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X-Ray Crystallographic Structure Analysis of Cyanobacterial Photosystem I at 2.5 Å Resolution

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Introduction

Water-oxidizing photosynthesis requires two membrane-embedded protein-pigment complexes, photosystem I (PS I) and photosystem II (PS II), for light-dependent reduction of $NADP^+$ by water. In PS I, the energy of sun-light is used to oxidize cytochrome c_6 or plastocyanin on the lumenal and to reduce ferredoxin or flavodoxin on the stromal side of the thylakoid membrane. Single crystals of PS I isolated from the thermophilic cyanobacterium *Synechococcus elongatus* (Fromme and Witt, 1998) are suitable for X-ray crystallographic analysis, limited to a medium resolution of 4 Å until recently (Schubert et al., 1997). The corresponding structural model already contained the complete set of cofactors involved in primary charge separation and electron transfer across the thylakoid membrane (Klukas et al., 1999a), called the electron transfer chain (ETC), and a large portion of the chlorophyll molecules which constitute the core antenna of PS I. Furthermore, 8 membrane intrinsic protein subunits were assigned tentatively to the electron density map, and first models of the polypeptide backbones of the stromal subunits PsaC and PsaE in the PS I complex were derived (Klukas et al., 1999b) using structural data of related proteins (Adman et al., 1976; Falzone et al., 1994). The structural model lacked any details about the chemical nature of interactions between the building blocks, *i.e.* the protein subunits and the organic and inorganic cofactors of the complex. In addition, chlorophyll molecules could only be modelled as symmetric porphyrins, such that an assignment of the transition dipolar moments, which need to be known in order to calculate excitation energy transfer rates on the basis of the Förster theory (Förster, 1948), was impossible. Further improvement of the crystal quality and success in finding conditions suitable for cryogenic data collection finally resulted in the first crystallographically refined structure of PS I at 2.5 Å resolution (Jordan et al., 2001). The refined crystal structure now provides the first basis for understanding the numerous intermolecular interactions, almost within the whole PS I complex.

Materials and Methods

PS I isolated from *Synechococcus elongatus* was crystallized as a trimer (Fromme and Witt, 1998) and shock-frozen in liquid propane using sucrose as a cryoprotectant. Diffraction data of native and heavy atom derivative crystals were collected at 100K (space group $P6_3$, $a = b = 281.0$ Å, $c = 165.2$ Å) at beamline ID2B (ESRF, Grenoble) and phases determined by the method of multiple isomorphous replacement including anomalous scattering (MIRAS). An initial model was built interpreting an electron density map at 3.5 Å resolution, and the

model completed and refined at 2.5 Å resolution employing standard difference Fourier syntheses and refinement procedures (Jordan et al., 2001) implemented in the program CNS (Brünger, 1998).

The structural model of the PS I monomer contains 11 biochemically characterized protein subunits. Only for the peripheral membrane-intrinsic subunit PsaK could the amino acid sequence not be assigned to the electron density map. A twelfth polypeptide, containing a single transmembrane α-helix, was found and named PsaX, because its electron density could be well interpreted using the N-terminal sequences of polypeptides of the same name identified in PS I from the thermophilic cyanobacteria *Synechococcus vulcanus* (Koike et al., 1989) and *Anabaena variabilis* (Ikeuchi et al., 1991). 96 chlorophyll molecules were located in the electron density map and the orientations of their $\mathbf{Q_x}$ and $\mathbf{Q_y}$ dipolar moments assigned. Of 22 carotenoids modelled as *β*-carotene, 16 were found in all-*trans* and five in different *cis* configurations, whereas one was modelled as an incomplete molecule. Four lipid molecules, of which three are phosphatidyl glycerol and one is monogalactosyl diglyceride, turned out to be integral constituents of the PS I complex. The set of cofactors is completed by three $[Fe_4S_4]$ clusters and two phylloquinone molecules, and a putative Ca^{2+} ion was identified, located at the interface between adjacent PS I monomers. The model further consists of 201 water molecules.

