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Biochimica et Biophysica Acta 1655 (2004) 222–230
BIOCHIMICA ET BIOPHYSICA ACTA
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Review

The stable tyrosyl radical in Photosystem II: why D?

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Received 25 June 2003; received in revised form 13 October 2003; accepted 13 October 2003

Abstract

Two redox-active tyrosines are present in Photosysytem II, the water-oxidizing enzyme. While the tyrosine that is kinetically competent in electron transfer, TyrZ, may also have a role in the enzyme mechanism, the second tyrosine, TyrD, has a stable radical and is not directly involved in the redox chemistry associated with enzyme function. Nevertheless, reasonable mechanistic roles for TyrD have been postulated that satisfy desires to rationalise the presence of this cofactor, or, in English, we think we know what it does. First, the TyrD radical acts an oxidant of the Mn cluster in the lowest state of the redox accumulation cycle (i.e., S_0), providing potential benefits in maintaining the cluster in the more stable higher valence states. This redox role may also be important during Mn assembly and indeed overreduced forms of the Mn cluster appear to be oxidised by $TyrD^{\cdot}$. Second, the proton generated by the TyrD radical is thought to remain in its vicinity having an electrostatic influence on the location and potential of the chlorophyll cation, P^{+}. This effect may be important for the kinetics of TyrZ oxidation and may provide a significant thermodynamic boost to the enzyme. In addition, through its electrostatic influence, $TyrD^{\cdot}(H^{+})$ may confine the highly oxidising cation P^{+} to the chlorophyll nearest to TyrZ, thereby accelerating TyrZ oxidation and restricting the potentially damaging redox chemistry to one side of the reaction centre: the disposable D1 side. This second role, evidence for which is beginning to emerge, constitutes a new role for a redox-active tyrosine in biology: as a positive charge generator in a hydrophobic environment. In this short review, we focus on work relevant to these two roles.

Keywords: Photosystem II; Tyrosine D; Redox reaction; Tyrosyl radical; G.T. Babcock

1. Introduction

The thesis work of G.T. Babcock and associated papers [1–5] established that the two unidentified species Z and D acted as electron donors in the water oxidising enzyme, Photosystem II (PSII), both giving rise to organic free radicals detectable by EPR. These two species have been the focus of much research since that time [reviews 6,7]. They have been identified [8–12], localised [8,9,13,14], and studied with very sophisticated spectroscopic methods so that much is known about their spin densities [15–18], their kinetics [1,2,19–21], their pH dependences [reviews 6,7,20–22], their orientation [23], their environments [24–28], etc. Their functions, however, remain incompletely understood. For TyrZ, it is generally agreed that it acts as a highly oxidising intermediate in electron transfer playing an important role in water oxidation [29,30]. As yet, however, its potential role as abstractor of protons from water [31–33] remains to be clearly demonstrated. TyrZ remains the focus of much attention and debate (see other articles in this volume). For the less glamorous sister, TyrD, most references are followed by the convenient but somewhat inaccurate phrase "with unknown function" or words to that effect. This is despite the fact that because of its long lifetime, the TyrD radical is much easier to study and has consequently been studied more extensively than the more elusive TyrZ radical. The aim of this short paper is to review current views of the role of TyrD in PSII.

2. Historical stuff

The situation, as deduced mainly through Babcock's early work, was as follows: Z and D had virtually identical spectroscopic characteristics and yet were kinetically very distinct (see Fig. 1), with the Z radical being short-lived and

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doi:10.1016/j.bbabio.2003.10.016

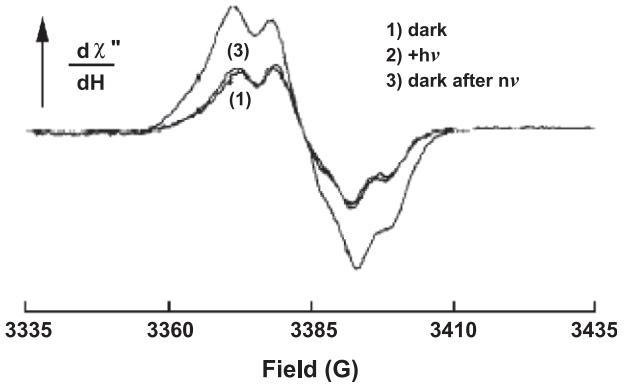

Fig. 1. Two radical species, one (D) stable in the dark, the other (Z) short-lived and visible under illumination in Mn-depleted PSII. The Z radical is almost identical to that from D and is superimposed on the D radical under illumination. This figure was taken from Ref. [71] and was one of the first reports of these signals without the contaminating chlorophyll radical from PS1.

the D radical being stable [1–3]. The explanation for this barely credible situation became evident when the symmetry of the purple bacterial reaction centre was discovered in the early X-ray crystallography studies (reviewed in Ref. [34]).

