Analysis of the Reaction Coordinate of Photosynthetic Water Oxidation by Kinetic Measurements of 355 nm Absorption Changes at Different Temperatures in Photosystem II Preparations Suspended in Either H_2O or D_2O[†]

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ABSTRACT: Flash-induced absorption changes at 355 nm were measured at different temperatures within the range of 2 °C ≤ ϑ ≤ 25 °C in dark-adapted PS II core complexes from spinach [O_2 evolution rate: 1500 ± 100 μmol of O_2 (mg of Chl)$^{-1}$ h^{-1}] that were dissolved either in H_2O- or in D_2O-containing buffer. Comparative measurements were performed at 20 °C in H_2O- or D_2O-containing suspensions of PS II membrane fragments [O_2 evolution rate: 600 ± 40 μmol of O_2 (mg of Chl)$^{-1}$ h^{-1}]. The results obtained reveal the following: (a) The activation energies of the individual redox steps in the water oxidizing complex (WOC) are dependent on the redox state S_i with $E_A(S_1 \rightarrow S_2) = 14$ kJ/mol, $E_A(S_2 \rightarrow S_3) = 35$ kJ/mol, and $E_A(S_3 \dashrightarrow S_0 + O_2) = 21$ kJ/mol for $\vartheta > 11$ °C, 67 kJ/mol for $\vartheta < 11$ °C in PS II core complexes dissolved in H_2O; (b) replacement of exchangeable protons by deuterons causes only minor changes (≤15%) of the activation energies; and (c) the rate constants of these reactions in PS II core complexes are characterized by H/D isotope ratios, $k_i(H)/k_i(D)$, of 1.6, 2.3, and 1.5 for the transitions $S_1 \rightarrow S_2$, $S_2 \rightarrow S_3$, and $S_3 \dashrightarrow S_0 + O_2$, respectively. The corresponding values of PS II membrane fragments are 1.3, 1.3, and 1.4. Based on these results and corresponding E_A data reported in the literature for PS II membrane fragments from spinach [Renger, G., & Hanssum, B. (1992) *FEBS Lett. 299*, 28−32] and PS II particles from the thermophilic cyanobacterium *Synechococcus vulcanus* Copeland [Koike, H., Hanssum, B., Inoue, Y., & Renger, G. (1987) *Biochim. Biophys. Acta 893*, 524−533], the reaction coordinate of the redox sequence in the WOC is inferred to be almost invariant to the evolutionary development from cyanobacteria to higher plants. Furthermore, the rather high activation energy of the $S_2 \rightarrow S_3$ transition provides evidence for a significant structural change coupled with this reaction. Implications for the mechanism of photosynthetic water oxidation are discussed.

Photosynthetic water cleavage into dioxygen and metabolically bound hydrogen takes place in a multimeric protein complex referred to as photosystem II (PS II)[1] that is anisotropically incorporated into the thylakoid membrane. The overall process comprises three different types of reaction sequences: (a) photooxidation of a special Chl *a* component (symbolized by P680) and subsequent stabilization of the primary charge separation by rapid electron transfer from $Pheo^{-\bullet}$ to a specially bound plastoquinone (Q_A) [for a review, see Renger (1992)]; (b) cooperation of 4 strongly oxidizing redox equiv in a manganese-containing unit, the water oxidizing complex (WOC), that leads to oxidation of two water molecules into dioxygen and four protons [for reviews, see Debus (1992) and Renger (1993)]; and (c) cooperation of 2 reducing equiv giving rise to plastoquinone reduction to plastoquinol under proton uptake [for a review, see Crofts and Wraight (1983)].

The structural and functional organization of reaction sequences (a) and (c) resembles that of the corresponding processes taking place in the reaction centers of anoxygenic purple bacteria [for a review, see Michel and Deisenhofer (1988)], except for the markedly different properties of P680 [for a discussion, see Renger (1993) and van Gorkom and Schelvis (1993)]. In contrast, reaction sequence (b) is unique for PS II of all oxygen-evolving photosynthetic organisms. The basic principles of the reaction pattern of (b) have been resolved about 25 years ago by the fundamental work of Joliot and Kok and their co-workers [for a review, see Joliot and Kok (1975)].

Reaction sequence (b) can be described by eq 1:

$$Y_Z^{OX} S_i + w_{i+1} H_2O \xrightarrow{k_i} Y_Z S_{i+1-4\delta_{i3}} + n_i H^+_{lumen} + \delta_{i3} O_2 \quad (1)$$

where k_i is the rate constant, Y_Z^{OX} is the redox active tyrosine of polypeptide D1 (Debus et al., 1988; Metz et al., 1989) that acts as intermediary redox group for the stepwise oxidation of the WOC by $P680^{+\bullet}$, n_i and w_{i+1} are the numbers of protons released and substrate water molecules bound, respectively, in the redox transition $S_i \rightarrow S_{i+1}$, S_i represents the redox state of the WOC with $i = 0$ under normal turnover

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[1] Abbreviations: β-DM, dodecyl *N*-β-maltoside; 2,6-DCBQ, 2,6-dichloro-*p*-benzoquinone; EDTA, ethylenediaminetetraacetate; MES, 2-(*N*-morpholino)ethanesulfonic acid; Pheo, pheophytin; PS II, photosystem II; P680, primary electron donor of PS II; Q_A, primary plastoquinone acceptor of PS II; S_i, redox state of the water oxidizing complex; WOC, water oxidizing complex; Y_Z, redox active tyrosine of polypeptide D1.

conditions, and δ_{i3} is the Kronecker symbol, i.e., $\delta_{i3} = 1$ for $i = 3$, otherwise zero.

This formulation tacitly implies that S_4 does not exist as the highest oxidation state of the manganese cluster in the WOC because Y_Z^{OX} is assumed to cause an oxidant-induced reduction of S_3 to $S_0 + O_2$ [Renger et al., 1994; see also Rappaport et al. (1994)]. Apart from this particular problem, a number of mechanistic questions of fundamental relevance are still far from being answered [for a list, see Renger (1987, 1993)].

Recently the redox active tyrosine Y_Z has been proposed to act as a hydrogen abstractor from the substrate water in the WOC (Babcock, 1995). This idea suggests the possibility of significant kinetic H/D isotope exchange effects. The latest results revealed that the kinetics of reaction sequence (a) remain virtually invariant to extensive H/D exchange (Vasiliev et al., 1996). Likewise, a vanishingly small effect was observed for the nanosecond kinetics of $P680^{+\bullet}$ reduction by Y_Z in systems with a functionally competent WOC (Karge et al., 1996). In marked contrast to that, however, an almost 3-fold retardation of $P680^{+\bullet}$ reduction by Y_Z is observed after rapid replacement of H by D in PS II membrane fragments deprived of a functionally competent WOC (Christen et al., 1997). The redox transitions in the WOC itself exhibit isotope effects of k_H/k_D ranging from 1.1 to 3.0 (Renger et al., 1994; Lydakis -Simantiris et al., 1995; Karge et al., 1996) as a function of the redox state S_i. However, the data so far available do not lead to a consistent feature of the H/D isotope exchange effect in different PS II preparations.

