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A hydrogen-atom abstraction model for the function of Y_Z in photosynthetic oxygen evolution

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Abstract

Recent magnetic-resonance work on Y_Z suggests that this species exhibits considerable motional flexibility in its functional site and that its phenol oxygen is not involved in a well-ordered hydrogen-bond interaction (Tang et al., submitted; Tommos et al., in press). Both of these observations are inconsistent with a simple electron-transfer function for this radical in photosynthetic water oxidation. By considering the roles of catalytically active amino acid radicals in other enzymes and recent data on the water-oxidation process in Photosystem II, we rationalize these observations by suggesting that Y_Z functions to abstract hydrogen atoms from aquo- and hydroxy-bound manganese ions in the $(Mn)_4$ cluster on each S-state transition. The hydrogen-atom abstraction process may occur either by sequential or concerted kinetic pathways. Within this model, the $(Mn)_4/Y_Z$ center forms a single catalytic center that comprises the Oxygen Evolving Complex in Photosystem II.

Introduction

Amino acid side chain radicals have been shown to play essential functional roles in a number of enzyme systems (see Sigel and Sigel 1994 for reviews). In general, these species appear to operate as either hydrogen-atom abstractors or as electron-transfer cofactors. The tyrosyl species in Photosystem II, Y_Z and Y_D , are representative of this class of radical cofactor. Although they occur at symmetry related positions in the D1 and D2 polypeptides that form the core of Photosystem II (Debus et al. 1988; Vermaas et al. 1988; Koulouligiotis et al. 1995), they are differentiated sharply, in terms of function (Barry 1993; Hoganson and Babcock 1994; Diner and Babcock 1995). Y_Z operates in a linear sequence to interface the photochemistry that occurs at P680 with the multi-electron, water-splitting chemistry in which the $(Mn)_4$ cluster participates. Y_D ,

on the other hand, undergoes redox chemistry but is not involved in the catalytic events associated with water oxidation.

Here, we present magnetic resonance data on Y_Z that provide insight into its dynamics and local protein environment and supplement detailed investigations of the radical that have been reported elsewhere (Tang et al. 1995; Tommos et al. 1995). These observations are compared to and contrasted with analogous data in the literature for and Y_D for other tyrosyl radicals (Bender et al. 1989; Hoganson and Babcock 1992; Tang et al. 1993; Tommos et al. 1993; Mino and Kawamori 1994; Rigby et al. 1994; Warncke et al. 1994; Tang et al. 1995). The comparison shows that pronounced distinctions occur in both hydrogen bonding and phenol head group motion in this class of radicals that can be correlated with function. Y_Z and Y_D can be distinguished on this basis, from which we conclude

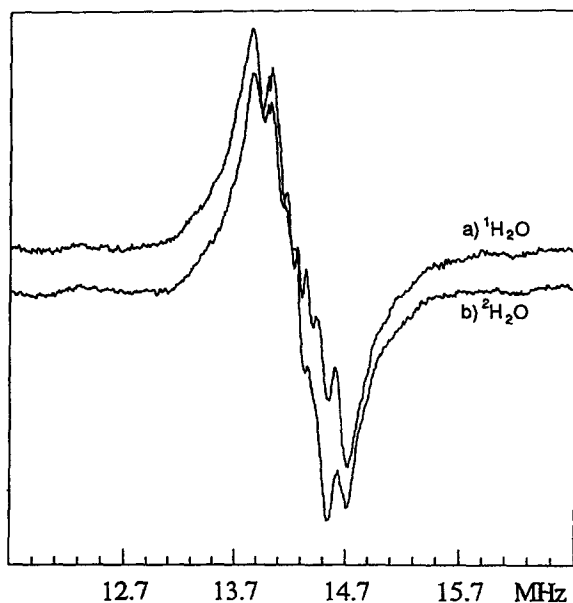


Fig. 1. ENDOR spectra of Y_Z in the Y_D -less mutant of *Synechocystis* in (a) $^1\text{H}_2\text{O}$ or (b) $^2\text{H}_2\text{O}$. Both spectra are in derivative mode and were detected by continuous-wave techniques. The microwave frequency was 9.399 GHz, the magnetic field was 0.3305 T, the microwave power was 2.0 mW, the radiofrequency power was 150 W at 15 MHz, frequency modulation was 50 kHz, time constant was 0.2 s, the temperature was 110 K, and the proton Larmor frequency was 14.3 MHz.

that, while Y_D appears tailored to a pure electron-transfer function, Y_Z has characteristics expected of a hydrogen-atom abstractor. This conclusion is combined with those from recent work from other laboratories on $(\text{Mn})_4/Y_Z$ distance estimates, Photosystem II proton release, and directed mutagenesis to generate a Y_Z hydrogen-atom abstraction model for water oxidation.

