Review

Amino acid residues involved in the coordination and assembly of the manganese cluster of photosystem II. Proton-coupled electron transport of the redox-active tyrosines and its relationship to water oxidation

Bruce A. Diner *

CR&D, Experimental Station, E.I. du Pont de Nemours and Co., Wilmington, DE 19880-0173, USA

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Abstract

The combination of site-directed mutagenesis, isotopic labeling, new magnetic resonance techniques and optical spectroscopic methods have provided new insights into cofactor coordination and into the mechanism of electron transport and proton-coupled electron transport in photosystem II. Site-directed mutations in the D1 polypeptide of this photosystem have implicated a number of histidine and carboxylate residues in the coordination and assembly of the manganese cluster, responsible for photosynthetic water oxidation. Many of these are located in the carboxy-terminal region of this polypeptide close to the processing site involved in its maturation. This maturation is a required precondition for cluster assembly. Recent proposals for the mechanism of water oxidation have directly implicated redox-active tyrosine YZ in this mechanism and have emphasized the importance of the coupling of proton and electron transfer in the reduction of $Y^*_Z$ by the Mn cluster. The interaction of both homologous redox-active tyrosines $Y_Z$ and $Y_D$ with their respective homologous proton acceptors is discussed in an effort to better understand the significance of such coupling. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

Polypeptides D1 and D2 constitute the heart of the photosystem II (PSII) reaction center and are responsible for the coordination of nearly all of the redox cofactors of this photosystem. With the construction of psbA and psbD deletion host strains of Synechocystis 6803 and Chlamydomonas reinhardtii, it became possible to probe, using site-directed mutagenesis, these coordination sites within D1 and D2, respectively. Among the most novel of these cofactors is the Mn cluster, which coupled to redox-active tyrosine, $Y_Z$, is responsible for photosynthetic water oxidation. Several groups [1–7] have extensively targeted residues on the luminal sides of the D1 and D2 polypeptides, considered the most likely sites for manganese coordination and for function of the redox-active tyrosines, $Y_Z$ (D1-Tyr161) [8,9] and $Y_D$ (D2-Tyr160) [10,11] (Synechocystis numbering used here and throughout). The influence of these mutations on the assembly, coordination and function of...
the Mn cluster and on the coupled electron and proton transfer of Y\textsubscript{Z} and Y\textsubscript{D} will be described below.

2. Assembly and coordination of the Mn cluster

2.1. High affinity binding site

There is considerable evidence in favor of a high affinity site associated with the binding of the first Mn involved in the assembly of the Mn cluster. This site has been investigated in a number of ways as a function of the Mn(II) concentration including: competition with diphenylcarbazide for electron donation to PSII [12,13], measurement of the rate of reduction of Y\textsubscript{Z} using EPR [14-16] or optical spectroscopy [17]; and measurement, using the chlorophyll fluorescence yield [15,18,19], of the extent of blockage of charge recombination between Q\textsubscript{A} and the electron donor side (Fig. 1).

Apart from the direct measurement by EPR of free Mn(II) in equilibrium with bound metal ion by Ono and Mino [15] (K\textsubscript{d} \approx 10 \muM, pH 6.5), these methods do not truly measure a dissociation constant, K\textsubscript{d}, but rather reflect the ability of Mn(II) to be bound and oxidized during the lifetime of the charge separated state or to compete with another electron donor. Thus, in these cases, the oxidation of Mn(II) is a function of the rate of diffusion of Mn(II) to the site, the affinity of Mn(II) for the site with Y\textsubscript{Z} oxidized and its rate of oxidation by Y\textsubscript{Z}. The concentration of Mn(II) to block half of the centers for charge recombination is approximately 1 \muM at pH 5.7-6.5 [15,17,18], decreasing by approximately a decade per pH unit between pH 5 and 7, and pH-independent above pH 7 [15]. Measurement of the rate of reduction of Y\textsubscript{Z} shows kinetics that are pseudo first order in the concentration of Mn(II) up to 3 \muM at pH 6.5 [15] and 10-20 \muM at pH 5.7 [17], indicating that below these limits the rate of Y\textsubscript{Z} reduction is diffusion-limited. Ono and Mino [15] have shown that the capacity of this high affinity site is limited to one Mn(II). Binding of additional Mn(II) requires concentrations that are at least 3-fold higher.

This process and the competition of Mn(II) with diphenylcarbazide for electron donation have been interpreted as indicating the participation in a high affinity binding site of histidine [15,16], suggested to be D1-His337 and D2-His336 (Synechocystis numbering) [13], or of carboxylic acid containing residues [16] suggested to be D1-Asp170 [12] in Mn(II) binding.

2.2. D1-Asp170

Extended directed mutagenesis at D1-170 has shown that the residue at this site (an Asp in wild type (WT)) has considerable influence on the rate of Mn(II) binding and oxidation and on the ability to assemble a fully functional Mn cluster [17-22]. A succession of saturating light flashes, given at a frequency of 1.67 [18] or 20 Hz [19], to whole cells of the mutant Synechocystis strains indicated a progressive quenching in the first few flashes of the chlorophyll fluorescence yield detected at 50 \mu s after each actinic flash. Those strains, most impaired in O\textsubscript{2} evolution, showed the most marked quenching and an accelerated rate of recombination in the presence of DCMU, indicative of an enhanced concentration of P680\textsuperscript{+} and therefore of an impairment in PSII donor side electron transfer. Nixon and Diner [17,18] have shown that the mutations at this site impact the ability to bind and oxidize Mn(II) following extraction of the Mn cluster from PSII core complexes. An apparent K\textsubscript{m} was determined by measuring, as a function of Mn(II) concentration, the blockage of charge recombination between Q\textsubscript{A} and the donor side following a single saturating light flash. Mn(II) was shown to reduce Y\textsubscript{Z} with the K\textsubscript{m} for doing so in-

Fig. 1. Manganese binds reversibly to PSII centers in the Y\textsubscript{Z} or Y\textsubscript{Z} state. Oxidation of Mn(II) to Mn(III) by Y\textsubscript{Z} stabilizes Q\textsubscript{A} by slowing the rate of charge recombination.

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creasing and the in vivo oxygen activity decreasing with increased $pK_a$ of the replacing residue. As an example, the second order rate constant for the reduction of $Y_2^*$ by Mn(II) decreases 100-fold upon replacement of Asp with Ser at D1-170 [17]. This mutant is completely inactive for oxygen evolution. Chu et al. [21], however, have shown that the accessibility of manganese to the donor side of PSII in vivo was greatly enhanced by insertional inactivation of the psbO gene that encodes the 33 kDa extrinsic polypeptide. The above cited lack of effect of this polypeptide on the in vitro $K_d$ ($K_m$) [14,15] would imply that in vivo the 33 kDa extrinsic polypeptide impedes access to the high affinity binding site rather than having a direct influence on the affinity of this site. The above observations imply that Asp170 is involved in the binding of the very first Mn to form the water-oxidizing complex. These measurements could not, however, distinguish whether the D1-Asp170 acts as a gatekeeper, controlling the access of Mn(II) to its coordination site, or is actually a ligand to the oxidized Mn(III).

