Kinetics of S_2 and S_3 Reduction by Tyrosine Y_D and Other Endogenous Donors As a Function of Temperature in Spinach PS II Membrane Fragments with a Reconstituted Plastoquinone Pool†

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ABSTRACT: The characteristic period four oscillation patterns of oxygen evolution induced by a train of single-turnover flashes were measured as a function of temperature in dark-adapted photosystem II (PS II) membrane fragments that were reconstituted with native plastoquinone-9 (PQ-9) by a recently developed procedure [Kurreck, J., Seeliger, A. G., Reifarth, F., Karge, M., & Renger, G. (1995) *Biochemistry 34*, 15721−15731]. The following results were obtained: (a) within the range 0−35 °C, the probabilities of misses (α) and double-hits (β) and the dark population of redox state S_1 exhibit similar dependencies on the temperature; (b) below a characteristic temperature ϑ_c these parameters remain virtually independent of temperature, above ϑ_c ($\vartheta_c = 20$ °C for α and β; $\vartheta_c = 30$ °C for S_1) the values of α and β increase whereas S_1 decreases; and (c) the dark decay of S_2 and S_3 via fast and slow kinetics owing to reduction of the water oxidase by Y_D and other endogenous electron donor(s), respectively, exhibits comparatively strong temperature dependencies with the following activation energies: $E_A(S_2^{fast}) = 60 \pm 10$ kJ/mol, $E_A(S_3^{fast}) = 55 \pm 10$ kJ/mol, $E_A(S_2^{slow}) = 80 \pm 5$ kJ/mol, and $E_A(S_3^{slow}) = 75 \pm 5$ kJ/mol. These values of PQ-9 reconstituted PS II membrane fragments are very similar to those that were previously reported for thylakoids [Messinger, J., Schröder, W. P., & Renger, G. (1993) *Biochemistry 32*, 7658−7668]. These findings reveal that the reaction coordinates of feeding electrons by endogenous electron donors into the water oxidizing complex (WOC) that attains the redox states S_2 and S_3 is virtually invariant to Triton X-100 treatment used in the isolation procedure of PS II membrane fragments from thylakoids. Implications of these findings are discussed.

Photosynthetic water oxidation to molecular oxygen and the coupled release of four protons into the thylakoid lumen takes place within a manganese-containing functional unit referred to as water oxidizing complex (WOC)[1] [for reviews, see Debus (1992) and Renger (1993)]. The overall process comprises a sequence of four univalent oxidation steps (Kok et al., 1970) that is energetically driven by the strongly oxidizing $P680^{\bullet+}$ formed during the primary steps of light-induced charge separation [for a review, see Renger (1992)]. A redox active component Y_Z identified by site directed mutagenesis as Tyr 161 of polypeptide D1 in *Synechocystis* sp. PCC 6803 (Debus et al., 1988a; Metz et al., 1989) functionally connects the WOC with $P680^{\bullet+}$. In a compact manner the four-step univalent oxidative pathway in the WOC with Y_Z^{OX} as oxidant can be summarized by eq 1:

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

where k_i is the rate constant, n_i and w_{i+1} are the numbers of protons released and substrate molecules bound, respectively, during the redox transition $S_i \rightarrow S_{i+1}$ within the WOC, S_i represents the redox state with i = number of oxidizing redox equivalents stored in the WOC, and δ_{i3} is the Kronecker symbol, i.e., $\delta_{i3} = 1$ for $i = 3$, otherwise zero. This formulation tacitly implies that Y_Z^{OX} is assumed to cause an oxidant-induced reduction of S_3 to S_0 under formation of molecular oxygen (Renger et al., 1994; Rappaport et al., 1994). Furthermore, it has to be emphasized that S_0 ($i = 0$) is the lowest redox state under steady state illumination while in thoroughly dark adapted samples the WOC is virtually entirely in S_1 (Vermaas, 1984) and under special conditions "super-reduced" states with $i < 0$ (e.g., S_{-1}, S_{-2}) can be populated [for a discussion, see Messinger and Renger (1993)].

