

Review Article

# Tyrosyl Radicals in Enzyme Catalysis: Some Properties and a Focus on Photosynthetic Water Oxidation†

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Enzymes that require a redox-active amino acid for catalysis or function have emerged as a distinct class of proteins. For the tyrosine-based radical enzymes, we show that the spin-density distribution in the radical follows an odd alternate pattern that is invariant to within 10% across the class. General properties of the radical enzymes are summarized from which we conclude that their essential role in catalysis is to initiate substrate metabolism by hydrogen-atom abstraction. These ideas are extended to the Y<sub>Z</sub> and Y<sub>D</sub> tyrosines in Photosystem II and a radical-based hydrogen-atom abstraction model for water oxidation is discussed. Differences in rates of oxidation of Y<sub>Z</sub> and Y<sub>D</sub> by the reaction-center chlorophyll, P680<sup>+</sup>, under various conditions, are considered and rationalized on the basis of changes in reorganization energy induced by the local protein structure and by the presence or absence of the (Mn)<sub>4</sub> cluster that binds substrate water.

## 1. Introduction

The general topic of radicals in biology can be quickly broken down into two distinct subject areas: the study of radicals that provoke non-specific chemistry that is ultimately deleterious to the cell and the study of radicals that are essential for enzyme catalysis. Destructive radical chemistry has been recognized for a considerable time and has been associated with the aging process and cell death.<sup>1</sup> In general, radicals produced from molecular oxygen are considered to be the principal reactants in these processes and the use of antioxidants (ascorbic acid, β-carotene, vitamin E) as a means by which to control their concentrations in the cell has been popular-

ized. The effectiveness of such an antioxidant regimen, however, is currently under debate in the medical community.

The second class of radicals, that is, those essential for proper biological function, has been recognized somewhat more recently. In this class, physiologically essential flavin, quinone, and chlorophyll radicals have been known since the 1950s (e.g., Ref. 2), primarily because their redox potentials are typically in the –200 to +400 mV vs. NHE range at neutral pH. Consequently, these species are relatively stable and can be detected by conventional spectroscopic methods. Catalytically essential amino-acid radicals also belong to this class. The first of these, the tyrosyl radical in the aerobic ribonucleotide reductase (RNR) from *E. coli*, was only discovered in the 1970s.<sup>3</sup> The realization of the widespread occurrence of amino-acid radicals in enzyme catalysis is still more recent and an area of considerable current interest and research activity.<sup>4</sup> The underlying reason as to why this is only occurring now lies in the redox instability of

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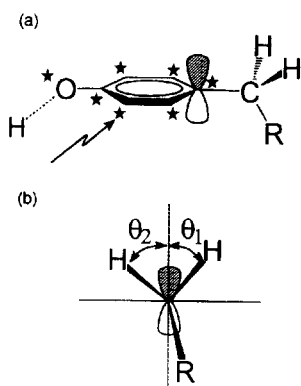
amino-acid radicals: the least oxidizing, the thiyl radical, has a potential upwards of +800 mV.<sup>5</sup> Consequently, these species typically occur only as transient radicals during the catalytic cycle and more sophisticated spectroscopic and/or trapping methods must be used to detect and characterize them. There are exceptions to this generalization and it is not surprising that the first two amino-acid radicals in this class, Y<sub>122</sub> in RNR and Y<sub>D</sub> in PSII, both occur as stable tyrosyl radicals in their protein environments.

Amongst the catalytically essential redox-active amino acids, glycyl, thiyl, tryptophanyl, and tyrosyl are the most prevalent.<sup>4</sup> In this article, we will focus initially on the tyrosyl members of this class, in order to understand aspects of their control and spectroscopy. We will then summarize emerging functional and structural themes in the radical enzyme class and conclude by applying these insights to the redox-active tyrosines, Y<sub>D</sub> and Y<sub>Z</sub>, in PSII to understand their roles in the O<sub>2</sub>-evolving process.

