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Direct observation of the immediate electron donor to chlorophyll-a_{II}^{+} (P-680^{+}) in oxygen-evolving photosystem II complexes

Resolution of nanosecond kinetics in the UV

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Flash-induced absorption changes in the UV have been monitored with nanosecond time resolution in oxygen-evolving photosystem II complexes from *Synechococcus* sp. At 260 nm, the oxidation of the immediate donor to Chl-a_{II}^{+} (P-680^{+}) in the 10–500 ns range could be observed directly. The difference extinction coefficient for its oxidation is estimated to be 6000–8000 $M^{-1} \cdot cm^{-1}$ at 260 nm. The observed kinetics for the oxidation of the donor is identical with the reduction kinetics of Chl-a_{II}^{+} as monitored at 824 nm. At 320 nm, the reduction of Q_A was found to be faster than 1 ns.

Oxygen evolution; Photosystem II; P-680; Electron transfer; Absorbance difference spectroscopy; (*Synechococcus* sp.)

1. INTRODUCTION

Photosynthetic oxygen evolution is driven by the successive extraction of four electrons from the water oxidizing enzyme system S [1] by photooxidized Chl-a_{II} (P-680), the primary electron donor of PS II. The reduction kinetics of Chl-a_{II}^{+} depend on the oxidation state (S_0–S_3) of the enzyme system S, i.e. on the number of the extracted electrons. The half-life times are in the range between 20 and 250 ns [2]. The oxidation kinetics of the S states, however, are considerably slower, namely in the order of tens to hundreds of microseconds [3–5]. Because of this gap in kinetics between Chl-a_{II} and S, the electron transfer between both involves at least one intermediate, D. Based on an observed biphasic nanosecond reduction of Chl-a_{II}^{+} in states S_2 and S_3, it has been proposed that there may be two electron carriers, D_1 and D_2, arranged in series between Chl-a_{II} and S [2] (for a recent discussion see [6]).

Since the electron transfer to Chl-a_{II}^{+} takes place in the nanosecond time range it has proved to be difficult to observe the oxidation of the secondary donor D (or the chain D_1-D_2) directly. Therefore, much interest was focused on PS II with inactivated water oxidizing enzyme. Under such condi-

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Abbreviations: Chl, chlorophyll; D, electron donor to Chl-a_{II}^{+} in O_2-evolving PS II; 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; Z, electron donor to Chl-a_{II}^{+} in PS II devoid of its O_2-evolving capability

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tions, Chl-$a_{II}^{\dagger}$ is reduced in the microsecond time range [7] by a donor called Z, which may be a modified form of D. Z^+ has been characterized by its EPR spectrum, called signal II_f [8], and by its optical difference spectrum in the UV [9–12]. It was interpreted to be a tightly bound plastoquinone molecule [13]. From the coinciding kinetics of the reduction of Chl-$a_{II}^{\dagger}$ and the oxidation of Z, respectively, it has been concluded that Z is the immediate donor to Chl-$a_{II}^{\dagger}$ in PS II inactive in oxygen evolution [12,14].

For oxygen-evolving PS II it has been shown that a donor component with an EPR spectrum (signal II_{vf}) [15] and optical difference spectrum [12,16] similar to the one of Z^+/Z oxidizes S. In neither of these studies, however, could the oxidation kinetics of this species be resolved, so that it remained an open question whether it is the immediate donor to Chl-$a_{II}^{\dagger}$ or whether another carrier operates between Chl-a_{II} and this species.

In this paper we describe measurements of absorption changes in the UV in purified and optically clear oxygen-evolving PS II complexes, using a flash photometer with highly improved time resolution. For the first time, the nanosecond kinetics of the oxidation of the immediate electron donor to Chl-$a_{II}^{\dagger}$ could be observed directly.

2. MATERIALS AND METHODS

Oxygen-evolving PS II complexes were prepared from the thermophilic cyanobacterium *Synechococcus* sp. according to Schatz and Witt [17] (SB12 extract), and further purified by differential sucrose density centrifugation according to Rögner et al. ([18], there called 'SG-1'). The samples contained buffer A (10 mM Mes/NaOH, pH 6.5, 10 mM $CaCl_2$, 5 mM $MgCl_2$), 0.05% (w/w) β-dodecyl-D-maltoside, 1.2 M sucrose and, where indicated 1 mM $K_3(Fe(CN)_6)$. They were characterized by a steady-state O_2-flash yield of about 2.5×10^{-3} O_2 per Chl and flash corresponding to 100 Chl/PS II active in O_2-evolution. Estimation of the antenna size based on the amount of photo-reducible Q_A yielded 60 Chl/PS II. Hence, approx. 60% of the reaction centers are active in O_2-evolution.

