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Biochimica et Biophysica Acta 1411 (1999) 86–91

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

Minireview

Photosynthetic water oxidation: a simplex-scheme of its partial reactions

Michael Haumann, Wolfgang Junge *

Abteilung Biophysik, FB Biologie/Chemie, Universität Osnabrück, D-49069 Osnabrück, Germany

Received 4 February 1999; accepted 22 February 1999

Photosynthesis of green plants, algae and cyanobacteria is powered by sunlight. It uses water as ultimate donor of electrons for the reduction of CO_2 to yield carbohydrates. Photosystem II (PS II), a water–plastoquinone oxidoreductase, is the key enzyme of *oxygenic* photosynthesis. It is an integral protein of the thylakoid membrane. PS II is *functionally* bipartite, its photosynthetic reaction centre (RC II) which mediates the primary charge separation is linked to the oxygen evolving complex (OEC) [1,2] which reacts with bound water (see Fig. 1). The net reaction of water oxidation:

$$2H_2O - 4e^- \rightarrow O_2 + 4H^+ \quad (1)$$

operates at an extremely positive midpoint potential of +0.93 eV vs NHE (at the physiological pH of 5 in the lumen of thylakoids) [3]. The oxidising side of RC II, is even more electropositive, $\geq +1.1$ eV [4]. The large redox span of about 1.25 eV between the primary electron donor in RC II, P_{680}, and the primary acceptor (Q_A), a plastoquinone, is unparalleled by any other reaction centre [5]. In relation to the energy of a quantum of red light, 1.82 eV (wavelength 680 nm), the efficiency of PS II has been driven to the limits.

Many membrane proteins in photosynthesis and respiration are now fairly well characterised. PS II and, above all, its OEC-portion has remained a tough challenge. The gross structure of the central D1/D2 moiety of PS II is known at a resolution of 8 Å from electron microscopy/diffraction [6]. It bears similarities with the L/M subunits of the reaction centre of purple bacteria and with PS I [7,8]. It is obvious, however, that the intriguing function of the OEC relies on those features which are unique in PS II and have no counterpart in other photosynthetic reaction centres. Further structural analysis of PS II by X-ray crystallography is therefore urgently needed.

The smallest isolated unit which is capable of oxygen evolution contains the D1/D2-heterodimer, at least four further polypeptides, and a total of about 40 chlorophyll molecules [9] (see Fig. 1). D1/D2 hosts the photochemically active chlorophyll molecule(s). P_{680}, the primary electron donor, reduces Q_A, the first of two bound plastoquinone molecules via pheophytin *a*. P_{680}^+ is, in turn, reduced by the OEC. The latter comprises at least a tetra-atomic manganese cluster (Mn_4) and a redox active tyrosine residue (D1Tyr161), named Y_Z. It has been proposed that their midpoint potentials are adjusted by bound Cl^- and Ca^{2+} [10,11], ionic cofactors, which seem to be essential for water oxidation. Y_Z and some ligands to Mn_4 are located on the D1 subunit [12]. Apparently, the D1-protein carries major portions of both functions of PS II, the photochemical (RC II) and the catalytic one (OEC).

Abbreviations: ENDOR, electron nuclear double resonance; EPR, electron paramagnetic resonance; EXAFS, extended X-ray absorption fine structure; FTIR, Fourier transform infrared spectroscopy; MN_4, tetra-atomic manganese cluster; OEC, oxygen evolving complex; P_{680}, primary donor in PS II; PS II, Photosystem II; Q_A, bound plastoquinone in PS II; RC II, photosynthetic reaction centre of PS II; XANES, X-ray absorption near edge structure; Y_Z, tyrosine 161 on subunit D1 of PS II

