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Optical characterization of the immediate electron donor to chlorophyll a_{II}^{+} in O_2-evolving photosystem II complexes

Tyrosine as possible electron carrier between chlorophyll a_{II} and the water-oxidizing manganese complex

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The number and chemical nature of the electron carrier(s) between Chl a_{II} and the water-oxidizing enzyme, S, were analyzed through flash-induced absorption changes in the UV with nanosecond time resolution. (i) At all wavelengths where the reaction of the donor with Chl a_{II}^{+} has been characterized, this donor is oxidized in the nanosecond time range in exact accordance with the reduction kinetics of Chl a_{II}^{+}. The donor is in turn re-reduced with $t_{1/2} > 10$ μs, i.e. in the range where S is oxidized. From this time course it is concluded that there exists only one electron carrier between Chl a_{II}^{+} and S. (ii) The UV-difference spectrum due to the electron transfer from the immediate donor to Chl a_{II}^{+} in the nanosecond time range in O_2-evolving PS II complexes is characterized by a maximum around 260 nm and smaller minimum around 310 nm. This spectrum is identical with that observed for the reaction of the donor with Chl a_{II}^{+} in the microsecond time range in Tris-treated PS II. Therefore, the donors in both reactions must be of the same chemical nature. (iii) This result, together with the well-established similarity of EPR signal II_f of the oxidized donor in Tris-treated PS II to the EPR signal II_s, recently assigned to Tyr-160 of the D2 protein of PS II [(1988) Proc. Natl. Acad. Sci. USA 85, 427–430], provides strong evidence that the immediate donor to Chl a_{II}^{+} in water-oxidizing PS II is also a tyrosine. (iv) It is shown that the UV-difference spectra of the oxidation of the immediate donor in O_2-evolving as well as that of Tris-treated PS II complexes are similar to the in vitro difference spectrum of the oxidation of tyrosine in water. This independent result supports the conclusion that the donor is a tyrosine.

Photosystem II; P-680; Tyrosine; Water oxidation; Flash absorption spectroscopy; (*Synechococcus* sp.)

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Abbreviations: Chl, chlorophyll; D, component in photosystem II, characterized by EPR signal II_{slow}; Fecy, $K_3(Fe(CN)_6)$; FWHM, full width at half maximum; Mes, 4-morpholineethanesulphonic acid; PS II, photosystem II; Q_A, primary quinone acceptor of PS II; S, water-oxidizing enzyme system; TyrOH, tyrosine; Z, intermediate electron carrier in PS II, characterized by EPR signal $II_{very\ fast}$; Z_{Tris}, electron donor to Chl a_{II}^{+} in PS II inhibited in water oxidation, characterized by EPR signal II_{fast}

1. INTRODUCTION

Water oxidation in photosynthesis begins with the light-induced transfer of an electron from Chl a_{II} (P-680) [1,2] to the first stable acceptor, Q_A [3,4]. The oxidized Chl a_{II} extracts electrons ultimately from water. For the respective evolution of one O_2 molecule and the oxidation of two H_2O molecules, four consecutive electron extractions from the water-oxidizing enzyme S [5,6] are performed by four Chl a_{II} turnovers. A catalytic manganese cluster in S cycles hereby through four different oxidation states characterized by optical difference spectroscopy [7–9a].

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An intermediate electron carrier between S and Chl a_{II}, Z, which accepts electrons from the water-oxidizing enzyme S, has been observed by EPR [10] and optical spectroscopy [7,11]. The oxidized form of this component giving rise to EPR signal II_{vf} is reduced by S with half-lives between 30 μs and 1.5 ms in accordance with the kinetics of the S-state transitions [10,12,13,8]. The time resolution of the EPR and optical measurements performed so far (≥ 2 μs) turned out to be too low by two orders of magnitude in order to determine whether this component reduces Chl a_{II}^{+} directly. Re-reduction of Chl a_{II}^{+} occurs namely with half-lives within the range 20–250 ns [14,15], depending on the redox state of S [16].

