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Water Oxidation in Photosystem II: From Radical Chemistry to Multielectron Chemistry†

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The basic reactions in photosynthesis involve the light-driven disruption of stable chemical bonds and transfer of the electrons in these bonds to form reactive intermediate species. Ultimately, the free energy trapped in this process is used by the organism to drive the synthesis of proteins, nucleic acids, and other complex biomolecules. Schematically, these events can be represented as

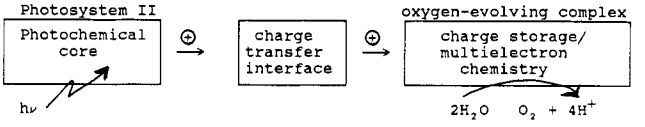
low energy electrons (stable substrate) $\xrightarrow{h\nu}$ high energy electrons (reactive intermediates) $\xrightarrow{\text{spontaneous}}$ biochemical reactions → plant growth and reproduction

In higher plants, algae, and cyanobacteria the low-energy electron source is water, which is oxidized to molecular oxygen with the injection of four electrons into the photosynthetic system:

$$2H_2O \rightarrow O_2 + 4H^+ + 4e^-$$

In developing the capacity to metabolize water, these organisms overcame two major difficulties. First, water is a very stable molecule, hence its abundance, and a reactive, unstable species must be created within the organism to oxidize it. Access to this highly oxidizing species must be controlled so that it is directed at water and not at components vital to the organism itself. Second, the photochemistry that occurs in photosynthesis is one-electron photochemistry; that is, one absorbed photon generates one oxidizing equivalent. Water chemistry, as indicated above, is a four-electron process, and a means by which to combine the oxidizing power of four absorbed photons is necessary. Moreover, this has to be done in such a way that reactive intermediates in the water oxidation process, such as hydroxyl radical or peroxide, are not released into the cell.

The photochemical and electron-transfer reactions that occur in the membrane-bound structure that evolved to overcome these obstacles, the photosystem II/oxygen-evolving complex (PSII/OEC),[1] can be represented as



Photosystem II forms the photochemical core of the system: upon light absorption PSII generates oxidizing equivalents or holes at sufficiently high potential to oxidize water. These oxidizing equivalents, designated as ⊕, are transferred one at a time through the charge-transfer interface to the oxygen-evolving complex where the four equivalents required in the water oxidation process are accumulated. Accordingly, the OEC can exist in five redox states depending on the number of stored oxidizing equivalents. These redox states are designated S_0–S_4, where the subscript denotes the charge storage state, with S_4 being the most oxidizing and capable of oxidizing water. In many respects the OEC acts as a capacitor, and the charge-transfer reactions that precede water splitting have a marked dependence on the S state of the complex.

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[1] Abbreviations: PSII, photosystem II; OEC, oxygen-evolving complex.

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The PSII/OEC exists as a discrete, isolable entity in the photosynthetic membrane [for reviews see Ghanotakis and Yocum (1985), Babcock (1987), Andréasson and Vänngård (1988), Renger (1988), and Brudvig et al. (1989)], and there is no indication of charge-transfer communication between different PSII/OEC units. Thus, a remarkable aspect of this complex, which has emerged as a general theme in the organization of photosynthetic electron-transfer assemblies, is the rapid departure from free radical chemistry. That is, the charge separation that occurs in the photochemical core necessarily generates one-electron oxidized and reduced species. The oxidizing and reducing equivalents carried by these radical intermediates are quickly combined at staging components or "gates", and subsequent reactions occur from these multielectron oxidized or reduced species. This gating activity usually takes place within the complex that contains the photochemical core and provides catalytic flexibility, as well as protection from reactive radical species. In the water-splitting process, this function is particularly apparent. The OEC acts as a four-electron gate, and radical reactions in water oxidation are avoided. This perspective aims at using recent results on the structure and function of the PSII/OEC to provide insight into the underlying principles of its operation.

Biochemical Organization of the Photosystem II/Oxygen-Evolving Complex

The close association of the photochemistry that produces the oxidizing equivalents necessary for water oxidation and the catalytic site at which water splitting occurs was suggested by the flashing light, oxygen-evolution measurements of Joliot, Kok, and their respective co-workers (Joliot & Kok, 1975). These experiments led to the concept of the charge storage center with its S_n valence states and to the tight physical coupling between PSII photochemistry and water oxidation at the charge storage center. As the structure of the photosynthetic membrane was elucidated, the expectation developed that both functions are incorporated in a single membrane-bound complex. This expectation was confirmed when Stewart and Bendall (1979) isolated an oxygen-evolving PSII complex from a thermophilic bacterium; Yocum and co-workers (Berthold et al., 1981) provided an extremely convenient, high-yield procedure by which to disintegrate the photosynthetic membrane and recover O_2-evolving PSII complexes. This latter development provided the starting material for a range of biochemical and biophysical experiments that have generated detailed insight into the organization and function of the PSII/OEC.

From a biochemical perspective, the PSII/OEC complex has been remarkably easy to resolve. Thus, a variety of smaller assemblies has been isolated following selective detergent solubilization of individual polypeptides. This situation has allowed rapid progress to be made in understanding the subunit composition of the complex and the functions of the specific polypeptides in the light absorption, charge separation, charge storage, and water oxidation reactions. While a considerable effort is needed to complete these assignments and, in particular, to understand the molecular basis for various activities associated with the PSII/OEC, there is enough information available to construct reasonable working models for the polypeptide and cofactor organization. Figure 1 shows such a model. The redox cofactors that are required for photochemistry and oxygen evolution are bound into a membrane-spanning complex that contains both extrinsic and intrinsic polypeptides. Several of these cofactors are indicated in Figure 1 and include the photochemically active chlorophyll P_{680}, a pheophytin initial electron acceptor, the quinone

