Hypothesis

A MODEL FOR THE MOLECULAR MECHANISM OF PHOTOSYNTHETIC OXYGEN EVOLUTION+

G. RENGER

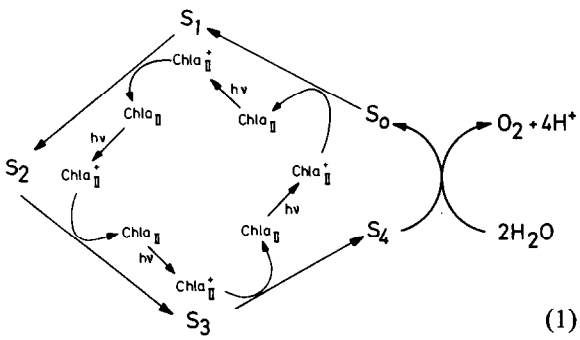
Max Volmer Institut für Physikalische Chemie und Molekularbiologie der Technischen Universität, Straße des 17. Juni 153, D-1000 Berlin 12, Germany

Received 10 June 1977
Revised version received 11 July 1977

1. Introduction

Water cleavage into molecular oxygen and protons, by electron abstraction, plays a central role in the conversion of sunlight into useful chemical energy in higher photoautotrophic organisms.

Irrespective of mechanistic details, water oxidation to oxygen requires the generation and cooperation of four redox equivalents of sufficient oxidizing power. Both functions are realized by different operational units. The formation of the positive charges occurs at a special chlorophyll-*a*, designated as Chl-a_{II} [1,2], via the primary photochemical processes within the reaction center of system II. For the indispensible cooperation a special storage device is necessary, which was found to be charged up sequentially by Chl-a^{+}_{II} via univalent electron transfer reactions, until after the accumulation of four redox equivalents oxygen is evolved (Kok mechanism, [3]). Accordingly, the general reaction scheme of this operational unit, referred to as the water-splitting enzyme system Y, can be described by eq. (1):



(1)

where S_i symbolizes the charge accumulation state and index $i = 0, \ldots 4$ reflects the number of stored positive charges.

Despite recent progress in photosynthesis research the molecular mechanism of photolytic water oxidation remains an unresolved problem. If one takes the properties of the free intermediate species of a 4-step univalent water oxidation (OH-radical, H_2O_2, superoxide radical) as a guide line [4], at least four fundamental questions have to be answered for an understanding of photosynthetic water cleavage:

1. In which step is the dioxygen bond achieved?
2. Which molecular mechanism realizes the 'taming' of the highly reactive intermediates, in order to prevent destruction of sensitive biological material and a rapid discharge of the higher S_i-states?
3. What is the chemical nature of these states?
4. What is the structural and functional arrangement of the storage places within the water-splitting enzyme system Y?

In the present paper a hypothetical model is proposed which might answer some of these questions. Furthermore it is discussed how the postulates introduced here agree with the available experimental data.

+Dedicated to the memory of my brother – Arnulf Renger (November 16, 1938 – April 26, 1977)

2. The postulated model

The model assumes that water oxidation occurs in a special microscopic 'reaction vessel', the water-splitting enzyme system Y, whose reaction properties are described by three postulates:

1. Based on earlier suggestions [5,6] the reactive intermediates of the sequential one-step water oxidation to oxygen are assumed to be stabilized at functional groups which contain manganese as the central ion.

2. Oxygen is ultimately formed via the oxidation of complexed superoxide by a 1-electron donor M, whose oxidized form M^+ is stable in the dark. The complexed oxygen is spontaneously released by an exergonic ligand–ligand exchange with two water molecules.

3. The preformation of the dioxygen bond occurs at the level of hydrogen peroxide, which is binuclearly complexed by two functional manganese groups.

