The Watersplitting System of Photosynthesis

I. A Postulated Model

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For the natural donor of photosynthetic photosystem II an operational model is postulated. This model combines a definite structural pattern of two types of electron donors (octahedral arrangement of 6 secondary donors around a primary donor) with conformational state dependent electron transfer probability. It is shown that the model provides an explanation of many experimental data, especially the kinetics of the "priming" reactions of the watersplitting system can be quantitatively described.

In the electron transport pathway of photosynthesis electrons are transferred from H_2O to $\text{NADP}^\oplus$ (s. fig. 1). The oxidation of water is initiated by the excited Chla_{II} ^{1, 2}. Within this process the elec-

trons are transferred from H_2O via Y to plastoquinone³⁻⁵. The electron transfer from H_2O to PQ is limited by a reaction with a half life time $\tau_{1/2} = 6 \cdot 10^{-4} \text{ s}$ ⁶⁻⁸.

The enzyme system Y which mediates the oxidation of water is unknown.

It was found that manganese is an essential component of Y⁹⁻¹³. Further investigations led to the conclusion that chloride¹⁴⁻¹⁶ is necessary for the function of the water-splitting system, too. It is possible to substitute water as natural electron donor by artificial e-donors like ascorbat¹⁷ or paraphenylendiamine¹⁸ or diphenylcarbazide¹⁹. According to JOLIOT²⁰ a water cleavage occurs only on a double oxidized electron donor. In respect to the kinetic behaviour it was elucidated that a "priming" is necessary for the activation of the water splitting

Fig. 1. Simplified electron transfer system in photosynthesis⁸. $\text{NADP}^\oplus$ = nicotinamide adenine dinucleotide phosphate, Chla_{I} = chlorophyll a_I, PQ = plastoquinone pool, Chla_{II} = chlorophyll a_{II}, Y = natural electron donor system for Chla_{II} , (Cl) = chloride, (Mn) = manganese.

* CCCP = carbonylcyanide-m-chlorophenylhydrazine.

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system²¹⁻²³. JOLIOT²⁴ found that the "priming" of the watersplitting system is characterized by a specific kinetic behaviour.

The system Y is very sensitive against irreversible destruction by temperature²⁵, pH²⁶, UV-irradiation²⁷ or tris-washing²⁸.

Furthermore it was found that CCCP* influences directly the active states of Y produced by the "priming" reactions^{29,30}. JOLIOT²⁴ explained his results about the kinetics of the "priming" reactions by the assumption that each photochemical centre of system II includes two electron donors which are alternately connected with the electron acceptor. After each photoact a switch occurs with an efficiency of about 85 per cent.

In the presented paper firstly a model for the enzymatic complex Y is proposed on a molecular basis. Then it will be shown that the postulates of the model primarily introduced axiomatically are compatible with the experimental data and that the kinetic behaviour of the model describes quantitatively the experimental results of JOLIOT²⁴.

The Model

The following assumptions are made:

1. The natural donor of photosystem II is a highly ordered metalloprotein system, in the following named the donor cell.
 2. In the donor cell there are two different types of donors (s. fig. 2):
- A. the primary donor D which is directly connected with the photoreaction centre (Chla_{II}) of system II. This arrangement is only a symbolized description indicating the fact that in a photo-process at photosystem II an electron transfer from the primary electron donor D to the primary electron acceptor PQ is induced by Chla_{II}.
 - B. Six identical secondary donors Mn are arranged octahedrally around the primary donor D. Mn symbolizes one functional group of the metalloprotein system.

Fig. 2. Model of the donor cell. D = primary donor, Chla_{II} = chlorophyll a_{II}, PQ = plastoquinone, Mn = secondary donor (functional group), top: spacial figure, bottom: equatorial view, arrows indicate the electron flow from the secondary donors Mn to the primary donor D.