For Tris-treatment, the SB12 extract was incubated with 800 mM Tris-HCl, pH 8.3, for 15 min, centrifuged as in [18] and dialyzed against buffer A. Before measurements 0.05% (w/w) β-dodecyl-D-maltoside and 1 mM $K_3(Fe(CN)_6)$ were added. Both types of PS II complexes were optically very clear, which is of great advantage for measurements in the UV.

Absorption changes at 824 nm were measured as described in [2]. In the UV range the measuring light was taken from a xenon flash (EG&G FXP-850Q), that discharged 50 J in a 60 μs (FWHM) flash, shaped by a home-built electrical network. Broadband interference filters from Schott between the flash tube and the sample assured that sample excitation from the measuring light was negligible. The measuring light was focused and detected by a Si-photodiode FND 100Q from EG&G, which was protected by a narrow band (approx. 8 nm FWHM) interference filter from Schott and 3 mm of colored glass U330 from Hoya. The photocurrent in the plateau of the xenon flash was balanced by a d.c. offset current fed to the load resistor using a model 5525 Bias Tee from Picosecond Pulse Labs. The signal was amplified (HP461A from Hewlett Packard and 7A13 differential amplifier plug-in from Tektronix in an A7704 mainframe). The vertical monitor output of the oscilloscope was fed into a Biomation 6500 transient recorder (2 ns/point) with Nicolet 1170 averager. The electrical bandwidth was approx. 1 kHz to 50 MHz. The profile of the measuring flash and a small (<20% of the maximal signal amplitude) fluorescence artifact were recorded separately and subtracted from the signals. Samples were excited by 3 ns (FWHM) laser flashes at 532 nm from a frequency-doubled Nd/YAG laser (YG441 from Quantel). The laser energies were chosen to yield 80–90% saturation for measurements in the UV and approx. 50% saturation at 824 nm. The repetition rate was 1 Hz.

For measurements with a time resolution of approx. 1 ns (inset of fig.2 only), the sample was excited by non-saturating pulses of 35 ps duration from a mode-locked, frequency-doubled Nd/YAG laser (YG471 from Quantel). The optical set-up was the same as described above, but the signal was delayed (1100A from Hewlett Packard), amplified (TV83/10408C from Telemeter) and registered with a 7912AD transient recorder from Tektronix with 7A29 plug-in. The overall electrical bandwidth was d.c. to approx. 300 MHz.

3. RESULTS AND DISCUSSION

Fig.1A shows the time course of the absorption change observed at 260 nm in oxygen-evolving PS II complexes. A fast bleaching, which is limited by the time resolution of the detection system used for this measurement ($t_{10-90} \simeq 10$ ns), is followed by an absorption increase in the time range between 10 and 500 ns. The absorption change remaining at the end of the time scale in fig.1A does not decay significantly up to 1.6 μs after the flash (not shown). The amplitude of the initial absorption decrease is due to the formation of Chl-$a_{II}^{+}Q_A^{-}$. It corresponds to a $\Delta\epsilon$ of approx. -17000 $M^{-1}\cdot cm^{-1}$ (calculated on the basis of 60 Chl/PS II and 85% saturation). Since the $\Delta\epsilon$ of Chl-a_{II} $\longrightarrow$ Chl-a_{II}^{+} is small (-2000 $M^{-1}\cdot cm^{-1} < \Delta\epsilon < 0$) ([12] and Gerken et al., to be published), the fast bleaching reflects essentially the reduction of Q_A [19]. Q_A^{-} is stable on a time scale of tens of microseconds [11,20]. The absorption increase between 10 and 500 ns is therefore attributed to the electron transfer from the secondary donor(s) to Chl-a_{II}^{+}. After inactivation of oxygen evolution by Tris washing, this kinetics disappears (fig.1B), as expected from the fact that Chl-a_{II}^{+} reduction is slowed down to the microsecond range by this treatment [7]. If Q_A is in the reduced state prior to the flash, the signals attributed to the formation of Q_A^{-} and the oxidation of the immediate donor should be absent. This is indeed the case, as demonstrated in fig.1C (no artificial acceptor, white background light).

The absorption increase in the time range between 10 and 500 ns at 260 nm under normal conditions (fig.1A) corresponds to $\Delta\epsilon \simeq 8000$ $M^{-1}\cdot cm^{-1}$ (calculated on the basis of 100 Chl/PS II active in O_2 evolution and 85% saturation). Taking into account that the contribution of $\Delta\epsilon$ (Chl-a_{II}^{+}/Chl-a_{II}) is small (see above), the absorption increase is caused for the major part by the oxidation of the donor(s) to Chl-a_{II}^{+}. The extinction coefficient for its oxidation is in the range 6000 $M^{-1}\cdot cm^{-1} < \Delta\epsilon < 8000$ $M^{-1}\cdot cm^{-1}$ at 260 nm.