On the basis of the above postulates the following reaction scheme for photosynthetic water oxidation is proposed:

$$M \xrightarrow{\oplus} M^+ \qquad (2a)$$

$$(H_2O)^* \xrightarrow{\oplus} (OH)^* + H^+ \qquad (b)$$

$$(OH)^* + (H_2O)^* \xrightarrow{\oplus} (H_2O_2)^{**} + H^+ \qquad (c)$$

$$M^+ + (H_2O_2)^{**} \xrightarrow{\oplus} \left[(O_2 \cdots H)^{**}\cdots M^+ + H^+\right] \longrightarrow (O_2)^{**} + 2H^+ + M \qquad (d)$$

$$(O_2)^{**} + 2\,H_2O \longrightarrow O_2 + 2(H_2O)^* \qquad (e)$$

where ⊕ denotes an oxidizing redox equivalent produced by Chl-a_{II} and transferred to the water-splitting enzyme system Y; one or two asterisks symbolize, mono- or binuclear complexation, respectively, at functional manganese groups.

The reaction scheme described by eq. (2a–e) suggests two basic problems:

i. By which molecular mechanism are the species complexed at the functional manganese groups?

ii. How does the complexation modify the reactive and energetic properties of the different redox states between water and oxygen?

With respect to the first question two modes of interaction have to be considered [7]: an inner sphere complexation directly at the central manganese ion or an indirect binding via an appropriate ligand. As the interaction with d-orbitals of transition metal ions was shown to play a pivotal role in the complexation of hydrogenperoxide, superoxide and oxygen [8], the direct complexation by manganese appears more probable.

Additionally, it is postulated that a charge delocalization between the central manganese ion and the corresponding ligand [9] is essential for the complexation.

Concerning the second problem, the complexation is assumed to modify the reactivity of the intermediates as well as the energetics of the various 1-electron transfer steps of eq. (2a–d). The changes in the properties of the complexed intermediates, compared to the corresponding free species, are so much pronounced that only a rather formal analogy exists between both types of reactants. Therefore, $(OH)^*$, $(H_2O_2)^{**}$ and $(HO_2 \rightleftharpoons H^+ + O_2^-)^{**}$ are referred to as 'cryptohydroxyl' radical, 'cryptohydroperoxide' and 'cryptosuperoxide', respectively [7]. The immense differences in the reactivity are obvious. Whereas the free hydroxyl radical is known to be the most potent oxidant known to chemistry, which reacts with many organic compounds at rates near the diffusion controlled theoretical limit, the complexed analog in the water-splitting enzyme system Y, $(OH)^*$, appears to be stable under normal physiological conditions (as $(OH)^*$ formally corresponds to the S_2-state in Kok's terminology, the lifetime of $(OH)^*$ amounts to a few seconds [3,10]. Lifetimes of the same order of magnitude have been found also for $(H_2O_2)^{**}$, which is analogous to S_3 of Kok's model.

Drastic changes due to the complexation are also postulated to occur in the energetics of the different

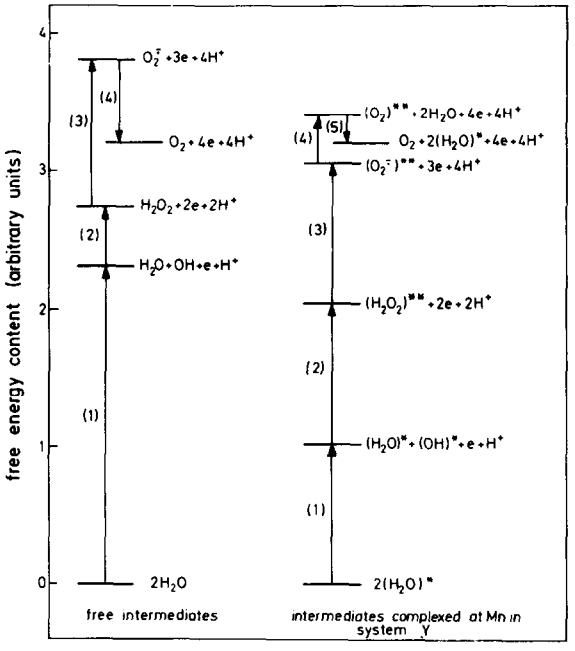


Fig.1. Schematic representation of the energetics of the 4-step univalent water oxidation. Left side: reaction pathway via free radical intermediates. Right side: reaction pathway in the photosynthetic water-splitting enzyme system Y according to the present model. The states H_2O and $(H_2O)^*$ are arbitrarily chosen to define the zero point of the energy scale. It must be clearly emphasized that the absolute energies of both states are not equal.

