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Minireview

Current perceptions of Photosystem II

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Abstract

In the last few years our knowledge of the structure and function of Photosystem II in oxygen-evolving organisms has increased significantly. The biochemical isolation and characterization of essential protein components and the comparative analysis from purple photosynthetic bacteria (Deisenhofer, Epp, Miki, Huber and Michel (1984) J Mol Biol 180: 385–398) have led to a more concise picture of Photosystem II organization. Thus, it is now generally accepted that the so-called D1 and D2 intrinsic proteins bind the primary reactants and the reducing-side components. Simultaneously, the nature and reaction kinetics of the major electron transfer components have been further clarified. For example, the radicals giving rise to the different forms of EPR Signal II have recently been assigned to oxidized tyrosine residues on the D1 and D2 proteins, while the so-called Q_{400} component has been assigned to the ferric form of the acceptor-side iron. The primary charge-separation has been meaured to take place in about 3 ps. However, despite all recent major efforts, the location of the manganese ions and the water-oxidation mechanism still remain largely unknown. Other topics which lately have received much attention include the organization of Photosystem II in the thylakoid membrane and the role of lipids and ionic cofactors like bicarbonate, calcium and chloride. This article attempts to give an overall update in this rapidly expanding field.

1. Introduction

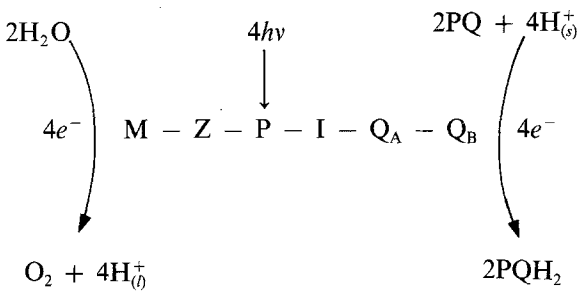
This article provides an update on current perceptions of Photosystem II (PS II) structure and function since the overall topic was last reviewed in this journal (van Gorkom 1985). During the interim time, many review articles have appeared in the literature on various aspects of PS II. Some of the more recent of these articles include the following: the light-harvesting components (Zuber et al. 1987, Peter and Thornber 1988), the primary photochemistry and reducing-side reactions (Diner 1987, Velthuys 1987, Mathis and Rutherford 1987, Rich and Moss 1987, Diner and Petrouleas 1988), the oxidizing-side reactions (Renger 1987a, b, Babcock 1987), the cofactor requirements (Homann 1987, Brudvig 1988), the protein components (Andersson and Åkerlund 1987, Green 1988), the molecular biology (Gray 1987, Chitnis and Thornber 1988, Shinozaki et al. 1989), organization in the membrane (Staehlin 1987) and general reviews (Anderson 1987, Witt 1987, Andrèasson and Vänngård 1988). The reader is referred to these articles for additional details.

Photosystem II can be defined as that part of oxygenic photosynthesis which catalyzes the photoinduced transfer of electrons from water to plastoquinone (PQ), i.e.

$$4H^+_{(s)} + 2PQ + 2H_2O \xrightarrow[\text{PS II}]{4h\nu} 2PQH_2 + O_2 + 4H^+_{(l)}. \quad (1)$$

In this formulation, in order to denote the vectorial organization of the reaction sequence across the

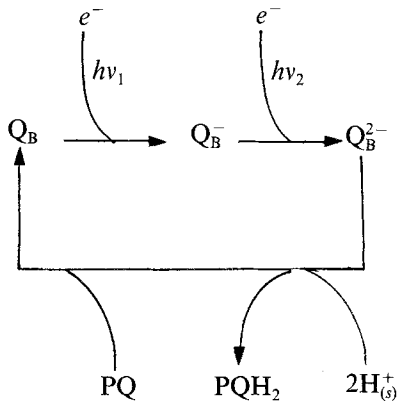
thylakoid membrane, the proton release and uptake are designated by subscripts (*l*) and (*s*), respectively, from the luminal (*l*) to the stromal (*s*) side. The minimal scheme to describe the PS II reaction sequence is typically given as follows:



(2)

where P is the primary electron donor chlorophyll (Chl) *a* molecule (i.e. P680) that is activated by a light quantum, I, the primary electron acceptor molecule, which is considered to be a pheophytin (Pheo) *a*, Q_A, the first bound plastoquinone acceptor, Q_B, the second bound plastoquinone acceptor, Z, the first electron donor to P^+ and M, the manganese-containing component that is involved in water oxidation and the release of O_2.

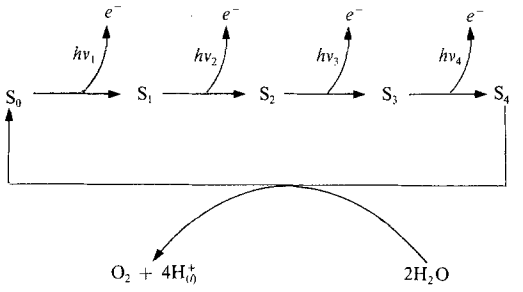
In order to couple the one-electron process of the primary reactants (P and I) with the two-electron process needed for PQ reduction and the four-electron process involved in H_2O oxidation (O_2 evolution), PS II has evolved unique binary and quartenary gating functions on the reducing and oxidizing sides, respectively. Thus, on the reducing side, Q_B is defined as a specially bound PQ molecule which undergoes a two-step reduction process (Velthuys 1981), i.e.



(3)

Upon one photochemical event of the primary reactants, the bound PQ becomes reduced to a semiquinone anion which is stabilized by the surrounding protein matrix. Upon a second photochemical event, the bound semiquinone becomes fully reduced, protonated and released from the Q_B binding site as plastoquinol (PQH_2). The binary cycle repeats itself as another PQ molecule binds at the Q_B site.

The quartenary gating function on the oxidizing side of PS II was at first elegantly demonstrated in the O_2-flash yield measurements of Joliot and coworkers (Joliot et al. 1969). As it is now well-established, the O_2 produced by a sequence of brief light flashes displays a damped oscillatory pattern with a periodicity of four beginning after the third flash in dark-adapted samples. Kok and coworkers (Kok et al. 1970) described the O_2-flash yield pattern in terms of a S-state cycle which is driven by successive photoactivations of PS II, i.e.



(4)

In Kok's original formulation, each S_n state (for $n = 0$–3) represented a charge distribution state of PS II, the advancement of which was governed by the ability of the primary reactants to undergo a charge separation upon photoactivation. The S_4 state was defined as that state of PS II which reacted to release O_2 and to regenerate the S_0 state. In more modern usage, the S_n states are often used in reference to the number of electrons extracted from the manganese-containing component on the oxidizing side of PS II (that is, M, as defined in Reaction Sequence 2 above).

Other reaction components of PS II which may interact with the flow of electrons from water to PQ, but are not designated in Reaction Sequence 2 above, include: cytochrome *b*559 (cyt *b*559), a dark-stable free radical component which gives rise to EPR Signal II_{slow} and usually has been denoted as

D^+, a non-heme iron (Fe) which is associated with the reducing-side quinone acceptors, carotenoids, monomeric forms of Chl *a* and a second Pheo *a* molecule. Details on these components will be expanded in the following sections.

2. Structural aspects of Photosystem II

The most significant advancements in PS II research since 1980 have been in the biochemical characterization. More than 22 polypeptides have been suggested to be associated with PS II (Masojidek et al. 1987). One possible scheme to describe the organization of PS II is shown in Fig. 1. In this hypothetical picture, PS II is subdivided into four functional parts: (1) the Reaction Core, which contains the primary reactants, the O_2-evolving components and the PQ-reducing components, (2) a Regulatory Cap, which is made up of a set of hydrophilic proteins that are extrinsically bound to the luminal surface of the membrane, (3) a Proximal Antenna and (4) a Distal Antenna. In the following sections each of these functional parts is discussed along with the overall organization of PS II in the thylakoid membrane.

2.1. Reaction core

Within the last two years, the primary reactants and the reducing-side components of PS II have been assigned to the so-called D1 and D2 intrinsic proteins of PS II (D1 $\approx$ 32 kDa and D2 $\approx$ 34 kDa, where 'D' refers to 'diffuse bands' on Coumassie-stained urea SDS-PAGE gels). Originally, this assignment was based on homology arguments for the gene sequences between the D1 and D2 proteins and the L and M subunits of the reaction center complex of purple bacteria (see Hearst 1987). The recent isolation of a D1/D2/cyt

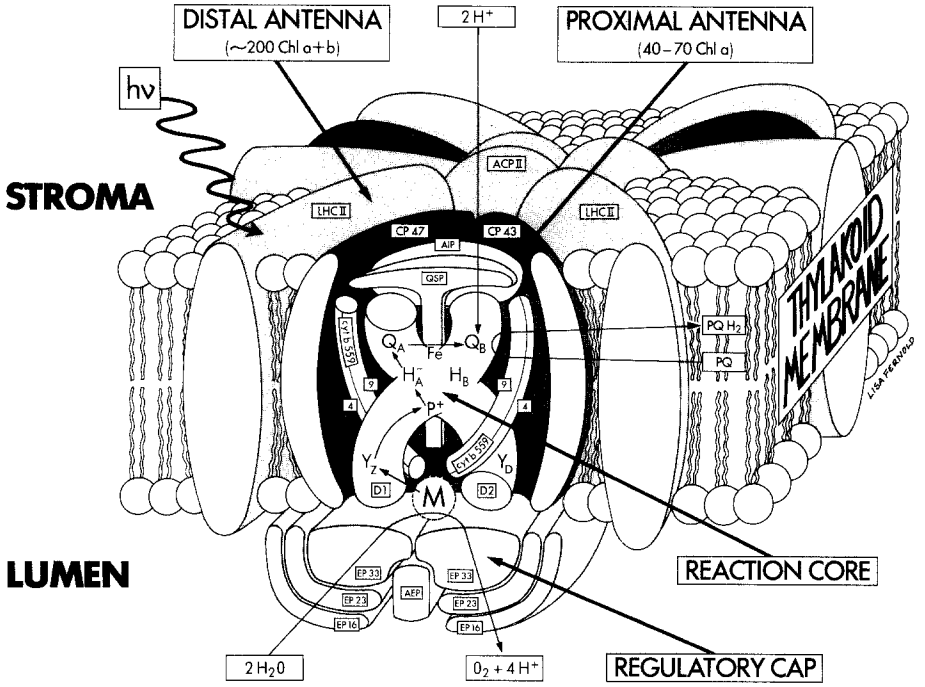


Fig. 1. A schematic representation of the functional organization of Photosystem II. The definition of symbols are as follows: P, the primary electron donor chlorophyll (Chl) (P680); H_A, the primary electron acceptor pheophytin (Pheo); H_B, a second Pheo; Q_A and Q_B, the first and second quinone electron acceptors, respectively; Fe, a non-heme iron atom; Y_Z, the tyrosine first electron donor to P; Y_D, a second tyrosine; M, the manganese-containing component involved in oxygen evolution; cyt *b*559, cytochrome *b*559 heterodimer; D1 and D2, Reaction Core subunits, QSP, quinone-shielding protein; AIP, accessory intrinsic proteins; EP 33, EP 23 and EP 16, extrinsic proteins of the Regulatory Cap; AEP, accessory extrinsic proteins; CP 47 and CP 43, Chl proteins of the Proximal Antenna; ACP II, accessory Chl proteins of the Distal Antenna; and LHC II, the Chl *a*/*b* light-harvesting complex. See the text for details.

*b*559 complex (Nanba and Satoh 1987, Barber et al. 1987, Seibert et al. 1988b) which contains a minimal amount of Chl and Pheo and which can undergo photochemistry (Danielius et al. 1987, Takahashi et al. 1987, Chapman et al. 1988, Wasielewski et al. 1989) has greatly added support to this assignment. Furthermore, both reducing-side and oxidizing side properties of PS II are located on the same protein (i.e. on D1) (Metz et al. 1986, Bishop 1987, Ikeuchi and Inoue 1987). Finally, the chemical identity of the dark-stable free radical giving rise to EPR Signal II_{slow} (i.e. D^+) has recently been assigned to a tyrosine residue (Barry and Babcock 1987) located on the D2 protein (Debus et al. 1988a, Vermaas et al. 1988a). The first electron donor to P^+, which gives rise to EPR Signal $II_{very\ fast}$ (i.e. Z), is most likely a tyrosine residues located on the D1 protein (Debus et al. 1988b, B. Diner, personal communication).

Due to these recent developments, we will depict the components Z and D as Y_Z and Y_D, respectively (Hoganson and Babcock 1988), where 'Y' is the biochemical symbol for tyrosine. Likewise, we will use the designation H_A for the primary electron acceptor molecule, where 'H' has been commonly used for the photosynthetic bacteria as the single letter notation for pheophytin. Consequently, a second Pheo *a* molecule (Nanba and Satoh 1987) will be designated as H_B. Thus, in Fig. 1, the primary reactants (P^+ H_A^-), the quinone electron acceptors (Q_A and Q_B), the associated non-heme iron (Fe), and the tyrosine electron donor to P^+ (Y_Z) are shown to be located on a D1/D2 heterodimer within the Reaction Core. Although no experimental evidence exists at present, the second Pheo *a* (H_B) and the dark-stable tyrosine radical (Y_D^+) may be located on a functionally separate part of the heterodimer. A separate 'non-functional arm' is known to exist in the purple bacteria (Michel et al. 1986). The D1/D2 heterodimer also contains β-carotene and some forms of Chl *a* other than P (Nanba and Satoh 1987) which are not depicted in Fig. 1. The additional Chl *a* may represent monomeric forms located between P and the pheophytins, as is known to be the case in purple bacteria.

The D1/D2 heterodimer is known to span the mebrane. Initially, the D1 subunit (originally called the Q_B-protein) was inferred to have 7–8 transmembrane segments based on its primary structure (see Kyle 1985). However, functional and sequence homologies with the L/M heterodimer of *Rhodopseudomonas viridis* (Deisenhofer et al. 1985), in combination with the analysis of amino-acid changes in herbicide-resistant plants (see Trebst and Depka 1985), led to the suggestion that both the D1 and D2 subunits have five transmembrane segments each. The latter folding pattern has received support in recent antibody studies (Sayre et al. 1986, Andersson et al. 1987). Apparently, the conformation of the D1 subunit is modulated depending on whether the Q_B site is occupied or not (Trebst et al. 1988). Both the D1 and D2 subunits can be phosphorylated (Millner et al. 1986, Ikeuchi et al. 1987, Marder et al. 1988).

A variety of spectroscopic methods have been used to investigate the nature and relative positions of the electron transfer components in the Reaction Core. In general, studies of the electric fields generated by the electron-transfer reactions show that the components are organized vectorially across the membrane, with the quinone acceptors towards the stromal side and P towards the luminal side (see Witt 1987).

The state of aggregation of P (whether it is Chl monomer or dimer) is controversial. Based on redox properties (Davis et al. 1979), optical (Mathis and Setif 1981, den Blanken et al. 1983) and EPR data (Rutherford et al. 1981) it is likely that the hole of the cationic form and the triplet state of P obtained on recombination reside on a monomeric species. It could be that P is a pair of Chl *a* molecules which interact strongly in the singlet excited state and weakly in the triplet state or when it is a cation. Studies on the triplet state of P indicate that the Chl is oriented with its macrocycle parallel to the membrane plane (Rutherford 1985a), markedly different to what is found in the purple bacteria (Tiede and Dutton 1981). Possibly, the triplet state of P in PS II migrates to an accessory monomeric Chl with a different orientation (Rutherford 1985b).

Studies of the electric fields generated by the electron transfers from P to the primary acceptor, H_A, and from H_A^- to the first quinone acceptor, Q_A, indicate that the distances between P and H_A and between H_A and Q_A are approximately equal (Trissl et al. 1987) [however, see (Meiburg et al. 1983)]. The orientation of H_A in PS II (Ganago et al. 1982)

is similar to what is observed in purple bacteria, where the Q_Y transition moment is parallel to the membrane normal. Resonance Raman spectroscopy shows that, as in purple bacteria, the 9-keto carbonyl of H_A is hydrogen-bonded to a glutamic residue (Glu130 of D1) (Moënne-Loccoz et al. 1989).

The electrochromic bandshifts in the blue and green parts of the Pheo optical spectrum when Q_A is reduced are stronger than when Q_B is reduced. This indicates that Q_A^- is closer to a Pheo molecule than Q_B^- (Schatz and Van Gorkom 1985). The EPR signals from the $Fe^{2+}Q_A^-$ complex (see Rutherford and Zimmerman 1984) are very similar to those observed in purple bacteria. This suggests that a 7 Å distance between the iron and the quinone ring of Q_A is conserved in PS II (see Mathis and Rutherford 1987).

The distance between P and Y_Z has been estimated to be 10–15 Å based on time-resolved EPR data (Hoganson and Babcock 1989). Since no change in the membrane potential was observed to accompany the Y_Z to P^+ electron transfer (Trissl et al. 1987), this electron transfer may occur laterally in the membrane. For the Y_D component, EPR studies indicate that the normal of its aromatic plane makes an angle of 60° with the membrane normal (Brok et al. 1986). A weak magnetic interaction between Y_D^+ and the manganese in the M component is detectable (de Groot et al.1986, Styring and Rutherford 1988, Isogai et al. 1988) (however, see Innes and Brudvig 1989), suggesting that the distance between these two components is large (30–40 Å) (Evelo et al. 1989). The distances from the stromal and luminal membrane surfaces to Y_D^+ have been estimated to be 26 and 27 Å, respectively (Innes and Brudvig 1989).

In addition to the L and M subunits, the reaction center complex of purple bacteria contains a third polypeptide called the H-subunit. The H-subunit does not bind any of the electron transfer components, but is probably important in optimizing the conformation of the L/M heterodimer (Deisenhofer et al. 1985). A similar situation may exist in PS II (Renger 1986). In Fig. 1, a cap-like structure is depicted above the acceptor side of the D1/D2 heterodimer. It is simply designated as QSP for 'quinone shielding protein'. Based on gene sequence homologies, a phosphorylatable protein with an apparent molecular mass of 9–10 kDa (Farchaus and Dilley 1986) has been suggested to be the PS II analog to the H-subunit (Packham 1988). The plastid-encoded gene for this protein yields a primary translation product of 73 amino acid residues with a calculated molecular mass of 7.8 kDa in spinach (Hird et al. 1986, Westhoff et al. 1986). The predicted primary structure reveals a single transmembrane segment and a large hydrophilic extension at the N-terminus which would presumably be located towards the stromal side of the membrane. There is about a 20% homology between this protein and the H-subunit. However, this protein has only been isolated from intact appressed thylakoid membranes and has not been associated with the Reaction Core. Nevertheless, its phosphorylation at threonine 2 on the N-terminal hydrophilic extension (Michael and Bennett 1987) is followed by a slight inhibition of O_2 evolution (Packham 1987) which is possibly mediated via an altered equilibrium between Q_A and Q_B (Hodges et al. 1987, Packham et al. 1988). Alternatively, another protein having an apparent molecular mass of 22 kDa has been implicated in the regulation of PS II reactions (Ljungberg et al. 1986a). This protein exhibits both hydrophilic and hydrophobic characteristics.

Although the similarities between PS II and purple bacteria are remarkable, there must also be very large differences since the purple bacteria do not evolve O_2. Thus, PS II contains manganese which is required for O_2 evolution (see Amesz 1983). The exact location of the manganese is unknown, since a functionally active manganese-containing protein has not yet been identified. However, there is indirect evidence based on mutant studies that the amount of bound manganese in PS II correlates inversely with a modified 34–36 kDa (Bishop 1987) [which is probably the precursor protein of D1 (see Gray 1987)]. It has been suggested that the functionally active manganese binds to the D1/D2 heterodimer (Coleman and Govindjee 1987, Sauer et al. 1988, Dismukes 1988). It is clear that the functionally active manganese is situated towards the luminal side of the membrane, probably within a restricted compartment (Miller and Cox 1984) which is formed between the Reaction Core and the extrinsic 33 kDa protein of the Regulatory Cap (see below)

(Seibert et al. 1988a, Yamamoto 1988). Until more information becomes available, the manganese-containing component, M, is simply depicted in Fig. 1 in such a way that it can be ligated to any number of the protein components.

