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Review

Photosynthetic water oxidation: the role of tyrosine radicals

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Abstract

This mini-review outlines the involvement of the tyrosine electron carriers, Y_D and Y_Z, in the mechanism of electron transfer from water to P680. We discuss our data and put forward our ideas on the role of Y_D and Y_Z.

Keywords: Photosynthesis; Oxygen evolution; Photosystem II; Water oxidation; Tyrosine; Manganese

1. Introduction

Photosystem II (PSII) is the membrane–protein complex that catalyses electron transfer from water to plastoquinone (see Refs. [1,2] for reviews). As outlined elsewhere in this special issue, PSII was one of Jerry Babcock's main areas of research, and he was particularly associated with work on the tyrosine electron carriers. These carriers donate electrons to the oxidized form of the reaction centre chlorophyll *a*, P680. P680$^+$ extracts an electron from the Mn-containing water-oxidizing complex (WOC) via tyrosine Y_Z, which is tyrosine-161 (cyanobacterial numbering) on the polypeptide D1 (PsbA). Sequential electron extraction cycles the WOC through the S-state cycle (named S_0 to S_4). During the S_3 to S_0 transition, the S_4 transient state spontaneously decays to S_0, releasing one molecule of oxygen. In the dark, the S_2 and S_3 states decay within a few minutes to S_1, the dark stable state. On prolonged dark adaptation, the S_0 state is oxidized to S_1 by a second redox-active tyrosine, Y_D, tyrosine-160 on the D2 (PsbD) polypeptide. The kinetics of reduction of P680$^+$ by Y_Z and subsequently $Y_Z^{\bullet}$ (the neutral radical) by the WOC are dependent on the S-state. Four protons are released to the lumenal side of the thylakoid membrane during the S-state cycle. The stoichiometry of the proton release is dependent on pH and on the type of PSII preparation used. The proton release into the bulk phase takes place within tens of microseconds after the photo-excitation of the reaction centre. This means that proton release precedes the actual S-state transition, and is coupled to P680$^+$ reduction and Y_Z oxidation, rather than to Y_Z reduction and the oxidation of water itself. In this paper we will outline our current results involving Y_D and Y_Z and also our view of their role in the mechanism of water oxidation.

2. A brief history

Tyrosine radicals were first observed as "Signal II" in PSII using electron paramagnetic resonance spectrometry (EPR), although the origin of signal II was not identified for many years (see below). Signal II was also one of the first EPR signals to be observed in photosynthesis [3], but it was not until the early 1970s that real progress was made in further characterization of Signal II. Jerry Babcock was heavily involved with these studies, publishing several papers in this area. One of these papers describes Signal IIvf, now recognized as the transient signal from $Y_Z^{\bullet}$ in oxygen-evolving centres [4].

Until the late 1980s Signal II was thought to be a plastoquinone-like radical. Then isotopic labeling and mutagenesis experiments (see Refs. [1,2,5–7] for discussion), again heavily involving Jerry's group, showed that the components involved were two tyrosine residues, and that these were located on the core PSII polypeptides D1 (PsbA) and D2 (PsbD).

Abbreviations: PSII, photosystem II; EPR, electron paramagnetic resonance; LBHB, low barrier hydrogen bonds; WOC, water oxidising complex

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By the late 1980s, it was therefore known that Y_Z constituted the link between P680 and the WOC [6]. Y_Z was thought to increase the efficiency of charge separation by acting as a buffer between P680 and the WOC during the relatively slow, multiple electron chemistry of water oxidation. In contrast, the other tyrosine, Y_D, was stable in the oxidised state for hours, was able to slowly undergo redox reactions with the lower S states of the WOC in the dark, but was not involved in the main electron transfer pathway in PSII.

The location of Y_Z was further clarified in the early 1990s as a result of the identification of EPR signals from interactions between Y_Z and the Mn cluster of the WOC [1,2,8–11]. These interaction signals were originally observed in PSII where oxygen evolution was inhibited (see Refs. [1,2] for reviews). In recent years we have found that similar signals could be observed in oxygen-evolving PSII [12–14]. The nature of the interaction placed Y_Z close to the Mn cluster and resulted in new models for water oxidation, including the H-atom abstraction model from Jerry Babcock's group [7,15,16]. This model in particular has been used as the benchmark against which new data and results are examined. The early X-ray structure data have confirmed the position of Y_Z [17,18] and today Y_Z is thought to be involved in the mechanism of water oxidation itself.

3. EPR studies

Oxidation of tyrosine in aqueous solution at neutral pH releases the phenoxyl proton to produce the neutral tyrosine radical, $Y^{\bullet}$. The E_m of the neutral tyrosine radical is one of the least oxidising among those of the common amino acids. Neutral tyrosine radicals may therefore be formed in proteins because they will not easily oxidise their protein environments.