For the functional group behaviour the following assumptions are made:

- a) The centre of the functional group is a manganese ion in a definite valence state (this state is not known).
 - b) The ligands around the central manganese ion are arranged in a special configuration. There exist two different stable conformational states, C₁ and C₂, and two labile conformational states C'_{lab} and C''_{lab}. (In fig. 3 conformation state C₁ is indicated by broken circles, C₂ by winded half circles and the two labile conformation states C'_{lab} and C''_{lab} by half circles.)
3. Excitation of Chla_{II} leads to the electron transfer from D to plastoquinone. The generated D⁺ rapidly oxidizes one of the functional groups according to the electron transition probability determined by the conformational state of the ligand shell.
 4. The electron transfer probability from D⁺ to a functional group in the case of the conformational states C'_{lab} or C''_{lab} or C₂ is much higher than in the

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²² P. JOLIOT, Biochim. biophysica Acta [Amsterdam] **102**, 116 [1965].

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²⁹ G. RINGER, Naturwissenschaften **56**, 370 [1969].

³⁰ G. RINGER, in preparation.

conformational state C_1 . That means: If a functional group has the conformational state C'_{lab} or C''_{lab} or C_2 this group is oxidized in a photoreaction via D, whereas the functional groups in the conformational state C_1 do not react. In the reduced state of a functional group only the conformational state C_1 exists.

5. A conformation change is only possible by interaction of two oxidized functional groups located in the same donor cell. If the two interacting groups are neighbours the rapid transition $C_1 \rightarrow C''_{lab}$ occurs. If the two interacting groups are not neighbours only the rapid transition $C_1 \rightarrow C'_{lab}$ takes place. The conformational states C'_{lab} or C''_{lab} relax instantaneously to the C_1 state at the end of the interaction caused by the reduction of one of the two oxidized functional groups.

If in the donor cell with two $Mn^\oplus(C'_{lab})$ functional groups and four $Mn(C_1)$ -functional groups one of the $Mn^\oplus(C'_{lab})$ -groups is oxidized to $Mn^{2\oplus}$ the instantaneous conformation transition $C''_{lab} \rightarrow C_2$ occurs. The conformational state C_2 relaxes slowly to the C_1 state after the ending of the interaction.

6. A water cleavage is possible only on a double oxidized secondary donor $Mn^{2\oplus}$. The conformational state of the $Mn^{2\oplus}$ -functional group does not influence the watersplitting reaction.

7. The system of the primary electron transfer from D to PQ has no influence on the redox and conformation behaviour of the secondary donor system. Furthermore there is no interaction between different donor cells in respect to the reactivity of these secondary donors.

From these postulates the following reaction scheme can be derived (fig. 3).

In a dark adapted donor cell E_i the first turnover induced by excitation of $Chla_{II}$ leads to the oxidation of one functional group $Mn(C_1)$ to $Mn^\oplus(C_1)$ (see eq. 1), via D. A donor cell with five $Mn(C_1)$ and one $Mn^\oplus(C_1)$ functional groups is symbolized by A_1 .

The second turnover now taking place in an A_1 -donor cell causes the oxidation of one of the functional groups with the same probability (according to postulates 4 and 6) for the six functional groups. The probability amounts $\frac{1}{6}$ for the oxidation of the $Mn^\oplus(C_1)$ -functional group to $Mn^{2\oplus}(C_1)$ and $\frac{5}{6}$ for the oxidation of a $Mn(C_1)$ -functional group to $Mn^\oplus(C_1)$ (see eq. 2). A donor cell with five $Mn(C_1)$ and one $Mn^{2\oplus}(C_1 \text{ or } C_2)$ is symbolized by Z_1 , a

Fig. 3. Scheme for the reactions of the donor cell. The primary donor D is symbolized by a point in the centre of the octahedron, the secondary donors are indicated by points equipped with the following symbols for the conformational states: broken circles for C_1 , half circles for C'_{lab} and C''_{lab} , winded half circles for C_2 . The relaxation reactions of the states A_1 , A_2 and A_3 are not presented in this figure.

donor cell with two $Mn^\oplus(C'_{lab} \text{ or } C''_{lab})$ and four $Mn(C_1)$ is indicated by A_2 . According to postulate 7 the $Mn^{2\oplus}$ -functional group oxidizes water thereby being reduced to $Mn(C_1)$ (see eq. 5).

In the A_2 -donor cell the probability is $\frac{4}{5}$ that the two $Mn^\oplus$ groups are contiguous (therefore the rapid transition $C_1 \rightarrow C''_{lab}$ takes place) and $\frac{1}{5}$ that the two $Mn^\oplus$ groups are not contiguous (therefore the rapid transition $C_1 \rightarrow C'_{lab}$ occurs).

