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Temperature dependence of $P680^+$ reduction in O_2-evolving PS II membrane fragments at different redox states S_i of the water oxidizing system

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The electron transfer kinetics from Z to $P680^+$ was analyzed as a function of temperature in the range of $248 < T < 295$ K by measuring absorption changes induced at 830 nm by a laser flash train in dark adapted O_2 evolving PS II membrane fragments from spinach. It was found: (i) that the kinetics of $P680^+$ reduction and their dependence on the redox state S_i of the catalytic site of water oxidation are only slightly affected by temperature within the physiological range of $270 < T < 295$ K. (ii) In the dark relaxed state S_1 the electron transfer from Z to $P680^+$ exhibits an activation energy of the order of 10 kJ/mol in $248 < T < 295$ K. (iii) In the 2nd and subsequent flashes of the train the ability for a stable charge separation between $P680^+$ and Q_A^- markedly decreases below -10°C. This phenomenon is assumed to be due to a strong effect of temperature on the electron transfer from Q_A^- to Q_B. The results are briefly discussed in relation to possible effects of structural changes in the D-1/D-2 polypeptide complex on the reaction coordinate of electron transfer steps in PS II.

$P680^+$-reduction kinetic; PS II membrane fragment; Water oxidation; Temperature dependence; S_i state

1. INTRODUCTION

Photosynthetic water oxidation takes place in the PS II complex at a manganese containing catalytic site via a four step univalent redox reaction sequence driven by the photooxidation of a special chlorophyll *a*, referred to as P680. P680 and the catalytic site of water oxidation are functionally coupled via a redox component Z (for recent review, see [1,2]). Based on recent findings [3] and model considerations [4,5] P680, Z and the catalytic site of water oxidation are inferred to be incorporated into two membrane bound polypeptides designated as D-1 and D-2. It is postulated that P680 is coordinatively bound by two histidines (His-198) of the transmembrane helices IV of D-1 and D-2, respectively, while Z is very likely to be a tyrosine residue (Y-161) located in transmembrane helix III of D-1 [6]. The amino acid residues acting as ligands of the first coordination sphere of the catalytic manganese cluster are not yet identified. Models have been proposed for manganese binding to D-1/D-2 [7]. Recent findings support a location in D-1 [8]. The possible binding of all functional groups indispensible for water oxidation to the same protein matrix would suggest an intimate functional interdependence in the reactivity of these redox centers, because the rate of electron transfer reaction is extremely sensitive to the spatial configuration of the reactants [9,10]. Indeed, by using the most powerful ADRY-agent [11], 2-(3-chloro-4-trifluoromethyl) anilino-3,5-dinitrothiopene (ANT 2p), it was shown that the $P680^+$ reduction kinetics depend on the redox state S_i of the catalytic site of water oxidation [12]. Later, refined measurements at markedly higher time resolution confirmed this basic conclusion [13]. A distortion of the polypeptide structure can

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lead to significant kinetic changes as shown by the drastic retardation of $P680^+$ reduction after destruction of the functional integrity of the catalytic site of water oxidation [14,15]. Accordingly, kinetic data provide an invaluable tool for monitoring structural/functional changes in the D-1/D-2 complex. A fine tuning of structural changes and the molecular dynamics of the apoprotein as reflected by the electron transfer rate might arise due to variation of the ambient temperature. Therefore in this study we analyzed the effect of temperature on the $P680^+$ reduction and its dependence on the redox state S_i of the catalytic site of water oxidation in the physiologically relevant range.

