Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochimica et Biophysica Acta 1655 (2004) 195–204
BIOCHIMICA ET BIOPHYSICA ACTA
BBA
www.bba-direct.com

Review

Coupling of electron and proton transfer in oxidative water cleavage in photosynthesis

G. Renger*

Max-Volmer-Laboratory of the Institute of Chemistry, Technical University Berlin, PC 14, Straße des 17 Juni 135, D-10623 Berlin, Germany

Received 23 April 2003; received in revised form 23 July 2003; accepted 23 July 2003

Abstract

This minireview addresses questions on the mechanism of oxidative water cleavage with special emphasis on the coupling of electron (ET) and proton transfer (PT) of each individual redox step of the reaction sequence and on the mode of O–O bond formation. The following topics are discussed: (1) the multiphasic kinetics of Y_Z^{ox} formation by $P680^{+\bullet}$ originate from three different types of rate limitations: (i) nonadiabatic electron transfer for the "fast" ns reaction, (ii) local "dielectric" relaxation for the "slow" ns reaction, and (iii) "large-scale" proton shift for the μs kinetics; (2) the ET/PT-coupling mode of the individual redox transitions within the water oxidizing complex (WOC) driven by Y_Z^{ox} is assumed to depend on the redox state S_i: the oxidation steps of S_0 and S_1 comprise separate ET and PT pathways while those of S_2 and S_3 take place via proton-coupled electron transfer (PCET) analogous to Jerry Babcock's hydrogen atom abstractor model [Biochim. Biophys. Acta, 1458 (2000) 199]; (3) S_3 is postulated to be a multistate redox level of the WOC with fast dynamic equilibria of both redox isomerism and proton tautomerism. The primary event in the essential O–O bond formation is the population of a state $S_3(P)$ characterized by an electronic configuration and nuclear geometry that corresponds with a complexed hydrogen peroxide; (4) the peroxidic type $S_3(P)$ is the entatic state for formation of complexed molecular oxygen through S_3 oxidation by Y_Z^{ox}; and (5) the protein matrix itself is proposed to exert catalytic activity by functioning as "PCET director". The WOC is envisaged as a supermolecule that is especially tailored for oxidative water cleavage and acts as a molecular machine.

Keywords: O–O bond formation; Primary donor; Redox active tyrosine; Redox isomerism; Tautomerism; Water cleavage

1. Introduction

The essential steps of photosynthetic water cleavage take place within the multimeric protein complex of Photosystem II (PS II) that acts as water–plastoquinone oxidoreductase and is anisotropically incorporated into the thylakoid membrane [1]. Recently, great achievements have been made in unraveling the structural features and the spatial arrangement of the cofactors by crystallization of PS II core complexes from thermophilic cyanobacteria *Thermosynechococcus* (*T.*) *elongatus* and *Thermosynechococcus vulcanus* and X-ray structure analysis. At present, structural data are available at a resolution of 3.8 [2] and 3.7 Å [3], respectively.

Two functional elements of PS II are specific for oxygen-evolving species and unique among all photosynthetic organisms: (a) a special chlorophyll-*a* complex P680 with the exceptionally strong oxidizing power of its cation radical $P680^{+\bullet}$ and (b) a catalytic unit (symbolized by WOC) for oxidative cleavage of two water molecules into molecular oxygen and four protons that are released into the thylakoid lumen.

The "inner sanctum" of the water oxidizing complex (WOC) is a redox active Mn_4–Ca unit embedded into the heterodimeric protein matrix of polypeptides D1 and D2 that also carries all other cofactors required for this process (for reviews, see Refs. [1,4,5]). In addition to the D1/D2 heterodimer the large lumen exposed loops E of polypeptides CP43 and CP47 and the extrinsic 33 kDa proteins are essential constituents for the assembly and/or stability of the WOC and regulation of the system (see Ref. [6] and references therein). These components are present in all oxygen-evolving organisms while two additional regulatory extrinsic subunits were replaced by entirely new proteins during evolution (see Ref. [7] and references therein).

* Tel.: +49-30-314-22794; fax: +49-30-314-21122.
E-mail address: renger@pc-109ws.chem.tu-berlin.de (G. Renger).

0005-2728/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbabio.2003.07.007

The oxidative water cleavage occurs via a pathway referred to as Kok cycle [8]. This "clock" comprises five redox states of the WOC referred to as S_i ($i=0,...4$). The sequence of oxidation steps is energetically driven by $P680^{+\bullet}$ with Tyr residue 161 of polypeptide D1 (vide infra) acting as redox mediator [1,4,5]. The overall reaction pattern summarized in Fig. 1 shows not only the redox states of the Kok-cycle but in addition "superreduced" states S_{-i}. At present, states down to S_{-3} are well characterized (see Ref. [9] and references therein); preliminary evidence for S_{-4} and S_{-5} has also been reported [10] but remains to be confirmed by further studies. These "superreduced" states are assumed to be involved as intermediates in the pathway of photoactivation [11,12].

The unraveling of three points is of key relevance for a deeper understanding of the mechanism of oxidative water cleavage (for a more detailed list, see Ref. [13]): (i) the coupling between electron and proton transfer steps of both Y_Z^{ox} formation and subsequent oxidative S_i state transitions, (ii) the electronic configuration and nuclear geometry of each redox state S_i, and (iii) the reaction coordinate for oxygen–oxygen bond formation.

This minireview (for a personal perspective on the history of our growing knowledge, see Ref. [14]) addresses three aspects on the basis of our current stage of knowledge and with special emphasis of the seminal work of Jerry Babcock on the functional role of Y_Z, its identification and the properties of Y_Z^{ox}. The following topics will be discussed by using the scheme of Fig. 1 as a guiding line: (a) kinetics and mechanism of Y_Z^{ox} formation with $P680^{+\bullet}$ as oxidant, (b) kinetics and mechanism of S_i state oxidation by Y_Z^{ox} and (c) hypothesis of S_3 as to be a multistate redox level that acts in its peroxidic type form $S_3(P)$ as entatic state for formation of complexed O_2.

2. Kinetics and mechanism of Y_Z^{ox} formation with $P680^{+\bullet}$ as oxidant

The unraveling of the nature of Y_Z and its identification as tyrosine residue Y161 of polypeptide D1 has been achieved by Jerry Babcock et al. [15–17]. He also showed by EPR studies that reduction of $P680^{+\bullet}$ and oxidation of Y_Z exhibit coincidental kinetics in PS II preparations deprived of an intact WOC and therefore Y_Z is acting as direct reductant of $P680^{+\bullet}$ [18]. This conclusion was confirmed by complementary measurements of transient optical absorption changes in the UV/NIR region [19]. Using the latter approach at higher time resolution, the kinetical coincidence of $P680^{+\bullet}$ reduction and Y_Z oxidation was also detected in samples with an intact WOC [20].