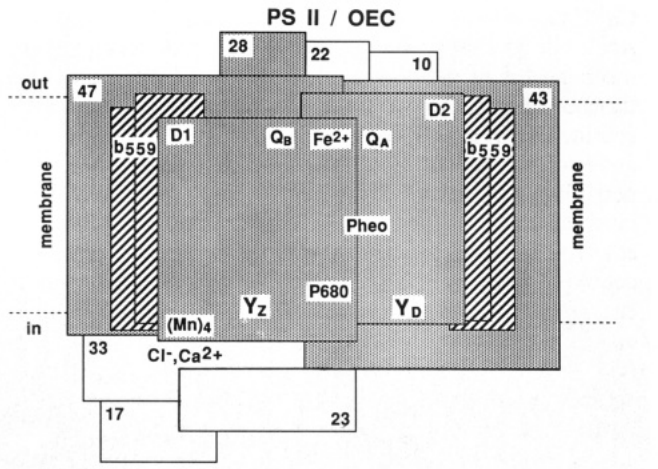

FIGURE 1: Model for the structure of the photosystem II/oxygen-evolving complex. Masses (in kilodaltons) are indicated for each polypeptide except for D1 and D2, which have masses of approximately 34 and 32 kDa, respectively, and cytochrome *b*-559, which occurs as a heterodimer of 4- and 9-kDa polypeptides. Chlorophyll binding subunits are stippled, and cytochrome *b*-559, which probably has two copies per PSII, is dashed. The 17-, 23-, and 33-kDa polypeptides are peripheral to the membrane; all other subunits are membrane-spanning. P_{680} is the reaction center chlorophyll, Pheo is an intermediate pheophytin electron acceptor, Q_A and Q_B are quinone acceptors, and Y_Z and Y_D are redox-active tyrosines. Other chlorophyll and pheophytin chromophores, which are associated with the PSII/OEC, are not shown.

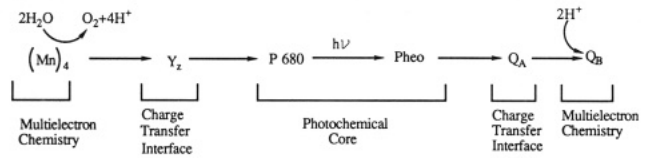

FIGURE 2: Sequence of electron transfers that occur in the PSII/OEC. On the acceptor side, the two-electron reduced species Q_BH_2 dissociates from its D1 binding site and diffuses to the cytochrome b_6f complex where further redox chemistry and proton translocation occur.

electron acceptors Q_A and Q_B, and the ferrous ion that is associated with these species. Four manganese atoms are involved in the charge storage/water oxidation site. This center is linked to P_{680} by a redox-active tyrosine, Y_Z, which functions as the charge-transfer interface. A second redox-active tyrosine, Y_D, and cytochrome *b*-559 are also present but do not appear to operate intimately in the main PSII/OEC functions. In terms of electron-transfer sequence and the transition from radical to multielectron chemistry, a simple linear chain best describes the situation (Figure 2). The kinetics of these reactions are considered in more detail below; before doing this, we discuss briefly the polypeptides and non-redox-active cofactors, Ca^{2+} and Cl^-, shown in Figure 1.

Approximately 12 different polypeptides are involved in the PSII/OEC; at least 3 of these are peripheral, the other 9 are membrane-spanning. The extrinsic polypeptides—the 17-, 23-, and 33-kDa subunits in Figure 1—appear to serve at least two functions. The first is to facilitate binding of the inorganic cofactors that are necessary for water-splitting chemistry. These include Cl^- and Ca^{2+}, in addition to the manganese ions at the site of charge storage and water oxidation chemistry [for review, see Homann (1987) and Babcock (1987)]. The function of Cl^- is not well understood, although the fact that amines, which bind to manganese (Beck & Brudvig, 1988; Britt et al., 1989) and inhibit O_2 evolution, are competitive with Cl^- provides support for the idea that the anion ligates manganese in some of the S states in a mechanistically important way (Sandusky & Yocum, 1984, 1986). The role of

Ca^{2+} is even more unclear. That this species is required for stable charge storage in the Mn ensemble is apparent, but the mechanism by which Ca^{2+} operates and the specific S-state transitions that are affected by its depletion are matters of considerable controversy [e.g., Ono & Inoue (1989), Boussac and Rutherford (1988), and dePaula et al. (1986)]. Interesting possibilities include models in which Ca^{2+} is either directly involved with manganese in a heteronuclear cluster (Bonadies et al., 1989; Penner-Hahn et al., 1989) or occurs with a unique coordination sphere but in close proximity to the manganese ensemble (Rutherford, 1989). The second function of the extrinsic subunits, and particularly of the 33-kDa polypeptide (Ghanotakis et al., 1984), is to isolate the endogenous redox-active species in the complex from the aqueous milieu. Owing to the likelihood of rearrangements occurring in the intrinsic polypeptide core upon removal of the soluble subunits [e.g., Hoganson et al. (1989)], this function has been more difficult to study quantitatively. Nonetheless, achieving such isolation was the key to the successful construction of the water oxidation system, and the erection of a diffusion barrier to limit access of unwanted reductants to the intermediate S states in water splitting seems to be part of the strategy.

Turning to the intrinsic polypeptides in Figure 1, the D1/D2 heterodimer forms the photochemical core by binding the chlorophylls (P_{680}), the pheophytins, and quinones that mediate the light-driven charge separation reaction (Nanba & Satoh, 1987). The sequence and secondary structure analogies that exist between D1/D2 and the corresponding L/M complex that comprises the bacterial reaction center core have been well documented [e.g., Hearst (1986) and Michel and Deisenhofer (1988)]. The pseudo-C_2 symmetry in protein structure and chromophore arrangement that occurs in the bacterial reaction center appears to be preserved in D1/D2 and has been shown to be a useful tool in designing experiments to study PSII (Debus et al., 1988a,b; Vermaas et al., 1988). This topic and the related issue of whether and where the pseudo-C_2 symmetry breaks down in the O_2-evolving system are considered in detail below.