2. MATERIALS AND METHODS

PS II membranes were prepared from spinach thylakoids according to the method of Berthold et al. [16] with a modification described in [17]. In order to decrease the sample scattering that is essential for highly resolved measurements of 830 nm absorption changes, 0.05% of dodecyl-*N*,*N*-dimethylaminonio-3-propanesulfonate (SB-12) was added. The detergent addition did not significantly affect the oxygen evolution capacity. Glycerol (33 vol%) or 44% ethylene glycol was added as protective agent which simultaneously reduced the freezing point of the suspension. The standard reaction mixture contained: PS II membrane fragments (chlorophyll concentrations: 200 μM if not otherwise stated in figure legends), 100 μM phenyl-*p*-benzoquinone (Ph-p-BQ) as electron acceptor, 20 mM Mes, pH 6.5, about 0.05% SB-12 and 33 vol% glycerol or 44% ethylene glycol. Stored frozen PS II membrane fragments were thawed and transferred to the suspension in the dark. The samples were kept 5 min at room temperature before cooling. The time for thermal equilibration was in the range of 5–15 min. The 830 nm absorption changes were measured with the equipment described in [18]. Optical pathlength: 4 cm, time between saturating laser flashes (FWHM: 3 ns, λ = 532 nm) of the train: 1 s.

3. RESULTS

As the rate of $P680^+$ reduction does not only depend on the spatial configuration of $P680^+$ and Z but also on the redox state S_i of the catalytic site for water oxidation, a variation of temperature could affect the kinetics via two parameters: (i) the activation process of the electron transfer step itself; (ii) the modification of the S_i-state dependence. In order to separate these effects, the $P680^+$ reduction has to be measured as a function of temperature at a known S_i state population ($i = 0,....3$). This problem can be solved by measuring the relaxation of absorption changes at 830 nm induced by a train of saturating laser flashes in dark-adapted samples. Fig.1 shows the traces obtained at -1.5°C (bottom) and at $+22$°C (top) in PS II membrane fragments from spinach. The relaxation of the absorption changes, $\Delta A_n^{830}(t)$, reflects the $P680^+$-reduction kinetics after the n-th flash. A numerical analysis reveals that the data can be properly described by a triexponential decay according to eqn 1:

$$\Delta A_n^{830}(t) = \Delta A^{830}(0) \cdot \sum_{\mu=1}^{3} a_\mu e^{-R_\mu t} \quad (1)$$

The detected initial amplitude at $t = 0$ (at a time resolution of about 5 ns the rise kinetics due to $P680^+$ formation cannot be resolved), $\Delta A^{830}(0)$, is independent of n. A satisfying fit of the relaxation at -1.5°C can be achieved with the rate constants $k_1 = 1.7 \times 10^7\ s^{-1}$, $k_2 = 3 \times 10^6\ s^{-1}$ and $k_3 < 7 \times 10^5\ s^{-1}$. The same rate constants also provide a proper description of the data obtained at $+22$°C provided that a k_1 value of $5 \times 10^7\ s^{-1}$ (relaxation mainly after the 1st and 5th flash) is used in addition to $k_1 = 1.7 \times 10^7\ s^{-1}$. The dependence of the $P680^+$-reduction kinetics on the redox state S_i is reflected by the amplitude factor a_μ in the form of eqn 2:

$$a_\mu = \sum_{i=0}^{3} C_{\mu,i} \cdot [S_i] \quad (2)$$

where $[S_i]$ represents the probability of a competent catalytic site being in the redox state S_i. Within the framework of Kok's model [19] a satisfactory description of the a_μ values according to eqn 2 can be achieved with the following relations: $C_{1,0} \approx C_{1,1}$ and $C_{1,2} \approx C_{1,3} \ll 1$; $C_{2,0} \approx C_{2,1} \approx 0$ and $C_{2,2} \approx C_{2,3}$; $C_{3,0} \approx C_{3,1} = 0$ and $C_{3,2} \approx C_{3,3}$. This analysis tacitly implies that the small contribution of microsecond kinetics to the overall relaxation after the first flash is caused by a minor fraction (<20% of the total number) of systems II which lack a functionally intact catalytic site. Accordingly, a_μ oscillates as a function of flash number as illustrated in fig.2 for the data obtained at -1.5°C. Similar results were obtained at $+22$°C (not shown). The data of fig.1 and their numerical fit permit two very interesting conclusions. (i) The dependence of the $P680^+$-reduction kinetics on the redox state S_i of the catalytic site of water oxidation appears to be almost independent of the

