crystallization. Recombinant DNA techniques are used to overproduce individual peripheral proteins that can be used for structural studies (Falzone et al., 1994).

Our understanding of how PSI is put together and disassembled is rather limited. The pathway of PSI assembly can be inferred from in vitro reconstitution experiments and from phenotypes of cyanobacterial mutants. Initially, PsaA and PsaB form a heterodimer; its stability is dependent on the correctly formed F_X cluster. Similarly, cubane iron-sulfur clusters at both F_A and F_B are needed for assembly of PsaC into PSI (Yu et al., 1995c). Assembly of PsaD and PsaE is dependent on the presence of PsaC in the complex. The sequence in which the hydrophobic proteins and cofactors are added is not known. Molecular chaperones may assist integration of intrinsic proteins. Assembly of PsaF into thylakoids involves soluble chaperones and Sec-like translocation machinery (Karnauchov et al., 1994). The PSI assembly may also require proteins that transport and add cofactors. These components have not been discovered. A striking feature of PSI biogenesis is that it is turned over at a considerably lower rate than PSII. In fact, the half-life of any PSI protein is difficult to determine because of the high stability of this complex. Unlike PSII, photochemical events in PSI do not lead to continuous turnover of its proteins. The stable nature of the PSI complex and the highly electronegative redox potentials of the PSI electron transfer centers seem to play crucial co-dependent roles in the evolution of this complex. A clue to the stability of PSI comes from the structural organization of the complex. The electron transfer chain in PSI is surrounded by a wall of transmembrane helices (Fig. 2C), which in turn is padded by small hydrophobic subunits (Fig. 2B). Protection on the reducing side is provided by the peripheral proteins. PSI is indeed a photosynthetic complex that is built right. It may represent a triumph in nature's relentless pursuit to build an efficient and durable photoconversion machinery.

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