Apart from Y_Z^{OX} as the unique oxidant of sequence eq 1, other endogenous redox groups can interfere with different redox states S_i of WOC. In analogy to Y_Z in D1, the D2 polypeptide also contains a redox active tyrosine symbolized by Y_D and identified as Tyr 160 in *Synechocystis* sp. PCC 6803 (Debus et al., 1988b; Vermaas et al., 1988). Although almost symmetrically arranged with respect to P680, the tyrosine residues Y_D and Y_Z exhibit quite different properties. The redox potential of Y_D/Y_D^{OX} is about 250 mV below that of Y_Z/Y_Z^{OX} (Boussac & Etienne, 1984). Therefore, Y_D^{OX} does not permit oxidation of the WOC in redox states S_i with $i \geq 1$ but Y_D rather leads to reduction of S_2 and S_3 (Styring & Rutherford, 1987). Except for reactions with Y_D/Y_D^{OX} the redox states S_2 and S_3 undergo a slow decay into S_1 via back reactions with the acceptor side, probably

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[1] Abbreviations: PSII, photosystem II; PQ-9, plastoquinone-9; WOC, water oxidizing complex; ADRY, acceleration of the deactivation reactions of the water-splitting enzyme system; β-DM, β-dodecyl maltoside; Y_D, redox active tyrosine of polypeptide D2; Y_Z, redox active tyrosine of polypeptide D1; D1, D2, polypeptides of photosystem II; S_i, redox state i of the WOC; MES, 2-(*N*-morpholino)ethanesulfonic acid; Q_A, Q_B, quinones of the acceptor side of PS II.

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involving Y_Z and P680 as deduced from measurements of delayed light emission (Rutherford & Inoue, 1983) and thermoluminescence (Rutherford et al., 1984).

In addition to these endogenous redox groups the reaction pattern of the WOC can be significantly modified by specifically acting exogenous compounds like hydrophobic ADRY-type substances (Renger, 1972; Hanssum et al., 1985) and hydrophilic reductants like NH_2OH and NH_2NH_2 [see Messinger et al. (1991) and Messinger and Renger (1993) and references cited therein].

Recently, the kinetics of the S_2 and S_3 decay by endogenous donors and the S_0 oxidation by Y_D^{OX} have been thoroughly analyzed in isolated thylakoids (Vass et al., 1990; Messinger et al., 1993). Correspondingly detailed studies are lacking for PS II membrane fragments because the rather limited size of the endogenous plastoquinone (PQ) pool in this sample type hampered the use of the characteristic period four oscillation of oxygen evolution induced by a train of single turnover flashes as an analytic tool [for a discussion, see Messinger et al. (1993)]. Recent studies, however, revealed that the PQ pool can be restored to high levels so that oscillation patterns are obtained comparable with those of isolated thylakoids (Kurreck et al., 1995, 1997). Therefore, in the present study PQ-9 reconstituted PS II membrane fragments were used in order to analyze the reductive relaxation of S_2 and S_3 by endogenous redox groups and the temperature dependence of these reactions.

MATERIALS AND METHODS

PSII membrane fragments were isolated from spinach according to the procedure of Berthold et al. (1981) with slight modifications (Völker et al., 1985). After the final isolation step, the PSII membrane fragments were resuspended in a weakly buffered medium [10 mM MES/NaOH (pH 6.5), 15 mM NaCl, 4 mM $MgCl_2$, and 400 mM sucrose] to chlorophyll concentrations of about 5 mg/mL.

Plastoquinone-9 (PQ-9) was prepared according to a method described by MacMillan et al. (1995) with some modifications (Kurreck et al., 1995). The reconstitution procedure was done as outlined in Kurreck et al. (1995) with some changes to reconstitute larger amounts. An increase of the pool size by a factor of about 2.5 was achieved. It has to be emphasized that this reconstitution procedure does not affect the value of the maximum oxygen evolution rate measured under saturating continuous light (Kurreck et al., 1995). $Y_D^{OX}S_1$ PS II membrane fragments were obtained by excitation of 100 μL of a suspension (chlorophyll concentration about 1 mM) with one flash at 0 °C and subsequent dark incubation on ice for 2 h (Messinger & Renger, 1990).

Flash-induced O_2 oscillation patterns were measured with a modified Joliot-type electrode (Joliot, 1972) that keeps the temperature at the electrode and the buffer reservoir constant within ±0.2 °C (Messinger & Renger, 1990). The pH of the flow buffer was adjusted at each temperature to pH 6.5. All measurements of this study were performed with samples of PQ-9 reconstituted PSII membrane fragments at a chlorophyll concentration of 1 mM. 10 μL of these suspensions was transferred to the Pt electrode and incubated for 2 min to achieve precipitation and thermal equilibration. The polarization of −650 mV was switched on 30 s before excitation with a train of short [full width at half-maximum = 3 μs] saturating xenon flashes separated by a dark time of 500 ms. The flow buffer contained 50 mM MES/NaOH (pH 6.5), 20 mM $CaCl_2$, 10 mM $MgCl_2$, and 300 mM mannitol.

