Volume 240, number 1,2, 153–158 FEB 06513 November 1988

Analysis of the electron transfer from Pheo$^-$ to Q_A in PS II membrane fragments from spinach by time resolved 325 nm absorption changes in the picosecond domain

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Received 22 September 1988

Absorption changes at 325 nm (ΔA_{325}) induced by 15 ps laser flashes ($\lambda = 650$ nm) in PS II membrane fragments were measured with picosecond time-resolution. In samples with the reaction centers (RCs) kept in the open state (P I Q_A) the signals are characterized by a very fast rise (not resolvable by our equipment) followed by only small changes within our time window of 1.6 ns. In the closed state (P I Q_A^-) of the reaction center the signal decays with an average half-life time of about 250 ps. It is shown that under our excitation conditions ($E = 2 \times 10^{14}$ photons/cm^2 per pulse) subtraction of the absorption changes in closed RCs (ΔA_{325}^{closed}) from those in open RCs (ΔA_{325}^{open}) leads to a difference signal which is dominated by the reduction kinetics of Q_A. From the rise kinetics of this signal and by comparison with data in the literature it is inferred that Q_A becomes reduced by direct electron transfer from Pheo$^-$ with a time constant of about 350 ± 100 ps.

Photosystem II; Electron transfer; Q_A-reduction kinetics; Pheophytin; Charge separation

1. INTRODUCTION

The absorption of light by the antenna pigments of photosynthesizing organisms is followed by a sequence of very fast processes like exciton migration, exciton trapping, primary radical pair formation and subsequent stabilization of the charge separation (for recent reviews see [1,2]). In photosystem II of higher plants this reaction sequence can be summarized by

$$\text{Chl}^* \text{ P I } Q_A \underset{k_{-t}}{\overset{k_t}{\rightleftharpoons}} \text{Chl P}^* \text{ I } Q_A \underset{k^*}{\overset{k_{PC}}{\rightleftharpoons}} \text{Chl P}^+\text{I}^-Q_A \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{Chl P}^+\text{I } Q_A^-$$

where Chl* symbolizes the excited chlorophyll molecules of the antenna, P the photoactive chlorophyll *a* (P680), I the intermediary redox carrier(s) and Q_A the primary plastoquinone acceptor. k_i are the rate constants of the above mentioned processes. Water cleavage caused by light-induced generation of electrons and holes in PS II is only possible if the primary charge separation can be sufficiently stabilized by rapid electron transfer from I$^-$ to Q_A [3]. If Q_A^- stays reduced the radical pair P680$^+$I$^-$ rapidly recombines either via excited singlet state formation and subsequent decay or by

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Abbreviations: ΔA_{325}, absorption changes at 325 nm; Car, carotenoid; Chl, chlorophyll; I, intermediary electron acceptor(s); Pheo, pheophytin; PS II, photosystem II; Q_A, primary plastoquinone acceptor; Z and Z^{ox}, reduced and oxidized form of the electron donor to P680

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00145793/88/$3.50

radiationless reaction into the ground state [4–9]. There is convincing evidence for a Pheo molecule acting as the primary acceptor of PS II [10] but the reaction pattern of the charge stabilization is not yet completely clarified. Based on the functional and structural analogy between PS II and the reaction centers of purple bacteria [11], $Pheo^-$ is anticipated to reduce Q_A directly in a single electron transfer step whereas redox titration experiments suggested the existence of intermediary redox components [12]. In this study we attempted to solve this problem by measuring the reduction kinetics of Q_A via flash-induced absorption changes at 325 nm and comparison with the reoxidation kinetics of $Pheo^-$ recently reported in the literature [5,6]. The data obtained support the idea of a direct reduction of Q_A by $Pheo^-$.

