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Review

Eight steps preceding O–O bond formation in oxygenic photosynthesis—A basic reaction cycle of the Photosystem II manganese complex

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Abstract

In oxygenic photosynthesis, water is split at a Mn_4Ca complex bound to the proteins of photosystem II (PSII). Powered by four quanta of visible light, four electrons and four protons are removed from two water molecules before dioxygen is released. By this process, water becomes an inexhaustible source of the protons and electrons needed for primary biomass formation. On the basis of structural and spectroscopic data, we recently have introduced a basic reaction cycle of water oxidation which extends the classical S-state cycle [B. Kok, B. Forbush, M. McGloin, Cooperation of charges in photosynthetic O2 evolution- I. A linear four-step mechanism, Photochem. Photobiol. 11 (1970) 457–475] by taking into account also the role and sequence of deprotonation events [H. Dau, M. Haumann, Reaction cycle of photosynthetic water oxidation in plants and cyanobacteria, Science 312 (2006) 1471–1472]. We propose that the outwardly convoluted and irregular events of the classical S-state cycle are governed by a simple underlying principle: protons and electrons are removed strictly alternately from the Mn complex. Starting in I_0, eight successive steps of alternate proton and electron removal lead to I_8 and only then the O–O bond is formed. Thus not only four oxidizing equivalents, but also four bases are accumulated prior to the onset of dioxygen formation. After reviewing the kinetic properties of the individual S-state transition, we show that the proposed basic model explains a large body of experimental results straightforwardly. Furthermore we discuss how the *I*-cycle model addresses the redox-potential problem of PSII water oxidation and we propose that the accumulated bases facilitate dioxygen formation by acting as proton acceptors.

Keywords: Manganese complex; Oxygen evolution; Photosynthesis; Proton release; Kok cycle; Water oxidation

1. Introduction

Driven by the absorption of four photons, plants and cyanobacteria produce the atmospheric dioxygen by the splitting of water according to the net equation:

$$2H_2O + 4\ \text{photons} = >4H^+ + 4e^- + O_2, \qquad (1)$$

where e^- denotes the electrons extracted from water (water oxidation), which eventually serve as reducing equivalents in the carbon fixation chemistry of the Benson–Calvin cycle [1]. The locus of water splitting is the oxygen-evolving complex (OEC) [2–7]. It comprises a tetra-manganese complex bound to the proteins of photosystem II (PSII) and a nearby tyrosine residue (Y_Z). After absorption of a photon by a chlorophyll of PSII, an electron is transferred from the primary chlorophyll donor (P680) via a specific pheophytin to a firmly bound plastoquinone (Q_A) and subsequently to the secondary quinone acceptor (Q_B) which, when doubly reduced and protonated, can leave its binding site (Fig. 1A). At the donor side of PSII, Y_Z is oxidized by $P680^+$ before the Mn complex advances in its reaction cycle (Fig. 1B), which is the subject of the present contribution and discussed further below. Four turnovers of the

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Abbreviations: EPR, electron paramagnetic resonance; ET, electron transfer; FTIR, Fourier-transform infrared (spectroscopy); EXAFS, extended X-ray absorption fine-structure; PS, photosystem; P680, primary chlorophyll donor; Y_Z, Tyr 160/161 of the D1 protein of PSII; XANES, X-ray absorption near-edge structure; XAS, X-ray absorption spectroscopy

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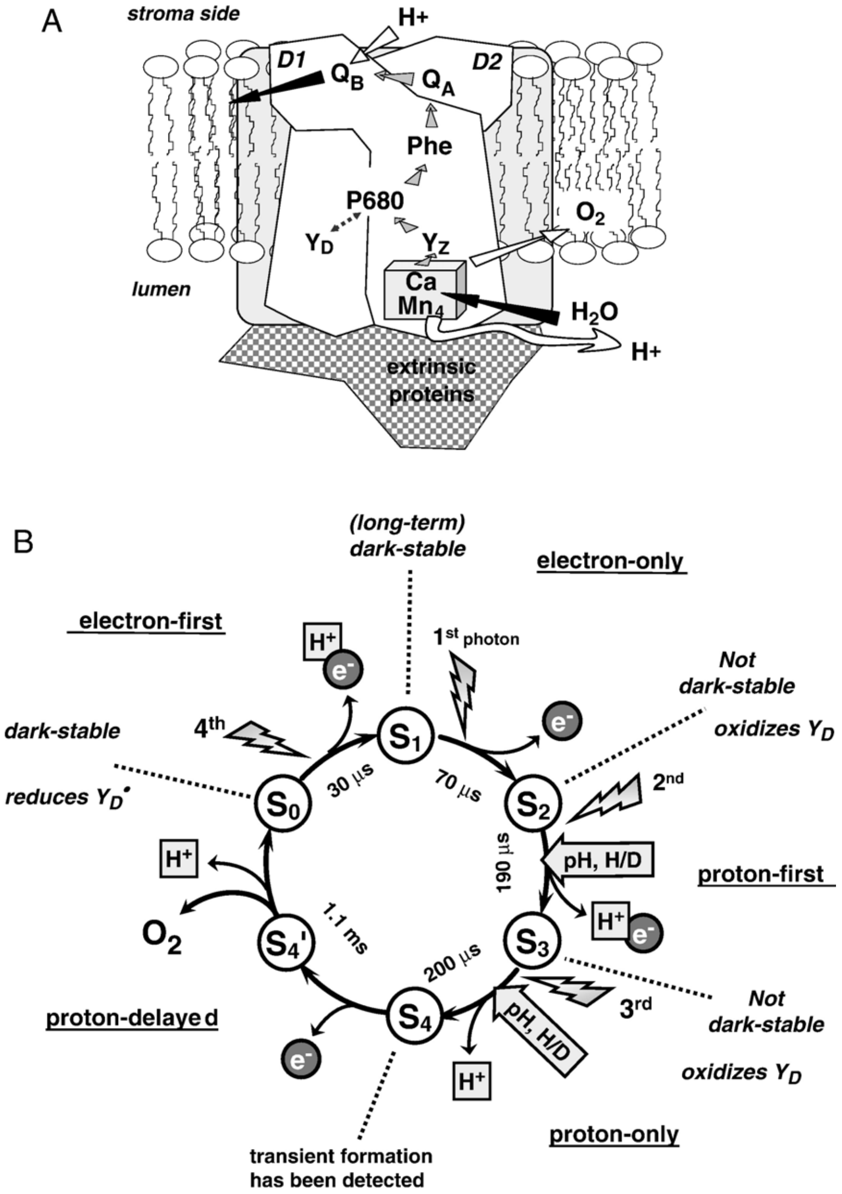