Indeed, perhaps the biggest surprise in that structural model was that the purple bacterial reaction centre was made up of two symmetrical subunits containing matching cofactors, with one side that was functional in charge separation and the other side nonfunctional. The two sides of the reaction centre had clearly evolved from an ancestor with two identical (functional) subunits [34]. At that time, it was already clear from spectroscopic studies that the purple bacterial reaction centre must have globally the same structure as PSII (e.g., Refs. [35,36]). The discovery of the heterodimeric nature of the purple bacterial reaction centre thus provided not only a model for the structure of PSII but also an explanation for anomalous properties of Z and D; that is, they were symmetrically identical "cofactors" located on two homologous reaction centre subunits in PSII (Fig. 2). This was demonstrated experimentally using site-directed mutagenesis [8–11].

3. The TyrD-less mutant: identity and location of the redox tyrosines

The site-directed mutagenesis work replaced Tyr-160 of the D2 subunit with a Phe. This eliminated the EPR signal from the D radical while the Z radical and enzyme activity were still present in the modified PSII [8,9]. This tyrosine had been targeted because this potentially redox-active amino acid had become the main suspect in the hunt for Z and D for a number of reasons. (1) Precedence for the occurrence of tyrosine radicals in enzymes had recently appeared in RNR [37]. (2) The alternative candidate, a semiquinone cation, was shot full of experimental holes, with extraction experiments, isotopic labelling experiments, spectroscopic measurements all coming out negative [12,29]. (3) The folding model for the PSII reaction centre based on the bacterial reaction centre provided no evidence of quinone binding sites but symmetrical tyrosines were conserved in all sequences of D1

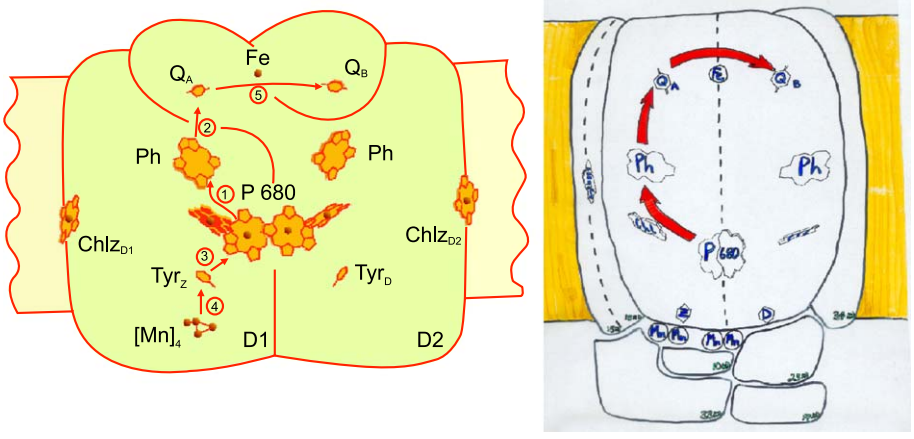

Fig. 2. A current and a vintage model of PSII showing the symmetrical position of TyrZ and TyrD (Z and D in the older model). The current model is based on that from the X-ray crystallography of PSII [66]. The 1985 model was based on first crystallographic model of the cofactors of the purple bacterial reaction centre [72] and while the author (AWR) has dabbled in models that are in retrospect less pleasing, this basic model has varied little since that time [73,74]. The model of the purple bacterial reaction centre protein had not yet been published and the focus was firmly on cofactors. Note that the core polypeptides were coyly unlabelled and the true role of the 34 kDa, a.k.a. the B-protein or D1, had not been realised. Other features of interest: the ambiguity in the chemical nature of Z and D, the asymmetry of the Mn ions, cyt b_{559} being on the wrong side (an arbitrary choice until recently). The extrinsic polypeptides are not too embarrassing except for the 10-kDa polypeptide (whatever happened to the 10 kDa?). Note also that the main difference in the cofactors between 1985 and 2003 is the separation between the two central chlorophylls but evidence for a monomeric structure and a surprising orientation for the triplet of P680 already existed and were being rationalised in the model based on the bacterial homology [35,73]. The older model is scanned from the original overhead transparency shown by AWR in an overview talk on oxygen evolution given at meeting in the USA in August 1985 organised by G.T. Babcock.

and D2 [38,34]. (4) A tyrosine was found to be situated between P and the cytochrome donor in *Rhodopseudomonas viridis* (reported by H. Michel at the Providence Photosynthesis Congress 1986; see Ref. [34]), and while in retrospect this is probably irrelevant to the situation in PSII, it was an important trigger in the hunt for symmetrical tyrosines in PSII [9]. The first two or three factors listed above were also relevant to the isotopic labelling study in Babcock's lab that first definitively identified the radicals as arising from tyrosine [12]. This work gave particular impetus to related site-directed mutagenesis project from that group [8].