Because of the rapid conformation transitions in the A_2 -donor cell there are two $Mn^\oplus(C'_{lab})$ -functional groups and four $Mn(C_1)$ -functional groups in

the donor cell with a probability of $\frac{4}{5}$ and two $\text{Mn}^\oplus(\text{C}'_{\text{lab}})$ -functional groups and four $\text{Mn}(\text{C}_1)$ -functional groups in the donor cell with a probability of $\frac{1}{5}$.

If a turnover in an A_2 -donor cell takes place, one of the two $\text{Mn}^\oplus(\text{C}'_{\text{lab}} \text{ or } \text{C}''_{\text{lab}})$ is oxidized to $\text{Mn}^{2\oplus}$ (postulate 4) producing a Z_2 -donor cell which contains one $\text{Mn}^{2\oplus}(\text{C}_2 \text{ or } \text{C}'_{\text{lab}})$ -functional group, one $\text{Mn}^\oplus(\text{C}_2 \text{ or } \text{C}'_{\text{lab}})$ -functional group and four $\text{Mn}(\text{C}_1)$ functional groups (see eq. 3). The $\text{Mn}^{2\oplus}$ -functional group cleaves water thereby being discharged to $\text{Mn}(\text{C}_1)$. Then the $\text{Mn}^{2\oplus} - \text{Mn}^\oplus(\text{C}_2 \text{ or } \text{C}'_{\text{lab}})$ functional group interaction ceases and the rapid conformation transition $\text{C}'_{\text{lab}} \rightarrow \text{C}_1$ occurs (postulate 6), whereas the C_2 -conformational state is relatively stable.

The Z_2 -donor cell therefore reacts with a probability of $\frac{1}{5}$ to an A_1 -donor cell and with a probability of $\frac{4}{5}$ to a donor cell A_3 , which contains one $\text{Mn}^\oplus(\text{C}_2)$ -functional group and five $\text{Mn}(\text{C}_1)$ functional groups (see eq. 6).

According to postulate 4 in an A_3 -donor cell one turnover generates the Z_1 -donor cell (see eq. 4). In the dark the donor cells in the states A_1 , A_2 and A_3 slowly relax to E_i (see eqs. 7, 8, 9).

The scheme is summarized in fig. 4.

Fig. 4. Scheme (1) – (9).

$k_{\text{D}}^{(i)}$ are the relaxation constants with $i = 1, 2, 3$. According to the results of JOLIOT^{20, 23} these constants are of the order of 10^{-1} s^{-1} (assuming first order reactions).

k_{O_2} is the pseudo first order rate constant for the dark reaction between the states Z_1 or Z_2 and H_2O (the concentration of H_2O is assumed to be constant).

Since the rate limiting step in the electron transport from H_2O to PQ has a half life time of

$6 \cdot 10^{-4} \text{ s}$ ^{7, 8} it follows that $k_{\text{O}_2} \geq 1100 \text{ s}^{-1}$.

Further investigations of SINCLAIR³¹ led to the conclusion that $k_{\text{O}_2} \geq 5000 \text{ s}^{-1}$.

The proposed model for the donor system of Chla_{II} is based on postulates concerning the architecture of a hypothetical manganoprotein complex and on postulates regarding the relationship between conformational states and electron transfer probabilities. From these assumptions a reaction scheme is derived which implies a characteristic kinetical pattern of behaviour.

First it must be examined whether this reaction scheme describes the experimental results obtained by JOLIOT²⁴ for the kinetic behaviour of the donor system Y.

Then it remains to show that the postulates stated axiomatically are compatible with the experimental facts so far known.

a) Kinetics

The experimental results of JOLIOT²⁴ obtained by the excitation of photosynthesis with a sequence of short flashes ($\sim 10^{-5} \text{ s}$), which are separated from each other by a time $t_{\text{d}} = 300 \text{ ms}$, are depicted in fig. 5 (open circles).

Fig. 5. Oxygen molecules per flash and per Chla_{II}, full circles: calculated values for excitation with short flashes (duration $\ll 6 \cdot 10^{-4} \text{ s}$) and dark times between the flashes t_{d} in the range $150 \text{ ms} \leq t_{\text{d}} \leq 500 \text{ ms}$; open circles: experimental results of JOLIOT²⁴ (flash duration $\sim 10^{-5} \text{ s}$, dark time $t_{\text{d}} = 300 \text{ ms}$).

