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Mini review

The role of manganese in photosynthetic water oxidation

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Summary. The process of photosynthetic water oxidation to dioxygen under proton release takes place via a sequence of four univalent redox steps in a manganese-containing unit. In this mini-review the current state of knowledge is briefly described with special emphasis on the following topics: (a) the nature of the catalytic site, (b) the structure of the redox chemistry of the manganese-containing active site, (c) the ligand structure and the entry of substrate water into the redox cycle, and (d) problems of the stoichiometry of proton release coupled with individual redox steps and the possible role of other cofactors (Cl^-, Ca^{2+}).

Key words: Photosynthesis – Photosystem II – Water oxidation – Plastoquinone – Manganese

Introduction

The 'invention' of a biomolecular device to dissociate water into dioxygen and metabolically bound hydrogen by using visible light was the cornerstone of the evolutionary development of all higher organisms in the early biosphere. This event, which occurred in photosynthesizing organisms about 2–3 billion years ago, had two consequences of paramount importance: (a) it allowed the huge water pool on the earth surface to become available as a hydrogen source for the biosphere, and (b) the resulting formation of dioxygen as a photosynthetic 'waste' product led to the present day aerobic atmosphere (including the important protective ozone layer). The latter event opened the road for the much more efficient exploitation of the free energy content of food through the aerobic respiration of heterotrophic organisms (for a review see Renger 1983).

The overall process for the cleavage of water during photosynthesis can be simply described as follows:

$$2\,H_2O + 2\,T \xrightarrow{h\nu} O_2 + 2\,H_2 \cdot T \qquad (1)$$

where T represents a molecule which chemically binds hydrogen. This process is carried out through the cooperation of three integral protein complexes that are anisotropically incorporated into the thylakoid membranes of oxygenic photosynthetic organisms as shown in the scheme of Fig. 1. The three complexes are defined as follows.

(a) *Photosystem II (PS II).* This complex catalyzes the light-induced water cleavage into dioxygen and a moderately reducing form of bound hydrogen. In this part of the reaction sequence, plastoquinone (PQ) serves as the hydrogen acceptor molecule, i.e.

$$2\,H_2O + 2\,PQ \xrightarrow[PS\ II]{h\nu} O_2 + 2\,PQH_2. \qquad (2)$$

(b) *Photosystem I (PS I).* This complex catalyzes the light-induced increase of the reducing power of the metabolically bound hydrogen up to a level that is only 0.1 V below that of molecular H_2 at pH = 7. In this case $NADP^+$ serves as the terminal acceptor:

$$1\,H^+ + 2\,PC_{red} + NADP^+ \xrightarrow[PS\ I]{h\nu} 2\,PC_{ox} + NADPH \qquad (3)$$

where PC = plastocyanin.

(c) *The cytochrome* b_6/f *unit.* This complex facilitates the transfer of reducing equivalents from PS II to PS I, in which both PQH_2 and plastocyanin act as the mobile electron carriers between the complexes:

$$PQH_2 + 2\,PC_{ox} \xrightarrow[cyt\ b_6/f]{} PQ + 2\,PC_{red} + 2\,H^+. \qquad (4)$$

The above electron transfer reactions are coupled with protolytic reactions. Consequently, the anisotropic arrangement of the PS II, PS I and cytochrome b_6/f complexes leads to the formation of a transmembrane difference in the protonic electrochemical potential. The free energy of this potential difference is then used for the generation of ATP, which is catalyzed by the ATP synthase complex (not explicitly shown in Fig. 1; for a review see Gräber 1987).

Transition metals play an essential role as redox-active centers in the photosynthetic electron transfer