It is important to note that Y_Z is never the only reductant of $P680^{+\bullet}$ because $Q_A^{-\bullet}$ always competes as electron donor to $P680^{+\bullet}$. Therefore, the kinetics strongly depend on the functional state of Y_Z and integrity of the WOC. If Y_Z^{ox} stays oxidized, $P680^{+\bullet}$ becomes reduced by $Q_A^{-\bullet}$ with a half lifetime of 100–200 μs [21,22]. It represents a dissipative electrogenic back reaction [23,24] and exhibits virtually no kinetic H/D isotope exchange effect [25]. An indispensable consequence of this dissipative pathway are incomplete S_i redox transitions of the WOC. A recent study reveals that $P680^{+\bullet}$ $Q_A^{-\bullet}$ recombination accounts to a large extent if not completely for the probability of misses (α) in the physiological pH range of the lumen (neutral to moderately acidic)

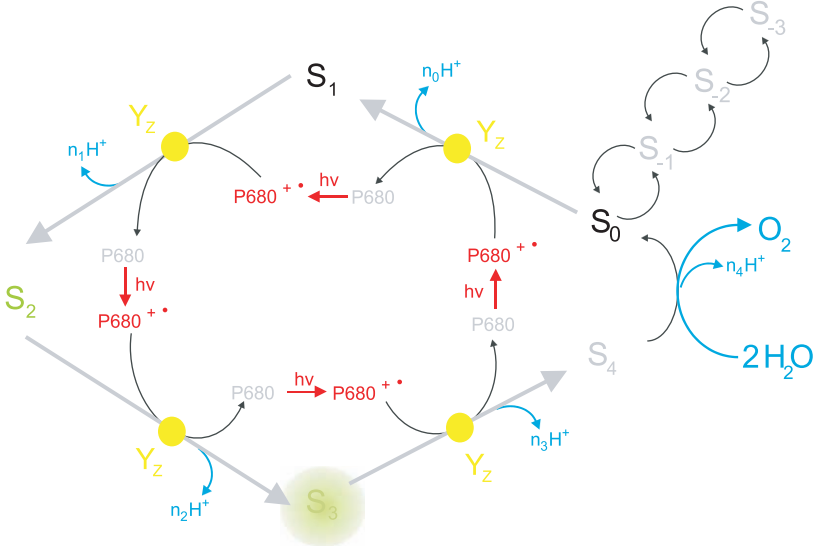

Fig. 1. Extended Kok-cycle of oxidative water cleavage. The photooxidation of P680 is marked in red, the redox mediator Y_Z in yellow and substrate ($2H_2O$)/products ($O_2/4H^+$) in blue, the metastable redox states S_2 and S_3 in green and superreduced states (S_{-1}, S_{-2}, S_{-3}) in grey. Recently, evidence has been presented for the population of S_{-2} and S_{-3} in frost-sensitive plants under cold stress [113]. For the sake of simplicity, the slow dark relaxation reactions of S_0, S_2 and S_3 are omitted (for a review, see Ref. [1]).

[26]. Minimum α-values of 6–8% were observed in intact spinach thylakoids at 4 °C and pH 6.5 [27]. Interestingly, virtually the same low α-values were recently found in thylakoids from *T. elongatus* but in this case the α-minimum is attained at 25 °C rather that at 4 °C [28]. The $P680^{+\bullet}$ reduction by Y_Z is characterized by three striking features: (i) a surprisingly complex multiphasic time course in the ns/μs time domain [29–31], (ii) a marked dependence on the redox state S_i of the WOC [29,31,32], and (iii) a drastic change of the reaction coordinate in samples lacking a functionally competent WOC as reflected by a marked kinetic H/D isotope exchange effect [25,33] and a comparatively high activation energy [33–35]. In the following, only the characteristics of systems with an intact WOC will be briefly discussed.

In general, basically two types of mechanisms can be responsible for the strikingly multiphasic kinetics of $P680^{+\bullet}$ reduction by Y_Z: (i) sample heterogeneity in the time domain of the reaction, or (ii) homogeneous process including different types of sequential rate limitation. The former mechanism has been discussed in terms of slow transitions (order of 10 ms) between particular conformational states [36] but the latter appears to be more likely when taking into account the different kinetic H/D isotope effects (vide infra) and the similarity of the kinetic pattern in different sample preparations. Therefore, the following considerations will be based on the second mechanism. Essentially three types of kinetics are relevant for further considerations: (i) "fast" ns-component (20–50 ns), (ii) "slow" ns-component (300–600 ns) and (iii) μs-components.

2.1. Mechanism of $P680^{+\bullet}$ reduction by Y_Z via "fast" ns kinetics

The extent of the "fast" ns kinetics depends on the oxidation states S_i of the WOC with markedly larger normalized amplitudes in S_0 and S_1 than in S_2 and S_3 [31,32]. In the dark-adaptated state S_1 [37] $P680^{+\bullet}$ reduction by Y_Z is dominated by "fast" ns-kinetics with 20 ns lifetime originally reported in Ref. [38]. The most interesting feature of this "fast" ns component is its rather small activation energy of about 10 kJ/mol [32] in the range of 248–295 K. A similarly weak temperature dependence was found at all S_i-states of the WOC in $271 < T \leq 298$ K [39]. This general phenomenon of small E_A values was later confirmed by others [40]. However, the authors of Ref. [40] now question their own data for the special case of the WOC attaining S_2 and report a value of about 25 kJ/mol for E_A [41]. Our recent data (P. Kühn, H.-J. Eckert and G. Renger, unpublished results) are not in favor with this result. There is no reason for a drastic revision of our original conclusions on comparatively small E_A values, in marked contrast to the statement of Ref. [41].

Based on the rather small E_A value a mechanism was discussed for the "fast" ns kinetics where the proton of the OH group of Y_Z is shifted within a hydrogen bond to a nearby base X [32]. Several studies provided convincing evidence that His 190 of polypeptide D1 is of key relevance for Y_Z oxidation by $P680^{+\bullet}$ [42–46]. Although most studies were performed with systems lacking an intact WOC, the results suggest that His 190 is the most likely candidate to act as base X for proton transfer in the "fast" ns reaction. Analyses within the framework of the Marcus theory of nonadiabatic electron transfer [47] led to the conclusion that the "fast" ns kinetics is limited by electron transfer [35,48]. This idea is supported by the lack of a kinetic H/D isotope exchange effect [49] and the reorganization energy λ of 0.5–0.6 eV [35,50]. As a consequence, the proton shift within the presumed hydrogen bond has to take place in <20 ns, and questions arise on its nature. In the most simple scenario, the phenolic proton is directly attached to one N-atom of His 190. Depending on the structure, two different configurations have to be considered [26]. In the case of a conventional hydrogen bond the pK values of Y_Z and H190 have to be properly tuned by the protein matrix so that ΔpK is significantly smaller than 4.0 to satisfy the requirements of the transition state theory for a proton shift in <20 ns (see Ref. [51] for details). Alternatively, at a short enough distance between the electronegative atoms sharing the hydrogen in the bridge, a unique functional element is established referred to as low-barrier hydrogen bond (LBHB) that is a new feature in understanding the catalysic mechanism of several enzymes (for a review, see Ref. [52]). It is important to note that the concept of an LBHB is not restricted to the singular case of a direct H-bonding between Y_Z and His 190 but it could also be established by including water molecule(s). This has been nicely illustrated for a concerted nonclassical transfer of two or three protons along hydrogen bond bridges in aspartate proteases [53]. Detailed density function theory (DFT) calculations in model systems revealed that analogous phenomena most likely occur also in superoxide dismutases [54,55]. One very attractive water species as a hydrogen bonding link between amino acid residues is the Zundel-ion $H_5O_2^+$ [56] that exhibits quite different behavior in different environments. It can switch from a normal hydrogen bond with O—O distances >2.7 Å to a LBHB configuration with a distance of <2.5 Å, depending on the charge distribution with the surrounding groups. Under some conditions, a rather abrupt transition can occur between both states [57].

It has to be emphasized that the idea of one or two water molecules forming a structurally well-defined link of hydrogen bonds between Y_Z and His 190 is not in contradiction with the conclusion that the environment around Y_Z is rather "dry" in systems with intact WOC and becomes "wet" after destruction of the WOC [35,48]. The key point is a well-defined array of one or two water molecules which drastically differs from that of bulk water with large structural fluctuations.

Recently observed new light-induced EPR signals were interpreted as to reflect Y_Z oxidation at liquid helium temperature [58,59] and taken as indirect evidence for the

phenolic proton of Y_Z being part of a LBHB bond [59]. However, regardless of the strength of the arguments, these findings do not permit any straightforward extrapolation to a functional competent PS II at room temperature because already minor distance changes owing to freezing can drastically alter the hydrogen bond character (vide supra).

At present, sound conclusions can be neither drawn on the geometry of the hydrogen bond nor on its possible LBHB character under physiological conditions. Detailed FTIR studies for samples with full oxygen evolution capacity and structural data of much higher resolution are indispensable prerequisites for further reliable considerations.