1-electron transfer steps, as is depicted in fig.1. Two basic assumptions are made:

i. The ligand–ligand exchange is an irreversible exergonic process with an absolute ΔG of the order of 10–20 kJ/mol, thus giving rise to an average overvoltage of 25–50 mV. (The average overvoltage is related to the average free energy required for the abstraction of one electron from water, i.e., $-\Delta G/4$ F.) However, the electron transfer steps are practically reversible. Thus, the irreversibility of photosynthetic wateroxidation is ascribed to this exchange reaction.

ii. The intermediates complexed at functional manganese groups are strongly bound with stabilization energies of the order of about 100–120 kJ/mol for the mononuclear complexed 'cryptohydroxyl' radical, and 70–80 kJ/mol for the binuclear complexed species 'cryptohydroxide' and 'cryptosuperoxide'. That means, the reactions

$$(OH)^* + H_2O \longrightarrow OH + (H_2O)^*$$

$$(H_2O_2)^{**} + 2H_2O \longrightarrow H_2O_2 + 2(H_2O)^*$$

$$(O_2^-)^{**} + 2H_2O \longrightarrow O_2^- + 2(H_2O)^*$$

are endergonic with ΔG 100–120 kJ/mol and 70–80 kJ/mol, respectively.

These assumptions lead to a characteristic energetic pattern for the consecutive 1-electron transfer reactions (1)–(4) in system Y (eq. (2a–d)), which clearly differs from that obtained for the free intermediate reaction sequence. Accordingly, each 1-electron abstraction step leading from complexed H_2O to 'cryptosuperoxide' needs an oxidizing redox equivalent with a midpoint potential $E_{m,7} \geq +1.0$ V. Thus, the midpoint potential of Chl-a_{II}^+/Chl-a_{II} has to be $E_{m,7} \geq +1.0$ V. The last redox step of eq. (2d), namely the oxidation of 'cryptosuperoxide' by M^+ is assumed to be of the order of +0.3–0.4 V (step 4, fig.1), including the overvoltage term of the exchange reaction, eq. (2e), which is hypothesized to be +0.1–0.2 V. Hence, the midpoint potential, $E_{m,7}$, of the redox couple M^+/M should be at least +0.3–0.4 V. This estimation of the redox potential would make the high potential cytochrome b_{559} a likely candidate for the substantiation of the donor substance M. However, there are a number of experiments which indicate, that cytochrome b_{559} is not directly involved into the process of photosynthetic water oxidation [11–13]. Therefore, the chemical nature of M remains to be clarified. It is essential to note, that according to the present model the redox reaction of M is not coupled with a protonation/deprotonation process. The redox free energy deliberated by the oxidation of M with Chl-a_{II}^+ is assumed to be used for an as yet unresolved process.

3. The stoichiometry between dioxygen and proton release

The overall reaction of water oxidation includes

also the liberation of 4 protons/molecule oxygen. Two modes of stoichiometric coupling are to be considered, depending on the mechanism:

i. If the charging of the water-splitting enzyme system Y up to state S_4 is not accompanied by deprotonation reactions, the proton release would be coupled only with the ultimate step which leads to oxygen formation. In this case in dark-adapted algae or chloroplasts, the protons liberated due to water oxidation under excitation with a train of single-turnover flashes, should give rise to a synchronous oscillatory pattern coinciding with that of oxygen evolution.

ii. If on the other hand, the intermediates of water oxidation represented by the S_i-states are acids with pK-values well below the physiological pH, then the oscillatory pattern would not be identical for the evolution of protons and oxygen, respectively.