In contrast to the conditions of water oxidation, in Tris-treated PS II complexes in which water oxidation is inactivated, the immediate electron donor Z_{Tris}, reducing Chl a_{II}^{+} over the microsecond time range [17,18], is well characterized by an EPR spectrum designated signal II_f [19,20] and an optical difference spectrum in the UV [11,21–23]. The EPR spectrum of the Z_{Tris} radical (EPR signal II_f) is identical with that of a species D (EPR signal II_{slow}) [19,24] which has been shown recently to be a tyrosine radical in position Tyr-160 of the D2 polypeptide [25–27]. (D is, however, not directly involved in the process of water oxidation but plays a role in some regulatory activities in PS II [28].) The identity of these two EPR spectra suggests that Z_{Tris} is also a tyrosine. It was proposed that this tyrosine is Tyr-161 of the D1 polypeptide [26] based on the similarity of the amino acid sequence of D1 and D2 [29,30]. It was suggested that Z in O_2-evolving PS II might also be this tyrosine and the donor to Chl a_{II}^{+} [26]. The identity of Z_{Tris} with Z, however, has not been well established. The EPR spectrum of signal II_{vf} of Z^{+} is rather noisy and allows no conclusive proof of identity with signal II_f of Z_{Tris}^{+} and II_s of D^{+} [31]. Furthermore, it has been reported that the optical difference spectra of Z^{+}/Z (measured 2 μs after a short flash)