Fig. 1. Photosystem II and the intricate properties of the classical S-state cycle. (A) Scheme of the arrangement of essential redox-factors in PSII. (B) S-state cycle of Bessel Kok extended by S_4-formation as proposed in [8]. The properties of the individual S-states and S-state transitions are schematically indicated. The electrons are transferred from the Mn complex to the Y_Z-radical which is formed after absorption of a photon by electron transfer to $P680^+$. The protons are, most likely, removed from the Mn complex or its ligand environment. The halftimes of the respective S-state transitions are indicated as determined in [8] by time-resolved X-ray experiments.

above electron-transfer chain induced by four sequential photon-absorption events are required for water oxidation and dioxygen formation according to Eq. (1).

Water oxidation by PSII is a process of fundamental importance for the atmosphere (O_2 production) and biosphere (primary biomass formation) of the Earth. Furthermore, it is foreseeable that biotechnological and biomimetic approaches for light-driven H_2-production from water need to become a cornerstone in the worldwide endeavor to move from fossil fuels to hydrogen-based fuel technologies. In this realm, elucidation of the mechanism of photosynthetic water oxidation by PSII – including the light-driven assembly of the Mn_4Ca complex, photodestruction, photoprotection, and repair mechanisms – is of prime interest.

The crystallographic models of intact PSII complexes [9–13] represent a long-awaited breakthrough in research on photosynthetic water oxidation. Rooting in an initiative of Horst Tobias Witt, in 2001 Berlin researchers have presented a crystallographic model which revealed the positions of the PSII redox-factors [9]. In 2004, James Barber and his coworkers in London crowned their strive for a PSII structure [14–17] by an almost complete structural model of PSII [11]. They presented the first atomic-resolution model of the PSII manganese complex and its ligand environment [11], which strongly has influenced the progress in the field. Inter alia Barber and coworkers could prove that the tetra–manganese complex is a pentanuclear Mn_4Ca complex. The proposed Mn_3Ca-cubane motif [11] represents an appealing structural building block which could be present in at least some of the intermediate states of the reaction cycle (unpublished results of our group).

We note that a definitive structural model of the Mn_4Ca complex cannot be concluded from the crystallographic data alone, inter alia because of Mn reduction by X-ray exposure during data collection [18–20] and the coupled loss of Mn-(μ-O)$_2$-Mn motifs [20]. Results obtained by X-ray absorption spectroscopy [21–26], FTIR [27–31], EPR [32–38], mass spectroscopy [39,40], and computational methods [41–43] eventually may provide the complementary information which is needed for a complete model that includes the location of water molecules, protons, and oxidation equivalents. The above and other spectroscopic techniques (see, e.g., [44–51] and section 2.1) also may provide the needed insight into the reaction dynamics, i.e. the structural changes associated with advancement in the reaction cycle.

Apart from the internal structure of the Mn complex, several other aspects of the crystallographic model are of relevance for considerations on the mechanism of photosynthetic water oxidation.

(i) The Mn complex is surrounded by a protein matrix which separates the complex from the aqueous bulk phase. This protein matrix prohibits an instantaneous equilibration (i.e., in the ps or ns time domain) of the substrates (2 H_2O) and products (4 H^+, O_2) with the aqueous bulk phase.

(ii) There are, most likely, paths for proton release and water uptake which connect the lumenal phase to the Mn complex. Barber and coworkers [11,52] and others [12,53] proposed a proton transfer path which starts at the Asp 61 which neighbors the monomeric Mn ion in the model of Barber and coworkers. The rate-limiting steps in the proton movements towards the lumenal bulk phase seem to be relatively slow (>100 µs), as discussed further below, rendering the Mn complex well 'insulated' with respect to incidental proton loss or uptake. Thus, the transfer of a proton to the lumenal bulk phase may either occur before or after oxidation of the Mn complex by the Y_Z-radical, but not simultaneously.

(iii) The imidazole sidechain of Y_Z, which after formation of the $Y_Z^{\bullet}$ radical oxidizes the Mn complex, is at a distance of about 7 Å to the nearest Mn ion rendering direct abstraction of a H-atom from water ligated to Mn unlikely. Furthermore, there are no indications for a proton channel connecting Y_Z to the aqueous phase [11,53]. This finding in conjunction with spectroscopic data (see further below) suggests that the $Y_Z^{\bullet}$ formation is *not* followed by release of the imidazole proton to the bulk phase and subsequent abstraction of a proton from the substrate water by $Y_Z^{\bullet}$. Rather the imidazole proton may reversibly shift within a 'rocking' hydrogen bond towards the near His_{190} [54].

The points (i) to (iii) are not only implied by the crystallographic models but they are also in agreement with a large body of biochemical and biophysical results obtained in the last two decades. On this basis we conclude that, conceptually, the Mn complex can be considered to be an entity which is well separated from the lumenal bulk phase. The Y_Z-residue is close to the Mn complex but most likely does not abstract protons from the substrate water molecules. Thus we consider Y_Z not to be an intrinsic part of the catalytic center which directly facilitates the elementary chemical steps involved in O–O bond formation.

