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Rapid Report

Comparison of primary electron transfer in Photosystem II reaction centres isolated from the higher plant *Pisum sativum* and the green alga *Chlamydomonas reinhardtii*

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Abstract

We have used ultrafast spectroscopy to compare the photochemistry of Photosystem II (PS II) reaction centres isolated from the higher plant pea, *Pisum sativum*, and a green alga, *Chlamydomonas reinhardtii*. The apparatus now used for these experiments includes a highly sensitive multichannel detector and a magic angle configuration for the pump and probe beam polarisations. For both organisms, identical time constants were obtained for both the formation of the radical pair $P680^+Ph^-$, and charge recombination from this state. We conclude that the use of the organism *Chlamydomonas reinhardtii* is well suited to future studies of the primary photochemistry of genetically mutated PS II reaction centres.

Key words: Photosystem II; Charge separation; Charge recombination; Reaction center; (*C. reinhardtii, P. sativum*)

Photosystem II (PS II) is the only photosynthetic reaction centre capable of using solar energy to oxidise water. This ability results from the high oxidising potential generated by the primary photochemical reactions of PS II. PS II is a central component of the photosynthetic apparatus of a wide range of organisms, including higher plants, algae and cyanobacteria. A PSII reaction centre (the D1/D2/cytochrome *b*-559 complex) has been isolated from higher plants [1,2]. This complex binds only 4–6 chlorophylls, thus greatly facilitating spectroscopic studies of its primary photochemistry. Optical excitation of this reaction centre results in primary charge separation, leading to the oxidation of the PS II primary electron donor P680 and the reduction of a pheophytin molecule. The secondary electron transfer steps of quinone reduction and tyrosine oxidation do not occur in the isolated PS II reaction centre. The primary photochemistry of this complex is therefore limited to formation of the radical pair state $P680^+Ph^-$ and charge recombination pathways from this state (reviewed in Ref. [3]).

There have been several recent studies of the primary photochemistry of PS II reaction centres isolated from higher plants. Several groups have reported a time constant of approximately 3 ps for the primary charge separation, with slower 20–50 ps components being assigned to energy transfer processes [4–8]. In contrast, our own experiments have indicated that charge separation occurs with an effective time constant of 21 ps [9,10], and is preceded by a sub-picosecond equilibration of excitation energy between P680 and other reaction centre chlorins [11]. We have also observed slower energy transfer processes, but have found that these can be avoided by photoselective excitation of P680 [12]. Other studies have characterised multiphasic charge recombination processes on the nanosecond time scale [13,6].

In spectroscopic studies of bacterial reaction centres, site-directed mutagenesis has been found to be a

Abbreviations: PS II, Photosystem II; P680, primary electron donor of PS II; Ph, pheophytin.

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useful tool in identifying important residues of the protein matrix involved in the primary photochemistry of this complex [14,15]. Site-directed mutagenesis has also been successfully applied to the study of the secondary electron transfer reactions in PS II [16]. To date, however, studies of the primary photochemistry of PS II have been limited to reaction centres isolated from higher plants. Since higher plants are not readily amenable to genetic manipulation, it is highly desirable to study the primary photochemistry of PS II reaction centres isolated from organisms which are easy to manipulate genetically.

The majority of work involving site-directed mutagenesis of PS II has been restricted mainly to two organisms, the cyanobacterium *Synechocystis* 6803, and the unicellular green alga *Chlamydomonas reinhardtii*. We report elsewhere on the isolation of PS II reaction centres from *Synechocystis* and a study of their photochemistry [17]. Here we report a study of the primary photochemistry of reaction centres isolated from *Chlamydomonas*. While site-directed mutagenesis of *Chlamydomonas* is less exploited than for *Synechocystis,* there is a particularly high degree of similarity in the amino acid sequence of the D1 and D2 polypeptides of *Chlamydomonas* and higher plants [18]. Furthermore, the light harvesting and thylakoid membrane systems, for higher plants and *Chlamydomonas*, are similar, in contrast to those of cyanobacteria, such as *Synechocystis*.