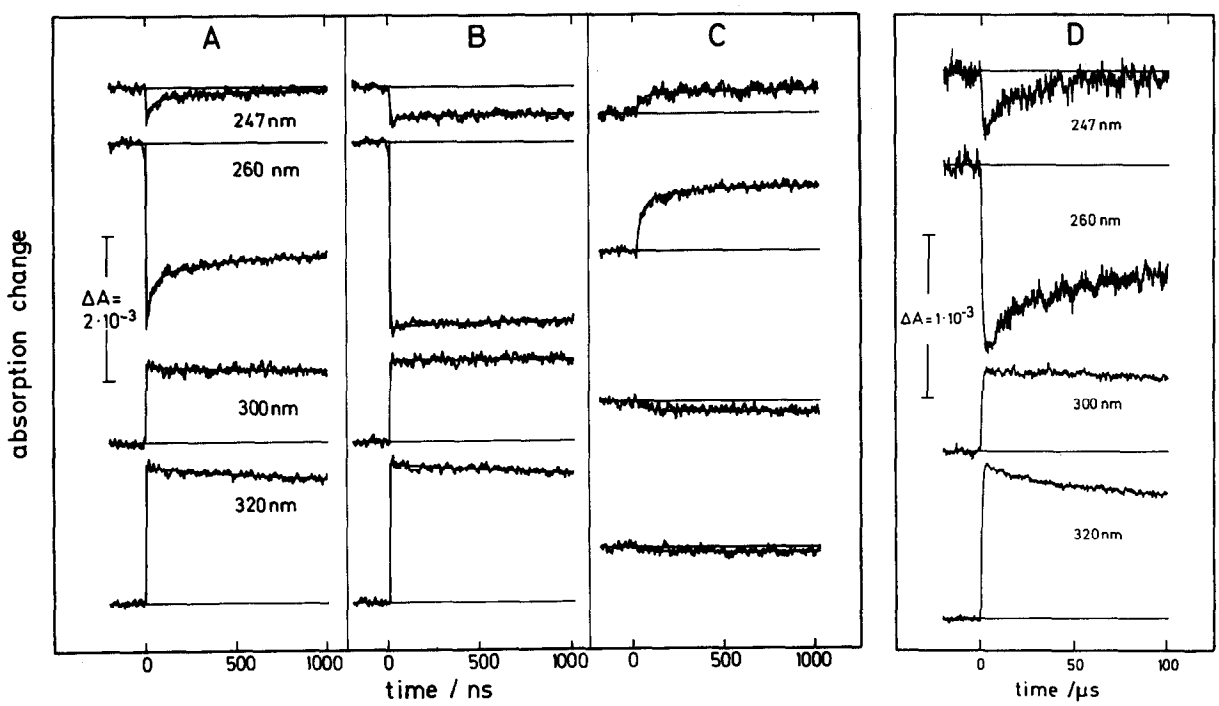

Fig.1. Time courses of UV-absorption changes at different wavelengths: (A) O_2-evolving PS II complexes at pH 6.5 in the presence of 1 mM Fecy, 60 μM Chl, optical path 0.21 cm, average of 2048 measurements; fresh samples were used after 512 flashes. (B) Tris-treated PS II complexes at pH 5.5 measured on a nanosecond time scale in the presence of 1 mM Fecy, 60 μM Chl; other details as in A. (C) Difference A – B. (D) Tris-treated PS II complexes at pH 5.2 measured on a microsecond time scale in the presence of 1 mM Fecy and 1 mM ferrocyanide, 50 μM Chl, optical path 0.11 cm, 2048 measurements averaged at 247 nm, 512 at 260 nm, 128 at 300 and 320 nm. The solid lines represent the results of fitting procedures (see text).

and Z^+_{Tris}/Z_{Tris} show systematic deviations in the wavelength region 275–340 nm [11]. It has not even been shown that Z reduces Chl a^+_{II} directly; i.e. it is an open question as to whether other electron carriers are engaged in the gap between 2 μs (fastest detection of Z^+) and 20 ns (fastest re-reduction phase of Chl a^+_{II}).

Recently, we were able to monitor absorption changes at 260 nm with nanosecond time resolution due to the oxidation of the immediate donor [32]. In order to clarify the points raised above, we report here on the UV-difference spectrum of the electron-transfer reaction from the immediate donor to Chl a^+_{II} in water-oxidizing and Tris-washed PS II complexes.

2. MATERIALS AND METHODS

Oxygen-evolving PS II complexes from *Synechococcus* sp. were prepared according to Schatz and Witt [33] and further purified [34]. The samples contained 20 mM buffer (Mes/NaOH at pH 6.5 or succinic acid/NaOH at pH 5.2 and 5.5), 20 mM $CaCl_2$, 10 mM $MgCl_2$, 0.05% (w/w) β-dodecyl-D-maltoside, about 1 M sucrose and the donors/acceptors indicated in the figure legends. They were characterized by a steady-state O_2-flash yield of about 2.5×10^{-3} O_2/Chl per flash, corresponding to 100 Chl/PS II active in O_2 evolution. Approx. 2/3 of the reaction centers were active in O_2 evolution, as was estimated by comparison with the amount of photo-reducible Q_A [21]. Tris-treated complexes were obtained by incubation of O_2-evolving complexes with 800 mM Tris-HCl (pH 8.3) in the light for 15 min and subsequent gel filtration using a column (Sephadex G-25) which was equilibrated with the buffer finally used in the measurement.

Absorption changes induced by 532 nm laser flashes (3 ns full-width at half-maximum, approx. 8 mJ/flash, expanded to a spot of approx. 5 cm^2) were measured with a time resolution of about 7 ns (t_{10-90}) as described in [32]. A total sweep of up to 5 μs was accessible, using a xenon flashlamp as measuring light source. The same set-up was used for measurements in the 100 μs time range, with the following modifications: The measuring light from a DC xenon arc lamp (XBO 150 W/1 from Osram) was pulsed by means of a shutter (open for 10 ms). The detector (S1723-02 Si-photodiode from Hamamatsu, loaded with 5 kΩ) was coupled to a TEK AM502 amplifier from Tektronix. The overall electrical bandwidth was DC–150 kHz. Excitation flashes were given at 0.8 Hz.

Signals measured on a nanosecond time scale were fitted to the function $\Delta A(t) = 2.8\alpha\exp(-\ln 2t/20\text{ ns}) + \alpha\exp(-\ln 2t/200\text{ ns}) + \beta\exp(-\ln 2t/840\text{ ns}) + \gamma$, where α, β and γ are parameters. Kinetics with fixed half-lives of 20 and 200 ns (ratio of initial amplitudes 2.8 : 1) were chosen, since signals attributed to the re-reduction of Chl a^+_{II} (monitored under repetitive excitation at 824 nm) could be best adapted with these phases [18].