These site-directed mutagenesis experiments [8,9] were important not only for identifying the tyrosines, both D directly and Z as a corollary (the identity of Z was later confirmed by mutagenesis [10,11]), and for establishing the validity of the D1/D2 folding model for PSII, but also for showing that TyrD was not essential for oxygen evolution in PSII. The question was thus raised: why does TyrD exist in all reaction centres studied?

From the evolutionary view point, the presence of redox-active tyrosines on both sides of the reaction centre indicated that the ancestral homodimer also contained the tyrosine and therefore that this ancestor could generate high oxidation potentials [39,40]. It has even been suggested that ancestral Mn binding sites could have existed on both sides of the reaction centre perhaps capable of oxidizing water [39,40]. Furthermore, the conservation of TyrD in all existing PSII species indicates that TyrD must play some specific role beneficial for PSII function. To understand that role, we first look to the redox reactions of the tyrosine.

4. TyrD as an electron donor

Early studies showed that although stable for days in the intact enzyme, the TyrD radical nevertheless decayed slowly in darkness and could be eliminated by the certain biochemical treatments such as dark incubation at high pH, the addition of some kinds of electron donors, etc. [1,22,41]. Subsequent illumination results in reappearance of TyrD and this process has thus been studied in some detail [1,20,21,22,41,42].

In the functional enzyme, the TyrD radical could be formed in the seconds time scale upon illumination [1]; this occurred at the expense of the charge equivalents stored on the charge accumulation system (i.e., the S_2 or S_3) (reaction: $TyrD(red) + S_2 \rightarrow TyrD(ox) + S_1$) [1,42]. The intrinsic electron donation rate of TyrD to P^+ in the functional enzyme is probably much slower than that of TyrZ, and TyrD oxidation presumably occurs though the electron transfer equilibria existing between the Mn cluster, TyrZ, P680, and TyrD [43]. Given that $TyrD^{\cdot}$ seems to have the lowest potential of these components, the equilibria are tipped towards its formation. And although its electron donation rate is slow, its potential and the equilibria in which it is involved result in its quantum yield of formation being high, at least at low light intensities. Under normal functional conditions then, any TyrD that is reduced (see below) will be oxidised upon the onset of illumination and it is clear that normal enzyme function occurs in the presence of the TyrD radical.

In PSII lacking Mn, TyrD oxidation occurs but its rate depends greatly on the pH [21]. At pH values above 7.7, the electron donation rate is very rapid ($t_{1/2}$ 200 ns in the majority of centres). It appears that this is at least as fast as TyrZ donation under the same conditions. Below pH 7.7, the donation rate is very slow with donation occurring in the millisecond time scale. This pH effect is explained in terms of the protonation state of the TyrD His-190 system [21].

From the folding model [8,10], computer modelling [44,45], site-directed mutagenesis, and spectroscopy [25,26,46], it is reasonably well established that $TyrD^{\cdot}$ is H-bonded to His-190. Babcock et al. [29] suggested that rapid Tyrosine D oxidation and reduction occurred with the H-bonded proton "rocking" between tyrosine and a base (see Fig. 3). The pH effect on TyrD can be explained in terms of the protonation of the proton acceptor at pH values lower than pH 7.7, thereby making Tyr oxidation more difficult [21]. The work with Mn-less PSII helps understand tyrosine redox chemistry [21] but may also be relevant to real life since the Mn-less state occurs when PSII reaction centres are made or repaired. The rapid donation seen at high pH may also be of physiological relevance since it is possible that assembly processes take place in a pH environment higher than that normally experienced by the functional enzyme. Indeed, during greening, assembly obviously must occur before water oxidation (and thus before acidification of the