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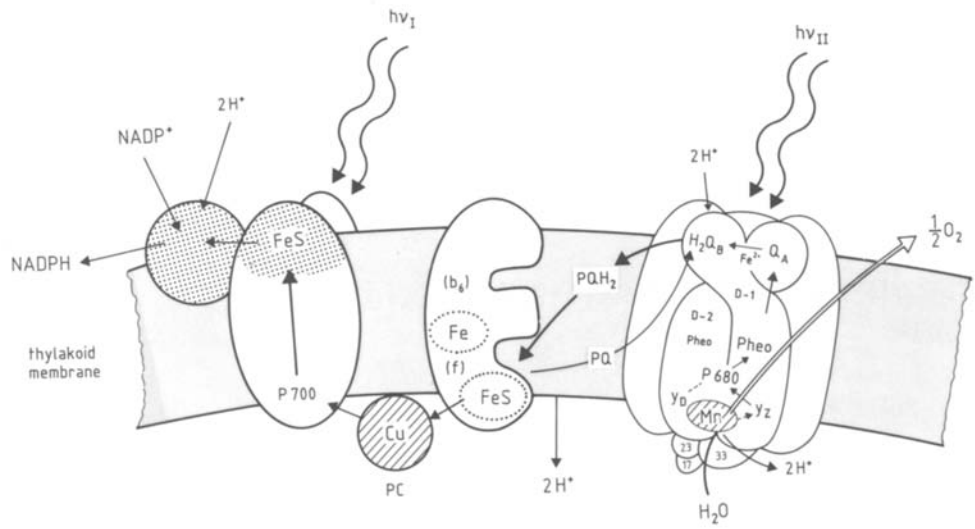


Fig. 1. General scheme of the photosynthetic electron transport from water to $NADP^+$. The transition metal centers participating in the reaction sequence are symbolized by hatched areas (manganese, copper) and dotted areas (iron). For further details see text

reactions: manganese is an indispensable part of PS II which catalyzes the actual oxidation of water, and iron, in the form of low-potential $(Fe\text{-}S)_x$ clusters, is directly involved in the formation of bound hydrogen as NADPH at PS I. In addition, iron is also an integral part of the cytochrome b_6/f complex (in both of the cytochromes and in the form of an unusual high-potential Rieske Fe-S center) while copper is the redox-active group of plastocyanin. Figure 1 schematically summarizes the sites of the functional transition metal centers.

The key steps of the photosynthetic dissociation of water take place in PS II. Therefore, this short communication will be restricted to the reactions of PS II with special emphasis on the functional and structural organization related to the water oxidation chemistry itself.

Structure and function of PS II

Within the PS II complex, the overall reaction sequence is comprised of three different parts (for reviews see Babcock 1987; Renger 1987a; Rutherford 1989; Hansson and Wydrzynski 1990).

The first part is the light-induced generation of strongly oxidizing and moderately reducing redox equivalents. This process is a π-electron-mediated reaction taking place in the picosecond time domain. A special chlorophyll-*a*-containing component, referred to as P680, ejects an electron in its excited singlet state ($^1P680^*$), with pheophytin *a* (Pheo-*a*) acting as primary acceptor. In order to permit reaction with water, the spin-coupled primary radical pair that is formed, $^1[P680^+Pheo^-]$, requires stabilization by subsequent electron transfer from $Pheo^-$ to a special non-covalently bound plastoquinone molecule (Q_A^-) that is magnetically coupled to a high-spin Fe^{2+} ion. This non-heme high-spin Fe^{2+} ion, however, is not indispensable for stable charge separation. Its role remains to be clarified and will not be discussed further here (for a review see Diner and Petrouleas 1988). Interestingly, Q_A acts only as one-electron component, switching between the quinone and semiquinone form under normal conditions. The reaction sequence can be described by Eq. (5) (for a recent review see Renger 1991):

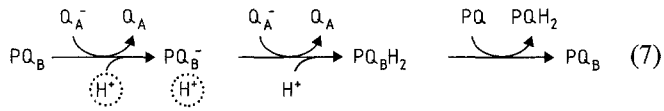
$$^1P680^*PheoQ_A \xrightarrow{\approx 3\ ps} {}^1[P680^+Pheo^-]Q_A \xrightarrow{\approx 300\ ps} P680^+PheoQ_A^- \quad (5)$$

The second part is the cooperative reaction of four oxidizing redox equivalents with two water molecules. This reaction leads to dioxygen and the release of four protons into the inner space (lumen) of the thylakoids:

$$4\oplus + 2\,H_2O \longrightarrow O_2 + 4\,H^+_{in}. \quad (6)$$

The mechanism and structural organization of this part of the reaction sequence is the topic of this mini-review.