Recently, however, Campbell et al. [23] used parallel mode EPR to examine the coordination environment of the photooxidized manganese in the WT and two D1-170 mutants. Classical perpendicular mode EPR in X-band is best suited to the detection of spin transitions in half integer spin systems that satisfy the selection rules $\Delta M_s = \pm 1$. Parallel mode EPR in X-band, however, offers the possibility of studying integral spin systems with $S \geq 1$ where the spin transitions satisfy the selection rules $\Delta M_s = \pm 2$ and higher. This latter technique is therefore well-suited to examine the coordination environment of Mn(III) (high spin $S = 2$), the expected product of the photooxidation of Mn(II) in Mn-depleted PSII core complexes reconstituted with one Mn/reaction center.

PSII core complexes from *Synechocystis WT*, and the D1-Asp170Glu and His mutants, all of which are photoautotrophic (His weakly so), were reconstituted in the dark with 1.2 Mn(II) per center and frozen under illumination using liquid $N_2$. Parallel mode EPR signals were obtained in the WT and D1-Asp170His mutants. The WT centers showed a six-line signal with a hyperfine splitting of $\sim 45$ G that was only visible in parallel mode, showing no signal in perpendicular mode. This signal clearly arises from Mn(III) as it closely resembles that observed for Mn(III) in superoxide dismutase [24] and for a Mn(III)(salen) complex in the presence of $N$-methylmorpholine $N$-oxide (NMO) [25]. In the case of the D1-Asp170His mutant, a Mn(III) signal was obtained that closely resembles a synthetic Mn(III)salen complex in the absence of NMO [23,25]. While others are possible, the simplest explanation of these results is that the Mn(III) is hexacoordinate in the presence of D1-Asp170 but pentacoordinate in D1-Asp170His. In the case of the D1-Asp170Glu mutant, no signal was observed in parallel mode. However, a signal was observed in perpendicular mode consistent with the generation of the $S = 3/2$, Mn(IV) [23]. Apparently the presence of the glutamate at D1-170 was able to stabilize Mn(IV) to the point where a double oxidation of Mn(II) could occur. This observation is also consistent with the increased lifetime of the S2 state of the Mn cluster [26] in whole cells of this mutant ($t_{1/2}$ decay $= 65 \pm 5$ s) compared to WT ($t_{1/2}$ decay $= 20 \pm 5$ s) [18]. The Mn(IV) could not be generated in either the WT or the D1-Asp170His cases. The effect of the His mutation on the S2 state lifetime is smaller than that of Glu with an S2 lifetime of $45 \pm 5$ s ($t_{1/2}$). These observations indicate a direct influence of the residue at D1-170 on the coordination environment and redox behavior of the bound Mn. It is likely then that this residue is involved in the coordination of Mn in this first step of the assembly of the Mn cluster. Whether this residue remains a ligand to the fully assembled complex is unclear, though the effect of the mutation on the S2 state lifetime, the inability to observe a S2 state multiline in the D1-Asp170Glu mutant [27] and X.-S. Tang, unpublished observation), and the modification by this mutation of the carboxylate region of the S2–S1 FTIR difference spectrum [27] would imply a close if not coordinated interaction with the Mn cluster. However, Chu et al. [5] have reported that the D1-Asp170Val *Synechocystis* mutant is weakly photoautotrophic and that, while not photoautotrophic, the Ile and Leu mutants were capable of O2 evolution but at diminished rates compared to WT. This observation implies that D1-Asp170 is not absolutely required for either the assembly or the function of the Mn cluster. Its function may be replaced either by a nearby coordinating residue or by a water molecule or coordinating anion. As we
will see below, there are no known D1 residues, identified by directed mutagenesis, where there is not some amino acid replacement able to show some level of assembly and function of the Mn cluster.

2.3. D2-Tyr160

Another residue that has been implicated in Mn cluster assembly is one that is assuredly remote from the location of the cluster. This is D2-Tyr160, the residue responsible for redox-active tyrosine, Y_D [10,11]. Dismukes and collaborators (Ananyev et al., submitted) have shown that in a mutant where this residue has been replaced by Phe [28], there is a decrease in the efficiency of photoactivation of the Mn cluster. This observation is consistent with the slowed photoautotrophic growth of the D2-Tyr160-Phe mutant. Tamura and Cheniae [29] have demonstrated the existence of two distinct light-dependent steps in the photoactivation process. A first binding and photooxidation of Mn(II) to form state L1 is followed by the binding of a second Mn(II) to form an intermediate, L2, which is unstable unless followed by a second photostep. The product is a stable intermediate, L3. Its formation is followed by the addition of two additional Mn(II) which are ultimately oxidized to the S0 state, the most reduced state of the Kok-Joliot cycle [26]. This state likely corresponds to the manganese redox state II,III,IV, IV [30,31], for a total of five photooxidations. One possible explanation for the role of Y_D is that this species, previously oxidized and long lived, contributes to the oxidation of the nascent cluster. This stored oxidizing equivalent could then be contributed to the L2 unstable intermediate to produce the more stable L3. Such a mechanism would obviate the need for a double photoevent that might be a rare occurrence in nature for photoautotrophs growing under dim light conditions.