The PS II Reaction Core also contains at least one heme, cyt *b*559 (see Cramer et al. 1986). It has long been known that cyt *b*559 can be photooxidized at 77 K, but its physiological role in PS II remains elusive. Although cyt *b*559 can exist in high- and low-potential forms, the conversion between these does not correlate with activity changes (Briantais et al. 1985, Ghanotakis et al. 1986, Bergström and Franzén 1987). Likewise, the kinetics of cyt *b*559 oxidation do not correlate with the overall kinetics of the main reaction sequence in PS II. Recently, it has been suggested that cyt *b*559 may have a photoprotective role (Thompson and Brudvig 1988) or that it could be involved in a proton pump (Arnon and Tang 1988) in connection with a cyclic electron flow around PS II (Falkowski et al. 1986).

The apoprotein of cyt *b*559 consists of two subunits with apparent molecular masses of 9 and 4 kDa. Each subunit contains a single histidine residue. Spectroscopic evidence shows that heme coordination in cyt *b*559 is *bis*-histidine (Babcock et al. 1985). Consequently, it is usually suggested that the heme binds across the two subunits. The most recent information indicates that there are two copies of the cyt *b*559 apoprotein per PS II unit and that the apoprotein spans the membrane with at least one heme located towards the stromal side (Tae et al. 1988). In Fig. 1, two cyt *b*559 heterodimers are shown to be tightly coupled to the D1/D2 heterodimer.

The core of PS II may contain other intrinsic proteins with apparent molecular masses of 24 kDa (Ljungberg et al. 1984), 22 and 10 (10.8) kDa (Ljungberg et al. 1986a, Lautner et al. 1988), 7–6.5 and 3.7 kDa (Schröder et al. 1988), 4.8 (4.15) kDa (Ikeuchi and Inoue 1988) and 2 (4.28) kDa (Murata et al. 1988) (values within parenthesis have been calculated from gene sequences). The function of these proteins is not certain at present, but some may serve as structural components needed to optimize the Reaction Core conformation and others may merely represent protein remnants that are not needed for PS II function. These additional proteins are simply represented in Fig. 1 as a blob labelled AIP, for 'accessory intrinsic proteins'.

2.2. Regulatory cap

In another contrast to the purple bacteria, PS II contains what is defined in Fig. 1 as a Regulatory Cap. It is comprised of a set of hydrophilic proteins that have apparent molecular masses of about 33, 23 and 16 kDa. These components are designated as EP 33, EP 23 and EP 16, where EP stands for 'extrinsic protein'. The Regulatory Cap proteins are extrinsically bound to the luminal surface of the membrane (see Andersson and Åkerlund 1987) and are usually assigned functions in regulating the ionic requirements for O_2 evolution (i.e. the chloride and calcium requirements) or in controlling the exchange of reactants with the manganese center (see Section 3.4). The genes for these proteins give predicted processed translation products of 247 (Tyagi et al. 1987), 186 and 149 (Jansen et al. 1987) amino acid residues and calculated molecular masses of 26.5, 20.2 and 16.5 kDa for the EP 33, EP 23 and EP 16, respectively. The EP 33, EP 23 and EP 16 are present in equimolar amounts (Murata et al. 1984, Andersson et al. 1984b). However, it is still controversial whether they exist as one or two copies per PS II unit, although crosslinking studies favor the latter possibility (Milner et al. 1987). In Fig. 1 two copies of each are depicted. The Regulatory Cap may contain another extrinsic protein as well, which has an apparent molecular mass of 5 kDa (Ljungberg et al. 1986b). In Fig.1, possible additional extrinsic proteins in the Regulatory Cap are designated as AEP, for 'accessory extrinsic proteins'.

Based largely on cross-linking (Bowlby and Frasch 1986, Machold 1986, Milner et al. 1987, Enami et al. 1989), protease (Isoagai et al. 1985) and immunological studies (Ljungberg et al. 1984, Ivy and Berg 1985, Aoki et al. 1986, Camm et al. 1987), the EP 33 can link directly with the Reaction Core and Proximal Antenna components, whereas the two smaller proteins EP 23 and EP 16 appear to shield the EP 33 from the luminal space and can link to the LHC II complex of the Distal Antenna. Antibodies to CP 47 of the Proximal Antenna (see below) and D1 will not bind to the PS II complex unless EP 33 is removed (Bricker et al. 1988, Sayer

et al. 1986). Thus, the hydrophilic extensions of CP 47 and D1 (Alt et al. 1984) may constitute a border region with the EP 33 (Andersson et al. 1987).

It has been suggested that the binding site of the EP 33 on the luminal surface of the membrane is positively charged, while the binding site of the EP 23 is negatively charged (Isogai et al. 1987). This is related to the removal of these proteins by various types of salt washes (see Andersson and Åkerlund, 1987). Recent electron-microscopy studies show that the extrinsic proteins protrude from the membrane surface. The removal and reconstitution of them leads to a loss and recovery of multimeric (tetrameric) structures on the ESs surface (i.e. the luminal side of appressed membranes) of freeze-etched PS II membrane preparations (Simpson and Andersson 1986, Seibert et al. 1987, Bassi et al. 1988a). After removal of the extrinsic proteins, dimeric structures are observed which may indicate two-fold symmetry in the remaining parts of PS II or that PS II exists as dimeric units in the membrane. In another electron-microscopy study, a top view of isolated PS II core complexes lacking the extrinsic proteins was found to be triangular shaped and could represent half of a dimeric unit (Irrgang et al. 1988).

2.3. Proximal antenna

The light-harvesting array for PS II as indicated in Fig. 1 is divided into two major parts, the Proximal Antenna and the Distal Antenna. The Proximal Antenna has a long-wavelength absorption band essentially matching that of the primary donor, and therefore functions to couple energy transfer from the bulk of the light-harvesting pigments in the Distal Antenna to the Reaction Core. The possible modes of energy transfer among the light-harvesting pigments have recently been reviewed (van Grondelle 1985, Pearlstein 1987).

The Proximal Antenna consists of two pigment-protein complexes, often called CP 47 and CP 43 (or CPa-1 and CPa-2, where CP stands for 'Chl-Protein complexes') (see Green 1988). These two proteins have apparent molecular masses of 45–51 and 40–45 kDa, respectively. They both consist of single subunits with possibly seven transmembrane segments containing 11–12 His residues, presumably involved in Chl binding, and a large hydrophilic segment towards the luminal side (Alt et al. 1984, Morris and Herrmann 1984). Only the CP 43 can be phosphorylated (Ikeuchi et al. 1987). Each protein binds 20–25 Chl *a* and about 5 β-carotene, but no Chl *b* or Pheo. Most of the Chl is oriented with the Q_X transition moment parallel to the membrane normal (Breton and Katoh 1987).

Recent low-temperature fluorescence and absorption spectroscopy have revealed several dissimilarities between CP 43 and CP 47 (Breton and Katoh 1987, van Dorssen et al. 1987). The fluorescence at 77 K peaks at 685 and 695 nm for CP 43 and CP 47, respectively, as was shown earlier (Nakatani et al. 1984). In addition to its main absorption at 669 nm, CP 43 also exhibits a band at 682 nm polarized perpendicular to the membrane normal. The absorption and linear dichroism in the red region of CP 47 is even more complex with bands at 660, 668, 677 and 690 nm. The latter band is probably associated with a single Chl *a* molecule with an unusual orientation of its Q_Y transition moment parallel to the membrane normal. It was tentatively assigned to the species responsible for the low-temperature fluorescence band F695. In earlier work the F695 band was suggested to arise from the photoactive Pheo (Breton 1982) and therefore the CP 47 was believed to harbor the primary reactants (see Green 1988). However, with the recent developments now assigning the primary reactants to the D1/D2 heterodimer, this is evidently not the case. The presence of the Chl species responsible for the F695 band would mean that CP 47 contains an energy trap deeper than the primary reactants themselves.

The orientations of the β-carotenes are different between CP 43 and CP 47, which those of CP 43 being largely parallel, and those of CP 47 perpendicular, to the membrane normal. The absorption maxima of the β-carotenes of CP 43 were found to be blue-shifted by 10–15 nm as compared to those of CP 47 (Breton and Katoh 1987).

The Proximal Antenna is apparently tightly coupled to the Reaction Core. The CP 47 and CP 43 proteins are always present in O_2-evolving preparations of PS II from higher plants. Recently, it has been shown that electron transfer from exogenous donors to Q_A can take place in the absence of CP 43 (Yamaguchi et al. 1988) but that the ability to reduce Q_A is lost when both CP 43 and CP 47 are removed (Akabori et al. 1988). Inactivation

of the genes for CP 47 or CP 43 by site-directed mutagenesis prevents or reduces, respectively, the assembly of the Reaction Core (Vermaas et al. 1988b). At present, the Proximal Antenna cannot be totally excluded from having functions in either direct binding or regulation of the electron transport components.

2.4. Distal antenna

The Distal Antenna contains accessory pigments at variable amounts with which the organism can extend its light absorption to other wavelengths and respond to varying conditions of light intensity and spectral distribution. The Distal Antenna as diagrammed in Fig. 1 is shown to consist of at least two parts, the LHC II and the ACP II. The LHC II is the classical light-harvesting Chl *a*/*b* complex, which is involved in the stacking of membranes (see Staehlin 1986, Anderson 1987) and the partitioning of energy between PS II and PS I (see Briantais et al. 1986). The LHC II contains several separable, oligomeric components (as many as six in spinach) consisting of proteins with apparent molecular masses of 25 and 27 kDa (see Peter and Thornber 1988). It has a Chl *a*/*b* ratio of 1.2 $\pm$ 0.2 and contains xanthophyll as the major carotenoid. About 30% of the total LHC II can be phosphorylated by membrane-bound kinase which is activated by high levels of reduced PQ. Part of the phosphorylated LHC II then constitutes a 'mobile' fraction which dissociates from PS II and migrates from the appressed to the non-appressed regions of the thylakoid membrane, where it is proposed to increase the light-harvesting capability of PS I (Larsson et al. 1987, Bassi et al. 1988b). The energy coupling between the LHC II, both the 'mobile' and 'non-mobile' parts, and the Reaction Core is also dependent upon the membrane surface charge (Zucchelli et al. 1988).

There are a number of other Chl proteins associated with the PS II Distal Antenna that are not part of the LHC II. We loosely denote these as ACP II in Fig. 1, for 'accessory Chl proteins'. Among these components are CP 29 (see Green 1988) and the recently discovered CP 26 and CP 24 (Dunahay and Staehlin 1986, Bassi et al. 1987) (the numbers after 'CP' denote the apparent molecular masses in kDa). These Chl proteins are separable from LHC II and generally have a slightly higher Chl *a*/*b* ratio of 2–3. None of these proteins are phosphorylated. The components of the ACP II may be more closely coupled to the Proximal Antenna and the Reaction Core and may serve as a 'linker' or 'anchor' for the LHC II proteins. One suggestion has been that these Chl proteins may participate in the dissipation of excess excitation energy arriving at the Reaction Core as a means to counteract photoinhibition (Bassi et al. 1987). In higher plants, the total Distal Antenna contains about 200 Chl (*a* + *b*) molecules per PS II unit.

1.3. Organization of PS II in the thylakoid membrane

Crucial to the overall organization of PS II within the thylakoid membrane are the inherent lipids (see Murphy 1986, Gounaris et al. 1986, Anderson 1987). The lipids constitute about 25–30% of the total thylakoid mass. The neutral galactolipids monogalactosyldiglyceride (MGDG) and digalactosyldiglyceride (DGDG) make up the major fraction (75%), while the remaining fraction consists of the negatively charged lipids phosphatidylglycerol (PG) (10%) and sulphoquinovosyldiacylglyceride (SQDG) (10%), and a small amount of other phospholipids (5%). The lipid distribution in thylakoid membranes is most likely asymmetric, both transversely across the membrane as well as laterally. The asymmetric transverse distribution is difficult to determine, but studies with rightside- and inside-out thylakoids indicate that about 60% of the total neutral galactolipids occurs towards the stromal side of the membrane (Sundby and Larsson 1985). Thus, the luminal side of the membrane may contain more of the other lipid components and hence would be more negatively charged. The lateral asymmetric distribution of lipids is more easily determined, since appressed and non-appressed regions can be spearated. The appressed regions have a higher MGDG/DGDG ratio and an enrichment of anionic lipids relative to the non-appressed region. In the appressed regions the lipids make up only 14% of the membrane mass, while in non-appressed regions they make up 40%.

It has been found that the addition of DGDG or the phospholipid phosphatidylcholine (PC) stimulates O_2-evolution activity of PS II-enriched

samples, while the anionic lipids SQDG and PG inhibit activity (Gounaris et al. 1983). Also, it was shown that the degree of saturation of the membrane lipids influences PS II activity (Horváth et al. 1987). Although it has been shown that the processed D1 subunit is post-translationally palmitoylated (Mattoo and Edelman 1987), there has been no direct evidence for specific association of membrane lipids within PS II. It has been observed, however, that the D1 subunit is more resistant to glutaraldehyde crosslinking than the D2 subunit and cyt *b*559, unless the sample is perturbed with specific detergents (i.e. glucosides having 7–9 carbon atom alkyl chains) (Adir and Ohad 1987). This suggests that the D1 confirmation might be stabilized by interactions with adjacent saturated lipids containing 7–9 carbon atom chains and would imply that the lipids may serve an important role at the heart of the Reaction Core. In support of this conclusion are the observations that lipids are essential for a stable charge separation between P^+ and Q_A^- (Jordan et al. 1983, Eckert et al. 1987b, Akabori et al. 1988) and that addition of linolenic acid blocks electron transport at Q_A (Warden and Csatorday 1987). Likewise, lipids are probably essential for the O_2-evolving step itself since, of the S-state transitions only the S_3 to S_4 to S_0 step shows a break in an Arrhenius plot (Koike et al. 1987), characteristic of a phase transition. Finally, the O_2-evolving step can be specifically inhibited by lauroyl choline, a lipid analog (Wydrzynski et al. 1985).

The division of thylakoid membranes into appressed and non-appressed regions is associated with an asymmetric distribution of PS II (see Murphy 1986, Gounaris et al. 1986, Staehlin 1986, Anderson, 1987). Thus, it is believed that PS II is primarily located in the appressed (or granal) regions and PS I in the non-appressed (or stromal) regions, although some PS II exists in the non-appressed regions as well. Functional heterogeneity of PS II described as α and β units (see Black et al. 1986) and arguments concerning PS II to PS I stochiometry (McCauley and Melis 1986, Chylla et al. 1987) may be related to the asymmetric distribution of PS II. For example, in non-appressed regions, it may be that PS II units are being newly synthesized or reprocessed such that the acceptor side is altered. It is known that the D1 subunit turns over rapidly (see Kyle and Ohad 1986), perhaps in response to photoinhibition at the Q_B site (Kirilovsky et al. 1988, Ohad et al. 1988) and/or at the primary reactants (Arntz and Trebst 1986, Cleland et al. 1986), and that the 34.5 kDa precursor protein to the D1 subunit is processed for incorporation into the thylakoid membrane in the non-appressed regions (see Gray 1987).

There may be additional protein components associated with PS II other than those defined in the previous section. For example, a 62 kDa protein has recently been identified in intact thylakoid samples and was suggested to be involved in a chloride channel through the membrane (Vambutas and Beattie 1987). Since the chloride requirement for photosynthesis is believed to be restricted to PS II (see Section 3.4), it would be interesting to determine whether this protein is associated with PS II. Often 60 kDa proteins are observed in PS II preparations, but these are usually considered to be aggregates of D1/D2 and/or smaller proteins (Steinmetz et al. 1986, Satoh et al. 1987).

The functional organization of PS II as depicted in Fig. 1 can be used to illustrate the properties of various PS II preparations. Thus, the entire PS II complex as shown would represent the PS II-enriched inside-out thylakoid vesicles (i.e. the appressed regions of the membranes) (Åkerlund and Andersson 1983) and the oxygen-evolving detergent-treated PS II preparations (Berthold et al. 1981, Kuwabara and Murata 1982). The PS II complex as shown but excluding the Distal Antenna, with or without ACP II components, would represent the first non-oxygen-evolving core preparations (see Yamada et al. 1985) and the later oxygen-evolving core preparations (Ikeuchi and Inoue 1985, Tang and Satoh 1985, Satoh et al. 1985, Franzén et al. 1986, Ghanotakis et al. 1987), which have various degrees of damage on the reducing side. Finally, the Reaction Core itself would correspond to the newly developed D1/D2/cyt *b*559 preparations, which currently show damages on both the oxidizing and reducing sides and are relatively unstable (Nanba and Satoh 1987, Barber et al. 1987, Seibert et al. 1988b, Ghanotakis et al. 1989).

Although spinach represents the most commonly used higher plant source for PS II preparations, there is also a wealth of literature on the use of preparations from the prokaryotic cyanobacteria.

The function of PS II in these organisms is basically the same as in higher plants, but there are some major structural differences (see Glazer 1984, Staehlin 1986, Zuber et al. 1987). The cyanobacteria do not form appressed regions of thylakoid membranes and thus do not have the same type of separation of PS II and PS I as in higher plants. In addition, the thylakoid membranes are also believed to be the site of the respiratory chain. The organization and composition of the Distal Antenna in cyanobacteria are very different as well. The cyanobacteria do not contain Chl *b* but use phycobilins instead as the accessory light-harvesting pigments. These pigments are organized into well-defined structures called phycobilisomes which protrude from the membrane surface and are connected to the Reaction Core complexes within the membrane via special anchor proteins.

The proteins of the Proximal Antenna and Reaction Core in cyanobacteria are similar to those found in higher plants (see Bricker et al. 1986). However, in the Regulatory Cap, the EP 16 and EP 23 are not present (Stewart et al. 1985a). Instead, another extrinsic protein with an apparent molecular mass of 9–10 kDa may be important in function (Stewart et al. 1985b, Rolfe and Bendall 1989). Highly O_2-evolving PS II preparations can be made from cyanobacteria using zwitterionic detergents with 12 carbon atom long chains (e.g. LDAO and SB 12) but these preparations usually require the presence of a high osmoticum (e.g. glycerol) and high concentrations of Ca^{2+} ions in order to exhibit maximum activity. As in higher plants, electron microscopy of PS II particles from cyanobacteria indicate dimeric structures (Rögner et al. 1987). Certainly, the optical properties of cyanobacterial PS II preparations have greatly assisted in the spectroscopic characterization of the PS II primary photochemistry (Schlodder et al. 1984) and the ability to perform site-directed mutagenesis in cyanobacteria (e.g. with *Synechocystis* sp. PCC 6803) was essential for the identification of Y_D as a tyrosine residue in the D2 protein (Debus et al. 1988a, Vermaas et al. 1988a).

3. Functional aspects of photosystem II

A division of PS II into structural and functional aspects may seem artificial since these concepts are so strongly interrelated. However, in the following sections, emphasis will be put on the more mechanistic details such as the nature of the reactants involved, their relative ordering in the sequence of electron transfer reactions and the kinetics and regulation of these reactions. Topics covered will include (1) electron transfer via the primary acceptor, H_A, to the first quinone acceptor, Q_A, (2) the two-electron quinone gate, Q_B, (3) electron transfer via the tyrosine components, Y_Z and Y_D, and (4) the manganese-containing four-electron oxygen gate, M.

3.1. Electron transfer via the primary acceptor, H_A, to the first quinone acceptor, Q_A

As indicated in Section 2.1 above, there are many structural similarities between the Reaction Cores of PS II and purple bacteria and it is natural to expect also a functional resemblance. At present, our understanding of the early electron transfer events in purple bacteria is particularly detailed (see Kirmaier and Holten 1987). To a large extent this has been achieved through the application of time-resolved optical spectroscopy to reaction center preparations. In contrast, similar studies of PS II have been hampered by the fact that the absorption bands of the chromophores in the Reaction Core are not as well separated spectrally as they are in purple bacteria and also by the lack of pure preparations. However, the recent development of the D1/D2/cyt *b*559 preparation (Nanba and Satoh 1987), which is free of antenna Chl, has opened up a new perspective in PS II research in this respect.