The third part is the cooperative reaction of two reducing redox equivalents with plastoquinone. This reaction takes place at a special plastoquinone binding site (Q_B site) located in polypeptide D1 of the PS II complex. Plastoquinone noncovalently binds to the Q_B site and is subsequently reduced in two univalent redox steps with Q_A^- as electron donor. Only the semiquinone form (Q_B^-) is comparatively strongly bound, while the fully reduced plastoquinone, after its protonation, is rather labile and readily exchanges with another PQ molecule, thereby completing the reaction cycle at the Q_B site (for a review see Crofts and Wraight 1983):



(7)

All functional redox groups participating in the reaction sequences described by Eqs. (5) and (7) are assumed to be bound within a heterodimer of polypeptides D1 and D2 of PS II (Trebst 1986; Michel and Deisenhofer 1988). The location of the catalytic site of water oxidation, however, is not yet known; but different lines of evidence support the idea that it is also located in the D1/D2 heterodimer (for a recent review see Vermaas and Ikeuchi 1990).

Photosynthetic water oxidation

General scheme

The generation of the sufficiently oxidizing redox equivalents needed for water oxidation occurs via the one-electron transfer from ^{1}P680* to Pheo (Eq. 5) but the process of water oxidation to dioxygen requires the co-operation of four such redox steps (Eq. 6). Therefore, an appropriate mechanism is necessary to permit the oxidation of water with $P680^+$ as the ultimate oxidant. The fundamental work of Kok, Joliot and co-workers (for a review see Joliot and Kok 1975) revealed that the coupling between $P680^+$ and water oxidation occurs via the intermediary accumulation of oxidizing redox equivalents referred to as S_i states (with i = number of accumulated equivalents). Upon excitation of dark-adapted samples with a train of saturating short flashes, a characteristic period-four oscillation pattern of the oxygen yield arises with pronounced maxima after the 3rd, 7th, 11th, etc. flash (a typical trace is shown at the top of Fig. 2). This result showed that the singly oxidized state S_1 is thermodynamically stable in the dark. This has been confirmed within the physiological pH range by recent studies (Renger and Hanssum 1988).

Early kinetic measurements indicated that $P680^+$ reduction occurs in the nanosecond (and microsecond) range. The relative amplitudes of the components with different life times depend on the redox state S_i (Gläser et al. 1976; Brettel et al. 1984). This behavior is only slightly dependent on temperature within the physiological range (Eckert and Renger 1988). Since the reduction kinetics of $P680^+$ are faster by about three orders of magnitude than the accumulation steps of the oxidizing redox equivalents, an intermediary redox carrier was inferred to connect the water-oxidizing entity with $P680^+$ (Babcock et al. 1976; Dekker et al. 1984a; Renger and Weiss 1986). This intermediary component has recently been identified by site-directed mutagenesis as tyrosine-161 of the polypeptide D1 in *Synechocystis* PCC 6803 (Debus et al. 1988; Metz et al. 1989). It is often symbolized in the literature by Y_Z. The involvement of Y_Z in the PS II reaction sequence reveals that the protein matrix not only provides the proper ligation for prosthetic groups but can also actively participate in redox reactions.

Based on the above description, a general functional organization for photosynthetic water oxidation can be summarized as shown on the bottom of Fig. 2. The scheme at the bottom does not contain information on the actual chemical mechanism of water oxidation. In order to resolve this problem, the electronic configuration and the nuclear geometry of the different S_i states need to be known. Despite recent progress, this goal is far from being achieved, as is illustrated by the following questions and their fragmentary answers which are summarized in the subsequent sections of this paper.

a) What is the nature of the catalytic site of water oxidation?

b) What is the nuclearity of the functional manganese complex and its electronic configuration in the different S_i states, and why is S_1 the thermodynamically stable state?

c) What are the nature and the redox properties of the structural ligands to the functional manganese? Which polypeptide(s) provide(s) the ligands and how are the reaction coordinates regulated by the protein matrix?

d) At which redox state S_i does the substrate water bind and at which redox level of the substrate is the essential O-O bond formed?

e) What is the stoichiometry of the intrinsic proton release pattern?

f) What is the mechanistic role of cofactors like Ca^{2+} and Cl^-?