Another useful approach for addressing mechanistic questions is the analysis of the reaction coordinates by measuring the rate constants of the individual redox steps in the WOC (see eq 1) as a function of temperature. Analysis within the framework of the Marcus theory [for reviews, see de Vault (1984) and Marcus and Sutin (1985)] of data obtained for PS II preparations from the thermophilic cyanobacterium *Synechococeus vulcanus* Copeland (Koike et al., 1987) and in PS II membrane fragments from spinach (Renger & Hanssum, 1992) led to the conclusion that the $S_2 \rightarrow S_3$ redox transition is accompanied by a significant structural change. This idea is highly supported by the drastic retardation of the S_3 reduction by NH_2OH and NH_2NH_2 compared with that of S_2 (Messinger & Renger, 1990; Messinger et al., 1991). The latest EXAFS data (Yachandra et al., 1996) provide direct evidence for this conclusion. It was therefore concluded that a nuclear rearrangement takes place in S_3. The proposed structural change was postulated to reflect the formation of an S_3 state with peroxidic type electronic configuration and nuclear geometry. Furthermore, this state is assumed to equilibrate with a state of complexed OH^-, thus giving rise to a redox isomerism in S_3 (Renger, 1993).

Recently it was shown that in PS II core complexes from spinach the rate of Y_Z^{OX} reduction by S_3 is slower by a factor of 3−4 compared with that of thylakoids and PS II membrane fragments while the other redox transitions in the WOC ($i = 0, 1, 2$) remain virtually unaffected (van Leeuwen et al., 1993a). A very similar feature was observed for PS II membrane fragments that were deprived of the extrinsic 18 and 23 kDa proteins by washing with NaCl (Dekker et al., 1984a). PS II membrane fragments and PS II core complexes provide a suitable material for comparative studies of the reaction coordinates of the WOC. The present study describes thorough analyses of two problems: (i) kinetic H/D isotope exchange effects on the univalent oxidation steps of the WOC in PS II membrane fragments and PS II core complexes from spinach, and (ii) the temperature dependence of these reactions in PS II core complexes dissolved either in H_2O- or in D_2O-containing buffer.

MATERIALS AND METHODS

PS II membrane fragments were prepared using the procedure developed by Berthold et al. (1981) with slight modifications outlined in Völker et al. (1985). In order to reduce scattering for the measurements of UV absorption changes, the samples were washed with a buffer solution containing 500 μM EDTA for 5 min and centrifuged. The pellet was resuspended in buffer medium. The oxygen evolution rates of the PS II membrane fragments under saturating CW light were 600 ± 40 μmol of O_2/(mg of Chl)$^{-1}$ h^{-1} in the presence of 0.5 mM 2,6-dichloro-*p*-benzoquinone (2,6-DCBQ). PS II core complexes have been purified according to a modified protocol as described previously by Haag et al. (1990). In order to achieve a high extent of H/D exchange without loss of oxygen evolution activity, a new protocol has been developed. At first, the Chl concentration of a freshly prepared sample has been adjusted to 100−200 μg/mL in a buffer containing 20 mM MES−NaOH, pH 6.5, 10 mM NaCl, 20 mM $CaCl_2$, 0.025% w/v β-dodecyl maltoside, and 35% w/v sucrose at 4 °C in the dark. Definite volumes of this concentrated sample were dropped into liquid nitrogen under moderate green light and then lyophilized in the dark and cold for 24 h. Finally, dried samples were redissolved on ice in either sterile twice-deionized water or D_2O (Eurisotop, 99.9%). It is important to note that foaming should be avoided during all the steps. Reversibility of D_2O/H_2O exchange has been achieved by equilibrium dialysis against the same buffer system.

It has to be emphasized that the oxygen evolution capacity remained virtually unaffected by this treatment. In both H_2O and D_2O samples the light-saturated oxygen evolution rates were found to be 1500 ± 100 μmol of O_2 (mg Chl)$^{-1}$ h^{-1} in the presence of 0.5 mM 2,6-DCBQ as electron acceptor.

Absorption changes at 355 nm induced in dark-adapted PS II core complexes by excitation with a train of single-turnover flashes were monitored with a home-built laser flash photometer with a conventional photoshutter technique for obtaining pulses with a few millisecond duration of the measuring light beam (Renger & Weiss, 1983). The intensity of these pulses was kept at a value that gives rise to an actinic effect of no more than 5% of PS II excitation. A laser flash (FWHM = 9 ns) frequency of 10 Hz was used in order to eliminate effects due to the rapid decay of redox states S_2 and S_3 in PS II core complexes (Gleiter et al., 1993; van Leeuwen et al., 1993a). The signals were monitored with an electrical bandwidth of 100 kHz. In all measurements, the cuvette temperature was kept constant within 0.1 °C by using a Peltier element (Renger & Hanssum, 1992).

Other assay conditions are given in the figure legends.

RESULTS

Rate Constants of the Univalent Steps of Electron Abstraction from the WOC by Y_Z^{OX} as a Function of Temperature. In general, two basically different methods are currently used to determine the rate constants k_i of the redox transitions

summarized by eq 1: (i) monitoring of the reduction kinetics of Y_Z^{OX} by time-resolved EPR measurements (Babcock et al., 1976; Lydakis-Simantiris et al., 1995; Razeghifard et al., 1997); or (ii) flash-induced absorption changes in the UV region (Dekker et al., 1984b; Renger & Weiss, 1986; Rappaport et al., 1994). Both methods are complementary and have "pro's and con's" but lead basically to the same results [for a compilation of data, see Table 1 of the latest report presented by Razeghifard et al. (1997)]. In the present study, laser flash-induced absorption changes were measured at 355 nm where the difference extinction coefficient of Y_Z^{OX}/Y_Z is close to 0 (Dekker, 1985; Weiss & Renger, 1986) so that the turnover of Y_Z does not lead to significant contributions to the absorption changes reflecting the reactions. In any case, the magnitude of $\Delta\epsilon(Y_Z^{OX}/Y_Z)$ does not affect the kinetics but only the extent of the absorption changes that are ascribed to the reactions of eq 1.

In addition to these absorption changes, the turnover of the quinones Q_A and Q_B at the acceptor side of PS II and the reduction of exogenous acceptors can give rise to interferences. Therefore, a short analysis of the possible effects is required before presenting and discussing the results obtained. In general, the later contributions can be kinetically separated.

The flash-induced formation of Q_A^- occurs with $\tau = 300$ ps (Eckert et al., 1988; Bernarding et al., 1994). This reaction gives rise to an "instantaneous" rise that is limited by the time resolution of the equipment in the microsecond/millisecond region and can easily be separated from the absorption changes that reflect the reactions of eq 1.

A more serious problem is the separation of components owing to the reactions that are induced by Q_A^-. Fortunately enough, the situation is comparatively simple for PS II core complexes. These preparations are lacking a functionally fully competent Q_B site (Gleiter et al., 1993), and therefore any affect owing to binary oscillations caused by Q_A^- reoxidation with Q_B (Q_B^-) is eliminated. This has been convincingly shown by a thorough analysis of the acceptor side reactions in these samples (van Leeuwen et al., 1993a). Q_A^- was found to be oxidized by exogenous acceptors (K_3-[$Fe(CN)_6$] plus DCBQ) with kinetics characterized by a half-lifetime of the order of 10 ms. Somewhat slower values of about 15 ms were found in another study under slightly different conditions (Karge et al., 1996). Regardless of these details, the kinetics are much slower (by a factor of more than 10) than those of the S_i-state transitions up to S_3. Based on this finding and the similarity of $\Delta\epsilon_{355}(S_2/S_1)$ and $\Delta\epsilon_{355}$-(S_3/S_2) (van Leeuwen et al., 1993b) with $\Delta\epsilon 355(Q_A^-/Q_A)$ (Schatz & van Gorkom, 1985), it follows that any significant kinetic interference by contributions from the acceptor side can be neglected. This conclusion is also valid for samples dissolved in D_2O. Only for S_3 oxidation by Y_Z^{OX} a kinetic separation is required as described in Karge et al. (1996).