PS II reaction centres were isolated from a Photosystem I and ATPase deficient mutant of *Chlamydomonas reinhardtii* as described by Alizadeh et al. [19] and from pea seedlings (*Pisum sativum*) as described previously [20]. For the time-resolved fluorescence spectroscopy, the PS II reaction centres were resuspended in a buffer containing 50 mM Tris (pH 7.2), 200 mM NaCl and 2 mM dodecyl maltoside; for the time-resolved absorption measurements, the buffer contained 20 mM Bis-Tris (pH 7.2), 20 mM NaCl, 10 mM $MgCl_2$ and 1.4 mM dodecyl maltoside. Experiments were conducted under anaerobic conditions as described previously [13,9], and samples contained 30 μg and 5–8 μg chlorophyll for the transient absorption and fluorescence measurements, respectively.

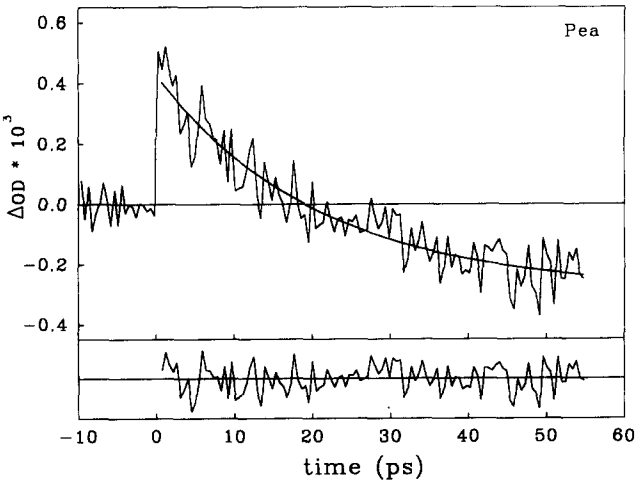

Fig. 1. Kinetics of the transient absorption change observed at 544 nm, following excitation of pea reaction centres using 694 nm pulses. Also shown is the best fit to the data (using a 21.5 ps exponential component and a non-decaying component) and the residuals. Time-resolved data were obtained simultaneously at 128 wavelengths between 510 and 595 nm using a multichannel detector.

Femtosecond transient absorption measurements were carried out at 295 K and as previously [9], except for two important differences: (1) data were obtained using a home-built multichannel detector [12] rather than a single wavelength detector. This enabled data to be collected much more rapidly (total data collection time for the data shown for *Chlamydomonas* PS II reaction centres was 60 min) and was essential given (i) the limited stability of PS II reaction centres [13], (ii) the limited amounts of *Chlamydomonas* reaction centres available and (iii) the small amplitude of the transient absorption signals associated with charge separation in PS II [9,10]; (2) data were collected with the polarisation of the probe beam rotated by 54.7° relative to the pump. This 'magic angle' configuration avoids any contributions to the data from depolarisation processes. All measurements were repeated a number of times to assess reproducibility; the error margins quoted in this paper are one standard error.

Picosecond time-resolved fluorescence lifetimes were measured using the technique of single photon counting. The apparatus and analysis of the fluorescence decays were as described by Booth et al. [13].

The polypeptide composition of the *Chlamydomonas* PS II reaction centre preparation is very similar to that from higher plants (see Alizadeh et al. [19]). The pigment stoichiometry of both preparations is approximately 6 chlorophyll *a*:2 pheophytin *a*:1 cytochrome *b*-559:1–2 β-carotenes [19,21].

The charge separation process was monitored by observing the bleaching of the pheophytin Q_x-absorption band. This particular spectral feature can be most unambiguously assigned, amongst the several different absorption changes which have been found to accompany charge separation in PS II reaction centres [9,10]. Data were collected on a 0–70 ps time scale and using 694 nm excitation pulses to excite P680 preferentially; Fig. 1 shows a typical decay. Fig. 2a shows the results of kinetic global analyses of these data collected for higher plant PS II reaction centres, using the multichannel detector and the 'magic angle' configuration. As previously, it was found that the data on this time scale could be fitted well by a component with a 21.5 ± 1 ps lifetime and a non-decaying component. Spectra of the amplitudes of these two components (kinetic spectra) are shown in Fig. 2a, while Fig. 3