The formulation of the reaction scheme of eq. (2a–d) tacitly assumes that, despite of the modification of their properties due to complexation, the intermediary stages $(H_2O^+)^*$ and $(H_3O_2^+)^{**}$ have pK-values low enough for complete dissociation into a proton and (OH)* and $(H_2O_2)^{**}$, respectively. Therefore, according to this mechanism the release of protons and oxygen are not synchronized. This model predicts that within the formalism of Kok's scheme the transition $S_0 \longrightarrow S_1$, eq. (2a), is not accompanied by proton liberation, the reactions $S_1 \longrightarrow S_2$ and $S_2 \longrightarrow S_3$, eq. (2b,c), release 1 H^+, whereas in the last step $S_3 \dashrightarrow (S_4) \longrightarrow S_0$, eq. (2d,e) one molecule of oxygen and two protons are produced.

It must be clearly emphasized that the model predicts only the 'intrinsic' proton release pattern. If there exist nonfunctional protonizable groups in the interior of the water-splitting enzyme system Y (e.g., amino acid residues) which are accessible to the bulk aqueous phase only under special conditions (e.g., by a dynamic structural change of Y coupled with the H_2O–O_2-exchange of eq. (2e)) then the 'intrinsic' pattern would not be detected by indicator methods acting outside of system Y. Furthermore, the 'intrinsic' pattern would also be masked, if protons are released into different spaces [14].

Data about the proton release pattern of the photosynthetic water oxidation became available recently. In their first study Kok and Fowler came to the conclusion that the oscillatory patterns for oxygen and proton evolution coincide [15]. However, in a later communication, this was reported not to be the case [16]. Recent data [17] obtained by measurements of oxygen production and proton release, due to water oxidation in spinach chloroplasts under repetitive flash group excitation conditions in the presence of the ADRY-reagent ANT 2s [9,18], confirm the latter conclusion. In fig.2 the experimental data from ref.

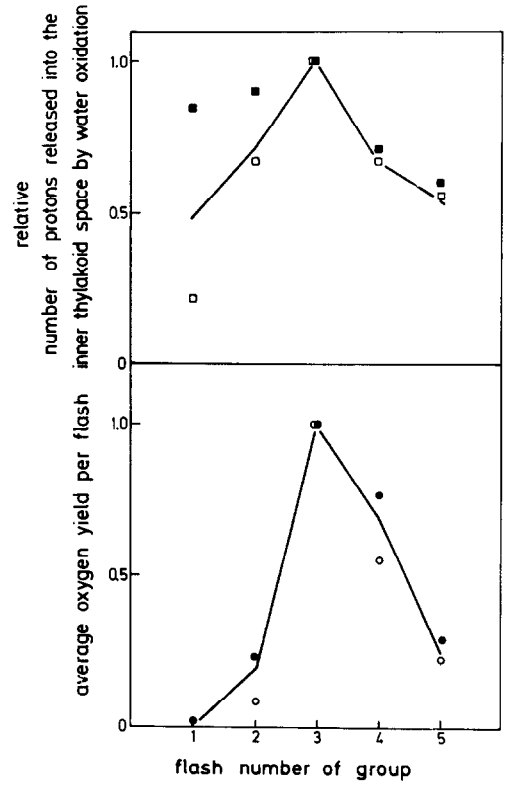


Fig.2. Oscillatory pattern of oxygen evolution and proton release due to system II electron transport with water as electron donor in a flash train of single turnover flashes. The curves give the theoretical values obtained for the present model with the additional assumption of a dark oxidation degree of M to be 0.65 and average probabilities of missings and double hits of 0.1 and 0.05, respectively. The experimental data indicated by squares and circles are redrawn from ref. [3,10] (open circles), ref. [16] (open squares) and ref. [17] (closed symbols). The activity in the third flash is used for normalization.

