Functional implications on the mechanism of the function of photosystem II including water oxidation based on the structure of photosystem II

Petra Fromme^{1,2*}, Jan Kern¹, Bernhard Loll³, Jacek Biesiadka³,
Wolfram Saenger³, Horst T. Witt¹, Norbert Krauss^{3,4} and Athina Zouni¹

¹Max-Volmer-Laboratorium für Biophysikalische Chemie und Biochemie, Technische Universität Berlin,
Strasse den 17 Juni 135, 10623 Berlin, Germany

²Department of Chemistry and Biochemistry, Arizona State University, Tempe, AZ 85287-1604, USA

³Institut für Chemie/Kristallographie, Freie Universität Berlin, Takustrasse 6, 14195 Berlin, Germany

⁴Institut für Biochemie, Universitätsklinikum Charité, Medizinische Fakultät der Humboldt Universität zu Berlin,
Monbijoustrasse 2, D-10117 Berlin, Germany

The structure of photosystem I at 3.8 Å resolution illustrated the main structural elements of the water-oxidizing photosystem II complex, including the constituents of the electron transport chain. The location of the Mn cluster within the complex has been identified for the first time to our knowledge. At this resolution, no individual atoms are visible, however, the electron density of the Mn cluster can be used to discuss both the present models of the Mn cluster as revealed from various spectroscopic methods and the implications for the mechanisms of water oxidation. Twenty-six chlorophylls from the antenna system of photosystem II have been identified. They are arranged in two layers, one close to the stromal side and one close to the lumenal side. Comparing the structure of the antenna system of photosystem II with the chlorophyll arrangement in photosystem I, which was recently determined at 2.5 Å resolution shows that photosystem II lacks the central domain of the photosystem I antenna, which is discussed in respect of the repair cycle of photosystem II due to photoinhibition.

Keywords: photosystem II; crystal structure; electron transfer; water oxidation

1. INTRODUCTION

Photosynthesis is the main process on earth, which converts the light energy from the sun into chemical energy. Three types of organisms are able to perform oxygenic photosynthesis: plants, algae and cyanobacteria. An ancestor of cyanobacteria may have been introduced into a eukaryotic cell early in their evolution, leading to the development of the photosynthetic plant organelle, the chloroplast. Cyanobacteria are widely distributed throughout the world and still produce more than 30% of the oxygen in the atmosphere. The main constituents of the photosynthetic machinery are well conserved between plants and cyanobacteria. The latter are used as model systems for the investigation of the structure and function of the large protein complexes, PSI and PSII, which catalyse the first and most important step of photosynthesis, the light-induced charge separation (for a review on photosynthesis see Witt (1996)). The two photosystems function in series, coupled by a pool of plastoquinone, the cytochrome *b₆f* complex and the soluble electron carrier

plastocyanin. In cyanobacteria, cytochrome *c*₆ can, under some conditions, substitute for plastocyanin.

Protons are pumped across the membrane by the *b₆f* complex. They lead to the establishment of a transmembrane proton gradient, together with the protons released during water oxidation at the inside (lumen) of the thylakoids and the protons consumed by ferredoxin-NADP⁺-oxidoreductase during NADP⁺ reduction at the stromal side of the thylakoid membrane. The proton gradient and the electrical potential, introduced by the charge separation, drive ATP synthesis. NADPH and ATP are high-energy molecules, which are consumed in the dark reaction of photosynthesis to fix carbon dioxide, and to synthesize sugars and all further constituents of the cells. The electrons for the whole process are provided by PSII in the process of water oxidation, where oxygen is evolved and provided for respiration, and is the energy source for all higher non-photosynthetic organisms, including humans. In respect of photosynthesis, oxygen is a by-product and, due to its high oxidation potential, even dangerous for the cells.

The recently determined X-ray structural model of PSII provides the first insight into the structure of the water-splitting mechanism in PSII. This provides the basis for a discussion of the functional aspects of the mechanisms of water oxidation, electron transfer and light capture.

* Author for correspondence (pfromme@asu.edu).