At first glance, the situation is somewhat more complicated for PS II membrane fragments. Although slightly modified compared with thylakoids (Renger et al., 1986), the Q_B site remains virtually intact, and the binary oscillations of the acceptor side reactions can take place. However, there exists a possibility to circumvent this problem. If Q_B^- becomes reoxidized between the flashes by an exogenous acceptor, then after each flash the same acceptor side reaction takes place, i.e., $Q_A^-Q_B \rightarrow Q_AQ_B^-$, which is characterized by kinetics of a few hundredths of microseconds (Bowes & Crofts, 1980; Weiss & Renger, 1984). Therefore, these kinetics are not easy to separate from those of the reactions $Y_Z^{OX}S_1 \rightarrow Y_ZS_2$ and $Y_Z^{OX}S_2 \rightarrow Y_ZS_3$. A simple way to eliminate this complication is to measure at a wavelength where $\Delta\epsilon_\lambda(Q_A^{-\bullet}/Q_A) \sim \Delta\epsilon_\lambda(Q_B^{-\bullet}/Q_B^-)$. This condition is sufficiently satisfied at 355−360 nm (Schatz & van Gorkom, 1985). As a consequence, for measurements with PS II membrane fragments, it is necessary to use experimental conditions where Q_B^- becomes oxidized between the flashes. This goal is achieved by again using K_3[$Fe(CN)_6$] plus DCBQ as electron acceptor. The reaction with these components is slow enough (on the order of a few milliseconds) to avoid significant kinetic interference.

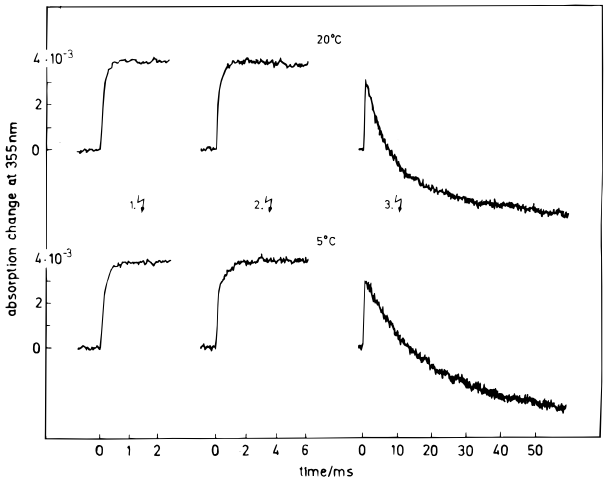


FIGURE 1: Absorption changes induced at 355 nm by a train of three laser flashes in dark-adapted PS II core complexes dissolved in H_2O buffer. The suspension contained sample material at a chlorophyll concentration of 25 μg/mL, 10 mM NaCl, 20 mM $CaCl_2$, 20 mM MES/NaOH, pH 6.5, 0.025% dodecyl β-maltoside, and 250 μM 2,6-dichloro-*p*-benzoquinone plus 200 μM K_3[$Fe(CN)_6$] as electron acceptor. 40 signals were averaged in the cases of the first and second flashes while the averaging implies 55 signals for the third flash; time between the flashes 100 ms, electrical bandwidth 100 kHz, other conditions as described under Materials and Methods. The measurements were performed either at 20 °C (top traces) or at 5 °C (bottom traces).

As a resumé of the above-mentioned consideration, the flash-induced 355 nm absorption changes measured under the assay conditions described under Materials and Methods and the figure legends permit a satisfactory separation of the kinetics of the S_i-state transition from those of acceptor side reactions.

Figure 1 shows typical traces that are induced by excitation of dark-adapted PS II core complexes with a train of laser flashes. The traces at the top were measured at 20 °C, those at the bottom at 5 °C. All absorption changes exhibit a fast unresolved rise that is due to $P680^{+\bullet}Q_A^{-\bullet}$ formation. In addition to this feature, the absorption changes induced by the first and second flashes exhibit a pronounced slower rise that can be kinetically resolved and is ascribed to reactions in the WOC (*vide supra*). On the other hand, after the third flash, the unresolved rise is followed by a pronounced decay. The WOC almost entirely populates redox state S_1 in sufficiently dark-adapted samples [see Seeliger et al. (1997) and references cited therein]. As a consequence, the resolved time courses of the 355 nm absorption changes in the microsecond/millisecond time domain reflect the redox

Table 1: Half-Lifetimes, $t_{1/2}$, at 20 °C and H/D Isotope Ratio of Rate Constants, k_i(H)/k_i(D), of Equation 1 with $i = 1$, 2, and 3 in PS II Core Complexes from Spinach Dissolved either in H_2O or in D_2O

transition in WOC	half-lifetimes $t_{1/2}$		k_i(H)/k_i(D)
	H_2O	D_2O	
$S_1 \rightarrow S_2$	75 ± 10 μs	120 ± 10 μs	1.6 ± 0.3
$S_2 \rightarrow S_3$	225 ± 25 μs	500 ± 40 μs	2.3 ± 0.4
$S_3 \rightarrow (S_4) \rightarrow S_0 + O_2$	4.1 ± 0.3 ms	6.0 ± 0.5 ms	1.5 ± 0.2

transitions $S_1 \rightarrow S_2$ (first flash), $S_2 \rightarrow S_3$ (second flash), and $S_3 \rightarrow\rightarrow S_0 + O_2$ plus acceptor side contribution (third flash).

An inspection of the data readily shows that the kinetics of the transients ascribed to $Y_Z^{OX}S_2 \rightarrow Y_ZS_3$ and $Y_Z^{OX}S_3 \rightarrow\rightarrow Y_ZS_0 + O_2$ are markedly retarded at 5 °C while the $Y_Z^{OX}S_1 \rightarrow Y_ZS_2$ transition (first flash) appears to be less sensitive to temperature. These temperature effects will be analyzed in more detail in a subsequent paragraph. Another striking feature is the more pronounced lag phase of the relaxation kinetics after the third flash that is observed at 5 °C. A similar phenomenon has been observed in PS II particles from the thermophilic cyanobacterium *Synechococcus vulcanus* Copeland (Koike et al., 1987). This lag phase was taken as evidence for a reaction sequence taking place during electron transfer from S_3 to Y_z (Rappaport et al., 1994). However, it has to be emphasized that owing to misses and double hits the 355 nm absorption changes induced by the third flash not only are the composite of contributions reflecting the Q_A reduction to $Q_A^{-\bullet}$ and the reduction of Y_Z^{OX} by S_3 but also contain a significant contribution due to the $S_2 \rightarrow S_3$ transition. The observed lag phase critically depends on the extent of the $S_2 \rightarrow S_3$ transition during the third flash. A detailed analysis of this phenomenon and possible implications will be outlined in a forthcoming paper.