Materials and methods

The procedures for model tyrosyl radical generation, spinach and *Synechocystis* Photosystem II isolation, Y_Z trapping, and $^1\text{H}_2\text{O}/^2\text{H}_2\text{O}$ exchange follow those described elsewhere (Hoganson and Babcock 1992; Tang et al. 1995; Tommos et al. 1995). The transient ENDOR methodology is described in detail by Hoganson and Babcock (1995) and the electron spin echo envelope modulation (ESEEM) techniques used here are analogous to those used in a recent study of Y_D in *Synechocystis* by Warncke et al. (1994).

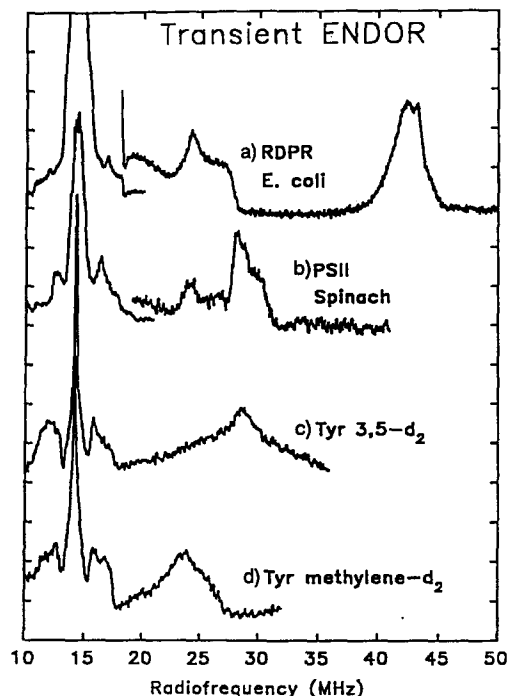


Fig. 2. ENDOR spectra of tyrosyl radicals. All spectra are in absorption mode and were obtained using the transient ENDOR detection scheme (Hoganson and Babcock 1995). (a) Ribonucleotide diphosphate reductase, *E. coli*, 12 K; (b) Photosystem II Y_D , spinach, 12 K; (c) ring 3,5-deuterated tyrosine in $^2\text{H}_2\text{O}/\text{NaO}^2\text{H}$ glass, 121 K; (d) methylene-deuterated tyrosine in $^2\text{H}_2\text{O}/\text{NaO}^2\text{H}$ glass, 121 K. The radicals in spectra c and d were generated by UV illumination.

Results

Figure 1 shows continuous-wave ENDOR spectra for Y_Z trapped in PS II particles from a Y_D -less mutant of *Synechocystis* 6803. In the upper trace, the particles were suspended in an $^1\text{H}_2\text{O}$ -containing buffer; in the lower trace, $^1\text{H}_2\text{O}$ has been exchanged for $^2\text{H}_2\text{O}$. There are significant changes in the Y_Z spectrum in the close-coupling region (± 1 MHz) around the ^1H Larmor frequency ($\nu_{1\text{H}} = 14.3$ MHz for the spectra in Fig. 1) upon solvent isotope exchange, which demonstrates that the Y_Z site and its close environment (< 5 Å) are accessible to solvent and contain exchangeable protons (see also, Tang et al. 1995). In contrast, we have observed no discernible differences in the spectra beyond those in the matrix region, consistent with both ENDOR (Tang et al. 1995; Tommos et al. 1995) and FTIR (Bernard et al. 1995) data that have been interpreted to indicate the absence of a well-ordered hydrogen bond to the phenol oxygen. The hydrogen-bond status of Y_Z clearly differs from that of Y_D , for which