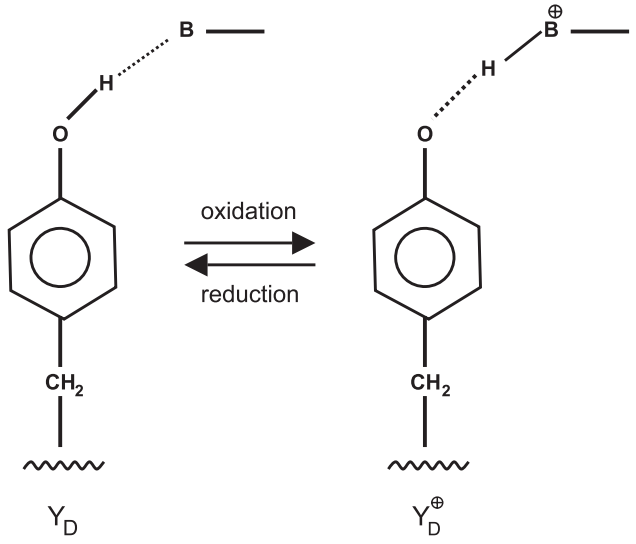

Fig. 3. The so-called "rocking proton" model for proton coupled electron transfer for tyrosine redox chemistry as presented by Babcock et al. [29].

lumen) and in other circumstances, assembly of the enzyme may occur in a location away from the occurrence of water oxidation [47]. It is thus possible that the local pH is higher in the areas where PSII is assembled and thus rapid TyrD donation could potentially occur during this process.

Even at low pH, TyrD oxidation occurs at low quantum yield in Mn-less reaction centres [20,21] and given its stability, it inevitably accumulates and is present in nearly all the centres after a short illumination. It seems likely then that assembly of the Mn cluster takes place in the presence of the TyrD radical [48,49]. It has been suggested that electron donation to P^+ early in photoactivation could play some kind of protective role [48], but we consider that a one-off, irreversible electron donation would be of limited protective value.

5. TyrD˙ as an oxidant: a role for TyrD˙ in Mn oxidation

From the early work on flash-induced O_2 evolution, it was established that dark-adaptation of the enzyme resulted in the majority of the centres accumulating in the stable state S_1 (i.e., with one charge equivalent stored), with a smaller fraction (25%) being in the S_0 state (i.e., no charge equivalents stored) [50]. Vermaas et al. [51] showed that long dark adaptation of PSII resulted in a conversion of the S_0 state into the S_1 state resulting in a 100% S_1 sample. The nature of the species oxidising S_0 to S_1 was not established. A clue to the nature of this reaction came from the observation that a fraction of S_2 state formed upon exciting the 100% S_1 sample decayed rapidly in the dark (1–2 s) and this fraction was about equal to the number of centres that had been converted from the S_0 state [51]. The rapid decay

