Photochemistry in the isolated Photosystem II reaction-centre core complex

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The photochemistry of the isolated Photosystem II reaction-centre core from pea and the green alga *Scenedesmus* was examined by e.s.r. Two types of triplet spectrum were observed in addition to the spin-polarized reaction-centre triplet previously identified. The additional triplet formed on continuous illumination at 4.2 K was attributed to a monomeric phaeophytin molecule. The second triplet, which was stable in the dark at 4.2 K following illumination, was assigned to the radical pair Donor⁺I⁻. This provides evidence that an electron donor to chlorophyll P680 is present on the polypeptide D₁–polypeptide D₂–cytochrome *b*-559 core complex.

INTRODUCTION

In the PSII reaction centre of oxygenic photosynthetic organisms, an electron is transferred across the membrane from the reaction-centre chlorophyll, P680, to a phaeophytin, I, and then to two quinones, Q_A and Q_B. The PSII reaction-centre complex consists of six polypeptides: 47 kDa, 43 kDa, two 32 kDa polypeptides termed D₁ and D₂ plus the 9 kDa and 4 kDa polypeptides of cytochrome *b*-559 [1,2]. D₁ and D₂ were proposed to contain the reaction-centre chlorophyll, P680, and initial electron acceptors because of sequence homology between these polypeptides and the L and M subunits of the purple-bacterial reaction centre [1]. Experimental support for this proposal was provided by Nanba & Satoh [3], who isolated a reaction-centre pigment–protein core complex containing only the D₁ and D₂ polypeptides and cytochrome *b*-559. This core complex was shown to be photochemically active by demonstration of the photoreduction of phaeophytin [3–8]. A light-induced spin-polarized triplet signal has also been detected by e.s.r. in this preparation [4]. The triplet species originates from a radical-pair recombination between the oxidized primary donor chlorophyll P680⁺ and the reduced phaeophytin acceptor I⁻ [9]. Redox titrations of the reaction-centre preparation using the spin-polarized triplet signal confirmed the lack of a quinone electron acceptor but suggested that the non-haem iron still present may act as an electron acceptor when oxidized [10].

Recent evidence from experiments using isotope substitution and amino acid replacement suggests that the electron donors D and Z may be tyrosine residues arranged symmetrically in polypeptides D₁ and D₂ [11,12]. Experiments were therefore designed to examine the reaction-centre core for evidence of electron donation to P680. Investigations were carried out on reaction centres isolated from both peas and the LF1 mutant of the alga *Scenedesmus obliquus*.

MATERIALS AND METHODS

PSII was prepared by the method of Ford & Evans [13] from greenhouse-grown pea *Pisum sativum* var. Feltham First. The PSII was resuspended and stored at 77 K in

20 mM-Mes/NaOH buffers, pH 6.3, containing 5 mM-MgCl₂, 15 mM-NaCl and 20 % (v/v) glycerol.

Scenedesmus obliquus LF1 was grown heterotrophically in the dark and membranes were prepared as in refs. [14] and [15]. Cells were collected by centrifugation and disrupted by the use of 0.5 mm glass beads and a Bead Beater (Biospec Products, Bartlesville, OK, U.S.A.) [15]. Thylakoids were collected by centrifugation at 40000 *g* for 30 min and PSII particles were prepared as for pea. PSII from the LF1 mutant has a low manganese content and the water-oxidation system is absent. This was attributed to the loss of a processing step with D₁, which results in a large polypeptide unable to bind manganese or the polypeptides involved in water oxidation [15,16].

The PSII reaction-centre core was isolated by the method based on that of Nanba & Satoh [3] as modified in ref. [6]. PSII particles were incubated in 1 M-Tris/HCl buffer, pH 8.8, for 1 h at 0 °C in room light to remove the hydrophilic polypeptides. The membranes were collected by centrifugation at 40000 *g* for 30 min at 4 °C. The membrane pellet was resuspended in 50 mM-Tris/HCl buffer, pH 7.2, and the reaction-centre core was prepared by using Triton X-100 detergent and Fractogel TSK DEAE-650(S) column chromatography as in refs. [3] and [6]. The fractions eluted from the Fractogel TSK DEAE-650(S) column were assayed for purified reaction centre by using e.s.r. measurement of spin-polarized triplet and SDS/polyacrylamide-gel electrophoresis for polypeptide composition.

E.s.r. measurements were performed with a JEOL X-band spectrometer with 100 kHz field modulation and an Oxford Instruments cryostat. Samples (0.3 ml) of the PSII reaction centre were placed in 3 mm-diameter calibrated quartz tubes, dark-adapted for 15 min and frozen to 77 K in the dark. Chlorophyll concentrations of reaction centres were 40 µg/ml. Illumination in the cryostat was provided by a 150 W light-source directed by a light-guide.