2.4. D1 C-terminal processing protease

There is a critical step in the assembly of the Mn cluster that does not involve a redox event. This is the proteolytic removal of the carboxy-terminal residues of the preD1 polypeptide by the D1 C-terminal processing protease [32–34], the encoding gene of which has now been cloned from Synechocystis [35], spinach [36] and Scenedesmus [37]. The enzyme has been purified to homogeneity from the latter two [36,37]. This proteolytic processing of preD1 is absolutely required for oxygen evolution [37–39]. Either 8, or 9 or 16 residues are removed in Chlamydomonas, all but two photosynthetic eukaryotes, and the cyanobacteria, respectively, with processing occurring between residue D1-Ala344 and D1-345 [40–42]. An 18-mer C-terminal extension mutant of Synechocystis was also photoautotrophic [42]. Apart from Chlamydomonas, the one exceptional eukaryote is Euglena, which from its psbA gene sequence (encoding D1) does not appear to have a C-terminal extension on the D1 polypeptide [43,44]. Failure to carry out proteolytic processing of the D1 extension allows only partial assembly of the Mn cluster, resulting in the binding of only 1–2 Mn/center [37,45]. Mutants which either lack the D1 protease [39,46] or in which the scissile bond has been modified to block processing [21,42,47] are unable to evolve oxygen and to grow photosynthetically. It has been shown, as in the case of Euglena, however, that Synechocystis (SΔ) [42] and Chlamydomonas [48,49] mutants in which the extension has been deleted, and that consequently express the mature form of D1, are able to assemble the Mn cluster and grow photosynthetically. That almost all oxygenic photoautotrophs express the D1 polypeptide with an extension [50] implies that it plays an important role either in the assembly of the reaction center or of the Mn cluster. In support of this idea, the Synechocystis SΔ truncation mutant grows at rates that are indistinguishable from WT at low light intensity, but grows less well at high light. This observation implies a rate limitation that is more marked in the SΔ strain where D1 is turning over rapidly. The residues that comprise the extension are not highly conserved, though there is considerable similarity in the last four residues of the sequence. The sequence is perfectly conserved in all known expressed psbA genes up to 14 residues to the amino side (P side) of the scissile bond [50]. This conservation is probably more a reflection of the constraints put on the coordination of the Mn cluster than on C-terminal processing. Consistent with this picture, the replacement of D1-Asp342 with Val was not photoautotrophic but still allowed processing, suggesting a need for water oxidation but not for protease recognition [42]. None of the truncated ver-
sions of D1 produced by introducing stop codons at D1-335, 342, 343, or 344 was able to evolve oxygen [21,42]. Replacements of D1-Ala344 with Gly, Met, Ser, and Val in the Sα background were all photoautotrophic [42]. The Tyr and Lys mutants at this site were non-photosynthetic but still evolved oxygen at 10 and 20% of WT levels, respectively. These observations and the requirement for C-terminal processing at Ala344 led Nixon et al. [42] to propose that the free carboxyl group of the C-terminal alanine might itself serve as a ligand to the Mn cluster.

Site-directed mutations have been introduced on the carboxy (Pβ) side of the scissile bond in an effort to better define the selectivity of the enzyme. Mutations introduced in place of D1-Ser345 including Cys, Gly, Phe and Val in Chlamydomonas [51] were all photoautotrophic [51]. In Synechocystis, replacements of this same residue by Arg and Ala allowed processing and were photoautotrophic, while the Pro replacement blocked processing and was not [21,42]. Synthetic peptide substrates (19-mers) modeled on the C-terminal 19 amino acids of the D1 polypeptide of spinach and bearing mutations at the site corresponding to D1-Ala345 (P1′ position) showed Vmax for processing by the spinach enzyme in vitro in the following order Ala, Ser, Phe, Cys > Gly > Val > Pro [47]. On the amino side, replacement of Leu343 (P2 position) with Ala eliminated in vitro processing [47], suggesting the need for a hydrophobic residue at this position.

D1 protease is nuclear encoded with signal sequences that direct it to the thylakoid lumen [37,46,52]. Its import into the lumen has been shown to be sensitive to inhibitors of the sec and ApH-dependent protein translocation complexes [53]. The mature protease seems to have no target other than preD1 in that the Synechocystis Sα mutant is insensitive to inactivation of ctpA, the gene that encodes D1 protease (Dexter Chisholm, personal communication). D1 protease appears to be a serine type protease with an unusual serine-lysine catalytic dyad [37]. The X-ray crystallographic structure of the Scenedesmus obliquus enzyme has been solved to 1.8 Å [54].

2.5. Other critical residues

Using site-directed mutagenesis, several groups have homed in on a number of residues in the D1 polypeptide as being particularly critical for oxygen evolving activity (for a recent review see [7]). In addition to those residues mentioned above, these include His190 [1,4,5,55–58], His332 [4,6,59], Glu333 [1,4,6], His337 [1,4,6], Glu333 [1,4,6], Glu334 [1,4,6]. Interestingly, neither the mutations in the C-terminal region of D1 nor the processing of the C-terminal extension has any influence on the ability to bind and oxidize the first Mn in the assembly of the cluster [42]. These observations indicate that the C-terminal residues do not participate in the coordination of the first bound Mn, though they likely contribute to the coordination of those bound later on in the assembly process.

2.5.1. Histidine

It was shown using ESEEM (electron spin echo envelope modulation) of 14N and 15N globally labeled PSII core complexes from Synechococcus sp. that there is a 5 MHz signal arising from 14N coupled to the S2 state multiline signal [60]. Labeling of Synechocystis PSII core complexes with [15N]histidine with only the two imidazole nitrogens labeled [61] resulted in the loss of the ESEEM signal, indicating the presence of at least one His ligand to the Mn cluster in the S2 state. A similar ESEEM study using histidine15N-labeled solely at the pi imidazole nitrogen of His [62] showed the S2 ESEEM spectrum to be indistinguishable from that of global 14N, indicating that coordination of Mn occurs solely through the tau nitrogen. This conclusion was strengthened by a recent FTIR study on similarly [15N]His labeled PSII core complexes [63]. In addition, chemical modifiers have also implicated histidine in Mn coordination [13,16].

Three histidines located on the luminal side of the reaction center have been shown to be critical for oxygen evolution. These are D1-His190, 332 and 337.
The D1-His190 mutations [1,4,5,55–58] are with only two known exceptions, Arg and Lys, unable to evolve oxygen [7]. None are photoautotrophic. This residue plays an important role in the coupling of proton and electron transfer in the oxidation of tyrosine, Y\(_Z\) (see below). It is difficult to distinguish between this role and that as a possible ligand to Mn as being critical for \(O_2\) evolution.

Numerous mutations have been constructed at D1-His332 [4,6,59]. Many of these show highly impaired (Gln and Ser) [4] or no rates of oxygen evolution and appear unable to assemble a functional Mn cluster [4,6,7]. A recent report [59] on the site-directed mutation D1-His332Glu has shown that while assembled in many centers, the Mn cluster can only advance beyond the S2 state with an 8000-fold reduced quantum yield. The mutation also shows a modified S2 state multiline signal. A photoautotrophic suppressor has been isolated from a mutant in which D1-His332 was replaced by Gly (W.J. Coleman, J. Wang and B.A. Diner, personal communication). This mutant retains the Gly replacement and has no compensatory mutations elsewhere in the coding region of the \(psbA\) gene. This is the only known photoautotrophic mutant at this site.

Many mutations at D1-His337 also appear to be unable to assemble a functional Mn cluster [1,4,6]. The Arg, Gln, Phe [6] and Ser [4] (Diner, personal communication) replacements are photoautotrophic while the Asp, Glu, Asn [6] and Leu [6,42] replacements are non-photoautotrophic but evolve oxygen. While less impaired than the D1-His332 mutations, these observations imply that both D1-His332 and D1-His337, while important, are individually not required for oxygen evolution and photoautotrophic growth. In those mutations where the replacing residue cannot act as a ligand, either a nearby residue, a water molecule or a small anion has succeeded in functionally replacing His332 or His337. In addition, unlike those mutations constructed at D1-Asp170, the mutations at neither D1-His332 nor 337 appear to affect the Mn binding site involved in the first step of cluster assembly [4].

### 2.5.2. Carboxylate containing residues

Three carboxylates have also been proposed as potential Mn ligands. These are D1-Glu333, D1-Asp342 and the C-terminal carboxylate of D1-Ala344.