2. MATERIALS AND METHODS

Oxygen-evolving PS II fragments were prepared from spinach according to the procedure described in [13] with modifications as described in [14]. The picosecond transient absorption measurements were performed using the pump-probe technique as described in detail elsewhere (Wiese, N., Bernarding, J. and Eichler, H.J., in preparation). The samples were excited at a repetition rate which could vary between 400 Hz and 4 MHz by 650 nm pulses (E = 2 nJ/pulse before attenuation, FWHM: 15 ps) from a cavity dumped dye laser (Spectra Physics type 375) synchronously pumped by a mode-locked argon ion laser (Spectra Physics type 171). A small fraction of the pulse energy was frequency doubled to 325 nm thereby providing the probe pulses at this wavelength (E = 2 pJ/pulse). At the point of overlap in the flow cuvette the pump and probe beams had a diameter of about 20 μm. The time delay between pump and probe pulse was adjustable between −300 and 1600 ps by means of a computer controlled step motor. The intensity of the probe beam was monitored by photomultipliers before (reference) and after the sample. In order to account for fluctuations of the probe beam the signals were fed into a difference amplifier. Further noise reduction was achieved by modulation of the pump-pulse train (200 Hz) and lock-in amplification of the output signal of the difference amplifier and signal averaging in a computer. The sample was pumped through a flow cuvette (optical pathlength: 0.5 mm) at a flow rate which was high enough for an exchange of the excited sample volume between two consecutive pump pulses at repetition rates up to 10 kHz.

The sample suspension contained: PS II particles at a concentration of 550 μg chlorophyll/ml, 20 mM Mes/NaOH (pH 6.5), 1 mM $K_3Fe(CN)_6$, and up to 0.1% sulfobetaine (SB12) was added to reduce particle scattering. Measurements of flash-induced 830 nm absorption changes (see [15] for details) revealed that sulfobetaine at this low concentration did not significantly affect the $P680^+$ reduction kinetics during the measuring time (not shown). The samples were kept at a temperature of about 4°C.

The reaction centers could be converted into the closed state (P680 I Q_A^-) either by an increase of the repetition rate of the laser to 800 kHz or 4 MHz or by illumination with strong cw-light from a He-Ne laser.

3. RESULTS AND DISCUSSION

Fig.1 shows typical traces of absorption changes at 325 nm (ΔA_{325}) in PS II membrane fragments with the reaction centers (RCs) kept either in the open (P680 I Q_A) or closed state (P680 I Q_A^-). The rise time of ΔA_{325} in all signals is instrument limited. The signal in fig.1A was obtained at a pulse repetition rate of 8 kHz where the sample volume excited by the pump beam was exchanged between two consecutive pulses, thus keeping most of the RCs in the open state. Under this condition the signal hardly decays within our time window of 1.6 ns. Although the difference spectrum of Q_A^- formation is characterized by its absorption maximum at 325 nm [16], the time course of the signal of fig.1A does not directly reflect the reduction kinetics of Q_A because they are obviously obscured by overlapping absorption changes due to other reactions giving rise to rapid transients. Therefore, for the resolution of these kinetics, two problems have to be solved: (i) identification of the non-decaying part of the absorption changes and (ii) proper separation of rapidly relaxing absorption changes that counterbalance the rise kinetics of Q_A reduction. This goal can be achieved by comparison with absorption changes induced in samples with closed reaction centers (P680 I Q_A^-).

Regardless of the mode of P680 I Q_A^- formation (see section 2) the flash-induced absorption changes at 325 nm exhibit a fast decay with an average half-life time of about 250 ps (fig.1B,C). However, significant differences exist for the occurrence of a background contribution as reflected by an offset signal. If the RCs are closed by a 100-fold increase of repetition rate (800 kHz) an offset arises of similar extent as the amplitude of ΔA_{325} in samples with open RCs (cf. fig.1A and B). This offset is absent if the state P680 I Q_A^- is achieved by strong cw background illumination. This phenomenon is understandable because the application of a lock-in detection technique eliminates any background that is not modulated with the chopping frequency of the pump pulse