The above mentioned considerations reveal that—regardless of mechanistic details—structural distortions can significantly alter modes of coupling between electron and proton transfer, as clearly illustrated by a marked increase of the kinetic H/D exchange effect and the activation energy in samples lacking a competent WOC [25,33–35]. Therefore, results obtained with this sample type do not provide a sound basis for mechanistic conclusions. In particular, the discovery of stoichiometric coupling between Y_Z oxidation and release of one H^+ into the aqueous bulk phase in Tris-washed inside–out thylakoids [60] does not necessarily reflect the proton pathway in PS II complexes with an intact WOC (vide infra).

2.2. Mechanism of $P680^{+\bullet}$ reduction in "slow" ns kinetics

Compared with the "fast" ns relaxation, the "slow" ns kinetics exhibit an opposite dependence on S_i with smaller normalized amplitudes in S_0 and S_1 than in S_2 and S_3 [31,32]. Accordingly, the two different ns kinetics are not reflections of sample heterogeneity and questions arise on the origin of the multiphasic kinetics. It is now well accepted that protein dynamics and cascades of relaxation processes are of key relevance in regulating the kinetics of redox reactions of biological organisms in general [61], and in particular in bacterial reaction centers [62] and PS II [63,64]. Therefore, the "slow" ns kinetics have been assumed to originate from relaxation processes that shift the redox equilibrium between $P680^{+\bullet}Y_Z$ and $P680Y_Z^{ox}$ towards the latter state [65]. Since both types of ns kinetics do not exhibit a H/D exchange effect [49], the rate-limiting relaxation reaction(s) of the "slow" ns kinetics are inferred to reflect a dielectric response near the reactants rather than a "large" scale proton shift. The activation energy of this process is not yet resolved. A former study presents virtually S_i-independent E_A values in the range of 8.2–14.4 kJ/mol (within the experimental error) [40]. This finding is in line with our recent results for the "slow" ns kinetics in PS II core complexes from spinach (P. Kühn, H.J. Eckert and G. Renger, unpublished results). Recently, however, the activation energy has been reported to be rather small (< 10 kJ/mol) when the WOC attains S_0 and S_1 but drastically increases to 25–30 kJ/mol in the case of S_2 and S_3 [41]. Further studies are required to clarify this point.

2.3. Mechanism of $P680^{+\bullet}$ reduction via μs kinetics

Apart from the reduction by $Q_A^{-\bullet}$ (vide supra), μs kinetics with lifetimes in the range of 30–50 μs were found that exhibit a characteristic period four oscillation [29,31,32]. A breakthrough in understanding their origin was achieved by the finding that replacement of exchangeable protons by deuterons significantly affects the oscillation pattern of the μs kinetics [26,66,67]. Accordingly, relaxation processes within a hydrogen bond network are most likely rate-limiting for the "35 μs kinetics". This idea is supported by recent findings using a Y160F mutant of *Chlamydomonas reinhardtii* [68]. Basically two alternative structures can be considered for the dimension of proton delocalization: (i) capture inside the protein matrix or (ii) release into the lumenal bulk phase. The second mode of removing the proton from Y_Z upon its oxidation by $P680^{+\bullet}$ is an essential postulate of Jerry Babcock's hydrogen atom abstractor model presented in 1995 [69]. Although this is a very attractive model and a stoichiometric H^+ release into the outer bulk phase has been found in Tris-washed inside–out vesicles [60], the proton transport pathways in PS II complexes with an intact WOC are still not clarified and is a matter of controversial discussions [48,70–72]. Directly related to this problem is the question as to what extent the mode of proton delocalization and eventual release into the lumen depends on the redox state S_i of the WOC (see Section 3).

2.4. Reaction pattern of $P680^{+\bullet}$ reduction by Y_Z and regulatory effects

Fig. 2 summarizes the reaction sequence of $P680^{+\bullet}$ reduction by Y_Z in PS II complexes with intact WOC. This scheme implies that protein dynamics and mode of hydrogen bonding essentially affect the overall kinetics. Likewise, it is easily understandable that at distances of 7–8 Å between Y_Z and the manganese cluster [2,3] the WOC in different redox states modulates the kinetics of Y_Z^{ox} formation (see above description). Among different parameters of physiological relevance (temperature, pH, water content) the role of Ca^{2+} is of special interest because it is not only an essential constituent of the WOC (see Ref. [73] and references therein) but in general also a very important component of a great variety of signaling and regulatory proteins (see textbooks of biochemistry).

Ca^{2+}-specific effects on $P680^{+\bullet}$ reduction kinetics are induced by different treatments. In mildly trypsinized PS II membrane fragments the extent of μs kinetics is drastically enhanced at the expense of ns kinetics. This effect is largely and specifically reversed by addition of Ca^{2+} [50,74,75]. Low pH/citrate treatment of PS II core complexes gives rise to a similar phenomenon [76]. It was concluded that removal of the single Ca^{2+} from the Mn_4–Ca unit of the WOC disturbs the tuning of the apparent p*K* of base X that is hydrogen-bonded with Y_Z [76]. However, this proposal is conclusive only if the applied treatment exclusively removes the single

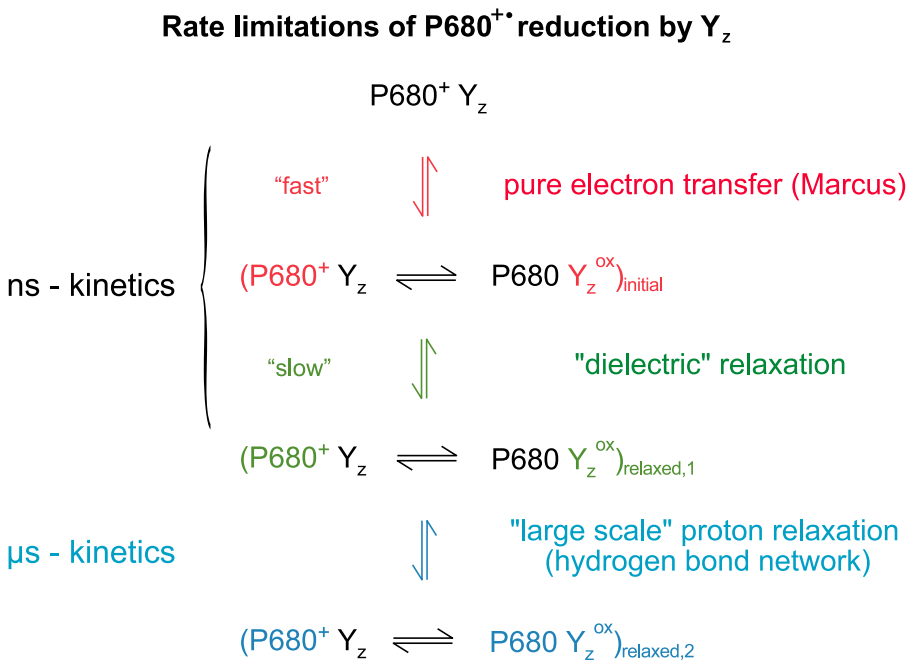

Fig. 2. Scheme of $P680^+$ reduction by Y_Z. The different types of kinetics and their limitations by nonadiabatic electron transfer, "dielectric" and "large-scale" proton relaxations are marked in red, green and blue, respectively (for further details, see text).

Ca^{2+} from the WOC and resuspension in Ca^{2+}-containing buffer causes nothing more than reinsertion of the cation into its empty site. In this respect, it has to be emphasized that the general problem of ambiguity with conclusions gathered from data of treated samples has been emphasized in many reports and is perfectly illustrated by a recent study on the Cl^- effect [77] (see also Section 3). Therefore, it appears worth considering a multiple mode of Ca^{2+} action [78], especially by taking into account the proposed role of Ca^{2+} in regulation of localized versus delocalized coupling of membrane energization by ΔpH [79]. An illustrative example for fine-tuning of the reactivity of cofactors has been recently reported for horseradish peroxidase C where binding of two endogenous Ca^{2+} at distances of >10 Å from the catalytic site is required for maintaining the structural integrity of the functional geometry of the heme [80].