The Structure

The Polypeptide Subunits

Nine of the PS I subunits contain at least one transmembrane α-helix. The central core of the membrane intrinsic structure is formed by the PsaA/PsaB heterodimer of pseudo-C_2 symmetry, to which the remaining seven smaller subunits are peripherally attached: PsaL, PsaI and PsaM at the side proximal to the central C_3 symmetry axis of the PS I trimer, PsaF and PsaJ at the distal side, and PsaK is attached peripherally to PsaA in the direction of the long axis of the PS I monomer. Each of the membrane spanning subunits is involved in binding of the chlorophyll and/or carotenoid cofactors which constitute the PS I core antenna, at least indicating an important part of their function: to provide the protein matrix for the complex arrangement of cofactors. An unusual structural feature is found in PsaF, which contains a kinked α-helix, entering the inner-membrane region from the stromal side with its N-terminal end and leaving it at its C-terminus on the same side of the membrane, thereby penetrating this region by only one third of the total membrane depth. Neither for this segment nor for the extended α-helical structures of PsaF on the lumenal side are the functions known at the moment. Only two of the PS I cofactors, the iron-sulfur clusters F_A and F_B, are bound to an extrinsic subunit – PsaC, which is located on the stromal side of the thylakoid membrane. As expected, this subunit shares striking similarities with bacterial $2[Fe_4S_4]$ ferredoxins, but contains C- and N-terminal extensions in contact with the membrane intrinsic subunits and an insertion connecting the iron-sulfur cluster binding motifs, which is directed towards the stromal surface of the complex. The centre of gravity of PsaC approximately coincides with the pseudo-C_2 axis of PsaA/PsaB. The second and third stromal subunit, PsaD and PsaE, which are mostly folded into β-sheet structures and most probably form the docking site for ferredoxin or flavodoxin together with PsaC, are not only involved in ionic interactions and hydrogen bonds with PsaC, but are also in direct contact to each other. Although the centres of gravity of PsaD and PsaE are located on opposite sides of the pseudo-C_2 axis, these direct contacts are made possible by the C-terminal loop segment of PsaD, which crosses PsaC at its stromal surface and thereby gets into close contact with PsaE. The specific interactions formed by this loop may be of particular importance for the proper assembly of the stromal subunits into the PS I complex.

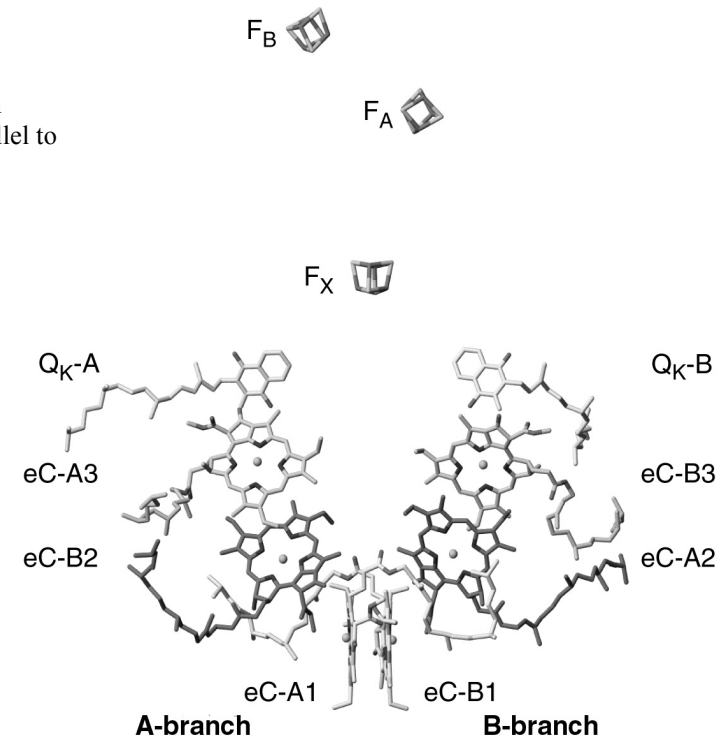


Fig. 1: Cofactors of the electron transfer chain (ETC), view parallel to the membrane plane