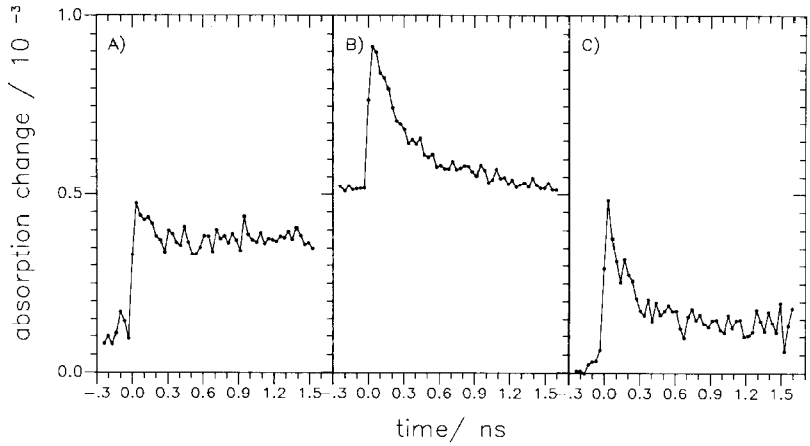

Fig.1. Laser-flash-induced absorption changes at 325 nm as a function of time in PS II membrane fragments at an excitation repetition rate of either 8 kHz (A,C) or 800 kHz (B). In signal C the sample was illuminated with strong cw light from a He-Ne laser. Experimental conditions were as described in section 2.

train. Therefore, the offset observed reflects the absorbance difference between the steady state reached at the repetition rate of the actinic flashes (the excitation by the weak probe pulse is negligible) and the dark state. At 800 kHz the reaction centers attain predominantly the redox state $Z^{ox}P680\ I\ Q_A^-$ since the recombination of $P680^+I^-Q_A^-$ to P680 I Q_A^- is markedly faster [4–9], while Q_A^- reoxidation and Z^{ox} reduction are slow compared to the repetition rate [17–21]. As $\Delta\epsilon(Z^{ox}/Z)$ is markedly smaller than $\Delta\epsilon(Q_A^-/Q_A)$ [16,22], the offset should mainly reflect the absorption change due to Q_A^- formation. However, in addition to these redox transitions excited states have to be considered also. Singlets can be neglected because of their short life times (for a recent review, see [23]). Likewise, chlorophyll triplets are expected to decay fast enough due to the rapid triplet-triplet energy transfer to carotenoids [24]. In contrast, carotenoid triplets (^{3}Car) cannot be neglected because at 800 kHz the excitation rate is faster than ^{3}Car decay [25,26]. In order to check this point, the offset extent was measured as a function of the actinic flash energy and of the flash repetition rate. The data depicted in fig.2 and 3c reveal a clear saturation type behaviour, indicating that carotenoid triplets do got significantly contribute to the offset. Therefore, the offset mainly reflects the formation of Q_A^- (see above). This conclusion is supported by the fact that the maximum extent of the offset corresponds to the reduction of one Q_A per 400 chlorophylls based on an $\Delta\epsilon(Q_A^-/Q_A)$ value of 13000 $(M \cdot cm)^{-1}$ [16]. This ratio is in close agreement with calculations based on measurements of 830 nm absorption changes (not shown).

The absorption changes induced at low repetition rates in samples with open RCs (fig.1A) are a superimposition of possible contributions from: (i) the lowest excited singlet state of antenna chlorophylls (Chl*); (ii) the triplet states ^{3}Chl formed by intersystem crossing and/or by radical pair mechanism; (iii) the formation and decay of $P680^+$ and I^-, and (iv) the formation of Q_A^- by electron transfer from I^-. Contributions due to the oxidation of Z by $P680^+$ and the formation of ^{3}Car occur on a time scale long compared to the time window of fig.1 and are therefore excluded. $P680^+$ can be neglected since $\Delta\epsilon(P680^+/P680)$ [22,27] is small compared to $\Delta\epsilon(Q_A^-/Q_A)$ [16]. Likewise, the contribution of ^{3}Chl is small because of its low yield of formation [24] and the comparatively small $\Delta\epsilon(^3Chl/Chl)$ at 325 nm [28]. This conclusion is confirmed by the complete relaxation of the transient absorption changes in closed RCs (fig.1B). ^{3}Chl should give rise to a non-decaying contribution within our time window of 1.6 ns because the life time of ^{3}Chl is ≥ 10 ns. This is not the case. In samples with open RCs the ^{3}Chl yield should be even lower [24]. The transient signals shown in fig.1A are therefore determined by contributions from Chl*, $Pheo^-$ and Q_A^-.