At least three of the other intrinsic polypeptides in Figure 1, the 47-, 43-, and 28-kDa polypeptides, are also known to bind chlorophyll. The 47-kDa polypeptide can be isolated along with D1, D2, and cytochrome *b*-559 in a discrete unit. Recent work has shown that these particles from two-dimensional lattices can be studied by electron microscopy and that, despite the fact that this preparation lacks endogenous quinone [Petersen et al., 1989; see also Diner et al. (1988b)], a tyrosine radical (presumably either Y_Z^+ and Y_D^+) can be generated by light in the presence of an exogenous acceptor. This latter finding contrasts with the situation in more highly resolved D1/D2/*b*-559 preparations for which tyrosine radical generation has not been reported. That the 47-kDa polypeptide may play additional roles in PSII beyond a simple light-harvesting function is also suggested by reports that the 33-kDa peripheral polypeptide can be cross-linked to it with retention of both manganese and O_2-evolution activity [Enami et al., 1989; Bricker et al., 1988; see also Gounaris et al. (1988)]. The 28-kDa polypeptide binds chlorophyll *a* and had been isolated in pure form (Ghanotakis et al., 1987). It has also been implicated in maintaining the native Q_B binding site on the D1 polypeptide (Bowlby et al., 1988). The 22- and 10-kDa polypeptides, which apparently do not bind cofactors, also appear to influence events in the vicinity of the quinones as their removal from PSII preparations correlates with increased accessibility of Q_A^- to exogenous acceptors. The other intrinsic polypeptides in PSII, the 4 and 9 kDa, provide histidine ligands, one from each polypeptide, to the heme of cyt *b*-559 (Babcock et al., 1985). Recent work has shown that both *b*-559 hemes are likely to be oriented to the outside of the membrane (Tae et al., 1988).

Electron-Transfer Kinetics in the PSII/OEC

The simple linear scheme for PSII electron transfer shown in Figure 2 is now generally accepted. In this section we focus on the kinetics of these reactions as they occur under conditions that are close to physiological. At low temperatures, the S-state oxidation reactions become slow reactive to those of competing pathways, and interesting alterations to the scheme in Figure 2 occur. In one of these alternative pathways, cytochrome *b*-559 is oxidized by way of a redox-active chlorophyll through Y_Z^+ and/or P_{680}^+. This pathway is postulated to have physiological significance in protecting PSII from photoinhibition, to which it is especially sensitive owing to the highly oxidizing conditions that are necessary for water oxidation (Thompson & Brudvig, 1988). This model is well suited to experimental test; the details of this pathway, however, will not be considered further here.

Primary Charge Separation and Electron Removal in PSII. The analogy to the photochemical reactions and structures that occur in the photosynthetic bacteria has led to rapid progress in understanding the primary charge separation in PSII (Michel & Deisenhofer, 1988). Schatz et al. (1988) predicted a formation time of $\sim$3 ps for P_{680}^+ $Pheo^-$ in open reaction centers, which has recently been observed by Wasielewski et al. (1989). An interesting aspect to this reaction is that this primary charge-separated state is close in energy to the precursor P_{680} electronic excited state (Van Gorkom, 1985; Schatz et al., 1988). This circumstance leads to several consequences: the primary photochemistry is reversible, with P_{680} serving as a shallow trapping state for excitons in the antenna pigment bed; the exciton decay kinetics are trap-limited rather than diffusion-limited, and the trapping time is a function of the antenna size. Mathis and co-workers (Hansson et al., 1988) have considered similar effects in discussing their observation that the P_{680}^+ $Pheo^-$ lifetime and yield are functions of the antenna size in various PSII preparations [see also Schlodder and Brettel (1988)]. Schatz et al. (1988) note that the interrelationship between antenna size and trapping time allows PSII to use the antenna system for exciton storage; that is, the antenna serves as a buffer for the slower electron-transfer steps that follow charge separation. This strategy was cited as increasing the overall efficiency of charge separation. As noted below, the use of kinetic buffers to increase stable charge separation efficiencies is also apparent in the reactions that precede O_2 evolution.

The electron transfer from $Pheo^-$ to Q_A occurs in the 300–600-ps range and has been resolved by studying both the oxidation of $Pheo^-$ (Nuijs et al., 1986; Schatz & Holzwarth, 1987) and the reduction of Q_A (Eckert et al., 1988). This time appears to be slightly slower than the 200-ps $BPheo^-$ to Q_A transfer in bacterial centers (Michel & Deisenhofer, 1988). This difference has also been observed recently in direct, time-resolved photovoltage measurements of the primary charge separation event (Trissl & Leibl, 1989). In the same study, the dielectric distance between Pheo and Q_A was found to be shorter in PSII than in the bacterial reaction centers. The slowing down of the $Pheo^-/Q_A$ reaction, despite the shorter distance, was attributed to a larger reorganization energy accompanying Q_A reduction in the oxygen-evolving organisms relative to the bacteria.

The final electron-transfer event in the shuttling of electrons out of PSII involves the transition to multielectron chemistry

Table I: Stoichiometries and Kinetics for Reactions Preceding O_2 Evolution

reaction	S state prior to photon absorption				ref
	S_0	S_1	S_2	S_3	
$Y_ZP_{680}^+ \rightarrow Y_Z^+P_{680}$ (ns)	20	20	40, 280	40, 280	Meyer et al., 1989
$Y_Z^+S_n \rightarrow Y_ZS_{n+1}$	30 μs	100 μs	300 μs	1.0–1.2 ms	Babcock et al., 1976; Dekker et al., 1984b
H^+ release	1	0	1	2	Saphon & Crofts, 1977
H^+ appearance kinetics	250 μs		220 μs	1.2 ms	Forster & Junge, 1985
formal charge[a]	0	0	+1	+1	
$S_n \rightarrow S_{n-1}$ (min)		stable	3–3.5	3.5–4	Styring & Rutherford, 1988
T_{freeze} (K)[b]	220–225	135–140	230	235	Styring & Rutherford, 1988
E_a (kJ/mol)[c]		9.6	26.8	15.5 (T >289 K), 59.4 (T <289 K)	Koike et al., 1987

[a] The formal charge is determined by considering proton release and the number of oxidizing equivalents stored in the OEC; below pH 5.3 protonation of an endogenous group occurs that also influences the formal charge; see Meyer et al. 1989). [b] T_{freeze} is defined as the temperature at which the indicated S-state transition is half-inhibited. [c] Determined in a thermophilic cyanobacterium; the exact kinetics in this system vary slightly from those in higher plant preparations, but the general characteristics of the various reactions appear to be maintained.

in the $Q_A^- \rightarrow Q_B$ transfer. The Q_B^- species remains tightly bound in its binding site and is reduced to the Q_B^{2-} species in a second electron transfer. The kinetics of the oxidation of the Q_A^- depend on the redox state of Q_B: Q_B^- is formed in ~200 μs whereas Q_B^{2-} is formed at about half this rate [see Crofts and Wraight (1983) for a review of this topic and of the fate of Q_BH_2 after it leaves its binding site].