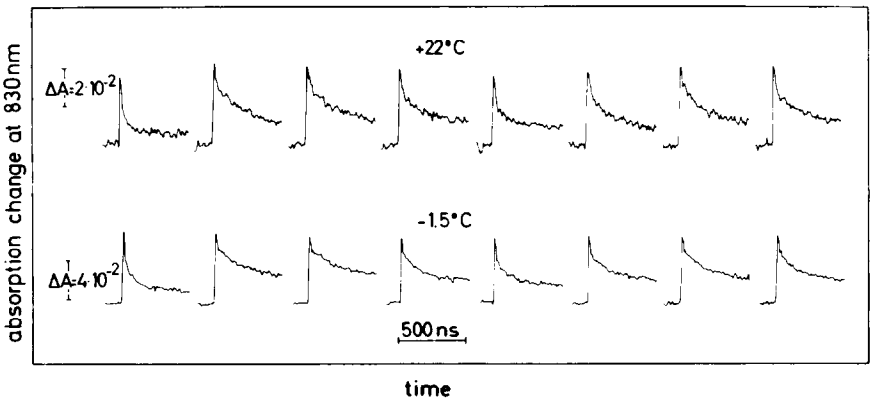

Fig.1. Absorption changes at 830 nm as a function of flash no. and time in dark-adapted O_2 evolving PS II membrane fragments from spinach at 22°C (top) and at −1.5°C (bottom). The measurements at 22°C were performed with a suspension of 100 μM chlorophyll. 15 signals were averaged in a Tektronix 7912 digitizer. Other experimental details as described in section 2.

temperature within the physiological range where the univalent redox transitions $S_i \rightarrow S_{i+1}$ are not kinetically blocked for all $i = 0,\ldots,3$. (ii) The reduction kinetics of $P680^+$ exhibit only a comparatively small temperature dependence in the region $270 < T < 295$ K. Therefore, the reduction kinetics of $P680^+$ measured under repetitive flash excitation is almost T-independent in the physiological temperature range as was recently shown for PS II membrane fragments from cyanobacteria [20] and higher plants [21].

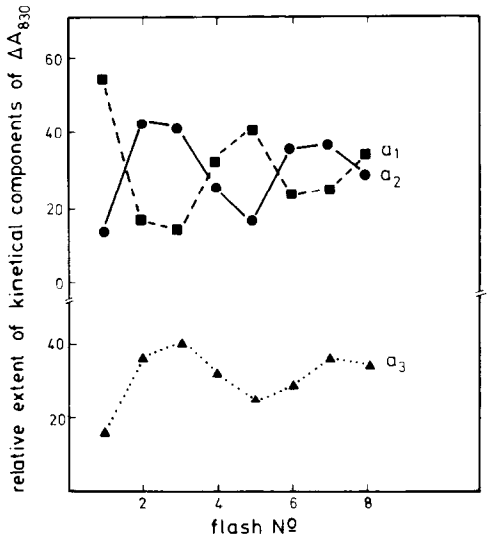

Fig.2. Amplitudes of the decay kinetics in the nanosecond range as a function of flash no. in dark-adapted O_2 evolving PS II membrane fragments at −1.5°C. The a_μ values ($\mu = 1, 2, 3$) were obtained by a triexponential fit of the data depicted in fig.1 (bottom) with fixed rate constants of $k_1 = 1.7 \times 10^7\ s^{-1}$, $k_2 = 3 \times 10^6\ s^{-1}$ and $k_3 < 7 \times 10^5\ s^{-1}$.

At lower temperatures a markedly different pattern is observed as is shown in fig.3. The most interesting phenomenon is the significant decrease of the detected initial amplitudes of the 830 nm absorption changes, $\Delta A_n^{830}(0)$, induced by flash numbers $n \geq 2$ at −25°C, while $\Delta A_1^{830}(0)$ remains unaffected at this temperature (see fig.3, right side, top). The decrease of $\Delta A_2^{830}(0)$ as a function of temperature is depicted in fig.3, right side, bottom. It reveals a comparatively steep decline below −15°C. At the temperature limit of our equipment (−25°C) $\Delta A_2^{830}(0)$ is about 40–50% of $\Delta A_2^{830}(0)$ at room temperature. As $\Delta A_1^{830}(0)$ remains invariant to a decrease of temperature down to −25°C, the reaction sequence Z P680 Pheo $Q_A \xrightarrow{h\nu} Z^{ox}$ P680 Pheo Q_A^- is only marginally affected in this range.