In PS II preparations where an antenna is present, the rate of the primary charge separation will be determined by the migration and trapping of the excitation energy, a process which takes place on the 100 ps timescale (Trissl et al. 1987, Schatz et al. 1987, Hodges and Moya 1988, Kischkoweit et al. 1988). However, for the recently developed D1/D2/cyt *b*559 preparation the time constant for the primary charge separation ($P^* \rightarrow P^+H_A^-$) has now been measured and found to be 3.0 $\pm$ 0.6 ps based on the risetime of the $P^+H_A^-$ absorption band around 820 nm (Wasielewski et al. 1989). A photochemical hole-burning study of the D1/D2/cyt *b*559 preparation at 4.2 K gave a value of 1.9 $\pm$ 0.2 ps (Jankowiak et al. 1989), while a model

calculation of excitation energy trapping in PS II resulted in a similar value of 3 ps (Schatz et al. 1988). These rates for the primary charge separation are close to those reported for purple bacteria (see Kirmaier and Holten 1987).

The charge separation is further stabilized by the rapid transfer of the electron away from H_A^-. When Q_A is oxidized, this electron transfer occurs with a time constant of 250–300 ps in a spinach PS II core preparation (Nuijs et al. 1986) (based on the decay of an absorption band around 655 nm ascribed to the electrochromic effect of H_A^- on a nearby Chl). Slower kinetics with a time constant of 510 ± 50 ps were observed in the thermophilic cyanobacterium *Synechococcus* sp. (Schatz et al. 1987).

There are several reports to indicate the possible presence of other intermediates between H_A and Q_A (see Diner 1986, Mathis and Rutherford 1987). However, it was concluded from double-flash optical studies that no high-yield acceptor exists which could stabilize an electron for more than a few ns when Q_A is prereduced (Brettel et al. 1985). Recently, the time constant for Q_A reduction was inferred to be 350 ± 100 ps in a time-resolved UV study (Eckert et al. 1988a). Thus, these results seem to exclude the possibility of an intermediate between H_A and Q_A in the major electron pathway.

When the forward electron transfer is blocked by reduction of Q_A, either chemically or by preillumination, there is an increase in the fluorescence yield from the PS II antenna. The origin of this so-called variable fluorescence as well as the related question of the yield and lifetime of the radical pair ($P^+H_A^-$) formed under these conditions is at present controversial. The theories discussed can be summarized with a simple kinetic model (van Gorkom 1985, Schatz and Holzwarth 1986, Mathis 1987), in which it is assumed that $P^+H_A^-$ is in equilibrium with the antenna Chl singlet excited state (A*): $A^* \leftrightarrow P^+H_A^-$. Originally, it was suggested that the variable fluorescence is a luminescence resulting from the $P^+H_A^-$ charge recombination with the energy reexciting the Chl (see Klimov and Krasnovskii 1981). Alternatively, the above equilibrium might be much displaced to the left, either because of a large antenna or because of a lower possibility for the $P^*H_A \rightarrow P^+H_A^-$ charge separation when Q_A is reduced. In this model, the variable fluorescence is a direct fluorescence from excited antenna Chl with little involvement of the radical pair (Trissl et al. 1987, Schatz et al. 1988).

In a number of recent reports, the radical pair formation was studied directly using time-resolved flash-absorption spectroscopy. Large variations in the yield and lifetime of $P^+H_A^-$ were found between different PS II preparations. For the D1/D2/cyt *b*559 complex the observed absorption changes suggested a significant yield of flash-induced pair that subsequently decayed with an 1/*e*-lifetime of *ca.* 40 ns (Danielius et al. 1987, Takahashi et al. 1987). Time-resolved fluorescence studies revealed a component with a similar lifetime (Seibert et al. 1988b, Mimuro et al. 1988, Hansson, Janot and Moya, unpublished observations). Thus, for these particles the original Klimov model seems applicable, although it should be emphasized that the D1/D2/cyt *b*559 preparations used in these studies lack Q_A and, thus, the acceptor side properties may be altered.

In more intact PS II preparations (in the presence of Q_A^- and an antenna) the radical pair decays faster. Very rapid decay kinetics with half-lifetimes less than 500 ps were reported for O_2-evolving PS II-enriched membranes and these were found to slow down to half-lifetimes of 2.7 (70%) and 15 ns (30%) after repeated light/dark cycles in the presence of $Na_2S_2O_4$ (Eckert et al. 1987a). In a study of a series of PS II preparations differing in antenna size, the half-lifetimes of $P^+H_A^-$ ranged from 3 to 32 ns. The shorter values were obtained from the preparations with a larger antenna (Hansson et al. 1988). A radical-pair 1/*e*-lifetime of *ca.* 11 ns was found in an O_2-evolving PS II particle from *Synechococcus* sp. (Schlodder and Brettel 1988). In the two latter studies, the yield of radical pair was estimated to be 40–75%. Significantly lower yields were inferred from a combined fluorescence and absorbance study of the above-mentioned particle from *Synechococcus* sp. (Schatz et al. 1987) and from a photovoltage study of pea chloroplasts (Trissl et al. 1987).

The observed yields and lifetimes of the radical pair show a trend towards smaller values with increasing antenna size. This is in accordance with a displacement of the above-mentioned equilibrium to the left, but generally, the radical-pair lifetimes are longer than the lifetime of the variable component in the fluorescence decay (1/*e*-lifetimes, 1–2.5 ns). To account for the discrepancy between absorbance and fluorescence studies, an extension

of the simple equilibrium model above was recently proposed (Schlodder and Brettel 1988). In this model, which is similar to an earlier model for purple bacteria (Woodbury and Parson 1984), the radical pair is assumed to relax irreversibly to an energetically more favourable conformation. It was concluded that the radical pair recombination does not give rise to the variable fluorescence (Schlodder and Brettel 1988).

The recombination of the radical pair in PS II units with Q_A reduced or absent can also occur via an intermediate triplet state of P (^{3}P) with a lifetime of about 100 μs. The triplet formed in such a radical-pair mechanism gives rise to an EPR signal with a characteristic spin polarization pattern (see Rutherford 1985b). Indeed, the observation of such an EPR signal in the D1/D2/cyt *b*559 preparation conclusively showed that a primary charge separation can occur in this particle (Okamura et al. 1987). With flash-absorption spectroscopy it was found that the quantum yield of ^{3}P increased from 23% at 276 K to 80% at 10 K (Takahashi et al. 1987), similarly to what has been found for purple bacteria (Schenck et al. 1982). Formation of the triplet state of a carotenoid can also be observed, but with a very low, essentially temperature independent quantum yield (Takahashi et al. 1987, Schlodder and Brettel 1988). Thus, although it is known that at least one [possibly two (van Dorssen et al. 1987)] β-carotene molecule is bound to the Reaction Core (Nanba and Satoh 1987), the triplet energy is not transferred to it.

3.2. The two-electron quinone gate, Q_B

As discussed in the previous section, H_A^- most likely reduces Q_A directly. Under normal conditions Q_A is a one-electron acceptor (see Crofts and Wraight 1983, Mathis and Rutherford 1987). The formation of Q_A^- is not accompanied by a protonation of the semiquinone. Instead, the electron is transferred to Q_B, which occurs in the 100–200 μs timerange. Although a proton uptake may be associated with the formation of Q_B^-, the protonation event most likely occurs at a protein group close to the Q_B site and not on the semiquinone. Upon the second reduction to Q_B^{2-}, which occurs in the 400–500 μs timerange, a true protonation occurs, forming bound plastohydroquinone (Q_BH_2). Since the binding affinity for Q_BH_2 is low, another PQ molecule from the pool can readily displace it.

A certain proportion of Q_B^- (*ca.* 30%) is stable in the dark. The loss of Q_B^-, when not driven forward by the light reactions, occurs via charge recombination with the M component. For M in the S_2 and S_3 states, this recombination reaction occurs with half-life times of about 30 s (Rutherford et al. 1984), while for M in the S_1 state the loss of Q_B^- takes hours (Hideg and Demeter 1985).

The unique electron transfer events that take place at the Q_A and Q_B sites depend upon the special binding of the PQ molecules to the protein. The PQ molecules at both the Q_B (Wydrzynski and Inoue 1987) and the Q_A (Diner et al. 1988) sites can be extracted and replaced with other quinone molecules, leading to altered acceptor-side properties. There is a wealth of literature to show that many herbicides interact with PS II at the Q_A/Q_B level (see Renger 1986). Indeed, radioactively labelled herbicides were originally used to tag the D1 protein and identify it as the location of the Q_B site (see Bühman et al. 1987). Upon addition of a herbicide inhibitor, such as DCMU, electron transfer from Q_A to Q_B is blocked. It is thought that the inhibitor molecule binds at or close to the Q_B site in competition with PQ binding, although recent evidence suggests that there may be two herbicide binding sites in the Reaction Core (Hsu et al. 1986, Giardi et al. 1988, Vasil'ev et al. 1988). Nevertheless, in the presence of an inhibitor, with Q_B in the semiquinone state, a reverse electron transfer back to Q_A takes place, i.e. $Q_AQ_B^-$ + inhibitor $\leftrightarrow$ Q_A^-(inhibitor) + PQ. The lifetime of Q_A^- under this condition depends upon the availability of the oxidizing charges on the M component (Koike et al. 1986).

Closely associated with Q_A and Q_B is a non-heme Fe^{2+} ion which interacts magnetically with the semiquinones, rendering them EPR invisible at room temperature. However, the non-heme iron is detectable by Mössbauer spectroscopy (see Diner and Petrouleas 1988). At low temperatures, EPR signals similar to the FeQ signals from purple bacteria can be seen at $g = 1.8$ and 1.9 (see Rutherford and Zimmermann 1984). In a thermophilic cyanobacterium, an unusual signal at $g = 1.6$ was observed in addition to the $g = 1.8$ signal (McDermott et al. 1988). In contrast to the purple bacteria, the Fe^{2+} in PS II can be oxidized (e.g. by

ferricyanide), giving rise to an Fe^{3+} EPR spectrum with g-values at about 8 and 5.5 (Petrouleas and Diner 1986, Zimmermann and Rutherford 1986, Aasa et al. 1988). The Fe^{3+} EPR signal disappears when the sample is illuminated. Redox titrations give a midpoint potential of 400 mV for the Fe^{2+}/Fe^{3+} redox couple. This valued correlates well with the midpoint potential of a component, termed Q_{400}, that has been proposed to accept electrons directly from Q_A^- in a DCMU-insensitive path (see Diner and Petrouleas 1988). It has been demonstrated that flash-induced semiquinones of some exogenous quinone acceptors (such as phenyl-*p*-benzoquinone) bound at the Q_B site can also oxidize the iron to Fe^{3+}. This reductant-induced oxidation of the Fe^{2+} follows a periodicity of two in a sequence of flashes (Zimmerman and Rutherford 1986).

Bicarbonate has a major effect on the acceptor side of PS II (see van Rensen and Snel 1985, Govindjee and Eaton-Rye 1986, Blubaugh and Govindjee 1988a). In the absence of HCO_3^-, electron transfer from Q_A to Q_B is slowed down, although this effect may be induced by formate which is used in the HCO_3^- depletion procedure (Jursinic and Stemler 1986). In any event, under HCO_3^--depleting conditions (using formate) the reduction of Q_{400} or its ability to form is slowed down, the oxidation of the Fe^{2+} ion is blocked and changes occur in the environment surrounding the Fe^{2+} ion, as determined by Mössbauer spectroscopy (see Diner and Petrouleas 1988). Although there appears to be several sites at which HCO_3^- can bind in PS II (see Blubaugh and Govindjee 1988b), one of these sites has recently been suggested to be the Fe^{2+} ion, involved in the protonation of Q_B^{2-} (van Rensen et al. 1988, Eaton-Rye and Govindjee 1988, Blubaugh and Govindjee 1988a, B. Diner, personal communication).

Although the Fe^{2+} ion may assume an important function on the acceptor side, it is too premature at this time to assign an exact function. It may only contribute in optimizing the conformation of the acceptor side. For example, replacement of the Fe^{2+} ion in purple bacteria with any number of divalent transition metal does not significantly alter the rate of electron transfer from Q_A to Q_B (Debus et al. 1986).

Numerous types of acceptor-side heterogeneity have been reported in the literature (see Black et al. 1986). This heterogeneity has been variously described in terms of non-B-type acceptors, Q_1, Q_2, X_a and, more recently, A_q (Jursinic and Dennenberg 1988). The non-B-type acceptors may be associated with PS II β units which are believed to be located in the non-appressed regions of the membranes (see Section 2.5). The very high midpoint potential measured for these acceptors (higher than that for the PQ pool) would correlate with the lack of characteristic period-two oscillation. Recently, analyses of O_2 and delayed-fluorescence flash-yield patterns reveal two different types of period-four behavior which may be related to the different acceptor-side properties of PS II α and β units (Delrieu and Rosengard 1988). The other cases of acceptor heterogeneity represent proposed additional acceptor components in PS II prior to Q_B. This acceptor heterogeneity, however, may be a consequence of experimental conditions and/or inhomogeneous PS II samples. Certainly, it is known that various extents of damage occurs on the acceptor side among different PS II preparations (see Section 2.5). Clarification of acceptor-side heterogeneity will most likely require further biochemical advances to produce well-defined and homogeneous PS II preparations.

3.3. Electron transfer via the tyrosine components, Y_Z and Y_D

The oxidized form of Y_Z (the physiological electron donor to P^+ as depicted in Fig. 1) was originally identified as a light-induced kinetic component of the well-known EPR Signal II (see Babcock 1987). In intact, O_2-evolving PS II preparations it is designated as the organic radical giving rise to the flash-induced Signal $II_{very\ fast}$. A chemically identical, though separate, organic radical giving rise to the dark-stable Signal II_{slow} is designated as Y_D in Fig. 1. At first, these organic radicals were thought to be specially bound plastohydroquinone cations (O'Malley and Babcock 1984) and considerable amount of spectroscopic evidence was used to support this assignment (Dekker et al. 1984b, O'Malley et al. 1984, Rutherford 1985, Brok et al. 1985, Brok et al. 1986). However, quantitation of the plastoquinone content of PS II preparations revealed too few plastoquinone molecules present to account for all the proposed quinone com-

ponents on both the oxidizing and reducing sides (Takahashi and Katoh 1986, de Vitry et al. 1986). This conflict led to a re-evaluation and to the current assignment of Signal II as oxidized forms of tyrosine (Barry and Babcock 1987, 1988). Subsequent studies using site-directed mutagenesis (Debus et al. 1988a, Vermaass et al. 1988a) appear to establish firmly that Signal II_{slow} arises from the oxidized form of Tyr160 in the D2 subunit. Optical (Gerken et al. 1988) and EPR (Hoganson and Babcock 1988, 1989) evidence have since strongly indicated that Signal $II_{very\ fast}$ also arises from an oxidized form of tyrosine. More recent site-directed mutagenesis studies indicate that it is Tyr161 in the D1 subunit (Debus et al. 1988b, B. Diner, personal communication).

The reduction of P^+ during normal electron transport in PS II must necessarily be fast since charge recombination of $P^+Q_A^-$ occurs in about 100 μs (see Conjeaud and Mathis 1986). The reduction kinetics of P^+ are quite complex, being multiphasic and dependent upon the S states. Based on optical kinetic measurements, for the S_0 and S_1 states, P^+ is reduced with a major phase at about 20 ns and a minor phase of 35 μs and for the S_2 and S_3 states, with major phases at about 50 and 250 ns and again a minor phase at 35 μs (Brettel et al. 1984). The S-state dependence of the submicrosecond kinetics can be understood in terms of coulombic effects of the charge stored on M, but the origin of the slow 35 μs kinetics (whose amplitude is also S-state dependent) is still controversial (see van Gorkom 1985).

Due to kinetic limitations ($\geqslant 2\,\mu$s) in early studies using time-resolved EPR and UV spectroscopy to monitor Y_Z^+ directly (Boska and Sauer 1984, Dekker et al. 1984b, Weiss and Renger 1986), it was difficult to show that the oxidation kinetics of Y_Z followed directly the reduction kinetics of P^+. The uncertainty in the oxidation kinetics of Y_Z left open the possibility that another component could exist between Y_Z and P. Indeed, the biphasic nanosecond reduction kinetics for P^+ in the S_2 and S_3 states were interpreted to indicate that another component did exist (Brettel et al. 1984). With recent improvements in time resolution to the nanosecond time range for UV measurements, the oxidation kinetics of Y_Z was shown to follow exactly the reduction kinetics of P^+ (Gerken et al. 1988). The biphasicity in the reduction kinetics of P^+ was reinterpreted to indicate different protonation states of M. Recent results using improved time-resolved EPR also support the above UV results (Hogansson and Babcock 1988). Thus, it is generally agreed that Y_Z is the immediate electron donor to P^+.

Upon elimination of O_2 evolution, the kinetics of P^+ reduction is altered from the ns to the μs time range, with half-lifetimes dependent upon the experimental conditions. In Tris-treated samples, for instance, after the first excitation following dark-adaptation, the P^+ reduction kinetics exhibit pH-dependent half-lifetimes of 2–40 μs due to electron donation from the oxidizing side. In subsequent flashes, P^+ becomes reduced through a recombination with Q_A^- having a half-lifetime of 120 μs (Conjeaud and Mathis 1986). Similar treatments to inhibit O_2 evolution convert EPR Signal $II_{very\ fast}$ to the so-called Signal II_{fast}, which has identical spectral characteristics but a slower rise kinetics (Boska et al. 1983, Bock et al. 1988, Hoganson and Babcock 1989). The rise kinetics correlate with the P^+ reduction kinetics (Conjeaud and Mathis 1986, Weiss and Renger 1986, Bock et al. 1988). Thus, it seems likely that Y_Z donates electrons directly to P^+ under these conditions as well.

Although Y_D is now chemically identified, its role in PS II is obscure (see Mathis and Rutherford 1987). The kinetics of formation and disappearance of Y_D^+ are far too slow to have a direct role in the main electron transport sequence. However, evidence has been provided that Y_D can donate electrons to M in the S_2 and S_3 states after 1 or 2 light flashes (see Vermaas et al. 1984) while Y_D^+ can accept electrons from M in the S_0 state in the dark (Styring and Rutherford 1987, Nugent et al. 1987). The specific modification of the Tyr160 in the D2 subunit by site-directed mutagenesis reduces the photoautotrophic growth of the cyanobacterium, although the number of intact PS II units in the mutants remained normal with respect to the wild type (Vermaas et al. 1988a). This suggests that an intact Y_D may be needed for PS II O_2 evolution.

3.4. The four-electron oxygen gate, M

3.4.1. Manganese requirement

In Section 1 we defined M as that component which governs the S states, i.e. as electrons are sequen-

tially extracted from M, PS II advances to the next higher S state. In the main path of electrons through PS II the reduction kinetics of Y_Z^+ (as well as of P^+ — see above) are modulated by the S-state turnover. The half-lifetimes for the S-state transitions of M as determined by UV measurements (Dekker et al. 1984c, Renger and Weiss, 1986, Saygin and Witt 1987) are: S_0 to S_1, 30–50 μs; S_1 to S_2, 40–110 μs; S_2 to S_3, 100–350 μs; S_3 to S_4 to S_0, 1.0–1.5 ms. The kinetic parameters for Y_Z^+ reduction are: S_0 to S_1, ≈ 50 μs; S_1 to S_2 ≈ 50 μs; S_2 to S_3, 400–600 μs; S_3 to S_4 to S_0, 1–1.3 ms (Boska and Sauer 1984, Cole and Sauer 1987). Thus, it seems clear that Y_Z^+ is reduced directly by the M component. There is some recent evidence to indicate a transitory state (that is not photo-induced) between S_2 and S_3 (Koike and Inoue 1987). Such a state was originally described by Bouges-Bocquet (see Wydrzynski 1982). The final S_3 to S_4 to S_0 transition is taken to be rate-limited by electron transfer (see Babcock 1987); however, it has recently been suggested that O_2 release (and not water oxidation) may be the overall rate-limiting step (Plijter et al. 1988) [see also (Meunier and Popovic 1988)].