## 2. Control of radical reactivity – spectroscopic insights

Owing to the strongly oxidizing potential of the tyrosyl radical (~ +1000 mV) and ability to promote atom-abstraction chemistry, means by which to control its reactivity so that potentially damaging, spurious reactions are minimized during catalysis, must be in force. Fig. 1 summarizes several potential methods by which

1. Access limitation
2. Spin density distribution (★)
3. Ring conformation ( $\theta_1$ ,  $\theta_2$ ) and ring mobility ( $\Delta\theta$ )
4. Hydrogen bonding (---H)
5. Covalent modification ( $\rightleftharpoons$ )



Spectroscopic (magnetic resonance) probes:

1. Tyrosine isotope labeling
2. Substrate isotope labeling
3. Spectral simulation
4. Electron spin echo (ESE) spectroscopy
5. Electron-nuclear double resonance (ENDOR) spectroscopy

Fig. 1. Upper: possible mechanisms for control of radical reactivity in catalytically essential, redox-active, amino-acid-containing proteins; lower: experimental probes that have been used to study amino-acid radical proteins.

radical enzymes may achieve this critical control function as well as the labeling and electron magnetic spectroscopic strategies that we have used to test these possibilities. The spectroscopic approaches are necessarily sophisticated, owing to the fact that, in general, the tyrosyl radicals are effectively immobilized in their protein environment. Consequently, powder spectra are observed and hyperfine coupling information on individual protons is lost in the anisotropically broadened EPR lineshape. As a result, continuous-wave and pulsed, double-resonance techniques and specific labeling are usually necessary to resolve the overall hyperfine coupling pattern.

The upper part of Fig. 2 shows an example of the partially resolved but significantly broadened single resonance spectra that are observed from tyrosyl radicals in proteins, in this case from the tyrosyl in galactose oxidase (GO).<sup>6</sup> This spectrum is typical of those observed from tyrosyl radicals and reflects delocalization of the unpaired spin through the phenol headgroup of the tyrosyl side chain and subsequent coupling of unpaired electron spin density to  $\alpha$ - and  $\beta$ -protons on the ring. EPR spectra are generally quantitatively uninterpretable in the absence of additional information on the specific hyperfine tensor components for the individual protons coupled to the unpaired electron spin. We have shown that ENDOR spectroscopy is particularly useful in determining these tensor components, and the lower part of Fig. 2 shows the application of this technique to the tyrosyl radical in galactose oxidase. The  $\alpha$ -protons at the ring 2,6 (*meta*) and 3,5 (*ortho*) positions are characterized by rhombic hyperfine tensors that reflect contact and dipolar coupling to unpaired electron spin at the immediate carbon to which they are bonded as well as through-space coupling to spin density at other carbons in the ring and at the phenol oxygen.<sup>7,8</sup> The  $\beta$ -CH<sub>2</sub> protons at the *para* position are characterized by more axial tensors and reflect both the unpaired electron density at the ring C-4 (*para*) position and their dihedral angles relative to the  $\rho_\pi$  orbital at this carbon, according to the well-known relation,  $A_\beta = \rho_{C_4} (B_0 + B_2 \cos^2 \theta)$ , where  $A_\beta$  is the isotropic hyperfine coupling to the  $\beta$ -proton,  $\rho_{C_4}$  is the unpaired electron spin density at the ring *para* carbon,  $B_0$  is a constant with a value close to zero,  $B_2$  is a constant with a value of 162 MHz, and  $\theta$  is the dihedral angle of the  $\beta$ -proton relative to the C<sub>4</sub> p<sub>z</sub> orbital.<sup>7,9,10</sup>

Table 1 summarizes the assignments for the hyperfine tensors that we have made for the tyrosyl radical in galactose oxidase as well as those for several other tyrosyl radicals that have been studied. In general, the coupling to the 2,6 (*meta*)  $\alpha$ -protons is relatively weak, reflecting low unpaired spin density at these positions; the 3,5 (*ortho*)  $\alpha$ - and 4 (*para*)  $\beta$ -protons are more strongly coupled, indicating higher spin densities at these positions. For all of the tyrosyl radicals studied thus far, the same basic pattern of spin densities is found and is summarized in Fig. 3. In our initial work on the tyrosyl radical in RNR,<sup>8</sup> we had suggested that the unpaired