Therefore, the marked reduction of $\Delta A_n^{830}(0)$ for $n \geq 2$ reflects a drastic temperature effect on a secondary electron transfer reaction. This rises questions about the nature of this highly temperature-sensitive reaction step. Different lines of indirect evidence led to the conclusion that the electron transfer from Q_A^- to Q_B becomes blocked below −30°C [22,23], whereas the redox transitions at the catalytic site of water oxidation exhibits S_i-state-dependent temperature effects [23–25]. A blockage of S_1 oxidation by Z can be excluded as being responsible for the $\Delta A_2^{830}(0)$

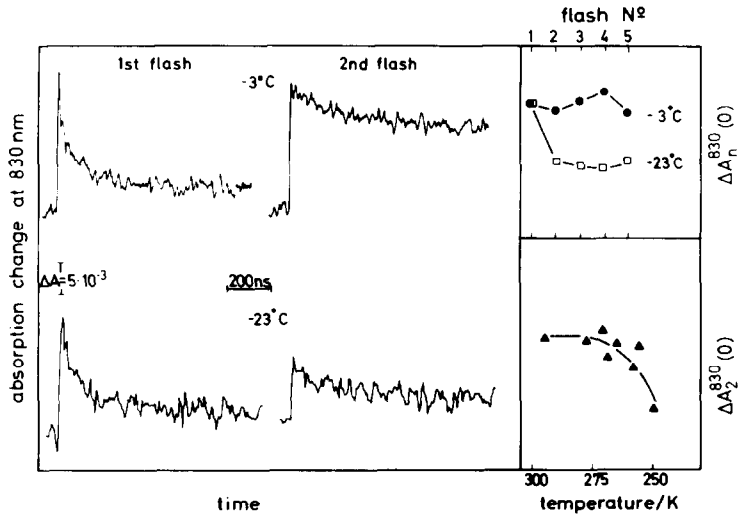

Fig.3. Absorption changes at 830 nm as a function of time in dark-adapted O_2 evolving PS II membrane fragments at −3°C and −23°C. Detected initial amplitude induced by the 2nd flash $\Delta A_2^{830}(0)$, as a function of temperature (bottom, righthand side). Experimental details as described in section 2. 15 signals were averaged.

decrease because it exhibits a markedly lower temperature barrier [23,24,26]. Furthermore, a specific blockage of Z^{ox} reduction gives rise to a back reaction between $P680^+$ and Q_A^- [27] which does not lead to a reduction of $\Delta A_n^{830}(0)$ but only to a retardation of the kinetics for $n \geq 2$. Based on these findings the decline of $\Delta A_n^{830}(0)$ with decreasing temperatures is inferred to reflect the inhibition of Q_A^- reoxidation by Q_B. The origin of this phenomenon is either a temperature-dependent structural change at the Q_B site which affects the binding and/or orientation of the secondary plastoquinone or a 'freezing' of vibrational modes that are essential for the electron transfer from Q_A^- to Q_B.