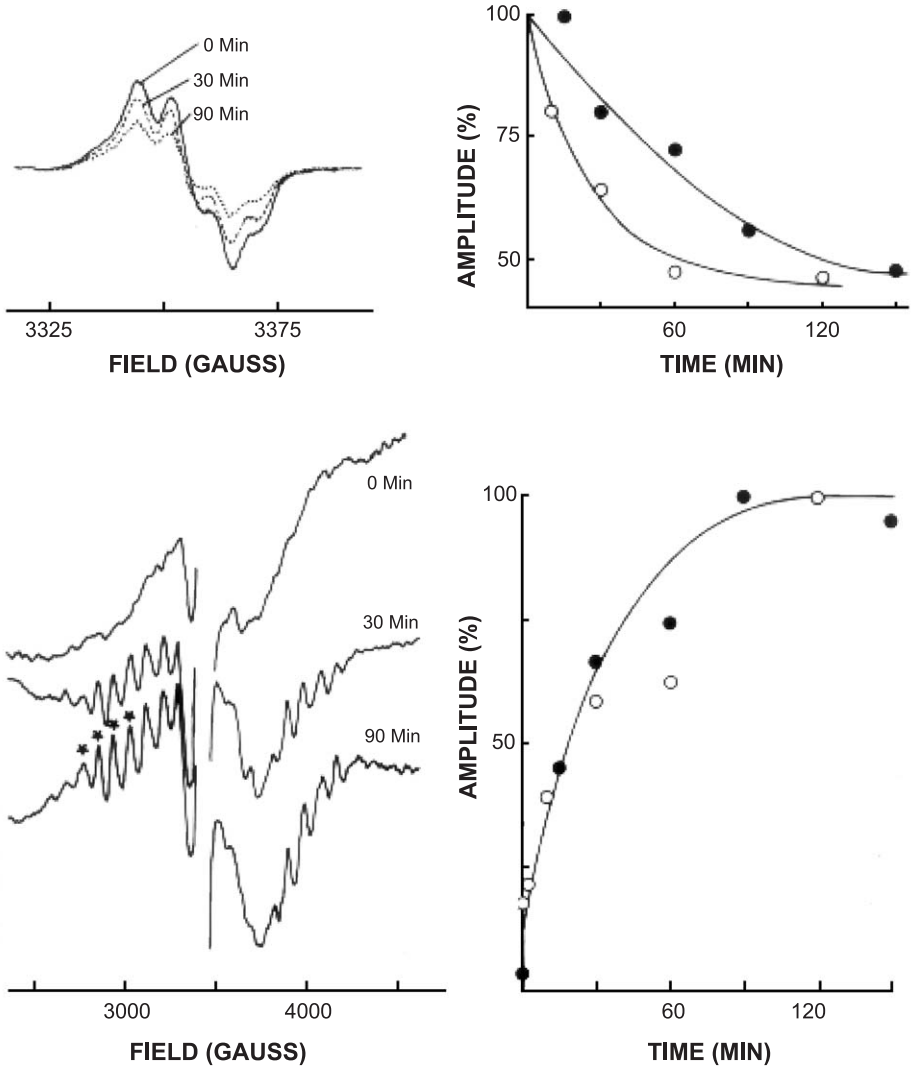

Fig. 4. Tyr D oxidises the Mn cluster in S_0 up to the S_1 redox state as shown by EPR. PSII was given three flashes to generate the S_0 state and the TyrD˙ was found to decay rapidly (A, B) relative to that in other S states. The open and closed symbols in B represent PSII membranes and thylakoids, respectively; the rate difference reflects their pH (pH 6 and 7.5, respectively). At the same time, S_1 is formed from S_0, as monitored by generation of the S_2 multiline upon illumination at 200 K (C, D). This figure is taken from Ref. [53].

of S_2 looked like the TyrD donation rate to S_2 as reported earlier. It seemed possible then that $TyrD^{\cdot}$ might be the oxidant of S_0: $TyrD^{\cdot}$ $S_0 \rightarrow$ TyrD S_1 and subsequently the reduced TyrD would be available for donating electrons to the Mn cluster in S_2: TyrD $S_2 \rightarrow TyrD^{\cdot}$ S_1. This model, which was first put forward as a hypothesis [52], was demonstrated experimentally using EPR [53] (Fig. 4).

The discovery that $TyrD^{\cdot}$ could oxidise the Mn cluster in the S_0 state provided a role for this radical [53]. It was suggested that the Mn cluster in S_0, the lowest redox state of the charge accumulation cycle, may be less stable than the other states, perhaps having MnII valence states, and that it would be beneficial to maintain the cluster at a higher valence level [53]. This was not unreasonable given that work with model complexes indicated that low valence Mn clusters were less stable. It was further suggested that if $TyrD^{\cdot}$ could oxidise S_0 to S_1; then, it seemed likely that it could oxidise the Mn in the lower valence states occurring during the process by which four Mn II ions are bound, oxidised, and assembled into the active Mn cluster, the so-called photoactivation [53]. Subsequently, Messinger and Renger [54] obtained evidence indicating that $TyrD^{\cdot}$ could oxidise some of the overreduced states of the Mn cluster obtained by chemical reductants. This could be taken as further support for the idea that Tyr could play a redox role in photoassembly of the Mn complex.

This work also placed the redox potential of the TyrD/$TyrD^{\cdot}(H^+)$ couple between those of the S_1/S_2 and S_0/S_1 [53] corresponding to the estimation made from direct oxidation of the radical with iridate (760 mV), a value that must be considered approximate because of the chemical modification of the EPR signal by the oxidant [55]. By studying the pH effects on TyrD reactions, Vass and Styring [56] made more specific estimates of the Em of the TyrD/$TyrD^{\cdot}$ couple. However, reliable absolute estimates of the redox potential of TyrD are still required.

6. Other TyrD radical reduction reactions

Under normal conditions, $TyrD^{\cdot}$ is well protected from reductive attack from the outside and it is stable for many hours [29]. When extrinsic polypeptides are removed, along with the Mn and Ca ions, then the $TyrD^{\cdot}$ seems to become more exposed to the outside and it seems to be able to decay more rapidly, particularly at high pH, presumably due to reductive attack (see Ref [22]).

There are reports of $TyrD^{\cdot}$ being involved in recombination reactions with electrons from Q_A^- under some conditions. This may be responsible for recombination luminescence and the so-called C-band of thermoluminescence has been attributed to this reaction under some conditions [57]. It is possible that the decay of $TyrD^{\cdot}$ over long periods of dark adaptation in the intact enzyme may be due in part to recombination reactions. When PSII is treated with hydrophilic reductants with low redox potentials, it is quite likely that the sequestered $TyrD^{\cdot}$ is reduced via Q_A reduction and a "charge recombination" reaction.