$Y_D^{\bullet}$ and $Y_Z^{\bullet}$ can be observed by EPR as neutral radicals of very similar line shape distinguishable by their microwave power saturation (relaxation) characteristics and decay kinetics. We have studied these tyrosine radicals by a variety of EPR techniques including ENDOR (Electron Nuclear Double Resonance) spectroscopy [19]. EPR spectra of $Y_D^{\bullet}$ and $Y_Z^{\bullet}$ are anisotropically broadened, with small g anisotropy and partially resolved hyperfine structure. The line shapes of these spectra are dominated by two large proton hyperfine couplings (to one β-proton and the protons at the 3 and 5 positions) and are relatively insensitive to the smaller couplings of the system. In oxygen-evolving samples the $Y_Z^{\bullet}$ radical is not observable below 230 K. Although this was thought to be due to the low temperature inhibition of Y_Z deprotonation, we now think this is due to an interaction with the Mn cluster of the WOC that broadens the $Y_Z^{\bullet}$ line shape at lower temperatures. When it interacts with the different S states (i.e. S_X $Y_Z^{\bullet}$), the $Y_Z^{\bullet}$ radical is broadened considerably [8,9,12,14], forming a "split signal" with features either side of the normal radical region of the EPR spectrum. It also shows much faster relaxation characteristics, and the line shape varies due to differences in the interacting Mn species found in different S states.

4. Y_D

Y_D does not have an essential role in electron flow in assembled PSII. From a study of equilibrium constants, the E_m of $Y_D/Y_D^{\bullet}$ has been estimated to be + 750 mV [20]. It has been suggested that Y_D is involved in the photoactivation of the WOC, which is the light-driven process of Mn^{2+} oxidation and complex assembly. It may also be required to oxidise Mn in S_0, maintaining the integrity of the Mn cluster [1,2,21,22]. The function of Y_D may be linked to the properties of neutral tyrosine radicals mentioned above, i.e. it might represent a way in which "stray" oxidising equivalents can be stored with minimal damage to the protein. Evidence indicates that D2 H190 forms a hydrogen bond to Y_D, which is located in a relatively hydrophobic pocket (see Refs. [1,21]). We have investigated the reduction kinetics of $P680^+$ during the S-state transitions. Y_D is 35 Å from Y_Z, and yet has a significant influence on proton-coupled electron transfer events in the vicinity of Y_Z [23]. This finding emphasises the delicacy of the proton balance that PSII has to achieve during the water splitting process.

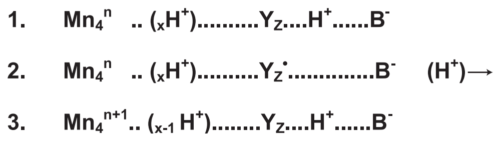
5. Y_Z

P680 photooxidation provides the driving force for water oxidation and most of the oxidising power must be used to oxidise water. This requires a short electron transfer chain with precise control of redox potential and reorganisation energy. A large fraction of $P680^+$ is reduced by Y_Z in nanoseconds (20–40 ns for S_0, S_1; 50–250 ns for S_3, S_4) but slower phases also occur [23]. This suggests a role for proton coupled electron transfer processes, in which proton migration is required to complete reduction of $P680^+$, resulting in a progressive change in the equilibrium between $Y_Z/Y_Z^{\bullet}$ and $P680/P680^+$ towards $Y_Z^{\bullet}$ formation. The E_m of the $Y_Z/Y_Z^{\bullet}$ couple is estimated to be + 950–1000 mV [20].

$Y_Z^{\bullet}$ reduction by the WOC is also S-state dependent taking 3–250 μs, 30–140 μs, 100–600 μs and ~ 1.5 ms, respectively, for the four S-state transitions between S_0 and S_4 [2,7]. It is likely that an interaction occurs between Y_Z and D1 H190 (see Ref. [21]). In many models, H190 is proposed as being the base that can accept the proton released from Y_Z on oxidation [2,7,15,16].

6. Y_Z and water oxidation

It has been proposed that Y_Z is involved in the water oxidation process itself, as a hydrogen atom abstractor, or in separate or coupled proton/electron transfer from the WOC



Scheme 1. Hydrogen atom abstraction by Y_Z. During oxidation a proton is lost from Y_Z. $Y_Z^\bullet$ then extracts an H-atom from the Mn complex, resetting the cycle.