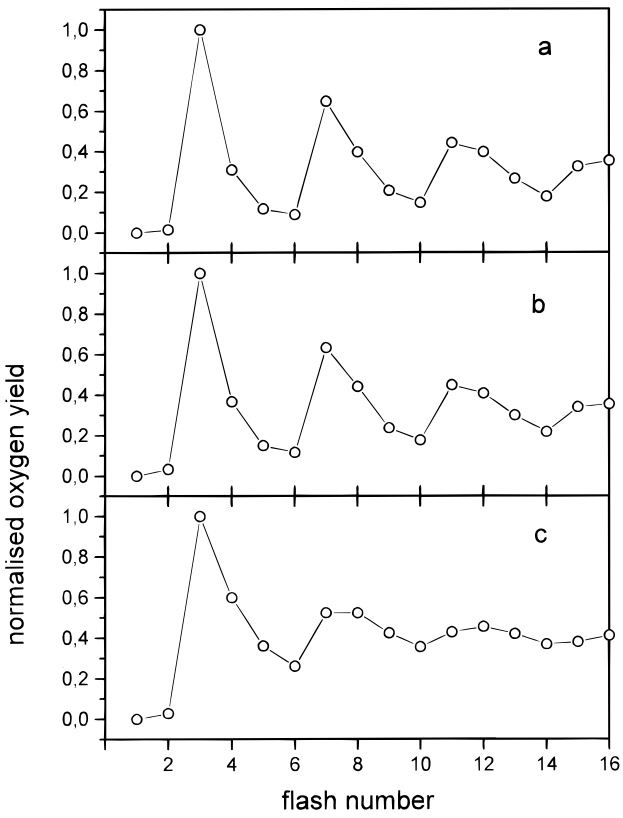

FIGURE 1: Normalized oxygen yield as a function of flash number in dark-adapted spinach PQ-9 reconstituted PS II membrane fragments in the $Y_D^{OX}S_1$ state illuminated by a train of single-turnover flashes at 2 (a), 20 (b), and 35 °C (c) at pH 6.5. The oxygen yield of each flash was normalized to the maximum yield of the third flash. Experimental conditions as described in Materials and Methods.

The probabilities of misses, double-hits, and the apparent initial S_i state populations, $[S_i]_0$, of dark-adapted samples were determined by a least-squares fit method comparing the relative oxygen yields of the first 16 of the train with calculated sequences [on the basis of the conventional Kok model, see Kok et al. (1970)] as outlined in Messinger et al. (1991). The S_2 and S_3 lifetimes were measured in the conventional way (Joliot & Kok, 1975) by exciting dark-adapted samples with one (S_2 formation) or two (S_3 formation) preflashes and monitoring the O_2 yield pattern induced by the flash train given at various dark times t_d after the preflashes. These patterns were deconvoluted into normalized S_i state populations within the framework of the conventional Kok model by the use of a least-squares fit method, taking misses and double-hits of the normal sequence (2 Hz) as fixed values. The rate constants were derived from curve fitting of S_2 or S_3 population as a function of t_d with a biexponentional decay model.

RESULTS

Effects of Temperature on the Oscillation Pattern of Flash-Induced Oxygen Evolution

Figure 1 depicts the characteristic period four oscillation of the oxygen yield induced by a train of single-turnover flashes in dark-adapted Y_D^{OX} (S_1) samples of PQ-9 reconstituted PSII membrane fragments at 2, 20, and 35 °C. An inspection of these traces readily reveals that the oscillation

patterns measured at 2 and 20 °C are virtually the same while at 35 °C a pronounced damping arises. Within the framework of Kok's model, and taking into account the observation that practically all WOCs attain the redox state S_1 after sufficiently long dark adaptation (Vermaas et al., 1984), the enhanced damping can be phenomenologically explained by an increase of the probabilities of misses (α) and double-hits (β) at higher temperatures.