## Tyrosine Radical in Galactose Oxidase

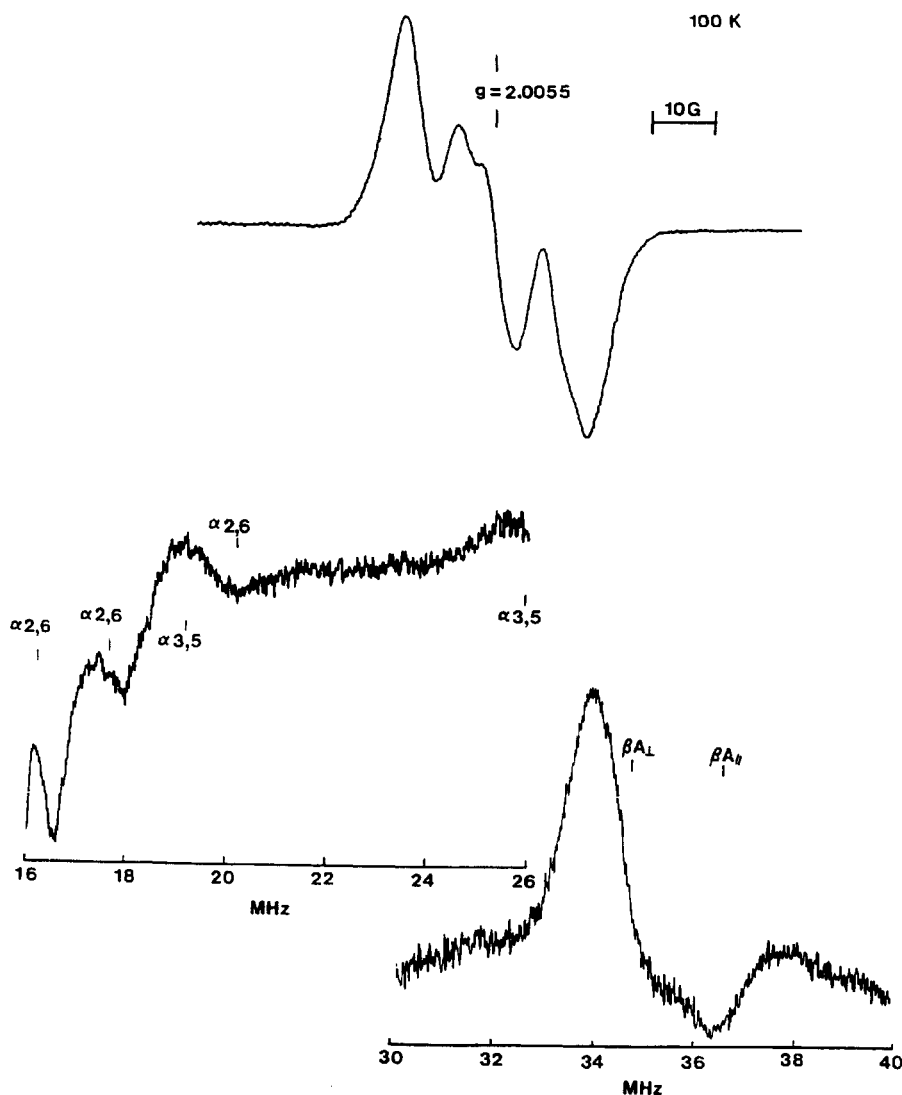


Fig. 2. X-Band EPR (upper) and ENDOR (lower) spectra of the tyrosyl radical in galactose oxidase (see Ref. 6 for details).

spin density at the phenol oxygen and at the C-4 position were significantly lower and higher, respectively, than the values at these positions in Fig. 3. We have recently returned to the  $Y_{122}$  radical in this enzyme and addressed the oxygen spin density directly by  $^{17}\text{O}$  labeling;<sup>10</sup> the results showed that our earlier estimates of the spin at this position and at C-4 were incorrect and that the values in Fig. 3 are appropriate for the RNR radical as well.

ENDOR spectroscopy also has proved to be a valuable technique for characterizing the hydrogen-bonding status of the phenol oxygen in tyrosyl radicals.<sup>8,11,12</sup> Our results show that the phenol oxygen in the  $Y_D$  tyrosine in PSII and in the tyrosyl radical in *apo*-galactose oxidase is hydrogen bonded; for  $Y_{122}$  in RNR and  $Y_{385}$  in prostaglandin synthase (PGHS), on the other hand, the tyrosyl phenol oxygen is not encumbered by hydrogen-bonding

interactions. In the case of the  $Y_D$  radical and of  $Y_{122}$  in RNR, these ENDOR-based conclusions on the hydrogen-bonding status of the phenol oxygen have been confirmed by high-field EPR studies.<sup>13</sup> Our results show, further, that hydrogen-bond interactions perturb the basic spin-density pattern in Fig. 3 only slightly: in the presence of a hydrogen bond, the spin density at the phenol oxygen decreases by ca. 10% while that at the C-4 position increases by the corresponding amount. These values can be rationalized reasonably well by considering the orbital energy level diagram for tyrosyl radical (Ref. 14, see also Refs. 15,16).