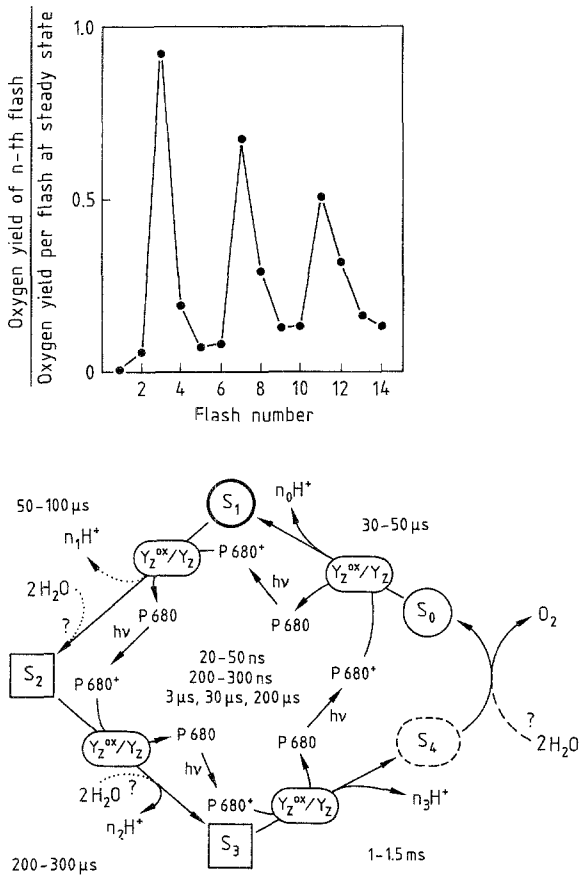


Fig. 2. Average oxygen yield per flash as a function of flash number in dark-adapted thylakoids at pH = 7.0 and 7° C (top, Messinger and Renger, unpublished results) and functional scheme of four-step univalent water oxidation. Typical half-life of the electron transfer processes from Y_Z to $P680^+$ and from the water-oxidizing complex to Y_Z^{ox} are shown in the center and at the corners of the figure, respectively (bottom). For further details see text

The nature of the catalytic site

In a generalized form, photosynthetic water oxidation can be described by the following scheme (Renger et al. 1990):

$$Y_Z^{ox} + HSU(Mn)_i \rightarrow Y_Z + HSU(Mn)_{i+1} + n_i H_{in}^+ \quad (8)$$

$$WOS(2H_2^*O) + HSU(Mn)_{i=4} \rightarrow HSU(Mn)_{i=0} + WOS(O_2) + (4 - \sum_i n_i \cdot m)H_{in}^+ \quad (9)$$

$$WOS(O_2) + 2\,H_2O \rightarrow WOS(2\,H_2^*O) + O_2 + mH_{in}^+ \quad (10)$$

where $i = 0, 1, \ldots 4$ represents the number of oxidizing redox equivalents stored in the manganese-containing hole storage unit HSU(Mn), n_i = number of protons released into the lumen in each individual redox step and m = number of protons released during the O_2/substrate water exchange at the catalytic water-oxidizing site (WOS), H_2^*O symbolizes bound substrate water of an unknown protonation state which depends on the properties of WOS as Lewis acid.

The above formulation raises a key question: are the functional entities HSU(Mn) and WOS structurally distinguishable units? The existence of an independent WOS unit would provide a functional separation of the hole storage process from a concerted four-electron water oxidation. In this case, the HSU(Mn) would be protected from intermediate interactions with water molecules (Wydrzynski et al. 1985), in order to prevent undesirable, deleterious side reactions (e.g. peroxide formation, see below). On the other hand, if one accepts that the rate of S_3 oxidation by Y_Z^{ox} is almost the same as the dioxygen release (1–2 ms, see Joliot and Kok 1975; Lavergne 1990), then the overall process including the concerted abstraction of four electrons from two water molecules under dioxygen-bond formation (Eq. 9) and the exchange reaction (Eq. 10) at the WOS would need to occur with a rate as fast or faster than the individual redox step of a one-electron abstraction from $HSU(Mn)_{i=3}$ (Renger and Weiss 1983). For kinetics reasons, therefore, one could assume that the HSU and WOS functions are exerted by the same unit. In this case, a binuclear manganese cluster is the most simple array to satisfy this condition as postulated previously (Renger 1978, 1987b). This idea is supported by the recent syntheses of a binuclear μ-peroxo-bridged (in addition to two μ-oxo bridges) manganese complex. This complex decomposes to release O_2 upon water addition (Bossek et al. 1990).