Hole Transfer and Accumulation Prior to Water Oxidation. The analogy to electron transfer in bacterial reaction centers clearly breaks down in the reactions that transfer holes out of the oxidized reaction center chlorophyll. In PSII, P_{680}^+ is reduced by a tyrosine residue (Y_Z in Figure 2; Barry & Babcock, 1987; Debus et al., 1988a,b; Gerken et al., 1988) whereas a cytochrome is the electron source in the non-oxygen-evolving systems. This difference clearly reflects the difference in function. In PSII, the oxidizing power generated in producing P_{680}^+ ($E_m^{0\prime} \approx 1.2$ V) must be maintained and directed eventually at water. Tyrosine oxidation, which produces the highly oxidizing tyrosine radical ($E_m^{0\prime} \approx 1.0$ V), is a means by which to achieve this. The reduction potentials generated photochemically in the bacterial reaction center are significantly lower, and cytochrome oxidation provides an energetically economic means by which to achieve stabilization of the primary charge separation.

Table I summarizes current data on the half-times and, where available, the temperature dependencies of the various electron-transfer and proton release events that occur as the PSII/OEC builds the oxidizing potential required for H_2O decomposition. Examination of these data reveals that the electron-transfer kinetics depend on both the protonation state in PSII and the oxidation state of the manganese complex. This is summarized in Table I under the "formal charge" heading; for the $S_0 \rightarrow S_1$ transition, for example, the oxidizing equivalent stored in the OEC is neutralized by the proton released during this S-state advance, and thus the formal charge in the PSII/OEC is the same in the S_0 and S_1 states. Witt and co-workers have recently carried out an examination of the pH dependence of the $Y_ZP_{680}^+ \rightarrow Y_Z^+P_{680}$ reaction (Meyer et al., 1989). Their results indicate that the protonation state of an acid/base group ($pK_a = 5.3$) also influences the kinetics of this reaction: at pH values below the pK_a of this group the $Y_ZP_{680}^+$ electron transfer is retarded compared to its kinetics at pH values above the pK_a. The biphasic time course of the $Y_ZP_{680}^+$ reaction for complexes in the S_2 and S_3 states (Table I) was attributed to heterogeneity in the sample with respect to the protonation state of this group. Witt and co-workers consider that the formal charge dependence of the redox reactions in PSII reflects direct electrostatic effects; protein rearrangements that accompany an S-state advance also provide an explanation for this behavior.

The rate of the $Y_ZP_{680}^+ \rightarrow Y_Z^+P_{680}$ reaction correlates well with the formal charge concept. The $S_nY_Z^+ \rightarrow S_{n+1}Y_Z$ reactions, however, depend more strongly simply on the oxidation state of the cluster, slowing down by roughly a factor of 3 for each additional oxidizing equivalent stored in the S ensemble. Babcock et al. (1976) noted early on that the half-time of the reduction of Y_Z^+ during the $S_3 \rightarrow S_0$ transition corresponded to the release kinetics of the O_2 formed on this transition and concluded that the electron-transfer, rather than water chemistry, rate limits the oxygen-evolution process. This conclusion accorded well with the lack of a significant deuterium isotope effect in the water oxidation process (Sinclair & Arnason, 1974). Recent work by Van Gorkom and co-workers (Plijter et al., 1988), however, has suggested that the early measurements of O_2 release kinetics may be in error and that O_2 remains bound for approximately 50 ms after its formation on the $S_3 \rightarrow S_0$ transition. Resolution of this issue is necessary.

The nature of the individual S-state transitions and the factors that influence their kinetics are beginning to be understood more clearly. X-ray absorption spectroscopy indicates a relatively high average manganese oxidation state (~+3) for the relaxed, low S-state ensemble (Sauer et al., 1988; Penner-Hahn et al., 1989). Data from a variety of spectroscopic approaches including optical absorption, EPR, X-ray absorption, and NMR [for reviews see Babcock (1987), Andreasson and Vanngard (1988), and Brudvig et al. (1989)] suggest that manganese oxidation is involved in most, if not all, of the S-state transitions. The optical data are probably the most direct in this context and, on the basis of near-UV absorption changes that are similar for each S-state advance [with the possible exception of the $S_0 \rightarrow S_1$ transition; see Lavergne (1989)], have been interpreted to indicate that the manganese ensemble acts as a linear charge accumulator as the OEC moves to the S_4 state (Kretschmann et al., 1988; Lavergne, 1989; Dekker, 1989). The other spectroscopies generally support this notion [see, for example, Evelo et al. (1989a)], with the important exception of $S_2 \rightarrow S_3$. For this transition, there is no evidence for a manganese valence change in the X-ray absorption edge, and both NMR and EPR fail to detect alterations in manganese valence as judged by relaxation measurements. These results are somewhat less direct than the optical work, however, and this situation, coupled with the fact that the $S = {}^1/_2$ multiline EPR signal of the S_2 state disappears upon formation of S_3, suggests that manganese oxidation, perhaps accompanied by structural rearrangement, does occur on this transition.