In the blue region, we observed a kinetic component not related to the electron transfer in PS II with a half-life of about 840 ns; the parameter β should represent this component, while γ denotes long-lived processes. Signals measured on a total sweep of 200 μs (in fig.1D only 120 μs) were fitted to $\Delta A(t) = \alpha\exp(-\ln 2t/13\ \mu\text{s}) = \beta\exp(-\ln 2t/200\ \mu\text{s}) + \gamma$, with α, β and γ as parameters. The half-lives of 13 and 200 μs represent the forward electron transfer from Z_{Tris} to Chl a^+_{II} under the conditions chosen, and the back reaction from Q^-_A to Chl a^+_{II}, respectively.

3. RESULTS

Fig.1A shows the time courses of flash-induced absorption changes measured in O_2-evolving PS II complexes in the nanosecond range at different wavelengths in the UV. The initial amplitudes correspond to the primary charge separation Chl $a_{II} \cdot Q_A \rightarrow$ Chl $a^+_{II} \cdot Q^-_A$. As Q^-_A remains reduced up to microseconds, the observed absorption changes in the nanosecond range are essentially due to electron transfer reactions at the donor side of PS II.

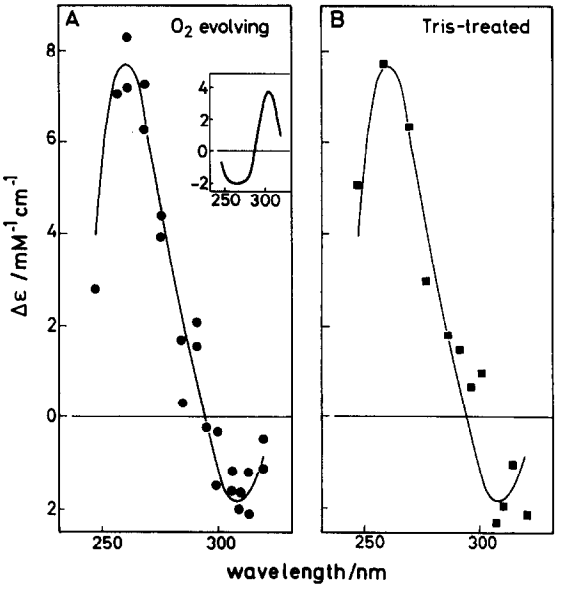

Fig.2. (A) Difference spectrum of the reduction of Chl a^+_{II} and the oxidation of its immediate donor in O_2-evolving PS II complexes. Absorption changes at 1 μs after the flash, obtained as outlined in the text for fig.1C, are depicted. The $\Delta\epsilon$ scale was evaluated on the basis of 100 Chl/PS II active in O_2 evolution and 80% saturation. (Inset) Difference spectrum of Chl a^+_{II}/Chl a_{II} (see text). (B) Spectrum of the extrapolated initial amplitudes of the 13 μs phase observed in Tris-treated PS II complexes at pH 5.2, attributed to the reduction of Chl a^+_{II} and the oxidation of its donor, Z_{Tris}. The $\Delta\epsilon$ scale was evaluated on the basis of 80% saturation and the observation that the forward electron transfer occurred in 2/3 of the reaction centers. (For an explanation of the fitting procedure see section 2).