Of the mutations constructed at D1-Glu333 [1,4,6], only D1-Glu333Gln is weakly photoautotrophic [6,42]. Mutations at this site impair assembly of the Mn cluster [6,7]. Of the mutations constructed at D1-Asp342, only D1-Asp342Glu is photoautotrophic [6] but Asn shows up to 70% of WT rates of \(O_2\) evolution [4,6]. Here too, there appears to be an inability to stably assemble the Mn cluster [7]. The mutations at D1-Glu333, at D1-Asp342 and D1-Ala344Stop (see above) and D1-Ser345Pro (see above) appear not to affect the Mn binding site involved in the first step of cluster assembly [4].

Extensive mutagenesis has been carried out on the Asp, Asn, Glu, Gln and His residues located on the luminal side of the D2 polypeptide, most of which remain photoautotrophic [1,2]. While D2-Glu69 [64] has been suggested as having a role in water oxidation, this residue is likely too far from the Mn cluster given what we now know of reaction center folding [65–67] and the location of the Mn cluster within 7–9 Å of redox-active tyrosine \(Y_\Phi\) (D1-Tyr161) (see below) and ~30 Å [68] from redox-active tyrosine, \(Y_D\) (D2-Tyr160). The large hydrophilic loops of CP43 and CP47 appear to be important for the binding of the 33 kDa extrinsic polypeptide (for reviews see [69,70]) and consequently influence the affinity for the essential cofactors, \(Ca^{2+}\) (for review see [71,72]) and \(Cl^-\) (for review see [71–73]), and the stability of the Mn complex (for review see [70,71]).

With the exception of D1-Asp170, the enormous work that went into engineering and characterizing the mutations at these sites has not as yet given a clear answer as to their roles as Mn ligands. However, the evidence is considerably weaker for other PSII polypeptides to participate in direct manganese coordination. The review in this series by Rick Debuss discusses this evidence in more detail. It is likely that, with the many ligands involved in the coordination of the Mn cluster, the replacement of any one coordinating residue by another, at the same or at a neighboring position, or by a small ligand like water or \(Cl^-\) might allow the complex to function, even if suboptimally. In the absence of an X-ray crystal structure, isotopic labeling techniques combined with site-directed mutations are more likely to give a clear answer as to ligand identity. Such efforts are
underway and are limited only by the ability to generate sufficient numbers of centers with assembled complexes to be able to characterize, using magnetic resonance techniques or FTIR, spectroscopic signals with adequate signal/noise.

3. Redox-active tyrosines

3.1. A neutral radical in PSII

There is a rapidly growing body of work on amino acid radicals participating in metal-based enzyme catalysis (for review see [74]). This effort has had considerable influence on the recent thinking about water oxidation in PSII (see below), particularly with regard to the role of redox-active tyrosine, YZ and the coupling of the oxidation of the Mn cluster with proton transfer.

The thermodynamic cycle below (Fig. 2) shows the relation between the pKa values and the reduction potentials of tyrosine in aqueous solution. With a pKa of −2 for the oxidized radical [75], a pKa for the reduced form of 10.3 and a reduction potential for the redox couple TyrO+/TyrO− of 0.72 V vs. NHE [76], the reduction potential of the redox couple TyrOH+/TyrOH can be calculated to be 1.45 V (see also [77]). Even if there were some modification of these numbers in the reaction center, this reduction potential is likely to remain well above that of the primary electron donor, P680+/P680 (E′ = 1.12 V) [78].

The absence of a strong isotropically coupled exchangeable proton in the cw ENDOR, ESE-ENDOR and ESEEM spectra of YZ* [28, 79, 80] and YD* [81–84] and the isotropic g value measured for the tyrosyl radicals indicate that it is the neutral (g = 2.0045) and not the cation radical (g = 2.0033) that is formed upon tyrosine oxidation [85]. Thus, the phenolic proton must be transferred either before, during or at some point shortly after the oxidation. In PSII centers containing an intact Mn cluster, the predominant nanosecond phases of oxidation of YZ by P680+ show virtually no deuterium kinetic isotope effect [86–88] and a very small activation energy (2.4 kcal/mol) [89] under conditions where the tyrosine is known to be protonated prior to its oxidation [90]. Because the formation of the cation radical would impose a substantial activation barrier and would cause the oxidation to be proton-limited, it is likely that the phenolic proton is transferred prior to the electron.

In Mn-depleted PSII core complexes, there is a more substantial deuterium kinetic isotope effect of 1.7–3.6 [57, 91, 92] and activation energy (7–11 kcal/mol) [91, 93] for the same reaction, where YZ is known to be protonated prior to oxidation [94]. That under these conditions (pH 5–7), YZ oxidation is not electron transfer-limited (B. Diner et al., in preparation) implies the need to dissociate the phenolic proton prior to or coincident with electron transfer to P680+. This conclusion is reinforced by the disappearance of the kinetic isotope effect at higher pH, where there would be an appreciable concentration of tyrosinate preceding photoexcitation [91, 92] (but see [57]).

3.2. Coupled proton and electron transfer in water oxidation

The conjuncture of a number of ideas and observations led Babcock [95, 96] and Britt [97, 98] and their collaborators to take a new, insightful look at the mechanism of water oxidation. The redox-active tyrosine YZ was proposed to act as both an electron and a proton (or H+) sink, accepting at the same time an electron from the Mn centers and a proton from a coordinated water or hydroxo-ligand. The ideas and observations that have contributed to and that have supported this model include: (a) the proposal by Krishtalik [99, 100] of the coupling of water oxidation
to the protonation of a base, the $pK_a$ of which were redox-dependent; (b) the appreciation that tyrosine could fulfill this role with its radically different $pK_a$ values in the oxidized and reduced states (see above); (c) the demonstration that $Y^*_Z$ and the Mn cluster were physically close [97,101], 7–9 Å between the centers of spin density [102–104]; (d) the demonstration that $Y^*_Z$ has considerably greater motional flexibility than does $Y^*_D$, potentially allowing the former to deliver and pick up a proton to/from different locations [79,83,105]; (e) new knowledge of the lowering of the bond dissociation energies for water and hydroxide O–H when bound to inorganic Mn complexes (77–87 kcal/mol) [106,107] and of O–H in tyrosine (87 kcal/mol) [108,109], making $^1H$ transfer, from the first to the second, exothermic; (f) the appreciation of the generality of $^1H^*$ transfer to oxygen in metalloradical enzymes [96,110]; and (g) the sensitivity of the vibrational modes of $Y_Z$ to the S state transitions of the cluster [90] and inversely, the spin state of the cluster ($g = 4.1$ versus $g = 2$ multiline) to the redox state of $Y_Z$ [111], both indicative of a hydrogen-bonded interaction between the cluster and $Y_Z$.

