

S10-005

Coupling of electron and proton movement in photosynthetic water oxidation

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Keywords: photosynthetic water oxidation, hydrogen bond network, H/D isotope exchange, activation energies, redox isomerism

Introduction

Photosynthetic oxidation of two water molecules to molecular oxygen and four protons takes place at a manganese containing unit - the water oxidizing complex (WOC) - within the multimeric Photosystem II (PS II) complex that acts as a water-plastoquinone-oxidoreductase. The overall process comprises three types of redox sequences: (i) light induced generation of a sufficiently stabilized ion radical pair with a strongly oxidizing cation $P680^{+}$ and a moderately reducing anion Q_A^{-} [for a review see (Renger, 1992)], (ii) formation of PQH_2 via a sequence of two one-electron transfer steps with Q_A^{-} as reductant [for a review see (Lavergne and Briantais, 1996)] and (iii) water oxidation via a sequence of four redox reactions energetically driven by $P680^{+}$ and a residue Y_Z of polypeptide D1 acting as mediator [for a review see (Renger, 1999) and references therein]. The kinetics of the individual reactions of sequence (iii) has been resolved and were found to be surprisingly complex for the reduction of $P680^{+}$ by Y_Z while the oxidation step in the WOC by Y_Z^{ox} can be satisfactorily described by monoexponentials except of the last one leading to formation and release of molecular oxygen [see (Renger, 1997) and references therein]. In the present communication the mechanism of these redox processes will be analyzed especially with respect to electron and proton coupling.

Mechanism of $P680^{+}$ reduction by Y_Z

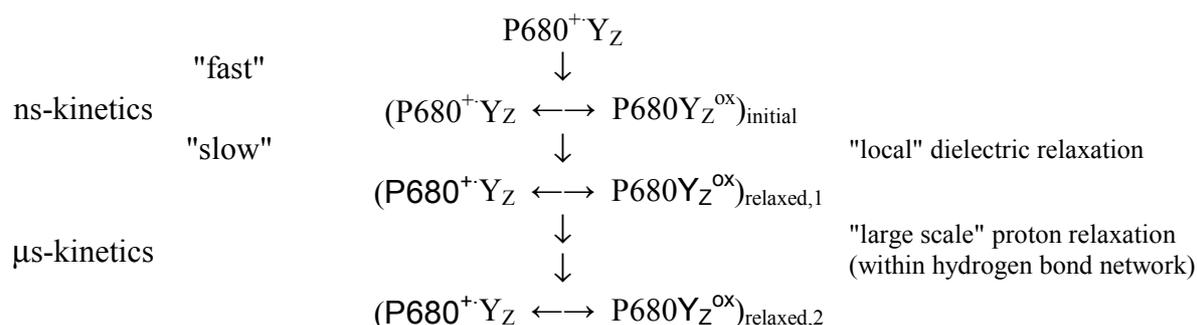
The multiphasic kinetics of electron transfer between the two redox groups $P680^{+}$ and Y_Z bound to the same protein matrix can be explained by two different mechanisms: (i) a heterogeneous population of different conformational states of PS II within the macroscopic ensemble or (ii) a sequence of events depending on relaxation processes within the protein matrix of each individual PS II complex. For the sake of simplicity the additional complexity owing to $P680^{+}$ reduction by components other than Y_Z will be ignored and the overall kinetics separated into three distinctly different components: "fast" and "slow" ns and μ s-kinetics. A heterogeneity of the ensemble cannot be the dominant factor responsible for the multiphasic kinetics because the extent of the normalized amplitudes of the three different components are almost independent of the structural complexity of the sample material (thylakoids, PS II membrane fragments and PS II core complexes from spinach) but strongly dependent on the redox state S_i of the WOC (Christen and Renger, 1999, Brettel et al., 1984, Eckert and Renger, 1988). Therefore, mechanism (ii) determines the complex kinetics of $P680^{+}$ reduction by Y_Z .