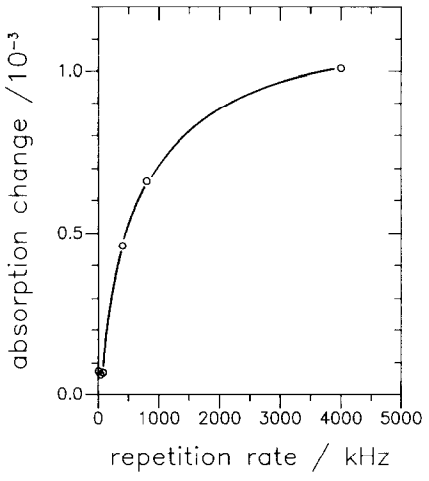

Fig.2. Signal offset of laser-flash-induced absorption changes at 325 nm, measured 200 ps before the actinic laser flash of maximum intensity, as a function of the repetition rate.

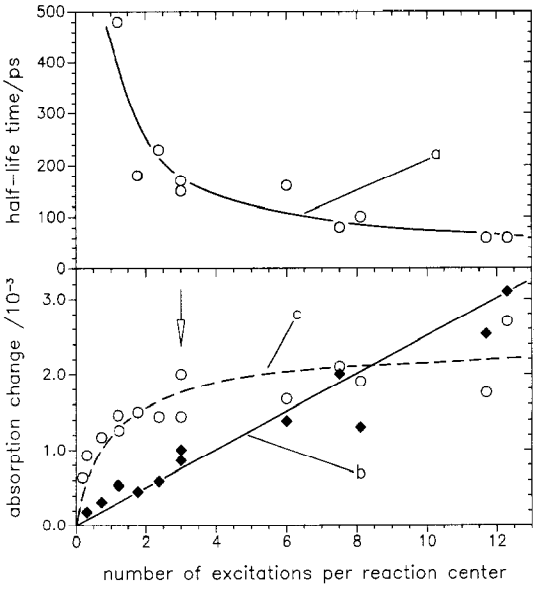

Fig.3. Average half-life time (a), initial amplitude of the transient absorption changes (b) and signal offset (c) as a function of the number of excitations per 300 chlorophyll molecules induced by the actinic laser pulse. The repetition rate was 4 MHz. The arrow indicates the pulse energy ($E = 2 \times 10^{14}$ photons/(cm^2)) used for the measurements of the signals of fig.1.

In open RCs, $Pheo^-$ becomes re-oxidized with a time constant of about 250–500 ps [5,6] and the decay kinetics of Chl* is also clearly faster than 1 ns [23]. Therefore, the non-decaying part of the signal, measured 1.5 ns after the exciting flash, is predominantly ascribed to Q_A^-. This assignment is supported by the similar magnitude of the non-decaying absorption changes in fig.1A and the extent of the offset observed at high-frequency excitation (fig.1B). These considerations imply that in fig.1A the rise kinetics of Q_A^- is concealed by the superimposed decay kinetics of $Pheo^-$ and Chl*.

In closed RCs the absorption changes at 325 nm are considered to be due to Chl* only since the contribution of the radical pair $P680^+I^-$ is expected to be rather small (10–20% of the decaying signal in fig.1B is calculated if one assumes a $\Delta\epsilon(P680^+I^-)$ of about 5000 $(M \cdot cm)^{-1}$ [29] and a yield of radical pair formation of $\lesssim$30–50% [7,9]). In agreement with this, the initial amplitude of the transient signal in closed RCs does not saturate but increases linearly with the laser energy (fig.3, curve b). Therefore, a satisfying elimination of the singlet state contribution can be achieved by subtraction of the absorption changes at 325 nm measured in closed RCs (fig.1B) from those in open RCs (fig.1A) if the Chl* life time is the same in both states of the RCs. This is not the case if the samples are excited with picosecond laser pulses of low pulse energy. Under these conditions, the Chl* life time is markedly longer in the closed than in the open state of the RC [23]. However, at high-photon densities, annihilation processes become the predominant decay path of Chl* and this life time difference disappears as reflected by fluorescence decay measurements [7]. Fig.3a shows that the Chl* life time is significantly shortened at the pulse energy used for the measurements of the signals of fig.1. This clearly indicates that this pulse energy is high enough to largely suppress life time differences of Chl* between samples with open and closed RCs, respectively.