Structure and redox chemistry of the manganese-containing active site

It is well known that each PS II, functionally competent in water oxidation, contains four manganese atoms. However, not all of the manganese may be involved in the catalytically active redox center. There are different lines of evidence for a heterogeneity in the manganese pool associated with PS II (for detailed discussion see Renger 1987a, b). Attempts to clarify the nuclearity of the manganese have been made by EPR and EXAFS (extended X-ray absorption fine structure) studies. The currently favored models are either two different binuclear manganese centers (Guiles et al. 1990) or a trinuclear manganese cluster together with a mononuclear Mn center (Hansson et al. 1987; George et al. 1989; Penner-Hahn et al. 1990).

The first information on the redox state of the functional manganese became available with the discovery of a low-temperature $g = 2$ multiline EPR signal (Dismukes and Siderer 1981), which characterizes S_2 (Brudvig et al. 1983). The analysis of this signal led to the conclusion that in S_2 the manganese cluster exists in a mixed valence state. Likewise, XANES (X-ray absorption near-edge structure) measurements revealed that the redox transition $S_1 \rightarrow S_2$ involves at least the abstraction of an electron from manganese. Based on EPR and XANES data, in comparison with synthetic model compounds, the S_2 state is inferred to be a Mn(III)Mn(IV) mixed-valence state either as a binuclear center or located within a trinuclear cluster (for review see Guiles et al. 1990; Penner-Hahn et al. 1990; Vänngard et al. 1990). The formation of S_2 also gives rise to a low-temperature EPR signal centered at $g = 4.1$. This signal could be due to the oxidation of a monomeric Mn(III) to Mn(IV) which forms a redox equilibrium with the proposed trinuclear manganese cluster that would give rise to the $g = 2$ multiline EPR signal (Vänngard et al. 1990).

In contrast to the $S_1 \rightarrow S_2$ transition, which appears to be primarily localized to the metal center, the situation is less clear for the other S-state redox transitions. XANES data, measurements of the water proton NMR relaxation and of the power saturation of the EPR signal of the redox active tyrosine in polypeptide D_2 in its oxidized form, Y_D^{ox} (this component is not indispensable for water oxidation, see Vermaas et al. 1988), suggest that the electron abstraction due to the $S_2 \rightarrow S_3$ transition may involve a ligand rather than a manganese oxidation (for review see Rutherford 1989; Guiles et al. 1990; Vänngard et al. 1990). It has been suggested that a histidine ligand becomes oxidized when S_3 is formed (Boussac et al. 1990). However, the data used to support this idea was obtained in modified samples and it remains to be determined whether a histidine oxidation also occurs under in vivo conditions (vide infra).

Ultraviolet-absorption changes were previously interpreted as an indication of a manganese-centered redox reaction on each step through the sequence $S_0 \rightarrow S_1 \rightarrow S_2 \rightarrow S_3 \rightarrow S_4$ (Dekker et al. 1984b; Saygin and Witt 1987). Apart from the inherent problems which prevent an unambiguous deconvolution of the data (Renger and Hanssum 1988), the broad, structureless ultraviolet band, which is observed in these studies, is not specific enough to permit a true distinction between a ligand- and a metal-centered oxidation, as discussed recently (Renger 1987b). In an earlier report (Renger 1978) it was suggested that the $S_0 \rightarrow S_1$ transition may not involve manganese oxidation. The absence of significant ultaviolet-absorption changes above 290 nm on the $S_0 \rightarrow S_1$ transition might support this idea (Lavergne 1989). Other data favor a manganese oxidation of the type Mn(II)→Mn(III) (Rutherford, 1989, Guiles et al., 1990). Compared with the other transitions, the couple S_1/S_0 exhibits a markedly lower redox potential (Rutherford 1989; Vass and Inoue 1991). This gives rise to a significantly higher redox gap with respect to the $P680^+/P680$ couple. It was recently discussed that this