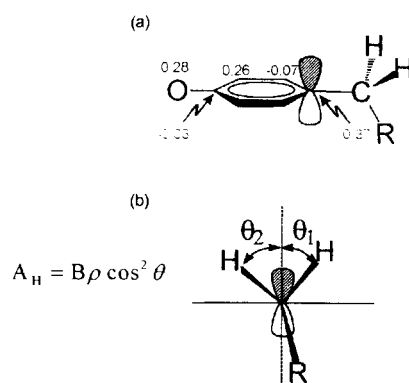
An important conclusion from this work is that the striking variation in cw-EPR lineshapes that is observed in tyrosyl radical enzymes and models can be attributed to variations in the dihedral angle that describes the geometry of the *b*-methylene group relative to the phenol

Table 1. Hyperfine tensors of tyrosyl radicals.<sup>a</sup>

Atom/position	RNR	GO	Y <sub>D</sub> (PSII)	Y <sub>Z</sub> (PSII)	PGHS <sup>b</sup>	Aqueous glass
H 3,5 x	-26.7		-26.4	-26.8	-25.7	-25.4
H 3,5 y	-8.4	-8.4	-7.9	-8.4		-7.2
H 3,5 z	-19.6	-21.6	-19.5	-19.5	-19.5	-19.5
H 2,6 x	5.0	4.8	4.2	5.0	4.9	
H 2,6 y	7.6	6.8	7.1	7.5	7.1	6.5
H 2,6 z	2.1	2.4		1.3		
H meth	61.2	43.4	31.5	35.4	69	3.0
H meth ⊥	53.7	39.8	27.2	29	58.2	2.3
H meth	2.1	11.4	9.8	7.9		
H meth ⊥	-4.5		4.4	1.4		
H bond	n.a.					
H bond ⊥	n.a.	3.1	3.0			
O    (z)	-125					-111
References	8,10	6	9,38,47-49	12		10,17,18

<sup>a</sup> Hyperfine tensor components given in MHz.

<sup>b</sup> Shi, W., Hoganson, C. W., Espe, M., Bender, C. J., Babcock, G. T., Kulmacz, R. J., Palmer, G., Tsai, A.-L., unpublished.



- All radicals (RNR, GO, Y<sub>D</sub>, PGHS, model) show same basic odd alternant pattern
- Insensitive to presence (Y<sub>D</sub>, apo-GO) or absence (RNR, PGHS) of H-bond
- Strong variation in dihedral ( $\theta_1/\theta_2$ ) angles:
 

RNR: $\theta_1 = 17^\circ$	GO: $\theta_1 = 34^\circ$
$\theta_2 = 77^\circ$	$\theta_2 = 86^\circ$
- Static heterogeneity in  $\theta_1/\theta_2$  can be detected and quantified

Fig. 3. Summary of spectroscopic results on tyrosyl-radical-containing enzymes and model compounds.

head group (Fig. 3 and Table 1), not to variations in spin-density distribution. Moreover, during the course of our investigations of the magnetic-resonance properties of tyrosyl radicals, we noticed that the <sup>2</sup>H-ESEEM lineshapes for specifically labeled  $\beta$ -CD<sub>2</sub> radicals and the corresponding continuous-wave and transient ENDOR features for the  $\beta$ -protons were variable.<sup>9,12,17</sup> A detailed investigation of this phenomenon showed that the extent of rotation mobility of the phenol headgroup, with respect to the C<sub>4</sub>-C<sub>β</sub> bond, was the source of this phenomenon. As the barrier to rotation about this bond decreases, heterogeneity in the dihedral angles for the  $\beta$ -protons, relative to the C<sub>4</sub> p<sub>x</sub> orbital, increases, and,

accordingly, the magnitude of the isotropic coupling to the  $\beta$ -protons shows increased dispersion. An analysis of the <sup>2</sup>H-ESEEM spectra provided detailed quantitative insight into the rotational mobility: both the barriers to rotation and the relative populations of the rotamers can be deduced.<sup>17,18</sup> This analysis shows that the distribution can be surprisingly large. For a tyrosyl model compound trapped in a low-temperature matrix, for example, rotational heterogeneity of  $\pm 30^\circ$  is apparent.<sup>17</sup> The analysis also demonstrates the utility of electron magnetic-resonance approaches in deducing the influence of the local environment on the details of the dynamics of the radical.