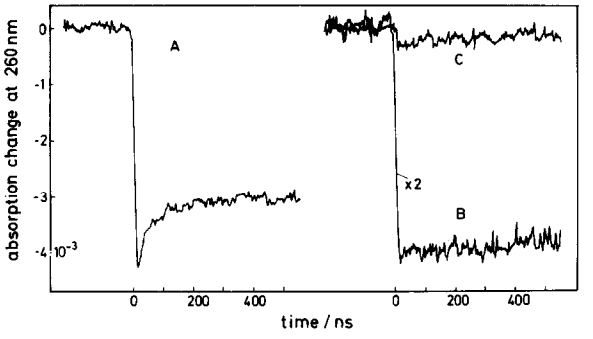

Fig.1. Time-courses of the absorption changes at 260 nm; (A) O_2-evolving SG-1 complexes, 80 μM Chl, 1 mM $K_3(Fe(CN)_6)$, average of 1024 measurements; (B) Tris-treated complexes, 50 μM Chl, 1 mM $K_3(Fe(CN)_6)$, average of 1024 measurements; (C) sample as in trace A, but in the absence of $K_3(Fe(CN)_6)$ and under white background illumination in order to accumulate Q_A^{-}, average of 512 measurements. Optical path, 0.22 cm. The signal in B is reproduced 2 times enlarged compared to A and C to compensate for the 2 times lower concentration of reaction centers (as determined from the absorption change at 320 nm).

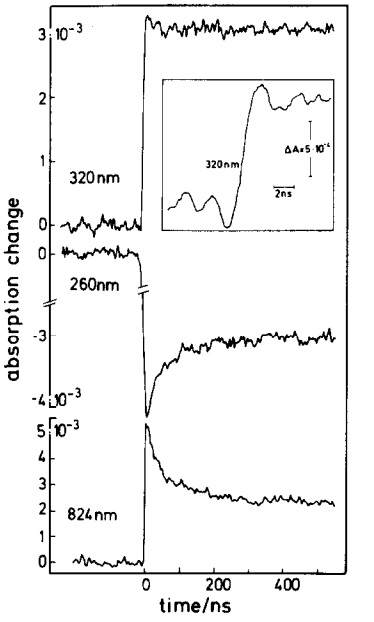

Fig.2. Time-courses of absorption changes at different wavelengths in O_2-evolving SG1 complexes: (top and center) 80 μM Chl, 1 mM $K_3(Fe(CN)_6)$, optical path 0.22 cm, average of 1024 measurements; (bottom) 25 μM Chl, 1 mM Fecy, optical path 5 cm, average of 64 measurements. (Inset) Time-course of absorption changes at 320 nm induced by laser flashes of 35 ps duration, recorded with a time resolution of approx. 1 ns; 170 μM Chl, 1 mM $K_3(Fe(CN)_6)$, optical path 0.12 cm, average of 64 measurements. Note that the energy of the excitation flashes was lower than in the other experiments.

Fig.2, upper trace, presents the absorption changes measured in an oxygen evolving sample at 320 nm. The signal shows a fast rise to a level which is stable in the time range examined. This signal is essentially due to the formation of Q_A^-, which is related to a characteristic absorption increase at 320 nm [19]. The absence of significant kinetics in the 10–500 ns range at 320 nm indicates that absorption changes due to the electron transfer from D (or the chain D_1-D_2) to Chl-a_{II}^+ are negligible at 320 nm.

We also measured the absorption changes at 320 nm using laser flashes of 35 ps duration and a faster detection system. The observed signal rises with $t_{10-90} \simeq 1.2$ ns (fig.2, inset), corresponding to the time resolution of the apparatus. This indicates that Q_A is reduced in less than 1 ns, which is compatible with the time of a few hundred picoseconds found for the reoxidation of photo-reduced pheophytin, the presumed intermediate between Chl-a_{II} and Q_A [21,22].

The new possibility to monitor small absorption changes in oxygen-evolving PS II in the UV with nanosecond time resolution gives direct access to the oxidation kinetics of the electron donor(s) in PS II and should hence also allow one to decide on the number of carriers involved in electron transfer from S to Chl-a_{II}^+. The existence of two carriers, D_1 and D_2, arranged in series, has been proposed as an explanation for the biphasic nanosecond reduction kinetics of Chl-a_{II}^+ in the S_2 and S_3 states [2]. An alternative explanation, which requires only one donor, has been proposed recently [23]. At around pH 7, there may co-exist two different protonation states of S, related to differing reduction kinetics of Chl-a_{II}^+.