RESULTS AND DISCUSSION

Fig. 1(a) shows the spin-polarized reaction-centre triplet detected by e.s.r. in the reaction-centre core preparation under continuous illumination at 4.2 K [4,9].

Fig. 1. E.s.r. spectra of triplets formed in the PSII core preparation

(a) Pea PSII reaction centres were examined under continuous illumination at 4.2 K. The AEEAAE polarization pattern (g -values 2.22, 2.162, 2.066, 1.955, 1.874 and 1.826) is indicated and the additional peaks are arrowed (g -values 2.265 and 1.799). (b) Pea PSII reaction centres were examined at 4.2 K in the dark following illumination for 8 min at 4.2 K. The dark-stable triplet is arrowed (g -values 2.038 and 1.964). (c) Fractogel TSK DEAE-650(S) column fraction containing PSII reaction-centre core plus 47 kDa, 43 kDa and light-harvesting polypeptides was examined under continuous illumination at 4.2 K. The light-induced triplet is arrowed. E.s.r. conditions were microwave power 25 μ W, modulation width 1 mT and temperature 4.2 K. The constant broad signal near $g = 2.1$ was caused by copper in the e.s.r. cavity.

The AEEAAE polarization pattern of the spectrum is interpreted as originating from a radical-pair recombination between $P680^+$ and I^- [9]. The characteristic zero-field splitting parameters were $[D] = 0.0297 \text{ cm}^{-1}$ and $[E] = 0.0041 \text{ cm}^{-1}$ [4,9]. Only the fraction from the Fractogel TSK DEAE-650(S) column containing the isolated reaction-centre core formed the light-induced triplet without the use of reductant. The core preparation was also characterized by the lack of major changes at

Fig. 2. E.s.r. spectra showing the extreme low-field peaks of both light-induced triplets in the PSII core preparation

(a) Spectrum obtained in the dark at 4.2 K. (b) Spectrum obtained under continuous illumination at 4.2 K. The additional triplet is arrowed. (c) Illuminated-minus-dark difference spectrum. E.s.r. conditions were as given in Fig. 1 legend.

$g = 2$ upon illumination at 4.2 K. Illumination at 77 K and higher temperatures decreased the size of the spin-polarized triplet observed on subsequent illumination at 4.2 K and resulted in the generation of a $g = 2$ radical attributed to photodamaged centres or non-specific electron donation to P680.

Fractions containing reaction-centre core contaminated by 47 kDa, 43 kDa or light-harvesting polypeptides showed no spin-polarized triplet without reduction by dithionite and formed a large $g = 2$ radical during illumination at 4.2 K (result not shown). This suggests that the spin-polarized triplet was absent owing to the availability of an electron donor or acceptor in these fractions. A small triplet spectrum with a polarization

Fig. 3. E.s.r. spectra of reaction-centre triplets in a *Scenedesmus* LF1 PSII core preparation

(a) Spectrum obtained in the dark at 4.2 K. (b) Spectrum obtained under continuous illumination at 4.2 K. (c) Spectrum obtained in the dark following 8 min illumination at 4.2 K. The dark-stable triplet is arrowed. E.s.r. conditions were as given in Fig. 1 legend.

pattern and zero-field splitting similar to those shown by free phaeophytin *a* [17] was obtained in some of these fractions (Fig. 1c, arrowed).

An additional light-induced signal was seen in the reaction-centre core samples together with the spin-polarized triplet described above (Fig. 1a, arrowed). The signal was symmetrical about the $g = 2$ region with peaks observed above the high-field and low-field peaks of the reaction-centre triplet (Figs. 1a and 2). The pattern suggests the possibility that an additional spin-polarized triplet was present with $[D] = 0.0350 \text{ cm}^{-1}$ and with part of the spectrum hidden by the major reaction-centre triplet. A full spectrum would be needed to confirm the

absorption and emission pattern. The signal was only present under continuous illumination and the same conditions required to observe the major reaction-centre triplet. The $[D]$ value is larger than would be expected from a chlorophyll triplet [18] or PS1 [19,20] but similar to that obtained for monomeric phaeophytin *a* in solution, although with the different polarization pattern [17]. The additional triplet may represent reaction centres where P680 is damaged, i.e. has lost magnesium, forming a phaeophytin, or perhaps centres in which the excitation has migrated out of the reaction centre on to a nearby phaeophytin molecule. It is also possible that the reaction centre itself may be composed of a chlorophyll-plus-phaeophytin dimer rather than a chlorophyll dimer. This would explain the zero-field splitting data for the major PSII reaction-centre triplet being close to the monomer chlorophyll value, $[D] = 0.0273 \text{ cm}^{-1}$ [18]. The reaction-centre triplet in the purple-bacterial bacteriochlorophyll dimer is significantly narrowed from the monomer value [17].