In 1970, Kok proposed an ingenious five-step reaction cycle [55], the now classical S-state cycle (Fig. 1B). The OEC is driven through its reaction cycle by the absorption of four photons. Starting in the dark-stable S_1-state, an absorbed photon causes the removal of an electron from the OEC until the S_4-state is reached and, in the $S_4 \rightarrow S_0$ transition, dioxygen is formed; the fourth photon closes the reaction cycle. This model describes the accumulation of four oxidizing equivalents prior to the O–O bond formation, but protonation-state changes are not considered. It remains a lasting achievement of Gerald Babcock that he shifted the focus. In his influential hydrogen-atom abstraction model, the accumulation of oxidizing equivalents occurs by a Mn oxidation that is directly coupled to the deprotonation of substrate water coordinated to manganese [56]. At a molecular basis this would be realized by moving a proton and an electron jointly in form of a hydrogen atom to the previously formed $Y_Z^{\bullet}$. The simplicity of this elegant model is appealing. The same coupled extraction of four electrons would take place four times until the S_4-state is reached where (i) four oxidizing equivalents would have been accumulated and (ii) two water molecules would be completely deprotonated.

The H-atom abstraction model is difficult to reconcile with the now available crystallographic models and, as outlined below, its simplicity is not met by the experimental data on electron transfer and proton release (Fig. 1B). Here an alternative basic model is discussed that involves alternating withdrawal of protons and electrons from the Mn complex of PSII. The regular sequence of alternating proton–electron abstractions explains the intricate experimental results on irregular properties of the individual S-state transitions straightforwardly and also bears mechanistic implications. The model has been introduced briefly elsewhere [57]. It roots, inter alia, in insights gained from the crystallographic model (points (i) to (iii)) and in recent results on a reaction intermediate formed in the crucial $S_3 \rightarrow S_0$ transition [8,57–60].

2. Review of S-state-transition properties and proton release

2.1. Experimental approaches

Each light flash causes formation of the Y_Z-radical which subsequently accepts an electron from the Mn_4Ca-complex. Application of a sequence of saturating flashes of visible light and analysis of the time courses of electron transfer from the Mn_4Ca-complex to $Y_Z^{\bullet +}$ measured after the 1st to 4th flash facilitates determination of the rate constants of the transitions $S_1 \rightarrow S_2$ (1st flash), $S_2 \rightarrow S_3$ (2nd flash), $S_3 => S_4 \rightarrow S_0$ (3rd flash), and $S_0 \rightarrow S_1$ (4th flash). These rate constants have been determined by time-resolved EPR (by Sauer, Babcock, Pace and their coworker; [61,62]), UV/Vis by Witt, Junge, Renger, Van Gorkom, Lavergne and their coworkers [63–68], and recently X-ray absorption measurements [8,69]. By monitoring the small UV/vis absorption changes, systematic studies of temperature dependence, pH-influence and kinetic isotope effect (H_2O/D_2O) have facilitated a kinetic characterization of the transition between the four semi-stable S-states (see below). Proton release has been investigated using pH electrodes or UV/vis absorption changes of pH-indicating dyes (see [65,70–72] and refs. therein). Electrochromic shifts of UV/vis absorption bands (Stark effect) of PSII pigments have provided insights in changes of the net charge of the PSII manganese complex [73–76]. The results which have been obtained by time-resolved spectroscopic techniques are crucial for construction and evaluation of mechanistic models of photosynthetic water oxidation. Some experimental findings on the properties of the individual S-state transitions are summarized in the following.

2.2. First flash: $S_1 \rightarrow S_2$ transition

Starting in the dark-stable S_1-state, the first photon induces the $S_1 \rightarrow S_2$ transition which involves Mn oxidation [77,78] (see also [23] and references therein). The first proton-release studies were carried out at neutral pH and no proton release associated with the $S_1 \rightarrow S_2$ transition was detected [79,80,81,82,83]. Later non-integer and pH-dependent proton release has been found to be explainable by electrostatically triggered pK-shifts of peripheral bases ([65,70–72] and refs. therein). Electrochromic studies suggest that, irrespective of pH, no charge compensating deprotonation occurs at the Mn_4Ca-complex [73,84,85]. The $S_1 \rightarrow S_2$ transition is characterized by a relatively low activation energy of ~15 kJ/mol in H_2O and D_2O [86] (12 kJ/mol in [87]) and can proceed even at cryogenic temperatures [88,89]. The rate constant is pH-independent and the kinetic isotope effect is small ($k_D/k_H \leq 1.3$). In conclusion, the $S_1 \rightarrow S_2$ transition involves electron transfer from the Mn_4Ca-complex to $Y_Z^{\bullet}$, but no proton removal from the donor-side redox factors.

2.3. Second flash: $S_2 \rightarrow S_3$ transition

The second photon initiates the $S_2 \rightarrow S_3$ transition, which differs in its kinetic properties pronouncedly from the $S_1 \rightarrow S_2$ transition. The pH-independent, 'obligatory' release of one proton as well as the electrochromism data showing the absence of charge accumulation suggest that the proton is released from the Mn complex ([65,70–72,90] and refs. therein). The rate constant is pH-dependent and the kinetic isotope effect of the $S_2 \rightarrow S_3$ transition is sizable (temperature-dependent k_D/k_H-ratio of 1.4 to 2.) and is clearly greater than in the $S_1 \rightarrow S_2$ transition [86]. Therefore it has been concluded that the $S_2 \rightarrow S_3$ transition is 'kinetically steered' by proton movements [86]. The activation energy is clearly greater than in the $S_1 \rightarrow S_2$ transition (35 kJ/mol in H_2O and 45 kJ/mol in D_2O [86]) and the transition is inhibited at low temperatures [88,89]. Noteworthy, with respect to its kinetic characteristics the $S_2 \rightarrow S_3$ transition may resemble the recently described $S_3 \rightarrow S_4$ transition which has been assigned to proton release from the Mn complex [8,59]. We propose that in the $S_2 \rightarrow S_3$ transition, after formation of the Y_Z radical and before Mn oxidation, a proton is removed from the Mn complex or its immediate ligand environment. This proton release is the rate-limiting step and thus determines the experimentally accessed kinetic properties of the $S_2 \rightarrow S_3$ transition.