The difference signal, obtained by subtraction of signal B (closed RCs) from signal A (open RC), is shown in fig.4. In principle, the difference signal should be the sum of absorption changes due to formation and decay of I^- and the reduction of Q_A. The contribution of I^- would be maximal if its formation rate is fast in comparison to the electron transfer from I^- to Q_A. In this case the difference signal should exhibit an instant rise due to I^- for-

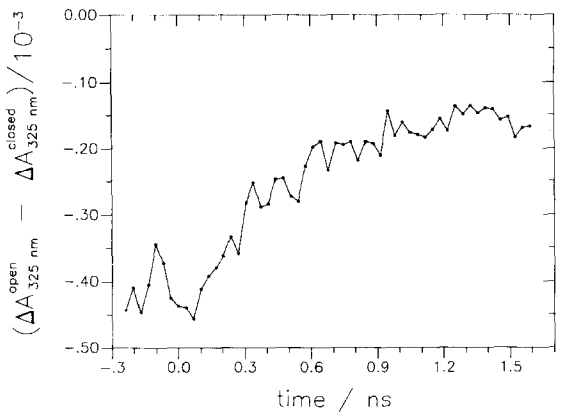

Fig.4. Difference $\Delta A^{open}_{325}(t) - \Delta A^{closed}_{325}(t)$ obtained by subtraction of laser-flash-induced absorption changes in closed reaction centers (fig.1B) from those in open reaction centers (fig.1A).

mation not resolvable by our equipment. Its extent is calculated to be about 40% of the maximum signal amplitude on the basis of $\Delta\epsilon(Pheo^-/Pheo) = 5000\ (M \cdot cm)^{-1}$ [29] and $\Delta\epsilon(Q_A^-/Q_A) = 13000\ (M \cdot cm)^{-1}$ [16]. This instant rise should be followed by a slower component due to the reduction of Q_A. An instant rise is obviously not observed in fig.4. This could indicate that either the formation of I^- is not fast in comparison to the I^- reoxidation (resulting in only a small transient concentration of I^-) or $\Delta\epsilon(I^-/I)$ is significantly smaller than 5000 $(M \cdot cm)^{-1}$ deduced from in vitro data at 325 nm [29]. These considerations show that the difference signal depicted in fig.4 is dominated by the reduction of Q_A. It exhibits a rise time of about 350 ps. Taking into account the uncertainties of the above mentioned separation procedure the electron transfer time from I^- to Q_A is inferred to be 350 ± 100 ps. Within the limits of experimental error this coincides with the reoxidation kinetics of $Pheo^-$ [5,6]. Therefore our data support the idea of a direct electron transfer from $Pheo^-$ to Q_A. However, we cannot rule out the possibility that the formation of $P680^+I^-$ is actually the rate limiting step of charge separation and that the electron transfer from I^- to Q_A occurs on a much faster time scale as proposed in [30]. In this case the data of fig.4 would indirectly reflect the kinetics of the formation of the radical pair $P680^+I^-$. A comparison with the rate constants of the corresponding processes in isolated reaction centers of purple bacteria [31], however, does not favor the idea of a drastically faster electron transfer from I^- to Q_A.

CONCLUSION

In PS II the stabilization of the primary charge separation occurs via a direct electron transfer from $Pheo^-$ to Q_A with a time constant of 350 ± 100 ps. This time constant resembling that of the corresponding step (from $BPheo^-$ to Q_A) in purple bacteria provides another interesting piece of evidence for the close similarity of the reaction centers of both types of organisms.

Acknowledgements: The authors would like to thank M. Müller for the preparation of the samples and Dr T. Wydrzynski for critical reading of the manuscript. Financial support from Deutsche Forschungsgemeinschaft (SfB 312) is gratefully acknowledged.