Regardless of the detailed mechanism, the currently available data indicate that Ca^{2+}-binding essentially affects structure and mode of hydrogen bonding, thus regulating not only the kinetics of $P680^{+\bullet}$ reduction by Y_Z, but also S_3 oxidation by Y_Z^{ox} (see next section).

3. Kinetics and mechanism of S_i state oxidation by Y_Z^{ox}

First kinetic information on the reduction of Y_Z^{ox} was obtained by Babcock et al. [81] by time-resolved EPR studies on signal II_{vf}. Despite the restricted time resolution, three important conclusions could be drawn: (a) the rate constants of the individual oxidation steps in the Kok cycle are in the range of $(1\ ms)^{-1}$ up to $\geq (100\ \mu s)^{-1}$; (b) the rate depends on the redox state S_i; and (c) the rate constant for Y_Z^{ox} reduction by the WOC in redox state S_3 virtually coincides with that of the release of molecular oxygen. It is worth mentioning that the latter finding was one of the cornerstones of Jerry's considerations that eventually led to the hydrogen atom abstractor model.

In marked contrast to its rather complex formation kinetics (see Section 2), Y_Z^{ox} induces individual oxidation steps in the WOC that can be satisfactorily described by a mono-exponential time course with the exception that the oxidation of S_3 often exhibits a small lag phase [82–86]. These reactions are characterized by comparatively small kinetic H/D isotope effects and activation energies with values that depend on S_i [87–91]. The data gathered from different sample types permit three conclusions on the reaction coordinates of the individual redox steps in the WOC: (i) there is virtually no change during evolutionary development from cyanobacteria to higher plants [92]; (ii) there exist no significant differences between mesophilic and thermophilic organisms; and (iii) apart from the conserved PS II-O subunit [7], the nature or even the presence of other extrinsic subunits are without effect, except of an influence on S_3 oxidation kinetics [35].

Data evaluation within the framework of the Marcus theory of nonadiabatic electron transfer [47] revealed that the reorganization energy of the oxidation step $Y_Z^{ox}S_2 \rightarrow Y_ZS_3$ is much larger (1.6 eV) than for S_0 and S_1 oxidation (0.6–0.7 eV) [11] and by using empirical rate constant–distance relationships [93] values of ≥ 15 Å are obtained for the van der Waals' distance between Y_Z and the redox-active Mn_4–Ca unit of the WOC [35]. These values

exceed distances of 7–8 Å gathered from the X-ray structure [2,3] by at least a factor of two. This "discrepancy" suggests that the redox transitions in the WOC are not rate-limited by the electron transfer step but rather represent triggered reactions, most likely owing to proton movement. It is one of the great impacts of Jerry Babcock's work to emphasize the paramount importance of proton-coupled electron transfer (PCET) in oxidative water cleavage. In his hydrogen atom abstractor model, all oxidation steps in the WOC are concerted PCETs driven by the tyrosyl radical $Y_Z^{\bullet}$ [48,69,94]. This conclusion appears to be supported by experiments where all S_i-state transitions are retarded by nearly the same factor of 3–5, when the native Ca^{2+} is replaced by its surrogate Sr^{2+} [95]. The important role of hydrogen bond network(s) for the WOC reactions has been recently nicely illustrated by an excellent report on the Cl^- effect. It provides convincing evidence that Cl^- probably participates in establishing a proton relay network by interaction with charged amino acid residues rather than being an integral constituent of the WOC [77]. However, the very attractive hydrogen atom abstractor model probably needs refinement by taking into account two striking differences in the nature of the S_i-state transitions: (i) $S_0 \rightarrow S_1$ and $S_1 \rightarrow S_2$ are metal-centered reactions, while $S_2 \rightarrow S_3$ is most likely either a ligand-centered oxidation (see Refs. [76,96] and references therein, but see also Ref. [97] for discussion of controversial conclusions), or the transition into a multistate redox level of the WOC as outlined here and in former reports [1,11]; and (ii) the stoichiometry of H^+ release coupled with S_0 and S_1 oxidation depends on several parameters (sample type, pH) while the oxidative S_3 formation leads under all conditions to the stoichiometric release of one proton (for reviews, see Refs. [70,71,98,99]). It is therefore concluded that the mode of coupling between electron and proton transfer differs for the S_0 and S_1 oxidation steps from that of S_2 and S_3 and only in the latter case a concerted PCET takes place. The pathway(s) could include water molecules as bridges (for detailed discussion, see Refs. [100,101]). If one accepts that S_2 formation is accompanied by accumulation of a positive charge (70,71,98,99 but see Ref. [94]), a reorientation of water dipole(s) can be induced that gives rise to a switch in the proton transfer pathway in a similar way as was recently proposed for cytochrome *c* oxidase [102]. This presumed "water dipole switch" giving rise to different geometries of the hydrogen bond network in the WOC might also be responsible for the characteristic S_i-state dependence of the kinetics of the "slowly" exchanging substrate water molecule with a striking retardation in the dark relaxed redox state S_1 [103] of the WOC.

4. Hypothesis of S_3 acting as entatic state for O—O bond formation and O_2 evolution

According to a widely accepted dogma the essential O—O bond is not formed before the WOC attains the

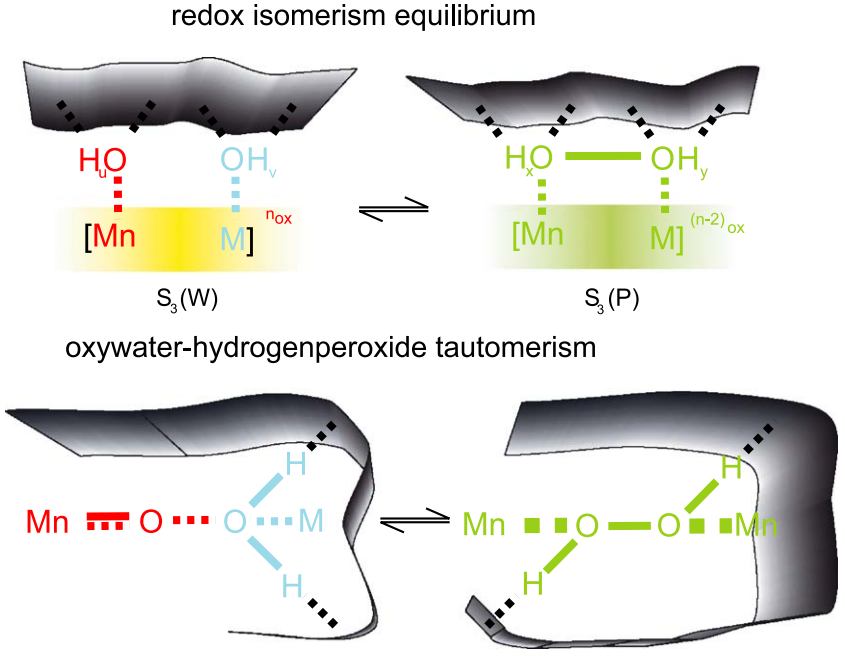

Fig. 3. Schematic representation of the proposed redox isomerism (top part) and oxywater-hydrogen peroxide tautomerism (bottom part) of the multistate redox level S_3 of the WOC. The electronic configuration (top) and protonation (bottom) of the presumed hydrogen-peroxide state is labeled in green, the asymmetric binding of substrate water in blue and red. M symbolizes a not yet assignable metal ion (either Mn or Ca), indices u, v, x and y in the top panel indicate hydrogen bonding of the substrate to the protein matrix, n_{ox} is the total oxidation state of the Mn_4–Ca unit. For the sake of simplicity, these indices are omitted in the bottom panel. Dynamic changes of the protein matrix coupled with the transitions between different forms of the S_3 equilibria are symbolized by differently shaped grey areas, the symbols S_3(W) and S_3(P) describe states where substrate water attains the formal redox levels of water and peroxide, respectively.