The Electron Transfer Chain (ETC)

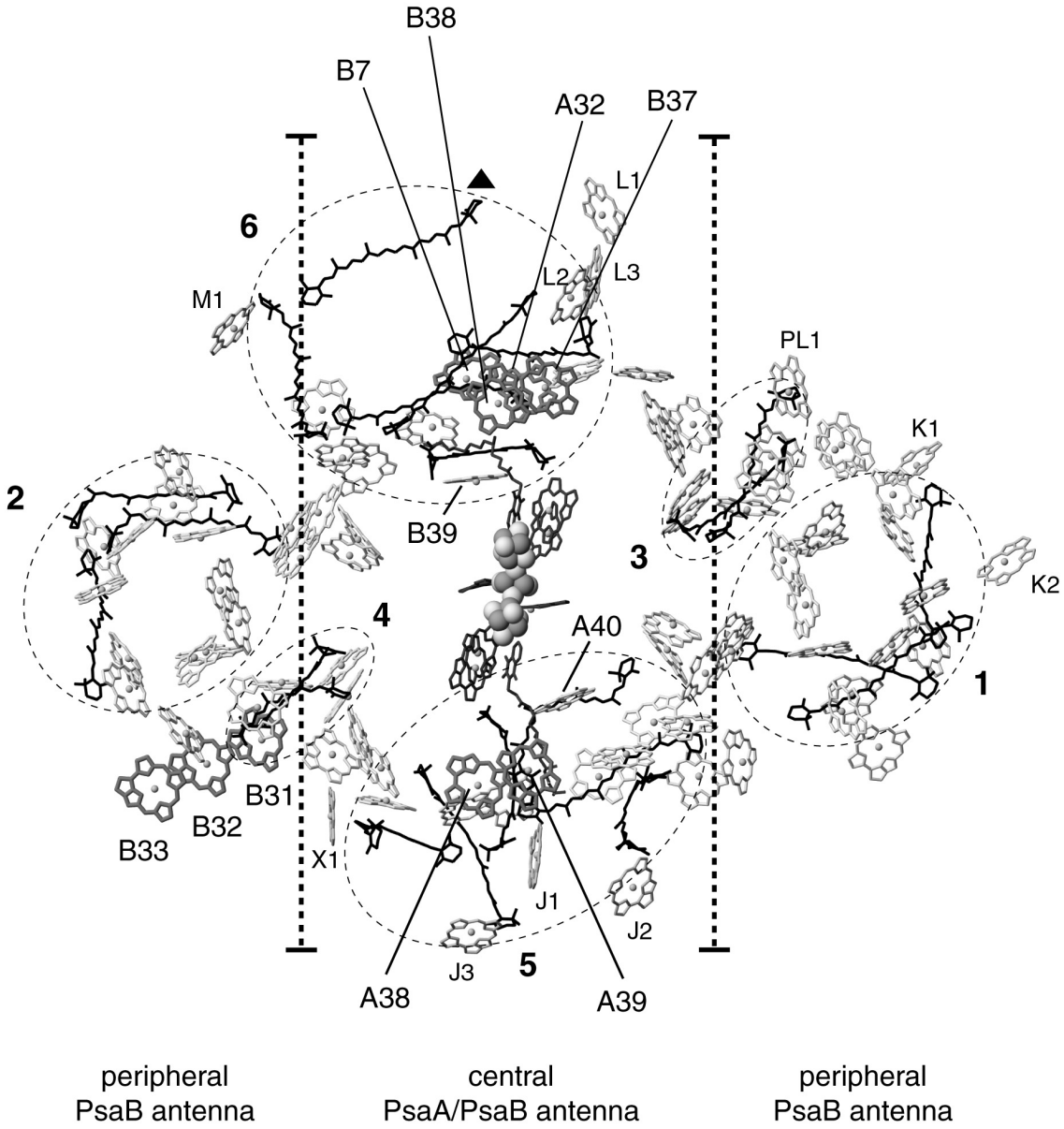
The membrane-intrinsic part of the ETC is characterized by the same pseudo-C_2 symmetry as the PsaA/PsaB heterodimer, which exclusively binds the corresponding cofactors. Three pairs of chlorophyll and one pair of phylloquinones are arranged in two branches, named A and B, and a single [Fe_4S_4] cluster is located on the pseudo-C_2 axis. The first chlorophyll pair eC-A1/eC-B1, next to the lumenal surface, was assigned to the primary donor P700, one or both chlorophyll *a* (Chl*a*) of the third pair eC-A3/eC-B3 should be identical with the primary acceptor A_0 and similarly one or both of the two phylloquinones Q_K-A and Q_K-B should be the secondary acceptor A_1. The chl of the second pair, eC-A2 and eC-B2, are located intermediate between the first and third. They are the only cofactors of the A-branch coordinated by PsaB and of the B-branch coordinated by PsaA. Their positions, intermediate between the primary donor and the putative primary acceptor chl in both branches, are different from but comparable with those of the 'accessory' bacteriochlorophylls in purple bacterial photosynthetic reaction centres (Lancaster et al., 2000). The chlorin planes of eC-A2 and eC-B2 are roughly parallel with and at close distances of ~3.8 Å to those of eC-B3 and eC-A3, respectively, which may indicate that they are engaged in electron transfer from P700 to A_0 and that the interacting chl of the second and third pair affect their spectroscopic and redox properties *vice versa*. The iron-sulfur cluster located on the pseudo-C_2 axis is F_X, coordinated by those two conserved cysteines each of PsaA and PsaB which had been predicted from the amino acid sequences (Fish et al., 1985). The assignment of the two iron-sulfur clusters located stromally of the thylakoid membrane, F_A to the cluster proximal and F_B to the cluster distal from F_X and closest to the stromal surface of PS I, was possible on the basis of mutational studies in which the cysteine ligands of these clusters in PsaC had been identified (Zhao et al., 1992).