Insight into the extent and functional implication of conformational change during the various S-state transitions is becoming available. Koike et al. (1987) and Styring and Rutherford (1988) have recently measured the temperature

dependencies of the individual S-state transitions. The former group used a thermophilic cyanobacteria for their work and obtained activation energies for three of the four S-state transitions as indicated in Table I. They found that the $S_1 \rightarrow S_2$ transition had a relatively small activation energy, which accords well with the observation that this transition persists to much lower temperatures than the other S-state changes (Table I). Styring and Rutherford interpreted the low inhibition temperature as reflecting the fact that $S_1 \rightarrow S_2$ is the only S-state advance that is not accompanied by proton release, suggesting that conformation rearrangements on this transition will be minimal. This interpretation, however, is at odds with the observation that the activation entropy is significantly larger for $S_1 \rightarrow S_2$ [−140 J/(mol·K)] than for $S_2 \rightarrow S_3$ [−85 J/(mol·K)], which indicates that conformational rearrangements to accommodate the increase in formal charge on the $S_1 \rightarrow S_2$ transition are of consequence (Koike et al., 1987). In agreement with this interpretation, de Paula et al. (1987) have noted that the conversion between the two EPR-detectable forms of the S_2 state, the g 4.1 and the multiline signals, proceeds with a significant negative activation entropy (~−90 J/mol·K) when the multiline is the product state.

The view of the S-state transitions that emerges from these considerations can be summarized as follows. The valence states of the four manganese that are involved in the OEC are primarily +3 and +4, with +3 occurring as the average value in the lower S states and advancing sequentially toward the +4 average in the higher S states. Water oxidation is probably a concerted event in the $S_3 \rightarrow S_0$ transition, although recent suggestions of H_2O_2 involvement in the OEC (Johansen, 1988; Wydrzynski et al., 1989) may indicate this species as an intermediate. Despite the apparent metal-centered oxidation character of the S-state transitions, however, there appear to be significant protein and/or cluster nuclear rearrangements on each of the transitions. Proton release is likely to be involved, and S-state-dependent Cl^- ligation to the manganese ensemble (Sandusky & Yocum, 1984; Rutherford, 1989) may also play a role in these nuclear activities.

Electron-Hole Recombination in PSII and the Necessity of Y_Z. At first glance, it would appear that water oxidation could proceed effectively with only the reaction center chlorophyll and the charge-accumulating/water-splitting manganese ensemble, that is, that the intermediacy of Y_Z is unnecessary in this process. The kinetic considerations above, however, combined with information on charge recombination rates in PSII, show clearly that Y_Z is vital to preserving high quantum yield in the O_2-evolving process.

Efficient photosynthesis requires that the initial products of primary charge separation, P^+ and Q^-, be engaged in productive electron transfer before deleterious P^+Q^- charge recombination can occur. This can be accomplished by removing the electron from Q^- or by filling the hole in P^+. In PSII the Q_A^- to Q_B electron transfer, like the analogous bacterial reaction, proceeds with a half-time of ~200 μs (Crofts & Wraight, 1983). In the oxygen-evolving system this time is essentially the same as the $P_{680}^+Q_A^-$ recombination time (Mathis & Rutherford, 1987; Hoganson & Babcock, 1989), which indicates that the net quantum yield would drop to 50% if the system relied on this reaction to preserve charge separation.

The relative sluggishness of Q_A reoxidation indicates that to achieve high quantum yield the hole on P_{680}^+ must be filled rapidly. From the discussion above, it appears that fairly substantial nuclear rearrangements are likely to accompany the S-state transitions, however, which suggests that the reorganization energies for these electron transfers are relatively large. Moreover, the average driving force for these transfers is small: the redox potential for P_{680}^+ is ~1.2 V, and the water/oxygen couple has a potential of 0.92 V at pH 5, the relevant pH in the photosynthetic water-splitting process. This combination, i.e., small driving force and large reorganization energy, is likely to limit the rates of hole transfer into the manganese ensemble, and indeed, Table I shows that these reactions proceed with times comparable to the $P_{680}^+Q_A^-$ recombination time. Even if these rates were increased by a factor of 10 by eliminating Y_Z, a significant departure from unit quantum yield in charge separation would occur. By introducing Y_Z, however, the photosynthetic system has avoided this pitfall. The forward rates of electron transfer from the tyrosine into P_{680}^+ are 3–4 orders of magnitude faster than $P_{680}^+Q_A^-$ charge recombination, which provides a quantum yield approaching unity. As importantly, the $Q_A^-Y_Z^+$ recombination reaction proceeds with a half-time that is approximately 2 orders of magnitude slower (~60 ms; Dekker et al., 1984a) than the $P_{680}^+Q_A^-$ recombination. Relative to the $Q_A^-Y_Z^+$ reaction, the productive $Y_Z^+S_n$ reactions are now fast, which ensures that high quantum yield is preserved all the way to the water-splitting site. Thus, the necessity of Y_Z^+ is clear: it functions as a hole storage tank to allow productive forward electron-transfer reactions to compete successfully with wasteful, but thermodynamically favored, charge recombination reactions.

C_2 Symmetry in Photosystem II and Identification of Y_Z and Y_D

In addition to Y_Z, photosystem II contains a second tyrosine free radical, Y_D^+ (Barry & Babcock, 1987). This species is stable in its free radical form and is not involved in the electron-transfer reactions that lead to water oxidation. The EPR line shapes of Y_Z^+ and Y_D^+ are identical, which indicates that the unpaired electron spin density distribution and the orientation of the tyrosine phenol ring with respect to the polypeptide backbone are identical for both species (Barry & Babcock, 1988). The orientation of the phenol ring, in particular, influences the EPR line shape, and differences in the orientation account for the fact that the spectra of Y_D^+ and Y_Z^+ are distinct from those of the tyrosine radicals that occur in ribonucleotide reductase and prostaglandin synthase (Bender et al., 1989).

This unusual and surprising circumstance, i.e., the occurrence in PSII of two tyrosine radicals, one active and the other inactive in electron transport, has been the key to their assignment to specific residues in PSII and to providing insight into the organization of the reaction center.