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Figure 1. Structural model of PSII at a 3.8 Å resolution (modified after Zouni *et al.* 2001a). View from the luminal side onto the membrane plane. The transmembrane α -helices are represented by columns. The α -helices assigned to individual proteins are depicted in different colours: (D1, yellow/green; D2, orange; CP47, red; CP43, purple; α - and β -subunits of cyt *b*559, green; tentatively assigned subunits PsbI, PsbK, PsbL and PsbX, blue; non-identified subunits, grey). The chlorophylls are represented by symmetrical porphyrin ring systems, the Fe atoms are shown as grey spheres. The Mn cluster is highlighted by a red circle and the Mn atoms are shown as red spheres.

2. STRUCTURE OF PHOTOSYSTEM II

The crystallization of PSII from the thermophilic cyanobacterium *Synechococcus elongatus* was reported in Zouni *et al.* (2000). The crystals formed were used to determine the structure of PSII by X-ray structure analysis at a resolution of 3.8 Å (Zouni *et al.* 2001a). The crystals were competent in oxygen evolution (Zouni *et al.* 2000) and their protein content has been analysed in detail (Zouni *et al.* 2001b). The latter analysis revealed the presence of the following 17 protein subunits in the crystals: PsbA to PsbO as well as PsbU, PsbV and PsbX. Fourteen of these subunits contain at least one transmembrane helix, so they can be regarded as integral membrane proteins. PsbO, PsbU and PsbV do not contain transmembrane α -helices and are located at the luminal side of the complex. In the structural model, at 3.8 Å resolution, the origin of 36 transmembrane helices was analysed in respect of the localization of the individual subunits, taking into account the available data from biochemical investigations (see figure 1 taken, with modifications, from Zouni *et al.*

(2001a)). The central domain of PSII, which carries the cofactors of the electron transfer chain, is formed by a cluster of 2×5 transmembrane helices, assigned to the protein subunits D1 (PsbA) and D2 (PsbD). The arrangement of these subunits in the transmembrane area resembles the structure of the L and M subunits of the RC of purple bacteria (Ermler *et al.* 1994; Deisenhofer *et al.* 1995) and, to a lesser extent, the structure of the C-terminal domain of the large subunits PsaA and PsaB of PSI (Jordan *et al.* 2001). This demonstrates that all photo-reaction centres may have evolved from a common ancestor as previously proposed (Blankenship 1992; Schubert *et al.* 1998). The similarity in the organization of D1/D2 and L/M subunits was directly observed, for the first time, in the structural studies of Rhee *et al.* (1998) and extended in the recent work of Hankamer *et al.* (2001a,b). The central cores of D1 and D2 are flanked by the antenna proteins CP47 (PsbB) and CP43 (PsbC). Each of these subunits consists of six transmembrane helices. Comparison with the structure of PSI reveals that the arrangement of the transmembrane helices of these proteins is very

Figure 2. Structural model of the luminal loops of D1 at 3.6 Å resolution. The loop structure of D1 is shown in yellow/green. The electron density of the Mn cluster is depicted at a contour level of 3σ , the electron density of TyrZ at 2σ . The AB-loop, the CD-helix and part of the C-terminal loop are located in proximity to the Mn cluster.

similar to the arrangement of the transmembrane helices in the N-terminal section of the PsaA/PsaB in PSI. This similarity has previously been demonstrated, based on the structural model of a subcomplex of PSII at 8 Å resolution, determined by electron crystallography (Rhee *et al.* 1998). The work of Hankamer *et al.* (1999) also anticipates the positioning of CP47/43 relative to the D1/D2 heterodimer based on low-resolution electron crystallography on intact PSII complexes. In the 3.8 Å X-ray structure, two helices, which are located in close proximity to helix A of D2, were assigned to the two proteins PsbE and PsbF, constituting the membrane-bound cytochrome *b559*. The remaining 12 transmembrane helices could not be unambiguously assigned at 3.8 Å resolution, but some proposals were made based on biochemical data.