The time course of the transients monitored after excitation with the first, second, and third flashes (see Figure 1, top) is not identical with that of the redox steps $S_1 \rightarrow S_2$, $S_2 \rightarrow S_3$, and $S_3 \rightarrow\rightarrow S_0 + O_2$, respectively, of the WOC because of the probability of misses (α) and double hits (β) that leads to an admixture of other S_i-state transitions to the overall kinetics that increases with the progress of the flash sequence. The values of α and β are also dependent on the flash number (Shinkarev & Wraight, 1993). However, the analysis of simulated kinetics revealed that the latter effect is comparatively small, and therefore the conventional Kok model (Kok et al., 1970) was used for data evaluation as described previously (Renger & Hanssum, 1992). The rate constants k_i gathered by this procedure for the redox steps described by eq 1 with $i = 1$, 2, 3 are summarized in Table 1. For the sake of convenience and easy comparability with values reported in the literature, the rate constants k_i are replaced in Table 1 by the corresponding half-lifetimes. The $t_{1/2}$ values obtained correspond with those reported by van Leeuwen et al. (1993a) for a similar type of preparation, thus confirming that in PS II core complexes only the reaction $Y_Z^{OX}S_3 \rightarrow Y_ZS_0 + O_2 + n_3H^+$ becomes significantly retarded (factor of 3−4) compared with untreated PS II membrane fragments (Dekker et al., 1984b, Renger & Weiss, 1986; Hoganson & Babcock, 1988; Rappaport et al., 1994), isolated thylakoids (Velthuys, 1981; Renger & Weiss, 1982), and thermophilic cyanobacteria (Koike et al., 1987).

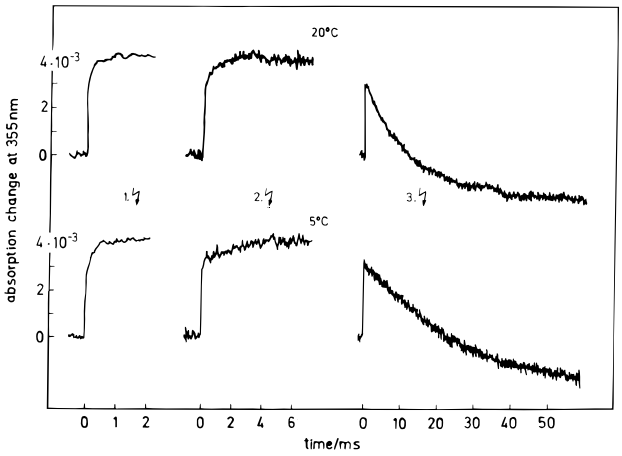


FIGURE 2: Absorption changes induced at 355 nm by a train of three laser flashes in dark-adapted PS II core complexes dissolved in D_2O buffer. Experimental conditions were the same as in Figure 1 except that D_2O was used as solvent instead of H_2O. The measurements were performed either at 20 °C (top traces) or at 5 °C (bottom traces) and pD 6.5.

H/D Isotope Exchange Effects on the Rate Constants of the Univalent Steps of Electron Abstraction from the WOC by Y_Z^{OX} in PS II Membrane Fragments and PS II Core Complexes. In order to achieve a thorough H/D isotope exchange, the experiments were performed with lyophilized PS II core complexes dissolved either in H_2O or in D_2O (see Materials and Methods). The Chl and buffer concentrations were carefully adjusted to that of the sample prior to lyophilization. Furthermore, the ionic strength was checked conductometrically, and the sucrose content was determined by refractometry. Typical traces of 355 nm absorption changes induced at 5 °C and 20 °C in dark-adapted samples by excitation with a train of laser flashes are shown in Figure 2. A comparison of the traces depicted in Figures 1 and 2 reveals a significant retardation of the kinetics that are ascribed to the electron transfer from the WOC in redox states S_2 and S_3 to Y_Z^{OX} while the other reactions seem to be less affected. Furthermore, the isotope effect is clearly seen at both temperatures.

The rate constants k_i gathered from the kinetic analysis of the 355 nm absorption changes measured at 20 °C in samples dissolved either in H_2O or in D_2O are compiled in Table 1. From these, data the kinetic H/D isotope exchange ratios k_i(H)/k_i(D) were calculated. The values obtained are summarized in the last column of Table 1.

A comparison with analogous measurements performed in PS II membrane fragments (Renger et al., 1994) revealed a close similarity of the values for k_1(H)/k_1(D) and k_3(H)/k_3(D) but a significant difference for the reaction $Y_Z^{OX}S_2 \rightarrow Y_ZS_3$. The latter effect could originate from the use of different sample material and therefore reflect changes of the hydrogen bond network. In order to address this problem, 355 nm absorption changes were measured in dark-adapted PS II membrane fragments. The traces obtained by excitation with the first and second flash are depicted in Figure 3, and the k_i(H)/k_i(D) ratios gathered from these data are compiled in Table 2. Based on the results of Tables 1 and 2, it is inferred that the H/D isotope exchange effect of $Y_Z^{OX}S_2 \rightarrow Y_ZS_3$ is significantly larger in PS II core complexes than in PS II membrane fragments while an analogous

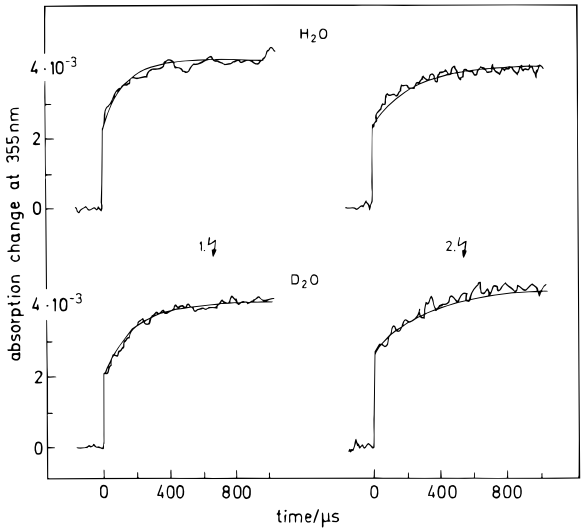


FIGURE 3: Absorption changes induced at 355 nm by two laser flashes in dark-adapted PS II membrane fragments suspended either in H_2O (top traces) or in D_2O (bottom traces). The suspension contained sample material at a chlorophyll concentration of 25 μg/mL, 10 mM NaCl, 20 mM MES/NaOH, pH 6.5, and 100 μM 2,6-dichloro-*p*-benzoquinone plus 700 μM $K_3[Fe(CN)_6]$ as electron acceptors. 200 signals were averaged in each case; time between the flashes 700 ms, electrical bandwidth 100 kHz.

Table 2: Half-Lifetimes, $t_{1/2}$, at 20 °C and H/D Isotope Ratio of Rate Constants, k_i(H)/k_i(D), of Equation 1 with $i = 1, 2$ in PS II Membrane Fragments from Spinach Suspended either in H_2O or in D_2O

	half-lifetimes $t_{1/2}$		
transition in WOC	H_2O	D_2O	k_i(H)/k_i(D)
$S_1 \rightarrow S_2$	85 ± 10 μs	110 ± 10 μs	1.3 ± 0.2
$S_2 \rightarrow S_3$	240 ± 25 μs	320 ± 35 μs	1.3 ± 0.2
$S_3 \rightarrow\!\!\!\rightarrow S_0 + O_2$	1.3 ± 0.2 ms[a]	1.8 ± 0.2 ms[a]	1.4[a] ± 0.3

[a] Data from Renger et al. (1994).

change is not observed for $Y_Z^{OX}S_1 \rightarrow Y_ZS_2$ and $Y_Z^{OX}S_3 \rightarrow\!\!\!\rightarrow Y_ZS_0 + O_2$.