It was found that the activation energy of the "fast" ns reaction is rather low (order of 10 - 15 kJ/mol) in both PS II membrane fragments (Eckert and Renger, 1988) and PS II core complexes from spinach (Kühn et al., 2001). An analysis of the data within the framework of

the Marcus theory (Marcus and Sutin, 1985) of nonadiabatic electron transfer led to the conclusion that this reaction is limited by the electron transfer with a reorganization energy of about 0.5 eV and a van der Waals distance of about 10 Å between Y_Z and $P680^+$ (Renger et al., 1998). It was postulated that the electron transfer is coupled with proton shift within a double well potential of a hydrogen bond between the OH-group of Y_Z and a basic group X (Eckert and Renger, 1988) now identified as His 190 of polypeptide D1 [see (Hays et al., 1999) and references therein].

The origin of the "slow" ns kinetics was a matter of debate. Two alternative explanations were proposed: i) electrostatic shift of ΔG owing to a positive charge of the WOC stored in oxidation states S_2 and S_3 (Brettel et al., 1984) or ii) a reaction that is conformationally gated probably via a change of the reorganisation energy λ (Renger et al., 1989). The former idea cannot explain the existence of "slow" ns kinetics after single flash excitation of dark adapted samples where neither S_2 nor S_3 are populated in the WOC [for a review see (Joliot and Kok, 1975)]. On the other hand, the proposal of a conformationally gated reaction through changes of λ gained strong support by recent theoretical considerations (Cherepanov et al., 2001). Based on the finding that both the "fast" and "slow" ns kinetics do not exhibit a kinetic H/D isotope exchange effect (Karge et al., 1996) it is concluded that the "slow" ns kinetics are kinetically limited by a comparatively short range of relaxation processes within the protein matrix that are not coupled with long range rearrangements of a hydrogen bond network.

The existence of μs kinetics gave rise to controverse discussions especially whether or not it occurs in PS II complexes with an intact WOC [see (Christen and Renger 1999) and references therein]. The properties of these reactions were thoroughly analyzed and it was found that they are markedly affected when exchangeable protons are replaced by deuterons both in thylakoids and PS II membrane fragments. The characteristic features of the μs kinetics and their dependence on the redox state S_i of the WOC led to the conclusion that these reactions are gated by relaxation processes that comprise a significant change of the hydrogen bond network (Schilstra et al. 1998, Christen and Renger 1999). It is not yet absolutely clear if this process extends into the region of the lumenal bulk phase. Regardless of this particular problem the above mentioned data and considerations lead to the following scheme for the mechanism of $P680^+$ reduction by Y_Z :



where the equilibria from top to bottom progressively shift towards Y_Z^{ox} . It is assumed that this pattern permits an optimization of the energetics of water oxidation.

Mechanism of water oxidation

In order to gain mechanistic information on water oxidation the reaction coordinates of each individual redox step induced by Y_Z^{ox} within the WOC was analyzed by measuring the dependence on temperature and the effect of replacement of exchangeable protons by deuterons [see (Karge et al., 1997) and references therein]. The thermal activation of these reactions was investigated in three types of PS II preparations with different structural

complexity and evolutionary origin whereas the isotope exchange effect was determined only in samples from higher plants [for a review see (Renger, 1997, 1999)]. The following table compiles the values of the activation energies $E_{A,i}$ and the kinetic isotope exchange effect $k_{H,i}/k_{D,i}$, where $k_{H,i}$ and $k_{D,i}$ are the rate constants for the oxidation of redox state S_i in samples suspended in H_2O and D_2O , respectively.

An inspection of this data reveals three striking features: i) the kinetic H/D isotope exchange effect and the temperature dependence are comparatively weak, ii) a characteristic break point temperature ϑ_c is observed in the Arrhenius plot for the S_3 oxidation in all sample types and iii) the temperature dependencies of the oxidation steps are not affected by the presence of the extrinsic regulatory proteins that were changed during the evolutionary development from cyanobacteria to higher plants. An evaluation of the kinetic data within the framework of the theory of nonadiabatic electron transfer (Marcus and Sutin, 1985) leads to reasonable λ -values of 0.6 - 0.7 eV for $S_0 \rightarrow S_1$ and $S_1 \rightarrow S_2$ but strikingly larger values of 1.6 eV for $S_2 \rightarrow S_3$ and 1.1 eV for S_3 -oxidation above ϑ_c (below ϑ_c markedly larger values are obtained) (Renger, 1997).