In order to study in more detail the effect of

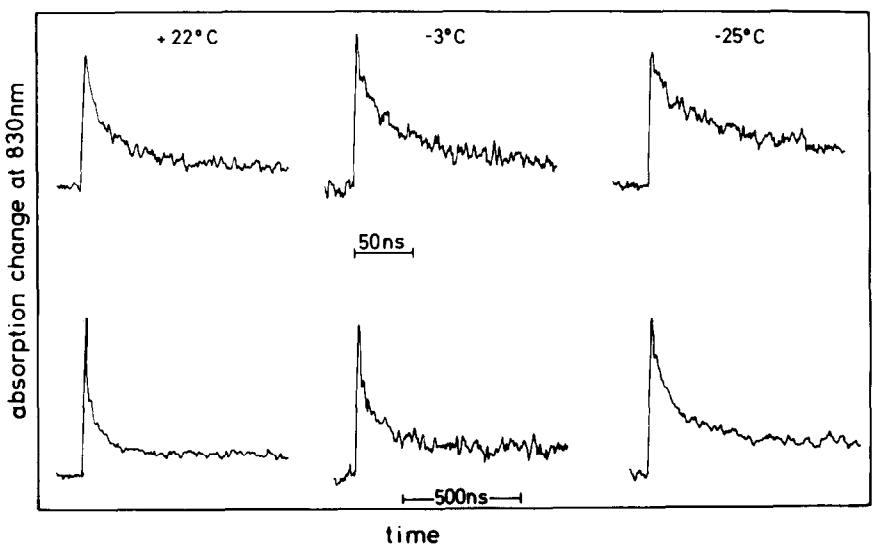

Fig.4. Absorption changes at 830 nm induced by the 1st flash of a train in dark-adapted O_2 evolving PS II membrane fragments as a function of time at different temperatures. Experimental details as described in section 2.

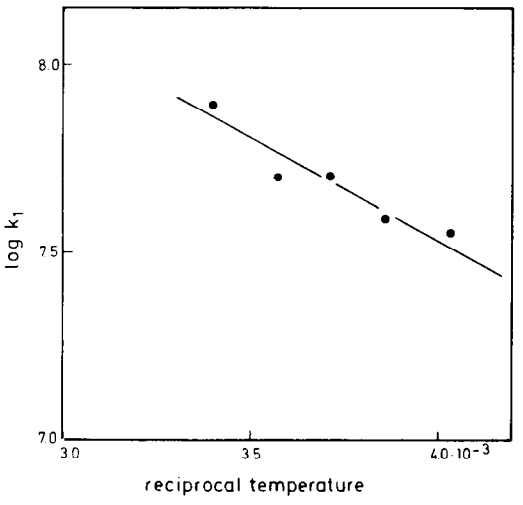

Fig.5. Rate constant k_1 of the decay of 830 nm absorption changes induced by the 1st flash in dark-adapted O_2 evolving PS II membrane fragments as a function of reciprocal temperature. Experimental details as described in section 2.

temperature on the electron transfer step from Z to $P680^+$ the 830 nm absorption changes caused by the first flash are depicted as a function of time at three different temperatures and two different time scales in fig.4. The traces reveal a slight temperature dependence, especially below $-3°C$. If the kinetics of the fast decay are approximated by an average rate constant the data depicted in fig. 5 as a functin of the reciprocal temperature are obtained. From this Arrhenius plot an activation energy of ~10 kJ/mol is obtained. It is interesting to note, that this comparatively small value is far below the activation energy of about 45 kJ/mol [28] for the highly retarded reaction between the same functional groups Z and $P680^+$ in samples deprived of their oxygen evolution capacity (see section 4).