Because of the rate of the limiting step in the electron transport between H_2O and PQ ($\tau_{1/2} = 6 \cdot 10^{-4} \text{ s}$ ^{7, 8}) excitation of photosynthesis with a short flash (duration $\ll 6 \cdot 10^{-4} \text{ s}$) only can lead to one turnover in Chla_{II}.

If in a sequence of short flashes additionally the time t_{d} between the flashes is long enough for com-

³¹ J. SINCLAIR, Biochim. biophysica Acta [Amsterdam] **189**, 60 [1969].

plete transport of electrons through the transport chain from H_2O to the terminal acceptor ($t_d \geq 150$ ms) each flash of the sequence induces one (and only one) e-transfer through the chain.

If on the other hand the time t_d is short enough, the relaxation reactions (7) – (9) can be neglected. According to the results of JOLIOT^{20, 23} this approximation is applicable for times $t_d \leq 500$ ms. Because the water cleavage must be faster than $6 \cdot 10^{-4}$ s the states Z_1 and Z_2 are completely converted (ep. (5) and (6)) at $t_d = 300$ ms. Therefore under the experimental conditions of JOLIOT²⁴ the following simplified reaction scheme is valid:

After a long dark time (a few minutes) all the donor cells are in the dark adapted state E_i . The excitation with the first flash leads to the reaction $E_i \rightarrow A_1$, no oxygen is produced. In the second flash A_1 is converted to A_2 and E_i accompanied by the evolution of $\frac{1}{12}$ O_2 per donor cell [eq. (11)]. In the third flash $\frac{5}{6}$ of all of the donor cells transit from A_2 to A_3 and A_1 thereby evolving oxygen [eq. (12)] and $\frac{1}{6}$ of all of the donor cells are converted from state E_i to A_1 , no oxygen is produced. Therefore the third flash produces $\frac{5}{12}$ O_2 per donor cell.

Before the excitation with the fourth flash $\frac{1}{3}$ of the donor cells are in the state A_1 , $\frac{2}{3}$ in the state A_3 . The fourth flash leads to the transition into $\frac{5}{18}$ A_2 and $\frac{13}{18}$ E_i -states [eq. (11) and (13)], $\frac{13}{36}$ O_2 are produced per donor cell. In the 5th flash $\frac{5}{18}$ of the donor cells (state A_2) are converted to $\frac{20}{90}$ A_3 , $\frac{5}{90}$ A_1 and $\frac{5}{36}$ O_2 [eq. (12)], whereas $\frac{13}{18}$ of the donor cells (state E_i) are transferred to $\frac{13}{18}$ A_1 , no oxygen is produced [eq. (10)].

Therefore in the 5th flash $\frac{5}{36}$ O_2 per donor cell are evolved.

In the same way it is possible to calculate the oxygen amount evolved by the 6th, 7th, ..., nth flash. The obtained results are depicted in fig. 5 (full circles).

The calculated values are in good agreement with the experimental results of JOLIOT²⁴. Therefore the postulated model covers the characteristic kinetical behaviour of the donor system of Chla_{II} .

b) Structure

The model postulates that at least six manganese are bound per donor cell. CHENIAE and MARTIN³² found experimentally that 6–12 manganese atoms are associated with each O_2 evolving centre. Similar results were obtained by ANDERSON et al.³³. About the nature of the primary donor the model is not restricted to any special substance. Therefore it could be possible that the primary donor also contains manganese. This assumption would explain the data of CHENIAE and MARTIN³² who found two different types of manganese existing in system II. Furthermore tris-washing^{34, 35} or heating lead to manganese-release. Experimental data of POSSINGHAM and SPENCER³⁶ show that manganese is tightly bound to the watersplitting system. These experimental facts are in correspondence with the postulates concerning the role of manganese in the enzyme system Y.

The model also assumes two different types of electron donors. The existence of at least two different electron donors can be derived from the results obtained with artificial electron donors for system II. It is obvious that the watersplitting system (in the presented model the system of secondary donors) can be completely destroyed, but the ability to transfer electrons from artificial electron donors via Chla_{II} remains*.