This finding reveals that the kinetic H/D isotope exchange effect of S_3 formation is slightly modified in PS II core complexes. The possible implications will be briefly outlined under Discussion.

For a comparison with k_1(H)/k_1(D) values gathered from EPR measurements, data are only available for PS II membrane fragments (Lydakis-Simantris, 1995). It is seen that very similar ratios are observed with both techniques (355 nm absorption changes and EPR) for $Y_Z^{OX}S_2 \rightarrow Y_ZS_3$ and $Y_Z^{OX}S_3 \rightarrow\!\!\!\rightarrow Y_ZS_0 + O_2$. On the other hand, a marked difference exists for the reactions $Y_Z^{OX}S_1 \rightarrow Y_ZS_2$. At present, it is difficult to find a simple explanation for this discrepancy. Further experiments are required to clarify this particular point.

Reversibility of the Kinetic H/D Exchange Effect. The H/D exchange was achieved by a rather thorough treatment. Therefore, the question arises as to whether the effect comprises protons that can be reversibly exchanged. In order to address this point, dialysis experiments were performed with samples dissolved in either H_2O or D_2O. The samples were dialyzed for 8 h in the dark (cutoff of dialysis membrane: 13−15 kDa) at 4 °C against a buffer solution in H_2O containing 35% w/v sucrose, 10 mM NaCl, 20 mM $CaCl_2$, 20 mM MES/NaOH, pH 6.5, and 0.025% (w/v) β-DM. The results obtained for the kinetics of Y_Z^{OX} reduction by S_3 are depicted in Figure 4. An inspection of these data reveals that virtually no change is observed when samples dissolved in H_2O are dialyzed against a buffer solution of H_2O. This finding shows that the dialysis does not affect the kinetics as expected for a sufficiently mild procedure. On the other hand, the dialysis of the D_2O sample against a H_2O buffer solution leads to an acceleration of the reaction up to the rate of the H_2O sample. Based on this result, the kinetic H/D exchange effect is inferred to comprise protons that are comparatively easy to exchange; i.e., the effect is reversible.

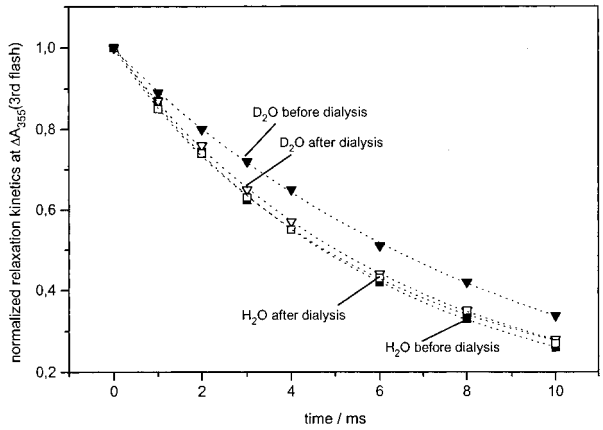


FIGURE 4: Normalized relaxation kinetics of 355 nm absorption changes induced by the third flash in samples that were dissolved either in H_2O or in D_2O and subsequently dialyzed against H_2O containing buffer. Squares and triangles represent data points obtained in samples suspended in H_2O and D_2O, respectively; closed symbols before and open symbols after dialysis against H_2O buffer. Other experimental conditions as described in Figure 1.

After having shown that kinetic H/D isotope exchange leads to a reversible retardation of the redox reactions in the WOC of both PS II membrane fragments (data not shown) and PS II core complexes (Figure 4), it now remains to be shown whether or not the reaction coordinates are modified in the latter compared with the former sample type. In order to investigate this problem, the temperature dependence of the rate constants was analyzed for PS II core complexes. In addition, temperature measurements were performed for samples dissolved either in H_2O or in D_2O.

Temperature Dependence of the Redox Steps in the WOC of Normal and Deuterated PS II Core Complexes. Flash-induced absorption changes at 355 nm were measured in dark-adapted samples at different temperatures in the range of $2 \leq \vartheta \leq 25$ °C where the WOC is only marginally affected by thermal instability. Typical traces obtained at 20 and 5 °C are shown in Figures 1 and 2. The deconvolution of the time courses within the framework of the Kok model was performed as described in Renger and Hanssum (1992) (vide supra). Figure 5 depicts the logarithm of rate constants k_i (for $i = 1, 2, 3$) as a function of the reciprocal temperature in PS II core complexes dissolved either in H_2O (closed symbols) or in D_2O (open symbols). These Arrhenius plots readily show that in both cases a qualitatively very similar pattern arises that is characterized by two striking features: (i) the redox transitions $Y_Z^{OX}S_1 \rightarrow Y_ZS_2$ and $Y_Z^{OX}S_2 \rightarrow Y_ZS_3$ of the WOC can be described by a straight line with a significantly steeper slope in the latter case; and (ii) the temperature dependence of the electron abstraction by Y_Z^{OX} from the WOC in redox state S_3 exhibits a

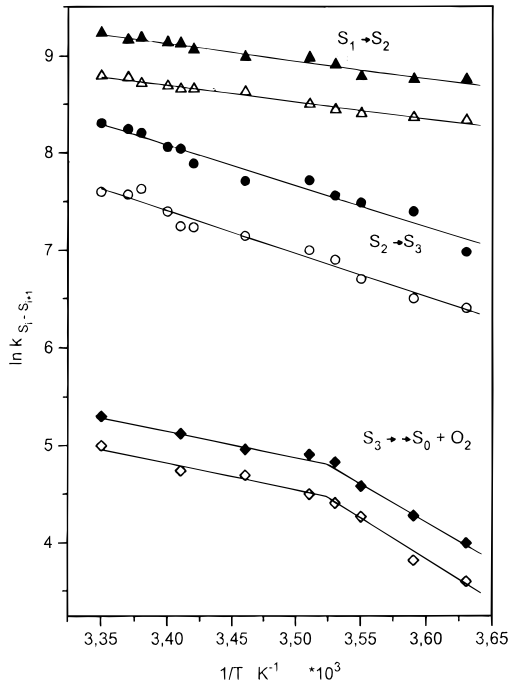


FIGURE 5: Rate constants k_i for $i = 1, 2, 3$ as a function of reciprocal temperature of PS II core complexes dissolved either in H_2O (closed symbols) or in D_2O (open symbols). Experimental conditions as in Figure 1. Ordinate: logarithmic.