When using a rate constant-distance relationship of nonadiabatic electron transfer (see e. g. Moser et al. 1995) van der Waals distances between Y_Z and WOC of $\geq 15 \text{ \AA}$ are obtained for the oxidation of S_1 (Renger et al., 1998). This value exceeds the distance of 7 \AA recently gathered from X-ray crystallographic analysis for the distance between the manganese cluster

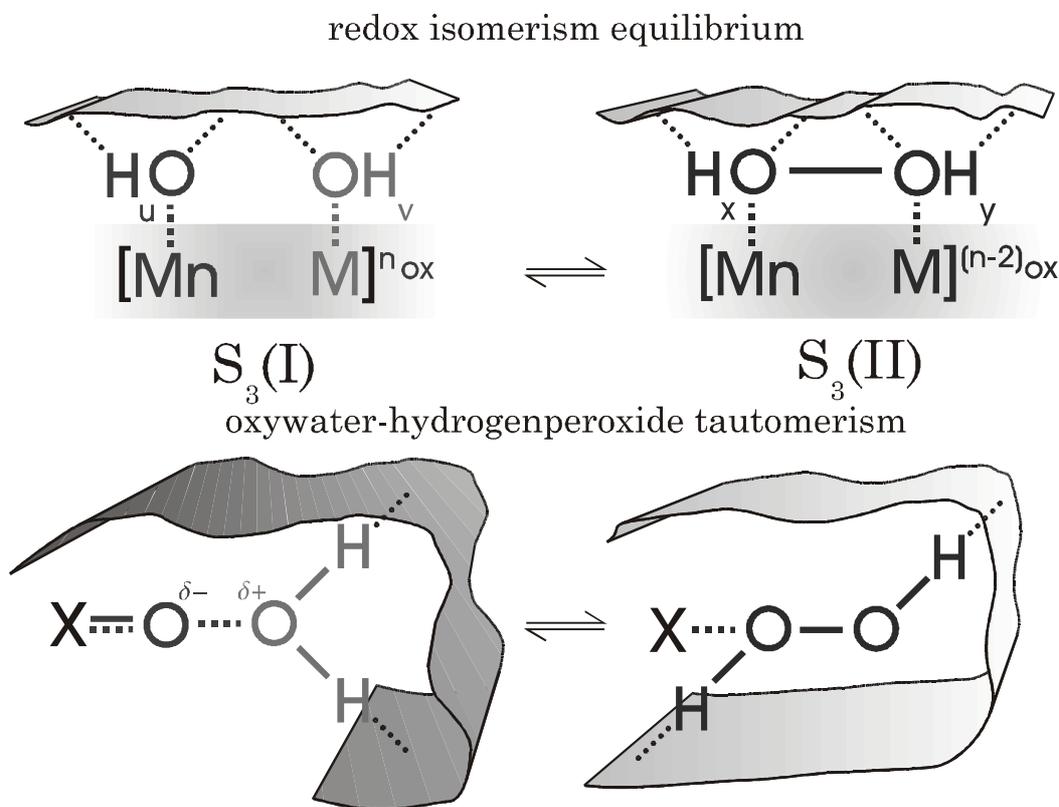
Reaction	Cyanobacteria	higher plants (spinach)			
	$E_{A,i}$ [kJ/mol]	Membrane fragments		Core complexes	
		$E_{A,i}$ [kJ/mol]	$k_{H,i}/k_{D,i}$	$E_{A,i}$ [kJ/mol]	$k_{H,i}/k_{D,i}$
$Y_Z^{\text{ox}} S_0 \rightarrow Y_Z S_1$	n.d.	5	n.d.	n.d.	n.d.
$Y_Z^{\text{ox}} S_1 \rightarrow Y_Z S_2$	9.6	12.0	1.3	14.8	1.6
$Y_Z^{\text{ox}} S_2 \rightarrow Y_Z S_3$	26.8	36.0	1.3	35.0	2.3
$Y_Z^{\text{ox}} S_3 \rightarrow Y_Z S_0 + O_2$	-	-	1.4	-	1.5
$\vartheta > \vartheta_c$	15.5	20.0	-	21.0	-
$\vartheta < \vartheta_c$	59.4	46.0	-	67.0	-
characteristic ϑ_c	+16°C	+6°C	-	+11°C	-
Extrinsic proteins	PSII-O PSII-V PSII-U	PSII-O PSII-P PSII-Q		PSII-O	