Upon perturbation or removal of the manganese from PS II, the S-state dependence in Y_Z^+ reduction, as well as all other period-four phenomena, is lost. Since manganese is a transition metal that can take on a number of oxidation states, functionally bound manganese in PS II has long been associated with the redox reactions involved in the M component (see Amesz 1983, Babcock 1987). Despite extensive studies, the exact location and functional operation of the manganese is still under considerable debate. It is generally agreed, however, that (1) stoichiometrically, four Mn ions are associated with each PS II unit, (2) at least some of the Mn ions are arranged in a multinuclear cluster, (3) valence state changes of the Mn ions are associated with the S-state transitions, and (4) the Mn ions are located within a restricted compartment towards the luminal side of the membrane.

The manganese stoichiometry has been determined by various extraction procedures (Yocum et al. 1981). For example, upon removal of the Regulatory Cap by treatment with $CaCl_2$, urea or $LaCl_3$, there is a variable release of the functional manganese (10–50%) depending upon the ionic conditions used during the extraction (Ono and Inoue 1984a, Miyao and Murata 1984a, b, Kuwabara et al. 1985, Ghanotakis et al. 1985, Imaoko et al. 1986). However, when a reductant is included during the extraction procedure, up to 90% of the manganese is solubilized (Ghanotakis et al. 1984b, Tamura and Cheniae, 1985) and this amount corresponds to about four Mn ions per PS II unit. The release of either half or all of the manganese under different extraction conditons implies that there are at least two manganese binding domains. Such heterogeneity in manganese binding has been implied in other studies (Allakhverdiev et al. 1986, Takahashi and Asada 1986, Tamura and Cheniae 1987, Isogai et al. 1988).

3.4.2. Arrangement and valence changes of manganese

Several spectroscopic methods, including X-ray absorption spectroscopy (XAS), near-infrared optical absorption and EPR spectroscopy, have recently been employed for investigations of the structural organization of the manganese in PS II. The information provided so far strongly suggests that at least some of the four Mn ions are arranged in a cluster. The XAS results obtained by Klein, Sauer and coworkers have recently been summarized (Sauer et al. 1988) and the reader is referred to this review for further details and references. A μ-oxo-bridged binuclear structure with a Mn–Mn distance of 2.70 Å was found to be consistent with the data, although more recent data does not exclude other types of bridging structures (George et al. 1989). A feature identified in a preliminary report as possible evidence for a second Mn–Mn distance of > 3 Å (Guiles et al. 1987) has been shown to exhibit significant dichroism (George et al. 1989). Simulations indicate that tetranuclear cubic (Brudvig and Crabtree 1986) or 'butterfly' (Vincent and Christou 1987) structures are unlikely to be present, while binuclear, trinuclear or highly distorted tetranuclear structures are all still possible (McDermott et al. 1988, George et al. 1989). The rapid development of model manganese complexes (Sheats et al. 1987, Bhula et al. 1988, Kulawiec et al. 1988, Matsushita et al. 1988, Wieghardt et al. 1988) should in the future help to clarify the organization of the Mn in PS II.

The XAS data also indicates that the ligands in the first coordination shell surrounding the Mn in PS II are light elements, predominantly O and N. A

study of an O_2-evolving PS II preparation from the thermophilic cyanobacterium *Synechococcus* sp. revealed that the Mn environment in this organism is very similar to that seen in spinach PS II (McDermott et al. 1988). No changes in the fine structure of the XAS spectra were found to accompany the S_1 to S_2 to S_3 transitions, indicating that no significant rearrangement of ligands occurs on these steps. However, for a state analogous to S_0, obtained by illumination of a NH_2OH-treated sample, indications of structural changes were found (see Sauer et al. 1988).

S-state-associated absorbance changes in the near IR have been observed in O_2-evolving PS II-enriched membranes from spinach (Dismukes and Mathis 1984). The absorption spectrum associated with the S_2 and S_3 states (see Dismukes 1986) was strikingly similar to intervalence charge-transfer bands observed for the Mn_2(III, IV) mixed-valence state in synthetic binuclear Mn complexes. However, in a subsequent study, only a much weaker absorption band could be seen. Based on theoretical considerations it was suggested that the observed absorption change could result from scattering changes (Velthuys 1988).

Two different light-induced EPR signals can be detected from the M component, a multiline signal centered at $g \approx 2.0$ and a featureless signal at $g = 4.1$ (see Dismukes 1986, Brudvig 1988). Most probably, both can be ascribed to manganese, as suggested from spectral similarities with low-molecular weight complexes and from correlations between their appearance and changes in the manganese X-ray absorption region (Goodin et al. 1984, Cole et al. 1987a). They are both associated with the S_2 state of M, as demonstrated from their oscillating behavior as a response to light flashes (Dismukes and Siderer 1981, Zimmermann and Rutherford 1986). Also, the disappearance of the multiline signal was shown to correlate with the decay of the S_2 state (Brudvig et al. 1983, Styring and Rutherford 1988b).

The multiline signal was originally assigned to a mixed-valence cluster of Mn ions (Dismukes and Siderer 1981). A single Mn ion can be ruled out from the lack of large-scale anisotropies in the signal (Hansson et al. 1984, 1987). Although small signal anisotropies can be detected (Rutherford 1985a), especially at a lower microwave frequency (Haddy et al. 1989), this will not alter the conclusion. Recent magnetic susceptibility data also rule out a single Mn ion (Sivaraja et al. 1989). Spinach grown on $^{15}NO_3$ showed a normal multiline signal, suggesting the absence of nitrogen in the first coordination sphere (Andréasson 1989). Temperature studies show that both the multiline (Hansson et al. 1984) and the $g = 4.1$ signals (de Paula et al. 1986) arise from ground state doublets. A recent proposal that the multiline signal may arise from an excited state doublet (de Paula et al. 1986) has not been confirmed (Hansson et al. 1987, Aasa et al. 1987, D. Stehlik, personal communication).

To account for the appearance of the EPR signals, two alternative models for the arrangement of the manganese in M have been proposed. The EPR signals may either arise from two different conformations of a tetranuclear Mn cluster (de Paula et al. 1986, Zimmermann and Rutherford, 1986b) or from a bi(tri)nuclear mixed-valence Mn complex (the multiline signal) in redox equilibrium with a monomeric Mn^{4+} ion (the $g = 4.1$ signal) (Hansson et al. 1987). A definitive conclusion does not seem possible based only on the presently available EPR data. Recently it was suggested that the $g = 4.1$ signal arises from an inactive conformation of M (Beck and Brudvig 1988). However, no correlation was found between the magnitude of this signal, abolished by small amounts of ethanol, and the steady-state or flash-induced O_2 evolution (L.-E. Andréasson, personal communication).

The appearance of the multiline EPR signal shows that the Mn ions have a mixed valence in the S_2 state but a definitive assignment of oxidation states is difficult. The lack of EPR signals from the other S states may be taken as evidence for oxidation-state changes of manganese, although other interpretations are possible. Whereas S_1 and S_3 might be difficult to detect since they are even-electron systems, it should be possible to observe S_0 and S_4. S_4 is too short-lived; for S_0, structural rearrangements may weaken the magnetic interactions, rendering the EPR signals highly anisotropic and difficult to detect. Such a rearrangement may also explain why, at low chloride concentrations, no multiline signal can be seen, although an apparent S_2 state may be attained (Ono et al. 1986).

Studies of the X-ray absorption-edge energies suggested an increase in the manganese oxidation state for an analogue S_0 state (see above) to S_1 and for the S_1 to S_2 but not for the S_2 to S_3 transitions

(see Sauer et al. 1988). Similar conclusions have also been drawn from studies of the relaxation properties of protons (Srinivasan and Sharp 1986a, b) and EPR Signal II_{slow} (Y_D^+) (Styring and Rutherford, 1988a). It is not clear where the additional oxidizing equivalent in S_3 resides, but it may be sufficiently close to the Mn ions to make the EPR signals disappear (Goodin et al. 1984).

A part of the flash-induced absorption changes in the UV region can be ascribed to the M component based on a characteristic period-four oscillation pattern. In one study, it was suggested that the absorption changes reflected Mn^{3+} to Mn^{4+} oxidations on all S-state transitions from S^0 to S_3 (Dekker et al. 1984a). The analysis of the data is complicated by interfering contributions from the acceptor side quinones (see Renger and Hanssum 1988) and several different interpretations exist. However, there now seems to be an agreement that the absorption change from the S_0 to S_1 transition is different from those due to the S_1 to S_2 and S_2 to S_3 transitions (Lavergne 1987, Saygin and Witt 1987, Kretschmann et al. 1988).

Taken together, the presently available spectroscopic data now seem to support that manganese is oxidized on the S_0 to S_1 and S_1 to S_2 transitions. However, it is still controversial whether the manganese is oxidized on the S_2 to S_3 transition. No information is available for the S_3 to S_4 transition. The XAS data suggest that manganese is predominantly in the +3 oxidation state in the S_1 state. This is consistent with the manganese extraction experiments (see previous subsection), which suggest that at least part of the manganese is stabilized in oxidation states higher than +2 in the dark, and with the general observations that manganese needs to be photoactivated (photooxidized) in order to be incorporated into its functional site (Callahan and Cheniae 1985, Callahan et al. 1986, Tamura and Cheniae 1987, Ono and Inoue 1987).

3.4.3. Protein interactions

The protein environment is essential for the overall water-oxidation reaction. Thus, the EP 33 of the Regulatory Cap is known to shield the manganese center and has also been called the 'manganese-stabilizing protein' (Kuwabara et al. 1987, Tyagi et al. 1987, Philbrick and Zilinskas 1988). However, perturbations to the Regulatory Cap can lead to various extents of inhibition of O_2 evolution (40–95%) without necessarily changing the properties of manganese. For example, removal of the Regulatory Cap proteins under high ionic conditions has no effect on the manganese environment as determined by XAS measurements (Cole et al. 1987b), does not prevent the formation of the S_2-state multiline EPR signal (Styring et al. 1987) and allows S-state turnover to occur up to S_3 (Ono and Inoue 1985, Vass et al. 1987), yet O_2 evolution is low or non-existent (depending upon the ion conditions) (Kuwabara et al. 1985, Tang and Satoh 1986, Ono and Inoue 1986) and the rate of O_2 release is markedly slowed down (Miyao et al. 1987). Recently, it has been shown that the two conserved cysteins in the EP 33 (Kuwabara et al. 1987, Philbrick and Zilinskas 1988) form a disulfide bridge which upon reduction alters the conformation such that the protein cannot bind and activate O_2 evolution (Tanaka and Wada 1988). Reoxidation of the reduced protein will subsequently lead to binding and restoration of O_2 activity. Mutational analysis of the cyanobacterial *Synechocystis* 6803 strain shows that the EP 33 is required for O_2 evolution activity and photoautotrophic growth (Philbrick and Zilinskas 1988).

Calcium and chloride are generally taken to be cofactors for PS II and are closely associated with the function of the Regulatory Cap proteins (see Critchley 1985, Homann 1987, Boussac and Rutherford 1988). Under certain conditions, removal of the EP 16 and EP 23 reduces the O_2 evolution activity by as much as 50%, but this loss in activity can be restored by the readdition of sufficient amounts of calcium and/or chloride. Such results indicate that the EP 23 regulates the binding affinities for these ionic cofactors (Andersson et al. 1984a, Ghanotakis et al. 1984a, Boussac et al. 1985). However, the nature of the calcium and chloride binding sites are still obscure and a matter of much controversy.

It is generally assumed that calcium has a specific structural role and it now seems that there are two Ca atoms tightly bound per PS II unit (Cheniae 1988, Ono and Inoue 1988a). At least one of these Ca atoms is essential for O_2 evolution activity (Ono and Inoue 1988a, Boussac and Rutherford 1988). A Ca^{2+}-binding, calmodulin-like protein with an apparent molecular mass of 13–15 kDa (but is not EP 16) has been isolated from PS II preparations (Sparrow and Evans 1984) while a Ca atom appears

to be associated with CP 29, a component of the ACP II (K. Irrgang, personal communication). However, there are many different opinions as to where the Ca^{2+} ions interact in the reaction sequence (i.e. on which S-state transition) (see Boussac and Rutherford 1988). Part of the controversy may be explained by the recent observation that the standard procedure for removing calcium from the sample (by extracting the EP 23 and subsequent EGTA treatment) does not actually remove the two tightly bound Ca^{2+} ions, despite the fact that addition of $CaCl_2$ to these samples increases O_2 evolution activity (Shen et al. 1988). Treatment of intact samples with chelators may influence the observed O_2 evolution (Wydrzynski and Renger 1986), which may occur through membrane surface charge effects on the oxidizing side (Dreschler and Neumann 1987).

Likewise, there are considerable controversies over where Cl^- ions interact in the reaction sequence (Itoh et al. 1984, Theg et al. 1984, Preston and Pace 1985, Imaoka et al. 1986, Damoder et al. 1986, Homann et al. 1986, Ono et al. 1986, Pistorius and Schmid 1987, Vass et al. 1987, Ono et al. 1987). The number of chloride binding sites is unknown, although there appears to be several (Sandusky and Yocum 1986, Coleman et al. 1987). However, the number of anion binding sites should not be based only on steady-state O_2 evolution or Hill-reaction measurements (Jursinic and Stemler 1988). Recently, a chloride binding site has been associated in close proximity to an iodination site on the D1 subunit (Ikeuchi et al. 1988).

Unlike the calcium requirement, the chloride requirement is not specific since other anions can replace chloride (in the order $Cl^- > Br^- \gg NO_3^- > I^-$). Generally, in order to observe a chloride dependence on O_2 evolution, samples must be pretreated with high concentrations of sulfate and/or high pH, conditions which are likely to complicate the interpretations of the results. Alkaline pH treament, for example, may have both reversible and irreversible effects (Cole et al. 1986, Vass et al. 1985, 1986, Schlodder and Meyer 1987) while sulfate effects may be variable depending upon the presence of EP 33 (Vass et al. 1987).

There are several suggestions that chloride ligates to the manganese ions directly and that this accounts for its effect on O_2 evolution activity (Critchley and Sargesson 1984, Damoder et al. 1986, Sandusky and Yocum 1986). However, there has been no direct evidence from XAS (see Sauer 1988) or the S_2-state multiline EPR measurements (Yachandra et al. 1986, Haddy et al. 1989) to indicate that chloride ligates to the manganese ions. In another hypothesis, chloride that is located in the proteinaceous compartment surrounding the manganese center facilitates deprotonation of the substrate H_2O molecules (Coleman and Govindjee 1987). This was based on pH-dependence studies which indicated a competitive behaviour between OH^- and Cl^- ions. However, changes in pH were recently found to have additional effects on the EP 23 (Homann 1988a, b). In view of the complications, it may be that many chloride effects are expressed indirectly through electrostatic interactions (Itoh and Uwano 1986) that alter protein conformations which affect the water-oxidation chemistry (Homann 1988c).

In the organization of PS II as depicted in Fig. 1, the Regulatory Cap forms a barrier between the manganese and the aqueous phase. Oxygen-17 (Wydrzynski et al. 1978) and proton (Sharp and Yocum 1980) NMR water relaxation measurements indicate that water exchange across the thylakoid membrane is slow (1–2 ms). Likewise, it has been found that the accessibility of exogenous electron donors to the manganese center, such as H_2O_2, NH_2OH and NH_2NH_2, increases when the Regulatory Cap is perturbed by the removal of the EP 23, EP 16 and/or EP 33 proteins (Ghanotakis et al. 1984b, Schröder and Åkerlund 1986, Berg and Seibert 1987, Frasch and Mei 1987, Mano et al. 1987). The Regulatory Cap may, in part, serve to control the exchange of reactants with the manganese center. This could be an advantage for the intact system to allow water to react only at the appropriate step so as to produce oxygen rather than other, potentially destructive water-oxidation intermediates.

3.4.4. Site of water-oxidation

Of fundamental importance for understanding the mechanism of water oxidation is a detailed knowledge of the electronic distribution and nuclear geometry within the M component among the various S states. The above sections clearly show that manganese is a central constituent. However, the oxidizing equivalents need not be restricted to

the manganese ions themselves, but could be located on redox active ligands, including water-oxidation intermediates, or on more distant redox groups within the protein matrix.

It is generally assumed that the manganese ions form the matrix on which two water molecules meet to form the dioxygen bond. Two lines of evidence based on the use of water analogues and isotopically labelled water indicate that water binds directly to manganese. Ammonia, which is isoelectronic with water, inhibits O_2 evolution and dramatically alters the S_2-state multiline EPR signal (Beck et al. 1986). Subsequent electron spin echo studies do show that ammonia binds directly to manganese in the S_2 state (Britt et al. 1989). However, EPR measurements also indicate that, although ammonia binds at two sites in both the S_1 and S_2 states, in neither state does the binding account for the observed inhibition of O_2 evolution (Andréasson et al. 1988). Rather, there is a separate NH_3 interaction on the S_3 to S_4 to S_0 transition (Ono and Inoue 1988b). It has been reported that the ammonia-induced modification of the S_2-state multiline EPR signal can be mimicked by Sr^{2+} ions, an unlikely water analogue (Boussac and Rutherford 1988).

In the second line of evidence, the linewidth of the S_2-state multiline EPR signal is found to increase with $H_2^{17}O$, indicating that oxygen ligates directly to the manganese center, interpreted either as peroxide, hydroxide or water itself, but not as superoxide or hydroxyl (Hansson et al. 1986, Andréasson et al. 1988). However, the isotope exchange conditions are not well-defined and it has been shown, for example, that μ-oxo bridges in iron complexes can undergo relatively fast isotopic exchange (Lippard 1988). In addition, the ^{17}O isotopic effect on the S_2-state multiline EPR signal are still observed in the presence of ammonia, which would suggest that there is at least one site in which ammonia and water do not compete with each other (Andréasson and Hansson, 1987, Andréasson et al. 1988). Line broadenings induced by D_2O have also been reported (Nugent 1987), but the effects are not easily reproducible (Yachandra et al. 1986, Haddy et al. 1989).

In general there are two views on how the water oxidation chemistry occurs, either as a concerted reaction on the S_3 to S_4 to S_0 transition or in a stepwise reaction which involves a bound water-oxidation intermediate formed prior to the S_3 to S_4 to S_0 transition. From a thermodynamic analysis, it has been argued that either two two-electron steps or one four-electron step are more likely than four single-electron step reactions (Krishtalik 1986). In the original Kok hypothesis, water could bind during any step in the S-state cycle, but water oxidation occurred as a concerted reaction on the final S_3 to S_4 to S_0 transition. Evidence has been obtained to support this view based on mass spectroscopic measurements using ^{8}O-labelled water. It was concluded that no non-exchangeable, partially oxidized water species are formed in the lower S states (Radmer and Ollinger 1986, Bader et al. 1987). Likewise, UV flash absorption measurements indicate a sequential oxidation of the manganese center through the S_3 state and an extraction of four electrons from two bound water species on the final S_3 to S_4 to S_0 transition (Saygin and Witt 1987). In this model, as in other (e.g. see Brudvig 1988), the concerted mechanism involves a peroxide-type intermediate during the O–O bond formation step.