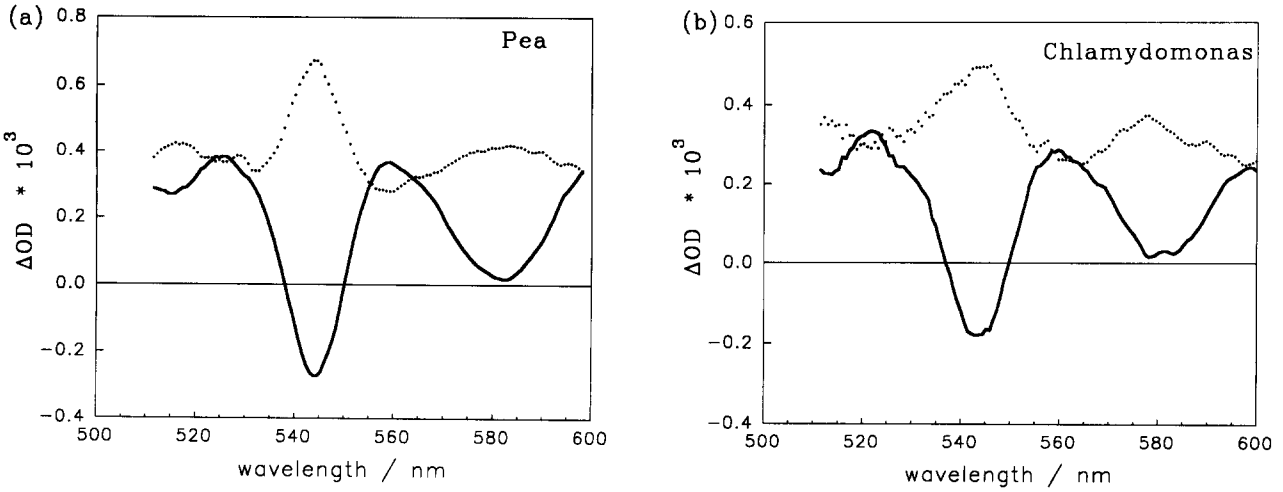

Fig. 2. Spectra of the amplitudes of two kinetic components resolved following excitation of PS II reaction centres isolated from (a) peas and (b) the green alga *Chlamydomonas*. The solid lines are spectra of a component which did not decay on the 0–70 ps time scale used for the experiment; this component is assigned to the radical pair state $P680^+Ph^-$. The dotted lines are spectra of a component with a lifetime of 21.5 ± 1 ps (pea reaction centres) or 21 ± 2 ps (*Chlamydomonas* reaction centres); this component is assigned to formation of the radical pair state.

shows the transient spectra at two time delays corresponding approximately to the spectra before and after the decay of the 21.5 ps component. These data are similar to those published previously using single wavelength detection [9], although they are much more detailed and precise.

The 100 ps spectrum (Fig. 3, solid line) is assigned to the radical pair state $P680^+Ph^-$ [9,10] with the minima at 544 nm and 582 nm being assigned to bleaching of the Q_x-absorption bands of pheophytin and P680, respectively. As previously [9], we find that the 21.5 ps component results in an increase in the amplitude of the pheophytin Q_x-bleach, and this component must therefore be associated with pheophytin reduction. The pheophytin Q_x-bleach present prior to the 21.5 ps component (i.e., in the 3 ps spectrum of Fig. 3) appears within 250 fs (data not published), and is assigned to excited singlet states of pheophytin formed by direct excitation or energy transfer faster than 250 fs. Using the magic angle polarisation conditions, 40–50% of the final pheophytin Q_x-bleach grows in with the 21.5 ps component, indicating that a lower limit of 40% of the total pheophytin reduction occurs with a 21.5 ps time constant. (The difference between the lower limit of 40% reported here, and that reported previously [9] probably arises from the different pump probe polarisation used in the two experiments.) We are only able to determine a lower limit from these data, rather than the actual proportion, due to contributions from pheophytin singlet states to the 544 nm bleach prior to the 21.5 ps component. As we have shown elsewhere, data collected over other spectral regions suggests that the actual proportion of radical pair formation associated with this 21.5 ps component is at least 75% (Ref. [10] and unpublished data).