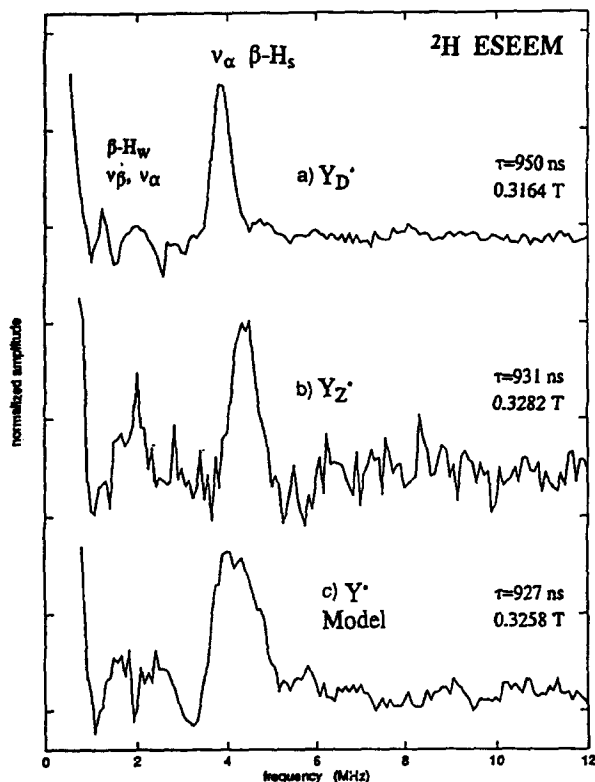


Fig. 3. ^2H -ESEEM spectra of (a) Y_D , (b) Y_Z , and (c) a model tyrosyl radical in a glass. The Y_D and Y_Z radicals were in *Synechocystis* PS II preparations. Typical spectrometer conditions were microwave frequency = 9.190 GHz, magnetic field = 0.3282 T, τ = 931 ns, ($\tau + T_0$) = 140 ns, microwave pulse power = 40 W (20 ns in duration at full-width, half maximum), pulse sequence repetition rate = 10 Hz, and T = 4K. At this field, $\nu_{2\text{H}} = 2.1$ MHz.

a variety of techniques have shown the occurrence of an exchangeable proton at a distance consistent with its forming a hydrogen bond to the Y_D phenol oxygen (reviewed in Barry 1993; Hoganson and Babcock 1994).

A further difference between the two tyrosyls in Photosystem II is illustrated by the magnetic-resonance spectra in Figs. 2 and 3. Figure 2 shows transient ENDOR spectra of the Y_{122} radical in ribonucleotide reductase (Fig. 2a), of Y_D (Fig. 2b), and of a model tyrosine radical specifically deuterated either at the 3,5 positions (Fig. 2c) or at the β -methylene positions (Fig. 2d). (In the ring-numbering system used here, the 3,5-ring carbons are *ortho* to the phenol oxygen and ring C_1 is bonded to the β -methylene carbon.) The spectra of the specifically labeled model radical confirm the 3,5- ^1H origin of the features in the 20–26

MHz region. In Figs. 2a–c, the highest frequency resonances arise from the more strongly coupled of the two β -methylene ^1H (Hoganson and Babcock 1994). In the transient ENDOR spectra, however, the spectral characteristics of these β - ^1H resonances are markedly different for the three radicals. For Y_{122} in RDPR and for Y_D in Photosystem II, this ^1H contributes sharp, well-resolved features at 40–45 MHz and from 27–31 MHz, respectively, reflecting the difference in β -methylene geometry for these two radicals. For the strongly coupled β - ^1H in the model, qualitatively different behavior is observed as its resonance is spread over 15 MHz with a single maximum at ~ 29 MHz.