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³³ J. M. ANDERSON, N. K. BOARDMAN and D. J. DAVID, *Biochem. biophys. Res. Commun.* **23**, 874 [1964].

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³⁵ K. YAMASHITA, M. ITOH, and K. SHIBATA, *Biochim. biophysica Acta* [Amsterdam] **189**, 133 [1969].

³⁶ D. SPENCER and J. V. POSSINGHAM, *Austra. J. Biol. Sci.* **13**, 441 [1960].

* Note added in proof: Recently it was shown by VERNON and SHAW [*Plant Physiol.* **44**, 1645 [1969]] that diphenylcarbazine only can donate electrons to photosystem II if the oxygen evolution system is inactivated. They concluded that in "intact chloroplasts photosystem II is not available to external agents and is 'covered up' by the enzymes responsible for oxygen evolution". This is in correspondence with the postulated model, where the primary electron donor D is covered up by the secondary donor manganese-protein system (the enzyme responsible for oxygen evolution).

The effect of chloride¹⁴⁻¹⁶ on the water splitting system is explicable if one assumes that chloride is an essential ligand of the central manganese ion.

Furthermore it was pointed out by HEATH and HIND³⁶ that chloride- and manganese-deficiency causes very similar effects on electron transport. Artificial electron donors like hydroxylamine can bypass the manganese and chloride deficiency block. Hydroxylamine is oxidized directly by the primary electron donor D. Therefore no "priming" was observed in the oxidation of hydroxylamine³⁸.

In contrast the effect of CCCP on the water splitting system²⁹ is located at the secondary donors Mn. It is assumed that CCCP accelerates the relaxation of the active states A_1 , A_2 , A_3 into the inactive state E_i (see eq. 7, 8, 9)³⁰.

It was found that an electron pool of PQ exists on the reducing (acceptor) side of $Chla_{II}$ ^{39, 40}. According to the model an electron pool with the same capacity is postulated also for the oxidizing (donor) side of $Chla_{II}$. Under normal conditions this pool is not observable since the rate limiting step in the electron transport between the donor- and the acceptor-pool is located on the acceptor side of $Chla_{II}$ ^{7, 8}.

This prevents the accumulation of positive charges on the donor side of $Chla_{II}$. There is no experimental evidence for the existence of a donor pool. Although the fluorescence measurements of MURATA⁴¹ are a hint for the existence of the donor pool further investigations are necessary for the unequivocal evidence of this pool.

From structural informations about the photosynthetic apparatus it can be concluded that system

II is more complicated⁴²⁻⁴⁴ in the architecture than system I. This could be caused by the structure of Y.

The phenomena discussed here show that the postulates about the structural organisation and the existence of two different electron donors are compatible with the experimental facts.

c) Conformational states

Up to now for the existence of special conformational states in the secondary donors of the water-splitting system there is no experimental evidence. But it is known that in other metallic enzymes the properties (redox behaviour, absorption, ESR-activity) are determined by the structure of the ligand shell⁴⁵⁻⁴⁹.

Furthermore it is known that conformation transitions can be very fast (10^{-7} s)^{50, 51}.

The electron transport probability is given by the shape and the height of the energy barrier⁵²⁻⁵⁴ between the primary donor and the functional groups. This is dependent on the geometry of the ligand shell.

The operational model described here is based on hypothetical assumptions. Unfortunately there is a complete lack of informations about the structure of Y.

However, it is hoped that this model which covers the kinetic behaviour and which is compatible with the facts so far known will stimulate such structural studies.

I am indebted to Prof. H. T. WITT for valuable discussions and criticism.

³⁷ R. L. HEATH and G. HIND, *Biochim. biophysica Acta* [Amsterdam] **189**, 222 [1969].

³⁸ P. BENNOUN and A. JOLIOT, *Biochim. biophysica Acta* [Amsterdam] **189**, 85 [1969].

³⁹ B. RUMBERG, P. SCHMIDT-MENDE, J. WEIKARD, and H. T. WITT, in: "Photosynthetic Mechanisms in Green Plants", Publ. 1145, Nat. Acad. Sci. Natl. Res. Council, Publ. **1963**, p. 18.

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