In order to analyze these temperature effects in more detail, the oscillation patterns were measured in intervals of 2 °C within the range 0−40 °C and evaluated within the framework of Kok's model. Figure 2 shows the probabilities of misses and double-hits and the dark population of S_1, $[S_1]_0$, as function of the temperature. The temperature dependence of α, β, and $[S_1]_0$ exhibits the same feature with a characteristic transition temperature ϑ_c of about 20 °C for α and β and about 30 °C for $[S_1]_0$. At values below ϑ_c the parameters α, β, and S_1 are virtually independent of temperature, while above ϑ_c a remarkable increase of α and β and a decrease of $[S_1]_0$ are observed with increasing temperature. Below the transition temperature, values of 0.10−0.12 and 0.020−0.022 (Figure 2, traces a and b) are gathered for the probabilities of misses and of double-hits, respectively, from the experimentally measured oscillation patterns of PQ-9 reconstituted PS II membrane fragments. This analysis also reveals that up to about 30 °C the WOC of dark-adapted samples populates exclusively the S_1 state (Figure 2c). At temperatures above 30 °C the normalized population of S_1 starts to decline and concomitantly an increasing fraction of the WOC's attains the apparent S_0 state. An analogous feature was recently observed when thylakoids are incubated in buffer solutions of high pH (Messinger & Renger, 1993). This phenomenon was shown to arise predominantly from S_2 and S_3 decay between the flashes owing to a fast reduction by Y_D. An analogous explanation would require that Y_D^{OX} becomes partly reduced when "$Y_D^{OX}S_1$" samples are incubated at temperatures above 30 °C. Alternatively the WOC itself could progressively populate the S_0 state. The data available do not permit an unambiguous assignment.

In general, the temperature range where the above mentioned effects can be analyzed is limited due to the thermal degradation and denaturation of the samples. Previous measurements of the absolute average oxygen yield per flash with a Clark type electrode have shown that dark incubation of isolated thylakoids for 3 min at elevated temperatures causes an irreversible loss of the oxygen evolution capacity. The onset of this effect is in the range of about 30 °C (Renger et al., 1989). Similar features were observed in PS II membrane fragments (data not shown). Therefore, in order to exclude a possible interference of effects other than a thermally induced increase of the rate constants and gather reliable results on the activation energies, only the results below 25 °C will be taken into account in the following analysis.

Activation Energies of S_2 and S_3 Decay by Endogenous Redox Components

The decay kinetics of S_2 and S_3 can be determined by varying the time between the first and second as well as between the second and the third flashes of the sequence as outlined previously [for a review, see Joliot and Kok (1975)]. In general, a biphasic decay is observed, in which the fast