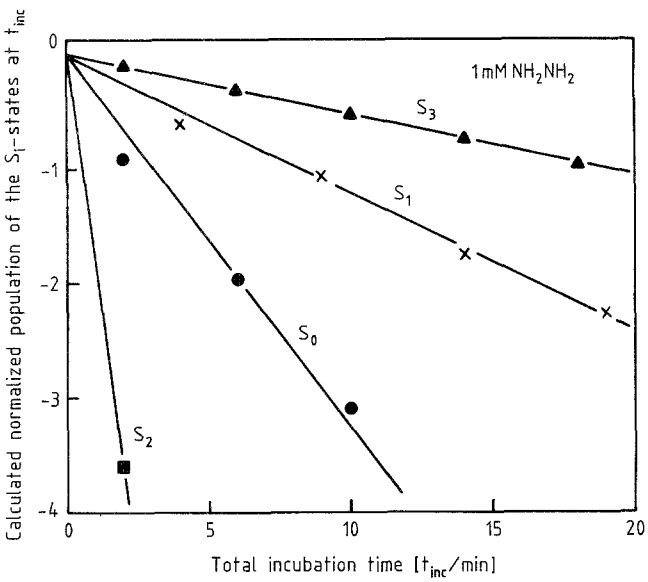


Fig. 3. Semilogarithmic plot of the calculated normalized concentration of the redox states S_i ($i=0,\ldots 3$) of the water-oxidizing system as a function of time in spinach thylakoids in the presence of 1 mM NH_2NH_2 (for details see Messinger and Renger 1990)

free energy is used to support structural changes of functional relevance (Renger et al. 1990).

In another approach to analyze the redox chemistry of water oxidation, the interaction of the S_i states with exogenous reductants can be studied. It was recently demonstrated that, for example, NH_2NH_2 phenomenologically introduces a donor pool in dark-adapted samples which leads to the formation of the formal redox states 'S_{-2}' (from S_0) and 'S_{-1}' (from S_1), as reflected by a two-digit phase shift in the flash-induced oxygen yield pattern (Renger et al. 1990). However, the surprising finding is that the redox state S_2 is very reactive to NH_2NH_2, whereas S_3 reacts rather sluggishly. The results obtained are depicted in Fig. 3. The slow reactivity of S_3 together with the high susceptibility of S_2 can be explained by the assumption that the $S_2 \rightarrow S_3$ state change transition does not lead to a stable manganese oxidation, but rather structural changes may take place in this transition which effectively forms a barrier against the reactivity with exogenous reductants. A similar situation might exist for S_0 and S_1, since S_0 appears to react faster with NH_2NH_2 than S_1 (Messinger and Renger, unpublished results).

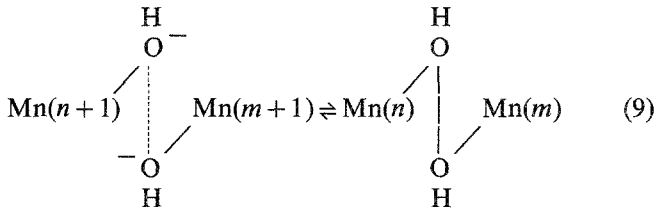
As a summary of our current knowledge, it seems clear that the $S_1 \rightarrow S_2$ transition involves a stable manganese oxidation state change, while $S_2 \rightarrow S_3$ probably leads to the oxidation of a ligand, including the possibility of the substrate itself. The $S_0 \rightarrow S_1$ transition might also comprise a manganese oxidation which differs from that of $S_1 \rightarrow S_2$.

Ligand structure and substrate water

EXAFS studies suggested that the first coordination sphere of the manganese in PS II is composed mainly of oxygen atoms with possible contributions of nitrogen atoms. It was inferred from the data that two manganese atoms at a distance of 0.27 nm are connected by two μ-oxo bridges and that other O(N)-containing ligands are located at a distance of 0.19 nm from Mn (for a review see Guiles et al. 1990 and references therein). Based on the assumption that the D1 and D2 polypeptides provide amino acid residues for the manganese ligation, different models have been described in the literature, postulating carboxylic acids (glutamate, aspartate) and histidine as likely ligands (for a review see Vermaas and Ikeuchi 1990). Attempts to identify specific amino acid residues by site-directional mutagenesis suggest that at least Asp-170 of polypeptide D1 is important for the stability of the functional manganese (Diner et al. 1990). Among the amino acid residues screened in polypeptide D2, at present only Glu-69 was shown to be of relevance (Vermaas and Ikeuchi 1990).

Apart from the postulated μ-oxo bridges and the above-mentioned amino acid residues, the question concerning the ligation of the substrate water needs to be considered. Experiments with ^{17}O- or ^{18}O-labeled water have not provided any conclusive answers, since rapid isotope exchange between the labeled water and other ligands cannot be excluded as interfering in the experiments performed to date (Renger 1987b). Therefore, it is not known at which redox state S_i the substrate water becomes a ligand or where the key O-O bond is formed.