Measurements on an extended time scale (not shown) revealed that when the final amplitude is reached, the signal remains stable up to at least 4 μs. The absorption changes might also contain minor contributions from triplet states of antenna pigments. In order to separate such signals and the absorption changes due to Q_A^-/Q_A, we measured the flash-induced absorption changes in Tris-treated complexes with the same set-up again on the nanosecond time scale (fig.1B). In Tris-treated complexes on this scale, Chl a_{II}^+ remains oxidized (half-life for reduction approx. 13 μs, see below) and, therefore, only the minor contributions of the triplet signals are superimposed on the stable signals. The signals measured with O_2-evolving complexes (fig.1A) minus those measured with Tris-treated complexes (fig.1B) at the same wavelength are shown in fig.1C. All traces obtained in this way could be adapted with the same double-exponential kinetics with half-lives of 20 and 200 ns, where the ratio of the initial amplitudes is 2.8:1 (see solid lines in fig.1C). These kinetics are the same as those reported for the re-reduction of Chl a_{II}^+ monitored at 824 nm [15,18] and are exclusively due to the re-reduction of Chl a_{II}^+ and the concomitant oxidation of its immediate donor in O_2-evolving PS II. The amplitude at 1 μs after the flash (i.e. when the reaction is already completed), read as a function of wavelength, represents the difference spectrum due to this reaction and is shown in fig.2A (the $\Delta\epsilon$ scale was evaluated as described in the legend).

Fig.1D demonstrates the flash-induced absorption changes measured in Tris-treated PS II complexes at pH 5.2 on the microsecond time scale. The changes with a half-life of 13 μs are attributed to the electron transfer Chl $a_{II}^+ \cdot Z_{Tris} \rightarrow$ Chl $a_{II} \cdot Z_{Tris}^+$. This reaction is more than 10-times faster than the back-reaction Chl $a_{II}^+ \cdot Q_A^- \rightarrow$ Chl $a_{II} \cdot Q_A$, which occurred in a fraction of the centers. The amplitude of the 13 μs phase determined by the fitting procedure (see section 2) is depicted as a function of the wavelength in fig.2B. A difference spectrum in the UV attributed to the electron-transfer reaction from Z_{Tris} to Chl a_{II}^+ in trypsinized (pH 7.5) PS II particles from spinach has been reported by Weiss and Renger [11]. In the wavelength region between 250 and 280 nm it is similar to our results (fig.2B), but deviates significantly within the range 280–320 nm. We have no explanation for this deviation.