* Corresponding author. Fax: +49-541-969-1221;
E-mail: junge@uos.de

PII: S0005-2728(99)00042-0

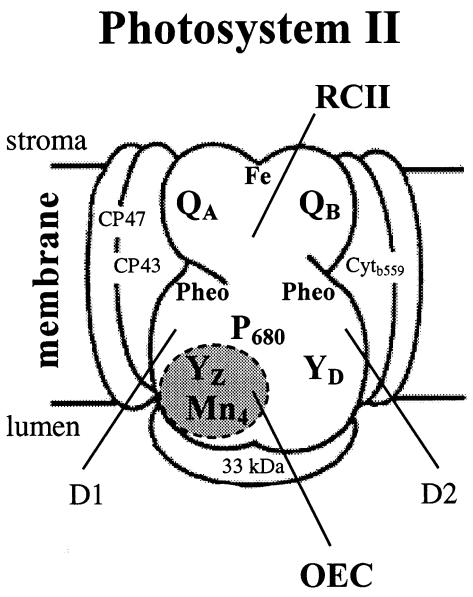

Fig. 1. Schematic drawing of the oxygen evolving core particle of Photosystem II (PS II) which incorporates a photochemical reaction centre (RC II) and the oxygen evolving complex (OEC). This complicated membrane protein is composed of at least seven different polypeptides. The central ones, D1 and D2, contain the redox cofactors of the photochemical activity, the chlorophyll *a*-entity (P_{680}), pheophytin *a* (Pheo) and plastoquinones (Q_A and Q_B). D1 contains the redox cofactors of the OEC, namely one tyrosine residue (Y_Z) and ligands to the tetra-manganese complex.

It has been a great advantage for studying the OEC that it is self-synchronised in the dark, so that it can be clocked through its partial reactions by short flashes of light. The OEC then steps through five increasingly oxidised states, S_0 to S_4. Only the last one liberates oxygen and is thereby reset to the state S_0, ready to undergo another reaction cycle. S_1 is the most stable state after dark adaptation. This model has been derived from the observation of (damped) oscillations of oxygen release with maxima after flashes no. 3, 7, 11, etc. [13]. Time-resolved mass spectrometry has revealed that both H_2O molecules yielding O_2 are rapidly exchangeable in S_2 and even in the penultimate state, S_3 [14]. This has raised the question whether (bound) water is only processed after the input of the fourth oxidising equivalent into the OEC.

P^+_{680}, which is formed in <1 ns [15], is reduced by Y_Z during each reaction step with half-rise times that range between 15 and 300 ns [16]. Y^{ox}_Z is, in turn, reduced in 30–300 μs in the first three steps and in $\cong 1$ ms in the final oxygen evolving one [17]. The first two oxidising equivalents (that are processed during $S_0 \Rightarrow S_1$ and $S_1 \Rightarrow S_2$) are stored on manganese itself as inferred from EPR- [18] and from X-ray spectroscopy (XANES) [19]. Both, S_0 and S_2 reveal a multiline EPR-signal that has been attributed to respective mixed-valence pairs, $Mn^{II}Mn^{III}$ in the former [20,21] and $Mn^{III}Mn^{IV}$ in the latter state ([18] and references therein). The chemical nature of the third equivalent (processed during $S_2 \Rightarrow S_3$) is under contention, due to conflicting results from optical and magnetic spectroscopy. Manganese [22,23], or one of its ligands, either histidine [10,24] or bound water [25,26], have been considered as candidates. During the fourth transition, $S_3 \Rightarrow S_4$, the oxidising equivalent is transiently stabilised as an oxidised tyrosine [27–29], Y^{ox}_Z. Its reduction *in milliseconds* coincides with the release of O_2. The coincidence has been one reason to postulate a role of Y^{ox}_Z in water oxidation [30,31] which is more active than just being an intermediate electron carrier between Mn_4 and P^+_{680}.

In artificial water oxidation the first univalent step to produce the hydroxyl radical requires a Gibbs energy of about 2.4 eV (pH 7, in aqueous medium), which is greater than the energy of one quantum of red light (1.8 eV). Renger [32] and Krishtalik [33] have argued that this severe thermodynamic and kinetic constraint might be overcome by coupling the first and the second step to yield a bound peroxide. The transient confinement of the intermediate reaction products in the enzyme pocket raises another complication. It diminishes the entropic stabilisation by dilution of the products. Accordingly, the so called 'configurational' potential (grossly speaking, the activation energy) of the overall reaction in the enzyme pocket can be much higher than the averaged +0.93 V (in water at pH 5). Krishtalik [33], who discussed this problem, has argued in favour of at least one hydrogen transfer step as a possible way to compensate the excess energy demand of the reaction. This has prompted the search for potential hydrogen acceptors in the OEC. Some authors, such as Britt and Babcock, have postulated that Y^{ox}_Z serves in this function [30,31]. Their view has been mainly based on the specific properties of Y^{ox}_Z, e.g., its hydrogen-bonding interactions and its rotational flexibility, as disclosed by EPR [34,35], ENDOR ([30]