Based on theoretical considerations, however, it has been suggested that in S_3 a peroxide intermediate could be formed at a binuclear manganese center (Renger 1978), probably as a redox equilibrium of the type:



(9)

The oxidation of such a peroxide state by Y_Z^{ox} could lead directly to binuclearly complexed dioxygen (Renger 1988; see also Fig. 4). This hypothesis is not proven experimentally but it was shown recently that flash-induced hydrogen peroxide can be formed by water oxidation in competition with the oxygen-evolving capacity in specially treated samples (Wydrzynski, Anayev, Klimov and Renger, unpublished results). However, the H_2O_2 was found to arise after a single flash, indicating its formation in the S_2 state. It remains to be determined whether this reaction is directly related to the oxidation of water to dioxygen (perhaps through a hydrolytic destabilization of a preformed peroxide state within the manganese cluster) or arises as a change in the water accessibility to the catalytic site and an altered water oxidation chemistry in the modified system. A scheme of photosynthetic water oxidation is shown in Fig. 4.

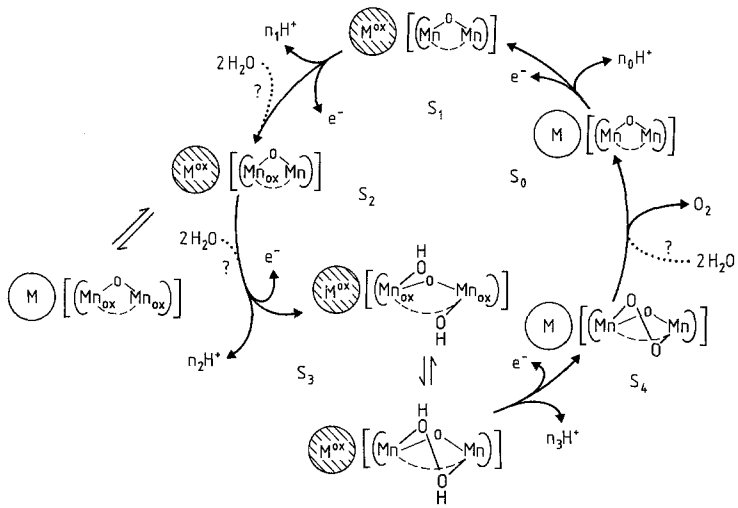


Fig. 4. Reaction scheme of photosynthetic water oxidation. For simplicity, only the manganese centers of the presumed binuclear catalytic site are presented. Likewise, only one bridging μ-oxo group is explicitly shown. The other ligands are symbolized by half ovals (dotted ovals represent further bridging ligands) around the Mn. As the stage of water entry into the redox cycle is unknown (see text), substrate ligation is only shown for S_3 and S_4. Component (or state) M is explained in the text. Oxidized states are symbolized by hatched areas. The coefficients n_i represent the number of protons released during redox transitions $S_i \rightarrow S_{i+1}$; their values are affected by the amino acids of the protein matrix (see text). The possibility of a redox equilibrium in S_3 is described by Eq. (9). Likewise, a redox equilibrium between Mn^{ox} and the binuclear manganese could arise in S_2 (Renger et al. 1987)

Comments on the stoichiometry of proton release and the possible role of other cofactors

Water oxidation to dioxygen is coupled with the release of four protons. At first glance, one might assume that the proton-release pattern provides information on the mechanism of photosynthetic water oxidation. However, the proton release per se does not provide direct information on the electronic configuration, i.e. whether it is the substrate water ligand or the manganese center which becomes oxidized in a particular redox step. On the other hand, a water oxidation step could take place without proton release if the H^+ is prevented from equilibration with the outer phase within the time scale of the monitoring system. It has been pointed out previously (Renger 1978) that the experimentally detected H^+-release pattern (referred to as extrinsic pattern) in PS II does not necessarily reflect the intrinsic H^+-release pattern. The protein matrix could give rise to H^+ release due to pK shifts of amino acid residues induced by a manganese oxidation. The widely accepted 1,0,1,2 stoichiometry (Förster and Junge 1985 and references therein), coupled with the redox transitions $S_0 \rightarrow S_1$, $S_1 \rightarrow S_2$, $S_2 \rightarrow S_3$ and $S_3 \rightarrow (S_4) \rightarrow S_0 + O_2$, respectively, could therefore be markedly affected by the protein. Data obtained with samples where the protein is modified by proteolysis are shown in Fig. 5 (Wacker et al. 1990). In the control samples a marked proton oscillation pattern is observed. It can be fitted by a 1,0,1,2 stoichiometry, but in most cases a 0,0,1,3 or even a non-