PSII contains three extrinsic subunits, which are located at the luminal side of the core complex: the 33 kDa protein (PsbO), the 12 kDa protein (PsbU) and the cytochrome *c550* (PsbV). The latter two subunits are unique to cyanobacteria, whereas PsbO is also present in the PSII complex of higher plants. At 3.8 Å resolution the main body of PsbO was identified as a β -barrel structure. PsbV was identified by the haem group, which could be positively identified by the high electron density of the Fe ion. The location of PsbU was not resolved at 3.8 Å resolution. However, the resolution of the electron density map was recently improved to 3.6 Å (Zouni *et al.* 2001b). In the improved model, all three extrinsic subunits could be located as well as large parts of the loop structures of D1/D2 and CP43 and CP47. The luminal loop structure of D1, as revealed from the improved structural model, is shown in figure 2 (from Zouni *et al.* (2001b) with modifications).

A total of 42 cofactors have been identified at 3.8 Å resolution: 26 antenna Chl bound to CP47/43, six chlorophylls coordinated by D1/D2 as well as the Mn cluster (consisting of four Mn ions), one non-haem iron located

at the D1/D2 interface on the stromal side of the membrane, and the two haem groups of cyt**b559** and cyt**c550**, respectively. In the improved structural model from (Zouni *et al.* 2001b), three additional chlorophylls, bound to CP43/CP47 were identified, so that the total number of chlorophylls increased to 29. The additionally assigned chlorophylls are all located at the periphery of the PSII complex.

3. FUNCTIONAL IMPLICATIONS ON THE ELECTRON TRANSPORT CHAIN

The positions of the cofactors of the ET chain as revealed at 3.8 Å resolution were confirmed at 3.6 Å resolution. Figure 3 shows the ET chain as revealed from the 3.8 Å structure. The acceptor side of PSII with the Pheo, the quinone and the non-haem iron resembles the structural arrangement of the ET chain in reaction centres of purple bacteria. This result is as expected, because the physical-chemical properties of these cofactors are quite similar in PSII and PbRCs. The important differences between PSII and the PbRC are however the redox potential of P_{680} , which is more than 1000 mV and the unique function of water oxidation.

First, we want to discuss the properties of P_{680} in respect of the structure. There are four Chl molecules located in the centre of the D1/D2 core, which are arranged in two symmetrically related pairs. These molecules had previously been found by electron crystallography (Rhee *et al.* 1998), although the angular orientation and the exact distance between these chlorophylls were not determined by electron crystallography, due to the limited resolution. The chlorophylls, named PD1 and PD2 are orientated parallel to the membrane, with a centre-centre distance of 10 Å. In both PbRC and PSI, there are two chlorophylls located at similar positions. However, the much greater distance between the two chlorophylls in PSII indicates that the two chlorophylls are not strongly excitonically coupled and therefore may be regarded as chlorophyll monomers. The second pair of chlorophylls is located at a distance of *ca.* 10 Å from the first pair of chlorophylls. The plane of the chlorin headgroup of these chlorophylls is tilted at an angle of 30° to the membrane plane. All four chlorophylls are equidistant. We will now discuss which of the four chlorophylls may be P_{680} , a question that was also explored in Witt *et al.* (2001).

In order to avoid a pre-assignment we changed the names of the four chlorophylls, and just numbered them as follows: P1($\approx$ PD1), P2($\approx$ PD2), P3($\approx$ ChlD1) and P4($\approx$ ChlD2). The arrangement is shown in figure 3b. First, we attempt to determine which of the four chlorophylls may correspond to P_{680}^+ .

To answer this question, structural information from spectroscopic investigations can be considered. The distance between P_{680}^+ and TyrZ has been determined by EPR investigations to be in the range of 7.9–0.2 Å (Lakshmi *et al.* 1999). Only the molecule P1 fulfils this requirement within the error limits, being located at a distance of 7.5 Å from TyrZ (the distance between Try=O and the closest Mn). Further evidence for the location of the spin density of P_{680}^+ on P1 is provided by pulse EPR experiments, determining the distance between P_{680}^+ and Q_A^- to be 27.7–0.7 Å (Zech *et al.* 1997, 1999). The dis-