Warncke and McCracken (1995) have explored the basis for this difference in resonant behavior for β - ^1H in tyrosyl radicals by spin echo methods and have shown that rotational mobility about the $\text{C}_\beta\text{--C}_1$ bond can occur to produce a dispersion in β -methylene ^1H dihedral angle (θ). Briefly, as the rotational barrier around $\text{C}_\beta\text{--C}_1$ decreases, the phenol head group samples an increasing range of dihedral angle; as the temperature is lowered for magnetic-resonance work, this distribution in θ is frozen in. Because the β - ^1H hyperfine couplings ($A_{1\text{H}\beta}$) are related to dihedral angle according to

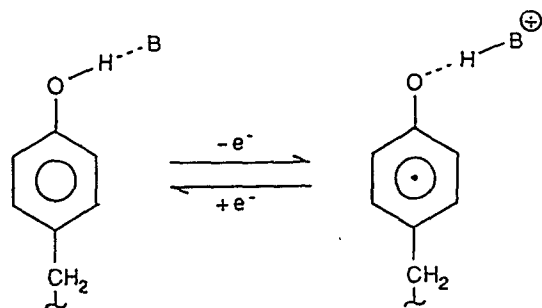
$$A_{1\text{H}\beta} = B_2 \rho_{\text{C}_1} \cos^2 \theta$$

where B_2 is a constant = 162 MHz and ρ_{C_1} is the unpaired electron-spin density at the ring C_1 position, dispersion in θ produces a range of $A_{1\text{H}\beta}$ couplings that broadens resonances due to the β -hydrogens. By using ^2H ESE techniques and specifically deuterated tyrosyl models, they were able to work out quantitative measures of dihedral angle variation from considerations of β - ^2H spin-echo lineshape. Figure 3 shows representative deuterium ESEEM spectra for Y_D , Y_Z , and a model tyrosyl radical in which the β -positions in all three radicals have been specially labeled with ^2H . In each spectrum, the feature in the 4 MHz region corresponds to the more strongly coupled β - ^2H (the lower resonant frequency for ^2H , relative to ^1H , reflects the lower magnetogyric ratio for ^2H). The full width at half maximum line widths ($\Delta\nu$) of the β - ^2H resonance in the three radicals vary significantly, which indicates varying degrees of rotational dispersion. For Y_D , $\Delta\nu = 0.48$ MHz; for Y_Z , $\Delta\nu = 0.71$ MHz; and for the model, $\Delta\nu = 1.21$ MHz. Error estimates based on measuring the β - ^2H linewidths in these samples at various τ values (at least nine different τ values were used for each

sample) sets the limits of uncertainty in $\Delta\nu$ at ± 0.03 MHz. Quantitative analysis indicates that θ dispersion increases from Y_D ($\Delta\theta \pm 4^\circ$), to Y_Z ($\Delta\theta \sim \pm 7^\circ$), to the model tyrosyl ($\Delta\theta \sim \pm 30^\circ$). For Y_D , the $\pm 4^\circ$ value is an upper limit; the uncertainty in the angular variation for Y_Z and the model tyrosine is $\pm 0.5^\circ$. A clear conclusion from this analysis is that there is considerably more rotational mobility in the Y_Z C_β - C_1 bond than in the analogous bond in Y_D .

Discussion

The results presented above and elsewhere (Mino and Kawamori 1994; Roffey et al. 1994; Bernard et al. 1995; Tang et al. 1995; Tommos et al. 1995) indicate that there are surprising differences between Y_D and Y_Z , in terms of their hydrogen bonding and dynamic behavior. The phenol head group of Y_D is rigid and essentially immobilized in a homogeneous conformation about the C_β - C_1 bond; its phenol oxygen is involved in a well-defined hydrogen-bond interaction with a nearby residue, most likely H-189 of the D2 polypeptide (Tang et al. 1993; Tommos et al. 1993). These observations, together with the likelihood that Y_D occurs in a hydrophobic environment (Svensson et al. 1991; Hoganson and Babcock 1994), are consistent with what would be expected for a pure electron-transfer cofactor – nuclear motion along the electron-transfer reaction coordinate is suppressed so that the reorganization energy (λ) is minimized. Small λ values maximize the rate of the electron-transfer process at a given driving force. This principle is clearly apparent in other pure electron-transfer cofactors such as the blue copper proteins and the cytochromes *b* and *c* (see Williams (1990) for a review). Thus, the ‘proton rocking’ model for the redox chemistry associated with Y_D (Scheme 1),



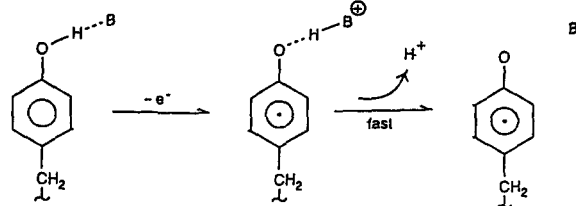
Scheme 1

is consistent with both structural and functional properties of Y_D (Babcock et al. 1989). The phenol proton of Y_D is hydrogen bonded to a nearby basic residue (H189(D2)); upon oxidation, the proton is retained in the site, the sense of the hydrogen-bond interaction is reversed, and nuclear motion is minimized.