The driving force for Mn oxidation would be enhanced by coupling the oxidation of a Mn center to the deprotonation of a Mn-bound water/hydroxide and the protonation of newly reduced $Y_Z$. Critical to the proposed formulation of the model has been the need to eliminate the abstracted proton to the thylakoid lumen. A key question then becomes what happens to the proton released upon oxidation of $Y_Z$. We will examine this question by first discussing the hydrogen bonding interactions that the redox-active tyrosines have with their surroundings, as these will constitute the first available proton acceptors.

3.3. Histidines D2-His189 and D1-His190 as proton acceptors

It has been known for some time that tyrosines $Y^*_Z$ and $Y^*_D$ are hydrogen-bonded, as indicated by the presence of a proton hyperfine interaction that is sensitive to $^2H/^{1}H$ exchange [28,80–82,92,112], by the value of the $g_S$ component of the anisotropic $g$-tensor [113], and by an FTIR $^2H/^{1}H$ isotope sensitive v(CO) vibrational mode (1497–1512 cm$^{-1}$) [94,114].

Early sequence comparisons [10] and modeling of PSII [65,115] suggested that a pair of homologous histidines D1-His190 and D2-His189 (Synechocystis numbering) might be in hydrogen-bonded contact with the redox-active tyrosines, $Y^*_Z$ and $Y^*_D$, respectively. It has now been clearly shown that D2-His189 acts as the direct proton acceptor to $Y_D$ (D2-Tyr160) [112–114,116,117]. Site-directed replacement of this histidine results in the loss of a hydrogen bond to the phenolic oxygen of $Y^*_D$ as detected by cw $^1H$-ENDOR [112]. This observation implied that in WT this histidine is hydrogen-bonded to $Y^*_D$. Pulsed $^{15}N$-ENDOR measurements following global $^{15}N$ labeling of PSII reaction centers showed two nitrogens to be magnetically coupled to $Y^*_D$ [117]. One of these ($A_{iso} = 0.8$ MHz) bears unpaired spin density (0.8%) of the tyrosyl radical. ESE-ENDOR of site-directed mutant, D2-His189Gln, and of $^{[15}N$]histidine labeled WT PSII core complexes showed this nitrogen to be the tau imidazole nitrogen of D2-His189 [117]. The strength of the isotropic coupling is consistent with this nitrogen serving as the source of the hydrogen bond detected in the cw ENDOR experiment. ESE-ENDOR of PSII core complexes labeled with $^{15}N$-labeled tyrosine [25] showed the second nitrogen ($A_{iso} = 0.66$ MHz, $A_{dip} = 0.09$ MHz) to be that of the tyrosyl peptide bond.

While ENDOR detects only the oxidized state of $Y_D$, FTIR measurements have shown that protonated reduced $Y_D$ is hydrogen-bonded to a neutral imidazole of histidine [114]. The site-directed mutant D2-His189Gln was used to show that the histidine in question is the one located at D2-189. If the imidazole were neutral when $Y_D$ was in the reduced state, then its protonation upon $Y_D$ oxidation would produce the imidazolium unless the pi proton of D2-His189 were in turn handed off to a secondary proton acceptor. Predictions, based on hybrid density functional calculations by O’Malley [118], of phenolic hydrogen bond distances, and relative spin densities on the phenolic oxygen, the C4 ring carbon and the hydrogen-bonded imidazole nitrogen (Fig. 3), do not provide a definitive choice between the neutral imidazole and the imidazolium. The parameters lie somewhere between the two extremes, but closer to the neutral imidazole. This would imply that there is an additional residue(s) in hydrogen-bonded contact with the pi imidazole nitrogen of D2-His189 that can
accept the pi proton upon protonation of the tau nitrogen.

Twenty-four hour incubation of PSII core complexes in D$_2$O at 4°C results in nearly 100% exchange of the Y$_D$ phenolic proton. Time domain ESEEM spectra of Y$_D$ following such exchange are best simulated by two approximately equivalent strength hydrogen (deuteron) bonds in Synechocystis [92]. Spinach Y$_Z$ is, however, best simulated by a single hydrogen bond [92]. As D2-His189 is the source of one of these in Synechocystis, what then provides the other? It has been proposed, based on modeling studies [66], that D2-Gln164 might be in hydrogen-bonded contact to D1-His190, the electron spin density distribution on this radical is practically identical to that of Y$_D$ [79].

Y$_D$ [25]. There remains, however, a puzzle as cw ENDOR [112], high field EPR [113] and FTIR [94] indicate the complete loss of the $^2$H/$^1$H exchangeable hydrogen-bonded proton following replacement of D2-His189 by Gln. If there were indeed two hydrogen bonds to Y$_D$, then both would have to be lost upon this single mutation to explain all of the above observations.

The relation between Y$_Z$ and its proton acceptor is considerably more complex than in the case of Y$_D$, despite the fact that D1 contains D1-His190, the homologue to D2-His189. Most of the studies on coupled proton and electron transfer associated with Y$_Z$ oxidation and reduction have been carried out on Mn-depleted PSII core complexes because of the much greater ease of measuring Y$_Z$ both kinetically and spectroscopically. The spectroscopic observations described below were obtained on such material unless indicated otherwise. High field, 245 GHz EPR, shows the $g_x$ component of the anisotropic $g$-tensor for Y$_Z$ (2.00750) to be considerably broadened (reflecting disorder), but close to that of WT Y$_D$ (2.00740) and far from that of the weakly or non-hydrogen-bonded D2-His189Gln (2.00832) [113]. By way of reference, the non-hydrogen-bonded tyrosyl radical of Escherichia coli ribonucleotide reductase shows a $g_x$ value of 2.00868 [113]. $^2$H ESE-ENDOR shows a deuterium dipolar hyperfine coupling of 470 kHz for Y$_Z$, similar to Y$_D$ and consistent with a strong hydrogen bond [80]. In the case of Y$_Z$, however, there is no well-resolved structure coming from the $I=1$ deuterium nuclear quadrupole interaction, indicating a disorder in the hydrogen bonding. A similar observation of disorder in Y$_Z$ hydrogen bonding is obtained using ESEEM [79] and cw ENDOR [28]. FTIR spectra reflect the strength of the hydrogen bond in the v(CO) mode of Y$_Z$ at 1512 cm$^{-1}$ as opposed to 1503 cm$^{-1}$ for Y$_D$ in WT and 1497 cm$^{-1}$ in the non-hydrogen bonding D2-His189Gln mutant [94] and 1498 cm$^{-1}$ for the non-hydrogen-bonded tyrosyl radical of E. coli ribonucleotide reductase [119]. The disorder in hydrogen bonding to Y$_Z$ is probably related to the greater conformational disorder observed in ESEEM spectra for Y$_Z$ compared to Y$_D$. This latter conclusion is based upon the greater variation in the dihedral bond angle between the tyrosine ring normal and one of the $\beta$-methylene C–H bonds, indicating a
greater flexibility in the orientation of the phenolic ring [79,120].