### 3. Functional and structural properties of radical enzymes

There is now sufficient information available in the literature to provide some generalizations on the properties of the radical enzyme class of proteins (e.g., Ref. 4). These are summarized in Fig. 4 and provide insights into both structural and functional properties of these proteins. The first generalization is that these enzymes generally are associated with a metal – typically, iron, copper, cobalt, or manganese.<sup>19</sup> In some instances, the metal does not occur, however, and is apparently replaced by redox-active organic cofactors such as *S*-adenosylmethionine. Functionally, however, the role of the cofactor is analogous to that of the metal (see below). These exceptions notwithstanding, we can classify the radical proteins as ‘metallo-radical enzymes’. Secondly, these enzymes can be divided into three different families, as indicated in Fig. 4, as the glycyI/thiyl group, the B<sub>12</sub>-dependent group, and the O<sub>2</sub>-dependent group. Specific examples of members of each of these three families are taken from Ref. 4 and given in the figure. Thirdly, these enzymes share structural and functional principles across the different families. Functionally, the metal center acts to generate the amino-acid radical and the radical, in turn, initiates catalysis by

### 1. Families

- Glycyl/thiyl radical enzymes  
examples: *formate pyruvate lyase, anaerobic ribonucleotide reductase, clostridial diol dehydratase*
- B<sub>12</sub>-dependent radical enzymes  
examples: *glutamate mutase, ethanolamine ammonia lyase*
- O<sub>2</sub>-dependent radical enzymes  
examples: *galactose oxidase, ribonucleotide reductase*

### 2. Functional and Structural Principles

- Metal catalyzes radical formation, which abstracts H atoms from substrate
- Metal center/radical/substrate usually in close physical proximity

### 3. Example: O<sub>2</sub>-dependent radical enzymes

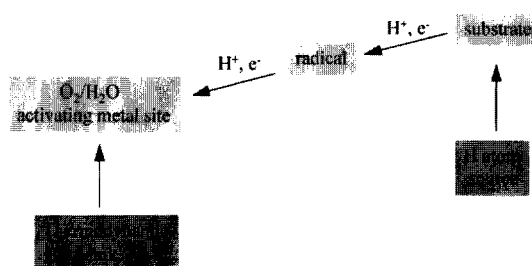


Fig. 4. Summary of various properties of the metallo-radical class of enzymes.

abstracting a hydrogen atom from substrate. There may be embellishments to this functional principle. For example, in pyruvate formate lyase, radical character on a glycyl residue is transferred to a cysteinyl side chain, which subsequently initiates catalysis.<sup>20</sup> In the aerobic RNR from *E. coli*, the radical that resides at the Y<sub>122</sub> position in the R2 subunit in the resting enzyme is thought to be mobilized upon substrate binding and transferred to the catalytically essential cysteine residue<sup>21,22</sup> in the R1 subunit, which then initiates conversion of ribonucleotides to deoxyribonucleotides. A similar mechanism has been proposed recently for the B<sub>12</sub>-dependent ribonucleotide reductase.<sup>23</sup> Nonetheless, the general scheme of 'metal generates radical and radical initiates catalysis by hydrogen-atom abstraction' remains the underlying principle. Accompanying this functional generalization is a related structural theme: the metal, the redox-active side chain, and the substrate-binding channel all are physically close in the enzyme structure. Again, there are exceptions, as in those proteins in which radical mobilization and migration occurs, but, in these cases, close proximity appears to be maintained between the metal and the initial radical and the ultimate catalytically active radical and the substrate. The operation of the radical relay is apparently a means by which to sequester radical activity and prevent non-specific side reactions when substrate is absent; by mobilizing radical character only upon substrate binding, the reactivity can be controlled and directed specifically toward the substrate. RNR provides the clearest example of this sequest-

ration principle.<sup>22</sup> Undoubtedly, the close physical proximity of the metal-radical-substrate triad is necessitated by the short tunneling distances accessible to protons and hydrogen atoms.

