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Photosynthetic water oxidation

A new chemical model

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A sequential four-step chemical model for the water oxidation process in photosystem II is presented, based on the observation that a peroxide-linked biquinone complex can be chemically formed as a result of hydroxide ion addition to quinone. In our model, the hydroxide ion intermediate is generated in photosystem II as a result of proton abstraction from water. In the model, the first two flashes of light raise the oxidation state of the bimanganese center, while the third and fourth flashes of light sequentially generate the peroxide-linked biquinone which is then directly oxidized by the bimanganese center to produce oxygen and regenerate quinone.

Photosystem II *Oxygen evolution mechanism* *Manganese* *Plastoquinone* *Cytochrome* b_{559}

1. INTRODUCTION

An understanding of the chemical mechanism by which green plants oxidize water to molecular oxygen continues to challenge but elude chemists and biologists. Photosynthetic water oxidation that occurs within the photosystem II complexes of higher plants has been the subject of many reviews [1–11]. All reviews now argue that oxygen evolution results from a sequential 4-step oxidation process in which the oxidizing equivalents produced by each photoact are conserved in a 'charge-storing' complex until sufficient oxidizing power has been accumulated for water oxidation to proceed. Each successive oxidation state of the water oxidizing complex is termed an S-state, S_n, where the subscript represents the number of charges that have accumulated. These S-states have been incorporated into a kinetic model often termed the 'oxygen clock' ([12], also see [11]):

$$S_0 \xrightarrow{h\nu} S_1 \xrightarrow{\eta\nu} S_2 \xrightarrow{\eta\nu} S_3 \xrightarrow{\eta\nu} [S_4] \longrightarrow$$
$$S_0 + O_2 + 4H^+$$

Despite detailed information on the kinetics of interconversion of these S-states, the chemical nature of each is still uncertain. The water-oxidizing complex has an absolute requirement for manganese [13], and current estimates suggest that a minimum of 4 manganese ions per photosystem II unit are required for normal function [14–17], although 2 of these can apparently be replaced with other metals [18–20]. Hence, contemporary models for the charge-storing system of photosystem II envisage a binuclear manganese complex as its functional component, in which charge is stored as increasing oxidation states of

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the manganese ions. The transition state immediately prior to oxygen evolution is postulated to involve the formation of an oxygen-oxygen bond between the 2 manganese ions [21]. Thus, the oxidized binuclear center of photosystem II is believed to act as a 'template' for the formation of the oxygen-oxygen bond [21–23]. Although the object of numerous investigations, the existence of such a manganese-dioxygen intermediate has not been confirmed, nor have binuclear models with associated water achieved formation of an oxygen-oxygen bond and subsequent evolution of molecular oxygen.

The recent discovery in our laboratories [24] that hydroxide ion can be oxidized by quinones to give a peroxide-bridged biquinone has prompted us to propose a new mechanism for the water-oxidation oxygen-evolution process of photosystem II. The oxygen-oxygen bond that forms between the 2 quinone molecules is oxidized by a binuclear manganese center (oxidized) to generate dioxygen and release the 2 quinones. In this report the chemistry associated with the proposed mechanistic path is outlined in a set of equations that are used to formulate a 4-step chemical model for the oxidation of water to dioxygen in photosystem II.

1.1. *The model*

Fig.1 outlines 2 schemes for the reduction of quinones in aprotic media [25]. The reduction of quinone to quinol, thought to occur on the acceptor side of photosystem II, is outlined on the left side of fig.1. In this case, an electron from the reaction center is transferred to a quinone (plastoquinone in the case of photosystem II) to generate its anion radical. Further reduction produces the completely reduced quinone. In the protic medium, that is typically found at the surface of the thylakoid membrane, the one-electron reduction steps of quinone would be coupled with proton addition to produce quinol (not shown).

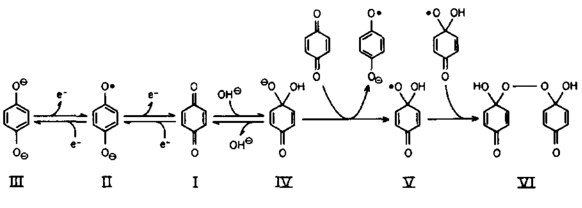

Fig.1. A simplified scheme for the reduction of quinone in an aprotic medium. The reduction of quinone by one-step electron additions is shown on the left, and its reduction by hydroxide ion to generate a peroxide-linked biquinone complex (VI) to the right. Quinone, I; semiquinone anion, II; quinol, III; hydroxide-adduct of quinone, IV; oxy-radical of hydroxide adduct, V; peroxy-linked biquinone, VI.