The most significant breakdown of the pseudo-C_2 symmetry of the membrane-intrinsic part of the ETC is found at P700, with eC-A1/eC-B1 forming a chlorophyll *a*´/chlorophyll *a* heterodimer with parallel chlorin planes and extended π-stacking. This arrangement would

correspond with a strong excitonic coupling of the chlorophylls and explain the red-shifted absorption of P700 with respect to the chl of the PS I bulk antenna. Asymmetry of P700 is also observed as regards the hydrogen bonding environment of the two chl. Hydrogen bonds are exclusively formed by PsaA to the chlorophyll *a´* eC-A1. For the remaining membrane-intrinsic cofactors of the ETC, the protein environment is symmetric as regards the axial ligands of the central Mg^{2+} ions of the chl, the hydrogen bonds and the π-stacking of the two quinones, Q_K-A and Q_K-B, with the indole rings of conserved Trp A697 and Trp B677, respectively. For the three chl within each branch, the axial ligands and hydrogen bonding environments are different. The atoms coordinating their central Mg^{2+} ions are N_ε of conserved histidines for eC-A1 and eC-B1, oxygen atoms of water molecules hydrogen bonded to consvered asparagines for eC-B2 and eC-A2, and most strikingly and never found in any chl binding protein so far, sulfur atoms of conserved methionines for eC-A3 and eC-B3. The pair eC-B2/eC-A2 is not involved in any hydrogen bond, whereas a single hydrogen bond is formed to each chl of the pair eC-A3/eC-B3. The $Mg^{2+}\cdots S$ interaction is unusual and should be weak according to the concept of hard and soft acids and bases. To which extent these different protein-chl interactions are responsible for fine-tuning of the redox potentials of the cofactors, is not clear up to now. The two quinones in PS I accept single hydrogen bonds each with the carbonyl oxygens in ortho position to the phytyl chains. The lack of a second hydrogen bond to the oxygen in para position may at least partially explain the negative shift of the redox potential of A_1 as compared to the electron acceptor Q_A in purple bacterial reaction centres with a symmetric hydrogen bonding environment of its quinone system (Lancaster et al., 2000).
The asymmetry of the P700 structure is well compatible with ENDOR data on $P700^{+\bullet}$, showing that ~80% of the spin density in the radical cation are probably located on the chlorophyll bound to PsaB (Käss et al., 2001), *i.e.* eC-B1, as the larger portion of spin density should be located on the chlorophyll lacking any hydrogen bonds according to an analysis of the analogous situation in purple bacterial reaction centres (Artz et al., 1997).