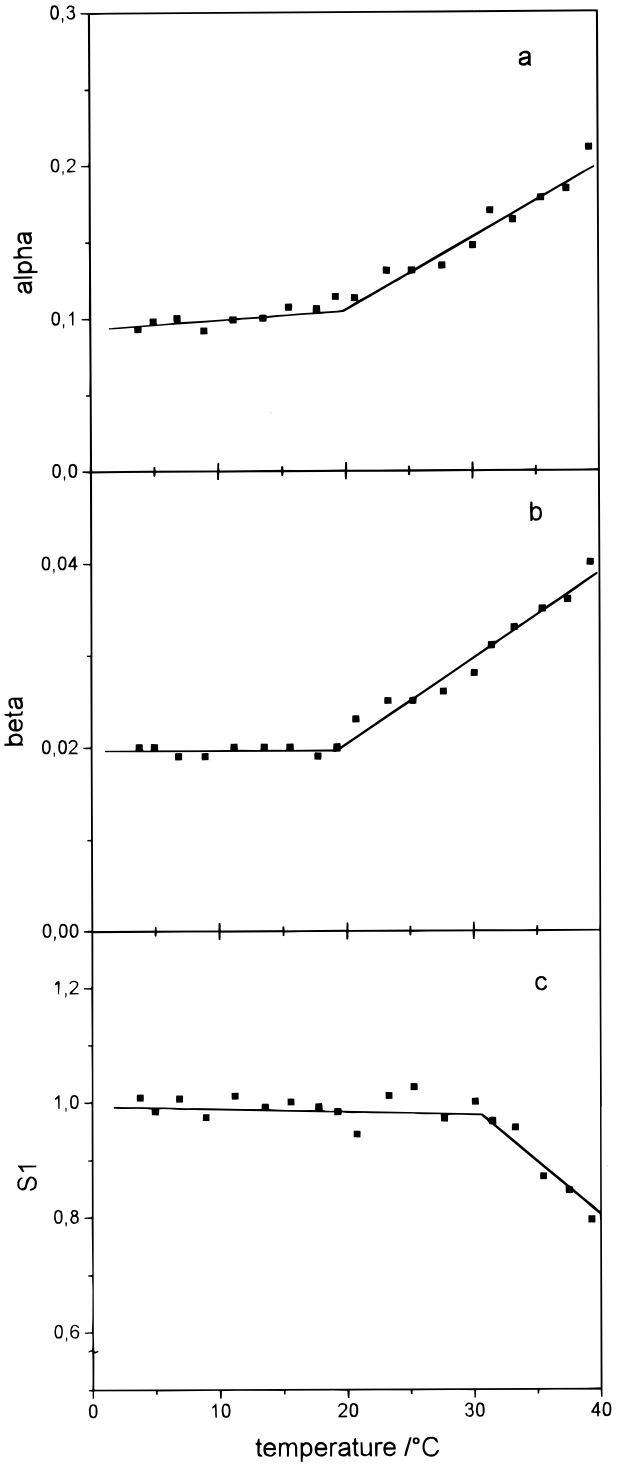

FIGURE 2: (a) Probabilities of misses (α), (b) double-hits (β), and (c) initial S_1 state population in dark-adapted PQ-9 reconstituted PS II membrane fragments as a function of temperature. For further details, see text.

phase reflects the reduction of S_2 and S_3 by Y_D and the slow phase represents the kinetics of the S_2 and S_3 decay by electron donation from the acceptor side and other endogenous reductants [for details see Messinger et al. (1993)]. Therefore, in order to permit the resolution of both kinetics, PS II membrane fragments were used that contained a significant fraction of Y_D in the reduced state. Figure 3 shows the biphasic decay of the states S_2 (traces a and b) and S_3 (traces c and d) at 3 and 20 °C. Two interesting features emerge from this data: (i) the relaxations are markedly slower at 3 °C compared with those at 20 °C as reflected by the different time domains of the four curves;