4. DISCUSSION

The results presented in this study indicate that the electron transfer from Z to $P680^+$ is only slightly affected by temperature in the range of $248 < T < 295$ K. Likewise, the dependence of $P680^+$ reduction on the redox state S_i of the catalytic site of water oxidation is almost invariant to temperature in the range $270 < T < 295$ K. On the other hand, drastic effects are observed for the electron transfer reaction from Q_A^- to Q_B. Both phenomena will be discussed briefly on the basis of a simplified theory of electron transfer in biological systems [9,10]. The rate constant of this process is determined by two parameters: (i) the electronic coupling and (ii) the Franck-Condon factor. The strength of electronic coupling between the states $P680^+$ Z and P680 Z^{ox} depends on the center to center distance of the redox components P680 and Z. A rough estimation of this effect can be given if one uses a scaling factor of about 10 nm^{-1} [10] for the dependence of electron transfer on the center distance between redox groups containing π-electron systems. On the basis of the ratio k_1/k_2, a shift of the P680-Z distance by about 0.17 nm is calculated for the difference between states S_0 or S_1 and S_2 or S_3, respectively, provided that the Franck-Condon factors remain invariant. Markedly larger distance changes would be required to explain the rate constant k_3 of $P680^+$ reduction. Therefore, the large difference between k_1 and k_3 probably comprises additional effects (vide infra). The Franck-Condon factor can be affected by a modification of the vibronic coupling (e.g. effect on the reorganization energy) and/or by S_i-state-dependent redox potential shifts $\Delta(\Delta G)$ between states $P680^+$ Z and P680 Z^{ox}. The regulation of $P680^+$ via structural effects might be supported by a recent report on different conformational states of the oxygen evolving complex in states S_0 and S_1 versus S_2 and S_3 [29]. The idea of a conformational regulation of electron transfer in biological systems is supported by recent findings indicating that the average distance between Q_A and P870 increases by $\gtrsim 0.1$ nm due to light-induced electron transport in reaction centers of photosynthetic bacteria [30]. Although these phenomena favor the idea of a modulation of the electron transfer from Z to $P680^+$ rate via conformational changes (for recent discussions see [1]) the possibility of $\Delta(\Delta G)$ shifts cannot be neglected and the relative contributions of both parameters remain to be clarified. Regardless of these unresolved mechanistic details the present analysis shows that in the physiological range of $270 < T < 295$ K neither $\Delta(\Delta G)$ nor the center distance between P680 and Z are markedly affected by temperature.

The temperature dependence of the electron

transfer kinetics from Z to $P680^+$ was analyzed in the whole range of $248 < T < 295$ K for the case that the catalytic site of water oxidation stays in the dark relaxed redox state S_1 [31]. A comparatively small activation energy of 10 kJ/mol was found. This value is very interesting for two reasons. (i) As Z is very likely a tyrosine residue of polypeptide D-1 [6] which can form a hydrogen bond it is attractive to speculate that the breaking of this bond during the electron abstraction by $P680^+$ essentially determines the activation energy of Z oxidation by $P680^+$. The activation energy of 10 kJ/mol is smaller than typical average energies of 20–25 kJ/mol for hydrogen bonds in proteins [32]. Therefore if the formation of the transition state comprises the breaking of a hydrogen bond, its strength is comparatively weak. Alternatively one might assume that the presumed hydrogen bond remains intact but becomes shifted within a double well potential: Tyr-$\underline{O}$-H....X ⇆ Tyr-$\dot{\underline{O}}$|... H-X^+, where X represents the amino acid residue which forms the above mentioned hydrogen bond with tyrosine. (ii) The activation energy of the electron transfer from Z to $P680^+$ markedly depends on the functional integrity of the catalytic site of water oxidation. In this study a value of ≈ 10 kJ/mol was observed for intact systems, whereas in samples deprived of their oxygen evolution capacity the activation energy was found to be about 45 kJ/mol [28]. This change of the activation energy fully accounts for the drastic retardation of the $P680^+$-reduction kinetics. Therefore, the question arises about the underlying mechanism of the marked change of the reaction coordinate. It is easily understandable that the damage of the catalytic site for water oxidation in the D-1 polypeptide leads to a structural change of the microenvironment of the functional D-1 tyrosine acting as Z. One might speculate about a change of hydrogen bonding of the tyrosine. In this respect it is interesting to note that in Tris-washed inside-out vesicles Z oxidation was shown to be coupled with a stoichiometric proton release [33]. So far, a corresponding effect (especially a fast transient proton release in systems undergoing S_1 oxidation to S_2 by Z^{ox}) has not been observed in samples fully competent in oxygen evolution. This could be either due to the limited time resolution of the methods used for the detection of protolytic reactions in PS II or due to a changed reaction coordinate of the deprotonation steps. This mechanistically very important question remains to be clarified.

In contrast to the slight temperature dependence of the donor side reactions the electron transfer from Q_A^- to Q_B appears to be very sensitive below a critical temperature of about 260 K. This indicates that dynamical structural changes are very important for this reaction as was shown previously for the corresponding reaction in purple bacteria [34] and discussed in [35]. The present study shows that measurements of the T dependence of electron transfer steps can provide a proper tool for analyzing the functional relevance of dynamical structural changes in PS II.

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