At the outset, we expected to see similar design principles implemented for Y_Z , as the conventional view of Photosystem II envisions this species as playing a pure electron-transfer role to effect rapid, quantum yield-preserving reduction of $P680^+$ and subsequent oxidation of the $(Mn)_4$ cluster. The results discussed here, however, show that this is not the case, which suggests that reconsideration of the conventional view of Y_Z function is necessary.

Several pieces of data relevant to such a reconsideration are available from other labs. Savéant (1993), in electrochemical work, has shown that phenol oxidation involves H-atom transfer, that is, concerted hydrogen ion and electron motion. We expect that this principle will be in force for Y_Z and that its oxidation will be coupled to its deprotonation. The results here, as well as those reported elsewhere (Bernard et al. 1995; Tang et al. 1995; Tommos et al. 1995), indicate that Y_Z is unlikely to be involved in a well-defined hydrogen-bond interaction, which suggests that the proton leaves the Y_Z site upon oxidation. One view of proton release during water-oxidation is consistent with this; each S-state transition is accompanied by stoichiometric proton release (Lübbbers et al. 1993). This phenomenon is obscured, in thylakoids and Photosystem II membranes, by the buffering action of amino acid side chains but is clearly observed in Photosystem II core preparations. Moreover, Haumann and Junge (1994) have shown that this proton release is coupled to the oxidation of Y_Z , not its reduction, in agreement with the considerations above. It has been shown, however, that H190(D1) is essential in promoting rapid reduction of $P680^+$ by Y_Z , as mutagenesis of this species slows the rate of the Y_Z $P680^+$ reaction by a factor of 200 (Diner et al. 1991; Tang et al. 1993; Kramer et al. 1994; Roffey et al. 1994). These observations suggest that H190(D1) plays an essential role in facilitating the concerted hydrogen-atom (i.e. coupled proton/electron) transfer that occurs upon Y_Z oxidation by functioning as an immediate, but transient, proton acceptor. Mutagenesis of this species, to remove its base function, would be expected to slow electron transfer by impeding the coupled proton release; in effect, the transient Y_Z/Y_Z redox potential would

increase. Taken together, these considerations suggest a 'proton sloughing' set of reactions upon Y_Z oxidation, as summarized in Scheme 2:

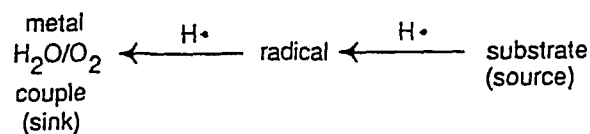


Scheme 2

In this sequence, we hypothesize that B represents H190(D1). The first step in the sequence represents concerted H-atom transfer, as oxidation and formation of the H-B bond are viewed as occurring simultaneously. In the second step, proton release from the site occurs on the μs time scale (Haumann and Junge 1994). We stress that the proton release in the second step is not viewed as occurring directly to bulk phase. Rather, we suggest that it occurs to other protein acid/base groups, which will, depending on the solution pH and intactness of the preparation, eventually deprotonate in a complex fashion to bulk phase (Lavergne and Junge 1993). The Y_Z environment is likely to be more hydrophilic than that of Y_D , as indicated by the occurrence of conserved carboxylic acid residues in D1 that do not occur in D2 (Svensson et al. 1990, 1991). These side chains may serve as secondary proton acceptors and provide the deprotonation pathway implied in the second step above that would facilitate proton efflux from the Y_Z site; conversely, their absence in D2 would confine the proton to the Y_D site, as indicated in Scheme 1.