On the right side of fig.1, an alternative reduction mechanism is outlined whereby semiquinone anion is generated by a hydroxide ion adduct of the quinone [$Q(OH)^-$] that reduces a second quinone. This electron transfer yields a semiquinone oxy-radical [species V, $^{\cdot}Q(OH)$] that dimerises to produce a peroxide-linked biquinone (VI, fig.1). The mechanism for the reduction of quinones by hydroxide ion in aprotic media (outlined in fig.1) has been developed on the basis of a study of anthraquinone in acetonitrile [24]. Hydroxide adducts of substituted quinones identical to species VI (fig.1) have been observed [6] and hydrogen peroxide, presumably the result of the hydrolysis of VI (fig.1), has been detected when hydroxide ion is added to benzoquinone [7]. We have observed essentially stoichiometric yields of semiquinone anion when hydroxide ion is added to a wide range of quinones, including α-tocopherolquinone and plastoquinone (unpublished).

We propose that such chemistry is utilized in photosystem II to bring about the photo-oxidation of water. The sequential chemical reactions that may occur under successive flashes of saturating irradiation, such that the reaction center of photosystem II would turnover once per flash, are shown in fig.2 and will be discussed for each S-state interconversion. This model scheme presents the chemistry that occurs upon interconversion of each S-state. It begins at the S_0 state, but all evidence clearly shows that the dark-adapted chloroplast rests in the S_1 state; this point will be returned to in the discussion.

1.1.1. S_0–S_1

Excitation of the photosystem II reaction center first generates reduced pheophytin ($\text{pheo}^{\cdot -}$) and oxidized chlorophyll ($\text{chl } a^{\cdot +}$) in a process commonly termed the primary charge separation (reaction a). Primary charge separation is thought to occur between a chlorophyll and a pheophytin molecule [28], although our model only requires an oxidized (chl $a^{\cdot +}$) and a reduced (pheo$^{\cdot -}$) species. The primary charge separation is stabilized when chl $a^{\cdot +}$ and pheo$^{\cdot -}$ react with other molecules on the oxidizing and reducing sides of photosystem II, respectively. This event is common to each of the S-state interconversions.

Pheo$^{\cdot -}$ reduces the secondary electron acceptor of photosystem II, a plastoquinone species termed Q_A, to produce $Q_A^{\cdot -}$ and regenerate pheophytin (reaction b). The one electron reduction of quinone was outlined in fig.1. $Q_A^{\cdot -}$ is then able to reduce further components of the noncyclic electron transport chain leading to photosystem I.

Simultaneous with Q_A reduction, chl $a^{\cdot +}$ oxidizes the bimanganese complex on the donor side of photosystem II from an Mn(II)-(III) to a Mn(III)-(III) oxidation state with regeneration of chlorophyll (reaction c). In this scheme, we show chl $a^{\cdot +}$ as the direct oxidant of the manganese center, although there is evidence in the literature for at least one additional component to mediate this electron transfer [10]. Extra components are of no consequence to this model at the present stage, but we will return to this point later.

Thus, the first flash of light to thylakoids in the S_0 state raises the oxidation state of the manganese center from an Mn(II)-(III) to a Mn(III)-(III) complex and generates reduced quinone.

1.1.2. S_1–S_2

During this transition, identical chemistry occurs as in the previous S-state conversion (S_0–S_1), except that the oxidation state of the manganese center is raised to an Mn(III)-(IV) state. The chemistry proposed in these first 2 steps is essentially the same as described in previous models; current data suggest that the transition to S_2 results in an Mn(III)-(IV) oxidation state [22].

1.1.3. S_2–S_3

The chemistry induced by the next flash of light differs from the previous steps and ultimately generates the quinone-hydroxide adduct (IV, fig.1) formed by the addition of hydroxide ion to quinone. We would postulate that the hydroxide ion required for this reaction is generated upon proton abstraction from water by a reduced basic intermediate formed in this step. In reaction d $Q_A^{\cdot -}$ reduces a component, here termed C^{III}, that acts as a cyclic electron carrier around photosystem II ultimately reducing chl $a^{\cdot +}$ in reaction g. It is this component (C) that is required to have a high affinity for protons in its reduced form so that it can abstract a proton from water thus generating hydroxide ion while mediating cyclic electron flow around photosystem II. This hydroxide ion is formed within the membrane as a bound intermediate and is never released as free OH$^-$. We speculate that C is cytochrome b_{559}, which has been suggested to act as a cyclic electron carrier and is thought to be an essential component for the normal functioning of water oxidation [29,30]. Also, reduced cytochrome b_{559} is known to have a higher affinity for protons and has been suggested to have such a proton-linked function in photosystem II [29,30].

The hydroxide ion generated in reaction d (fig.2) can react with a quinone associated with the oxidizing side of photosystem II, Q_W, to generate a hydroxide ion adduct, $Q_W(OH)^-$ (IV, fig.1). $Q_W(OH)^-$ then interacts with a second quinone,