In contrast to the view of a concerted reaction, several models consider that water molecules bound to the manganese center become partially oxidized (usually, for energetic reasons, to peroxide-type intermediates) particularly in the S_3 state (see Renger and Govindjee 1985). The measured proton release from the oxidizing side of PS II is known to be modulated in a pattern of 1, 0, 1, 2 as the S-state cycle progresses from S_0 to S_4 (see Förster and Junge 1985). Similarly, an intrinsic proton release interpreted from flash-induced electrochromic measurements yields the same stochiometry (Saygin and Witt, 1985). In the past, the proton release pattern has been used to support the partial oxidation of water during the S-state cycle. However, the mesaured proton release in the early S-state transitions may only arise from the dissociation of bound water molecules (Saygin and Witt 1987) or from membrane Bohr effects (Chandrasekar et al. 1986) or conformationally controlled proton transport processes (Renger et al. 1987) that are only indirectly coupled to the water-oxidation chemistry. On the other hand, the XAS and magnetic relaxation studies (see Section 3.4.2) which indicate that the manganese center is not sequentially oxidized through the entire S-state cycle, also has been used to support a stepwise

water-oxidation reaction. But in these measurements the ligand-centered redox chemistry that is implied need not necessarily involve bound water, but could be mediated through other ligands (Matsushita et al. 1988) often suggested to be redox active ligands of the protein matrix (Goodin et al. 1984, Renger 1988) such as histidines (Padhye et al. 1986). The water oxidation mechanism, therefore, still remains largely unknown.

Several reports have indicated that under certain inhibitory conditions (Wydrzynski et al. 1985, Eckert et al. 1988) and in certain sample preparations (Frei et al. 1988) [including salt-washed PS II samples where the EP 23 and EP 16 have been removed (Ono and Inoue 1984b)] electron flow through PS II after multiple turnover can be greater than what is accounted by the measured oxygen yield. A similar situation has recently been found in PS II samples suspended under low osmotic conditions (Wydrzynski et al. 1988). In this case [as well as possibly for salt-washed PS II samples (Schröder and Åkerlund 1986)] H_2O_2 appears to be formed, presumably via reactions on the oxidizing side of PS II. The possible production of H_2O_2 by PS II could imply that the M component is perturbed by these treatments in such a way that it leads to the release of partial water oxidation intermediates rather than oxygen.

4. Prospects for future research

Certainly, there have been many significant advances in PS II research in the last few years, which have led to our current perceptions of PS II structure and function. There remain, however, several important problems which will need to be addressed in the future. Some of the areas for future research are listed as follows:

(1) There is a need for a Reaction Core preparation which maintains a functional Q_A. This is important not only to establish the primary photochemistry, but also to unequivocally identify the location of all Reaction Core components.

(2) The role of the CP 47 needs to be clarified. Why, for example, does it appear to be essential for Q_A function as well as O_2 evolution if it does not bind any of the reaction components?

(3) The role of cyt *b*559 needs to be determined. This component must be tightly coupled to the Reaction Core for some reason.

(4) The protein matrix has become recognized as extremely important for the PS II reaction sequence. It will be necessary to know in the future whether redox active sites other than Y_Z and Y_D are also located in the protein matrix and how protein dynamics ultimately regulate PS II function.

(5) The role of the calcium and chloride cofactors needs to be clarified. It remains to be demonstrated whether these cofactors have a specific interaction in the reaction sequence or whether their effects are mediated through protein conformation changes. In this area of research electrostatic effects as well as the lipid structure of the membrane must be taken into consideration.

(6) Finally, despite all recent major efforts, the location and redox chemistry of the manganese ions must be further clarified. In addition, where is the site of the water oxidation chemistry — does it actually take place on the manganese ions or does it occur at some other location in the complex?

The future promises to be both fruitful and exciting in PS II research.

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References

Aasa R, Andréasson L-E, Lagenfelt G and Vänngård T (1987) A comparison between the multiline EPR signals of spinach and *Anacystis nidulans* and their temperature dependence. FEBS Lett 221: 245–248

Aasa R, Andréasson L-E, Styring S and Vänngård T (1989) The nature of the Fe(III) EPR signal from the acceptor-side iron of photosystem II. FEBS Lett 243: 156–160

Adir N and Ohad I (1987) Structural properties of the D1 and

surrounding photosystem II polypeptides as revealed by their interaction with cross-linking reagents. J Biol Chem 263: 283–289

Akabori K, Tsukamoto H, Tsukihara J, Nagatsuka T, Motokawa O and Toyoshima Y (1988) Disintegration and reconstitution of photosystem II reaction center core complex. I. Preparation and characterization of three different types of subcomplex. Biochim Biophys Acta 932: 345–357

Allakhverdiev SI, Shafiev MA and Klimov VV (1986) Effect of reversible extraction of manganese on photooxidation of chlorophyll P680 in photosystem II preparations. Photobiochem Photobiophys 12: 61–65

Alt J, Morris J, Westhoff P and Herrmann RG (1984) Nucleotide sequence of the clustered genes for the 44 kDa chlorophyll *a* apoprotein and the '32 kDa-like' protein of the photosystem II reaction center in the spinach plastid chromosome. Curr Genet 8: 597–606

Amesz J (1983) The role of manganese in photosynthetic oxygen evolution. Biochim Biophys Acta 726: 1–12

Anderson J (1987) Molecular organization of thylakoid membranes. In: Amesz J (ed) New Comprehensive Biochemistry, Vol 15, Photosynthesis, pp 273–297. Elsevier, Amsterdam

Andersson B and Åkerlund H-E (1987) Proteins of the oxygen evolving complex. In: Barber J (ed) Topics in Photosynthesis, Vol 8, The Light Reactions, pp 379–420. Elsevier, Amsterdam

Andersson B, Critchley C, Ryrie IJ, Jansson C, Larsson C and Anderson JB (1984a) Modification of the chloride requirement for photosynthetic O_2 evolution. The role of the 23 kDa polypeptide. FEBS Lett 168: 113–112

Andersson B, Larsson C, Jansson C, Ljungberg U and Åkerlund H-E (1984b) Immunological studies on the organization of proteins in photosynthetic oxygen evolution. Biochim Biophys Acta 766: 21–28

Andersson B, Sayre RT and Bogorad L (1987) Transbilayer organization of photosystem II proteins with special emphasis on the 32 kDa Qb-binding protein. Chemica Scripta 27B: 195–200

Andréasson L-E and Hansson Ö (1987) EPR studies of the oxygen-evolving system. The interaction with amines. In: Biggins J (ed) Progress in Photosynthesis Research, Vol 1, pp 503–510. Martinus Nijhoff, Dordrecht

Andréasson L-E and Vänngård T (1988) Electron transport in photosystems I and II. Ann Rev Plant Physiol Plant Mol Biol 39: 379–411

Andréasson L-E, Hansson Ö and von Schenck K (1988) The interaction of ammonia with the photosynthetic oxygen-evolving system. Biochim Biophys Acta 936: 351–360

Andréasson L-E (1989) Is nitrogen liganded to manganese in the photosynthetic oxygen evolving system? Biochim Biophys Acta 973: 465–467

Aoki K, Ideguchi T, Kakuno T, Yamashita J and Horio T (1986) Intermolecular relations of the photosystem II complex in spinach chloroplasts as detected by immunochemical assay. J Biochem 100: 875–882

Arnon DI and Tang GM-S (1988) Cytochrome *b*-559 and proton conductance in oxygenic photosynthesis. Proc Natl Acad Sci USA 85: 9524–9528

Arntz B and Trebst A (1986) On the role of the Q_B protein of PS II in photoinhibition. FEBS Lett 194: 43–49

Åkerlund H-E and Andersson B (1983) Quantitative separation of spinach thylakoids into photosystem II-enriched inside-out vesicles and photosystem I-enriched rightside-out vesicles. Biochim Biophys Acta 725: 34–40

Babcock GT (1987) The photosynthetic oxygen evolving process. In: Amesz J (ed) New Comprehensive Biochemistry, Vol 15, Photosynthesis, pp 125–158. Elsevier, Amsterdam

Babcock GT, Widger WR, Cramer WA, Oertling WA and Metz JG (1985) Axial ligands of chloroplast cyt *b*559: Identification and requirement for a heme-cross-linked polypeptide structure. Biochemistry 24: 3638–3645

Bader KP, Thibault P and Schmid GH (1987) Study on the properties of the S_3-state by mass spectrometry in the filamentous cyanobacterium *Oscillatoria chalybea*. Biochim Biophys Acta 893: 564–571

Barber J, Chapman DJ and Telfer A (1987) Characterization of a PS II reaction center isolated from the chloroplasts of *Pisum sativum*. FEBS Lett 220: 67–73

Barry AB and Babcock GT (1987) Tyrosine radicals are involved in the photosynthetic oxygen-evolving system. Proc Natl Acad Sci USA 84: 7099–7103

Barry AB and Babcock GT (1988) Characterization of the tyrosine radical involved in photosynthetic oxygen evolution. Chemica Scripta 28A: 117–122

Bassi R, Hoyer-Hansen G, Barbato R, Giacometti GM and Simpson DJ (1987) Chlorophyll-proteins of the photosystem II antenna system. J Biol Chem 262: 13333–13341

Bassi R, Ghiretti-Magaldi A, Tognon G, Giacometti GM and Miller KR (1988a) Two dimensional crystals in PS II membranes. In press

Bassi R, Rigoni F, Barbato R and Giacometi GM (1988b) Light-harvesting chlorophyll *a*/*b* proteins (LHCII) populations in phosporylated membranes. Biochim Biophys Acta 936: 29–38

Beck WF, de Paula JC and Brudvig GW (1986) Ammonia binds to the manganese site of the O_2-evolving complex of photosystem II in the S_2 state. J Am Chem Soc 108: 4018–4022

Beck WF and Brudvig GW (1988) Ligand-substitution reactions of the O_2-evolving center of photosystem II. Chemica Scripta 28A: 93–98

Berg SP and Seibert M (1987) Is functional manganese involved in hydrogen-peroxide-stimulated anomalous oxygen evolution in $CaCl_2$-washed photosystem II membranes? Photosynth Res 13: 3–17

Bergström J and Franzén L-G (1987) Restoration of high-potential cytochrome *b*-559 in salt-washed photosystem II-enriched membranes as revealed by EPR. Acta Chem Scand Ser B41: 126–128

Berthold DA, Babcock GT and Yocum CA (1981) A highly resolved, oxygen-evolving photosystem II preparation from spinach thylakoid membranes. FEBS Lett 134: 231–234

Bhula R, Gainsford GJ and Weatherburn DC (1988) A new model for the oxygen-evolving complex in photosynthesis. A trinuclear μ_3-oxo-manganese(III) complex which contains a μ-peroxo group. J Am Chem Soc 110: 7550–7552

Bishop NI (1987) Evidence for multiple functions of the intrinsic, 32–34 kDa chloroplast membrane polypeptide of *Scenedesmus* in photosystem II reactions. In: Weissner W, Robinson DG and Starr RC (eds) Algal Development, Molecular and Cellular Aspects, pp 149–155. Springer-Verlag, Berlin

Black MT, Brearley TH and Horton P (1986) Heterogeneity in chloroplast photosystem II. Photosynth Res 8: 193–207

Blubaugh DJ and Govindjee (1988a) Kinetics of the bicarbonate effect and the number of bicarbonate-binding sites in thylakoid membranes. Biochim Biophys Acta 936: 208–214

Blubaugh DJ and Govindjee (1988b) The molecular mechanism of the bicarbonate effect at the plastoquinone reductase site of photosynthesis. Photosynth Res 19: 85–128

Bock CH, Gerken S, Stehlik D and Witt HT (1988) Time resolved EPR on photosystem II particles after irreversible and reversible inhibition of water cleavage with high concentrations of acetate. FEBS Lett 227: 141–146

Boska M, Sauer K, Buttner W and Babcock GT (1983) Similarity of EPR signal II_f rise and P-680$^+$ decay kinetics in tris-washed chloroplast photosystem II preparations as a function of pH. Biochim Biophys Acta 722: 327–330

Boska M and Sauer K (1984) Kinetics of EPR signal II_{vf} in chloroplast photosystem II. Biochim Biophys Acta 765: 84–87

Boussac A, Maison-Peteri B, Etienne A-L and Vernotte C (1985) Reactivation of oxygen evolution of NaCl-washed photosystem-II particles by Ca^{2+} and/or the 24 kDa protein. Biochim Biophys Acta 808: 231–234

Boussac A and Rutherford AW (1988) Nature of the inhibition of the oxygen-evolving enzyme of photosystem II induced by NaCl washing and reversed by the addition of Ca^{2+} or Sr^{2+}. Biochemistry 27: 3476–3483

Bowlby NR and Frasch WD (1986) Isolation of a manganese-containing protein complex from photosystem II preparations of spinach. Biochemistry 25: 1402–1407

Breton J (1982) The 695 nm fluorescence (F_{695}) of chloroplasts at low temperature is emitted from the primary acceptor of photosystem II. FEBS Lett 147: 16–20

Breton J and Satoh S (1987) Orientation of the pigments in Photosystem II: Low-temperature linear-dichroism study of a core particle and of its chlorophyll-protein subunits isolated from *Synechococcus* sp. Biochim Biophys Acta 892: 99–107

Brettel K, Schlodder E and Witt HT (1984) Nanosecond reduction kinetics of photooxidized chlorophyll-a_{II} (P-680) in single flashes as a probe for the electron pathway, H^+ release and charge accumulation in the O_2-evolving complex. Biochim Biophys Acta 766: 403–428

Brettel K, Schlodder E and Witt HT (1985) Evidence for only one stable electron acceptor in the reaction center of photosystem II in spinach chloroplasts. Photobiochem Photobiophys 9: 205–213

Briantais J-M, Vernotte C, Miyao M, Murata N and Picaud M (1985) Relationship between O_2 evolution capacity and cytochrome *b*559 high-potential form in Photosystem II particles. Biochim Biophys Acta 808: 348–351

Briantais J-M, Vernotte C, Krause GH and Weis E (1986) Chlorophyll *a* fluorescence of higher plants, chloroplasts and leaves. In: Govindjee, Amesz J and Fork DC (eds) Light Emission by Plants and Bacteria, pp 540–586. Academic Press, New York

Bricker TM, Guikema JA, Pakrasi HB and Sherman LA (1986) Proteins of cyanobacterial thylakoids. In: Staehlin LA and Arntzen CJ (eds) Encyclopedia of Plant Physiology, New Series, Vol 19, Photosynthesis III, pp 640–652. Springer-Verlag, Berlin

Bricker TM, Odom WR and Queirolo CB (1988) Close association of the 33 kDa extrinsic protein with the apoprotein of CPal in photosystem II. FEBS Lett 231: 111–117

Britt RD, Zimmermann J-L, Sauer K and Klein M (1989) Ammonia binds to the catalytic Mn of the oxygen evolving complex of photosystem II: Evidence by electron spin echo envelope modulation spectroscopy. J Am Chem Soc 111: 3522–3532

Brok M, Ebskamp FCR and Hoff AJ (1985) The structure of the secondary donor of photosystem II investigated by EPR at 9 and 35 GHz. Biochim Biophys Acta 809: 421–428

Brok M, Horikx JTG and Hoff AJ (1986) An EPR study of signal II in oriented photosystem II membranes. FEBS Lett 203: 36–40

Brudvig GW (1988) Involvement of manganese in photosynthetic water oxidation. In: Que L (ed) Metal Clusters in Biology, ACS Symposium Series, in press. Am Chem Soc, New York

Brudvig GW, Casey JL and Sauer K (1983) The effect of temperature on the formation and decay of the multiline EPR signal species associated with photosynthetic oxygen evolution. Biochim Biophys Acta 723: 366–371

Brudvig GW and Crabtree RH (1986) Mechanism for photosynthetic O_2 evolution. Proc Natl Acad Sci USA 83: 4586–4588

Bühmann U, Herrmann C, Kötter C, Trebst A, Depka B and Wietoska H (1987) Inhibition and photoaffinity labeling of photosystem II by thiazolyliden-ketonitriles. Z Naturforsch 42c: 704–712

Callahan FE and Cheniae GM (1985) Studies on the photoactivation of the water-oxidizing enzyme. I. Processes limiting photoactivation in hydroxylamine-extracted leaf segments. Plant Physiol 79: 777–786

Callahan FE, Becker DW and Cheniae GM (1986) Studies on the photoactivation of the water-oxidizing enzyme. II. Characterization of weak light photoinhibition of PS II and its light-induced recovery. Plant Physiol 82: 261–269

Camm EL, Green BR, Allred DR and Staehelin LA (1987) Association of the 33 kDa extrinsic polypeptide (water-splitting) with PS II particles: immunochemical quantification of residual polypeptides after membrane extraction. Photosynth Res 13: 69–80

Chandrashekar TK, O'Malley PJ, Rodriguez T and Babcock GT (1986) ENDOR characterization and D_2O exchange in the Z^+/D^+ radical in photosystem II. Photosynth Res 10: 423–429

Chapman DJ, Gounaris K and Barber J (1988) Electron transport properties of the isolated D1–D2–cytochrome *b*559 photosystem II reaction centre. Biochim Biophys Acta 933: 423–431

Cheniae GM (1988) Heterogeneity of calcium binding sites in photosystem II. Plant Physiol, in press

Chitnis PR and Thornber JP (1988) The major light-harvesting complex of photosystem II: Aspects of its molecular and cell biology. Photosynth Res 16: 41–64

Chylla RA, Garab G and Whitmarsh J (1987) Evidence for slow turnover in a fraction of photosystem II complexes in thylakoid membranes. Biochim Biophys Acta 894: 562–571

Cleland RE, Melis A and Neale PJ (1986) Mechanism of photoinhibition: photochemical raction center inactivation in system II of chloroplasts. Photosynth Res 9: 79–88