2.4. Third flash: $S_3 \rightarrow S_4 \rightarrow S_4' \rightarrow S_0$ transition

Absorption of the third photon initiates the sequence of events leading to dioxygen formation and release with a halftime of about 1 ms. By time-resolved X-ray studies we have shown that within about 200 µs a kinetically resolvable intermediate is formed [8,59]; results obtained by other spectroscopic techniques are in agreement with this finding [86,91,92]. In [59] the experimental results on the $S_3 \rightarrow S_4$ transition are summarized and discussed in detail. Briefly, formation of the S_4-intermediate does not involve electron transfer from the Mn complex to the Y_Z radical but, most likely, a deprotonation at the Mn complex [8,59,91] (Fig. 2). We assigned the intermediate to the S_4-state of Kok's cycle, but due to its specific character the use of the S-state terminology may be inappropriate (see Supporting Online Material of [8] and [59,60]). In any event, formation of an essential, kinetically resolvable reaction intermediate does not occur by oxidation of the Mn complex but rather by a deprotonation step (Fig. 2, labeled by 'proton-only' in Fig. 1B).

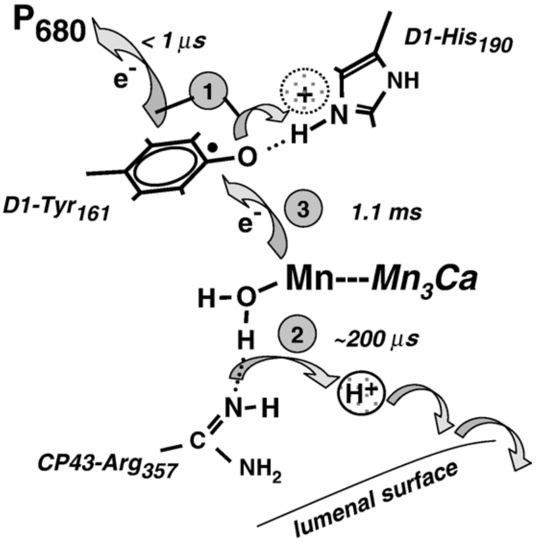

Fig. 2. Scheme for S_4-formation in the $S_3 \Rightarrow S_0$ transition (from ref. [8]). Three steps are considered: (1) Absorption of a photon is followed by Y_Z oxidation within less than 1 µs, a process coupled to a proton shift within the hydrogen bond to a histidine residue (His190 of the D1 protein). (2) The positive charge stemming from Y_Z oxidation promotes, with a halftime of about 200 µs, proton shifts and deprotonation of Arg 357 of the CP43 protein. This assignment of the deprotonating group is hypothetical, but plausible. The proton is moving in a bucket-brigade-type mechanism along a specific proton path towards the lumenal surface. (3) S_4-formation is followed by electron transfer to $Y_Z^{\bullet}$. This process formally corresponds to formation of the S_4' state in Fig. 1B, but is kinetically indistinguishable from Mn-reduction and O_2-formation (identical halftimes of ~1.1 ms).

The subsequent electron transfer from the Mn complex to Y_Z ($S_4 \rightarrow S_4'$ transition) is assumed to be the rate-limiting step in dioxygen formation ($t_{1/2}$ of about 1.2 ms); it is followed by the rapid onset of water oxidation and Mn reduction. The millisecond rate constant is pH-independent and exhibits a small ($k_D/k_H = \sim 1.4$ in [86]) or even negligible ($k_D/k_H < 1.2$ in [93]) kinetic isotope effect. It thus resembles the rate constant of the electron transfer in the $S_1 \rightarrow S_2$ transition in its kinetic properties. A difference lies in the clearly greater activation energy of the millisecond transition, which is possibly explainable by a greater reorganization energy due to more substantial structural changes of the manganese complex of PSII (see [21,94] and references therein). Time-resolved proton release measurements suggest that a proton is released from the Mn complex in the millisecond time domain [65,86,95]. Interestingly, in D_2O [86] or at pH-values around pH 6.3 [95] the proton release seems to be delayed with respect to the Mn-reduction and dioxygen formation.

2.5. Fourth flash: $S_0 \rightarrow S_1$ transition

The fourth photon closes the described cycle by promoting the $S_0 \rightarrow S_1$ transition. Investigations on this transition are hampered by S-state mixing resulting from the inevitable miss events on preceding flashes, possibly explaining that mutually not fully consistent pH-dependent proton stoichiometries have been reported [65,70–72]. Recently Schlodder and Witt detected a pH-independent release of a single proton by microelectrode measurements on PSII core complexes of the cyanobacterium *Synechococcus elongatus* [70]. Electrochromic measurements suggest that, irrespective of the lumenal pH, no charge is accumulated in the $S_0 \rightarrow S_1$ transition [73,84,85]. On the basis of this finding and in agreement with the pH-independent proton release reported in [70], we conclude that the $S_0 \rightarrow S_1$ transition most likely involves oxidation of the Mn complex as well as a charge-compensating deprotonation of or close to the Mn complex. As is the case for the electron transfer to the Y_Z radical in the $S_1 \rightarrow S_2$ transition, the rate constant seems to be essentially pH-independent and, in comparison to the $S_2 \rightarrow S_3$ transition, insensitive to H_2O/D_2O exchange; its activation energy is relatively low [86,87]. Thus the properties of the $S_0 \rightarrow S_1$ rate constant suggest an electron transfer which is not limited by proton movements, whereas the proton release data points towards a charge compensating deprotonation. In summary, the experimental results on the $S_0 \rightarrow S_1$ transition of the Mn complex can be rationalized by assuming a rate-limiting ET followed by a deprotonation step and proton release into the lumenal bulk phase.