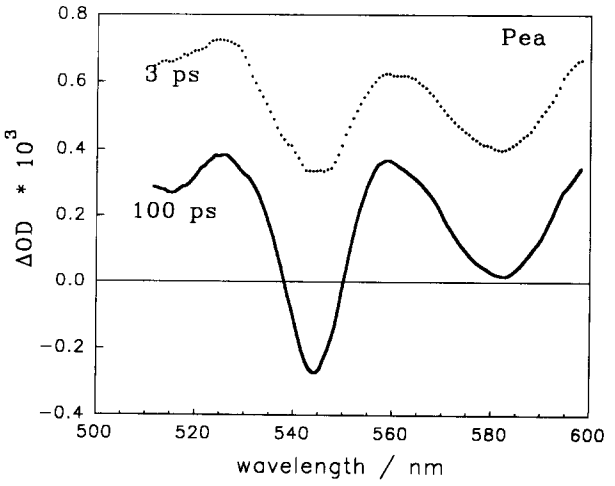

Fig. 3. Transient absorption spectra at time delays of 3 ps (dotted line) and 100 ps (solid line) for pea reaction centres; these spectra were calculated from the kinetic spectra shown in Fig. 2a. These spectra correspond approximately to the spectra before (3 ps) and after (100 ps) decay of the 21.5 ps component; the difference between them therefore corresponds to the dotted spectrum in Fig. 2a. These spectra demonstrate that the 21.5 ps component is associated with an increase in the bleaching of the pheophytin Q_x-absorption band at 544 nm.

Fig. 2b shows the results of identical experiments conducted with *Chlamydomonas* PS II reaction centres. Again, the data could be fitted well by two components, one with a lifetime of 21 ± 2 ps and one a non-decaying component. Comparing Fig. 2b with 2a demonstrates that the spectra of the amplitudes of these two components are almost indistinguishable from those obtained using higher plant PS II reaction

Table 1
Results of the analyses of the time-resolved fluorescence decays of higher plant and *Chlamydomonas* PS II reaction centres. The best fits of the fluorescence decays correspond to four exponentials. Data collection used a 280 ns time scale

Component	Lifetime (ns)	Relative fluorescence yield (% of total)
Higher plant PS II reaction centres		
F1	51	31 ± 1
F2	17	21 ± 1
F3	5	41 ± 1
F4	1.2	7 ± 1
Chlamydomonas PS II reaction centres		
F1	53	13
F2	14	16
F3	5	57
F4	1.2	14

centres. Specifically, the non-decaying component assigned to the radical pair state exhibits similar minima at 544 nm and 582 nm, while the spectrum of the 21 ps component has a similar maximum at 544 nm, indicating that this component also results in an approximate 40% increase in the pheophytin Q_x-bleach. Therefore not only are the time constants for pheophytin reduction indistinguishable within error between the *Chlamydomonas* and higher plant PS II reaction centre preparations, but the spectral changes associated with this electron transfer are also essentially indistinguishable.

It should be noted that the 21.5 ps and 21 ps observed time constants for radical pair formation do not correspond directly to an electron transfer rate constant, but are a complex combination of all the intrinsic rate constants for the associated energy and electron transfer processes [12].

Table 1 gives the results of the analyses of the fluorescence decays of the *Chlamydomonas* PS II reaction centre compared with that for higher plants. Similar lifetimes were obtained whether the data sets were analysed with the 5 ns component fixed or free running. The lifetimes for both preparations are essentially the same. For *Chlamydomonas*, the two long-lived fluorescence components, attributable to radical pair charge recombination [13], have lifetimes of 53 ns and 14 ns, compared with lifetimes of 51 ns and 17 ns obtained with higher plant PS II reaction centres. The 5 ns component is attributed primarily to non-functional chlorophyll uncoupled from primary charge separation [13]. The larger yield of this 5 ns component observed for the *Chlamydomonas* reaction centre, relative to the higher plant reaction centre, probably originates from a partial loss of activity of the *Chlamydomonas* reaction centre during this experiment. This loss of activity was not observed in subsequent transient absorption experiments following minor modifications to the isolation procedure and resuspension conditions. The observation of similar multiphasic charge recombination lifetimes for the two reaction centres suggests that both the energetics of the radical pair state, and the kinetics of the charge recombination pathways, are highly conserved between the two organisms.