formal redox state S_4 (including $S_3Y_Z^{ox}$). Based on early thermodynamic considerations [104,105], it was proposed that this key step of the overall process occurs at the electronic level of a peroxidic configuration and that this state can be formed already at S_3 [104]. Later, in a refined model S_3 was postulated to be a multistate redox level of the WOC comprising fast dynamic isomerism and oxywater–hydrogen peroxide tautomerism equilibria. Within the framework of this model schematically summarized in Fig. 3 (for details, see Ref. [100] and references therein) the peroxidic form $S_3(P)$ is unique. When Y_Z^{ox} is generated by $P680^{+\bullet}$, the equilibria of Fig. 3 spontaneously shift toward the peroxide form $S_3(P)$ that is assumed to be the entatic state (an entatic state resembles in its nuclear configuration the geometry of the transition state of an enzymatic reaction, see Ref. [106]) for formation of complexed O_2. Accordingly, Y_Z^{ox} becomes reduced simultaneously with the appearance of O_2 coordinated to the catalytic site in formal redox state S_0, followed by very rapid and exergonic product substrate/exchange [104] as described by the following equation:

$$Y_Z^{ox}\,S_3(P) \xrightarrow{k_{ox}} Y_ZS_0(O_2)_{complexed} \xrightarrow{2H_2O} Y_ZS_0 + O_2 + xH^+$$
$$\downarrow nH^+$$

This mechanism is in line with the virtual kinetic coincidence of Y_Z^{ox} reduction by S_3 and oxygen release [107].

As an extension of the current model it is further proposed that the structured microenvironment not only determines the reactive properties of the Mn_4–Ca unit as an apoprotein (for an illustrative example of tuning the properties of metal centers via hydrogen bonding in Mn- and Fe-superoxide dismutases, see Ref. [108]), but itself exhibits catalytic activity as a functional constituent of the WOC. It is assumed to act as a "PCET director" which favors the O—O bond formation. The most convincing experimental support for the hypothesis of an active role of the protein were recently published FTIR studies in PS II core preparations from *T. elongatus* [109]. Apart from the WOC, first evidence was presented for protein-driven oxygen enzymology even without specific cofactors. Experimental data support antibody catalyzed water oxidation by singlet oxygen ($^1\Delta_gO_2$) [110]. The reaction sequence probably involving H_2O_3 leads to H_2O_2 formation. Quantum mechanical calculations provide plausible mechanisms and especially offer an explanation for the role of the protein structure in the unexpected chemistry [111]. The wealth of "exotic" oxygen species like $HO_3^{\bullet}$ radicals during the pathway of antibody-catalyzed chemical modification of antigens (Ref. [112]) illustrates the role of particular protein motifs.

It has to be emphasized that the extended model considers the WOC as a supermolecule especially tailored for the delicate process of oxidative water cleavage including definite pathways for substrate entry and product release. As a consequence, the widely used conventional concept of cofactor and apoprotein is not pertinent for gaining a deeper understanding of the mechanism of this fascinating process.

Note added in proof

During the preparation of the proofs of this article two studies were reported that provide clear evidence for the existence of a definite water transport pathway from the catalytic site of cytochrome *c* oxidase into the bulk phase [114] and the decisive role of the water channel and a hydrogen bond network for a controlled and directed proton transport in the same enzyme [115]. These findings support the idea of the existence of defined substrate/product pathways also in the WOC.

Acknowledgements

I am very grateful to R. Steffen and P. Kühn for pre paring the computer graphics of the figures. The financial support by Deutsche Forschungsgemeinschaft is gratefully acknowledged.

References

[1] G. Renger, Molecular mechanism of water oxidation, in: G.S. Singhal, G. Renger, Govindjee, K.-D. Irrgang, S.K. Sopory (Eds.), Concepts in Photobiology: Photosynthesis and Photomorphogenesis, Kluwer Academic Publishing, Dordrecht, Narosa Publishing, Delhi, 1999, pp. 292–329.
[2] A. Zouni, H.T. Witt, J. Kern, P. Fromme, N. Krauß, W. Saenger, P. Orth, Crystal structure of photosystem II from *Synechococcus elongatus* at 3.8 Å resolution, Nature 409 (2001) 739–743.
[3] N. Kamiya, J.-R. Shen, Crystal structure of oxygen-evolving photosystem II from *Thermosynechococcus vulcanus* at 3.7 Å resolution, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 98–103.
[4] R.J. Debus, The manganese and calcium ions in photosynthetic O_2 evolution, Biochim. Biophys. Acta 1102 (1992) 269–352.
[5] V.K. Yachandra, K. Sauer, M.P. Klein, Manganese cluster in photosynthesis: where plants oxidize water to dioxygen, Chem. Rev. 96 (1996) 2927–2950.
[6] T.M. Bricker, L.K. Frankel, The structure and function of CP47 and CP43 in photosystem II, Photosynth. Res. 72 (2002) 131–146.
[7] I. Enami, S. Kikuchi, S. Fukuda, H. Ohta, J.R. Shen, Binding and functional properties of four extrinsic proteins of photosystem II from a red alga, *Cyanidium caldarium*, as studied by release–reconstitution experiments, Biochemistry 37 (1998) 2787–2793.
[8] B. Kok, B. Forbush, M. McGloin, Cooperation of charges in photosynthetic O_2 evolution: I. A linear four-step mechanism, Photochem. Photobiol. 11 (1970) 457–475.
[9] J. Messinger, G.R. Seaton, T. Wydrzynski, U. Wacker, G. Renger, The S_{-3} state of the water oxidase in photosystem II, Biochemistry 36 (1997) 6862–6873.
[10] J. Messinger, J.H. Robblee, U. Bergmann, C. Fernandez, P. Glatzel, S. Isgandarova, B. Hanssum, G. Renger, S.P. Cramer, K. Sauer, V.K. Yachandra, Manganese oxidation states in photosystem II, Proc. 12th Int. Congr. Photosynthesis, S10-019, CSIRO Publishing, Collingwood, Australia, 2001.