2.6. Irregular properties of the S-state transitions

The kinetic properties of the individual S-state transitions differ with respect to activation energy, pH-sensitivity and kinetic isotope effect, as described above. Only in the $S_2 \rightarrow S_3$ transition, the ET from the Mn complex to $Y_Z^{\bullet}$ seems to be 'kinetically steered' by a proton and only the $S_1 \rightarrow S_2$ transition can proceed at cryogenic temperatures. In the course of the $S_3 \rightarrow S_0$ transition, electron transfer and O–O bond formation ($S_4' \rightarrow S_0$ in Fig. 1B) likely is preceded by an essential deprotonation step ($S_3 \rightarrow S_4$ in Fig. 1B) and presumably followed by a second deprotonation step. The yield of the $S_3 \rightarrow S_0$ transition is pronouncedly pH-dependent [96], but the millisecond rate constant of dioxygen formation is pH-independent. For the transitions between the four semi-stable S-states (S_1, S_2, S_3 and S_0) the stoichiometry of proton release from the Mn complex or its immediate environment – the intrinsic proton release pattern – seems to be 0:1:2:1 (for $S_1 \rightarrow S_2$, $S_2 \rightarrow S_3$, $S_3 \rightarrow S_0$, and $S_0 \rightarrow S_1$). Furthermore, the S-states differ not only in their rate of formation, but also in the lifetime of the individual S-states and likely also in their equilibrium redox potential. Whereas the S_1-state is stable in the dark and the S_0-state lifetime may reach several hours, the lifetime of the S_2 and S_3 state is in the range of tens of seconds [97]. Slow oxidation of the S_0-state towards the S_1-state by the Y_D-radical is possible, whereas in the S_2- and S_3-state the Mn complex can oxidize Y_D resulting in $Y_D^{\bullet}$ formation [98]. Some aspects of the heterogeneity in the properties of the individual S-state transitions are schematically summarized in Fig. 1B.

3. Framework model of alternating proton and electron removal

3.1. The 'Mn-complex' entity

The intermediates states in the proposed basic reaction cycle of photosynthetic dioxygen formation are states of the *Mn complex*. A definition of the entity denoted as 'Mn complex' thus is required and provided in the following.

The Mn complex is the catalytic site of dioxygen formation. It consists not only of the four manganese, the single calcium ion and their bridging ligands, but also of the ligand environment of the five metals ions including all residues and water molecules that are directly involved in the elementary steps of dioxygen formation. Thus the Mn complex includes amino acid residues and water species directly ligated to manganese as well as crucial water molecules and residues that interact via hydrogen bonds with the immediate manganese ligands. The binding sites of the 'substrate water' molecules (and the bound substrate water itself) are considered to be part of the Mn complex. However, we explicitly assume (or define) that the Y_Z residue, even though close and possibly in hydrogen-bonding distance to a water ligand of the Ca ion, is not an intrinsic part of the Mn complex. (We deliberately use the term 'Mn complex' instead of 'oxgyen-evolving complex' because the latter also had been used to denote an entity including both the Mn complex and Y_Z.)

3.2. Outline of the model

A simple principle may be hidden underneath the outwardly complex and irregular events of the S-state cycle: *protons and electrons are removed strictly alternately from the Mn complex*. This assumption leads directly to a basic reaction cycle which extends the classical S-state cycle by taking into account explicitly the proton release from the Mn complex [60]. The corresponding reaction cycle comprises nine states and is shown in Fig. 3. Starting in the I_0-state, eight successive steps of strictly alternate proton and electron removal lead to the I_8-state where four electrons and four protons have been removed. Thus, four oxidizing equivalents as well as four bases (i.e., deprotonated groups) have been accumulated prior to O_2-release in the $I_8 \rightarrow I_0$ transition.

We emphasize that the *I*-states in the reaction cycle of Fig. 3 refer exclusively to states of the Mn complex. In Fig. 3, it is indicated where in the reaction sequence electrons and protons are removed from the *Mn complex*, where O_2 is released, and where the substrate water may enter the Mn complex. The *I*-cycle straightforwardly explains the kinetic data discussed in Section 2.1, as the reader may confirm by a step-by-step comparison of the properties of the classical S-state cycle (see Fig. 1B and Section 2) to the respective *I*-cycle transitions (Fig. 3).

3.3. Regularity of the I-cycle

The proposed sequence of alternating proton and electron removal from the Mn complex is appealing due to its regularity. How can it explain the intricate and strikingly irregular properties of the classical S-state cycle? The *I*-cycle is irregular with respect to the relation between *I*-cycle transitions and the classical S-state transitions. The number of *I*-cycle transitions per S-state transition differs; there are two in $S_0 \rightarrow S_1$, one in $S_1 \rightarrow S_2$, two in $S_2 \rightarrow S_3$, and four in $S_3 \rightarrow S_0$. (i) In the *I*-cycle model, in the $S_0 \rightarrow S_1$ transition first an electron is transferred from the Mn complex to $Y_Z^{\bullet +}$ ($I_1 \rightarrow I_2$) and then a proton is released to the lumenal bulk phase ($I_2 \rightarrow I_3$). However not in each of the classical S-state transitions the Mn complex is oxidized and one proton is subsequently released from the Mn complex into the lumenal bulk phase. (ii) Specifically in the $S_1 \rightarrow S_2$ transition, manganese oxidation without associated proton release is observed ($I_3 \rightarrow I_4$) so that exclusively in this S-state transition a positive charge is accumulated. (iii) We propose that in the subsequent S-state transition, i.e. $S_2 \rightarrow S_3$ ($I_4 \rightarrow I_5 \rightarrow I_6$) the sequence of oxidation and proton removal from the Mn complex is reversed (proton-first) in comparison to the $S_0 \rightarrow S_1$ transition (electron-first). (iv) In the transition between the semi-stable states S_3 and S_0, in the *I*-cycle scheme first a proton is released ($I_6 \rightarrow I_7$), then electron transfer ($I_7 \rightarrow I_8$) and dioxygen formation ($I_8 \rightarrow I_0$) takes place and only subsequently a second proton ($I_0 \rightarrow I_1$) is released. Thus in the $S_3 \rightarrow S_0$ transition, overall two protons are released and the positive charge of the Mn complex previously gained in the $S_1 \rightarrow S_2$ transition is lost.