tance determined by EPR fits quite well with the distance between Q_A and P1 (26 ± 1 Å), and the distance between Q_A and P2 (27 ± 1 Å). The distance between Q_A and P3 (22 ± 1 Å) is too short and the distance between Q_A and P4 (30 ± 1 Å) is too large to meet the required parameters. Therefore, it seems reasonable that P_{680}^+ is located on the molecule P1—at least at cryogenic temperatures, where the EPR experiments have been performed. However, this poses the question of why the neighbouring chlorophylls P2 and P3 are not oxidized instead of the TyrZ (redox potential of 1.0 V) by P1. Taking into account that Pheo $^+$ has a redox potential of 1.4 V we came to the conclusion that all four chlorophylls must have a high redox potential of between 1.0 and 1.3 V and may be able to perform the initial charge separation. This point has already been discussed in Witt *et al.* (2001) and Barber (2002). Is there an option to discriminate between the four chlorophylls using spectroscopy? There is only weak coupling between the four chlorin rings of the chlorophylls P1–P4, so there might be almost no spectral separation between them. Thus, in PSII, the absorption bands are largely overlapping and are centred between 670 and 680 nm. The antenna system can therefore provide each of the porphyrins with excitation energy followed by energy transfer between them. Therefore the excited state P_{680}^* may be delocalized, at least at room temperature, among all four chlorophylls.

Another question is on which molecule the triplet state of P_{680} may be localized. Optical spectroscopy investigations on partially orientated samples provide evidence that the triplet state $^3P_{680}$ is located on a chlorophyll molecule with an inclination of 30° to the membrane plane (van Mieghem *et al.* 1991). This requirement is fulfilled by P3 and P4. However, the triplet state must be primarily formed from the state P_{680}^+ located on P1, by intersystem crossing, therefore P4 can be excluded as a possible candidate and P3 remains as the most probable molecule for the location of $^3P_{680}$.

It is quite acceptable to assign the different forms of P_{680} to individual molecules, however, the key question still remains: what factors are involved in the shift of the redox potentials of all four chlorophylls to more than 1 V?

There are several factors which may increase the redox potential among the potential candidates: (i) strong H-bonding; (ii) protonation of the keto-group; (iii) 'unusual' coordination of the central Mg^{2+} atom; and (iv) a strong electrical field in the binding pocket. For molecules P1 and P2 we can exclude an 'unusual' coordination, because the improved 3.6 Å structure shows that P1 and P2 are coordinated by histidines of D helices (Zouni *et al.* 2001b), as is the case for the chlorophylls of P_{700} in PSI and the bacteriochlorophylls of the special pair in PbRC. The central ligand for the chlorophylls P3 and P4 and detailed knowledge of the protein-cofactor interaction for P1–P4 are still unknown, so the question of why P_{680} has such a high redox potential remains unanswered.

4. FUNCTIONAL IMPLICATIONS ON THE WATER-OXIDIZING COMPLEX

The special function of PSII, which has no counterpart in any other natural or artificial system, is the ability to oxidize water to O_2 and $4H^+$. PSII performs this reaction

by a cluster of four Mn ions bound to the proteins. This assembly is named the WOC. The reaction is performed in four steps: each charge separation event, driven by one absorbed photon, leads to a charge separation across the thylakoid membrane, forming P_{680}^+ , which extracts one electron from the WOC via the redox-active amino acid TyrZ. Oxygen is released when four electrons have been extracted from the WOC.

In the structural model at 3.8 Å resolution, a high electron density with the dimensions of $6.8 \text{ Å} \times 4.9 \text{ Å} \times 3.3 \text{ Å}$ was assigned to the Mn cluster and four Mn atoms have been tentatively placed within the electron density (see figure 4). The electron density is not spherical, but has a papaya-like structure so that the arrangement of the Mn ions resembles a 'Y'.

It should be emphasized that the assignment is still tentative, because no individual atoms can be resolved at this resolution. However, the shape of the electron density and the 3 + 1 arrangement is confirmed at 3.6 Å resolution.