If there is only one donor, its oxidation kinetics should correspond exactly to the reduction kinetics of Chl-a_{II}^+. In the case of two donors, D_1 and D_2, in series, the kinetics of the absorption changes due to the electron transfer from D_2 via D_1 to Chl-a_{II}^+ will only correspond exactly to the reduction kinetics of Chl-a_{II}^+ if the extinction changes $\Delta\epsilon(D_1^+/D_1)$ and $\Delta\epsilon(D_2^+/D_2)$ are identical at the wavelength studied. Therefore, in fig.2 we compare the kinetics of the absorption changes at 260 nm (redrawn from fig.1A) with the kinetics of Chl-a_{II}^+ reduction as monitored at 824 nm. Within the noise of the measurements, both kinetics are identical in the range from 10 to 500 ns. So, either there is only one carrier, D, or there are two carriers D_1 and D_2 with rather similar differential extinction coefficients at 260 nm. For a decision on the number of carriers an extension of the present studies to other wavelengths is required.

With respect to the question if the donor Z functioning in PS II with inactivated oxygen evolution is either a modified state of the normal immediate donor observed directly in this work, or an alternative donor, it is of interest to compare the differential extinction coefficients for the oxidation of these species. At 260 nm our value of $\Delta\epsilon$ = 6000–8000 $M^{-1} \cdot cm^{-1}$ agrees roughly with the reported values of about 8000 $M^{-1} \cdot cm^{-1}$ for the oxidation of Z [9–12]. Whether or not these species are identical will have to be decided when the complete absorbance difference spectrum for the oxidation of the normal immediate donor to Chl-a_{II}^+ becomes available.

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REFERENCES

[1] Kok, B., Forbush, B. and McGloin, M. (1970) Photochem. Photobiol. 11, 457–475.
[2] Brettel, K., Schlodder, E. and Witt, H.T. (1984) Biochim. Biophys. Acta 766, 403–415.
[3] Dekker, J.P., Plijter, J.J., Ouwehand, L. and Van Gorkom, H.J. (1984) Biochim. Biophys. Acta 767, 176–179.
[4] Renger, G. and Weiss, W. (1985) Biochem. Soc. Trans. 14, 17–20.
[5] Saygin, Ö. and Witt, H.T. (1987) Biochim. Biophys. Acta 893, 452–469.
[6] Witt, H.T., Schlodder, E., Brettel, K. and Saygin, Ö. (1986) Ber. Bunsenges. Phys. Chem. 90, 1015–1024.
[7] Conjeaud, H. and Mathis, P. (1980) Biochim. Biophys. Acta 590, 353–359.

[8] Babcock, G.T. and Sauer, K. (1975) Biochim. Biophys. Acta 376, 329–344.
[9] Dekker, J.P., Van Gorkom, H.J., Brok, M. and Ouwehand, L. (1984) Biochim. Biophys. Acta 764, 301–309.
[10] Diner, B.A. and De Vitry, C. (1984) in: Advances in Photosynthesis Research (Sybesma, C. ed.) vol.I, pp.407–411, Martinus Nijhoff/Dr W. Junk Publishers, Den Haag.
[11] Schatz, G.H. and Van Gorkom, H.J. (1985) Biochim. Biophys. Acta 810, 283–294.
[12] Weiss, W. and Renger, G. (1986) Biochim. Biophys. Acta 850, 173–183.
[13] O'Malley, P.J. and Babcock, G.T. (1984) Biochim. Biophys. Acta 765, 370–379.
[14] Boska, M., Sauer, K., Buttner, W. and Babcock, G.T. (1983) Biochim. Biophys. Acta 722, 327–330.
[15] Babcock, G.T., Blankenship, R.E. and Sauer, K. (1976) FEBS Lett. 61, 286–289.
[16] Dekker, J.P., Plijter, J.J. and Van Gorkom, H.J. (1987) in: Progress in Photosynthesis Research (Biggins, J. ed.) vol.I, pp.533–536, Martinus Nijhoff Publishers, Dordrecht, The Netherlands.
[17] Schatz, G.H. and Witt, H.T. (1984) Photobiochem. Photobiophys. 7, 1–14.
[18] Rögner, M., Dekker, J.P., Boekema, E.J. and Witt, H.T. (1987) FEBS Lett. 219, 207–211.
[19] Stiehl, H.H. and Witt, H.T. (1968) Z. Naturforsch. 23b, 220–224; and (1969) Z. Naturforsch. 24b, 1588–1598.
[20] Bowes, J.M. and Crofts, A.R. (1980) Biochim. Biophys. Acta 590, 373–384.
[21] Nuijs, A.M., Van Gorkom, H.J., Plijter, J.J. and Duysens, L.N.M. (1986) Biochim. Biophys. Acta 848, 167–175.
[22] Holzwarth, A.R., Brock, H. and Schatz, G.H. (1987) in: Progress in Photosynthesis Research (Biggins, J. ed.) vol.I, pp.61–65, Martinus Nijhoff Publishers, Dordrecht, The Netherlands.
[23] Meyer, B. (1987) Thesis, Technische Universität Berlin.