Cole J, Boska M, Blough NV and Sauer K (1986) Reversible and irreversible effects of alkaline pH on Photosystem II electron-

transfer reactions. Biochim Biophys Acta 848: 41–47
Cole J and Sauer K (1987) The flash number dependence of EPR signal II decay as a probe for charge accumulation in photosystem II. Biochim Biophys Acta 891: 40–48
Cole J, Yachandra VK, Guiles RD, McDermott AE, Britt RD, Dexheimer SL, Sauer K and Klein M (1987a) Assignment of the $g = 4.1$ EPR signal to manganese in the S_2 state of the photosynthetic oxygen-evolving complex. An X-ray absorption edge spectroscopy study. Biochim Biophys Acta 890: 395–398
Cole JL, Yachandra VK, McDermott AE, Guiles RD, Britt RD, Dexheimer S, Sauer K and Klein MP (1987b) Structure of the manganese complex of photosystem II upon removal of the 33-kilodalton extrinsic protein: An X-ray absorption spectroscopy study. Biochemistry 26: 5967–5973
Coleman WJ and Govindjee (1987) A model for the mechanism of cloride activation of oxygen evolution in photosystem II. Photosynth Res 13: 199–223
Coleman WJ, Govindjee and Gutowsky HS (1987) The location of the chloride binding sites in the oxygen-evolving complex of spinach photosystem II. Biochim Biophys Acta 894: 453–459
Conjeaud H and Mathis P (1986) Electron transfer in the photosynthetic membrane. Biophys J 49: 1215–1221
Cramer WA, Theg SM and Widger WR (1986) On the structure and function of cytochrome *b*-559. Photosynth Res 10: 393–403
Critchley C (1985) The role of chloride in photosystem II. Biochim Biophys Acta 811: 33–46
Critchley C and Sargeson AM (1984) A manganese-chloride cluster as the functional centre of the O_2 evolving enzyme in photosynthetic systems. FEBS lett 177: 2–5
Crofts AR and Wraight CA (1983) The electrochemical domain of photosynthesis. Biochim Biophys Acta 726: 149–185
Damoder R, Klimov VV and Dismukes GC (1986) The effect of Cl-depletion and X-reconstitution on the oxygen-evolution rate, the yield of the multiline manganese EPR signal and EPR Signal II in the isolated Photosystem-II complex. Biochim Biophys Acta 848: 378–391
Danielius RV, Satoh K, van Kan PJM, Plijter JJ, Nuijs AM and van Gorkom HJ (1987) The primary reaction of photosystem II in the D1-D2-cyt *b*559 complex. FEBS Lett 213: 241–244
Davis MS, Forman A and Fajer J (1979) Ligated chlorophyll cation radicals: Their function in photosystem II of plant photosynthesis. Proc Natl Acad Sci USA 76: 4170–4174
Debus RJ, Feher G and Okamura MY (1986) Iron-depleted reaction centers from *Rhodopseudomonas sphaeroides* R-26.1: Characterization and reconstitution with Fe^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+} and Zn^{2+}. Biochemistry 25: 2276–2287
Debus RJ, Barry BA, Babcock GT and McIntosh L (1988a) Site-directed mutagenesis identifies a tyrosine radical involved in the photosynthetic oxygen-evolving system. Proc Natl Acad Sci USA 85: 427–430
Debus RJ, Barry BA, Sithole I, Babcock GT and McIntosh L (1988b) Directed mutagenesis indicates that the donor to P_{680}^+ in photosystem II is tyrosine-161 of the D1 polypeptide. Biochemistry 27: 9071–9074
de Groot A, Plijter JJ, Evelo R, Babcock GT and Hoff AJ (1986) The influence of the oxidation state of the oxygen-evolving complex of Photosystem II on the spin-lattice relaxation time of Signal II as determined by electron spin-echo spectroscopy. Biochim Biophys Acta 848: 8–15
Deisenhofer J, Epp O, Miki K, Huber R and Michel H (1984) X-ray structure analysis of a membrane protein complex: electron density map at 3 Å resolution and a model of the chromophores of the photosynthetic reaction center from *Rhodopseudomonas viridis*. J Mol Biol 180: 385–398
Deisenhofer J, Epp O, Miki K, Huber R and Michel H (1985) Structure of the protein subunits in the photosynthetic reaction center of *Rhodopseudomonas viridis* at 3 Å resolution. Nature 318: 618–624
Dekker JP, Plijter JJ, Ouwehand L and van Gorkom HJ (1984a) Kinetics of manganese redox transitions in the oxygen-evolving apparatus of photosynthesis. Biochim Biophys Acta 767: 176–179
Dekker JP, van Gorkom HJ, Brok M and Ouwehand L (1984b) Optical characterization of photosystem II electron donors. Biochim Biophys Acta 764: 301–309
Dekker JP, van Gorkom HJ, Wensink J and Ouwehand L (1984c) Absorbance difference spectra of the successive redox states of the oxygen-evolving apparatus of photosynthesis. Biochim Biophys Acta 767: 1–9
Delrieu M-J and Rosengard F (1988) Characterization of two types of oxygen-evolving photosystem II reaction center by the flash-induced oxygen and fluorescence yield. Biochim Biophys Acta 936: 39–49
den Blanken HJ, Hoff AJ, Jongenelis APJM and Diner BA (1983) High-resolution triplet-minus-singlet absorbance difference spectrum of photosystem II particles. FEBS Lett 157: 21–27
de Paula JC, Beck WF and Brudvig GW (1986) Magnetic properties of manganese in the photosynthetic O_2-evolving complex. 2 Evidence for a manganese tetramer. J Am Chem Soc 108: 4002–4009
de Vitry C, Carles C and Diner BA (1986) Quantitation of plastoquinone-9 in photosystem II reaction center particles. Chemical identification of the primary quinone, electron acceptor Q_A. FEBS Lett 196: 203–206
Diner BA (1987) The reaction center of photosystem II. In: Staehelin LA and Arntzen CJ (eds) Encyclopedia of Plant Physiology, New Series, Vol 19, Photosynthesis III, pp 422–435. Springer-Verlag, Berlin
Diner BA and Petrouleas V (1988) Q_{400}, the non-heme iron of photosystem II iron-quinone complex. A spectroscopic probe of quinone and inhibitor binding to the reaction center. Biochim Biophys Acta 895: 107–125
Diner BA, de Vitry C and Popot J-L (1988) Quinone exchange in the Q_A binding site of photosystem II reaction center core preparations isolated from *Chlamydomonas reinhardtii*. Biochim Biophys Acta 934: 47–54
Dismukes GC (1986) The metal centers of the photosynthetic oxygen-evolving complex. Photochem Photobiol 43: 99–115
Dismukes GC (1988) The spectroscopically derived structure of the manganese site for photosynthetic water oxidation and a proposal for the protein-binding sites for calcium and manganese. Chemica Scripta 28A: 99–104
Dismukes GC and Siderer Y (1981) Intermediates of a polynuclear manganese center involved in photosynthetic oxidation of water. Proc Natl Acad Sci USA 78: 274–278
Dismukes GC and Mathis P (1984) A near infrared electronic

transition associated with conversion between S-states of the photosynthetic O_2-evolving complex. FEBS Lett 178: 51–54

Dreschler Z and Neumann J (1987) Membrane charge affecting electron donation to PS II in chloroplasts. Photosynth Res 13: 143–157

Dunahay TG and Staehlin LA (1986) Isolation and characterization of a new minor chlorophyll *a*/*b* protein complex (CP 24) from spinach. Plant Physiol 80: 429–434

Eaton-Rye JJ and Govindjee (1988) Electron transfer through the quinone acceptor complex of photosystem II after one or two actinic flashes in bicarbonate-depleted spinach thylakoid membranes. Biochim Biophys Acta 945: 248–257

Eckert H-J, Renger G, Bernarding J, Faust P, Eichler H-J and Salk J (1987a) Examination of fluorescence lifetime and radical pair decay in photosystem II fragments from spinach. Biochim Biophys Acta 893: 208–218

Eckert H-J, Toyoshima Y, Akabori K and Dismukes GC (1987b) The involvement of lipids in light-induced charge separation in the reaction center of photosystem II. Photosynth Res 14: 31–41

Eckert H-J, Wiese N, Bernarding J, Eichler H-J and Renger G (1988a) Analysis of the electron transfer from $Pheo^-$ to Q_A in PS II membrane fragments from spinach by time resolved 325 nm absorption changes in the picosecond domain. FEBS Lett 240: 153–158

Eckert H-J, Wydrzynski T and Renger G (1988b) The effect of DIDS, trifluoperazine and lauroylcholine chloride on $P680^+$ reduction and oxygen evolution. Biochim Biophys Acta 932: 240–249

Enami I, Miyaoka T, Mochizuki Y, Shen J-R, Satoh K and Katoh S (1989) Nearest neighbor relationships among constituent proteins of oxygen-evolving photosystem II membranes: binding and function of the extrinsic 33 kDa protein. Biochim Biophys Acta 973: 35–40

Evelo RG, Styring S, Rutherford AW and Hoff AJ (1989) EPR relaxation measurements of photosystem II reaction centers: Influence of S-state oxidation and temperature. Biochim Biophys Acta 973: 428–442

Falkowski PG, Fujita Y, Ley A and Mauzerall D (1986) Evidence for cyclic electron flow around photosystem II in *Chlorella pyrenoidosa*. Plant Physiol 81: 310–312

Farchaus J and Dilley RA (1986) Purification and partial sequence of the M_r 10,000 phosphoprotein from spinach thylakoids. Arch Biochem Biophys 244: 94–101

Förster V and Junge W (1985) Stoichiometry and kinetics of proton release upon photosynthetic water oxidation. Photobiochem Photobiophys 41: 183–190

Franzén L-G, Styring S, Etienne A-L, Hansson Ö and Vernotte C (1986) Spectroscopic and functional characterization of a highly oxygen evolving Photosystem II reaction center complex from spinach. Photobiochem Photobiophys 13: 15–28

Frasch WD and Mei R (1987) Hydrogen peroxide as an alternate substrate for the oxygen-evolving complex. Biochim Biophys Acta 891: 8–14

Frei R, Eckert H-J, Renger G and Bachofen R (1988) Preparation and characterization of photosystem II particles from a thermophilic cyanobacterium. Biochim Biophys Acta 932: 258–267

Ganago IB, Klimov VV, Ganago AO, Shuvalov VA and Erokhin YE (1982) Linear dichroism and orientation of pheophytin, the intermediary electron acceptor in photosystem II reaction centers. FEBS Lett 140: 127–130

George GN, Prince RC and Cramer SP (1989) The manganese site of the photosynthetic water-splitting enzyme. Science 243: 789–791

Gerken S, Brettel K, Schlodder E and Witt HT (1988) Optical characterization of the immediate electron donor to chlorophyll a_{II}^+ in O_2-evolving photosystem II complexes. Tyrosine as possible electron carrier betrween chlorophyll a_{II} and the water-oxidizing manganese complex. FEBS Lett 237: 69–75

Ghanotakis DF, Babcock GT and Yocum CF (1984a) Calcium reconstitutes high levels of oxygen evolution in polypeptide depleted photosystem II preparations. FEBS Lett 167: 127–130

Ghanotakis DF, Topper JN and Yocum CF (1984b) Structural organization of the oxidizing side of photosystem II. Exogenous reductants reduce and destroy the Mn-complex in photosystem II membranes depleted of the 17 and 23 kDa polypeptides. Biochim Biophys Acta 767: 524–531

Ghanotakis DF, Babcock GT and Yocum CF (1985) Structure of the oxygen-evolving complex of Photosystem II: calcium and lanthanum compete for sites on the oxidizing side of Photosystem II which control the binding of water-soluble polypeptides and regulate the activity of the manganese complex. Biochim Biophys Acta 809: 173–180

Ghanotakis DF, Yocum CF and Babcock GT (1986) ESR spectroscopy demonstrates that cytochrome *b*559 remains low potential in Ca^{2+}-reactivated, salt-washed PSII particles. Photosynth Res 9: 125–134

Ghanotakis DF, Demetriou DM and Yocum CF (1987) Isolation and characterization of an oxygen-evolving Photosystem II reaction center core preparation and a 28 kDa Chl-*a*-binding protein. Biochim Biophys Acta 891: 15–21

Ghanotakis DF, de Paula JC, Demetriou DM, Bowlby NR, Petersen J, Babcock G and Yocum CF (1989) Isolation and characterization of the 47 kDa protein and the D1–D2–cytochrome *b*559 complex. Biochim Biophys Acta 947: 45–53

Giardi MT, Marder JB and Barber J (1988) Herbicide binding to the isolated photosystem II reaction center. Biochim Biophys Acta 934: 64–71

Glazer AN (1984) Phycobilisome: A macromolecular complex optimized for light energy transfer. Biochim Biophys Acta 768: 29–51

Goodin DB, Yachandra VK, Britt DR and Sauer K (1984) The state of manganese in the photosynthetic apparatus. 3 Light-induced changes in X-ray absorption (*k*-edge) energies of manganese in photosynthetic membranes. Biochim Biophys Acta 767: 209–216

Gounaris K, Whitford D and Barber J (1983) The effect of thylakoid lipids on an oxygen-evolving photosystem II preparation. FEBS Lett 163: 230–234

Gounaris K, Barber J and Harwood JL (1986) The thylakoid membranes of higher plant chloroplasts. Biochem J 237: 313–326

Govindjee and Eaton-Rye JJ (1986) Electron transfer through photosystem II acceptors: Interaction with anions. Photosynth Res 10: 365–379

Gray JC (1987) Genetics and synthesis of chloroplast membrane proteins. In Amesz J (ed) New Comprehensive Biochemistry,

Vol 15, Photosynthesis, pp 319–342. Elsevier, Amsterdam
Green BR (1988) The chlorophyll-protein complexes of higher plant photosynthetic membranes or Just what green band is that? Photosynth Res 15: 3–32
Guiles RD, Yachandra VK, McDermott A, Britt DR, Dexheimer SL, Sauer K and Klein MP (1987) Structural features of the manganese cluster in different states of the oxygen evolving complex of photosystem II: An X-ray absorption spectroscopy study. In: Biggins J (ed) Progress in Photosynthesis Research, Vol 1, pp 561–564. Martinus Nijhoff, Dordrecht
Haddy A, Aasa R and Andréasson L-E (1989) S-band EPR studies of the S_2-state multiline signal from the photosynthetic oxygen-evolving complex. Biochemistry, in press
Hansson Ö, Andréasson L-E and Vänngård T (1984) Studies on the multiline EPR signal associated with state S_2 of the oxygen-evolving system. In: Sybesma C (ed) Advances in Photosynthesis Research, Vol 1, pp 307–310. Martinus Nijhoff/Dr W Junk Publishers, The Hague
Hansson Ö, Andréasson L-E and Vänngård T (1986) Oxygen from water is coordinated to manganese in the S_2 state of photosystem II. FEBS Lett 195: 151–154
Hansson Ö, Aasa R and Vänngård T (1987) The origin of the multiline and $g = 4.1$ electron paramagnetic resonance signals from the oxygen-evolving system of Photosystem II. Biophys J 51: 825–832
Hansson Ö, Duranton J and Mathis P (1988) Yield and lifetime of the primary radical pair in preparations of photosystem II with different antenna size. Biochim Biophys Acta 932: 91–96
Hearst JE (1987) Primary structure and function of the reaction center polypeptides of *Rhodopseudomonas capsulata* — the structural and functional analogies with the photosystem II polypeptides of plants. In: Staehelin LA and Arntzen CJ (eds) Encyclopedia of Plant Physiology New Series, Vol 19, Photosynthesis III, pp 382–389. Springer-Verlag, Berlin
Hideg E and Demeter S (1985) Binary oscillation of delayed luminescence: Evidence of the participation of Q_B^- in the charge recombination. Z Naturforsch 40c: 827–831
Hird SM, Dyer TA and Gray JC (1986) The gene for the 10 kDa phosphoprotein of photosystem II is located in chloroplast DNA. FEBS Lett 209: 181–186
Hodges M, Boussac A and Briantais J-M (1987) Thylakoid membrane protein phosphorylation modifies the equilibrium between photosystem II quinone electron acceptors Biochim Biophys Acta, 894: 138–145
Hodges M and Moya I (1988) Time-resolved chlorophyll fluorescence studies on pigment-protein complexes from photosynthetic membranes. Biochim Biophys Acta 935: 41–52
Hoganson CW and Babcock GT (1988) Electron transfer events near the reaction center in O_2-evolving photosystem II preparations. Biochemistry 27: 5848–5855
Hoganson CW and Babcock GT (1989) Redox cofactor interactions in photosystem II: Electron spin resonance spectrum of P_{680}^+ is broadened in the presence of Y_Z^+. Biochemistry 28: 1448–1454
Homann PH (1987) The relations between the chloride, calcium and polypeptide requirements of photosynthetic water oxidation. J Bioenerg Biomembr 19: 105–123
Homann PH (1988a) The chloride and calcium requirement of photosynthetic water oxidation: effects of pH. Biochim Biophys Acta 934: 1–13:
Homann PH (1988b) Chloride relations of photosystem II membrane preparations depleted of, and resupplied with, their 17 and 23 kDa extrinsic polypeptides. Photosynth Res 15: 205–220
Homann PH (1988c) Structural effects of Cl^- and other anions on the water oxidizing complex of chloroplast photosystem II. Plant Physiol 88: 194–199
Homann PH, Gleiter H, Ono T and Inoue Y (1986) Storage of abnormal oxidants Σ_1, Σ_2, and Σ_3 in photosynthetic water oxidases inhibited by Cl^--removal. Biochim Biophys Acta 850: 10–20
Horvath G, Melis A, Hideg E, Droppa M and Vigh L (1987) Role of lipids in the organization and function of photosystem II studied by homogeneous catalytic hydrogenation of thylakoid membranes in situ. Biochim Biophys Acta 891: 68–74
Hsu B-D, Lee J-Y and Pan R-L (1986) The two binding sites for DCMU in photosystem II. Biochem Biophys Res Commun 141: 682–688
Ikeuchi M and Inoue Y (1986) Characterization of O_2 evolution by a wheat photosystem II reaction center complex isolated by a simplified method. Disjunction of secondary acceptor quinone and enhanced Ca^{2+} demand. Arch Biochem Biophys 247: 97–107
Ikeuchi M and Inoue Y (1987) Specific ^{125}I labeling of D1 (herbicide-binding protein). An indication that D1 functions on both the donor and acceptor side of photosystem II. FEBS Lett 210: 71–76
Ikeuchi M, Plumley GF, Inoue Y and Schmidt GW (1987) Phosphorylation of photosystem II components, CP43 apoprotein, D1, D2 and 10 to 11 kilodalton protein in chloroplast thylakoids of higher plants. Plant Physiol 85: 638–642
Ikeuchi M and Inoue Y (1988) A new photosystem II reaction center component (4.8 kDa protein) encoded by chloroplast genome. FEBS Lett 241: 99–104
Ikeuchi M, Koike H and Inoue Y (1988) Iodination of D1 (herbicide-binding protein) is coupled with photooxidation of $^{125}I^-$associated with Cl^--binding site in photosystem II water-oxidation system. Biochim Biophys Acta 932: 160–169
Imaoka A, Akabori K, Yanagi M, Izumi K, Toyashima Y, Kawamori A, Nakayama H and Sato J (1986) Roles of three lumen-surface proteins in the formation of S_2 state and O_2 evolution in photosystem II particles from spinach thylakoid membranes. Biochim Biophys Acta 848: 201–211
Innes JB and Brudvig GW (1989) Location and magnetic relaxation properties of the stable tyrosine radical in photosystem II. Biochemistry 28: 1116–1125
Irrgang K-D, Boecher EJ, Vater J and Renger G (1988) Structural determination of the photosystem II core complex from spinach. Eur J Biochem 178: 209–217
Isogai Y, Yamamoto Y and Nishimura M (1985) Association of the 33-kDa polypeptide with the 43-kDa component in photosystem II particles. FEBS Lett 187: 240–244
Isogai Y, Nishimura M and Itoh S (1987) Charge distribution on the membrane surface of the donor side of photosystem II. Effect of the free radical relaxing agent dysprosium on the power saturation of EPR Signal II_s in PS-II particles. Plant Cell Physiol 28: 1493–1499

Isogai Y, Nishimura M, Iwaki M and Itoh S (1988) Location of manganese atoms in Photosystem II studied by EPR power saturation of Signal II_{slow}. Biochim Biophys Acta 936: 259–268

Itoh S, Yerkes CT, Koike H, Robinson HH and Crofts AR (1984) Effects of chloride depletion on electron donation from the water-oxidizing complex to the photosystem II reaction center as measured by microsecond rise of chlorophyll fluorescence in isolated pea chloroplasts. Biochim Biophys Acta 766: 612–622

Itoh S and Uwano S (1986) Characteristics of the Cl^- action site in the O_2 evolving reaction in PS II particles: Electrostatic interaction with ions. Plant Cell Physiol 27: 25–36

Ivey S and Berg SP (1985) The use of polyclonal antibodies to identify peptides exposed on the stroma side of the spinach thylakoid. Photosynth Res 6: 193–199

Jankowiak R, Tang D, Small GJ and Seibert M (1989) Transient and persistent hole burning of the reaction center of photosystem II. J Phys Chem 93: 1649–1654

Jansen T, Rother C, Steppuhn J, Reinke H, Beyreuther K, Jansson C, Andersson B and Herrmann RG (1987) Nucleotide sequence of cDNA clones encoding the complete '23 kDa' and '16 kDa' precursor proteins associated with the photosynthetic oxygen-evolving complex from spinach. FEBS Lett 216: 234–240

Joliot P, Barbieri G and Chabaud R (1969) Un nouveau modèle des centres photochimique du système II. Photochem Photobiol 10: 309–329

Jordan BR, Chow W-S and Baker AJ (1983) The role of phospholipids in the molecular oganization of pea chloroplast membranes: Effect of phospholipid depletion on photosynthetic activities. Biochim Biophys Acta 725: 77–86

Jursinic P and Stemler A (1986) Correlation between the binding of formate and decreased rates of charge transfer through the photosystem II quinones. Photobiochem Photobiophys 43: 205–212

Jursinic P and Stemler A (1988) Multiple anion effects on photosystem II in chloroplast membranes. Photosynth Res 15: 41–56

Jursinic P and Dennenberg R (1988) Thylakoid photosystem II activity supported by the non-quinone acceptor Q_{400} and an ancillary quinone acceptor A_q. Biochim Biophys Acta 935: 225–235