Scheme 2 indicates that the Y_Z site sloughs its proton upon oxidation; the postulated hydrogen-bonding interaction with H190(D1) lowers the redox potential of Y_Z and minimizes λ , thus preserving the high rate of electron transfer to $P680^+$. At the conclusion of this process, a tyrosyl radical, unencumbered by hydrogen bonds and with a good deal of motional lability, remains. A likely function for this radical in water oxidation is suggested by several bits of information. Inspection of the functional amino acid radical literature (Frey 1990; Sigel and Sigel 1994) indicates that the underlying catalytic role of these radical species involves hydrogen-atom abstraction from substrate. An oxygen-activating metal center serves to produce

the radical by using the water/oxygen couple as an electron sink. A generalized mechanism for this sequence of events is summarized in Scheme 3:



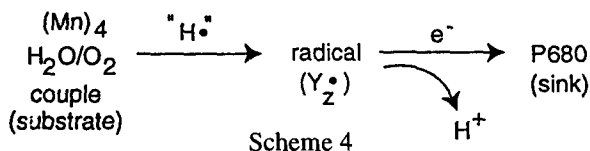
Scheme 3

Moreover, in those enzymes for which crystal structures are available (e.g. prostaglandin synthase, Picot et al. 1994), the oxygen-activating metal center is in close physical proximity ($< 10 \text{ \AA}$) to the redox active amino acid.

These insights carry over to Photosystem II in two contexts. First, the original EPR work on $S_n Y_Z$ reaction kinetics showed that the $S_3 Y_Z$ reaction rate-limited the water-oxidation chemistry, i.e., that O=O bond formation and dioxygen release were fast, compared to the reduction of Y_Z by S^3 (Babcock et al. 1976). This suggests that significant nuclear rearrangements occur upon the rereduction of Y_Z and that these raise λ sufficiently to manifest themselves by rate limiting the chemistry. The absence of a hydrogen bond to Y_Z implicates protonic motion as the source of the nuclear rearrangement. Second, Britt and co-workers have shown that the source of the split S_3 signal in Ca^{2+} -depleted Photosystem II preparations is a tyrosyl radical involved in a magnetic interaction with the manganese cluster (Gilchrist Jr. et al. 1995). By assuming that the interaction is dipolar, they were able to estimate a distance of $\sim 4.5 \text{ \AA}$ between the two paramagnets. A comparison of the details of the spectrum of the tyrosyl radical in the split S_3 species, which shows significantly broadened $\beta\text{-}^1\text{H}$ resonances relative to those for Y_D in the same sample, with that of Y_Z that we report here and elsewhere (Tommos et al. 1995) indicates similar motional lability, which provides support for the identification of the split S_3 tyrosyl with Y_Z .

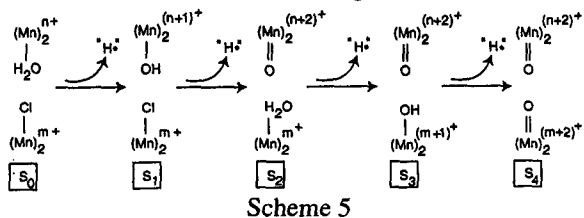
Taken together, these results suggest that strong analogies exist between Y_Z in Photosystem II and the other radical enzymes that have been studied. Relative to these other systems, Photosystem II appears simply to reverse the direction of hydrogen-atom flow: a water-activating metal center acts as substrate for a radical center generated by photochemistry at P680. Scheme 4 summarizes the process envisioned, where

the notation "H·" reflects the idea that H· transfer may be either sequential or concerted (see below):



The motional lability that we have detected and quantified for Y_Z is essential in this model, as the deprotonation/reprotonation reactions are viewed as directional. Y_Z deprotonates to H190(D1), which is likely to be oriented toward P680, whereas its reprotonation occurs from the H_2O (Mn)₄ center, which is likely to be more remote from the reaction center chlorophylls. Thus, in order to assure proper disposition of the phenol head group during the acid/base chemistry, motional flexibility is necessary. These geometric considerations are likely to be especially critical, in view of the short deBroglie wavelength and correspondingly short tunneling distances for hydrogen, relative to electrons, and is, most likely, the underlying physical basis for the theme of close physical proximity between metal and radical centers in radical enzymes.

To complete construction of the model, we suggest that hydrogen-atom abstraction chemistry occurs on each S-state transition, according to Scheme 5.