Fig. 4 gives an example of the operation of the O<sub>2</sub>-dependent metallo-radical enzymes. In this family, the function of the metal site is to bind dioxygen and undergo the appropriate redox chemistry to generate a metal-bound, activated oxygen species. The nature of this activated oxygen intermediate can vary considerably, as exemplified by the oxygen chemistry at the heme site in prostaglandin synthase, at the non-heme center in RNR, or at the copper site in galactose oxidase. In these systems, the activated oxygen species serves as the thermodynamic sink to provide oxidizing power to generate the amino-acid radical. Once generated, the radical then uses the substrate as a replenishing hydrogen-atom source in initiating catalysis.

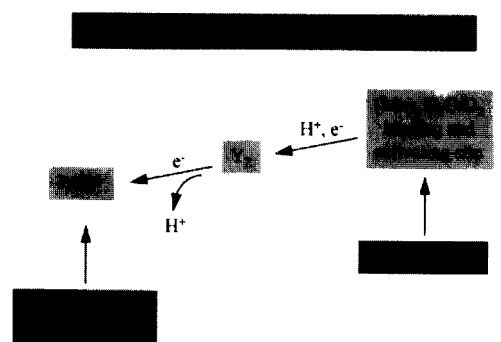
### 4. A case study: Y<sub>Z</sub> and Y<sub>D</sub> in photosystem II

Within the context of the overall scheme for metallo-radical enzymes summarized in Fig. 4, Photosystem II, the water-splitting photosystem in plant and algal photosynthesis, and its associated redox-active tyrosyl radicals, Y<sub>Z</sub> and Y<sub>D</sub>, appear to be anomalous. The Y<sub>Z</sub> species has been viewed as providing an electron-transfer interface between the PSII reaction center chlorophyll, P680, and the (Mn)<sub>4</sub> cluster that has been thought to be the site for water oxidation and oxygen-oxygen bond formation to produce molecular oxygen. The Y<sub>D</sub> tyrosine, which exists as a dark stable radical in oxygenic organisms, has no known function in water splitting (for reviews, see Refs. 24-26). The postulated role of Y<sub>Z</sub>, as a simple electron transfer cofactor, does not appear to allow an easy connection to the hydrogen-atom abstraction function of the side-chain radicals in Fig. 4.

Recent data, however, have allowed us to reconsider the role of Y<sub>Z</sub> in the water-oxidation chemistry that is catalyzed by Photosystem II. In a detailed study of the electron magnetic-resonance properties of Y<sub>Z</sub> in Mn-depleted PSII preparations from *Synechocystis*,<sup>12</sup> we showed that it had unexpected properties relative to Y<sub>D</sub>; in particular, it does not appear to have characteristics that would allow it to function optimally as a simple electron-transfer intermediate. Thus, our earlier work had shown that Y<sub>D</sub> is immobilized in its protein site<sup>9</sup> with a well-defined and ordered H-bond interaction between its phenol oxygen and a proton at approximately 1.85 Å.<sup>25</sup> Molecular modeling of the Y<sub>D</sub> site, furthermore, suggests that its local environment is more hydrophobic, compared with the Y<sub>Z</sub> site.<sup>27,28</sup> All of these characteristics – rigidity, well-ordered hydrogen bond, and hydrophobicity – are what would be expected for an optimized electron-transfer species, as they all work to lower the reorganization energy ( $\lambda$ ) associated with the electron-transfer process. Our work on Y<sub>Z</sub>, on the other hand, shows that, in the absence of manganese, its phenol head

group is mobile, that a hydrogen bond to its phenol oxygen is absent or disordered, and the modeling work suggested a more hydrophilic environment. These factors suggest that the reorganization energy associated with  $Y_Z$  oxidation is likely to be high, which would decrease its effectiveness as a pure electron-transfer mediator. We will return, below, to the issue of the reorganization energies associated with  $Y_D$  and  $Y_Z$  redox chemistry. Concurrent with our work on the properties of  $Y_Z$  and  $Y_D$ , Britt and coworkers showed that  $Y_Z$  was in much closer physical proximity with the  $(Mn)_4$  cluster than had been previously thought. They estimated the distance between  $Y_Z$  and  $(Mn)_4$  to be 4.5–7.0 Å (Ref. 29, see also Refs. 30,31).