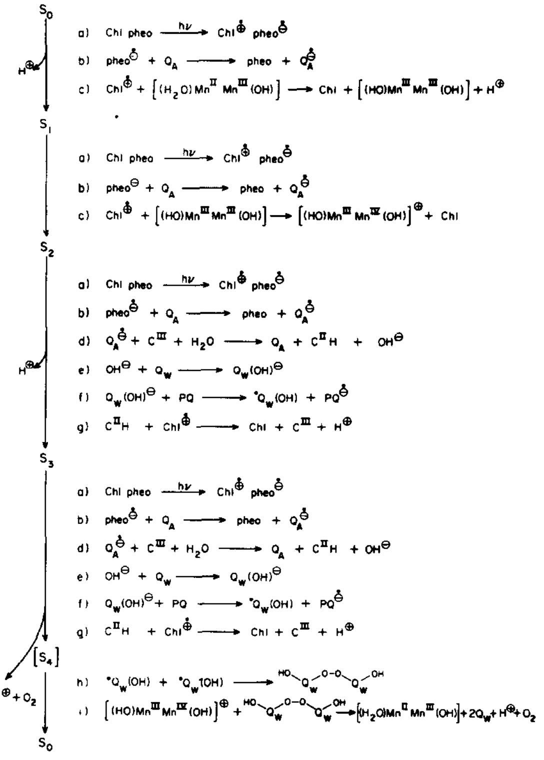

Fig.2. Chemical model and reaction sequence for the S-state interconversions of the water-oxidizing complex of photosystem II. See text for details.

probably plastoquinone, to generate semiquinone anion and the oxy-radical, $^{\cdot}Q_W(OH)$, as shown in reaction f (fig.2). The semiquinone anion can participate in noncyclic electron flow (see above, S_0–S_1). The oxy-radical (species V, fig.1), which must be reasonably stable with a sufficient lifetime for the final step (S_3–S_4) to occur, probably is bound to a protein in close association to a metal ion that confers stability (similar to the stabilization of quinone radicals proposed in other models, e.g. [22]).

1.1.4. S_3–S_4

The fundamental chemistry of the S_2–S_3 transition is repeated to generate a second oxy-radical (V, fig.1) so that in the meta-stable S_4 state there are 2 oxy-radicals that spontaneously dimerise to form a peroxide-linked biquinone (species VI, fig.1) in reaction h (fig.2). This species is oxidized by the manganese center [Mn(III)-(IV)] to give dioxygen and Q_W with the manganese center reduced to the Mn(II)-(III) oxidation state. Thus, the S_0 state is automatically re-established, ready for the next cycle.

2. DISCUSSION

The unique feature of this new chemical model for photosystem II is that the oxygen-oxygen bond is formed between 2 quinone molecules rather than between 2 manganese ions. The manganese center serves to oxidize a peroxide-linked biquinone species to produce molecular oxygen rather than act as a template for oxygen-oxygen bond formation.

The model includes essential roles for both cytochrome b_{559} and quinones in the water-oxidizing mechanism of photosystem II. The physiological role of cytochrome b_{559} at room temperature is still uncertain, but a proton-linked function in photosystem II has been proposed [29,30]. This idea has been extended to link hydroxide ion production (and its oxidation by quinone) to the water-oxidation mechanism. Although there is some evidence for an association between manganese and plastosemiquinone anion in photosystem II [31] and stable semiquinone complexes of manganese have been proposed as models for the manganese center of photosystem II [32,33], there are no conclusive experimental results to support this proposition. More recently, ESR data has suggested that plastoquinol acts as an intermediary electron carrier between the manganese ions and the reaction center of photosystem II [4]. Whether there is any relationship between these observations and the present model remains to be elucidated.

When subject to a train of saturating light flashes, thylakoids also exhibit a characteristic pattern of proton release from the water-oxidizing complex. As can be seen from fig.2, we predict a proton release pattern from the S_0–S_1: S_1–S_2:S_2–S_3:S_3–S_0 transitions of 1:0:1:2. Previous experiments [10] indicate proton-release patterns of either 1:0:1:2 or 0:1:1:2, but a proton release pattern of 1:0:1:2 is in accord with recent kinetic experiments [10].

The reaction sequence of the model (fig.2) is in close conformity to current data for the chemical nature of the S-states. However, evidence for such a sequence is not conclusive and it is possible that a different order of events occurs in photosystem II. For example, the S_1–S_2 and S_2–S_3 transitions could be interchanged so that the second flash of light generates hydroxide ion. Then the oxidation state changes of the manganese center would occur during the S_0–S_1 and S_2–S_3 transitions. Likewise, the S_0–S_1 and S_2–S_3 transitions could be interchanged. Although such modifications of the scheme are not supported by X-ray absorption edge data [22], flash-induced absorption changes indicate a change in manganese ion oxidation state during the S_2–S_3 transition [35].

Recent models on the structural organization of photosystem II, e.g. [10], could easily accommodate the present reaction sequence and chemistry. However, we would emphasize the central role of cytochrome b_{559} in mediating cyclic electron flow around photosystem II. The present model and its reaction sequence (fig.2) predict a site on the oxidizing side of photosystem II for the interaction of plastoquinone with Q_W that is analogous to the reducing side of photosystem II, where plastoquinone interacts with Q_A. Clearly, more work is required to elucidate the chemical mechanism of water oxidation that occurs in photosystem II. What we have presented is a viable model for the chemical aspects of photosystem II and one that includes numerous predictions that can be tested. We believe it provides a framework for the systematic characterization of the chemical sequences for the oxidation of water to dioxygen in photosystem II.

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