Kirilovsky D, Vernotte C, Astier C and Étienne A-L (1988) Reversible and irreversible photoinhibition in herbicide-resistant mutants of *Synechocystis* 6714. Biochim Biophys Acta 933: 124–131

Kirmaier C and Holten D (1987) Primary photochemistry of reaction centers from the photosynthetic purple bacteria. Photosynth Res 13: 225–260

Kischkoweit C, Leibl W and Trissl H-W (1988) Theoretical and experimental study of trapping times and antenna organization in pea chloroplasts by means of the artificial fluorescence quencher *m*-dinitrobenzene (DNB). Biochim Biophys Acta 933: 276–287

Klimov VV and Krasnovskii AA (1981) Pheophytin as the primary electron acceptor in photosystem 2 reaction centers. Photosynthetica 15: 592–609

Koike H, Siderer Y, Ono T and Inoue Y (1986) Assignment of thermoluminescence A band to $S_3Q_A^-$ charge recombination: sequential stabilization of S_3 and Q_A^- by a two-step illumination at different temperatures. Biochim Biophys Acta 850: 80–89

Koike H and Inoue Y (1987) A low-temperature-sensitive intermediate state between S_2 and S_3 in photosynthetic water oxidation deduced by means of thermoluminescence measurements. Biochim Biophys Acta 894: 573–577

Koike H, Hanssum B, Inoue Y and Renger G (1987) Temperature dependence of S-state transitions in a thermophilic cyanobacterium, *Synechococcus vulcanus* Copeland measured by absorption changes in the ultraviolet region. Biochim Biophys Acta 893: 524–533

Kok B, Forbush B and McGloin M (1970) Cooperation of charges in photosynthetic oxygen evolution: I. A linear four step mechanism. Photochem Photobiol 11: 457–475

Kretschmann H, Dekker JP, Saygin Ö and Witt HT (1988) An agreement on the quarternary oscillation of ultraviolet absorption changes accompanying the water splitting in isolated photosystem II complexes from the cyanobacterium *Synechococcus* sp. Biochim Biophys Acta 932: 358–361

Krishtalik LI (1986) Energetics of multielectron reactions. Photosynthetic oxygen evolution. Biochim Biophys Acta 849: 162–171

Kulawiec RJ, Crabtree RH, Brudvig GW and Schulte GK (1988) Modeling the oxygen-evolving center of photosystem II: Synthesis and characterization of a tetranuclear manganese carboxylate complex. Inorg Chem 27: 1309–1311

Kuwabara T and Murata N (1982) Inactivation of photosynthetic oxygen evolution and concomitant release of three polypeptides in the photosystem II particles of spinach chloroplasts. Plant Cell Physiol 23: 533–539

Kuwabara T, Miyao M, Murata T and Murata N (1985) The function of the 33 kDa protein in the photosynthetic oxygen-evolution system studied by reconstitution experiments. Biochim Biophys Acta 806: 283–289

Kuwabara T, Reddy KJ and Sherman LA (1987) Nucleotide sequence of the gene from the cyanobacterium *Anacystis nidulans* R2 encoding the Mn-stabilizing protein involved in PS II water oxidation. Proc Natl Acad Sci USA 84: 8230–8234

Kyle DJ (1985) The 32,000 dalton Qb protein of photosystem II. Photochem. Photobiol. 41: 107–116

Kyle DJ and Ohad I (1986) The mechanism of photoinhibition in higher plants and algae. In: Staehlin LA and Arntzen CJ (eds) Encyclopedia of Plant Physiology, New Series, Vol 19, Photosynthesis III, pp 468–475. Springer-Verlag, Berlin

Larsson UK, Anderson JM and Andersson B (1987) Variations in the relative content of the peripheral and inner light-harvesting chlorophyll *a*/*b*-protein complex (LHC II) subpopulations during thylakoid light adaptation and development. Biochim Biophys Acta 894: 69–75

Lautner A, Klein R, Ljungberg U, Reiländer H, Bartling D, Andersson B, Reinke H, Beyreuther K and Herrmann RG (1988) Nucleotide sequence of cDNA clones encoding the complete precursor for the '10 kDa' polypeptide of photosystem II from spinach. J Biol Chem 263: 10077–10081

Lavergne J (1987) Optical-difference spectra of the S-state transitions in the photosynthetic oxygen-evolving complex. Biochim Biophys Acta 894: 91–107

Lippard SJ (1988) Oxoverbrueckte polyeisenzentren in biologie und chemie. Angew Chem 100: 353–371

Ljungberg U, Åkerlund H-E, Larsson C and Andersson B (1984) Identification of polypeptides associated with the 23 and 33 kDa proteins of photosynthetic oxygen evolution. Biochim Biophys Acta 767: 145–152

Ljungberg U, Åkerlund H-E and Andersson B (1986a) Isolation and characterization of the 10 kDa and 22 kDa polypeptides of higher plant photosystem 2. Eur J Biochem 158: 477–482

Ljungberg U, Henrysson T, Rochester CP, Åkerlund H-E and Andersson B (1986b) The presence of low-molecular-weight polypeptides in spinach photosystem II core preparations. Isolation of a 5 kDa hydrophilic polypeptide. Biochim Biophys Acta 849: 112–120

Machold O (1986) Relationship between the 43 kDa chlorophyll-protein of PS II and the rapidly metabolized 32 kDa Q_B protein. FEBS Lett 204: 363–367

Mano J, Takahashi M and Asada K (1987) Oxygen evolution from hydrogen peroxide in photosystem II: Flash-induced catalytic activity of water-oxidizing photosystem II membranes. Biochemistry 26: 2495–2501

Marder JB, Telfer A and Barber J (1988) The D1 polypeptide subunit of the Photosystem II reaction centre has a phosphorylation site at its amino terminus. Biochim Biophys Acta 932: 362–365

Masojidek J, Droppa M and Horváth G (1987) Analysis of the polypeptide composition of grana and stroma thylakoids by two-dimensional gel electrophoresis. Eur J Biochem 169: 283–288

Mathis P (1987) Primary reactions of photosynthesis: Discussion of current issues. In: Biggins J (ed) Progress in Photosynthesis Research, Vol 1, pp 151–160. Martinus Nijhoff, Dordrecht

Mathis P and Setif P (1981) Near infra-red absorption spectra of the chlorophyll *a* cations and triplet state *in vitro* and *in vivo*. Isr J Chem 21: 316–326

Mathis P and Rutherford AW (1987) The primary reactions of photosystem I and II of algae and higher plants. In: Amesz J (ed) New Comprehensive Biochemistry Vol 15, Photosynthesis, pp 63–96. Elsevier, Amsterdam

Matsushita T, Spencer L and Sawyer DT (1988) Synthesis and characterization of binuclear manganese complexes: Redox models for the water oxidation cofactor of Photosystem II. Inorg Chem 27: 1167–1173

Mattoo AK and Edelman M (1987) Intramembrane translocation and posttranslational palmitoylation of the chloroplast of 32 kDa herbicide-binding protein. Proc Natl Acad Sci USA 84: 1497–1501

McDermott AE, Yachandra VK, Guiles RD, Cole JL, Dexheimer SL, Britt RD, Sauer K and Klein MP (1988) Characterization of the manganese O_2-evolving complex and the iron-quinone acceptor complex in photosystem II from a thermophilic cyanobacterium by electron paramagnetic resonance and X-ray absorption spectroscopy. Biochemistry 27: 4021–4031

McCauley SW and Melis A (1986) Quantitation of Photosystem II in spinach chloroplasts. Biochim Biophys Acta 849: 175–182

Meiburg RF, van Gorkom HJ and van Dorssen RJ (1984) Non-electrogenic charge recombination in photosystem II as a source of sub-millisecond luminescence. Biochim Biophys Acta 765: 295–300

Metz JG, Pakrasi HB, Seibert M and Arntzen CJ (1986) Evidence for a dual function of the herbicide-binding D1 protein in photosystem II. FEBS Lett 205: 269–274

Meunier PC and Popovic R (1988) Optimization of the bare platinum electrode as an oxygen measurement system in photosynthesis. Photosynth Res 15: 271–279

Michael HP and Bennett J (1987) Identification of the phosphorylation site of an 8.3 kDa protein from photosystem II of spinach. FEBS Lett 212: 103–108

Michel H, Epp O and Deisenhofer J (1986) Pigment-protein interactions in the photosynthetic reaction center from *Rhodopseudomonas viridis*. EMBO J 5: 2445–2451

Miller M and Cox RP (1984) Inhibition of photosynthetic oxygen evolution in non-vesicular preparations releases Mn^{2+} into a restricted aqueous compartment. Biochem Biophys Res Comm 119: 168–172

Millner PA, Marder JB, Gounaris K and Barber J (1986) Localization and identification of phosphoproteins within the photosystem II core of higher-plant thylakoid membranes. Biochim Biophys Acta 852: 30–37

Millner PA, Gogel G and Barber J (1987) Investigation of the spatial relationships between photosystem 2 polypeptides by reversible crosslinking and diagonal electrophoresis. Photosynth Res 13: 185–198

Mimuro M, Yamazaki I, Itoh S, Tamai N and Satoh K (1988) Dynamic fluorescence properties of D1–D2–cytochrome *b*559 complex isolated from spinach chloroplasts: analysis by means of the time-resolved fluorescence spectra in picosecond time range. Biochim Biophys Acta 933: 478–486

Miyao M and Murata N (1984a) Role of the 33 kDa polypeptide in preserving Mn in the photosynthetic oxygen-evolution system and its replacement by chloride ions. FEBS Lett 168: 281–286

Miyao M and Murata N (1984b) Effect of urea on photosystem II particles. Evidence for an essential role of the 33 kilodalton polypeptide in photosynthetic oxygen evolution. Biochim Biophys Acta 765: 253–257

Miyao M, Murata N, Lavorel J, Maison-Peteri B, Boussac A and Etienne A-L (1987) Effect of the 33 kDa protein on the S-state transitions in photosynthetic oxygen evolution. Biochim Biophys Acta 890: 151–159

Moënne-Loccoz P, Robert B and Lutz M (1989) A resonance Raman characterization of the primary electron acceptor in photosystem II. Biochemistry 28: 3641–3654

Morris J and Herrmann RG (1984) Nucleotide sequence of the gene for the P680 chlorophyll a apoprotein of the photosystem II reaction center from spinach. Nucl Acids Res 12: 2837–2850

Murata N, Miyao M, Omata T, Matsunami H and Kuwabara T (1984) Stoichiometry of components in the photosynthetic oxygen evolution system of photosystem II particles prepared with triton X-100 from spinach chloroplasts. Biochim Biophys Acta 765: 363–369

Murata N, Miyao M, Hayashida N, Hidaka T and Sugiura M (1988) Identification of a new gene in the chloroplast genome encoding a low-molecular-mass polypeptide of photosystem II complex. FEBS Lett 235: 283–288

Murphy DJ (1986) The molecular organization of the photosynthetic membranes of higher plants. Biochim Biophys Acta 864: 33–94

Nakatani HY, Ke B, Dolan E and Arntzen CJ (1984) Identity of the photosystem II reaction center polypeptide. Biochim Biophys Acta 765: 347–352

Nanba O and Satoh K (1987) Isolation of a photosystem II reaction center consisting of D1 and D2 polypeptides and cytochrome *b*559. Proc Natl Acad Sci USA 84: 109–112

Nugent JHA (1987) Water binding to the oxygen-evolving system of chloroplasts Effects of isotope substitution on the S_2-state EPR spectrum. Biochim Biophys Acta 893: 184–189

Nugent JHA, Demetriou C and Lockett CJ (1987) Electron donation in photosystem II. Biochim Biophys Acta 894: 534–542

Nuijs AM, van Gorkom HJ, Plijter JJ and Duysens LNM (1986) Primary-charge separation and excitation of chlorophyll *a* in photosystem II particles from spinach as studied by picosecond absorbance-difference spectroscopy. Biochim Biophys Acta 848: 167–175

O'Malley PJ and Babcock GT (1984) EPR properties of immobilized quinone cation radicals and the molecular origin of Signal II in spinach chloroplasts. Biochim Biophys Acta 765: 370–379

O'Malley PJ, Babcock GT and Prince RC (1984) The cationic plastoquinone radical of the chloroplast water splitting complex. Hyperfine splitting from a single methyl group determines the EPR spectral shape of signal II. Biochim Biophys Acta 766: 283–288

Oh-oka H, Tanaka S, Wada K, Kuwabara T and Murata N (1986) Complete amino acid sequence of 33 kDa protein isolated from spinach photosystem II particles. FEBS Lett 197: 63–66

Ohad I, Koike H, Schochat S and Inoue Y (1988) Changes in the properties of reaction center II during the initial stage of photoinhibition as revealed by thermoluminescence measurements. Biochim Biophys Acta 933: 288–298

Okamura MY, Satoh K, Isaacson RA and Feher G (1987) Evidence of the primary charge separation in the D1/D2 complex of photosystem II from spinach: EPR of the triplet state. In Biggins J (ed) Progress in Photosynthesis Research, Vol 1, pp 379–381. Martinus Nijhoff, Dordrecht

Ono T and Inoue Y (1984a) Ca^{2+}-dependent restoration of O_2-evolving activity in $CaCl_2$-washed PS II particles depleted of 33, 24 and 16 kDa polypeptides. FEBS Lett 168: 281–286

Ono T and Inoue Y (1984b) Reconstitution of photosynthetic oxygen evolving activity by rebinding of 33 kDa protein. FEBS Lett 166: 381–389

Ono T and Inoue Y (1985) S-state turnover in the O_2-evolving system of $CaCl_2$-washed photosystem II particles depleted of three peripheral proteins as measured by thermoluminescence. Removal of 33 kDa protein inhibits S_3 to S_4. Biochim Biophys Acta 806: 331–340

Ono T, Zimmermann JL, Inoue Y and Rutherford AW (1986) EPR evidence for a modified S-state transition in chloride depleted photosystem II. Biochim Biophys Acta 850: 193–201

Ono T and Inoue Y (1986) Effects of removal and reconstitution of the extrinsic 33, 24 16 kDa proteins on flash oxygen yield in Photosystem II particles. Biochim Biophys Acta 850: 380–389

Ono T and Inoue Y (1987) Reductant-sensitive intermediates involved in multi-quantum process of photoactivation of latent O_2-evolving system. Plant Cell Physiol 28: 1293–1299

Ono T, Nakayama H, Gleiter H, Inoue Y and Kawamori A (1987) Modification of the properties of S_2 state in photosynthetic O_2-evolving center by replacement of chloride with other anions. Arch Biochem Biophys 256: 618–624

Ono T and Inoue Y (1988) Discrete extraction of the Ca atom functional for O_2 evolution in higher plant PS II by a simple low pH treatment. FEBS Lett 227: 147–152

Ono T and Inoue Y (1988) Abnormal S-state turnover in NH_3-binding Mn centers of photosynthetic O_2 evolving system. Arch Biochem Biophys 261:

Packham NK (1987) Phosphorylation of the 9 kDa photosystem II-associated protein and the inhibition of photosynthetic electron transport. Biochim Biophys Acta 893: 259–266

Packham NK (1988) Is the 9 kDa thylakoid membrane phosphoprotein functionally and structurally analogous to the 'H' subunit of bacterial reaction centres? FEBS Lett 231: 284–290

Packham NK, Hodges M, Etienne AL and Briantais JM (1988) Changes in the flash-induced oxygen yield pattern by thylakoid membrane phosphorylation. Photosynth Res 10: 221–232

Padhya S, Kambara T, Hendrickson DN and Govindjee (1986) Manganese-histidine cluster as the functional center of the water oxidation complex in photosynthesis. Photosynth Res 9: 103–112

Pearlstein RM (1987) Structure and exciton effects in photosynthesis. In: Amesz J (ed) New Comprehensive Biochemistry, Vol 15, Photosynthesis, pp 299–317. Elsevier, Amsterdam

Peter GF and Thornber JP (1988) The antenna components of photosystem II with emphasis on the major pigment-protein LHC IIb. In: Sheer H and Scheider (eds) Photosynthetic Light-harvesting Systems — Structure and Function. W de Gruyter and Co, Berlin, in press

Petrouleas V and Diner BA (1986) Identification of Q_{400}, a high-potential electron acceptor of photosystem II, with the iron of the quinone-iron acceptor complex. Biochim Biophys Acta 849: 264–275

Philbrick JB and Zilinskas BA (1988) Cloning, nucleotide sequence and mutational analysis of the gene encoding the photosystem II manganese-stabilizing polypeptide of *Synechocystis* 6803. Mol Gen Genet 212: 418–425

Pistorius EK and Schmid GH (1987) Effect of chloride ions on the S-state distribution and deactivation kinetics in preparations of the cyanobacterium *Anacystis nidulans*. Biochim Biophys Acta 890: 352–359

Plijter JJ, Aalbers SE, Barends J-PF, Vos MH and van Gorkom HJ (1988) Oxygen release may limit the rate of photosynthetic electron transport; the use of a weakly polarized oxygen electrode. Biochim Biophys Acta 935: 299–311

Preston C and Pace RJ (1985) The S-state dependence of Cl^--binding to plant photosystem II. Biochim Biophys Acta 810: 388–391

Radmer R and Ollinger O (1986) Do the higher oxidation states of the photosynthetic O_2-evolving system contain bound H_2O? FEBS Lett 195: 285–289

Renger G (1986) Herbicide interactions with photosystem II: Recent developments. Physiol Veg 24: 509–521

Renger G (1987a) Mechanistic aspects of photosynthetic water cleavage. Photosynthetica 21: 203–224

Renger G (1987b) Biological exploitation of solar energy by

photosynthetic water splitting. Angew Chemi (Int Eng Ed) 26: 643–660

Renger G (1988) On the mechanism of photosynthetic water oxidation to dioxygen. Chemica Scripta 28A: 105–109

Renger G and Govindjee (1985) The mechanism of photosynthetic water oxidation. Photosynth Res 6: 33–55

Renger G and Weiss W (1986) Studies on the nature of the water oxidizing enzyme system. III. Spectral characterization of the intermediary redox states in the water-oxidizing enzyme system Y in photosystem II. Biochim Biophys Acta 850: 173–183

Renger G and Hanssum B (1988) Studies on the deconvolution of flash-induced absorption changes into the difference spectra of individual redox steps within the water-oxidizing enzyme system. Photosynth Res 16: 243–259

Renger G, Wacker U and Voelker M (1987) Studies on the protolytic reactions coupled with water cleavage in photosystem II membrane fragment from spinach. Photosynth Res 13: 167–189

Rich PR and Moss DA (1987) The reaction of quinones in higher plant photosynthesis. In: Barber J (ed) Topics in Photosynthesis, Vol 8, The Light Reactions, pp 421–446. Elsevier, Amsterdam

Rolfe SA and Bendall DS (1989) The role of an extrinsic 9 kDa polypeptide in oxygen evolution by photosystem II particles from *Phormidium laminosum*. Biochim Biophys Acta 973: 220–224

Rutherford AW (1985a) Orientation of EPR signals arising from components in photosystem II membranes. Biochim Biophys Acta 807: 189–201

Rutherford AW (1985b) How close is the analogy between the reaction centre of photosystem II and that of purple bacteria? Biochem Soc Trans 14: 15–17

Rutherford AW and Zimmerman JL (1984) A new EPR signal attributed to the primary plastoquinone acceptor of photosystem II. Biochim Biophys Acta 767: 168–175

Rutherford AW, Patterson DR and Mullet JE (1981) A light-induced spinpolarized triplet detected by EPR in photosystem II reaction centers. Biochim Biophys Acta 635: 204–214

Rutherford AW, Renger G, Koike H and Inoue Y (1984) Thermoluminescence as a probe of photosystem II. The redox and protonation states of the secondary acceptor quinone and the O_2-evolving enzyme. Biochim Biophys Acta 767: 548–556

Rögner M, Dekker JP, Boekema EJ and Witt HT (1987) Size, shape and mass of the oxygen-evolving photosystem II complex from the thermophilic cyanobacterium *Synechococcus* sp. FEBS Lett 219: 207–211