Table 3: Activation Energies, E_A, and Preexponental Factors of the Individual Redox Steps of Equation 1 with $i = 1, 2, 3$ in PS II Core Complexes Dissolved either in H_2O or in D_2O [the Corresponding Values Are Symbolized by (H) and (D), Respectively]

reaction	E_A(H) (kJ/mol)	A(H) (s^{-1})	E_A(D) (kJ/mol)	A(D) (s^{-1})
$Y_Z^{OX}S_1 \rightarrow Y_ZS_2$	14.8 ± 1	4.0×10^6	14.7 ± 1	2.4×10^6
$Y_Z^{OX}S_2 \rightarrow Y_ZS_3$	35.0 ± 2	5.35×10^9	37.1 ± 2	6.5×10^9
$Y_Z^{OX}S_3 \rightarrow\!\!\rightarrow Y_ZS_0 + O_2$				
$T > 280$ K	21.0 ± 1.5	8.9×10^5	24.5 ± 1.5	2.7×10^6
$T < 280$ K	67.0 ± 5	2.9×10^{14}	67.0 ± 5	1.8×10^{14}

pronounced breakpoint at a characteristic temperature ϑ of about 10 °C. These general features closely resemble those previously found for PS II particles from thermophilic cyanobacteria (Koike et al., 1987) and PS II membrane fragments from spinach (Renger & Hanssum, 1992) as will be further outlined under Discussion. Furthermore, the nearly parallel curves for H_2O- and D_2O-dissolved samples clearly show that replacement of exchangeable protons by deuterons causes only marginal effects on the activation energies of the individual redox steps in the WOC. For comparability and a more quantitative evaluation, the activation energies and preexponential factors were calculated from the plot of the data in Figure 5. The values obtained are compiled in Table 3. They confirm the qualitative conclusions.

DISCUSSION

For the sake of better illustration of the comparison of PS II membrane fragments and PS II core complexes from spinach, and PS II preparations from thermophilic cyanobacteria, Table 4 presents the activation energies, E_A, and the breakpoint temperatures ϑ_c, reported so far in the literature and in the present study. An inspection of the data compiled in Tables 1, 2, and 4 reveals that the reaction

Table 4: Activation Energies of the Reactions Equation 1 with $i = 1, 2, 3$ in PS II Particles from *Synechococcus vulcanus* Copeland (Koike et al., 1987), PS II Membrane Fragments (Renger & Hanssum, 1992), and PS II Core Complexes (This Study) from Spinach

	E_A (kJ/mol)		
		spinach	
reaction	*Synechococcus vulcanus* Copeland	PS II membrane fragments	PS II core complexes
$Y_Z^{OX}S_1 \rightarrow Y_ZS_2$	9.6	12.0	14.8
$Y_Z^{OX}S_2 \rightarrow Y_ZS_3$	26.8	36.0	35.0
$Y_Z^{OX}S_3 \rightarrow\!\!\rightarrow Y_ZS_0 + O_2$			
$\vartheta > \vartheta_c$[a]	15.5	20.0	21.0
$\vartheta < \vartheta_c$	59.4	46.0	67.0

[a] $\vartheta_c = 16$ °C (*Synechococcus*), 6 °C (PS II membrane fragments), and 11 °C (PS II core complexes).

coordinates of the individual redox steps that are induced by Y_Z^{OX} in the WOC (see eq 1) of different sample types are characterized by the following features (the transition $S_0 \rightarrow S_1$ is omitted because data were reported only for PS II membrane fragments): (a) the activation energies of the univalent reactions $Y_Z^{OX}S_1 \rightarrow Y_ZS_2$ and $Y_Z^{OX}S_2 \rightarrow Y_ZS_3$ are virtually identical in PS II membrane fragments and PS II core complexes from spinach while minor but discernible differences were found for both E_A and ϑ_c of the electron abstraction from the WOC in redox state S_3 that eventually leads to O_2 evolution; (b) comparable E_A values were observed for all three reactions, eq 1 with $i = 1$, 2, and 3 in PS II particles from the thermophilic cyanobacterium *Synechococcus vulcanus* Copeland, together with a shift of ϑ_c toward higher temperatures; and (c) the activation energies increase only slightly ($\leq$15%) when exchangeable protons are substituted by deuterons in PS II core complexes.

Based on findings (a) and (b), the general conclusion can be drawn that the reaction coordinates of the four-step univalent reaction pattern in the WOC remained almost invariant to the evolutionary development of oxygen-evolving organisms from thermophilic cyanobacteria to higher plants. Although thermophilic cyanobacteria are not the very first oxygen-evolving organisms, the above-mentioned finding implies that the WOC was already optimized at early stages of its "invention". Furthermore, it can be concluded that except for the 33 kDa protein which plays a key role in stabilizing the manganese cluster [for the latest review, see Seidler (1996)], the other extrinsic subunits are very likely without any effect on the reaction coordinates of electron abstraction from the WOC, possibly except for the last reaction leading to O_2 evolution.

Reaction Coordinate of Photosynthetic Water Oxidation. A schematic representation of the reaction coordinate of the redox steps within the WOC requires information on the energetics of the states that are involved in the four-step univalent redox sequence summarized by eq 1.

Values of the Gibbs energy difference between the redox states S_{i+1} and S_i, $\Delta G^\circ_{WOC,i}$, from direct redox titrations of the WOC are lacking. However, information is available from the literature on the driving forces of $P680^{+\bullet}$ reduction by Y_Z (Brettel et al., 1984), symbolized by $\Delta G_i^\circ(P680^{+\bullet}Y_Z/P680Y_Z^{OX})$, and the electron transfer steps from the WOC to Y_Z^{OX} (Vass & Styring, 1991; Vos et al., 1991), symbolized by $\Delta G_i^\circ(Y_Z^{OX}S_i/Y_ZS_{i+1})$. Furthermore, estimations have

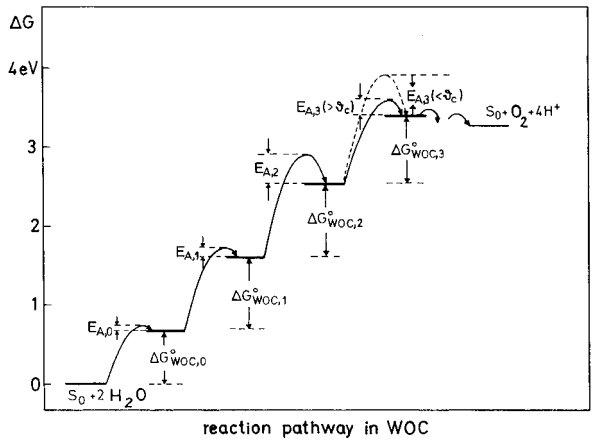


FIGURE 6: Generalized reaction coordinate of the four-step univalent water oxidation in the WOC of the photosynthetic apparatus (for details, see text).

been presented on the redox potential of P680/P680$^{+\bullet}$ (Jursinic & Govindjee, 1977; Klimov et al., 1979). Accordingly, $\Delta G^\circ_{WOC,i}$ can be calculated by (the sign of $\Delta G^\circ_{WOC,i}$ is positive, while the driving forces are negative)

$$\Delta G^\circ_{WOC,i} = \Delta G^\circ(\mathrm{P680/P680^{+\bullet}}) - \Delta G^\circ_i(\mathrm{P680^{+\bullet}Y_Z/P680Y_Z^{OX}}) - \Delta G^\circ_i(\mathrm{Y_Z^{OX}S_i/Y_ZS_{i+1}}) \quad (2)$$

Accordingly, the following values are obtained: $\Delta G^\circ_{WOC,0} \approx 0.75$ eV, $\Delta G^\circ_{WOC,1} \approx 1$ eV, $\Delta G^\circ_{WOC,2} \approx 1$ eV, and $\Delta G^\circ_{WOC,3} \approx 0.9$ eV. Except for $\Delta G^\circ_{WOC,0}$, these values are rather similar to a previous estimation (Renger, 1978). As the water oxidation requires a suitable energetic tuning, it is assumed that the comparatively large redox gap of about 250 meV of the reaction $Y_Z^{OX}S_0 \rightarrow Y_ZS_1$ is not owing to a loss process but the Gibbs energy is stored in another form (e.g., conformational energy) [for further discussion, see Renger et al. (1990)]. Together with the E_A values, the reaction coordinates of the WOC are obtained. The results depicted in Figure 6 reveal two striking features: (i) the electron abstraction from the WOC by Y_Z^{OX} requires increased activation energies when the oxidation state increases; and (ii) the Y_Z^{OX} reduction by S_3 that eventually leads to O_2 formation exhibits a drastic change at the characteristic temperature ϑ_c that probably reflects a state transition of S_3 (vide infra).