REFERENCES

[1] Pearlstein, R.M. (1982) in: Photosynthesis (Govindjee, ed.) vol.1, pp.293–330, Academic Press, New York.
[2] Renger, G. (1987) Angew. Chem., Int. Edn 26, 643–660.
[3] Renger, G. and Eckert, H.-J. (1980) Bioelectrochem. Bioenerg. 7, 101–124.
[4] Shuvalov, V.A., Klimov, V.V., Dolan, E., Parson, W.W. and Ke, B. (1980) FEBS Lett. 118, 279–282.
[5] Nijs, A.M., Van Gorkom, H.J., Plijter, J.J. and Duysens, L.N.M. (1986) Biochim. Biophys. Acta 848, 167–175.
[6] 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, Dordrecht.
[7] Eckert, H.-J., Renger, G., Bernarding, J., Faust, P., Eichler, H.-J. and Salk, J. (1987) Biochim. Biophys. Acta 893, 208–218.
[8] Takahashi, Y., Hansson, Ö., Mathis, P. and Satoh, K. (1987) Biochim. Biophys. Acta 893, 49–59.
[9] Hansson, Ö., Duranton, J. and Mathis, P. (1988) Biochim. Biophys. Acta 932, 91–96.
[10] Klimov, V.V. and Krasnovskii, A.A. (1981) Photosynthetica 15, 592–609.
[11] Michel, H. and Deisenhofer, J. (1988) Biochemistry 27, 1–7.
[12] Evans, M.C.W., Atkinson, Y.E. and Ford, R.C. (1985) Biochim. Biophys. Acta 806, 247–254.
[13] Berthold, D.A., Babcock, G.T. and Yocum, C.F. (1981) FEBS Lett. 167, 127–130.
[14] Völker, M., Ono, T., Inoue, Y. and Renger, G. (1985) Biochim. Biophys. Acta 806, 25–34.
[15] Eckert, H.-J., Wydrzynski, T. and Renger, G. (1988) Biochim. Biophys. Acta 932, 240–249.

[16] Dekker, J.P., Van Gorkom, H.J., Brok, M. and Ouwehand, L. (1984) Biochim. Biophys. Acta 764, 301–309.
[17] Robinson, H.H. and Crofts, A.R. (1983) FEBS Lett. 153, 221–226.
[18] Weiss, W. and Renger, G. (1984) FEBS Lett. 169, 219–223.
[19] Babcock, G.T., Blankenship, R.E. and Sauer, K. (1976) FEBS Lett. 61, 286–289.
[20] Dekker, J.P., Plijter, J.J., Ouwehand, L. and Van Gorkom, H.J. (1984) Biochim. Biophys. Acta 767, 176–179.
[21] Renger, G. and Weiss, W. (1986) Biochem. Soc. Trans. 14, 17–20.
[22] Weiss, W. and Renger, G. (1986) Biochim. Biophys. Acta 850, 173–183.
[23] Holzwarth, A.R. (1985) Photochem. Photobiol. 43, 717–725.
[24] Kramer, H. and Mathis, P. (1980) Biochim. Biophys. Acta 593, 319–329.
[25] Wolff, C. and Witt, H.T. (1969) Z. Naturforsch. 24b, 1031–1037.
[26] Mathis, P. (1969) in: Progress in Photosynthesis Research (Metzner, H. ed.) pp.818–822, H. Laupp, Tübingen.
[27] Borg, D.C., Fajer, J., Felton, R.H. and Dolphin, D. (1970) Proc. Natl. Acad. Sci. USA 67, 813–820.
[28] Seifert, K. (1968) Thesis, Technische Universität Berlin.
[29] Fujita, I., Davis, M.S. and Fajer, J. (1978) J. Am. Chem. Soc. 100, 6280–6282.
[30] Schatz, G.H. and Holzwarth, A.R. (1986) Photosynth. Res. 10, 309–318.
[31] Parson, W.W. and Ke, B. (1982) in: Photosynthesis: Energy Conversion by Plants and Bacteria (Govindjee, ed.) pp.331–385, Academic Press, New York.