[1,2,7,15,16,24–27]. A role in triggering the formation and release of oxygen has also been proposed, as the reduction of $Y_Z^\bullet$ during the S_3 to S_0 transition is the rate-limiting step in water oxidation [1,2,15,16,24–27]. These mechanisms recognise the importance of controlling the redox potentials of S-state intermediates. Some also suggest that control over the pK_a's of amino acids surrounding the WOC are important in order to maintain electroneutrality in the process [1,2,7,15,16]. This means that water oxidation is exquisitely sensitive to structural changes between preparations and treatments, which may alter water accessibility, hydrogen bonding, pK_a's, redox potentials, etc.

A leading model is the H-atom abstraction model [7,15,16] developed by Babcock and collaborators (outlined in Scheme 1). In this model and subsequent modified forms, $Y_Z^\bullet$ is an abstractor of hydrogen atoms from the WOC on each turnover. Charge distribution onto a hydrogen-bonding network leading to proton release is proposed to start at D1 H190, which accepts the proton from Y_Z.

An assumption made in some models such as the H-atom abstraction model is that Y_Z is initially protonated and loses this proton on oxidation. However Y_Z may be effectively deprotonated through strong hydrogen bonding or by acting as a ligand. There is no evidence that Y_Z is a ligand to calcium or manganese. Tunneling of hydrogen-bonded protons is thought to occur even at cryogenic temperatures [28–30] along low barrier hydrogen bonds (LBHB). This may allow proton movement in PSII from tyrosine Y_Z along a hydrogen bond at very low temperatures. Recently it was suggested that this could also occur with Y_D in Mn-depleted PSII at high pH allowing $Y_D^\bullet$ formation at cryogenic temperatures [22].

We have shown that the split EPR signals characteristic of $Y_Z^\bullet$ interacting with the Mn cluster can be detected in oxygen-evolving samples at cryogenic temperatures [12,14,31], suggesting that Y_Z in oxygen-evolving preparations can operate at 4 K, at least in some S states and some PSII reaction centres. The S_1 to S_2 turnover can also occur below 77 K [14]. S_2 $Y_Z^\bullet$ and S_1 $Y_Z^\bullet$ EPR signals can be obtained both in the forward direction using white light, and in the reverse direction by near-infrared illumination [12–14,32–34,37]. The near-infrared treatment is thought to directly excite an Mn^{3+} atom in the WOC and the excited state then oxidises Y_Z.

We therefore propose that Y_Z is involved in a permanent hydrogen bond(s) and that the proton moves between Y_Z redox states. Photooxidation of P680 starts the process, the oxidation of Y_Z requiring proton movement to complete the electron transfer in all PSII centres [23]. Changes in charge on the oxidised Y_Z site at physiological temperatures then cause proton release from the Mn complex by electrostatic effects. Y_Z would then be able to operate in some S states at temperatures where only short distance proton movements such as those along LBHB's can occur [29]. These properties explain the EPR [12,14,31] and electrochromic shift data (see Refs. [1,2,25]). The slower kinetics of Y_Z in inhibited or Mn-depleted samples could then be explained by the loss of the H-bond network and protonation of reduced Y_Z. This change in the environment of Y_Z, requiring a full deprotonation reaction on oxidation of Y_Z, would slow electron transfer.

In recent papers we have developed, together with Petrouleas and colleagues, a new model of S state turnover. We assume that water associated with the Mn cluster is in hydrogen-bonding contact with Y_Z. Electron spin echo envelope modulation (ESEEM) studies using D_2O [35,36] and $H_2^{17}O$ (Evans, M.C.W., Nugent, J.H.A. and Pace, R. unpublished data) show that water accesses the functional Mn complex and can be bound to it through the oxygen atom in the S_2 state. Recent experiments in our laboratory investigating the proposed S_1 $Y_Z^\bullet$ EPR signal generated by illumination at 4 K show strong proton modulation of the signal (Evans M.C.W. and Nugent J.H.A. unpublished data). Exchange of H_2O for D_2O partly replaces the proton modulation with ^2H modulation. The Larmor frequency modulations indicate the presence of exchangeable protons in close proximity to the redox centres giving rise to the signal. This may reflect either water bound to the Mn complex or that the hydrogen-bonding network involves either exchangeable protons or water. We proposed that an array comprising this water and adjacent water (or OH or O) ligands to Mn acts as an efficient proton translocation pathway [2,29].