In this model, the (Mn)₄ cluster consists of two magnetically coupled (Mn)₂ dimers that serve to anchor and position the substrate and to act as a redox pool to absorb oxidizing equivalents. Accordingly, the hydroxy and oxo ligands to the manganese dimers in the scheme carry formal charges of -1 and -2 , respectively. Chloride binds to one of the two dimer halves in the low S-states to block the site and prevent peroxide formation in S_2 , which might be likely if two (Mn)₂-OH species were allowed to form (Rutherford et al. 1992). Dioxygen formation occurs as two $\text{Mn}=\text{O}$ species, held in close physical proximity, condense to form the $\text{O}=\text{O}$ bond in S_4 and leave as dioxygen (see Naruta et al. 1994). The model is satisfying in postu-

lating a critical role for Cl^- in the water-splitting process and in accounting for the recent $^{16}\text{O}/^{18}\text{O}$ exchange work carried out by Messinger et al. (1995). These authors showed that one of the two oxygens in the S_3 state is only slowly exchangeable, consistent with Scheme 5, as we would expect a difference in exchange kinetics for the bound oxo and hydroxo species depicted in the S_3 state.

The $\text{Cl}^-/\text{H}_2\text{O}$ exchange that occurs on the $\text{S}_1 \rightarrow \text{S}_2$ transition in Scheme 5 is interesting, as it results in a net positive charge accumulating on the cluster in the S_2 state, which persists to S_3 . This behavior is roughly consistent with the long time behavior of the electrochromic shifts that occur upon S-state advance (Rappaport and Lavergne 1991). We realize, however, that chloride involvement is likely to be more complex than that shown in Scheme 5, as Cl^- is clearly involved in the S_2 state under certain conditions (Sandusky and Yocum 1986; Ono et al. 1986). Moreover, whether Scheme 5 can be developed to rationalize the details of both the extent and kinetics of the electrochromic shifts, which appear to correlate well with the net charge change determined by the balance of electrons and protons (Rappaport et al. 1994), remains to be determined.

Essential to the model presented here is the ability of the Y_Z tyrosyl radical to abstract a hydrogen atom from water or hydroxyl coordinated to the manganese cluster. The kinetic details of the hydrogen-atom abstraction process may involve either concerted H· transfer or sequential H^+ , e^- transfer (e.g. Foti et al. 1994); recent isotope effect measurements on the reduction of Y_Z by the high S states suggest that a process that is more sequential may be favored (Renger et al. 1994; Lydakis-Simantiris and Babcock, unpublished). Regardless of the details of the mechanistic pathway, however, evidence for the lack of a hydrogen bond to Y_Z and enhanced mobility of the ring around the $\text{C}_1-\text{C}_\beta$ axis, which we have obtained experimentally, are consistent with the requirements of the model and with each other. It is necessary to point out, however, several caveats associated with the model presented here. First, the magnetic resonance and FTIR work that has been carried out to suggest motional flexibility and the absence of an exchangeable proton has been done on core complexes lacking the manganese cluster. The work of Britt and co-workers indicates, however, that this mobility is at least conserved in the presence of the cluster under Ca^{2+} depleted conditions. Second, the lack of detection of a well-ordered, exchangeable

proton from the ENDOR of Y_Z is not absolutely compelling as an argument against hydrogen bonding. Such bonding in a disordered site could produce variations in hydrogen bond length that are not observable in ENDOR or ESE as discrete, well-defined resonances like those of Y_D . However, the requirements of the model could still be met if there were hydrogen bonding to Y_Z , but not in the conformation of the radical that abstracts the hydrogen atom. Moreover, disordered hydrogen bonding, as well as motional lability of Y_Z and the hydrophilicity of the Y_Z site, are inconsistent with a simple electron-transfer function for the side chain. Instead, they point to a more complex activity. Finally, the model postulated in Scheme 5 above, while providing a role for Cl^- in water oxidation, is mute on the Ca^{2+} requirement.

Despite these caveats, however, a hydrogen-atom abstraction model accounts well for several critical observations on the water-oxidation process. Moreover, it casts the PS II/OEC complex within the larger class of radical enzymes and provides a useful target for the design of additional experimental inquiries.

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