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 \( \text{H}_2\text{O} \) to \( \text{NADP}^\circ \) (s. fig. 1). The oxidation of water is initiated by the excited \( \text{Chl}_{\text{a1}} \). Within this process the electrons are transferred from \( \text{H}_2\text{O} \) via \( Y \) to plastoquinone \( ^3 \). The electron transfer from \( \text{H}_2\text{O} \) to \( \text{PQ} \) is limited by a reaction with a half life time \( \tau_{1/2} = 6 \times 10^{-4} \text{s} \). The enzyme system \( Y \) which mediates the oxidation of water is unknown.

Fig. 1. Simplified electron transfer system in photosynthesis \(^8\). \( \text{NADP}^\circ = \text{nicotinamide adenine dinucleotide phosphate} \), \( \text{Chl}_{\text{a1}} = \text{chlorophyll a1} \), \( \text{PQ} = \text{plastoquinone pool} \), \( \text{Chl}_{\text{a1}} = \text{chlorophyll a1} \), \( Y = \text{natural electron donor system for} \text{Chl}_{\text{a1}} \), \( (\text{Cl}) = \text{chloride} \), \( (\text{Mn}) = \text{manganese} \).

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\( ^* \text{CCCP} = \text{carbonylcyanide-m-chlorophenylhydrazone} \).

system. Joliot\textsuperscript{24} 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 temperature\textsuperscript{25}, pH\textsuperscript{26}, UV-irradiation\textsuperscript{27} or tris-washing\textsuperscript{28}.

Furthermore it was found that CCCP\textsuperscript{*} influences directly the active states of Y produced by the "priming" reactions\textsuperscript{29,30}. Joliot\textsuperscript{24} 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 Joliot\textsuperscript{24}.

**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\textsubscript{11}) 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\textsubscript{11}.

   B. Six identical secondary donors Mn are arranged octahedrally around the primary donor D. Mn symbolizes one functional group of the metalloprotein system.

3. Excitation of Chla\textsubscript{11} leads to the electron transfer from D to plastoquinone. The generated D\textsuperscript{0} 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\textsuperscript{0} to a functional group in the case of the conformational states C\textsubscript{lab} or C\textsubscript{lab} is much higher than in the

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conformational state $C_1$. That means: If a functional group has the conformational state $C_{lab}^\prime$ or $C_{lab}''$ 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 $Mn^{\circ}$($C_{lab}'''$)-functional groups and four $Mn(C_1)$-functional groups one of the $Mn^{\circ}$($C_{lab}'''$)-groups is oxidized to $Mn^{\circ \circ}$ 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 $Mn^{\circ \circ}$. The conformational state of the $Mn^{\circ \circ}$-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 Chl$C_{II}$ leads to the oxidation of one functional group $Mn(C_1)$ to $Mn^{\circ}$($C_1$) (see eq. 1), via $D$. A donor cell with five $Mn(C_1)$ and one $Mn^{\circ}$($C_1$) 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 $Mn^{\circ}$($C_1$)-functional group to $Mn^{\circ \circ}$($C_1$) and $\frac{2}{6}$ for the oxidation of a $Mn(C_1)$-functional group to $Mn^{\circ}$($C_1$) (see eq. 2). A donor cell with five $Mn(C_1)$ and one $Mn^{\circ \circ}$($C_1$ or $C_2$) is symbolized by $Z_1$, a donor cell with two $Mn^{\circ}$($C_{lab}'''$ or $C_{lab}''$) and four $Mn(C_1)$ is indicated by $A_2$. According to postulate 7 the $Mn^{\circ \circ}$-functional group oxidizes water thereby being reduced to $Mn(C_1)$ (see eq. 5).

In the $A_2$-donorcell the probability is $\frac{5}{6}$ that the two $Mn^{\circ}$ groups are contiguous (therefore the rapid transition $C_1 \rightarrow C_{lab}'''$ takes place) and $\frac{1}{6}$ that the two $Mn^{\circ}$ 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 $Mn^{\circ}$($C_{lab}'''$)-functional groups and four $Mn(C_1)$-functional groups in
the donor cell with a probability of $\frac{3}{5}$ and two Mn$^{5+}$ (C$^{ab}_1$)-functional groups and four Mn(C$^1$) -functional groups in the donor cell with a probability of $\frac{2}{5}$.

If a turnover in an A$_2$-donor cell takes place, one of the two Mn$^{5+}$ (C$^{ab}_1$ or C$^{ab}_2$) is oxidized to Mn$^{2+}$ (postulate 4) producing a Z$_2$-donor cell which contains one Mn$^{2+}$ (C$_2$ or C$^{lab}_2$)-functional group, one Mn$^{5+}$ (C$_2$ or C$^{lab}_2$)-functional group and four Mn (C$^1$) functional groups (see eq. 3). The Mn$^{2+}$-functional group cleavages water thereby being discharged to Mn(C$^1$). Then the Mn$^{2+}$ — Mn$^{5+}$ (C$_2$ or C$^{lab}_2$) functional group interaction ceases and the rapid conformation transition C$^{lab}_2$ — 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 $\frac{2}{5}$ to an A$_1$-donor cell and with a probability of $\frac{3}{5}$ to a donor cell A$_3$, which contains one Mn$^{5+}$ (C$_2$)-functional group and five Mn(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.