We therefore conclude that we are unable to resolve any differences between the primary photochemistry of the higher plant and algal PS II reaction centres. Identical lifetimes were obtained for both the charge separation and recombination reactions; moreover the spectral changes associated with charge separation were also found to be very similar. The similarity of the primary photochemistry of these two reaction centres is consistent with the high degree of homology of the amino acid sequences of the D1 and D2 polypeptides of these two organisms [18].

These results indicate that PS II reaction centres isolated from the green alga *Chlamydomonas reinhardtii* by the method of Alizadeh et al. [19] are appropriate for detailed spectroscopic study of the primary photochemistry of PS II. We have demonstrated that use of the multichannel detector allows us to obtain detailed kinetic information from small amounts of reaction centre sample. When applied to mutated strains of *Chlamydomonas*, this experimental approach should be very useful in determining the effects of point mutations of the D1 and D2 polypeptides upon the primary photochemistry of PS II.

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References

[1] Nanba, O. and Satoh, K. (1987) Proc. Natl. Acad. Sci. USA 84, 109–112.
[2] Barber, J., Chapman, D. and Telfer, A. (1987) FEBS Lett. 220, 67–73.
[3] Seibert, M. (1993) in The Photosynthetic Reaction Centre, Vol. 1 (Deisenhofer, J. and Norris, J.R., eds.), pp. 319–356, Academic Press, London.
[4] Wasielewski, M.R., Johnson, D G., Seibert, M. and Govindjee (1989) Proc. Natl. Acad. Sci. USA 86, 524–528.
[5] Seibert, M., Toon, S., Govindjee, O'Neil, M.P. and Wasielewski, M.R. (1992) in Research in Photosynthesis, Vol. II (Murata, N., ed.), pp.41–44, Kluwer, Dordrecht.
[6] Roelofs, T.A., Gilbert, M., Shuvalov, V.A. and Holzwarth, A.R. (1991) Biochim. Biophys. Acta 1060, 237–244.

[7] Schelvis, J.P.M., Van Noort, P.I., Aartsma, T.J. and Van Gorkom, H.J. (1994) Biochim. Biophys. Acta 1184, 242–250.
[8] Jankowiak, R., Tang, D., Small, G.J. and Seibert, M. (1989) J. Phys. Chem. 93, 1649–1654.
[9] Hastings, G., Durrant, J.R., Hong, Q., Barber, J., Porter, G. and Klug, D.R. (1992) Biochemistry 31, 7638–7647.
[10] Durrant, J.R., Hastings, G., Joseph, D.M., Barber, J., Porter, G. and Klug, D.R. (1993) Biochemistry 32, 8259–8267.
[11] Durrant, J.R., Hastings, G., Joseph, D.M., Barber, J., Porter, G. and Klug, D.R. (1992) Proc. Natl. Acad. Sci. USA 89, 11632–11636.
[12] Rech, T., Durrant, J.R., Joseph, D.M., Barber, J., Porter, G. and Klug, D.R. (1994) Biochemistry, submitted.
[13] Booth, P.J., Crystall, B., Ahmad, I., Barber, J., Porter, G. and Klug, D.R. (1991) Biochemistry 30, 7573–7586.
[14] Finkele, U., Lauterwasser, C., Zinth, W., Gray, K.A. and Oesterhelt, D. (1990) Biochemistry 29, 8517–8521.
[15] Nagarajan, V., Parson, W.W., Davis, D. and Schenk, C. (1993) Biochemistry 32, 12324–12336.
[16] Pakrasi, H.B. and Vermaas, W.F.J. (1992) in The Photosystems: Structure, Function and Molecular Biology (Barber, J., ed.), pp. 231–257, Elsevier, Amsterdam.
[17] Giorgi, L. et al. (1994) manuscript in preparation.
[18] Svensson, B., Vass, I. and Styring, S. (1991) Z. Naturforsch. 46C, 765–776.
[19] Alizadeh, S., Nixon, P.J., Telfer, A. and Barber, J. (1994) submitted.
[20] Chapman, D.J., Gounaris, K. and Barber, J. (1991) in Methods in Plant Biochemistry (Rogers, L., ed.), pp. 171–193, Academic Press, London.
[21] Gounaris, K., Chapman, D.J., Booth, P., Crystall, B., Giorgi, L.B., Klug, D.R., Porter, G. and Barber, J. (1990) FEBS Lett. 265, 88–92.