In conclusion, in the *I*-cycle scheme the irregularity in the properties of the classical S-state cycle is *not* removed by considering the experimental results (as summarized in section 2.) to be a misleading reflection of the real events at the Mn complex. Rather we explain the intricate experimental findings

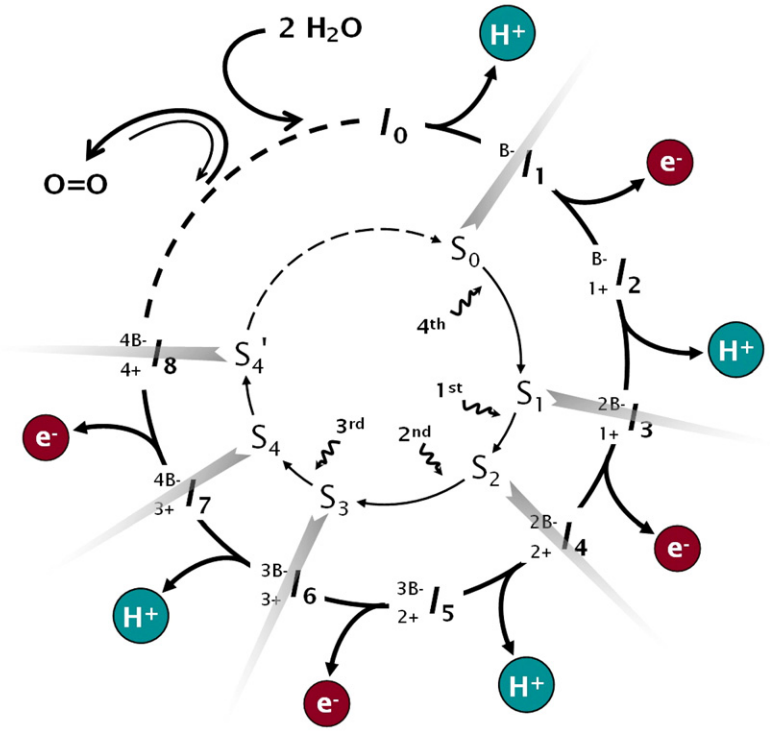

Fig. 3. Basic reaction cycle of the Mn complex of photosynthetic water oxidation. In eight steps protons and electrons are alternately removed from the Mn complex of PSII. The electrons are transferred to the Y_Z-radical; the protons are eventually released into the lumenal bulk phase. The left subscript and superscript indicate the number of oxidizing equivalents and bases, respectively, which have been accumulated by the Mn complex. (Notably, experiments at elevated oxygen pressure [100,101] suggest a possibly peroxidic intermediate in the $I_8 \rightarrow I_0$ transition, which is not indicated in the above scheme; it differs from all the depicted *I*-states [60,99,102]. Also distinct states acquired by exposure of PSII to exogenous reductants [103] or in the course of the photoactivation process [104] are not considered.)

by assuming that the irregularity in the events following the $Y_Z^{\bullet}$ formation merely obscures the intrinsically regular sequence of alternating removal of protons and electrons from the Mn complex.

3.4. Dual role of the Y_Z radical

The $Y_Z^{\bullet}$ radical is assumed to act not only as an electron acceptor, but also as an 'electrostatic promoter' [105] of crucial deprotonation reactions. In the reaction cycle of Fig. 3, the $Y_Z^{\bullet}$ state formed within less than 1 μs after photon absorption represents the starting point for the transitions $I_1 \rightarrow I_2$, $I_3 \rightarrow I_4$, $I_4 \rightarrow I_5$, and $I_6 \rightarrow I_7$. In the transitions $I_1 \rightarrow I_2$ and $I_3 \rightarrow I_4$, $Y_Z^{\bullet}$ formation is directly followed by electron transfer from the Mn complex. In the transitions $I_4 \rightarrow I_5$ and $I_6 \rightarrow I_7$, however, the positive charge of the $[Y_Z^{\bullet}\text{–H–His}_{190}]^+$ entity causes the release of a proton from the Mn complex to the bulk phase. Only subsequently ($I_5 \rightarrow I_6$ and $I_7 \rightarrow I_8$ transition, respectively) an electron is transferred from the Mn complex to $Y_Z^{\bullet}$. Evidence for a *proton-first ET* has been reported for the $S_3 \rightarrow S_4 \rightarrow S_4'$ transition (or $I_6 \rightarrow I_7 \rightarrow I_8$), as discussed further above. For the $S_2 \rightarrow S_3$ transition ($I_3 \rightarrow I_4 \rightarrow I_5$), the kinetic properties are compatible with an analogous deprotonation at the Mn complex preceding electron transfer to $Y_Z^{\bullet}$, but experimental proof for a kinetically resolvable I_4-intermediate is still lacking.

We emphasize that in the proposed model the $Y_Z^{\bullet}$ radical acts as an electron acceptor and in addition triggers proton release from the Mn complex, but it does not abstract a hydrogen atom from the Mn complex as has been proposed in the context of the hydrogen-atom abstraction model of Babcock and coworkers [56]. The $Y_Z^{\bullet}$ formation likely is coupled to a shift in the position of the phenoxyl proton towards the nearby His_{190} [54], but the proton is *not* released into the lumenal bulk phase [105]. Consequently, the $Y_Z^{\bullet}\text{–His}_{190}$ entity acquires a positive charge upon Y_Z oxidation and can electrostatically cause pK-shifts that facilitate proton release. Proton release from the Mn complex to the lumenal bulk phase necessarily involves a sequence of proton transfer steps. We propose that the release of peripheral protons precedes the deprotonation at the Mn complex and that the defect created by the initial release event is transferred in a bucket-brigade-type mechanism to the Mn complex.