and Y_Z (Zouni et al., 2001) by a factor of two. This discrepancy suggests that the metal centered redox reactions $S_0 \rightarrow S_1$ and $S_1 \rightarrow S_2$ are gated either by conformational changes or by a fast adiabatic activation reaction with negative entropy changes (Davidson, 2000). Based on the finding that the rate constant of all Y_Z^{ox} -induced redox steps in the WOC become retarded after replacement of Ca^{2+} by Sr^{2+} (Westphal et al., 2000) it is most likely that the reactions are conformationally gated by rearrangement of hydrogen bonds. The data are in line with the famous "hydrogen abstractor" model of Babcock (for a review see Tommos and Babcock, 1998). However, different coupling modes of electron and proton movement for the individual redox steps of the WOC cannot entirely be excluded (Renger, 2001).

Regardless of the sample type the activation energy of S_2 oxidation to S_3 is markedly larger giving rise to a reorganisation energy of about 1.6 eV (Renger et al., 1998). Furthermore, this reaction has been shown to be a ligand rather than metal centered process [for a review see

(Renger, 1999) and references therein]. Different lines of evidence led to the conclusion that S_3 is the entatic state of water oxidation where preformation of the essential O-O bond occurs at the level of a peroxidic state that is part of a rapid redox isomerism equilibrium. Furthermore, the oxywater-hydrogen peroxide tautomerism permits the balanced shift towards a symmetric peroxide via a fine tuning of proton movement. Both equilibria are schematically shown in the following figure (see also Renger, 2001).

The rapid redox equilibration between ligand and metal center(s) can comprise several states of different electronic configuration and nuclear geometry (as outlined in Renger, 2001). In the figure only the extreme states symbolized by $S_3(I)$ and $S_3(II)$ with complete electron localization on ligand or metal, respectively, are shown. Furthermore, this description also indicates that one or two manganese centers could be involved. The binding of the substrate molecules to the protein matrix is not yet clarified. Recently the first evidence for a water molecule with asymmetric hydrogen bonding has been presented by using FTIR difference spectroscopy. The asymmetry of the hydrogen bonds in this water molecule that is assumed to be coordinated to manganese increases when S_1 is oxidized to S_2 (Noguchi and Sugiura, 2000).



Schematic representation of the redox isomerism (top) and oxywater-hydrogenperoxide tautomerism (bottom). Hydrogen bonding to the protein matrix is symbolized by dotted lines, substrate coordination to the template by squares. The center M could be either a second manganese or a redox inert metal ion (Ca^{2+}) (for a discussion, see Renger 2001). For better illustration, in the lower part X represents the template for oxywater and hydrogen peroxide without specifying metal centers (see top part).

Any shift of the proton within a hydrogen bond gives rise to a change of the charge distribution. Therefore the indices u, v, x and y symbolize averaged protonation states of the two substrate molecules in $S_3(I)$ and $S_3(II)$, respectively. It is clear that minor changes can give rise to rather significant effects on the population of a symmetric hydrogen peroxide species. Therefore the oxidation of Y_Z is assumed to give rise to a drastic shift towards $S_3(II)$

followed by a two electron abstraction and substrate/product exchange



The overall process is limited by k_{ox} . This readily explains why all procedures that modulate k_{ox} give rise to the same effect on Y_Z^{OX} reduction and disappearance of S_3 .

In summary, a mechanism is proposed where $S_0 \rightarrow S_1$ and $S_1 \rightarrow S_2$ are manganese-centered oxidation steps whereas $S_2 \rightarrow S_3$ comprises ligand centered reactions as part of a redox isomerism equilibrium (as outlined in Renger 2001). Oxidation of Y_Z in complexes with WOC's staying in S_3 causes a rapid proton movement that triggers the immediate shift of both equilibria towards a symmetric hydrogen peroxide that is oxidized to complexed molecular oxygen followed by product/substrate exchange. This mechanism considers the WOC as a molecular machine that is especially tailored for water oxidation. Its fine tuning of concerted electron and proton transfer by a special protein matrix determines the energetics of the overall process and avoids the generation of deleterious intermediates.

Acknowledgements

The author would like to thank for all contributions of coauthors to the own papers compiled in the reference list. Likewise my thanks include many invaluable discussions with G. Christen, H.-J. Eckert, K.-D. Irrgang, J. Messinger and R. Steffen. The financial support by Deutsche Forschungsgemeinschaft (Sfb 498, TP C4) is gratefully acknowledged.

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