The UV spectra in fig.2A,B are practically iden-

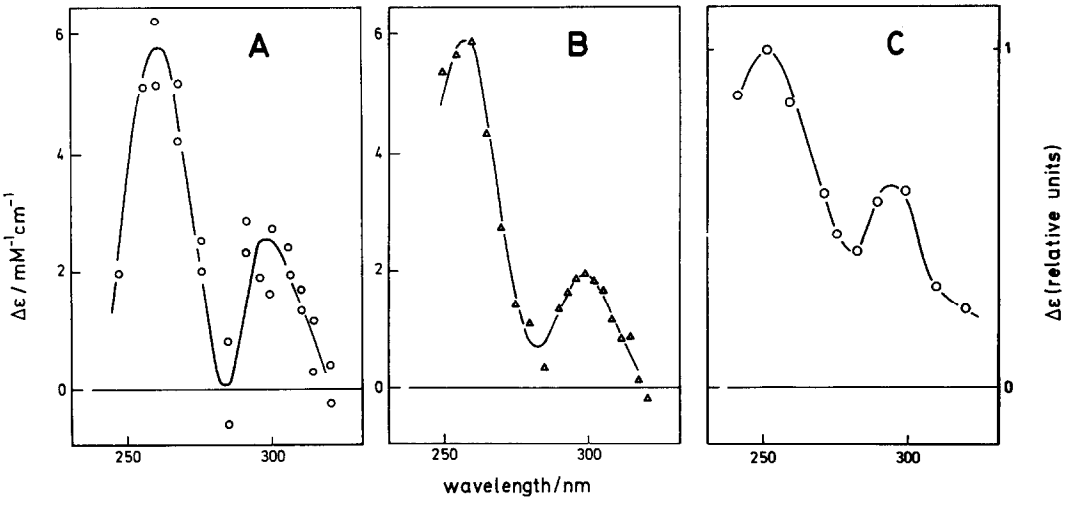

Fig.3. (A) Spectrum shown in fig.2A minus that of Chl a_{II}/Chl a_{II}^+ (inset in fig.2A, inverted). This is attributed to the extinction difference of the oxidized minus reduced form of the immediate donor, which reduces Chl a_{II}^+ in O_2-evolving PS II. (B) Difference spectrum of the oxidized minus reduced form of the donor Z_{Tris} which reduces Chl a_{II}^+ in Tris-treated PS II. Measured in the preparations used here according to the method in [21] (pH 8.3, 10 μM DCMU, 2.5 mM Fecy, 2.5 mM ferrocyanide). (C) In vitro difference spectrum taken from [37,38] attributed to the formation of the phenoxy radical TyrO$^\cdot$, which results from the loss of the phenolic hydrogen from tyrosine; measured with tyrosine in argon-flushed H_2O (pH 7) as absorption changes at 15 μs after a short flash. The scale of the ordinate in [37] is not calibrated in $\Delta\epsilon$ units, but the spectrum contains a smaller peak around 407 nm for which the extinction coefficient is known to be 2000–3200 $M^{-1} \cdot cm^{-1}$ [38]. This means that one unit in the $\Delta\epsilon$ scale in C corresponds to 5500–8800 $M^{-1} \cdot cm^{-1}$.

tical. The contribution due to the re-reduction of Chl a_{II}^{+} is the same for both spectra. The Chl a_{II}^{+}/Chl a_{II} difference spectrum (see inset, fig.2A) is clearly distinct from the total spectra in fig.2A,B. From the identity of the total spectra it follows that the difference spectrum for oxidation of the immediate donor to Chl a_{II}^{+} in O_2-evolving PS II is the same as that for oxidation of the donor in Tris-treated complexes. From this it can be concluded that both donors are of the same chemical nature.

Subtraction of the Chl a_{II}/Chl a_{II}^{+} difference spectrum (inset of fig.2A, inverted) from the spectrum shown in fig.2A yields the spectrum due to oxidation of the immediate donor in O_2-evolving PS II alone, being shown in fig.3A. The UV spectrum of Chl a_{II}^{+}/Chl a_{II} (Gerken et al., to be published) was obtained as the difference of the spectrum of Chl $a_{II}^{+}Q_A^{-}$/Chl $a_{II}Q_A$ minus Q_A^{-}/Q_A. As extinction changes caused by the reduction of Q_A are large (up to 17000 $M^{-1} \cdot cm^{-1}$) compared to those of Chl a_{II}^{+}/Chl a_{II}, the precision of the spectrum shown in fig.2A (inset) is only of the order of $\pm 1700\ M^{-1} \cdot cm^{-1}$. As a consequence, the precision of the spectrum shown in fig.3A is also reduced. The above conclusion drawn from fig.2A,B states that the spectrum for oxidation of the immediate donor in O_2-evolving complexes in fig.3A is the same as that for oxidation of the donor Z_{Tris}^{+}/Z_{Tris} in Tris-treated PS II complexes. The latter is well established from measurements on a millisecond to seconds time scale, taking advantage of the long lifetime of Z_{Tris}^{+} in PS II with inhibited O_2 evolution [21–23]. Fig.3B shows the Z_{Tris}^{+}/Z_{Tris} spectrum measured according to Dekker et al. [21] with the PS II complexes from *Synechococcus* used in this study (Gerken et al., to be published), which is in agreement with previously published data.

Regarding the main features, i.e. a maximum around 260 nm and a smaller one around 300 nm, the spectra due to oxidation of the donors in fig.3A,B are very similar.

4. DISCUSSION

Here, two main questions have been raised: (i) Are there one or more electron carriers active in the electron-transfer route between Chl a_{II} and the water-oxidizing enzyme complex, S? (ii) What is the chemical nature of the carrier(s) between Chl a_{II} and S?