More information concerning the distance between the Mn ions in its different S states is provided by EXAFS experiments (Yachandra *et al.* 1987; Cinco *et al.* 1999; Dau *et al.* 2001), leading to several models of PSII, which are in agreement with the EXAFS results. The C-shaped model, which was preferred and even arises in text books is not in agreement with the 3 + 1 arrangement of Mn as revealed from the X-ray structural model. However, several EXAFS models fulfil this 3 + 1 request. Two of them are shown in figure 5. An arrangement, where three of the four Mn atoms are arranged in triangular formation was also favoured by Carrell *et al.* (2002), because it fulfils the requirement of 'spin frustration' (spins are not able to achieve an anti-parallel orientation), which is necessary to get a confident interpretation of the EPR spectra in the different S states. Dismukes and co-workers exclude all linear or cubic arrangements, because the spins can be orientated anti-parallel in these geometries. The two models illustrated in figure 5 do not contradict any of the present results and are in good agreement with the recent interpretations of the electron density map.

There are many models for the mechanism of water oxidation giving rise to oxygen evolution and H^+ release (for example, Haumann *et al.* 1997b; Schlodder & Witt 1999; Messinger 2000; Noguchi & Sugiura 2000). One of the most striking models for the water-oxidation mechanism is the H-extraction model of Babcock and co-workers (Westphal *et al.* 2000). The principle was that a hydrogen atom is directly extracted from water molecule(s) bound to the Mn cluster by TyrZ. TyrZ has to be located in close proximity to the Mn cluster for this direct extraction mechanism to occur. The X-ray structural model shows that the distance between the keto-oxygen group and the Mn cluster is in the range of 7–8 Å, a distance which is too large to allow direct hydrogen abstraction. However, this finding does not exclude a mechanism where hydrogen atoms are extracted from the WOC by a water network, which might be located between TyrZ and the Mn cluster.

The next question, which has long been a matter for debate, is how and in which step, protons are released from the WOC. Several groups proposed an important role for His190, by a direct proton transfer from TyrZ (Tang *et al.* 1993; Berthomieu *et al.* 1998; Hays *et al.*

Figure 3. The cofactors of the electron transport chain in photosystem II. (a) The cofactors as named in Zouni *et al.* (2001). (b) Assignment of the cation radical and the triplet state of P_{680} to individual chlorophylls.

Figure 4. Electron density of the Mn cluster. The contour level shown is at 4σ . The four Mn atoms are tentatively placed within the electron density. (a) View along the membrane plane. (b) View from the luminal side onto the membrane plane.

Figure 5. Models of the Mn cluster previously derived from EXAFS investigations by Yachandra *et al.* (1987), which are in agreement with the present X-ray structural data by Zouni *et al.* (2001a,b) and the interpretation of EPR spectra, discussed in Carrell *et al.* (2002).

1998). In the present X-ray structural model (Zouni *et al.* 2001b), His190 is not in close proximity to TyrZ, so the question remains open. However, there are several protonable groups located close to TyrZ that could fulfil this function. Furthermore, a cluster of water molecules could mediate proton transfer between the Mn cluster and protonable amino acids, as discussed above for the H-extraction from TyrZ. Different patterns of proton release have been published during recent years (for example, Schlodder & Witt 1999; Haumann *et al.* 1997b; Messinger 2000; Noguchi & Sugiura 2000). In samples where the extrinsic subunits have been depleted, a 1 : 1 : 1 : 1 stoichiometry has been found, whereas in most of the publications on intact PSII complexes protons are released in a 1 : 0 : 1 : 2 pattern (see Haumann *et al.* (1997a) and references therein). The present X-ray structure shows that the Mn cluster is deeply buried within the protein, shielded from the aqueous phase both by the loops of the

Figure 6. A comparison of the antenna systems of PSI and PSII. (a) View from the stromal side onto the membrane plane (i) PSI, (ii) PSII. (b) View parallel to the membrane plane. (i) PSI: antenna chlorophylls coordinated by PsaA are shown in green, antenna chlorophylls coordinated by PsaB are shown in blue and the chlorophylls coordinated by the small membrane intrinsic proteins and the phospholipid are shown in yellow. The chlorophylls of the ET chain are depicted in red, the phyloquinones in white. The three FeS clusters are omitted from the figure for clarity. (ii) PSII: the antenna chlorophylls, which are supposed to be coordinated by CP47 are shown in green, antenna chlorophylls coordinated by CP43 are shown in blue and the two chlorophylls coordinated by D1 and D2 are shown in yellow. The chlorophylls and the pheophytins of the ET chain are depicted in red, the quinone Q_A is depicted in white. The two haem cofactors are shown in orange.