The Core Antenna System

The PS I core antenna consists of 90 Chl*a* and 22 carotenoid molecules. Each of the carotenoids is in contact (<3.6 Å) to the headgroup of at least one antenna Chl*a*, an arrangement which is suitable for both quenching of Chl*a* triplet states by the carotenoids and for efficient energy transfer from excited carotenoids to the Chl*a* molecules. An analysis of the distribution of the five different *cis*-carotenoids within the PS I structure does not provide a simple explanation for their functional roles at the moment.
The arrangement of the 79 antenna Chl*a* molecules which are bound to PsaA/PsaB may be divided into three domains. Two peripheral domains, where the Chl*a* molecules are arranged in two separated layers parallel to the membrane plane, are attached to a central domain where the Chl*a* molecules are located in all depths of the membrane. The structures of the peripheral domains might be in favour of excitation energy transfer within single layers, thereby being responsible for an efficient energy transfer to the central domain. The probability of excitation energy transfer from each of the inner Chl*a* of the central domain to the reaction centre might be comparable, compatible with existing kinetic models (Valkunas et al., 1995). The layer structure of the peripheral PsaA/PsaB antenna is not strictly obeyed by some of the 11 Chl*a* bound to the smaller subunits and a phospholipid.



To understand and to simulate the mechanisms of excitation energy transfer in PS I on the basis of the present crystal structure using the theory of Förster, it would be necessary to know the spectral properties of the Chl*a* molecules and the exact nature of their excitonic couplings. At the moment, only a few dimers and a single trimer of Chl*a* molecules can be identified for which the excitonic couplings are most probably stronger than for the remaining chlorophylls and which could belong to the so called 'red chlorophylls', which absorb light at longer wavelengths than P700. The trimer aC-B31/aC-B32/aC-B33 is suggested to contribute to the long-wavelength absorption of PS I as it consists of π-stacked Chl*a* molecules with roughly parallel $\mathbf{Q_X}$ and $\mathbf{Q_Y}$ transition dipolar moments, which should lead to strong excitonic couplings, and its whole structure strongly resembles crystal structures of chlorophyll derivatives (Kratky and Dunitz, 1977), with absorption maxima at 740 nm. The Chl*a* dimer aC-A32/aC-B7 is interesting because it is located at the monomer-monomer interface close to the C_3 axis. If the PS I trimer is separated into monomers, it could be that at least one Chl*a* of this pair would be lost, thus explaining the decrease in absorption of monomeric PS I from *Synechococcus elongatus* at 719 nm with respect to the corresponding PS I trimer (Pålsson et

al., 1998). The two Chl*a* pairs aC-A38/aC-A39 and aC-B37/aC-B38 are related by the pseudo-C_2 symmetry and are located at relatively short distances (~16 Å) from the 'connecting' Chl*a* molecules, aC-A40 and aC-B39, which seem to link the core antenna and the reaction centre of PS I.

Conclusion

The refined crystal structure of PS I provides a huge amount of detailed information about this complex system. In spite of this, many questions concerning the various functional aspects of PS I, as there are the uni- or bidirectionality of electron transfer along the two branches of the ETC, the spectral assignment of Chl*a* in the structure and the mechanism of exciton transfer, and the mechanisms of docking of the electron carrier proteins at the donor and acceptor sides of PS I, cannot be answered *ad hoc* using the structural model. Functional studies on site-directed PS I mutants designed on the basis of the present structure will hopefully help to elucidate many of these aspects.

Acknowledgements

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