For Y_Z^+ and Y_D^+ the similar EPR line shapes indicate that the polypeptide sequences in the vicinity of the two radicals are similar. Debus et al. (1988a,b) used this information plus the idea that analogies exist between PSII and the bacterial reaction center (Michel & Deisenhofer, 1988) to design directed mutagenesis experiments aimed at engineering out one or the other radical. The striking lessons apparent from the bacteria were, first, the near C_2 symmetric arrangement of both the L and M polypeptides and of the chromophores involved in charge separation and, second, the inactivity of one of the C_2 symmetric branches in charge separation. The occurrence of Y_Z/Y_D in PSII echoes this situation and suggested that the two tyrosines may occur in symmetry-related amino acid clusters on D1 and D2. Inspection of the sequences of these two polypeptides with these considerations in mind showed that only the tyrosine pair at the 160 (D2) and 161 (D1) positions fulfilled these criteria: in the folding scheme of D1 and D2

proposed by Trebst (1986) [see also Sayre et al. (1986)], these two tyrosines occur in symmetry-related positions in the C membrane-spanning helices of the two polypeptides; moreover, the four upstream and four downstream amino acids adjacent to the tyrosines are essentially conserved between D1 and D2, which is important in rationalizing the nearly identical spectral properties of the two radicals. Mutagenesis of these tyrosines to phenylalanine and subsequent spectral and functional analysis showed that Y_D corresponds to Tyr^{160} of D2 and that Y_Z is Tyr^{161} of D1 (Debus et al., 1988a,b). Vermaas and co-workers (1988) have obtained the same result for Y_D, and Metz et al. (1989) have recently mutagenized the D1 Tyr^{161} and agree with its assignment as Y_Z. These results also accord with iodination data that suggest that Y_D occurs in D2 and Y_Z in D1 [e.g., Ikeuchi and Inoue (1988) and Takashi and Satoh (1989)].

Two conclusions follow from these data. First, the analogy between PSII and the bacterial reaction center, in terms of C_2 symmetry considerations and in terms of active and inactive electron-transport branches, is considerably strengthened by the Y_Z/Y_D assignments. In fact, they show that experiments designed with these considerations in mind are a powerful tool in analyzing PSII. A caveat to this approach, which is also considered in the following section, is that the analogy should be used with prudence: PSII splits water whereas the bacterial reaction center does not; in addition, there are likely to be evolutionary embellishments, for example, in pigment composition (Dekker et al., 1989), that have occurred in PSII that are absent in the bacteria. Second, when Y_Z is mutagenized to phenylalanine, photosynthetic growth is absent, whereas when Y_D is deleted, photosynthetic growth continues (Debus et al., 1988,b). Thus, Y_D cannot substitute for Y_Z, and despite the apparent C_2 symmetry in PSII, the Y_D branch is functionally incompetent. Within the context of reaction center design and function these results indicate that the symmetry-related branches are not functionally redundant; i.e., one does not serve as a "backup" for the other.

That Y_D^+ is stable in its radical form is a very surprising observation. Its redox potential is expected to be extremely oxidizing—the corresponding Y_Z^+ species is sufficiently unstable to be able to drive the oxidation of water—and consequently one would expect a very short lifetime of the free radical. Recent experiments have provided a basis to interpret this behavior. Rodriguez et al. (1987) used ENDOR spectroscopy to show that the phenol oxygen in the radical is involved in a hydrogen bond interaction and that the hydrogen-bonded proton is extremely resistant to exchange with bulk solvent. Evelo et al. (1989b) have recently confirmed this observation in a series of spin-echo EPR measurements. The hydrogen bond in Y_D^+ contrasts in an interesting way with the situation in ribonucleotide reductase where the phenol oxygen of the neutral radical is clearly not involved in a hydrogen bond (Bender et al., 1989). Y_D^+ is thus well isolated from solvent, which explains its insensitivity to added reductants, and suggests that the phenol hydrogen may undergo a rocking motion upon redox change as indicated in Figure 3 [see also Eckert and Renger (1988)]. Whether such a situation occurs for Y_Z upon redox change is unknown, but it will be interesting to study the effect of deuterium exchange upon the redox kinetics of this species. These results support the idea, which can also be inferred from D1/D2 folding patterns, that Y_Z and Y_D are buried in the membrane and that the stability of Y_D^+ arises from simple isolation from redox-active species. Seclusion of Y_D^+ is also indicated in EPR relaxation measurements reported by Isogai et al. (1988) and by Innes and Brudvig (1989). The latter authors were able to estimate the distance between the paramagnet and the solution phase and found that Y_D^+ is centered essentially in the middle of the PSII membrane at least 25 Å from the nearer solution phase.

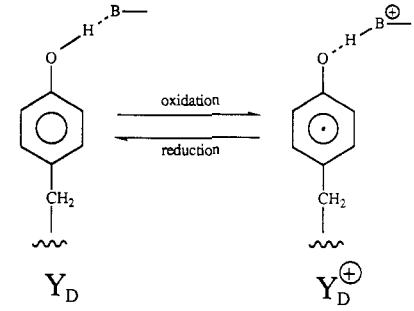

FIGURE 3: Proton rocking motion model for redox transitions in Y_D. In its reduced form (Y_D) the phenol proton is postulated to be hydrogen bonded to a base (e.g., histidine, amide N) in the vicinity of the tyrosine. Proton transfer from tyrosine to the base does not occur owing to the high pK_a (~10) of the tyrosine. Upon oxidation of the residue to form Y_D^+, the phenol become strongly acidic ($pK_a \approx -1$), and proton transfer to the base occurs to form the $H-B^+$ species. The proton back hydrogen bonds to the oxygen of the neutral radical. Evidence for the occurrence of the hydrogen-bonded proton has been found by both ENDOR (Rodriguez et al., 1987) and ESE (Evelo et al., 1989b) spectroscopies. The H_2O/D_2O exchange rate of the hydrogen-bonded proton is remarkably slow (Rodriguez et al., 1987), which leads to a model in which the proton rocks back and forth, depending on tyrosine redox state, between the phenol oxygen and the base.