and references therein, [36]) and FTIR [37]. For review see also [38]. One particular argument was related to the then assumed very short distance between manganese and Y_Z. An appropriate reaction mechanism has been derived [30,31], which is compatible with concepts of inorganic and theoretical chemistry [39,40] but relies on a set of experimentally testable assumptions. That it is still under contention, is owed to the following features. (1) The originally reported very small distance between manganese and Y_Z of only $\leq$0.45 nm [30] has been withdrawn. The same group now favours a distance of at least 0.9 nm [41] in agreement with other authors [42–44], whereas one group favours 1.5–2.0 nm [45]. (2) Most data on distances and on the hydrogen-bonded state of Y_Z were collected on samples that had been depleted of Mn_4 or Ca^{2+}. Such treatments deactivate oxygen evolution and affect the inner structure of the OEC [12,46] (see also [47] and references therein). Whether such results bear on the *intact* OEC is questionable. (3) It has been difficult to integrate into this concept data on proton transfer [48–55], electrogenic charge transfer [16,28,56–58], and proton-steered electron transfer [54,56,59,67,68] (see also review articles [60,61]). There is no evidence for the removal over a long distance of the phenolic proton, when Y_Z is oxidized by P_{680}^+ in intact PS II. Instead, the proton seems to stay in close vicinity to Y_Z^{ox} on a neighbouring base, both in oxygen evolving [28,29,59] and, under certain conditions, also in PS II with dysfunctional OEC [27,37,46,62,63,68]. This is not quite what one expects if Y_Z is charged up to act as a hydrogen acceptor (i.e., an acceptor of e^- *and* H^+) from water.

The above sketched discrepancies have prompted us to propose another reaction scheme for water oxidation which relies on a minimum set of active elements, is compatible with a wealth of data, and avoids *deus ex machina* elements. The scheme is illustrated in Fig. 2.

We assume that the OEC contains only three redox active cofactors, namely two out of the four manganese atoms plus one tyrosine, Y_Z. In the most reduced state of the OEC, S_0, the manganese atom in the distal position relative to Y_Z is in the Mn^{II}-state and the proximal one appears as Mn^{III}. One water molecule is assumed to be loosely bound to Mn^{III}. Y_Z is hydrogen-bonded to D1His190 [34,62,63] which results in UV/VIS-spectroscopical properties of $Y_Z^{\delta-}-H^{\delta+}$–His190 that are intermediate between the ones of free tyrosine (Y_ZH) and tyrosinate (Y_Z^-) [47]. The proton shift between Y_Z and His190 is a prerequisite for the ability of Y_Z to reduce P_{680}^+ *in nanoseconds* [32,47,63]. This holds for all transitions from S_0 to S_4. If this interaction is lost, as in Mn_4-depleted PS II at acid pH [46,47], in Ca^{2+}-depleted centres [64,65], or by certain point mutations at D1–His190 [62,63], the electron transfer to P_{680}^+ is slowed by orders of magnitude.

The first electron hole is rapidly moved on from $Y_Z^{\bullet}$ to the distal manganese. It is electrically compensated by the binding of a hydroxyl anion to yield the most symmetrical and stable state of the OEC, S_1. The abstraction of the second electron from Y_Z oxidises the distal manganese again, creating $Mn^{IV}Mn^{III}$. Our choice of the distal manganese as the target of the first two oxidation steps is tentative. It has been motivated by a distance of at least 0.9 nm between Y_Z and manganese in S_2 (see above) and by data on local electrochromism [58]. In the state S_2 there are two water molecules bound to the Mn_4-cluster, both exchangeable, one (here tentatively at the proximal Mn^{III}) is exchanged more rapidly (8 ms) than the other one (300 ms) [14].