3.5. Relation between electron and proton transfer

In the *I*-cycle model it is explicitly assumed that the electron and proton removal from the Mn-complex entity is a sequential, but not a concerted process. When discussing this aspect of the *I*-cycle model, it is important to discriminate between proton movements from the Mn-complex entity to the lumenal bulk phase, on the one hand, and proton-shifts or proton transfer within the Mn complex, on the other hand. Taking into account the separation of the Mn complex from the lumenal bulk phase, concerted ET and proton release *to the bulk* can be safely excluded so that these processes are necessarily sequential. However, it is conceivable that the ET from the Mn complex to the Y_Z radical is coupled to *internal* proton shifts within the Mn complex. Hence the possibility of electron transfer from the Mn complex to $Y_Z^{\bullet +}$ coupled to (i) a concerted proton movement at the Y_Z (shift of the proton in the hydrogen bond from O–Y_Z to N–His_{190}) and (ii) a concerted proton movement within the Mn complex, is not *per-se* excluded by the *I*-cycle scheme. In the $I_1 \rightarrow I_2$ and $I_3 \rightarrow I_4$ transitions, we do not see any need to invoke an internal concerted electron–proton process. In the $I_5 \rightarrow I_6$ and $I_7 \rightarrow I_8$ transitions, however, an internal base may be created in the preceding bulk-phase proton release which subsequently acts as a proton acceptor in an concerted electron–proton transfer. This important aspect needs to be addressed by further experimental and theoretical studies.

3.6. Mechanistic implications

The *I*-cycle model does not only explain the kinetic data straightforwardly, but also we can address two central mechanistic questions [106]: (1) the redox-potential problem and (2) the acceptor-base hypothesis.

3.6.1. The redox-potential problem

In four successive oxidation steps the Mn complex accumulates oxidizing equivalents. In each of these steps the oxidant is the $Y_Z^{\bullet}$-radical with a redox potential close to +1.1 V [44,107] implying an approximately constant potential also for the Mn complex in the ET to Y_Z (around +1.0 V). However, a redox-potential increase by 0.5 to 1 V is predicted for pure oxidation of the Mn complex that would impair subsequent oxidation of the complex by $Y_Z^{\bullet}$ ('redox-potential problem' described in [106]; see also [108] and references therein). In the *I*-state model, the potential increase is prevented by the four charge-compensating and potential-lowering deprotonation events that are interlaced with the oxidation steps. (From the viewpoint of protein electrostatics, the redox-potential problem is related to the energetic costs of extracting an electron from the already positively charged Mn complex.) The Mn complex is uncharged and its potential is below 1.1 V in the states I_1, I_3, I_5, and I_7 and thus prior to each of the four steps of electron transfer to $Y_Z^{\bullet}$. The redox potentials in the individual states of the I-cycle are schematically depicted in Fig. 4 and discussed in the following.

(i) In the S_0-state (I_1), the potential of the Mn complex is assumed to be below 1.1 V so that the ET to $Y_Z^{\bullet}$ in the $I_1 \rightarrow I_2$ transition is energetically feasible. The resulting Mn oxidation charges the Mn complex and raises its potential to a level which is prohibitively high (>> 1.1 V) for ET to $Y_Z^{\bullet}$. In the *I*-cycle model, the Mn oxidation which results in formation of the high-potential I_2-state is followed by a deprotonation step ($I_2 \rightarrow I_3$) which restores charge neutrality and lowers the Mn-complex potential to a value below 1.1 V in the semi-stable I_3-state (equals S_1). Thereby, Mn oxidation in the $S_1 \rightarrow S_2$ (or $I_3 \rightarrow I_4$) transition becomes feasible.

(ii) As opposed to the $S_0 \rightarrow S_1$ transition, in the $S_1 \rightarrow S_2$ transition the ET to $Y_Z^{\bullet}$ is not immediately followed by a potential-lowering proton release. This is the only transition between semi-stable S-states which is not

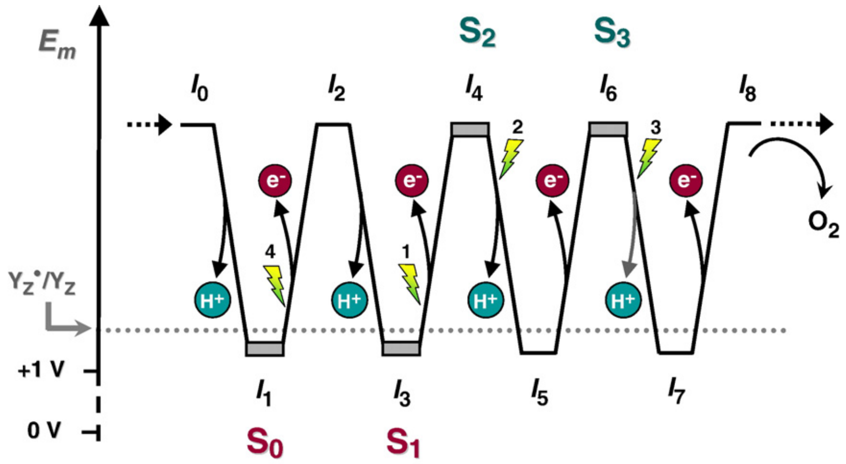

Fig. 4. Redox-potential scheme for the Mn complex. For each I-state, the potential for a *subsequent* oxidation by the $Y_Z^{\bullet}$-radical is indicated. In PSII, the oxidation steps can proceed only if the potential of the Mn complex is less positive than the indicated potential of the $Y_Z^{\bullet}/Y_Z$ redox couple.

associated with any proton release from the Mn complex (but pH-dependent deprotonation of peripheral bases [65]) and thus is described in the *I*-cycle model by a single transition. Consequently, in the semi-stable S_2-state (or I_4) the Mn complex is positively charged, in comparison to the S_0-state complex, and its potential is prohibitively high for immediate oxidation of the Mn complex by $Y_Z^{\bullet}$ ($>> 1.1$ V). How can the $S_2 \rightarrow S_3$ transition proceed?