Strangely, storage of samples containing Q_A^- at 77 K leads to loss of both Q_A^- and the $TyrD^{\cdot}$ radical [58,59]; somehow, the electron manages to find its way to the high potential state in a very slow reaction. The relevance of this phenomenon is not clear but spectroscopists working with frozen material often encounter it.

Treatment with formate and phosphate seems to destabilise the radical at relatively low pH. This may be due to a perturbation of the environment affecting the protonation reaction associated with reduction of the $TyrD^{\cdot}$ [60]. At pH 6 in the presence of phosphate/formate, the yield of TyrD oxidation is $\sim$ 50% after one flash and the decay of $TyrD^{\cdot}$ has a $t_{1/2}$ of some minutes, properties very similar to those of TyrD at higher pH (8.5) without formate/phosphate. This indicates that the formate/phosphate treatment affects the protonation state of the TyrD His pair and/or it environment, mimicking aspects of higher pH.

7. $TyrD^{\cdot}$ in photoactivation

As we pointed out above, the production of the TyrD-less mutants not only identified the radical but also showed that it was unnecessary for oxygen evolution. It was observed, however, that cultures grew more slowly, and it was suggested [8,9] that this could be related to photoactivation of the Mn cluster in line with the earlier suggestion [53]. The experimental proof of a role for TyrD in photoactivation came recently: Ananyev et al. [49] looked directly at the function of TyrD during photoactivation by using wild-type and the TyrD-less mutant. They found that indeed the mutant assembled the Mn cluster at a slower rate. It was not clear whether this was due to a redox role of the $TyrD^{\cdot}$. The authors went on to observe that oxygen evolution was affected in the mutant with O_2 evolution being less efficient under illumination with short nonsaturating pulses and it was concluded that the oxidation of the Mn cluster/TyrZ by P^+ has a lower yield in the TyrD-less mutant. This effect was attributed to an electrostatic role for $TyrD^{\cdot}$ (however, see Ref. [61]).

8. $TyrD^{\cdot}(H^+)$: an electrostatic role

In the rocking model for tyrosine redox reactions [29], it is assumed that the base picks up the proton released upon tyrosine oxidation and returns the proton upon tyrosine reduction. In the simplest model, the charge then remains in the proximity of the radical. Now it is possible that the charge is translocated away from the TyrD radical itself [28], but modelling indicates that the TyrD is in a hydrophobic environment [44,45], so migration of the proton far from the radical is not expected. The potential electrostatic influence of $TyrD^{\cdot}$, or more precisely, its

associated proton, has been discussed [62–64]. There are some spectroscopic features of chlorophyll (probably P), which have been attributed to an electrostatic influence of TyrD redox state [60] and there is a report of a slow down in the kinetics of $P^+Q_A^-$ recombination in the TyrD-less mutant that may arise from such an elelctrostatic effect [62,65]. However, the electrostatic effect of TyrD has received more attention recently [21,40,49,65] since ambiguities over the structure and location of the P^+ cation were resolved [64,66].

The current view is that P^+ is located on the two chlorophylls that are counterparts to the special pair of bacteriochlorophylls in the purple bacterial reaction centre [64]. As has been clear for many years, these chlorophylls (designated P_{D1} and P_{D2}) do not constitute a "special pair" or a "dimer" (see, e.g., Ref. [67]): their excitonic coupling is much too small for that [68–70]. Instead, the cation may be thought of as being distributed between the pigments in a redox equilibrium. Diner et al. [64] showed that P_{D1} was most likely the main bearer of the cation under the conditions of their experiment. Faller et al. [21] showed that the oxidation of TyrD occurred in $t_{1/2} = 183$ ns, at pH values above 7.7; while this was something of a surprise, it also indicated that in those centres where this occurred, the cation must be localised on P_{D2}. This led to the idea that before TyrD oxidation, the equilibrium distribution of the cation between P_{D1} and P_{D2} may favour P_{D2} [21,65].

This idea was further supported by the anomalous observation that the decay of the P^+ cation was just as rapid with (a) TyrD alone (i.e., in the TyrZ less mutant), (b) TyrZ in the presence of $TyrD^{\cdot}$, or (c) both of tyrosines available as donors. Given a and b, the donation rate should have been faster in the presence of two equivalent donors. An explanation for this was that TyrD donation dominated when both TyrZ and TyrD were available to donate and that this was due to the P^+ cation being located mainly on P_{D2}. However, on subsequent excitation in the presence of $TyrD^{\cdot}(H^+)$, the cation was electrostatically pushed onto P_{D1} (Fig. 5), thereby accelerating electron donation from TyrZ [21].