Where Does Pseudo-C_2 Symmetry in PSII Break Down—Location of the Manganese Ensemble

Like the bacterial reaction center subunits L and M, the PSII subunits D1 and D2 are each likely to contain five membrane-spanning helices (Sayre et al., 1986). The mutagenesis results above have established that Y_D and Y_Z occur in the third membrane-spanning helix, the C helix of the two polypeptides. On the basis of conserved amino acid residues and the bacterial analogy, most researchers identify the fourth and fifth membrane-spanning helices, the D and E helices of the polypeptides, as forming the binding site for P_{680}, the intermediate pheophytin acceptor, Q_A, Q_B, and the associated Fe^{2+} [e.g., Trebst (1986) and Michel and Deisenhofer (1988)]. Such a structure is consistent with a P_{680} Y_Z separation distance of 8–12 Å that has been estimated recently (Hoganson & Babcock, 1989).

Within this context two extreme models for the location of the manganese ensemble can be constructed as shown in Figure 4. In the first (Figure 4a), pseudo-C_2 symmetry is preserved in PSII as the manganese ensemble is incorporated roughly along the C_2 symmetry axis and derives ligands from amino acids near the carboxy termini of both D1 and D2. Dismukes (1988) and Rutherford (1989), among others, have argued in favor of such a structure. Consideration of the requirements for effective manganese function in its water-oxidizing capacity leads to the conclusion that the most likely ligands are carboxyl groups (Pecoraro, 1988), a conjecture supported by the failure to observe nitrogen hyperfine coupling in the S_2 multiline EPR signal (Andrêasson, 1989; Britt et al., 1989). Consequently, most speculation as to appropriate ligands centers on conserved aspartates and glutamates, and indeed, in the carboxy-terminal regions of D1 and D2 a number of these occur that are absent in the bacterial reaction center. In the second model (Figure 4b), the pseudo-C_2 symmetry is broken by manganese incorporation, which is located off the C_2 symmetry axis. Inspection of the amino acid sequences of D1 and D2 reveals that several

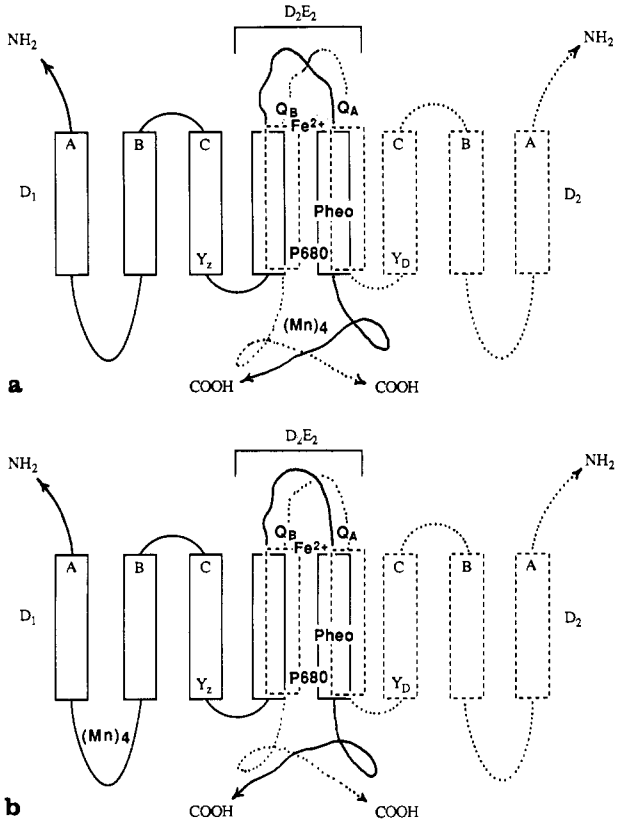

FIGURE 4: Models for the disposition of the Mn binding site in the PSII/OEC. The reaction center polypeptides D1 and D2, each with five membrane spanning helices (A–E), are shown. In analogy with the bacterial reaction center, the chromophores involved with primary charge are associated with the 4-helix D_2E_2 structure. The pseudo-C_2 symmetry axis is along the Fe^{2+}···P_{680} direction. In (a), the placement of the manganese cluster preserves pseudo-C_2 symmetry as it is postulated to occur near the –COOH termini of D1 and D2. In (b), incorporation of Mn is envisioned to occur in the AB interhelical region of the D1 polypeptide only, which disrupts pseudo-C_2 symmetry in the reaction center. An interesting speculation, not shown explicitly in (b), is that Ca^{2+} binds to the corresponding AB interhelical region in D2.

unique, conserved glutamates and aspartates occur in the AB interhelical loop in D1, which suggests this locus as a plausible Mn binding site in the model of Figure 4b.

Before examining the sparse experimental data that are useful in arguing for one or the other of these two extreme models, two points can be made. First, the organization of the manganese ensemble is not known. Most of the discussion on this issue suggests that all four metals are in close proximity and multinuclear clusters (n = 2–4) are considered as attractive structures for the ensemble [for reviews see Christou and Vincent (1987) Pecoraro (1988), and Brudvig et al. (1989)]. Recent EXAFS data appear to support this idea (George et al., 1989; Penner-Hahn et al., 1989). Nonetheless, other structures remain viable, particularly given the Ca^{2+} requirement for O_2 evolution (Bonadies et al., 1989; Rutherford, 1989), and this area is one of intense research activity. Second, the data supporting an association of manganese with only D1 and D2 are indirect [see, for example, Dismukes (1988)], and other PSII polypeptides, in particular the integral 47-kDa chlorophyll binding polypeptide and 33-kDa extrinsic polypeptide, may also be involved in supplying ligands to the metal ions.