The series of consecutive reactions and intermediate states (S_3', S_3'') after the third abstraction of an electron from the OEC in state S_2 deserve closer inspection. This reaction sequence is distinguished by three properties. (i) The electron transfer to $Y_Z^{\bullet}$ is slower than in the previous transitions and probably kinetically limited by a proton transfer (as apparent from a greater H/D-kinetic isotope effect, $\cong$2.5 instead of 1.1 and a larger activation energy ([29] and references therein). (ii) As soon as the electron hole has reached the OEC it is electrically compensated by proton release into the bulk (as evident from the absence of a long-lived electrochromic transient [10,16,28], and from a larger electrogenicity [57]). (iii) The final site of the oxidizing equivalent is a manganese ligand rather than manganese itself, debatable when considering XANES data alone [19,22], but it is clearly favoured by data on K_β-fluorescence (J. Messinger, personal communication)). Moreover, the fully expressed S_3-state may have some peroxide character [3,29].

At this point we refrained from invoking in this

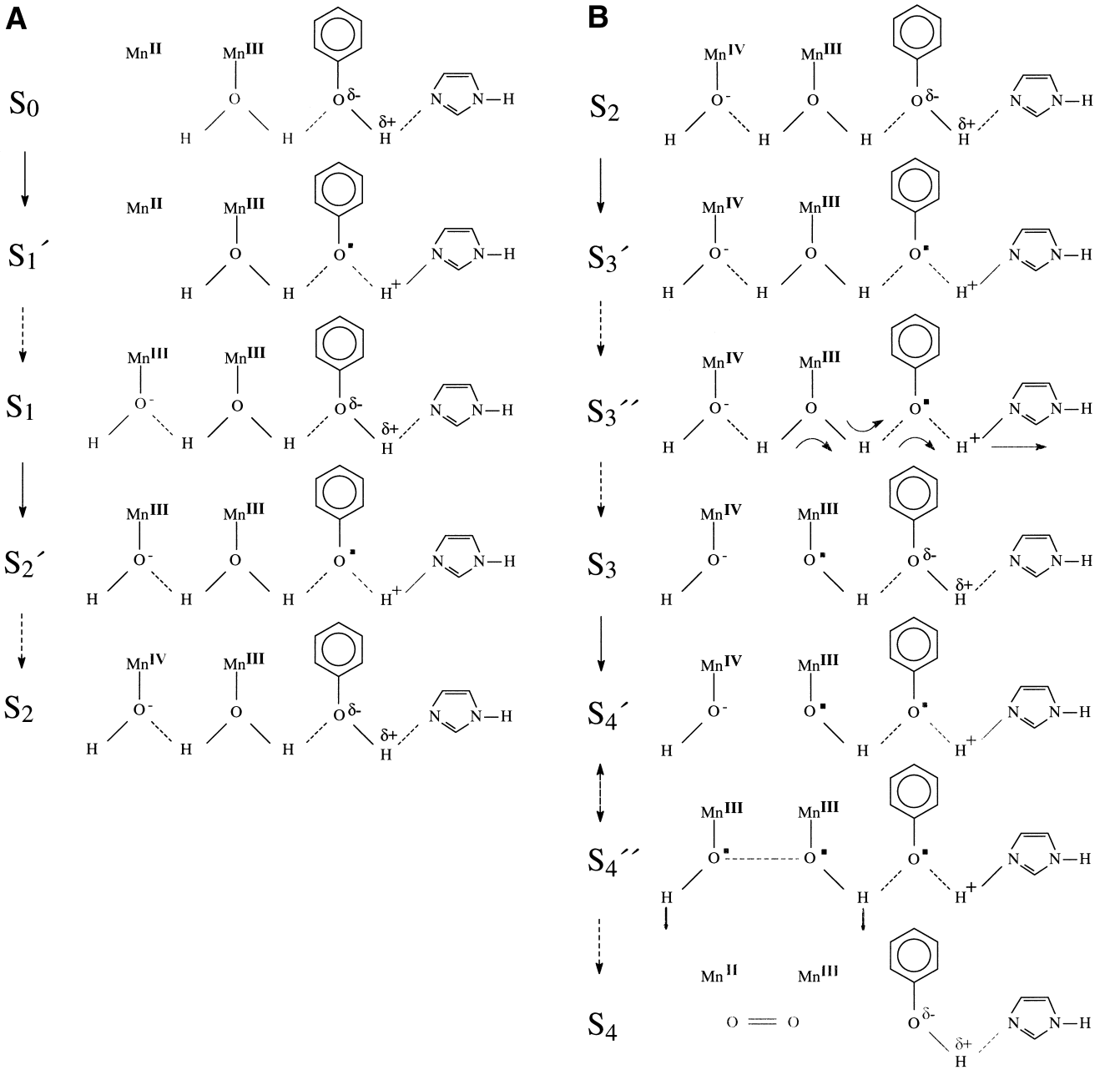

Fig. 2. Tentative model for the reaction sequence of photosynthetic water oxidation. The photochemically driven four-stepped progress involves five increasingly oxidised formal states ($S_0 \ldots S_4$). It is followed by oxygen evolution during the transition from S_4 back to S_0. Y_Z is shown as hydrogen-bonded to the proximal bound water, which implies a small distance between Y_Z and the proximal Mn. The reaction scheme is, however, independent of this assignment, as alternative pathways for the release of water protons are conceivable, different from the here suggested path through Y_Z.

transition another amino acid cofactor, X, as suggested in previous work (mainly on inhibited PS II) from several laboratories including our own one [10,24,66]. As the spectroscopic features are not very specific in this respect, X may simply be bound water. It is oxidised into a bound hydroxyl radical and then forms a pre-peroxide state together with the bound hydroxyl anion. The radical/manganese interaction quenches the multiline EPR-signal of the still existing mixed-valence manganese cluster. Still, both