As described in the previous section, Annayev et al. [49] also suggested that in the TyrD-less mutant, the absence of an electrostatic influence from $TyrD^{\cdot}(H^+)$ could have been responsible for the decrease in the efficiency of the assembly of the Mn cluster and O_2 evolution at low light intensity. Diner and Rappaport [65] estimated the electrostatic effect on the potential of a charge on or close to the TyrD–His pair would be 60–150 mV depending on the dielectric constant. This effect is certainly big enough to have an important effect on the equilibrium, which Diner et al. [64] estimated to favour P_{D1} by around 40 mV, an estimate made in the presence of $TyrD^{\cdot}$. In a TyrD-less PSII from *Chlamydomonas reinhardii*, Jeans et al. [61] found little effect on the flash dependence of P^+ reduction, indicating that the quantum yield of the S state cycle was little affected using flashing light (i.e., equivalent to low light intensities). However, they did see a slowing down of the slow phase

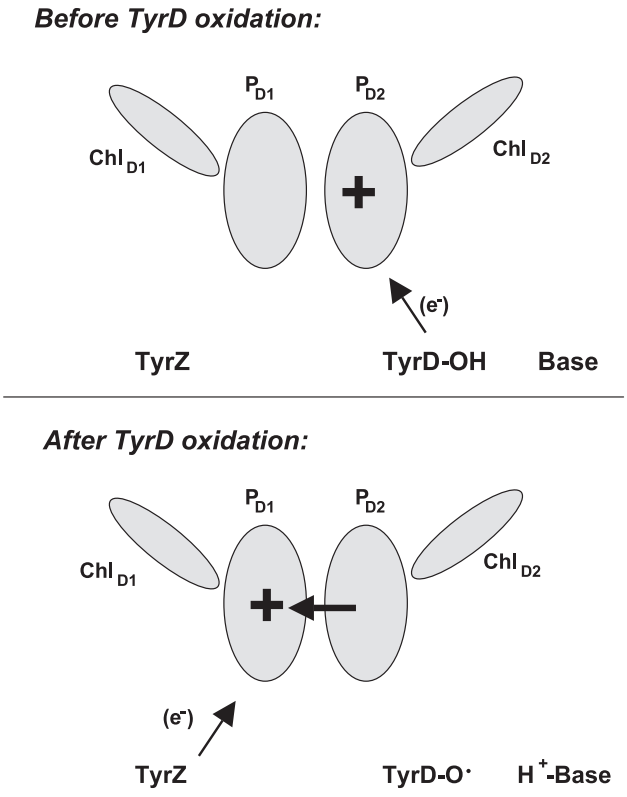

Fig. 5. An electrostatic role for $TyrD^{\cdot}$ in PSII. The location of the cation is suggested to be determined by in redox equilibrium between the central chlorophylls (mainly P_{D1} and P_{D2}). TyrD is oxidized by P_{D2}^+ and its proton remains in the vicinity. On the subsequent turnover, shown in the scheme, the proton has an electrostatic effect on the P^+ equilibrium pushing the cation towards P_{D1}, favouring the oxidation of TyrZ.

of P^+ reduction in the TyrD-less mutant. This could be related to the electrostatic effect of $TyrD^{\cdot}(H^+)$.

When we write $TyrD^{\cdot}(H^+)$, we do not specify the location of the proton, merely that it is in the environment of the tyrosyl radical. It could be located on the H-bonding partner of the tyrosine, His-190, or it could be another base perhaps one step further away (see Ref. [28]). The pH studies on TyrD oxidation kinetics and temperature dependence led to a model in which we proposed that the H-bonding partner to TyrD is protonated below pH 7.6. In the simplest model, this could be His-190 itself or alternatively a neighbouring base. In this model, at low pH, the redox state of the TyrD will not effect the charge state of its H-bonding partners while at higher pH, the oxidation of TyrD will result in the generation of a positive charge. In a TyrD-less mutant, it might then be predicted that at low pH, there would be little effect on the electrostatic environment while at higher values a marked effect would be expected.

The actual p*K* of the protonatable group may be expected to be affected by the removal of the TyrD. Assuming no effects from structural perturbations, the absence of the tyrosine from the H-bonded amino acid ensemble might be expected to lower the p*K* of the ionisable group. The role of TyrD as a charge generator may then be limited to those

conditions of pH where one of its H-bonding partners is unprotonated. When the enzyme is fully functional and the lumen is acidified, effects of the absence of TyrD will be less marked. Detrimental effects of the absence of TyrD may be more evident when photoactivation is taking place or before full acidification of the lumen.