As indicated above, there is little experimental data available that bear directly on the two extreme models in Figure 4. A non-O_2-evolving, low fluorescent mutant of *Scenedesmus*, LF1, has been extensively characterized and shown to be defective

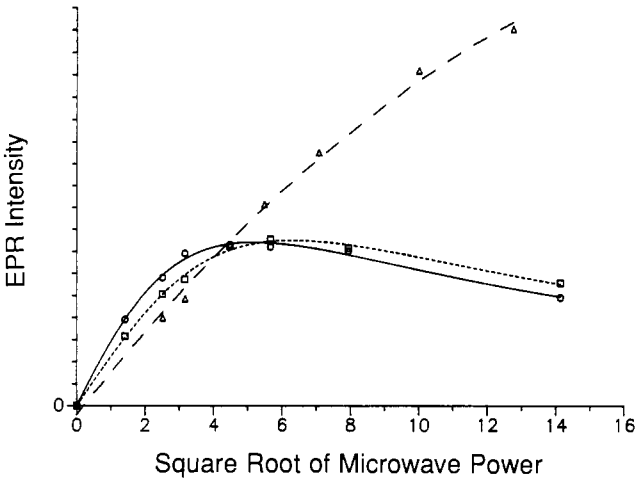

FIGURE 5: Effect of microwave power on the amplitude of the EPR signals of Y_Z^+ and Y_D^+ in O_2-evolving PSII membranes. The Y_Z^+ (△) and Y_D^+ (□) measurements were made by using a 1-Hz flash repetition rate; the Y_D^+ (○) measurement was performed in the dark. The curves have been normalized at 20 mW.

in assembling the manganese complex [see Rutherford et al. (1988)]. This has been linked to a failure to process the carboxy terminus of the D1 polypeptide (Diner et al., 1988a). As the authors point out, however, this effect, while supporting an association of Mn with D1, may well be indirect. Similarly, recent chemical modification studies have implicated histidine residues on D1 in manganese binding (Tamura et al., 1989). Although the suggestion of histidine ligation runs counter to the spectroscopic data discussed above, it may be rationalized if the multiline EPR signal arises from only two or three of the four Mn atoms in the complex. The authors in this study have not yet identified the specific histidines involved and thus discussed both the symmetric and asymmetric models in Figure 4 as viable alternatives.

Some insight into resolving the question posed in Figure 4 is available from EPR power saturation studies. Warden et al. (1976) showed that Y_Z^+ was much more strongly relaxed than Y_D^+ and attributed the effect to an interaction with the manganese ensemble that occurred for the former but not for the latter radical. This observation suggests an asymmetric disposition for the water-splitting site relative to the two tyrosines and has been studied in detail recently. The occurrence of different magnetic interactions between manganese and the two tyrosines has been confirmed by measuring the power saturation of the two organic radicals under identical flashing light conditions [Figure 5; see also Rutherford (1989)]. A weaker, S-state-dependent magnetic interaction has been observed for Y_D^+, and Evelo et al. (1989a) used this to estimate a distance of 30–40 Å between this radical and the Mn cluster. The small magnitude of the relaxation enhancement in these experiments, however, suggests that this estimate should be viewed with some caution. Hoganson and Babcock (1988) showed that the stronger Y_Z^+/Mn interaction does not broaden the Y_Z^+ spectrum appreciably, despite its increased relaxation rate, and suggested a distance somewhat greater than 15 Å between the two paramagnents. A more detailed analysis of this interaction is not possible without more specific information on the magnetic properties of the cluster. Taken together, these considerations provide some evidence, albeit weak, in favor of a departure from C_2 symmetry at the Mn cluster. Site-directed mutagenesis, which can now be carried out on both D1 and D2 (Debus et al., 1988a,b) and chemical modification studies are likely to provide a resolution to this question.

Conclusions

In the past decade the PSII/OEC has gone from being mysterious to being one of the most well-understood membrane proteins in biology. Several factors have facilitated this rapid progress. First, oxygen evolution has proven to be remarkably stable to biochemical manipulation, despite early indications to the contrary. This stability, coupled with the relative ease by which individual polypeptides can be removed from the complex, has allowed functional assignments for the various subunits to be made. It has also provided samples for biophysical analysis in which the PSII concentrations can approach 1 mM. This has significantly improved signal to noise ratios in some spectroscopies [e.g., Penner-Hahn et al. (1989)] and made others possible [e.g., the first magnetic susceptibility measurement on O_2-evolving PSII preparations has recently appeared (Sivaraja et al., 1989)]. Second, the crystallization of the bacterial reaction center, the realization of the analogies between it and PSII (Michel & Deisenhofer, 1988), and the demonstration of the relevance of constructing experiments on the basis of the anology (Debus et al., 1988a,b) have provided a "shortcut" to dissecting the oxygen-evolving system. Third, because the water-splitting process, like all photosynthetic reactions, can be initiated by light, kinetic studies over the picosecond to second time regime have become possible. Rate constants and their temperature dependencies are becoming available for the charge-transfer processes that lead from photochemical electron-hole separation to the actual water-splitting event. The relevant redox partners in these events have been identified, and the factors that influence the rate constants are beginning to emerge.

Despite this rapid evolution in our understanding of the PSII/OEC, many of its most interesting and novel aspects are nebulous. The physical arrangement of the chromophores remains uncertain, particularly with regard to the actual site of water oxidation. In a broader sense, the issue is in what areas the bacterial analogy will break down. This pertains not only to the location of the manganese cluster but also to the pigment composition and organization. Even more fundamentally, the PSII/OEC provides a vexing paradox. The arrangement of the redox components and the kinetics of the electron-transfer reactions that are involved in producing O_2 efficiently provide an excellent example of the economy of nature. The occurrence of Y_D and the demonstration that it is not a backup to the active charge-transfer pathway, on the other hand, seem to be counterexamples. Some insight into the resolution of this paradox comes from the observation that the Y_D-less mutant, while competent in O_2 evolution, grows at about one-third the rate of the wild type (Debus et al., 1988a). This suggests that the inactive branch in PSII, and perhaps in the bacteria as well, is involved in dynamic interactions that facilitate assembly (perhaps in a template role) or stabilize the reaction center once it is formed. Finally, with regard to water-splitting chemistry itself, we have little knowledge of mechanism. The majority of current work, both on the natural system itself and in new and stimulating inorganic model complexes (e.g., Pecoraro, 1988), is aimed at determining the structure of the complex. As progress in this effort continues, an appreciation of how the complex actually works should grow.

Added in Proof

Although Y_D and Y_Z occur as neutral radicals in their oxidized states as discussed in the text, we prefer to designate the radicals as Y_D^+ and Y_Z^+. The basis for this preference derives partly from the fact that the proton does not leave the radical site (see Figure 3) and partly from the convenience that the "+" notation provides in designating the redox state of the tyrosine.

Registry No. H_2O, 7732-18-5.

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