$^{15}$N ESE-ENDOR experiments aimed at detecting nitrogen coupling to Y$_Z^*$ show $^{15}$N hyperfine features that are analogous to those attributed to the peptide nitrogen of Y$_D^*$ [25]. In contrast, however, no strong isotropically coupled nitrogens are detected for Y$_Z^*$ upon either global $^{15}$N or $[^{15}$N]histidine labeling [25]. This observation would imply that Y$_Z$ and D1-His190 do not form a direct proton donor–acceptor hydrogen-bonded pair.

Several observations, however, argue that these two residues are nonetheless functionally coupled. These are: (1) site-directed mutations that replace His at D1-190 all result in a dramatic slowing of Y$_Z$ oxidation by P680$^+$ by as much as 1000-fold at pH = 9.0 [4,56–58,121–123], consistent with the loss of the preferred proton acceptor. This slowing can be partially reversed by the addition of exogenous bases (e.g. imidazole, ethanolamine) [58]; (2) FTIR spectra of Y$_Z^*/Y_Z$ [94], Y$_D^*/Y_D$ [114] and of model tyrosine with methyl imidazole as the hydrogen bond acceptor [114] all show features (a weak $\nu'_{\alpha}(CO)$ at 1271 cm$^{-1}$ and a strong $\delta(COH)$ at 1250–1255 cm$^{-1}$) that are consistent with Y$_Z$ hydrogen-bonded to a histidine side chain, presumably D1-His190; (3) FTIR difference spectra for both Y$_Z^*/Y_Z$ [94] and Y$_D^*/Y_D$ [114] show the loss upon Y$_Z$ (Y$_D$) oxidation of phenolic $\delta(COH)$ bending modes at 1238–1255 cm$^{-1}$ of what are likely C–N stretch modes of His at 1107 and 1095 cm$^{-1}$, respectively. These features also show a similar pH dependence (Catherine Berthomieu, personal communication) reflecting a structural interaction between Y$_Z$ and a His that is most likely associated with a proton transfer upon Y$_Z$ oxidation. The results are interpreted as a disappearance of this interaction upon formation of the tyrosinate with a $pK_a$ of 8–8.3 (but see [57]).

These seemingly contradictory observations between the $^{15}$N ESE-ENDOR on the one hand and the kinetic and FTIR data on the other can be reconciled as follows. Y$_Z$ and D1-His190 could interact intermittently, in a gated fashion, with the formation of a direct or indirect hydrogen-bonded link between them, involving water, amino acid side chains or both, the connectivity of which would determine the rate of proton transfer. Either, $[^{15}$N]His coupling to Y$_Z^*$ exists in too small a fraction of centers to be detectable by ESE-ENDOR or, because of the indirect nature of the link, no coupling would be observed as there would be no significant spin density on the imidazole tau nitrogen of D1-His190. It is also possible, given the disorder of the hydrogen-bonded interactions to Y$_Z^*$ and the low temperature (15 K) at which the ESE-ENDOR is conducted, that the linked state might be frozen out under these experimental conditions. This was not the case for Y$_D^*$, however, where the ESE-ENDOR spectra were measured at 11 K [117].

3.4. Kinetics of proton and electron transfer

Electron transfer, at room temperature, from Y$_Z$ to P680$^+$ in Mn-depleted PSII core complexes shows multiple kinetic components with deuterium isotope effects ranging from 1.7 to 3.6, depending on the kinetic resolution of the data and the way in which the data are deconvoluted [57,91,92]. In addition, there is a marked pH dependence for the rate of electron transfer from Y$_Z$ to P680$^+$, with $pK_a$ values for this process ranging from 7.0 to 8.3 depending on the material used, the presence or not of Ca$^{2+}$ and the manner in which the data are deconvoluted. As the rate increases with pH, from tens of microseconds at acidic pH to microseconds or submicrosecond at alkaline pH (as originally observed by Mathis and coworkers [124,125]), the deuterium isotope effect decreases. To zero at pH 9.5, according to Ahlbrink et al. [91] and Diner et al. [92]. To 1.7 according to Hays et al. [57]. While the detailed mechanisms differ, all authors agree that there is a decreased dependence of the overall P680$^+$ reduction rate on proton transfer as the pH becomes increasingly alkaline.

Were Y$_Z$ to be in direct hydrogen-bonded contact with D1-His190, then considering the substantial difference in $pK_a$ values ($\sim$4), we can consider the transition state barrier to be close to the thermodynamic energy difference between the initial (Tyr$_{OH}^–$ N-His) and final (Tyr$^\circ$–HN-His$^+$) states. The concentration ratio of the transition state to the ground state is the ratio of their acidity constants or $10^{\delta pK_a}$, where $\delta pK_a = pK_a[acceptor] – pK_a[donor]$ and the rate of decay of the transition state is $6.2 \times 10^{12}$ s$^{-1}$ [126]. If the rate were limited by the transfer of the phenolic proton from Y$_Z$ to an imidazole nitro-
gen of His190, then the electron transfer would be expected to occur at a rate that approximates $6.2 \times 10^{12} \text{ s}^{-1} \times 10^{\Delta pK_a}$, or $6.2 \times 10^{8} \text{ s}^{-1}$ [92]. As this rate is far faster than the rate observed for P680$^+$ reduction in Mn-depleted centers ($= 10^2 \text{ s}^{-1}$), the electron transfer should not be limited by proton transfer; however, the observations of the pH dependence of the deuteron isotope effect and of the rate of electron transfer imply that it is. In the gated model proposed above, the making and breaking of hydrogen bonds to allow direct or indirect hydrogen-bonded proton transfer between YZ and D1-His190 would be responsible for the deuteron isotope effect. Ultimately, once the pH became sufficiently alkaline such that YZ was in the tyrosinate form, there should no longer be a deuteron isotope effect. The deprotonation would no longer be rate limiting. There is disagreement as to what the $pK_a$ values measured for the deuteron isotope effect actually mean. The $pK_a$ of 7.0–8.3, mentioned above for the rate of YZ to P680$^+$ electron transfer, has been proposed by Ahlbrinck et al. [91] and Diner et al. [92], based on a similar $pK_a$ for the deuteron isotope effect and for the pH dependence of the rate of YZ oxidation, to come from the tyrosinate/tyrosine couple. The argument of the latter authors is further supported by oxidized minus reduced difference spectra. The UV Y$_Z^+$$-$YZ difference spectra measured at pH 6.1 and 9 are different, the former consistent with Y$_Z^+$$-$YZ and the latter with Y$_Z^+$$-$Y$_Z^-$. Likewise, FTIR difference spectra of the same PSII core complexes show a disappearance with an apparent $pK_a$ of 8–9 of the $\delta$(COH) bending mode of tyrosine at 1238–1255 cm$^{-1}$ and a signal at 1107 cm$^{-1}$, likely $\nu$(C–N) of imidazole (Catherine Berthomieu, personal communication). However, Hays et al. [57], who still observe a residual deuteron isotope effect ($k_{\delta}/k_{\gamma} = 1.7$) up to pH 10, attribute this $pK_a$ (7.5 in their hands) to the $pK_a$ of the tau N–H of the D1-His190. Below this $pK_a$, the histidine would need to deprotonate to allow proton transfer. These authors further propose that in WT, as in the D1-190 mutants lacking histidine, the $pK_a$ of the tyrosinate/tyrosine couple is on the order of 10.3. Mamedov et al. [127] have proposed a $pK_a$ of 8.1 for the same couple in the D1-His190 mutants, based on measurements of the pH dependence of Q$_A^+$ donor side charge recombination. This estimate may, however, be somewhat underestimated, as the rate of charge recombination does not directly measure $k_{\delta}$, the rate of YZ to P680$^+$ electron transfer, and would tend to become insensitive to $k_{\delta}$ once this rate became substantially faster than the intrinsic P680$^+$Q$_A^+$ charge recombination rate of 0.7 [9] to 5 ms$^{-1}$ [128].