Rutherford and Faller [40] pointed out that this role for TyrD as a generator of a positive charge in a hydrophobic environment was potentially important not only for accelerating the kinetics of TyrZ oxidation but also because after the first turnover it would localise the highly reactive chemistry almost exclusively on the D1 side of the reaction centre, thereby restricting any potential oxidative damage to the D1 protein. Since oxygenic photosynthetic species appear to have adopted a strategy of sacrificing and replacing D1 to overcome photodamage, then being able to limit this to D1 is a very significant economy measure and a good reason for maintaining TyrD throughout the course of evolution [40].

This idea predicts that the TyrD-less mutant would show enhanced photosensitivity. This is the case, but this cannot be considered as a validation since nearly every mutant of PSII shares this characteristic. However, it would be more telling if the TyrD-less mutant showed enhanced sensitivity of D2 compared to D1. This experiment has yet to be done.

The electrostatic effect of $TyrD^{\cdot}(H^{+})$ will not only influence the location of the cation but also raise the potential of P^{+} itself. The higher potential P^{+} in the presence of $TyrD^{\cdot}(H^{+})$ would of course have its own influence on kinetics and for that matter on photodamage. It is possible that having TyrD as a photoinducable stable charge generator provides an important thermodynamic boost to the system as a whole. Furthermore, there may be other advantages in being able to switch on the very highest potential states at the appropriate time during photoassembly of the Mn cluster.

This role for TyrD as a device for generating a positive charge within the hydrophobic core of a protein can be considered a new role for a redox tyrosine. The generation of a positive charge through the simple mutation of an amino acid to one with a cationic side chain would likely provide problems in the folding of this membrane protein (as pointed out to us by Peter Nixon), so the redox-active tyrosine is a nice solution to this difficulty [40].

9. More on the TyrD-less mutant

When the TyrD-less mutants were published showing that TyrD was not required for water oxidation, TyrD picked up the reputation of being an unnecessary cofactor, superfluous to PSII. The two roles for TyrD described above, redox and electrostatic, may be seen as merely improving the efficiency of the system rather than being essential for its existence. On the other hand, it seems that TyrD is so important that not a single species so far studied has encountered conditions during its evolution where TyrD has become dispensable. It thus seems possible that the roles of TyrD in improving the efficiency of PSII may be crucial for function.

Reinforcing this idea, it is of note that the TyrD-less mutants have been made and studied so far have been obtained in species that do not require PSII for growth and although the mutant grows photoautotrophically, it does so only very slowly [8,9,61]. The same modifications has recently been made in a species (*T. elongatus*) in which PSII is absolutely required for growth and great difficulty has been encountered in producing TyrD-less cells (M. Sugiura et al., in preparation). The cells are very sensitive to light and there appears to be a strong selection pressure to revert even under very low light intensity. We consider then that TyrD is very important for PSII. Of the two roles, the electrostatic role could be the more important but more experimentation is required before its true importance is determined.

10. Conclusion

So why D? TyrD can play a redox and very likely an electrostatic role. In its redox role, it can oxidise the Mn cluster when in low valence states and this maybe important for the stability and the photoassembly of the Mn cluster. In its less-demonstrated electrostatic role, it is assumed that the proton generated upon TyrD radical formation remains in its vicinity and tunes the redox potential and the location of the P^{+} cation. This redox tuning is of obvious importance for the efficiency of normal function but is also potentially important in restricting the highly oxidizing redox chemistry to the disposable, D1 side of the reaction centre, thus making the rapid D1 turnover a viable strategy against photodamage. The presence of TyrD in all species studied and the difficulties encountered in growing TyrD-less mutants in species that are obligatory phototrophs indicate that TyrD should not be considered as a mere evolutionary appendix providing occupational therapy for EPR spectroscopists but rather a cofactor with a sophisticated yet essential role in PSII.

Acknowledgements

We would like to thank our many collaborators who have worked with us on tyrosyl radical chemistry: G.T. Babcock, R. Ahlbrink, K. Brettel, R. Debus, Y. Deligiannakis, P. Dorlet, C. Goussias, O. Hansson, A.J. Hoff,. A. Ivancich, W. Junge, J. Lavergne, F. MacMillan, F. Rappaport, S. Styring, W. Vermaas, P. Setif, M. Sugiura, S. Un, and C. Zhang. We also acknowledge discussion with many of our colleagues particularly P. Nixon, J. Barber, and B.A. Diner. AWR gratefully acknowledges G.T. Babcock for 20 years of stimulating discussion on Z and D. . . and the rest.

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