(iii) The *I*-cycle scheme predicts that in the $S_2 \rightarrow S_3$ transition, $Y_Z^{\bullet}$ formation is followed by a proton release ($I_4 \rightarrow I_5$) which lowers the potential of the Mn complex and thereby facilitates the subsequent ET step ($I_5 \rightarrow I_6$). (The $I_5 \rightarrow I_6$-step may or may not be coupled to an internal proton transfer within the Mn complex, as discussed above.) The resulting semi-stable I_6-state corresponds to the classical S_3-state. The S_3-state is a charged, high-potential S-state ($E_m >> 1.1$ V) comparable to the S_2-state, but different from the low-potential S_0-state.

(iv) The transition between the semi-stable states S_3 and S_0 involves four transitions in the *I*-cycle scheme. In analogy to the $S_2 \rightarrow S_3$ transition, a potential-lowering proton release in the $I_6 \rightarrow I_7$ transition (or $S_3 \rightarrow S_4$) is the prerequisite for the ET step in the $I_7 \rightarrow I_8$ transition (or $S_4 \rightarrow S_4'$), which is kinetically not separable from dioxygen formation and release in the $I_8 \rightarrow I_0$ transition. In the subsequent $I_0 \rightarrow I_1$, water binding and reorganization of the Mn complex after dioxygen formation is associated with release of another proton. Thereby the positive charge acquired in the $S_1 \rightarrow S_2$ transition is lost, resulting in an uncharged, low-potential S_0-state.

In summary, about equal values of the Mn-complex redox potential in each ET to $Y_Z^{\bullet}$ does not imply equal potential in each of the four classical S-states. Of the four semi-stable S-states, the S_0 and S_1 are uncharged, low-potential states ($E_m \sim 1.0$ V) whereas the S_2 and S_3 are charged, high-potential states ($E_m >> 1.1$ V). This explains straightforwardly previously reported differences in the properties of the Mn complex in S_0 and S_1, on the one hand, and in S_2 and S_3, on the other hand. The low-potential S-states (S_0 and S_1) are long-term dark-stable whereas the high-potential states (S_2 and S_3) decay in the second and minute time domain by recombination with reducing equivalents at the PSII acceptor side (and presumably also by oxidation of external reductants). The Mn complex in the states S_2 and S_3 can oxidize Y_D, a redox-active tyrosine located in the D2-subunit of PSII, whereas the S_0-state complex can become oxidized to the S_1-state by $Y_D^{\bullet}$, the oxidized form of Y_D. Also the sensitivity to X-ray photoreduction (equally high in S_2 and S_3, but clearly lower in S_0 and S_1, see [20] and supporting material of [94]) likely represents a manifestation of the potential differences.

3.6.2. Accumulation of proton-acceptors

Since the Mn complex cannot rapidly exchange protons with the aequeous bulk-phase, the entropy gain resulting from 'dilution' of protons cannot support the elementary steps of dioxygen formation in the $S_3 \rightarrow S_0$ transition (see [106,109,110], Dau and Haumann, in preparation). Consequently, to account for the given energetic restraints [106,109,110], (i) either protons are removed from the substrate water molecules prior to I_8-formation or (ii) proton acceptors are 'prepared' in the proton-release steps of the *I*-cycle. Water exchange experiments suggest that deprotonation of substrate water does not occur in the $S_2 \rightarrow S_3$ transition [39,111,112], and supposedly not at any point prior to formation of the S_3-state. Thus we propose that in the I_8-state not only 4 oxidizing equivalents have been accumulated, but also 4 bases (B_1 to B_4 in the transitions $I_0 \rightarrow I_1$, $I_2 \rightarrow I_3$, $I_4 \rightarrow I_5$ and $I_6 \rightarrow I_7$, respectively). The subsequent O_2-formation step ($I_8 \rightarrow I_0$) involves the transfer of 4 electrons to high-valent Mn (Mn reduction) and 4 protons to the 4 acceptor bases. On basis of X-ray absorption data [21,94], we have proposed that B_2 and B_3 are oxygens in bridging position between Mn ions (μ-oxo bridges). This would be energetically particularly favorable in the O_2-formation step, as discussed in detail elsewhere [106]. The proton acceptor B_4 has been hypothesized to be a specific arginine residues (Arg-357 of the CP 43 protein [7,8,113]), but also the Asp-61 of the

D1 protein of PSII is a candidate. The formation of B_1 likely is related to the rearrangements within the Mn complex following O_2-release and subsequent water binding, but the chemical identity of B_1 is completely unknown.

We note that the basic mechanism of how the redox-potential problem is solved in PSII is directly implied by the I-cycle model. On the contrary, the above considerations on acceptor bases are not a necessary consequence of the reaction cycle of Fig. 3. The accumulation of *four* acceptor bases proposed by us – as opposed to substrate water deprotonation in the transitions from I_0 to I_8 – represents a working hypothesis which contrasts with the majority of past proposals on the mechanism of photosynthetic oxidation. Also the creation of acceptor bases on some transitions and deprotonation of substrate water on others is conceivable and has been proposed [7,21,113,114]. Further experimental and theoretical studies are required to clarify this central mechanistic question.

3.7. Concluding remark

The simple assumption of alternate extraction of electrons and protons from the Mn complex enables a straightforward explanation of experimental results and results in a basic mechanistic model of photosynthetic water oxidation. This framework model is largely based on experimental results but there remain hypothetical aspects, which should be scrutinized. The crucial challenge, however, now lies in elucidation of how the proposed basic model is realized at the atomic level. Obvious open questions relate to the chemical identity of the acceptor bases and the details of the proton movements. We believe that elucidation of the structure of *all nine* intermediates of the reaction cycle – including location of the water molecules, protonation states, and localization of oxidizing equivalents – eventually may facilitate an unambiguous answer to the question of how water is oxidized in oxygenic photosynthesis.

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