[11] G. Renger, Mechanistic and structural aspects of photosynthetic water oxidation, Physiol. Plant. 100 (1997) 828–841.
[12] T. Ono, Metallo-radical hypothesis for photoassembly of $(Mn)_4$-cluster of photosynthetic oxygen evolving complex, Biochim. Biophys. Acta 1503 (2001) 40–51.
[13] G. Renger, Water cleavage by solar radiation—an inspiring challenge of photosynthesis research, Photosynth. Res. 38 (1993) 229–247.
[14] G. Renger, Apparatus and mechanism of photosynthetic oxygen evolution: a personal perspective, Photosynth. Res. 76 (2003) 269–288.
[15] B. Barry, G.T. Babcock, Tyrosine radicals are involved in the photosynthetic oxygen-evolving system, Proc. Natl. Acad. Sci. U. S. A. 84 (1987) 7099–7103.
[16] R.J. Debus, B.A. Barry, G.T. Babcock, L. McIntosh, Directed mutagenesis indicates that the donor to $P680^+$ in Photosystem II is tyrosine-161 of the D1 polypeptide, Biochemistry 27 (1988) 9071–9074.
[17] G.T. Babcock, B.A. Barry, R.J. Debus, C.W. Hoganson, M. Atamian, L. McIntosh, U. Sithole, C.G. Yocum, Water oxidation in Photosystem II: from radical chemistry to multielectron chemistry, Biochemistry 28 (1989) 9557–9565.
[18] M. Boska, K. Sauer, W. Buttner, G.T. Babcock, Similarity of EPR signal II, rise and P-680^+ decay kinetics in tris-washed chloroplast photosystem II preparations as a function of pH, Biochim. Biophys. Acta 722 (1983) 327–330.
[19] W. Weiss, G. Renger, On the functional connection between the reaction center complex and the water oxidizing enzyme system Y, Biochim. Biophys. Acta 850 (1986) 173–183.
[20] S. Gerken, K. Brettel, E. Schlodder, H.T. Witt, Optical characterization of the immediate electron donor to chlorophyll a^+_{II} in O_2-evolving photosystem II complexes. Tyrosine as possible electron carrier between chlorophyll a_{II} and the water-oxidizing manganese complex, FEBS Lett. 237 (1988) 69–75.
[21] G. Renger, Ch. Wolff, The existence of a high photochemical turnover rate at the reaction centers of system II in Tris-washed chloroplasts, Biochim. Biophys. Acta 423 (1976) 610–614.
[22] J. Haveman, P. Mathis, Flash-induced absorption changes of the primary donor of photosystem II at 820 nm in chloroplasts inhibited by low pH or tris-treatment, Biochim. Biophys. Acta 440 (1976) 346–355.
[23] H. Conjeaud, P. Mathis, G. Paillotin, The effect of pH on the reduction kinetics of P-680 in tris-treated chloroplasts, Biochim. Biophys. Acta 48 (1979) 280–291.
[24] G. Renger, A rapid vectorial back reaction at the reaction centers of photosystem II in tris-washed chloroplasts induced by repetitive flash excitation, Biochim. Biophys. Acta 547 (1979) 103–116.
[25] G. Christen, M. Karge, H.-J. Eckert, G. Renger, The role of protonation steps in electron transfer reactions in Tris-treated PS II membrane fragments, Photosynthetica 33 (1997) 529–539.
[26] G. Christen, A. Seeliger, G. Renger, $P_{680}^{+\bullet}$ reduction kinetics and redox transition probability of the water oxidising complex as a function of pH and H/D isotope exchange in spinach thylakoids, Biochemistry 38 (1999) 6082–6092.
[27] J. Messinger, W.P. Schröder, G. Renger, Structure–function relations in photosystem II. Effects of temperature and chaotropic agents on the period four oscillation of flash induced oxygen evolution, Biochemistry 32 (1993) 7658–7668.
[28] S. Isgandarova, G. Renger, J. Messinger, Functional differences of photosystem II from *Synechococcus elongatus* and spinach characterized by flash-induced oxygen evolution patterns, Biochemistry 42 (2003) 8929–8938.
[29] M. Gläser, Ch. Wolff, G. Renger, Indirect evidence for a very fast recovery kinetics of chlorophyll-*a* II in spinach chloroplasts, Z. Naturforsch. 31c (1976) 712–721.
[30] G. Renger, H.J. Eckert, W. Weiss, Studies on the mechanism of photosynthetic oxygen formation, in: Y. Inoue, A.R. Crofts, A.R. Murata, N. Murata, G. Renger, K. Satoh (Eds.), The Oxygen Evolving System in Photosynthesis, Academic Press, Japan, 1983, pp. 73–82.
[31] K. Brettel, E. Schlodder, H.T. Witt, Nanosecond reduction kinetics of photooxidized chlorophyll-a_{II} (P-680) in single flashes as a probe for the electron pathway, H^+-release and charge accumulation in the O_2-evolving complex, Biochim. Biophys. Acta 766 (1984) 403–415.
[32] H.J. Eckert, G. Renger, Temperature dependence of $P680^+$ reduction in O_2-evolving PS II membrane fragments at different redox states S_i of the water oxidizing system, FEBS Lett. 236 (1988) 425–431.
[33] R. Ahlbrink, M. Haumann, D. Cherepanov, D. Bögershausen, O. Mulkidjanian, W. Junge, Function of tyrozine Z in water oxidation by Photosystem II: electrostatical promotor instead of hydrogen abstractor, Biochemistry 37 (1998) 1131–1142.
[34] S. Reinman, P. Mathis, Influence of temperature on photosystem II electron transfer reactions, Biochim. Biophys. Acta 635 (1981) 249–258.
[35] G. Renger, G. Christen, M. Karge, H.-J. Eckert, K.-D. Irrgang, Application of the Marcus theory for analysis of the temperature dependence of the reactions leading to photosynthetic water oxidation—results and implications, JBIC 3 (1998) 360–366.
[36] J. Lavergne, F. Rappaport, Stabilization of charge separation and photochemical misses in photosystem II, Biochemistry 37 (1998) 7899–7906.
[37] W.C.J. Vermaas, G. Renger, G. Dohnt, The reduction of the oxygen-evolving system in chloroplasts by thylakoid components, Biochim. Biophys. Acta 764 (1984) 194–202.
[38] J.A. van Best, P. Mathis, Kinetics of reduction of the oxidized primary electron donor of photosystem II in spinach chloroplasts and in chlorella cells in the microsecond and nanosecond time ranges following flash excitation, Biochim. Biophys. Acta 503 (1978) 178–188.
[39] G. Renger, H.J. Eckert, R. Hagemann, B. Hanssum, H. Koike, U. Wacker, New results on the mechanism of photosynthetic water oxidation, in: G.S. Singhal, J. Barber, R.A. Dilley, R.A. Govindjee, R. Haselkorn, P. Mohanty (Eds.), Photosynthesis: Molecular Biology and Bioenergetics, Narosa Publ. House, New Delhi, 1989, pp. 357–371.
[40] C. Jeans, M.J. Schilstra, D.R. Klug, The effect of temperature on $P680^+$ reduction kinetics during water splitting, in: G. Garab (Ed.), Photosynthesis and Effects, vol. II. Kluwer Academic Publishing, Dordrecht, 1998, pp. 1045–1048.
[41] C. Jeans, M.J. Schilstra, D.R. Klug, The temperature dependence of $P680^+$ reduction in oxygen-evolving Photosystem II, Biochemistry 41 (2002) 5015–5023.
[42] H.-A. Chu, A.P. Nguyen, R.J. Debus, Amino acid residues that influence the binding of manganese or calcium to Photosystem II: 1. The lumenal interhelical domains of the D1 polypeptide, Biochemistry 34 (1995) 5839–5858.
[43] F. Mamedov, R.T. Sayre, S. Styring, Involvement of histidine 190 on the D1 protein in electron/proton transfer reactions on the donor side of Photosystem II, Biochemistry 37 (1998) 14245–14256.
[44] C. Berthomieu, R. Hienerwadel, A. Boussac, J. Breton, B.A. Diner, Hydrogen bonding of redox-active tyrosine Z of Photosystem II probed by FTIR difference spectroscopy, Biochemistry 37 (1998) 10547–10554.
[45] A.-M.A. Hays, I.R. Vassiliev, J.H. Golbeck, R.J. Debus, Role of D1-His190 in proton-coupled electron transfer reactions in photosystem II: a chemical complementation study, Biochemistry 37 (1998) 11352–11365.
[46] A.-M.A. Hays, I.R. Vassiliev, J.H. Golbeck, R.J. Debus, Role of D1-His190 in the proton-coupled oxidation of tyrosine Y_Z in manganese-depleted Photosystem II, Biochemistry 38 (1999) 11851–11865.
[47] R.A. Marcus, N. Sutin, Electron transfer in chemistry and biology, Biochim. Biophys. Acta 811 (1985) 265–322.
[48] C. Tommos, G.T. Babcock, Oxygen production in nature: a light-driven metalloradical enzyme process, Acc. Chem. Res. 31 (1998) 18–25.