[3,16,17] are redrawn and compared with the theoretical predictions made on the basis of the present model. In order to account for the damping of the oscillatory pattern of oxygen evolution, Kok originally introduced an average missing probability $\bar{\alpha}$ and a double hit probability β [3]. Although refined mathematical treatments of this problem [19,20] indicate the limitation of Kok's assumptions, the present analysis will be simplified by application of this model. The theoretical curves in fig.2 are obtained for $\bar{\alpha}$ 0.1, β 0.05 and a degree of M-oxidation in the dark, 0.65 (i.e., $[S_0]$ 0.35 and $[S_1]$ 0.65). It is seen that with these values the experimental data of both oscillatory patterns, obtained under different assay and excitation conditions, are fairly well satisfied, except for the proton release of the first flash of the train. Where large scattering exists, the experimental data does not permit valid comparison.

In summary, at the present stage of knowledge the experimental data seem to fit the 'intrinsic' proton release pattern of the model. Further experiments, especially about the kinetic properties of proton release are required. Preliminary data (Junge and Ausländer, in preparation) indicate that the kinetics of proton release by the donor side of system II are rather complex depending on the charge accumulation state of the water-splitting enzyme system Y.

4. The functional and structural organization of the reactive groups within the water-splitting enzyme system Y

As the electron abstraction reactions eq. (2a–d), are ultimately caused by photooxidized Chl-a^+_{II}, two questions may be posed:

i. What is the functional connection between Chl-a_{II} and the water-splitting enzyme system Y?

ii. How are the reactions, eq. (2a–e), performed within system Y?

The mode of functional connection between Chl-a_{II} and the water-splitting enzyme system Y determines the kinetics as well as the efficiency of the electron transfer reactions symbolized by ⊕ in eq. (2a–d). For the interpretation of missing probabilities, in the order of 0.1–0.2, a statistical imperfection for these transfer processes was claimed to exist, which may be caused either by incomplete functional coupling [3] (photochemical losses or statistical blockage of a certain percentage of the reaction centers) or by a dynamic structural connection between Chl-a_{II} and system Y [21]. However, an oscillatory pattern was earlier shown to be reconcilable also with an electron transfer mechanism avoiding these imperfections, if specific structure–function correlations for system Y are introduced [5]. Therefore, the efficiency of the electronic coupling between Chl-a_{II} and system Y and its mode of regulation remain to be clarified.

The reduction kinetics of photooxidized Chl-a^+_{II} was shown to be at least triphasic [22] with amplitude ratios dependent on the charge accumulation state S_i of system Y and on the inner thylakoid proton concentration $[H^+]_{in}$ [23]. It was inferred that Chl-a_{II} and system Y are functionally connected via an electron carrier, denoted as D_1, the redox state of which (and eventually its protonation degree) depends on S_i and $[H^+]_{in}$.

Taking into consideration these effects, the simplest structure of the oxidizing side of system II would be a tetragonal (or pseudotetragonal–trigonal) array of the electron carriers D_1, M and two functional manganese ion groups. However, as the number of manganese ions is about 4–6/system Y [24], one can assume that 2–3 functional units, each containing two manganeses at a distance short enough to allow binuclear complexation for the peroxide and superoxide intermediates, are ordered around D_1 and M. D_1 is probably located at the periphery of the enzyme system Y, because it acts as connecting carrier to Chl-a_{II}. It is assumed that the arrangement of the functional groups leads to the formation of a structurally-ordered water cluster containing a small number of molecules, the properties of which differ from those of water in the bulk phases. If additionally the possibility of a functional cooperation of two system II reaction centers [25,26] is considered, then structural and functional organization schemes of the donor side of system II can be postulated, which explain the oscillatory patterns without the introduction of missing probabilities, as will be outlined in a forthcoming paper.

A last point, which remains to be discussed here,

is the electronic configuration of the functional manganese groups. The valence state of manganese in dark adapted algae and chloroplasts is unknown. On the basis of light activation experiments an oxidation state of +4 was inferred [27], but measurements of water, proton spin-lattice relaxation suggest, that manganese exists as a mixture of oxidation states, probably of +2 and +3 [28]. Recent data support the valence state +2 [29], so that it seems to be reasonable to assume, that the ground valence state of the functional manganese groups might be +2. However, in order to account for the EPR-properties (for discussion [29]) a special type of complexation and interaction should exist.