The remarkedly larger activation energy of the redox transition $Y_Z^{OX}S_2 \rightarrow Y_ZS_3 + n_2H^+$ can be explained in different ways. One simple and attractive interpretation is the assumption of a significant structural change that is coupled with this oxidation step. This idea is highly supported by the finding that the WOC in S_3 reacts much slower with exogenous hydrophilic reductants (NH_2NH_2, NH_2OH) than in S_2 (Messinger & Renger, 1990; Messinger et al., 1991). The latest EXAFS data provide direct evidence for a marked lengthening of Mn···Mn distances accompanying S_2 oxidation to S_3 while only very minor structural modifications take place during the transitions $S_0 \rightarrow S_1$ and $S_1 \rightarrow S_2$ of the WOC (Yachandra et al., 1996).

Kinetic H/D Isotope Effects: The Possible Role of Hydrogen Bonds. The results obtained in samples dissolved either in H_2O-containing or in D_2O-containing buffer and the dialysis experiments reveal that replacement of exchangeable protons by deuterons gives rise to a retardation of the electron transfer reaction eq 1 with $i = 1$, 2, and 3. Two features emerge from an inspection of the data compiled in Tables 1 and 2: (i) the kinetic H/D isotope exchange effects in PS II membrane fragments are in general comparatively small, with k_i(H)/k_i(D) ratios of 1.3−1.4 (Renger et al., 1994; this study), and similar values were obtained with PS II core complexes for $i = 1$ and 3; (ii) the redox transition $S_2 \rightarrow S_3$ exhibits a significantly enhanced k_2(H)/k_2(D) ratio of ≥2 (Karge et al., 1996; this study) for PS II core complexes.

Kinetic retardation owing to H/D isotope exchange can originate from different effects [for reviews, see Spicer and Poulter (1975) and Schowen and Schowen (1982)]. A primary isotope effect would comprise the break of an X−H bond. The wavenumber ν_{X-H}, of this bond can be calculated by [see Textbooks of Physical Chemistry (Levine, 1995)]

$$\nu_{X-H} = \frac{2k_BT \ln[k(H)/k(D)]}{hc[1 - (\mu_{X-H}/\mu_{X-D})^{1/2}]} \quad (3)$$

where μ_{X-H} and μ_{X-D} are the reduced masses of the bond in normal and deuterated samples and c is the velocity of light (the other symbols are the same as in former formulas).

Insertion of k_i(H)/k_i(D) ratios of 1.3, 1.6, and 2.3 into eq 3 leads to wavenumbers of ∼350 cm^{-1}, ∼650 cm^{-1}, and ∼1150 cm^{-1}, respectively. These values readily show that none of the redox reactions eq 1 in the WOC involves the break of a covalent −OH or −NH bond in the rate-limiting steps because the vibrations of these bonds are characterized by wavenumbers ≥3000 cm^{-1} [see Textbooks of Physical Chemistry, e.g., Levine (1995)]. Kinetic H/D isotope exchange ratios of 1.3−1.6 that are typical for the WOC (see Tables 1 and 2) are rather in line with the break of one to three hydrogen bridges because they exhibit typical values of 100−300 cm^{-1} (Jeffrey & Saenger, 1991). A primary isotope effect would imply a change of activation energy. However, the expected increase of E_A in deuterated samples is rather small for k(H)/k(D) of 1.3 ($\Delta E_A \approx 0.65$ kJ/mol) or 1.6 ($\Delta E_A \approx 1.2$ kJ/mol) so that an unambiguous decision cannot be achieved. A markedly larger k_2(H)/k_2(D) ratio was found for the transition $S_2 \rightarrow S_3$ in PS II core complexes. In the case of a primary isotope effect, an increase of the activation energy in the deuterated sample by about 2 kJ/mol is calculated for a k(H)/k(D) ratio of 2.3 (Table 1). Although the experimentally determined difference (2.1 kJ/mol) fits with the expected value, this correspondence should be considered only as a hint and not as proof because of the experimental error in the determination of the activation energies.

Alternatively, secondary isotope or solvent effects could also account for the observed decrease of the rate constants in deuterated samples (Schowen & Schowen, 1982). Furthermore, the possibility has to be considered that electron transfer steps are coupled with protolytic reactions including the protein matrix. In this respect, it is very interesting to note that isotope ratios of 1.4 have been observed for some electron transfer steps in the cytochrome *c* oxidase (Hallen & Nilsson, 1992) that catalyzes the reverse reaction sequence of water oxidation [for a review, see Babcock and Wickström (1992)]. The ratios of 1.4 were interpreted to reflect an interaction between redox intermediates [peroxidic state(s)] and protons of a basic group, BH, of the protein (Hallen &

Nilsson, 1992). The strikingly similar kinetic H/D exchange effects are in favor with the idea that substrate linkage to the protein matrix via hydrogen bonds also plays an important role in the WOC.

One surprising result of the present study is the significantly increased $k_2(H)/k_2(D)$ ratio in PS II core complexes. An analogous—even more pronounced—phenomenon has been recently discovered for the univalent electron transfer from Y_Z to $P680^{+\bullet}$. It was found that in samples with a functionally competent WOC the dominating nanosecond kinetics are virtually invariant to thorough H/D isotope exchange (Karge et al., 1996), while after destruction of the WOC a $k(H)/k(D)$ value of 2.7 was found for $P680^{+\bullet}$ reduction by Y_Z (Christen et al., 1997). This finding together with a 3-fold activation energy in the latter sample type (Eckert & Renger, 1988; Renger et al., unpublished results) led to the conclusion that the reaction coordinate of this process becomes significantly changed by modifications of the microenvironment of Y_Z that are of high functional relevance (Christen et al., 1997). In the case of the univalent oxidation step $S_2 \rightarrow S_3$ in the WOC of PS II core complexes, the changes are less severe because the activation energy is almost the same as that of PS II membrane fragments (see Table 1). It is therefore inferred that in PS II core complexes the network of hydrogen bridges near the catalytic site is slightly changed without a significant effect on the activation energy but giving rise to an increased kinetic H/D isotope exchange effect. This gentle modification of the WOC might also be responsible for the retardation by a factor of 3−4 of the rate of electron abstraction from S_3 by Y_Z^{OX} in PS II core complexes (van Leeuwen et al., 1993a).

Mechanistic Considerations. Two mechanistic aspects of photosynthetic water oxidation will be briefly addressed: (a) conventional electron transfer versus "hydrogen abstractor" model; and (b) the electronic configuration and nuclear geometry of S_3.