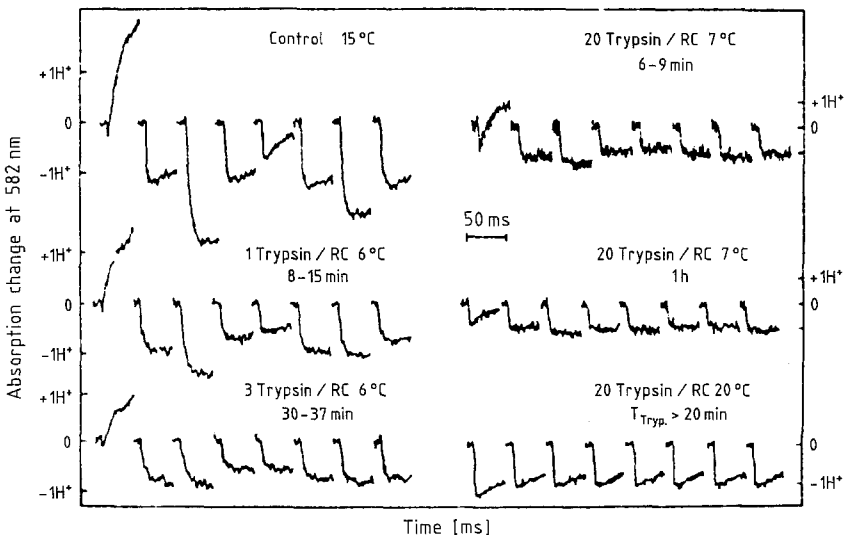


Fig. 5. Absorption changes at 582 nm reflecting the proton release (decrease) or uptake (increase) by dark-adapted PS II membrane fragments illuminated with a train of short saturating flashes. For further details see text and Wacker et al. (1990)

integer stoichiometry provides a better fit. Upon mild treatment with trypsin, the proton oscillation pattern is modified without an effect on the oxygen evolution. After destruction of the oxygen-evolution capacity by severe trypsin treatment, the oscillation pattern disappears and, in this case, the proton release reflects the deprotonation of oxidized Y_D^{ox} (Renger and Völker 1982). The data of Fig. 5 show that the protein matrix really affects the extrinsic proton-release pattern. Independent lines of evidence support this idea (Lübbers and Junge 1990; Lavergne and Rappaport 1990).

There are numerous reports in the literature of the effects by Ca^{2+} and Cl^- ions on the O_2-evolution activity. Such results are usually taken to indicate that both ions play a direct role as cofactors in the water oxidation chemistry (for reviews, see Homann 1987; Rutherford 1989; Govindjee 1989; Coleman 1990). However, the procedures used to generate a Ca^{2+} and/or Cl^- demand in activity may introduce secondary, artificial modifications which are compensated by the addition of these ions (Shen and Katoh 1990; Wydrzynski et al. 1990). Nevertheless, it seems clear that at least Ca^{2+} is inherently required to establish a functionally competent PS II, as indicated by the need for Ca^{2+} ions in the photoactivation of O_2 evolution (Ono and Inoue 1983; Tamura et al. 1989). Thus, Ca^{2+} ions may have a structural function that is necessary for the proper arrangement of the catalytic site (Rutherford 1989). Similarly, an inherent Cl^- ion requirement may also be only indirectly mediated through secondary, structural effects (Lindberg et al. 1990).

Concluding remarks

In conclusion, we have tried to summarize in this article the considerable information which has been gathered over the recent years on the process of water oxidation in photosynthetic plants. There is now broad agreement on the overall features of photosystem II where the water oxidation process occurs. It is clear, for example, that the transition-metal manganese plays a critical role in the mechanism and undergoes a redox change in at least one of the intermediary steps. Nevertheless, in spite of our current knowledge, important structural and mechanistic problems remain to be solved. These include the exact organization and ligand environment at the manganese within the protein matrix, the entry and binding of the substrate water to the catalytic site, the mechanism of the O-O bond formation and release of the high-yield dioxygen product. The future promises to provide many exciting challenges as we unravel the secrets of this most important biological process.

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