Acknowledgements

The author would like to thank Dr W. Ausländer and Professor Dr W. Junge for valuable discussions. The financial support by Deutsche Forschungsgemeinschaft and by E. R. P. Sondervermögen is gratefully acknowledged.

References

[1] Döring, G., Renger, G., Vater, J. and Witt, H. T. (1969) Z. Naturforsch. 24b, 1139–1143.
[2] Wolff, Ch., Gläser, M. and Witt, H. T. (1975) Proc. 3rd Int. Congr. Photosynthesis Res. Rehovot (Avron, M. ed) pp. 295–305, Elsevier, Amsterdam.
[3] Kok, B., Forbush, B. and McGloin, M. (1970) Photochem. Photobiol. 11, 457–475.
[4] George, P. (1965) in: Oxidases and Related Redox Systems (King, T. E., Mason, H. S. and Morrison, M. eds) Vol. 1, pp. 3–36, Wiley, New York, London, Sydney.
[5] Renger, G. (1970) Z. Naturforsch. 25b, 966–971.
[6] Renger, G. (1972) Physiol. Veg. 10, 329–345.
[7] Renger, G. (1977) in: Topics in Current Chemistry (Boschke, L. ed) Vol. 69, pp. 39–90, Springer, Berlin, Heidelberg, New York.
[8] Hanzlik, R. P. (1976) in: Inorganic Aspects of Biological and Organic Chemistry, Academic Press, New York, San Francisco, London.
[9] Renger, G. (1972) Eur. J. Biochem. 27, 259–269.
[10] Joliot, P., Joliot, A., Bouges, B., Barbieri, G. (1971) Photochem. Photobiol. 11, 287–305.
[11] Cox, R. P. and Bendall, D. S. (1972) Biochim. Biophys. Acta 283, 124–135.
[12] Phung Nhu Hung, S. (1974) Z. Pflanzenphysiol. 72, 389–394.
[13] Ben Hayyim, G. (1974) Eur. J. Biochem. 41, 191–196.
[14] Gould, J. M. and Izawa, S. (1974) Biochim. Biophys. Acta 333, 509–524.
[15] Fowler, Ch. F. and Kok, B. (1974) Biochim. Biophys. Acta 357, 299–309.
[16] Fowler, Ch. F. (1977) Biochim. Biophys. Acta 459, 351–363.
[17] Junge, W., Renger, G. and Ausländer, W. (1977) FEBS Lett. 79, 155–159.
[18] Renger, G. (1972) Biochim. Biophys. Acta 256, 428–439.
[19] Lavorel, J. (1976) J. Theor. Biol. 57, 171–185.
[20] Greenbaum, E. (1977) Photochem. Photobiol. 25, 293–298.
[21] Lavorel, J. (1976) FEBS Lett. 66, 164–167.
[22] Gläser, M., Wolff, Ch. and Renger, G. (1976) Z. Naturforsch. 31c, 712–721.
[23] Renger, G., Gläser, M. and Buchwald, H. E. (1977) Biochim. Biophys. Acta 461, 392–402.
[24] Cheniae, G. M. (1970) Ann. Rev. Plant Physiol. 21, 467–498.
[25] Siggel, U., Renger, G. and Rumberg, B. (1972) Proc. 2nd Int. Congr. Photosynthesis Res., Stresa (Forti, G., Avron, M. and Melandri, A. eds) Vol. 1, pp. 753–762, Dr W. Junk Publ., The Hague.
[26] Diner, B. (1974) Biochim. Biophys. Acta 368, 371–385.
[27] Radmer, R. and Cheniae, G. M. (1971) Biochim. Biophys. Acta 253, 182–186.
[28] Wydrzynski, T., Zumbulyadis, N., Schmidt, P. G. and Govindjee, (1975) Biochim. Biophys. Acta 408, 349–354.
[29] Siderer, Y., Malkin, S., Poupko, R. and Luz, Z. (1977) Arch. Biochem. Biophys. 179, 174–182.