Taken together, these two experiments suggested that a significant revision of the role of  $Y_Z$  in PSII and water oxidation was necessary. Britt's results showed that the radical is not sequestered in the protein remote from the water oxidation site. The effects of removing the  $(Mn)_4$  cluster, which includes a dramatic reduction in the rate of the  $P680^+/Y_Z$  electron transfer and a lowering of the redox potential of the  $Y_Z/Y_Z$  couple,<sup>32</sup> could be rationalized in terms of a disruption of the  $Y_Z$  site that produced the disordered structure detected in our magnetic-resonance studies. In considering the implications of these results, we suggested that they indicated that  $Y_Z$  functions in PSII in a fashion analogous to the radicals in the enzymes described in Fig. 4, i.e., as a hydrogen-atom abstractor.<sup>12,33,34</sup> The essence of this reformulation of the function of  $Y_Z$  is described in Fig. 5.  $P680^+$  acts as the thermodynamic sink and water as the substrate.  $Y_Z$



- Simple reversal of H atom current from that in  $O_2$ -dependent radical enzymes
- $(Mn)_4$  acts as substrate binding site and sink for oxidizing equivalents
- $H^+$ ,  $e^-$  abstraction from  $(Mn)_4$ -bound  $H_2O$  or  $OH^-$  on each S-state transition; rate-limits  $O=O$  bond formation on  $S_3/S_4/S_0$
- Overall water-splitting process at  $Y_Z/(Mn)_4$  active site is charge neutral; analogous to  $O_2$  reduction in cytochrome oxidase
- Rotational mobility in  $Y_Z$  phenol head group necessary for directional  $H^+$  uptake and release

Fig. 5. Model for photosystem II operation as a metallo-radical enzyme.

serves to abstract hydrogen atoms, either in a sequential or concerted way, from water and hydroxyl ligands bound to the  $(Mn)_4$  cluster. The metal center acts as the substrate-binding site and to delocalize oxidizing equivalents generated during the four successive photoacts that are required for water oxidation. Because of the coupled transfer of an electron and a proton on each step, the system maintains charge neutrality throughout the photochemical and chemical processes necessary for oxygen–oxygen bond formation and dioxygen release. Elsewhere, we have described, in more detail, a likely structure for the manganese ensemble and the mechanism by which the oxygen–oxygen bond forms.<sup>33,34</sup>

A key issue in the proposed hydrogen-atom abstraction model for PSII is the rate of electron transfer through  $Y_Z$ . Distance, driving force, and reorganization energy ( $\lambda$ ) are the main parameters in determining the kinetics of this process<sup>35–37</sup> and, in the concluding section of this article, we will consider the interplay of these factors for  $Y_D$  and for  $Y_Z$  in Mn-containing and -depleted preparations. We assume  $Y_D$  and  $Y_Z$  are symmetrically disposed, with respect to  $P680^+$ , and that Mn release does not change, dramatically, the  $P680-Y_D$  or  $P680-Y_Z$  distances.<sup>25</sup> In addition, we assume that Mn release does not perturb the  $Y_D$  site significantly, which is consistent with EPR and ENDOR studies of this center.<sup>38</sup> Two issues, then, need be addressed: (1) the decrease in  $P680^+$  reduction rate by  $Y_Z$  to  $\sim 10^5 s^{-1}$  when the  $(Mn)_4$  cluster is removed and (2) the difference in rate between  $Y_D$  ( $\sim 10^4 s^{-1}$ ) and  $Y_Z$  ( $\sim 10^8-10^7 s^{-1}$ ) reduction of  $P680^+$  in  $O_2$ -evolving preparations.<sup>25</sup>