In the presence of the intact Mn cluster pH $\cong$ 6, the electron transfer from YZ to P680$^+$ is much faster, occurring with the major kinetic components showing half times of 20–40 ns in the S0 and S1 states and 50–250 ns in the S2 and S3 states [129,161]. These components show very weak deuteron kinetic isotope effects [86,87], though more substantial isotope effects are observed on the microsecond time scale [87]. It has been proposed [130,131] that YZ may even be deprotonated (tyrosinate) to explain the high rate and lack of an isotope effect, though this proposal is in conflict with the observation of a $\delta$(COH) bending mode at 1252–1255 cm$^{-1}$ attributed to YZ at pH 6.0 in Mn-intact PSII [90,94]. It has also been proposed that YZ and D1-His190 might be in direct hydrogen-bonded contact in intact centers, with proton transfer occurring on the nanosecond time scale and where the electron transfer would no longer be proton-limited [132]. While this is not easy to determine in the absence of an X-ray crystal structure, measurements of Y$_Z^+$$-$^{15}N coupling in globally labeled acetate-treated [133] spinach samples in the S2 Y$_Z^+$ state did not show a signal that resembled the isotropic 0.8 MHz hyperfine coupling observed for Y$_Z$ [25]. This observation implies that direct hydrogen bonding to D1-His190 does not occur. However, there is the underlying assumption that acetate treatment would not perturb the YZ-His190 hydrogen-bonded interaction. It is clear from FTIR [94] that under conditions of Ca$^{2+}$ depletion [134], which like acetate treatment [133] allows generation of an S2Y$_Z^+$ state, there are vibrational modes of His that remain coupled to the oxidation of YZ. More likely, the hydrogen-bonded interaction between YZ and D2-His190 remains indirect but more ordered than when the Mn cluster is removed. While likely slower than direct hydrogen bonding, proton transfer in an ordered network would only need to be fast enough so as to be not rate limiting for electron transfer, to explain the absence of a deuteron isotope effect for the submicrosecond components of P680$^+$ reduction [87].
3.5. Fate of the $Y_Z$ phenolic proton

The question then arises as to what happens to the proton following oxidation of the tyrosines. We know that there is a dramatic difference in the $^2H/^{1}H$ exchange rates of the phenolic proton between $Y_D$ and $Y_Z$, showing a $t_{1/2}$ of about $9$ h [81,112] and $<2$ min [91,92,135,136], respectively. This slow access to solvent exchange for $Y_D$ is consistent with its location far from the lumenal surface bulk solvent interface 20 and 28 Å [137] and 26 and 40 Å [138] in the absence and presence of the 17, 23 and 33 kDa extrinsic polypeptides, respectively. In the case of $Y_D$, which is buried within the reaction center with slow access to bulk solvent, the released phenolic proton most likely remains locally bound. A comparison of the electronic structure (Fig. 3) with predictions made from hybrid density functional calculations mentioned above [118] would be consistent with the proton remaining at least partially localized on D2-His189 to form the imidazolium and in part shared with a nearby residue or residues.

In the hydrogen atom or electron plus proton abstraction model of water oxidation in its simplest form, the proton departing from $Y_Z$ would be transferred into the lumen leaving $Y_Z^*$ free to recoup a proton from a water bound to the Mn cluster. There are a number of arguments that have been put forward, that imply that the phenolic proton of $Y_Z$ may not be free to leave the reaction center during the lifetime of the radical. A chlorophyll band shift to the red of $\sim433$ nm has been observed to be associated with $Y_Z^*$ oxidation and the $S_1\rightarrow S_2$ state transition of the Mn cluster, if purely electrochromic, should have the same sign of the band shift as $Y_Z$ and the Mn cluster are thought to be in close proximity. Both show very similar red shifts in the Soret region with an inflection at $\sim433$ nm [139,140,145]; however, in contrast, in the $Q_y$ region of the chlorophyll a spectrum, the first shows a red shift and the second a blue shift [146]. The contrasting behavior of these signals between the Soret and the $Q_y$ region is surprising, but has been interpreted in the $Q_y$ region as reflecting differences in charge localization between $Y_Z^*-Y_Z$ and $S_2-S_1$ [146].

We have recently shown (Diner et al., in preparation) that site-directed mutations at D1-198, replacing the WT histidine with other residues, displace to the blue the Soret absorbance spectrum of the $P_A$ chlorophyll coordinated by this residue. This displacement of the absorbance maximum, as observed in the $P680^+-P680$ difference spectra, has allowed us to show that the cationic charge of $P680^+$ at room temperature is primarily localized on this chlorophyll. The chlorophyll band shift that accompanies the oxidation of $Y_Z$ has an inflection point at $433$ nm that coincides with the absorbance maximum of the $P_A$ chlorophyll. The inflection point is also displaced to the blue by the mutations at D1-198 to the same extent as observed for $P680^+-P680$. This observation indicates that the $P_A$ chlorophyll coordinated by D1-His198 is the primary sensor of the oxidation of $Y_Z$. The $Q_y$ transition moments of the $P_A$ and $P_B$ chlorophylls, coordinated by D1-His198 and D2-His197, respectively, are oriented parallel to the plane of the membrane [65,66,147,148]. The conclusion that $P_A$ is the spectroscopic probe for $Y_Z^*-Y_Z$ contrasts (see also [146]) with the theoretical arguments of Fajer and collaborators [149] wherein placing a positive charge near pyrrole ring I produces a red electrochromic shift in the chlorophyll $Q_y$ transition. Of the two special pair, $P_A$ and $P_B$, chlorophylls with their $Q_y$ transition moments aligned toward $Y_Z$ and $Y_D$, only the D2-His197 coordinated $P_B$ chlorophyll has the correct orientation to produce
an electrochromic red shift associated with YZ oxidation. The ability of Diner et al. (in preparation) to affix a spectroscopic marker to the P\textsubscript{A} chlorophyll coordinated by D1-His198 leaves little doubt that the chlorophyll coordinated by this ligand is the principal spectroscopic probe for Y\textsubscript{Z}\textsuperscript{2}\textsuperscript{−}–YZ. Consequently, these observations call into question either the proposed sign of the difference in dipole moments between the ground and excited states for P\textsubscript{A} [149] or the interpretation of the chlorophyll band shifts, associated with YZ oxidation, as being electrochromic in nature [91,142,143,146].