[49] M. Karge, K.-D. Irrgang, S. Sellin, R. Feinäugle, B. Liu, H.-J. Eckert, H.J. Eichler, G. Renger, Effects of hydrogen/deuterium exchange on photosynthetic water cleavage in PS II core complexes from spinach, FEBS Lett. 378 (1996) 140–144.
[50] G. Renger, H.-J. Eckert, M. Völker, Studies on the electron transfer from Tyr-161 of polypeptide D-1 to $P680^+$ in PS II membrane fragments from spinach, Photosynth. Res. 22 (1989) 247–256.
[51] A. Fersht, W. H. Enzyme Structure and Mechanism, Freeman, New York, 1985.
[52] W.W. Cleland, Low barrier hydrogen bonds in enzymatic catalysis, Arch. Biochem. Biophys. 382 (2000) 1–5.
[53] D.B. Northrop, Follow the protons: a low-barrier hydrogen bond unifies the mechanisms of the aspartic proteases, Acc. Chem. Res. 34 (2001) 790–797.
[54] Z. Smedarchina, W. Siebrand, A. Fernandez-Ramos, Q. Cui, Kinetic isotope effects for concerted multiple proton transfer: a direct dynamics study of an active-site model of carbonic anhydrase II, J. Am. Chem. Soc. 125 (2003) 243–251.
[55] H. Chen, S. Li, Y. Jiang, A density functional theory study on the intramolecular proton transfer in the enzyme carbonic anhydrase, J. Phys. Chem. A 107 (2003) 4652–4660.
[56] G. Zundel, Hydrogen bonds with large proton polarizability and proton-transfer processes in electrochemistry and biology, in: I. Rice, S.A. Rice (Eds.), Advances in Chemical Physics, vol. 111. Wiley, New York, 2000, pp. 1–219.
[57] M.E. Green, Partially charged H_5O_2 as a chemical switch: a bond order and atoms in molecules study of hydrogen bonding determined by surrounding groups, J. Phys. Chem. A 106 (2002) 11221–11226.
[58] J.H.A. Nugent, I.P. Muhiuddin, M.C.W. Evans, Electron transfer from the water oxidizing complex at cryogenic temperatures: the S_1 to S_2 step, Biochemistry 41 (2002) 4117–4126.
[59] C. Zhang, S. Styring, Formation of split electron paramagnetic resonance signals in Photosystem II suggests that tyrosine Z can be photooxidized at 5 K in the S_0 and S_1 states of the oxygen-evolving complex, Biochemistry 42 (2003) 8066–8076.
[60] G. Renger, M. Völker, Studies on the proton release of the donor side of system II. Correlation between oxidation and deprotonization of donor D1 in Tris-washed inside–out thylakoids, FEBS Lett. 149 (1982) 203–207.
[61] L.N. Christophorov, A.R. Holzwarth, V.N. Kharkyanen, F. van Mourik, Structure–function self-organization in nonequilibrium macromolecular systems, Chem. Phys. 256 (2000) 45–60.
[62] H.-W. Trissl, K. Bernhard, M. Lapin, Evidence for protein dielectric relaxations in reaction centers associated with the primary charge separation detected from *Rhodospirillum rubrum* chromatophores by combined photovoltage and absorption measurements in the 1–15 ns time range, Biochemistry 40 (2001) 5290–5298.
[63] L. Konermann, G. Gatzen, A.R. Holzwarth, Primary processes and structure of the Photosystem II reaction center: 5. Modeling of the fluorescence kinetics of the D_1–D_2–cyt-b_{559} complex at 77 K, J. Phys. Chem., B 101 (1997) 2933–2944.
[64] S. Vasilev, C.-I. Lee, G.W. Brudvig, D. Bruce, Structure-based kinetic modeling of excited-state transfer and trapping in histidine-tagged Photosystem II core complexes from *Synechocystis*, Biochemistry 41 (2002) 12236–12243.
[65] A. Mulkidjanian, Photosystem II of green plants: on the possible role of retarded protonic relaxation in water oxidation, Biochim. Biophys. Acta, (1999) 1–6.
[66] M.J. Schilstra, F. Rappaport, J.H.A. Nugent, C. Barnett, D.R. Klug, Proton/hydrogen transfer affects the S-state-dependent microsecond phases of $P680^+$ reduction during water splitting, Biochemistry 37 (1998) 3974–3981.
[67] G. Christen, F. Reifarth, G. Renger, On the origin of the "35 μs kinetics" of $P_{680}^{+\bullet}$ reduction in Photosystem II with an intact water oxidising complex, FEBS Lett. 429 (1998) 49.
[68] C. Jeans, M.J. Schilstra, N. Ray, S. Husain, J. Minagawa, J.H.A. Nugent, D.R. Klub, Replacement of tyrosine D with phenylalanine affects the normal proton transfer pathways for the reduction of $P680^+$ in oxygen-evolving photosystem II particles from *Chlamydomonas*, Biochemistry 41 (2002) 15754–15761.
[69] G.T. Babcock, The oxygen-evolving complex in photosystem II as a metallo-radical enzyme, in: P. Mathis (Ed.), Photosynthesis: From Light to Biosphere, Kluwer Academic Publishing, Dordrecht, 1995, pp. 209–215.
[70] F. Rappaport, J. Lavergne, Coupling of electron and proton transfer in the photosynthetic water oxidase, Biochim. Biophys. Acta 1503 (2001) 246–259.
[71] W. Junge, M. Haumann, R. Ahlbrink, A. Mulkidjanian, J. Clausen, Electrostatics and proton transfer in photosynthetic water oxidation, Philos. Trans. R. Soc. Lond. B 357 (2002) 1407–1418.
[72] C. Tommos, Electron, proton and hydrogen-atom transfers in photosynthetic water oxidation, Philos. Trans. R Soc. Lond. B 357 (2002) 1383–1394.
[73] V.K. Yachandra, Structure of the manganese complex in photosystem II: insights from X-ray spectroscopy, Philos. Trans. R. Soc. Lond. B 35z7 (2002) 1347–1358.
[74] M. Völker, H.J. Eckert, G. Renger, The modification of the donor side reaction pattern in PS II membrane fragments by trypsin and $CaCl_2$, in: J. Biggins (Ed.), Progr. Photosynth. Res., vol. 1. Martinus Nijhoff/Dr. W. Junk, Dordrecht, 1987, pp. 545–548.
[75] M. Völker, H.-J. Eckert, G. Renger, Effects of trypsin and bivalent cations on P 680^+-reduction, fluorescence induction and oxygen evolution in PS II-membrane fragments from spinach, Biochim. Biophys. Acta 890 (1987) 66–77.
[76] M. Haumann, W. Junge, Evidence for impaired hydrogen-bonding of tyrosine Y_Z in calcium-depleted Photosystem II, Biochim. Biophys. Acta 1411 (1999) 121–133.
[77] K. Olesen, L.-E. Andreasson, The function of the chloride ion in photosynthetic oxygen evolution, Biochemistry 42 (2003) 2025–2035.
[78] P. Kühn, N. Iwanowski, H.-J. Eckert, K.-D. Irrgang, H.-J. Eichler, G. Renger, Reaction coordinate of $P680^{+\bullet}$ reduction by Y_Z in PS II core complexes from spinach, Proc. 12th Int. Congr. Photosynthesis, Brisbane S13-024, 2001, www.publish.csiro.au/ps.
[79] R.-S. Pan, R.A. Dilley, Influence of Ca^{2+} on the thylakoid lumen violaxanthin de-epoxidase activity through Ca^{2+} gating of H^+ flux at the CF_0 H^+ channel, Photosynth. Res. 65 (2000) 141–154.
[80] M. Laberge, Q. Huang, R. Schweitzer-Stenner, J. Fidy, The endogenous calcium ions of horseradish peroxidase C are required to maintain the functional nonplanarity of the heme, Biophys. J. 84 (2003) 2542–2552.
[81] G.T. Babcock, R.E. Blankenship, K. Sauer, Reaction kinetics for positive charge accumulation on the water side of chloroplast photosystem II, FEBS Lett. 61 (1976) 286–289.
[82] J.P. Dekker, J.J. Plijter, L. Ouwehand, H.J. van Gorkom, Kinetics of manganese redox transitions in the oxygen-evolving apparatus of photosynthesis, Biochim. Biophys. Acta 767 (1984) 176–179.
[83] G. Renger, W. Weiss, Functional and structural aspects of photosynthetic water oxidation, Biochem. Soc. Trans. 14 (1986) 17–20.
[84] F. Rappaport, M. Blanchard-Desce, J. Lavergne, Kinetics of electron transfer and electrochromic change during the redox transitions of the photosynthetic oxygen evolving complex, Biochim. Biophys. Acta 1184 (1994) 178–192.
[85] P.J. van Leeuwen, C. Heimann, P. Gast, J.P. Dekker, H.J. van Gorkom, Flash-induced redox changes in oxygen evolving spinach Photosystem II core particles, Photosynth. Res. 38 (1993) 169–176.
[86] M.R. Razeghifard, R.J. Pace, Electron paramagnetic resonance kinetic studies of the S states in spinach PS II membranes, Biochim. Biophys. Acta 1322 (1997) 141–150.
[87] H. Koike, B. Hanssum, Y. Inoue, G. Renger, Temperature dependence of S-state transition in a thermophilic cyanobacterium, *Synechococcus vulcanus* Copeland, measured by absorption changes in UV region, Biochim. Biophys. Acta 893 (1987) 524–533.