For the first question, we know that removal of the  $(Mn)_4$  cluster lowers the redox potential of  $Y_Z$ ,<sup>32</sup> which, if distance and reorganization energy remain constant, predicts that  $Y_Z/P680^+$  electron transfer should accelerate. The decrease in rate actually observed indicates that the reorganization energy associated with  $Y_Z$  oxidation is a function of the presence of the  $(Mn)_4$  cluster and increases when the metals are removed. Three factors can be envisioned that could produce the observed increase in  $\lambda$ : (a) increased hydrophilicity in the local protein environment, (b) increased disorder in  $Y_Z$  and its hydrogen-bonding interaction, and (c) retention of positive charge, i.e., of the proton generated upon  $Y_Z$  oxidation, in the local protein environment.<sup>39,40</sup> For  $Y_Z$ , in either Mn-depleted or intact preparations, proton release is detected upon oxidation,<sup>41,42</sup> which suggests that (c), above, does not occur; proton release along the D1–H190 and D1–E189 route we have suggested for the intact system<sup>33</sup> may still occur when the  $(Mn)_4$  cluster is removed or, upon Mn-depletion, the  $Y_Z$  site may become sufficiently accessible to solvent that proton release occurs directly to bulk. Our ENDOR results discussed earlier suggest that (b) is certainly likely, and recent work<sup>43,44</sup> indicates that water has increased access to the  $Y_Z$  site in the absence of the metals. Thus, we can attribute the decrease in  $P680^+-Y_Z$  electron-transfer rate to an increase in water content about  $Y_Z$  and to an increase in

local disorder at the cofactor. The corollary to this analysis is that the  $Y_Z$  site in water splitting material is well ordered in its hydrogen-bonding interactions and that water, except that that is bound at the  $(Mn)_4$  substrate sites, is excluded. These structural conclusions are summarized in Figs. 6(a) [ $(Mn)_4$  intact] and 6(b) [ $(Mn)_4$  removed].

For the second question, we know that  $Y_D$  has a lower reduction potential than  $Y_Z$ ,<sup>25</sup> which favors faster reduction of  $P680^+$  by  $Y_D$ . Thus, with the assumption of a symmetric placement of  $P680^+$ , with respect to  $Y_Z$  and  $Y_D$ , the reorganization energy provides the only parameter capable of rationalizing the faster rate of  $P680^+$  reduction by  $Y_Z$  than  $Y_D$ . We have seen above, however, that  $Y_D$  is immobilized with a well-ordered hydrogen bond in a region of low dielectric, all of which favor a low reorganization energy.

Moreover, the  $Y_D$  structure and local environment are not perturbed, to a measurable extent, by removal of the  $(Mn)_4$  cluster.<sup>38</sup> Thus, the only means by which to rationalize the higher reorganization energy in  $Y_D$  is to suggest that the proton that is released upon  $Y_D$  oxidation is trapped on the H190 residue to which the tyrosine hydrogen bonds.<sup>45,46</sup> This suggestion is well supported by two observations: (1) the proton in the  $Y_D$  site exchanges extremely slowly ( $t_{1/2} > 24$  hs) with bulk phase<sup>25</sup> and (2) the D1-E189 residue, which appears to be associated with the  $Y_Z$  deprotonation pathway, is not conserved in the D2 sequence associated with  $Y_D$ ; rather, it is replaced by the acid/base inactive phenylalanine residue.<sup>27</sup> The postulated local structure and truncated proton translocation pathway about the  $Y_D$  site are illustrated in Fig. 6(c).

## 5. Concluding comments

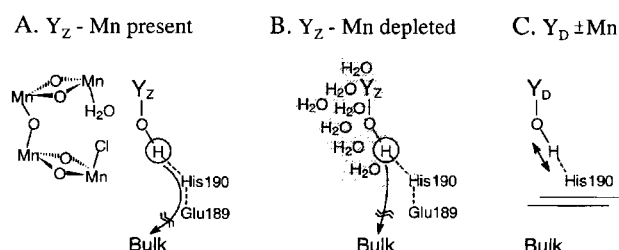
Control of radical reactivity is critical in radical enzymes. With the generation of the radical, the protein has created an energetic intermediate capable of promoting diverse chemical reactions. The function of this protein then becomes one of directing the chemistry toward the substrate. Access limitation, as in the case of RNR, is one means by which this is accomplished. In PSII, control of

reorganization energies by control of proton currents, of local order at the radical center, and of water access emerges as an additional mechanism for directing radical chemistry.

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**Fig. 6.** Models for the structure and local environments around  $Y_Z$  and  $Y_D$  in photosystem II: (a)  $Y_Z$  in native,  $O_2$ -evolving PSII preparations; (b)  $Y_Z$  in preparations in which the  $(Mn)_4$  cluster has been removed; local disorder about the  $Y_Z$  headgroup is indicated by stipling; (c)  $Y_D$  in either  $O_2$ -evolving or inhibited PSII preparations.

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