With respect to the number of carriers, information can be obtained as follows. On the one hand, oxidation of the immediate electron donor to Chl a_{II}^{+} was followed in this work by flash absorption spectroscopy from the very beginning. The oxidation kinetics could be adapted biphasically with half-lives of 20 and 200 ns (repetitive excitation) in exact accordance with the re-reduction kinetics of Chl a_{II}^{+}. The donor is, in turn, re-reduced with $t_{1/2} > 10\ \mu s$. On the other, the redox reaction of the intermediate electron carrier Z was followed by EPR measurements (signal II_{vf}) [10]. Z was found to be oxidized in $\leq 3\ \mu s$ (the rise time was instrument limited) [35] and re-reduced with half-lives of 30 μs to 1.5 ms [10] in accordance with the oxidation times of the S states and manganese, respectively [8,12,13]. We conclude from this time course that only one electron carrier functions between Chl a_{II} and the water-oxidizing enzyme S. This carrier is oxidized as the immediate donor to Chl a_{II}^{+} with a $t_{1/2}$ = 20–250 ns and remains in this state until re-reduced by S with $t_{1/2} = 30\ \mu s$ to 1.5 ms, depending on the oxidation states of S. We shall now discuss the chemical nature of this carrier functioning as an immediate donor to Chl a_{II}^{+}.

(i) A species, D, not engaged in electron transfer from H_2O to Chl a_{II}, has been shown to be a tyrosine residue in position 160 in the D2 polypeptide of PS II [25–27]. The EPR signal II_{slow} of D^{+} is identical with EPR signal II_{fast} of Z_{Tris}^{+}, the oxidized donor to Chl a_{II}^{+} in inactivated PS II [19,24]. It was therefore proposed that Z_{Tris} is also a tyrosine but in position 161 in the D1 polypeptide of PS II. It was suggested that Z in water-oxidizing PS II (EPR signal II_{vf}) is also a tyrosine and the donor to Chl a_{II}^{+} [26]. However, proof for this latter suggestion has not been given (see section 1). As a result of this work, it has been shown that the difference spectrum of the oxidation of Z_{Tris} is the same as that for oxidation of the immediate donor to Chl a_{II}^{+} in O_2-evolving complexes, indicating that the latter is also a tyrosine. Recently, Bock et al. [36] reported on an EPR spectrum in PS II complexes where O_2 evolution was inactivated by acetate, but completely reversible. From the result it was suggested that the immediate donor to Chl a_{II}^{+} in O_2-evolving PS II complexes might be a tyrosine.

(ii) The characteristics of the difference spectrum due to the oxidation of the immediate donor to Chl

a_{II}^{+} for O_2-evolving PS II (see fig.3A) and for Tris-treated PS II (see fig.3B) have been compared with the in vitro spectrum of the oxidation reaction of tyrosine. The in vitro difference spectrum $TyrO^{\cdot}/TyrOH$ (OH designates the phenolic hydroxyl group of tyrosine) in water is shown in fig.3C (taken from [37,38]). The main features of this difference spectrum are similar to those in fig.3A,B. This similarity lends support to the above-outlined suggestion in an independent way, i.e. that the immediate donor to Chl a_{II}^{+} in O_2-evolving PS II is a tyrosine. Since in water the pK value for H^+ release from $TyrO^{\cdot}H^+$ is beyond zero (p$K<0$) [39], it is very likely that in O_2-evolving PS II, H^+ release also occurs with the electron extraction; i.e. that the reaction Chl a_{II}^{+}TyrOH $\rightarrow$ Chl a_{II}TyrO$^{\cdot}$ + H^+ takes place. This H^+ should be re-attached when the oxidized donor is re-reduced by an electron from the water-splitting enzyme S. H^+ release and re-uptake concomitant with oxidation and reduction of the donor Z_{Tris} have been observed in inactivated PS II complexes [40,41] but have not as yet been demonstrated for water-oxidizing PS II.

In [16] two electron carriers between Chl a_{II} and S were proposed as an explanation for the biphasic nanosecond re-reduction kinetics of Chl a_{II}^{+} in states S_2 and S_3. On the basis of only one carrier between Chl a_{II} and S, the biphasic nanosecond kinetics in S_2 and S_3 need to be explained in a different way. Recent measurements at pH 7 provided evidence for the coexistence of two different protonation states of S. This would explain the biphasic nanosecond kinetics in an uncomplicated manner [42].

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