bound water molecules are exchangeable (30 and 500 ms) [14].

The final production of oxygen is initiated by the forth abstraction of an electron from the OEC, producing $Y_Z^{\bullet}$. It is apparent that the only other oxidisable component of the OEC is not the bound hydroxyl anion, but the $Mn^{III}Mn^{III}$-peroxide entity, a small fraction of which may be present already in state S_3 (see [29] and references therein). In state S_4'' peroxide is formed but firmly bound and/or so short-lived that it has remained undetected. We suppose that the low probability of the peroxide state (the equilibrium between states S_4' and S_4'' is poised towards S_4'), rather than the velocity of the electron transfer, limits the rate of oxygen evolution.

When considering the tentative reaction sequence in Fig. 2, some paradigms that have caused vivid controversy in the literature may appear questionable if not obsolete. Neither are amino acids, other than tyrosine Y_Z, needed as redox cofactors nor does Y_Z^{ox} necessarily release its phenolic proton into the bulk during each transition. What about the dichotomy between the two proposed roles of Y_Z^{ox}, namely the hydrogen acceptor [30,31,38] versus the electrostatic promoter [29,46]? In our model Y_Z possibly serves in both functions, as a hydrogen acceptor, but only during the transition $S_3' \rightarrow S_3''$, where the bound hydroxyl radical is formed, and as an electrostatic promoter which provides the final overpotential to push the reactions from S_3 to S_4, the final step which produces oxygen.

Despite of its reduction to a minimum set of elements the proposed reaction scheme appears compatible with most of the available data. With the semi-stationary states S_i now reasonably well understood, the obvious task for the future is twofold: to resolve (1) the atomic structure, and (2) the chemical nature of the intermediate states, S_i'.

Acknowledgements

We wish to thank our colleagues R. Ahlbrink, M. Hundelt, Drs. D. Cherepanov and A. Mulkidjanian for their contributions as well as Profs. G. Babcock and R. Debus, and H.H. Limbach for stimulating discussion. Our work was financially supported by the Deutsche Forschungsgemeinschaft (SFB 171-A2), the Fonds der Chemischen Industrie and the Land Niedersachsen.

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