membrane intrinsic subunits and the three extrinsic subunits PsbO, PsbU and PsbV. From this we can conclude that it is very unlikely that the proton release pattern in the intact PSII complex reflects the direct proton release from the Mn cluster; instead protonable groups will be involved in this process. Several proton pathways were proposed in the structure of the cytochrome oxidase (Konstantinov *et al.* 1997; Ostermeier *et al.* 1997), raising the question of whether special pathways for the substrate water or the products, in the form of O_2 and protons, might exist in PSII. At the present time, the resolution of the structural model does not allow the identification of ordered water molecules, but this would be an interesting question which may be answered by an improved X-ray structure in the future.

Site-directed mutagenesis of the luminal loops of D1 in PSII revealed several amino acids that could provide ligands to the Mn cluster, and it has been shown by several groups that mutants in these sequence regions affect the oxygen-evolving activity of PSII (see Debus (2001) and references therein). The recent X-ray structure analysis shows that part of the A/B loop, the loop between the end of the transmembrane helix C and the luminal CD helix, as well as the C-terminal region of D1, are located in close proximity to the Mn cluster (see figure 2; Zouni *et al.* 2001b). In addition to the loops of D1, part of the loop region of CP43 is also located in close proximity to the Mn cluster, whereas proteins D2 and CP43 are not located within a distance of 10 Å from the Mn cluster. Comparing the structural model with the mutants, described in Debus (2001 and references therein), the following amino acids could be possible candidates for the

liganding of the Mn cluster: Asp170, the C-terminal Ala344 as well as the sequence region around Asn335.

5. FUNCTIONAL IMPLICATIONS ON THE ANTENNA SYSTEM

In the recent structural model of PSII, 26 chlorophylls have been assigned to the core antenna system. Twelve chlorophylls are coordinated by CP43 whereas 14 chlorophylls are coordinated by CP47. The two proteins D1 and D2 which coordinate the cofactors of the ET chain only provide the ligands for two antenna chlorophylls, probably by His118 and His117 in helix B of D1 and D2, respectively (Lince & Vermaas 1998; Stewart *et al.* 1998).

The arrangement of antenna chlorophylls in PSII is shown in figure 6. The antenna chlorophylls are arranged in two layers, one close to the stromal side, the other close to the luminal side of the membrane. All chlorophylls are located at quite long distances from the ET chain. ChlZD1 and ChlZD2 are the two chlorophylls that are closest to the ET chain, at a distance of 22 Å from P3 and P4. This arrangement of antenna chlorophylls in PSII shows some similarities but also striking differences to the arrangement of antenna chlorophylls in PSI.

The arrangement of chlorophylls coordinated by CP43 and CP47 resembles, somewhat, the arrangement of chlorophylls in the peripheral antenna domain of PSI. In both cases, the chlorophylls are arranged in two layers close to the membrane surface. However, PSI contains a central domain of the antenna system, which surrounds the inner core of 10 transmembrane helices. There are *ca.* 50 chlorophylls and 12 carotenoids located in the central

antenna domain of PSI, whereas the structure of PSII shows that the corresponding structural region in PSII only harbours the two chlorophylls ChlZD1 and ChlZD2. This explains why the excitation energy transfer in PSI is more efficient than in PSII. We therefore speculate on the question of why PSII has not developed a central antenna domain during evolution. The well-known phenomenon of photoinhibition may be the reason for the lack of the central antenna domain in PSII. The D1 protein is degraded during the process of photodamage and repair in the PSII complex (see Aro *et al.* 1993; Spetea *et al.* 1999; Bailey *et al.* (2002) and references therein). During repair, the whole PSII complex has to be opened up. Damaged D1 is proteolytically degraded and replaced by a new D1 protein. In PSI, the majority of the 50 chlorophylls of the central domain is coordinated by the C-terminal domain of PsaA and PsaB, which corresponds structurally to the arrangement of D1/D2 in PSII. If PSII also would contain a central antenna domain, all of these chlorophylls would have to be replaced during D1 repair, which would be a significant waste of energy.