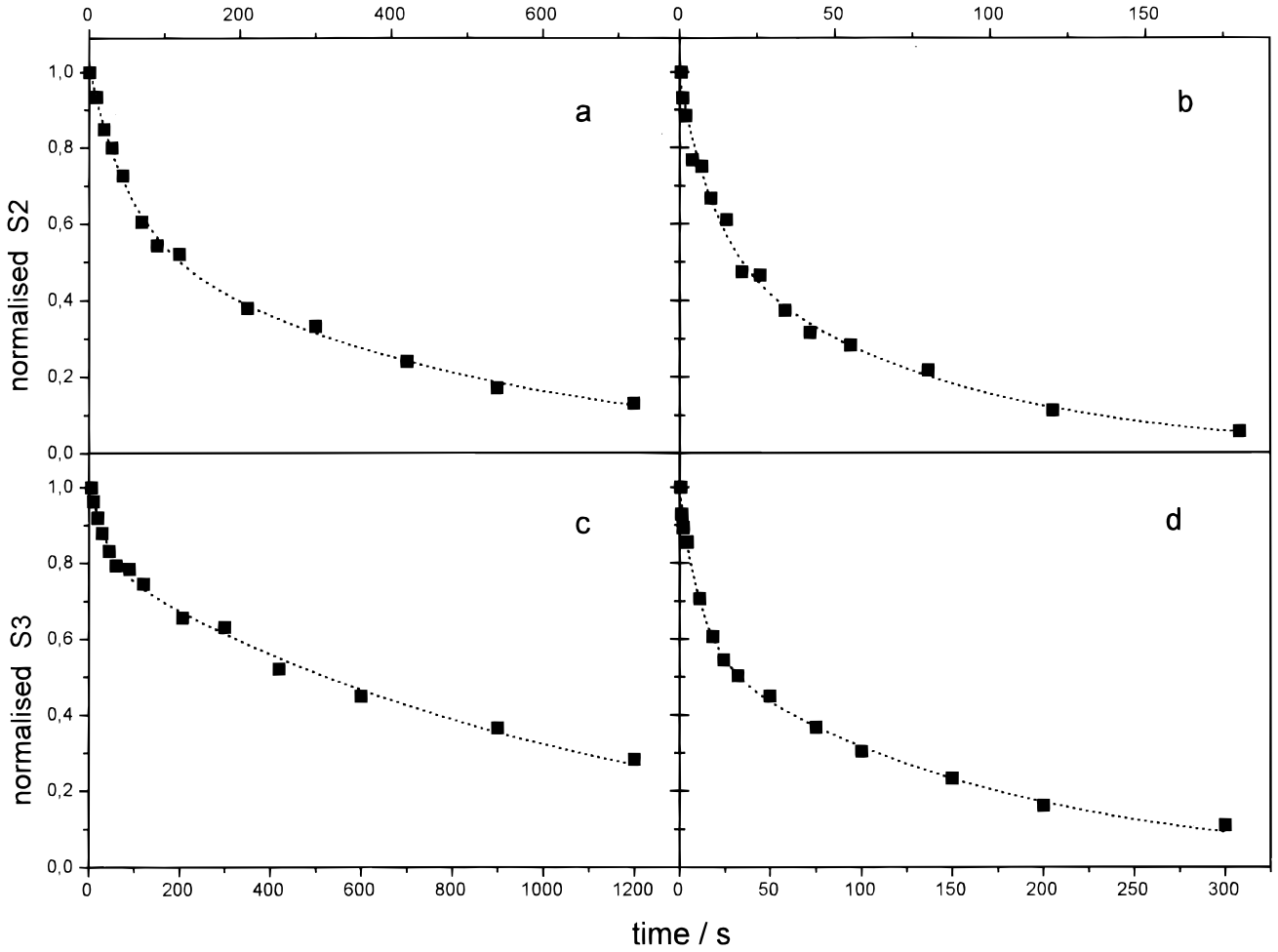

FIGURE 3: Normalized population of redox states S_2 and S_3 as a function of dark time between the first and the second or the second and the third flash of the sequence in PQ-9 reconstituted PS II membrane fragments at 3 °C (a, c) and 20 °C (b, d). The dotted curves represent the biexponential fit of the data (contents of Table 1).

Table 1: Normalized Extents (a_f, a_s) and Half-Lifetimes ($t^f_{1/2}$ and $t^s_{1/2}$) of the Fast (f) and Slow (s) Phases of S_2 and S_3 Decays at Different Temperatures in PQ-9 Reconstituted PS II Membrane Fragments at pH 6.5

temp	S_2 decay				S_3 decay			
(°C)	a_f	$t^f_{1/2}$ (s)	a_s	$t^s_{1/2}$ (s)	a_f	$t^f_{1/2}$ (s)	a_s	$t^s_{1/2}$ (s)
3	0.40	35	0.60	320	0.21	25	0.79	750
8	0.50	30	0.50	230	0.16	20	0.84	450
12	0.41	20	0.59	120	0.34	25	0.66	320
16	0.44	15	0.56	90	0.47	15	0.53	190
20	0.42	8	0.58	55	0.41	8	0.59	110

and (ii) the initial population are the same at both temperatures (not shown because for the sake of direct comparability the initial extrapolated populations at $t = 0$ are normalized to 1.0). The first feature is indicative of comperatively high activation energies of both the fast and the slow decay of S_2 and S_3 (vide infra).

The half-life times gathered from a fit of the experimental data by a two exponential decay kinetics are compiled in Table 1. An inspection of this data for S_2 reveals that the $t_{1/2}$ values vary between 8 s (20 °C) and 35 s (3 °C) for the fast phase and 55 s (20 °C) and 320 s (3 °C) for the slow phase. Likewise the half-life times of the dark relaxation reactions of S_3 vary between 8 s (20 °C) and 25 s (3 °C) for the fast reaction and 110 s (20 °C) and 750 s (3 °C) for the slow reaction. These findings show that the dark stability of S_2 and S_3 markedly increases at lower temperatures for both the fast and the slow decays. As a consequence of these properties it is clear that measurements at lower temperatures are much more suitable for analysis of the period four oscillation of the redox states S_i.

The activation energies can be gathered from an Arrhenius plot (Figure 4) of the rate constants as a function of reciprocal temperature. In all cases the experimental results are satisfactorily described by straight lines. It has to be emphasized that the error of the rate constants is larger for the fast decay component and that the activation energies are less precise for these reactions. The activation energies calculated from this plot are summarized in Table 2. Two features emerge from this data: (i) the activation energies for both the fast and the slow relaxation of S_2 and S_3 are rather large, and (ii) the thermal activation of the slow decay kinetics is markedly higher than that of the fast decay.

DISCUSSION

Recent progress achieved in reconstitution of a functionally competent endogenous PQ pool (Kurreck et al., 1995, 1997) opened the way to perform a detailed study on two characteristic properties of photosynthetic water oxidation in PS II membrane fragments: (i) period four oscillation of the oxygen yield induced by a train of single-turnover flashes in dark-adapted samples and (ii) reductive dark relaxation of redox states S_2 and S_3 in the WOC by Y_D and other endogenous electron donors. The results obtained with $Y_D^{OX}S_1$ samples of PQ-9 reconstituted PS II membrane fragments [see Materials and Methods] reveal that the