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\begin{align*}
E_i & \xrightarrow{h \nu} A_1, \\
A_1 & \xrightarrow{h \nu} \frac{3}{5} A_2 + \frac{2}{5} Z_1, \\
A_2 & \xrightarrow{h \nu} Z_2, \\
A_3 & \xrightarrow{h \nu} Z_1, \\
Z_1 & \xrightarrow{k_{o2}} E_i + \frac{2}{5} O_2, \\
Z_2 & \xrightarrow{k_{o2}} \frac{3}{5} A_3 + \frac{3}{5} A_1 + \frac{3}{5} O_2, \\
A_1 & \xrightarrow{k_{o1}} E_i, \\
A_2 & \xrightarrow{k_{o1}} A_1 \xrightarrow{k_{o1}} E_i, \\
A_3 & \xrightarrow{k_{o1}} A_1 \xrightarrow{k_{o1}} E_i.
\end{align*}
\]

Fig. 4. Scheme (1) — (9).

$k_{o1}^{(i)}$ are the relaxation constants with $i = 1, 2, 3$. According to the results of JOLIOT $^{20,23}$ these constants are of the order of $10^{-1} \text{s}^{-1}$ (assuming first order reactions).

$k_{o2}$ is the pseudo first order rate constant for the dark reaction between the states Z$_1$ or Z$_2$ and H$_2$O (the concentration of H$_2$O is assumed to be constant).

Since the rate limiting step in the electron transport from H$_2$O to PQ has a half life time of $6 \cdot 10^{-4} \text{s}^{7,8}$ it follows that $k_{o2} \gg 1100 \text{s}^{-1}$. Further investigations of SINCLAIR $^{31}$ led to the conclusion that $k_{o2} \geq 5000 \text{s}^{-1}$.

The proposed model for the donor system of Chl$_{III}$ 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 JOLIOT $^{24}$ 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.

\[a) \text{Kinetics}\]

The experimental results of JOLIOT $^{24}$ 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 $t_d = 300 \text{ ms}$, are depicted in fig. 5 (open circles).

![Fig. 5. Oxygen molecules per flash and per Chl$_{III}$, 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 ms $\leq t_d \leq 500$ ms; open circles: experimental results of JOLIOT $^{24}$ (flash duration $\sim 10^{-5} \text{s}$, dark time $t_d = 300 \text{ ms}$).](image)

Because of the rate of the limiting step in the electron transport between H$_2$O and PQ ($\tau_{o1} = 6 \cdot 10^{-4} \text{s}^{7,8}$) excitation of photosynthesis with a short flash (duration $\ll 6 \cdot 10^{-4} \text{s}$) only can lead to one turnover in Chl$_{III}$.

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].
The fourth flash leads to the transition into $\text{state } \text{A}_i \text{ and } \text{state } \text{E}_j \text{ accompanied by oxygen is produced}$. In the second excitation with the first flash leads to the reaction $\text{A}_2 \text{ is converted to } \text{A}_3$, and $\frac{1}{3}$ of all of the donor cells are converted to state $\text{E}_1 \text{ to } \text{A}_1$, no oxygen is produced. Therefore the third flash produces $\frac{1}{3}$ oxygen per donor cell.

Before the excitation with the fourth flash $\frac{1}{3}$ of the donor cells in the state $\text{A}_1 \text{ and } \text{state } \text{A}_2$. The fourth flash leads to the transition into $\frac{1}{3}$ of all of the donor cells transit from $\text{E}_2$ to $\text{A}_3$ and $\text{A}_4$ thereby evolving oxygen [eq. (12)] and $\frac{1}{3}$ of all of the donor cells are converted from state $\text{E}_1$ to $\text{A}_1$, no oxygen is produced. Therefore the third flash produces $\frac{1}{3}$ oxygen per donor cell.

Therefore in the 5th flash $\frac{1}{3} \text{O}_2$ 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 Joliot. Therefore the postulated model covers the characteristic kinetic behaviour of the donor system of Chl$\alpha_{11}$.

b) Structure

The model postulates that at least six manganese are bound per donor cell. Cheniae and Martin found experimentally that 6 – 12 manganese atoms are associated with each oxygen evolving centre. Similar results were obtained by Anderson et al. 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 Martin who found two different types of manganese existing in system II. Furthermore tris-washing or heating lead to manganese-release. Experimental data of Possingham and Spencer 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 Chl$\alpha_{11}$ remains.

Note added in proof: Recently it was shown by Vernon and Shaw (Plant Physiol. 44, 1645 [1969]) that diphenylcarbazide 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 chloride 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 HIND 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 hydroxylamine.

In contrast the effect of CCCP on the water splitting system 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).

It was found that an electron pool of PQ exists on the reducing (acceptor) side of \( 
\text{Chl}_{11} \) and \( 
\text{Chl}_{12} \). 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 \( 
\text{Chl}_{11} \).

This prevents the accumulation of positive charges on the donor side of \( 
\text{Chl}_{11} \). There is no experimental evidence for the existence of a donor pool. Although the fluorescence measurements of MURATA 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 complicated 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 shell.

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

The electron transport probability is given by the shape and the height of the energy barrier 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.

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