Looking at the structure of PSII the question arises, how can efficient energy trapping occur, when all antenna chlorophylls are located at a large distance from the pigments of the ET chain. A major player in the antenna system has yet to be identified: the carotenoids. A carotenoid content of 10–12 has been reported for the intact PSII complex of cyanobacteria. There is striking evidence from biochemical and biophysical investigations (Telfer *et al.* 1994; Pascal *et al.* 1999; Tracewell *et al.* 2001b) that carotenoids may fill the missing link between the core antenna system and the ET chain (Tracewell *et al.* 2001a). It was speculated that two of them may be located between the ChlZD1/ChlZD2 molecules and P3/P4. An indirect link between the antenna system and the ET chain by carotenoids would be logical, because dangerous and damaging destruction of antenna chlorophylls by the high oxidation potential of P_{680}^{+} can be avoided. It will be interesting to see where the carotenoids appear in an improved X-ray structure model, the elucidation of which is in progress.

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Discussion

S. Styring (*Department of Biochemistry, Lund University, Lund, Sweden*). Could you please tell us something about the structure around Y-D-His190 on the D2 protein so as to draw analogies for the structure between Y_Z-OH and His 190 on the D1 protein?

P. Fromme. This is an interesting question, we will have to look at the structure in detail to answer this question.

A. W. Rutherford (*Service de Bioénergétique, CEA Saclay, France*). I have one question. You pointed out that the regions of the D1 and CP43 proteins are within 10 Å of the Mn cluster. Does this mean that there are no other polypeptides within 10 Å of the catalytic centre, for example, the 33 kDa protein?

P. Fromme. As we can say so far, where only 80% of the backbone of the PsbO is assigned, there is no region of PsbO which is located within 10 Å distance to the Mn cluster.

R. D. Britt (*Department of Chemistry, University of California, Davis, CA, USA*). In a Mn-free preparation we have found using ENDOR that there is a strong coupling between Y_D and histidine but not in the case of Y_Z. So this may support your findings.

P. Fromme. Yes, in the present structural model, there is no His within 5 Å distance of Y_Z.

W. Junge (*Abteilung Biophysik, Universität Osnabrück, Osnabrück, Germany*). I have one question. At the XII International Photosynthesis Congress in Brisbane last year you did not assign the position of cyclopentanone rings to the electron densities of the inner chlorophylls. Can you do it now?

P. Fromme. Yes, for most of the chlorophylls the orientation of the transition dipole moment can be determined, because the electron density shows the location of five and six rings of the chlorine headgroup, however the electron density of some of the chlorophylls in the periphery are not so well defined, therefore there are still some doubts concerning the transition dipole moments of these chlorophylls.

W. Junge. As to the assignment of P_{680}^{+} to the P1-chlorophyll in the D1 protein, do you agree that it is only localized on this chlorophyll at low temperatures (e.g. liquid nitrogen), whereas it is delocalized at room temperature because there is little leeway in the redox potentials of the the four chlorophylls to differ? We have recently tagged this D1 chlorophyll and found the cation to be localized on P1 only at low temperature but delocalized at higher temperatures.

P. Fromme. The location on P1 is based on the EPR experiments performed at low temperature. I find it very reasonable that the cation might be delocalized at room temperature, perhaps even between all four chlorophylls.

C. Dismukes (*Department of Chemistry, Princeton University, Princeton, NJ, USA*). I like the funnel model that you propose for the Mn_4 cluster. However, do the X-ray data actually locate the position of the Mn atoms or only the

outer boundary of the heavy atoms? Also, do you mean to eliminate the other models like the bent butterfly model?

P. Fromme. Individual Mn atoms cannot be located at the present resolution. The bent butterfly model is also in agreement with the present electron density assigned to the Mn cluster.

GLOSSARY

EPR: electron paramagnetic resonance
 ET: electron transport
 EXAFS: extended X-ray absorption fine structure
 PbRC: photo reaction centre of purple bacteria
 PSI: photosystem I
 PSII: photosystem II
 RC: photo reaction centre
 WOC: water-oxidizing complex