Sandusky PO and Yocum CF (1986) The chloride requirement for photosynthetic oxygen evolution: factors affecting nucleophilic displacement of chloride from the oxygen-evolving complex. Biochim Biophys Acta 849: 85–93

Satoh K, Ohno T and Katoh S (1985) An oxygen-evolving complex with a simple subunit structure — 'a water-plastoquinone oxidoreductase' — from the thermophilic cyanobacterium *Synechococcus sp.* FEBS Lett 180: 326–330

Satoh K, Fujii Y, Aoshima T and Tado T (1987) Immunological identification of the polypeptide bands in the SDS-polyacrylamide gel electrophoresis of photosystem II preparations. FEBS Lett 216: 7–10

Sauer K, Guiles RD, McDermott AE, Cole JL, Yachandra VY, Zimmermann J-L, Klein MP, Dexheimer SL and Britt RD (1988) Spectroscopic studies of manganese involvement in photosynthetic oxygen evolution. Chemica Scripta 28A: 87–91

Saygin Ö and Witt HT (1985) Sequence of the redox changes of manganese and pattern of the changes of charges during water cleavage in Photosynthesis. Photobiochem Photobiophys 10: 71–81

Saygin Ö and Witt HT (1987) Optical characterization of intermediates in the water-splitting enzyme system of photosynthesis — possible states and configurations of manganese and water. Biochim Biophys Acta 893: 452–469

Sayre RT, Andersson B and Bogorad L (1986) The topology of a membrane protein: The orientation of the 32 kd Qb-binding chloroplast thylakoid membrane protein. Cell 47: 601–608

Schatz GH and van Gorkom HJ (1985) Absorbance difference spectra upon charge transfer to secondary donors and acceptors in photosystem II. Biochim Biophys Acta 810: 283–294

Schatz GH and Holzwarth AR (1986) Mechanisms of chlorophyll fluorescence revisited: Prompt or delayed emission from photosystem II with closed reaction centers? Photosynth Res 10: 309–315

Schatz GH, Brock H and Holzwarth AR (1987) Picosecond kinetics of fluorescence and absorbance changes in photosystem II particles excited at low photon density. Proc Natl Acad Sci USA 84: 8414–8418

Schatz GH, Brock H and Holzwarth AR (1988) A kinetic and energetic model for the primary processes in photosystem II. Biophys J 54: 397–405

Schenck CC, Blankenship RE and Parson WW (1982) Radical-pair decay kinetics, triplet yields and delayed fluorescence from bacterial reaction centers. Biochim Biophys Acta 680: 44–59

Schlodder E, Brettel K, Schatz GH and Witt HT (1984) Analysis of the Chl-a_{II}^+ reduction kinetics with nanosecond time resolution in oxygen-evolving Photosystem II particles from *Synechococcus* at 680 and 824 nm. Biochim Biophys Acta 765: 178–185

Schlodder E and Meyer B (1987) pH dependence of oxygen evolution and reduction kinetics of photooxidized chlorophyll *a*II (P-680) in Photosystem II particles from *Synechococcus* sp. Biochim Biophys Acta 890: 23–31

Schlodder E and Brettel K (1988) Primary charge separation in closed photosystem II with a lifetime of 11 ns. Flash-absorption spectroscopy with O_2-evolving photosystem II complexes from *Synechococcus*. Biochim Biophys Acta 933: 22–34

Schröder WP and Åkerlund H-E (1986) H_2O_2 accessibility to the Photosystem II donor side in protein-depleted inside-out thylakoids measured as flash-induced oxygen production. Biochim Biophys Acta 848: 359–363

Schröder WP, Henrysson T and Åkerlund H-E (1988) Characterization of low molecular mass proteins of photosystem II by N-terminal sequencing. FEBS Lett 235: 289–292

Seibert M, DeWit M and Staehlin LA (1987) Structural localization of the O_2-evolving apparatus to multimeric (tetrameric) particles on the luminal surface of freeze-etched photosynthetic membranes. J Cell Biol 105: 2257–2265

Seibert M, Cotton TM and Metz JG (1988a) Surface-enhanced Raman scattering spectroscopy: probing the lumenal surface of photosystem II membranes for evidence of manganese. Biochim Biophys Acta 934: 235–246

Seibert M, Picorel R, Rubin AB and Connolly JS (1988b) Spectral, photophysical and stability properties of isolated photosystem II reaction center. Plant Physiol 87: 303–306

Sharp RR and Yocum CF (1980) The kinetics of water exchange across the chloroplast membrane. Biochim Biophys Acta 592: 169–184

Sheats JE, Czernuszewicz RS, Dismukes GC, Rheingold AL, Petrouleas V, Stubbe JA, Armstrong WH, Beer RH and Lippard SJ (1987) Binuclear manganese (II) complexes of potential biological significance. J Am Chem Soc 109: 1435–1444

Shen J-R, Satoh K and Katoh S (1988) Calcium contents of oxygen evolving photosystem II preparations from higher plants. Effects of NaCl-treatment. Biochim Biophys Acta 933: 358–364

Shinozaki K, Hayashida N and Sugiura M (1988) *Nicotinana* chloroplast genes for components of the photosynthetic apparatus. Photosynth Res 18: 7–31

Simpson DJ and Andersson (1986) Extrinsic polypeptides of the chloroplast oxygen-evolving complex constitute the tetrameric ESs particles of higher plant thylakoids. Carlsberg Res Commun 51: 467–474

Sivaraja M, Philo JS, Lary J and Dismukes GC (1989) Photosynthetic oxygen evolution: Changes in magnetism of the water-oxidizing enzyme. J Am Chem Soc 111: 3221–3225

Sparrow RW and England RR (1984) Isolation of a calcium-binding protein from an oxygen-evolving photosystem II preparation. FEBS Lett 177: 95–98

Srinivasan AN and Sharp RR (1986a) Flash-induced enhancements in the proton NMR relaxation rate of photosystem II particles. Biochim Biophys Acta 850: 211–217

Srinivasan AN and Sharp RR (1986b) Flash-induced enhancements in the proton NMR relaxation rate of photosystem II particles: Response to flash trains of 1–5 flashes. Biochim Biophys Acta 851: 369–376

Staehlin LA (1986) Chloroplast structure and supramolecular organization of photosynthetic membranes. In: Staehlin LA and Arntzen CJ (eds) Encyclopedia of Plant Physiology, New Series, Vol 19, Photosynthesis III, pp 1–84. Springer-Verlag, Berlin

Steinmetz AA, Castroviejo M, Sayre RT and Bogorad L (1986) Protein PS II-G: an additional component of photosystem II identified through its plastid gene in maize. J Biol Chem 261: 2485–2488

Stewart AC, Ljungberg U, Åkerlund H-E and Andersson B (1985a) Studies on the polypeptide composition of the cyanobacterial oxygen-evolving complex. Biochim Biophys Acta 808: 353–362

Stewart AC, Siczkowski M and Ljungberg U (1985b) Glycerol stabilizes oxygen evolution and maintains binding of a 9 kDa polypeptide in photosystem II particles from the cyanobacteria, *Phormidium laminosum*. FEBS Lett 193: 175–179

Styring S and Rutherford WA (1987) In the oxygen evolving complex of photosystem II the S_0-state is oxidized to the S_1-state by D^+ (Signal II_{slow}) Biochemistry 26: 2401–2405

Styring S, Miyao M and Rutherford WA (1987) Formation and flash-dependent oscillation of the S_2-state multiline epr-signal in an oxygen evolving Photosystem-II preparation lacking the three extrinsic proteins in the oxygen-evolving system. Biochim Biophys Acta 890: 32–38

Styring S and Rutherford AW (1988a) The microwave power saturation of S II_{slow} varies with the redox state of the oxygen-evolving complex of photosystem II. Biochemistry 27: 4915–4923

Styring S and Rutherford AW (1988b) Deactivation kinetics and temperature dependence of the S-state transitions in the oxygen-evolving system of photosystem II measured by EPR spectroscopy. Biochim Biophys Acta 933: 378–387

Sundby C and Larsson C (1985) Transbilayer organization of the thylakoid galactolipids. Biochim Biophys Acta 813: 61–67

Tae G-S, Black MT, Cramer WA, Vallon O and Bogorad L (1988) Thylakoid membrane protein topography: transmembrane orientation of the chloroplasts cytochrome *b*559 psbE gene product. Biochemistry 27: 9075–9080

Takahashi M and Asada K (1986) Sizes of Mn-binding sites in spinach thylakoids. J Biol Chem 261: 16923–16926

Takahashi Y and Katoh S (1986) Numbers and functions of plastoquinone molecules associated with Photosystem II preparations from *Synechococcus* sp. Biochim Biophys Acta 848: 183–192

Takahashi Y, Hansson Ö, Mathis P and Satoh K (1987) Primary radical pair in photosystem II reaction center. Biochim Biophys Acta 893: 49–59

Tamura N and Cheniae G (1985) Effects of Photosystem II extrinsic proteins on microstructure of the oxygen-evolving complex and its reactivity to water analogs. Biochim Biophys Acta 809: 245–259

Tamura N and Cheniae G (1987) Photoactivation of the water-oxidizing complex in photosystem II membranes depleted of Mn and extrinsic proteins. I. Biochemical and kinetic characterization. Biochim Biophys Acta 890: 179–194

Tanaka S and Wada K (1988) The status of cysteine residues in the extrinsic 33 kDa protein of spinach photosystem II complexes. Photosynth Res 17: 255–266

Tang X-S and Satoh K (1985) The oxygen-evolving photosystem II core complex. FEBS lett 179: 60–64

Tang X-S and Satoh K (1986) Reconstitution of photosynthetic water-splitting activity by the addition of 33 kDa polypeptide to urea-treated PS II reaction center complex. FEBS Lett 201: 221–224

Theg SM, Jursinic PA and Homann PH (1984) Studies on the mechanism of chloride action on photosynthetic water oxidation. Biochim Biophys Acta 766: 636–646

Thompson LK and Brudvig GW (1988) Cytochrome *b*-559 may function to protect photosystem II from photoinhibition. Biochemistry 27: 6653–6658

Tiede DM and Dutton PL (1981) Orientation of the primary quinone of bacterial photosynthetic reaction centers contained in chromatophore and reconstituted membranes. Biochim Biophys Acta 637: 278–290

Trebst A and Depka B (1985) The architecture of photosystem II in plant photosynthesis. Which peptide subunits carry the reaction center of PS II? In: Michel-Beyerle ME (ed) Antennas and Reaction Centers in Photosynthetic Bacteria, pp 216–224. Springer-Verlag, Berlin

Trebst A, Depka B, Kraft B and Johanningmeier U (1988) The Q_B site modulates the conformation of the photosystem II reaction center polypeptides. Photosynth Res 28: 163–177

Trissl H-W, Breton J, Deprez J and Leibl W (1987) Primary electrogenic reactions of photosystem II as probed by the

light-gradient method. Biochim Biophys Acta 893: 305–319
Tyagi A, Hermans J, Steppuhn J, Jansson C, Vater F and Herrmann RG (1987) Nucleotide sequence of cDNA clones encoding the complete '33 kDa' precursor protein associated with the photosynthetic oxygen-evolving complex from spinach. Mol Gen Genet 207: 288–293
Vambutas V and Beattie DS (1987) Chloride-driven 3,3′-dipropylthiodicarbocyanine ($DiSC_3$-(5)) and tetraphenylphosphonium cation (TPP^+) uptake by thylakoids: inhibition of uptake by antibodies raised to the major polypeptides of the chloride efflux active particle(s). Biochim Biophys Acta 893: 69–74
van Dorssen RJ, Breton J, Plijter JJ, Satoh K, van Gorkom HJ and Amesz J (1987) Spectroscopic properties of the reaction center and the 47 kDa chlorophyll protein of photosystem II. Biochim Biophys Acta 893: 267–274
van Gorkom HJ (1985) Electron transfer in photosystem II. Photosynth Res 6: 97–112
van Grondelle R (1985) Excitation energy transfer, trapping and annihilation in photosynthetic systems. Biochim Biophys Acta 811: 147–195
van Rensen JJS and Snel JFH (1985) Regulation of photosynthetic electron transport by bicarbonate, formate and herbicides in isolated broken and intact chloroplasts. Photosynth Res 6: 231–246
van Rensen JJS, Tonk WJM and de Bruijin SM (1988) Involvement of bicarbonate in the protonation of the secondary quinone electron acceptor of photosystem II via the non-haem iron of the quinone-iron acceptor complex. FEBS 226: 354–351
Vasil'ev IR, Matorin DN, Lyadsky VV and Venediktov PS (1988) Multiple action sites for photosystem II herbicides as revealed by delayed fluorescence. Photosynth Res 15: 33–39
Vass I, Koike H and Inoue Y (1985) High pH effect on S-state turnover in chloroplasts studied by thermoluminescence. Short-time alkaline incubation reversibly inhibits S_3 to S_4 transition. Biochim Biophys Acta 810: 302–309
Vass I and Inoue Y (1986) pH dependent stabilization of $S_2Q_A^-$ and $S_2Q_B^-$ charge pairs studied by thermoluminescence. Photosynth Res 10: 431–436
Vass I, Ono T and, Inoue Y (1987) Stability and oscillation properties of thermoluminescent charge pairs in the O_2-evolving system depleted of Cl^- or the 33 kDa extrinsic protein. Biochim Biophys Acta 892: 224–235
Velthuys B (1981) Electron-dependent competition between plastoquinone and inhibitors for binding in photosystem II. FEBS Lett 126: 277–281
Velthuys B (1987) The photosystem II reaction center. In: Barber J (ed) Topics in photosynthesis, Vol 8, The Light Reactions, pp 341–378. Elsevier, Amsterdam
Velthuys B (1988) Spectroscopic characterization of the acceptor state Q_A^- and the donor state S_2 of photosystem II of spinach in the blue, red and near-infrared. Biochim Biophys Acta 933: 249–257
Vermaas WFJ, Renger G and Dohnt G (1984) The reduction of the oxygen-evolving system in the cloroplasts by thylakoid components. Biochim Biophys Acta 704: 194–202
Vermaas WFJ, Rutherford AW and Hansson Ö (1988a) Site-directed mutagenesis in photosystem II of the cyanobacterium *Synochocystis* sp. PCC 6803: Donor D is a tyrosine residue in the D2 protein. Proc Natl Acad Sci USA 85 8477–8481
Vermaas WFJ, Ikeuchi M and Inoue Y (1988b) Protein composition of the photosystem II core complex in genetically engineered mutants of the cyanobacterium *Synochocystis* sp. PCC 6803. Photosynth Res 17: 97–113
Vincent JB and Christou G (1987) A molecular 'double-pivot' mechanism for water oxidation. Inorg Chim Acta 136: L41–L43
Warden JT and Csatorday K (1987) On the mechanism of linolenic acid inhibition in photosystem 2. Biochim Biophys Acta 890: 215–223
Wasielewski MR, Johnson DG, Seibert M and Govindjee (1989) Determination of the primary charge separation rate in isolated photosystem II reaction centers with 500-fs time resolution. Proc Natl Acad Sci USA 86: 524–528
Weiss W and Renger G (1986) Studies on the nature of the water-oxidizing enzyme. II. On the functional connection betweeen the reaction-center complex and the water-oxidizing enzyme system Y in photosystem II. Biochim Biophys Acta 850: 173–183
Westhoff P, Farchaus JW and Herrmann RG (1986) The gene for the M_r 10,000 phosphoprotein associated with photosystem II is part of the *psbB* operon of the spinach plastid chromosome. Curr Genet 11: 165–169
Weighardt K, Bossek U, Nuber B, Weiss J, Bonvoisin J, Corbella M, Vitols SE and Girerd JJ (1988) Synthesis, crystal structures, reactivity, and magnetochemistry of a series of binuclear complexes of manganese (II), −(III), and -(IV) of biological relevance. The crystal structure of $[L'Mn^{IV}(\mu\text{-}O)_3Mn^{IV}L'](PF_6)_2 \cdot H_2O$ containing an unprecedented short Mn · · · Mn distance of 2.296 Å. J Am Chem Soc 110: 7398–7411
Witt HT (1987) Examples for the cooperation of photons, exitons, electrons, electric fields and protons in the photosynthesis membrane. New J Chem 11: 91–101
Woodbury NWT and Parson WW (1984) Nanosecond fluorescence from isolated photosynthetic reaction centers of *Rhodopseudomonas sphaeroides*. Biochim Biophys Acta 767: 345–361
Wydrzynski T (1982) Oxygen evolution in photosynthesis. In: Govindjee (ed) Photosynthesis: Energy Conversion by Plants and Bacteria, Vol I, pp 469–506. Academic Press, New York
Wydrzynski T and Renger G (1986) On the interpretation of the NMR water-proton relaxivity of photosynthetic membrane samples: ramifications in the use of EDTA. Biochim Biophys Acta 851: 65–74
Wydrzynski T and Inoue Y (1987) Modified photosystem II acceptor properties upon replacement of the quinone at the Q_B site with 2,5-dimethyl-p-benzoquinone and phenyl-p-benzoquinone. Biochim Biophys Acta 893: 33–42
Wydrzynski T, Marks SB, Schmidt PG, Govindjee and Gutowsky HS (1978) Nuclear magnetic relaxation by the manganese in aqueous suspensions of chloroplasts. Biochemistry 17: 2155–2162
Wydrzynski T, Huggins BJ and Jursinic PA (1985) Uncoupling of detectable O_2 evolution from the apparent S-state transitions in photosystem II by lauroylcholine chloride: possible implications in the photosynthetic water-splitting mechanism. Biochim Biophys Acta 809: 125–136

Wydrzynski T, Ångström J and Vänngård T (1989) H_2O_2 formation by photosystem II. Biochim Biophys Acta 973: 23–28

Yachandra VK, Guiles RD, Sauer K and Klein MP (1986) The state of manganese in the photosynthetic apparatus. 5. The chloride effect in photosynthetic oxygen evolution. Is halide coordinated to the EPR-active manganese in the O_2-evolving complex? Biochim Biophys Acta 850: 333–342

Yamada Y, Itoh N and Satoh K (1985) A versatile chromatographic procedure for purifying PS II reaction center complex from digitonin extracts of spinach thylakoids. Plant Cell Physiol 26: 1263–1271

Yamaguchi N, Takahashi Y and Satoh K (1988) Isolation and characterization of a photosystem II core complex depleted in the 43 kDa-chlorophyll-binding subunit. Plant Cell Physiol 291: 87–158

Yamamoto Y (1988) Analysis of the relationship between the extrinsic 30 kDa protein, manganese and oxygen evolution in the thylakoid of *Chlamydomonas reinhardtii* grown under manganese-deficient conditions. Biochim Biophys Acta 933: 165–171

Yocum CF, Yerkes CT, Blankenship R, Sharp RR and Babcock GT (1981) Stoichiometry, inhibitor sensitivity, and organization of manganese associated with photosynthetic oxygen evolution. Proc Natl Acad Sci USA 78: 7507–7511

Zimmerman J-L and Rutherford AW (1984) EPR studies of the oxygen-evolving enzyme of photosystem II. Biochem Biophys Acta 767: 160–167

Zimmerman J-L and Rutherford AW (1986a) Photoreductant-induced-oxidation of Fe^{2+} in the electron-acceptor complex of photosystem II. Biochim Biophys Acta 851: 416–423

Zimmerman J-L and Rutherford AW (1986b) Electron paramagnetic resonance properties of the S_2 state of the oxygen evolving complex of photosystem II. Biochemistry 25: 4609–4615

Zuber H, Brunisholz R and Sidler W (1987) Structure and function of light-harvesting pigment-protein complexes. In: Amesz J (ed) Photosynthesis, pp 233–271. Elsevier, Amsterdam

Zucchelli G, Garlaschi FM and Jennings RC (1988) Influence of electrostatic screening by cations on energy coupling between photosystem II reaction centers and the light-harvesting chlorophyll *a/b* protein complex II. Biochim Biophys Acta 934: 144–150