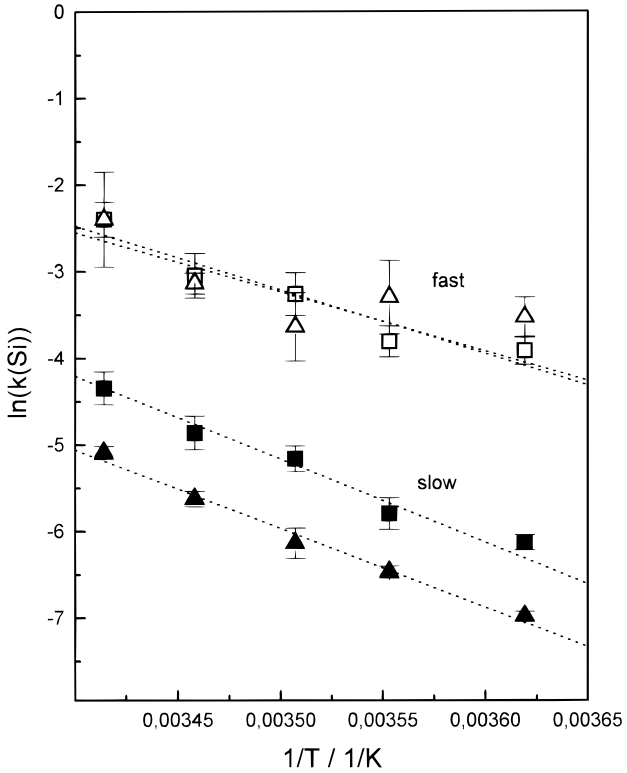

FIGURE 4: Semilogarithmic plot of the rate constants of the slow (closed symbols) and the fast (open symbols) S_2 (squares) and S_3 (triangles) decay as a function of reciprocal temperature in PQ-9 reconstituted PS II membrane fragments. The error bars are calculated from the maximal and minimal rate constants resulting from the deviations in the biexponentional fit curves.

Table 2: Activation Energies of Dark Relaxation Reactions in the WOC

reaction type	activation energy
S_2 and S_3 reduction by Y_D	$E_A(S_2^{fast}) = 60 \pm 10$ kJ/mol
	$E_A(S_3^{fast}) = 55 \pm 10$ kJ/mol
S_2 and S_3 reduction by other endogenous electron donors	$E_A(S_2^{slow}) = 80 \pm 5$ kJ/mol
	$E_A(S_3^{slow}) = 75 \pm 5$ kJ/mol

probabilities of misses (α) and double-hits (β) and the dark population of redox state S_1, $[S_1]_0$, gathered from data analysis within the framework of the conventional Kok model (Kok et al., 1970) exhibit a characteristic temperature dependence. Below temperatures ϑ_c of 20 and 30 °C the values of α, β, and $[S_1]_0$, respectively, remain virtually constant, but above ϑ_c the former parameters (α, β) start to increase while $[S_1]_0$ decreases with progressing temperature. Apart from somewhat smaller β values, which are fully consistent with a slight retardation of Q_A^- reoxidation by Q_B (Q_B^-) owing to the Triton X-100 treatment of PS II membrane fragments, the temperature dependence is strikingly similar to that of isolated thylakoids (Messinger et al., 1993). This feature leads to the conclusion that the mode of thermal tuning of α, β, and $[S_1]_0$ via structural changes and/or shifts of redox equilibria at the donor and acceptor side [for a more detailed discussion, see Messinger et al. (1993)] are not significantly affected by treatment with the detergent Triton X-100. Likewise, the reconstitution procedure of the PQ pool is also without effect. The possibility that the PQ-9 reconstitution procedure reverses effects caused by Triton X-100 appears to be highly unlikely and therefore will not be considered as a reasonable explanation.

The shape of the period four oscillation pattern and the values of α and β depend on donor *and* acceptor side reactions [for further discussion, see Renger and Hanssum (1988) and Shinkarev and Wraight (1993)]. Therefore, these parameters provide only limited information on the WOC.