We therefore favour a mechanism of S state turnover where tyrosine is hydrogen-bonded to a nearby base (Scheme 2). In the Y_Z reduced state the length of the hydrogen bond will have a normal distribution. At cryogenic temperatures the proton may be initially trapped either proximal or distal to Y_Z, depending on the environment and conditions near the WOC in each PSII reaction centre. Electron transfer from P680 to Y_Z is favoured when the hydrogen bond is long and the proton is at the maximum distance. In individual centres, fast (ns) electron transfer will occur in those centres where the length is maximal; it will show no deuterium isotope effect. These centres will also be able to oxidise Y_Z at cryogenic temperatures. In centres with the minimal bond length, electron transfer will be slowed until the proton moves away. Oxidation of P680 with the appearance of a positive charge will favour pushing the proton away, increasing the hydrogen bond length. These centres will show slower electron transfer rates with a deuterium isotope effect, and no electron transfer at 4 K. At room temperature, a distribution of rates and isotope effects is seen, reflecting the statistics of the hydrogen bond

1. $\mathrm{H^{+}_{n}.Mn_{4}^{n}}$ .... $(\mathrm{H^{+}})$ ..... $\mathrm{B^{-}}$..........$\mathrm{H^{+}}$.. $\mathrm{Y_{Z}}$ P680
2. $\mathrm{H^{+}_{n}.Mn_{4}^{n}}$ .... $(\mathrm{H^{+}})$ ..... $\mathrm{B^{-}}$........$\mathrm{H^{+}}$.... $\mathrm{Y_{Z}}$ ....$\mathrm{P680^{+}}$
3. $\mathrm{H^{+}_{n}.Mn_{4}^{n}}$ ....$(\mathrm{H^{+}})$.............. $\mathrm{B^{-}}$.....$\mathrm{H^{+}}$........ $\mathrm{Y_{Z}^{\bullet}}$ P680
4. $\leftarrow (\mathrm{H^{+}})\ \mathrm{.Mn_{4}^{n}}$ ....$(\mathrm{H^{+}})$.............. $\mathrm{B^{-}}$.....$\mathrm{H^{+}}$.......$\mathrm{Y_{Z}^{\bullet}}$ P680
5. $\mathrm{H^{+}_{n-1}.Mn_{4}^{n}}$ ...$(\mathrm{H^{+}})$................ $\mathrm{B^{-}}$.....$\mathrm{H^{+}}$.......$\mathrm{Y_{Z}^{\bullet}}$ P680
6. $\mathrm{H^{+}_{n-1}.Mn_{4}^{n+1}}$ .... $(\mathrm{H^{+}})$...... $\mathrm{B^{-}}$.........$\mathrm{H^{+}}$...$\mathrm{Y_{Z}}$ P680

Scheme 2. $\mathrm{Y_Z}$ as an electron-abstractor, proton repeller. $(\mathrm{H^+})$ = hydrogen bonding network. P680 is oxidised, displacing protons in the H-bond network and allowing $\mathrm{Y_Z}$ to complete the reduction of $\mathrm{P680^+}$. Oxidation of $\mathrm{Y_Z}$ causes further displacement in the H-bond network resulting in the loss of a proton from the Mn complex. Loss of the proton allows the Mn complex to reduce $\mathrm{Y_Z}$. The system is now reset for the next turnover.

length. Oxidation of $\mathrm{Y_Z}$ displaces the phenolic proton further towards the base. This in turn will influence the protons throughout the hydrogen bond network, resulting in ejection of a proton from the WOC. This reduces the redox potential of the Mn complex and activation barriers, allowing subsequent electron transfer from the Mn complex and re-reduction of $\mathrm{Y_Z}$. It is likely that the behaviour of the system is modified by changes on the Mn complex as the S state cycle progresses. A feature of the mechanism is a wave of proton movement following the changes in $\mathrm{p}K_\mathrm{a}$ induced by the initial oxidation of P680. The model (outlined in Scheme 2) proposes that electrostatic effects drive the process in an energetically finely tuned system. It accounts for the heterogeneity seen in PSII reactions. Slight modification of the WOC would cause significant changes to this system as seen experimentally. The strength of the hydrogen-bonding to $\mathrm{Y_Z}$ and the exact state of the hydrogen bond network will depend on the S state, the oxidation state of P680, the extent of deprotonation of the Mn cluster, and environmental factors etc., e.g. pH. $\mathrm{Y_Z}$ will be a good low-temperature donor in S states where the Mn cluster is deficient of protons, as observed for $\mathrm{S_1}$ [14,37]. $\mathrm{Y_Z^{\bullet}}$ is also readily formed by low temperature illumination of a proton-deficient S2′ state [12,32,33].

In summary, $\mathrm{Y_Z}$ acts in a relay, being rapidly oxidised by the reaction centre during which proton movement in a hydrogen bond network allows the oxidation of $\mathrm{Y_Z}$. This in turn lowers the redox potential in the Mn cluster by proton release, thus allowing $\mathrm{Y_Z}$ reduction by electron transfer from the Mn cluster. This role of $\mathrm{Y_Z}$ in the water-splitting process is described as an electron-abstractor, proton repeller mechanism (see Scheme 2 and Ref. [33]). Domino deprotonation through another network of proton acceptors leads to proton release into the lumen.

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