Other arguments in favor of proton retention are based upon measurements of proton release as a function of pH in O\textsubscript{2}-active and in Mn-depleted PSII core complexes. The extent of proton release associated with each of the S state advances has been found to be variable depending on the preparation, showing either substantial oscillations of period four or monotonous single proton release with each S state advance (for review see [150]). Furthermore, the rate of proton release slows with increasing pH in a manner that is consistent with the increased pK\textsubscript{a} of the groups showing proton release as the pH is increased and the increasing difference in pK\textsubscript{a} between the groups undergoing deprotonation and the pK\textsubscript{a} of the hydronium ion (−1.74) [151]. The rate of proton release slows by approximately one decade per pH unit between pH 5 and 7 in Mn-intact centers [91]. Between pH 6 and 8, the rate of proton release is similar for both Mn-intact and Mn-depleted centers. In contrast, under the same conditions, the rate of YZ oxidation increases with pH. In Mn-intact centers, the rate of proton release (hundreds of μs) is far slower than the rate of oxidation of YZ and occurs on the same time scale or slower even than the oxidation of the Mn cluster in the S0 and S1 states [87]. Rappaport and Lavergne [143] have observed in Mn-depleted centers that the pK\textsubscript{a} of a luminal surface protonatable group shifts from ≥9 to 6 upon oxidation of YZ. Practically no proton release occurred below pH 5.0. The pK\textsubscript{a} for the stoichiometry of proton release is, however, inconsistent with the deprotonation of tyrosine which, as mentioned above, has a pK\textsubscript{a} of ~2. This pK\textsubscript{a} is unlikely to be shifted by 8 pH units, or, if it were, it would not be of much use for redox-coupled deprotonation. These observations argue against the proton that first appears in solution as arising from the deprotonation of YZ, but instead favor its attribution to a Bohr proton. Only below pH 6 in Mn-depleted PSII cores do Ahlbrink and coworkers [91] see a rate of proton release that approximately matches that of YZ oxidation. This observation correlates with the loss in the same material of the Y\textsubscript{Z}\textsuperscript{2}\textsuperscript{−} associated electrochromic signal below pH 6.0. Rappaport and Lavergne [143], however, continue to see a fast phase in the relaxation of the electrochromic signal at pH 5.5 despite the loss in their hands of most of the proton release. Their interpretation is that there is a component of the electrochromic signal that is responsive to an electrostatic relaxation occurring in the submillisecond time range. There is also disagreement, on the extent of proton release in Mn-depleted centers at pH ≥6 among the different groups, which likely reflects differences in subunit composition between the PSII core preparations. These differences could affect the accessibility of YZ to the bulk medium and the nature of the surface protonatable groups that respond to YZ oxidation.

It has been argued [152] that there needs to be a sufficient stabilization of the released phenolic proton to render exothermic the oxidation of YZ by P680\textsuperscript{+}. A hydrogen-bonded acceptor chain consisting of Tyr-His-Glu\textsuperscript{−} is sufficient to do so. The authors do not go so far as to say that the proton needs to be released into the bulk solvent phase. Even if there were proton release accompanying both YZ and Y\textsubscript{D} oxidation, the fact that the reduction potential of Y\textsubscript{Z}/Y\textsubscript{D} is substantially lower than that of Y\textsubscript{Z}/Y\textsubscript{D} (see [153] and references therein) means that there are other ways to stabilize tyrosine oxidation outside of bulk solvent proton release. Furthermore, the ability to observe in Mn-intact thylakoid preparations no proton release at pH 5.5 for S1→S2 [154] and, on other steps, kinetics of proton release substantially slower than rates of Y\textsubscript{Z} oxidation that show deuterium isotope effects [87], would argue for a dissociation of tyrosine oxidation and proton release to the bulk phase. Indeed the chlorophyll band shift associated with the S1→S2 transition has been interpreted as having an electrochromic origin arising from a retained proton. It may be sufficient to delocalize the released proton without its being released to the lumen in order to benefit from the extraexothermicity associated with proton transfer from the Mn
cluster to YZ. Were this the case, then there would need to be a difference in pKa between the Mn cluster and the protonated base or a kinetic switch to assure that the proton delivered to YZ upon its reduction was from the cluster only. The observed mobility of YZ [79,98,120,142] could be consistent with such a switch. Alternatively, the extraxothermicity may not be required on every advance of the S states and the switch function or the location of the more acidic proton that reprotonates YZ could be variable and depend on the S state [98]. It is possible that the state of the Mn cluster could influence or control the pathway for YZ deprotonation, particularly as the Mn cluster is likely to be closer to the luminal surface of the membrane than YZ [155]. Were this the case, then the retention of a proton within the proton transfer pathway could raise the reduction potential of YZ/YZ++, allowing this oxidant to drive what might be more unfavorable oxidations in the S2 and S3 states. Such a model could also explain the lowered kzp equilibrium constant (YZP680+ = YZ(H+)+P680) [129,156]) and the slowed oxidation of YZ by P680+ in the S2 and S3 states relative to what is observed for S1 and S2 [129].

It is important to point out that where a water or hydroxo-proton dissociates from the Mn cluster upon manganese oxidation and YZ is reprotonated upon tyrosine reduction, it should not matter thermodynamically whether the proton that is ultimately released (assuming that it winds up in the same place) originated from YZ or from the Mn cluster. In the first case, the proton reprotonating YZ comes from the Mn cluster. In the second case, the reprotonating proton is the retained proton initially localized on YZ before its oxidation. In both cases, the initial and final states are the same. Consequently, in both cases, the driving force contributed by the difference in O–H bond dissociation energies between the Mn cluster and YZ is the same. The advantage to having YZ participating in the deprotonation of the Mn cluster, if not thermodynamic, might be principally a kinetic one, providing a lower barrier pathway for proton transfer from the Mn cluster.

As far as YZ oxidation is concerned, it is probably fair to say at this point that we do not really know where the YZ proton goes, except that D1-His190 is involved and that YZ and the Mn cluster are likely in hydrogen-bonded contact [90,111]. Further probing of the deprotonation pathway using new spectroscopic tools such as low frequency FTIR [157] and resonance Raman [158], accompanied by isotopic labeling and kinetic measurements of site-directed mutants affected in the immediate environment of the Mn cluster and YZ (e.g. D1-Glu189), are likely to provide new insights into the coupling of proton transfer and Mn and YZ oxidation/reduction.

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