The conventional view of electron transfer steps taking place at the manganese cluster of the WOC with Y_Z^{OX} as oxidant has recently been questioned and replaced by the idea that $P680^{+\bullet}$ leads to the formation of a neutral $Y_Z^{\bullet}$ radical which acts as abstractor of hydrogen atoms from H_2O (or HO^-) coordinated to manganese. Although very attractive at first glance, there are several experimental findings that are difficult to reconcile with this model [for a discussion, see Renger (1997)]. It is therefore questionable that the conventional view has to be abandoned, and it appears worth analyzing the electron transfer data within the framework of the Marcus theory [for reviews, see de Vault (1984) and Marcus and Sutin (1985)]. A detailed analysis of the PS II donor side that will be presented in a forthcoming paper (G. Renger, G. Christen, and K.-D. Irrgang, unpublished results) leads to the following results: (i) the $P680^{+\bullet}$ reduction by Y_Z is characterized by reorganization energies of 0.5 and 1.6 eV in samples with a WOC that is fully competent or destroyed, respectively, and the distance between these redox groups is 9 ± 2 Å in both sample types; (ii) the "reorganization energies" are of the order of 0.7 and 1.6 eV for the redox transitions $Y_Z^{OX}S_1 \rightarrow Y_ZS_2$ and $Y_Z^{OX}S_2 \rightarrow Y_ZS_3$, respectively; and (iii) the data for the manganese-centered oxidation of S_1 to S_2 lead to values of the order of 15 Å for the distance between Y_Z and the redox active manganese cluster. If one takes into account a possible effect of the protein structure (Gray & Winkler, 1996) the distance could be even larger.

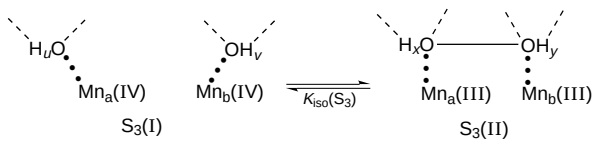
It has to be emphasized that the interpretation of reorganization energies gathered from a Marcus-type analysis is not straightforward for redox reactions in proteins [for a discussion, see Davidson (1996)]. In spite of this problem, the first result suggests that the environment of Y_Z is markedly altered (toward a more hydrophilic nature) when the WOC becomes destroyed. This conclusion is highly supported by the finding of a markedly increased H/D isotope exchange effect (Karge et al., 1996; Christen et al., 1997). Accordingly, the properties of Y_Z^{OX} in samples without a functionally competent WOC should be considered with care when drawing conclusions on the mechanism of photosynthetic water oxidation (Renger, 1997).

An interpretation of the physical meaning of the second result requires further studies, but it should be emphasized that the values obtained are consistent with the assumption of a significant structural change accompanied with the redox transition $S_2 \rightarrow S_3$.

The estimation of a distance of the order of 15 Å between the redox active manganese and Y_Z^{OX} is fully consistent with conclusions based on the results obtained by different methods, i.e., high-field EPR (Un et al., 1994), spin−lattice relaxation (Kodera et al., 1995), and local electrochromism (Mulkidjanian et al., 1996), but is at variance with a value of 4.5 Å gathered from electron spin echo−electron nuclear double resonance (Gilchrist et al., 1995). Two contradicting conclusions can be drawn from these findings: either all interpretations of data leading to larger distances are wrong and as a consequence of the present analysis the redox reactions in the WOC cannot be described by the Marcus theory, or the 4.5 Å value has to be revised and the univalent oxidation steps in the WOC are kinetically limited by electron transfer processes rather than changes of covalent bonds. At the present stage of knowledge, a definite answer to this most relevant question cannot be given, and therefore we will refrain from any further speculation (see also Note Added in Proof).

The second point to be addressed is the origin of the structural changes that are coupled with the $S_2 \rightarrow S_3$ transition. In an extension of previous proposals, it is assumed that in S_3 there exists a redox isomerism equilibrium that comprises two states, $S_3(I)$ and $S_3(II)$. The key point is the idea that $S_3(II)$ attains an electronic configuration and nuclear geometry that correspond with a peroxidic state while $S_3(I)$ contains two asymmetrically complexed water molecules in a partially deprotonated form (Renger, 1993).

Accordingly, the redox isomerism can be described by



where Mn_a and Mn_b are two manganese centers that are in the mixed-valence states Mn(III) Mn(IV) in redox state S_2 [for details, see Roeloffs et al. (1996) and references cited therein], u, v, x, and y are the numbers of protons covalently bound to the oxygen atom of each substrate molecule, hydrogen bonds are symbolized by thin dotted lines, the

interactions of oxygen atoms with manganese are represented by heavy dotted lines, and $K_{iso}(S_3)$ is the equilibrium constant.

In this model, the essential O−O bond is preformed in S_3(II) as an "entatic" state of the WOC for product formation. The oxidation of Y_Z by $P680^{+\bullet}$ in redox state S_3 of the WOC is assumed to shift the redox equilibrium toward S_3(II) followed by electron rearrangement that directly leads via oxidant-induced reduction of the manganese to the generation of complexed dioxygen (Renger, 1993).

It has to be emphasized that the existence of a peroxidic state in S_3 is easily reconcilable with the $^{16}O/^{18}O$ isotope exchange data of Messinger et al. (1995) provided that the redox isomerism equilibrium is sufficiently fast (order of 1 ms) and the substrate exchange rates at Mn_a and Mn_b differ by more than 1 order of magnitude (500 ms versus <30 ms).

The proposed redox isomerism also readily explains the finding of only minor shifts of the K-edge shift of manganese during the redox transition $S_2 \rightarrow S_3$ of the WOC (Roeloffs et al., 1996). An equilibrium constant of $K_{iso}(S_3) = 1$ is consistent with a zero net change of the oxidation state of manganese. Values of $K_{iso}(S_3) < 1$ [shift of the equilibrium toward S_3(I)] would lead to a partial increase of this net change, and the limit of 1 is reached for $K_{iso}(S_3) = 0$. In this respect, it has to be stressed that the equilibrium constant $K_{iso}(S_3)$ of the redox isomerism could exhibit a marked temperature dependence, and therefore measurements performed at $T < 10$ K do not necessarily reflect the situation in a functionally competent WOC at physiological temperatures [for a discussion of this fundamental problem, see Renger (1987)].

A two-state model of S_3 can also account for the characteristic breakpoint of the Arrhenius plot at ϑ_c [see Koike et al. (1987), Renger and Hanssum (1992), and Figure 5 of this study]. At present, it seems to be premature to discuss specific models in order to explain the change of the reaction coordinate, but the breakpoint phenomenon as a general feature has to be taken into account when considering possible pathways of O_2 formation in the WOC.

NOTE ADDED IN PROOF

After submission of this paper, two papers came to our knowledge that are of relevance for the topics to be discussed here: (i) the values of the kinetic isotope exchange effects and the activation energies in PS II core complexes from spinach are in line with the report of Bögershausen et al. (1996), except for the lack of the break point in the Arrhenius plot of S_3 oxidation owing to the limited temperature region analyzed in this above-mentioned study; and (ii) the assignment of the 4.5 Å value (gathered from measurement of the S_3* split signal) to the distance between Y_Z and the functional manganese has been questioned in the latest report of Astashkin et al. (1997). These authors rather prefer the idea that it reflects a distance between two organic radicals. In light of this interpretation, the apparent conflict on the distance between Y_Z and the manganese cluster might be irrelevant.

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