[88] G. Renger, B. Hanssum, Studies on the reaction coordinates of the water oxidase in PS II membrane fragments from spinach, FEBS Lett. 299 (1992) 28–32.
[89] O. Bögershausen, M. Haumann, W. Junge, Photosynthetic oxygen evolution: H/D isotope effects and the coupling between electron and proton transfer during transitions $S_2 \rightarrow S_3$ and $S_3 \rightarrow S_4 \rightarrow S_0$, Ber. Bunsenges. Phys. Chem. 100 (1996) 1987–1992.
[90] M. Karge, K.-D. Irrgang, G. Renger, Analysis of the reaction coordinate of photosynthetic water oxidation by kinetic measurements of 355 nm absorption changes at different temperatures in PS II preparations suspended in H_2O or D_2O, Biochemistry 36 (1997) 8904–8913.
[91] N. Lydakis-Simantiris, D.F. Ghanotakis, G.T. Babock, Kinetic isotope effects on the reduction of the Y_Z radical in oxygen evolving and tris-washed photosystem II membranes by time resolved EPR, Biochim. Biophys. Acta 1322 (1997) 129–140.
[92] G. Renger, Studies on structure and mechanism of photosynthetic water oxidation, in: G.A. Peschek, W. Löffelhardt, G. Schmetter (Eds.), The Phototrophic Prokaryotes, Plenum, New York, 1999, pp. 35–50.
[93] C.C. Page, C.C. Moser, X. Chen, P.L. Dutton, Natural engineering principles of electron tunnelling in biological oxidation-reduction, Nature 402 (1999) 47–52.
[94] C. Tommos, G.T. Babcock, Proton and hydrogen currents in photosynthetic water oxidation, Biochim. Biophys. Acta 1458 (2000) 199–219.
[95] K.I. Westphal, N. Lydakis-Simantiris, R.I. Cukier, G.T. Babcock, Effects of Sr^{2+}-substitution on the reduction rates of $Y_Z^{\bullet}$ in PS II membranes—evidence for concerted hydrogen-atom transfer in oxygen evolution, Biochemistry 39 (2000) 16220–16229.
[96] J. Messinger, J.H. Robblee, U. Bergmann, C. Fernandez, P. Glatzel, H. Visser, R.M. Cinco, K.L. McFarlane, E. Bellacchio, S.A. Pizarro, S.P. Cramer, K. Sauer, V. Yachandra, M.P. Klein, Absence of Mn-centered oxidation in the $S_2 \rightarrow S_3$ transition: implications for the mechanism of photosynthetic water oxidation, J. Am. Chem. Soc. 123 (2001) 7804–7820.
[97] C. Goussias, A. Boussac, A.W. Rutherford, Photosystem II and photosynthetic oxidation of water: an overview, Philos. Trans. R. Soc. Lond. B 357 (2002) 1369–1381.
[98] J. Lavergne, W. Junge, Proton release during the redox cycle of the water oxidase, Photosynth. Res. 38 (1993) 279–296.
[99] M. Haumann, W. Junge, Protons and charge indicators in oxygen evolution, in: D.R. Ort, C.F. Yocum (Eds.), Oxygenic Photosynthesis: The Light Reactions, Kluwer Academic Publishing, Dordrecht, 1996, pp. 165–192.
[100] G. Renger, Photosynthetic water oxidation to molecular oxygen: apparatus and mechanism, Biochim. Biophys. Acta 1503 (2001) 210–228.
[101] J.S. Vrettos, G.W. Brudvig, Water oxidation chemistry of photosystem II, Philos. Trans. R. Soc. Lond. B 357 (2002) 1395–1405.
[102] M. Wikström, M.I. Verkhovsky, G. Hummer, Water-gated mechanism of proton translocation by cytochrome *c* oxidase, Biochim. Biophys. Acta 1604 (2003) 61–65.
[103] W. Hillier, T. Wydrzynski, The affinities for the two substrate water binding sites in the O_2 evolving complex of photosystem II vary independently during S-state turnover, Biochemistry 39 (2000) 4399–4405.
[104] G. Renger, Theoretical studies about the functional and structural organization of photosynthetic oxygen evolution, in: H. Metzner (Ed.), Photosynthetic Oxygen Evolution, Academic Press, London, 1978, pp. 229–248.
[105] L.I. Krishtalik, Energetics of multielectron reactions. Photosynthetic oxygen evolution, Biochim. Biophys. Acta 849 (1986) 162–171.
[106] W. Kaim, B. Schwederski, Bioinorganic Chemistry: Inorganic Elements in the Chemistry of Life: An Introduction and Guide (Inorg. Chemistry), Wiley, 1995.
[107] M.R. Razeghifard, R.J. Pace, EPR kinetic studies of oxygen release in thylakoids and PSII membranes: a kinetic intermediate in the S_3 to S_0 transition, Biochemistry 38 (1999) 1252–1257.
[108] J. Maliekal, A. Karapetian, C. Vance, E. Yikilmaz, Q. Wu, T. Jackson, T.C. Brunold, T.G. Spiro, A.-F. Miller, Comparison and contrasts between the active site p*K*s of Mn-superoxide dismutase and those of Fe-superoxide dismutase, J. Am. Chem. Soc. 124 (2002) 15064–15075.
[109] T. Noguchi, M. Sugiura, Analysis of flash-induced FTIR difference spectra of the S-state cycle in the photosynthetic water-oxidizing complex by uniform ^{15}N and ^{13}C isotope labeling, Biochemistry 42 (2003) 6035–6042.
[110] P. Wentworth, L.H. Jones, A.D. Wentworth, X.Y. Zhu, N.A. Larsen, I.A. Wilson, X. Xu, W.A. Goddard, K.D. Janda, A. Eschenmoser, R.A. Lerner, Antibody catalysis of the oxidation of water, Science 293 (2001) 1806–1811.
[111] D. Datta, N. Vaidehi, X. Xu, W.A. Goddard, Mechanism for antibody catalysis of the oxidation of water by singlet dioxygen, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 2636–2641.
[112] P. Wentworth Jr., A.D. Wentworth, X. Zhu, I.A. Wilson, K.D. Janda, A. Eschenmoser, R.A. Lerner, Evidence for the production of trioxygen species during antibody-catalyzed chemical modification of antigens, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 1490–1493.
[113] M. Higuchi, T. Noguchi, K. Sonoike, Over-reduced states of the Mn-cluster in cucumber leaves induced by dark-chilling treatment, Biochim. Biophys. Acta 1604 (2003) 151–158.
[114] B. Schmidt, J. McCracken, S. Ferguson-Miller, A discrete water exit pathway in the membrane protein cytochrome *c* oxidase, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 15539–15542.
[115] T. Tsukihara, K. Shimokata, Y. Katayama, H. Shimada, K. Muramoto, H. Aoyama, M. Mochizuki, K. Shinzawa-Itoh, E. Yamashita, M. Yao, Y. Ishimura, S. Yoshikawa, The low-spin heme of cytochrome *c* oxidase as the driving element of the proton-pumping process, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 15304–15309.