More details on the properties of the WOC can be obtained by analyzing the individual redox steps that are induced by the redox active tyrosines Y_Z^{OX} and the couple Y_D/Y_Z^{OX}. It was recently shown that in PS II membrane fragments the reaction coordinates of the individual oxidation steps induced by Y_Z^{OX} (see eq 1) exhibit a characteristic pattern with activation energies which are strongly dependent on the redox state S_i of the WOC (Renger & Hanssum, 1992). Activation energies of 12 and 35 kJ/mol were found for the formation of S_2 and S_3, respectively. These values remain virtually the same after removal of several protein subunits by a solubilization procedure with β-dodecyl maltoside [Karge, M., Irrgang, K.-D., & Renger, G., submitted for publication] that permits the isolation of PS II core complexes with high oxygen evolution capacity (Haag et al., 1990). Likewise, in PS II particles from the thermophilic cyanobacterium *Synechococcus vulcanus* Copeland similar activation energies were observed (Koike et al., 1987). In marked contrast to the oxidative reaction pathway, the reduction of S_2 and S_3 exhibits a quite different kinetic pattern. These reactions are not only slower by orders of magnitude but also the temperature dependence is quite different. Two characteristics emerge from the results of the present study: (i) the activation energies are significantly larger than those of the corresponding oxidative reactions of S_2 and S_3 [see Renger and Hanssum (1992)], and (ii) within the experimental error, the E_A values are virtually the same for S_2 and S_3 reduction by Y_D while those of S_2 and S_3 formation by Y_Z^{OX} differ by a factor of about 3. The latter finding indicates that electron funneling from Y_D into the WOC in redox states S_2 and S_3 comprises a markedly different pathway than electron abstraction by Y_Z^{OX} from the WOC. In the case of WOC oxidation, direct electron transfer between the redox groups is very likely to occur because the distance between Y_Z^{OX} and the manganese cluster was recently estimated to be as short as 4.5 Å (Gilchrist et al., 1995). On the other hand, the distance between Y_D and the manganese cluster was reported to be in the range of 27−30 Å (Kodera et al., 1994; Hara et al., 1996). It therefore seems questionable that the electron transfer takes place without the participation of the redox groups Y_Z and P680 as intermediary redox carrier. A recent detailed analysis of electron transfer reactions in biological systems led to the conclusion that a variation of 20 Å in the distances between donors and acceptors in a protein matrix changes the rate constant by about 12 orders of magnitude (Moser et al., 1992). The actual ratio of oxidative formation of S_2 by Y_Z^{OX} and reductive decay by Y_D is of the order of 10^5 [see Renger and Weiss 1986 and data of Table 1]. A similar ratio ($\sim 5 \times 10^4$) is found for the corresponding reactions of S_3. These experimental data sharply contrast with a "theoretical" value of the order of 10^{12}. The theoretical value is certainly only a qualitative estimate because the Gibbs energy $\Delta G°$ and the reorganization energies have not been taken into account. In both cases the same type of redox groups is involved. Therefore, the reorganisation energies should not differ drastically even though the microenvironments of Y_Z and Y_D could be somewhat different [Tommos et al., 1995; Force et al., 1995; Tang et al., 1996; but see comments of Christen et al. (1997)]. The exothermicity of the reductive pathway is higher by

about 130 meV than that of the oxidative reaction [about 170 versus 40−50 meV, see Vass and Styring (1991) and Vos et al. (1991)]. These values, however, cannot explain a discrepancy by a factor of 10^7 [see Moser et al. (1992)]. Therefore, one could discuss that either the reported distances are wrong or that the reductive pathway involves Y_Z and P680 as intermediary redox groups. On the basis of the finding that the back reaction between S_2 and $Q_A^-(Q_B^-)$ also occurs via Y_Z, P680, and Pheo as intermediates [for a discussion see Rutherford et al. (1984) and references cited therein], the latter alternative appears to be much more plausible. This idea is in line with a previous report of Buser et al. (1992).

Another interesting conclusion can be drawn from the data compiled in Tables 1 and 2 and their comparison with recently reported values for the rate constants and activation energies of the reductive decay of S_2 and S_3 in thylakoids (Messinger et al., 1993). Closer inspection of these results reveals that within an error limit of 10% the activation energies are the same for the fast and slow decay in both sample types. Furthermore the half-life times of S_2 and S_3 reduction by Y_D at 20 °C differ by a factor of no more than 1.4. These findings readily show that the reaction coordinate of these reactions is affected neither by the treatment of thylakoids with Triton X-100 (Berthold et al., 1981) nor by the sonication step that is required for the PQ-9 reconstruction (Kurreck et al., 1995, 1997). Extrapolation of this feature to the oxidative reaction pathway (see eq 1) leads to the important conclusion that the properties gathered from sophisticated spectroscopic measurements in PS II membrane fragments widely reflect those of the WOC in the native state of PS II in the natural environment of the normal thylakoid membrane. This idea gains support by latest findings revealing that the pattern of activation energies of oxidative S_2 and S_3 formation by Y_Z^{OX} is only marginally, if at all, changed when PS II core complexes with high oxygen evolution activity are isolated by solubilization of PS II membrane fragments with β−DM (Karge, M., Irrgang, K.-D., & Renger, G., submitted for publication).

Apart from these mechanistic conclusions the data of the present study are of practical relevance for all experiments with PS II membrane fragments where the lifetimes of S_2 and S_3 and their temperature dependence have to be taken into account.

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