Fig. 1(b) shows the spectrum of core complex in the dark at 4.2 K following 8 min illumination at 4.2 K. During illumination a photoinduced signal near $g = 2$ was produced that was stable in the dark at 4.2 K and consisted of two peaks split by approx. 12 mT (Fig. 1b, arrowed). The size of the spin-polarized triplet was decreased by approx. 10% by this illumination period. The shape of the dark-stable signal and the magnitude of the splitting suggest that it arises through a magnetic interaction between two radicals. The signal is similar to one previously reported in $\text{Na}_2\text{S}_2\text{O}_4$ -reduced pea PSII particles [21], which was attributed to formation of Donor^+I^- through electron donation to P680^+ . This provides evidence for the presence of an electron donor, D or Z, in the core preparation. The low yield of this triplet suggests that electron donation of 4.2 K occurs with a low probability. The triplet was also present in preparations with additional polypeptides (Fig. 1c).

We also obtained a reaction-centre preparation from the LF1 mutant of *Scenedesmus obliquus* [15,16] that contains only D_1 , D_2 and cytochrome *b*-559 polypeptides.

The LF1 reaction-centre core showed the same e.s.r. characteristic as the pea preparation (Fig. 3). On continuous illumination the spin-polarized triplet was formed (Fig. 3b), and in the dark following illumination the dark-stable radical-pair triplet was observed (Fig. 3c, arrowed). Warming the sample to 77 K in the dark for 30 s and remeasuring at 4.2 K resulted in loss of the signal. This behaviour was noted in ref. [21] for the signal in reduced PSII membranes and can be attributed to recombination of the Donor^+I^- radical pair. The characteristics of electron donors D and Z, which have a high redox potential in PSII membranes [11,12], can be expected to change during purification as a result of the stripping away of polypeptides and greater exposure of the redox centre in the core preparation.

The microwave-power-saturation characteristics of the proposed Donor^+I^- triplet in both LF1 and pea core preparations were different from those observed in reduced PSII membranes. The maximum signal size was observed at $100 \mu\text{W}$ in reaction centres, contrasting with 30 mW [21] in PSII membranes. This may be due to the removal of the manganese in the water-oxidation system in the core preparation. The presence of manganese has been shown to alter the relaxation properties of the donor D, and its absence in the LF1 mutant [16] results

in a decrease in microwave-power-saturation characteristics of D. We therefore conclude that the dark-stable triplet arises from the Donor^+I^- radical pair and that this indicates that an electron donor to P680^+ was present in the PSII core preparation. Conclusive evidence would be provided by the isolation of the reaction-centre core from the PSII mutant [12] where the postulated tyrosine residue is replaced.

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REFERENCES

1. Michel, M. & Deisenhofer, J. (1988) *Biochemistry* **27**, 1–6
2. Trebst, A. (1987) *Z. Naturforsch. C* **42**, 742–750
3. Nanba, O. & Satoh, K. (1987) *Proc. Natl. Acad. Sci. U.S.A.* **84**, 109–112
4. Okamura, M. Y., Satoh, K., Isaacson, R. A. & Feher, G. (1987) in *Progress in Photosynthesis Research* (Biggins, J., ed.), vol. 1, pp. 379–381, Martinus Nijhoff, Dordrecht
5. Danielius, R. V., Satoh, K., Van Kan, P. J. M., Plijter, J. J., Nuijs, A. M. & Van Gorkom, H. J. (1987) *FEBS Lett.* **213**, 241–244
6. Barber, J., Chapman, D. J. & Telfer, A. (1987) *FEBS Lett.* **220**, 67–73
7. Takahashi, Y., Hansson, O., Mathis, P. & Satoh, K. (1987) *Biochim. Biophys. Acta* **849**, 49–59
8. Van Dorsen, R. J., Breton, J., Plijter, J. J., Satoh, K., Van Gorkom, H. J. & Ames, J. (1987) *Biochim. Biophys. Acta* **893**, 267–274
9. Rutherford, A. W., Paterson, D. R. & Mullet, J. E. (1981) *Biochim. Biophys. Acta* **635**, 205–214
10. Telfer, A., Barber, J. & Evans, M. C. W. (1988) *FEBS Lett.*, in the press
11. Barry, B. A. & Babcock, G. T. (1987) *Proc. Natl. Acad. Sci. U.S.A.* **84**, 7099–7103
12. Debus, R. J., Barry, B. A., Babcock, G. T. & McIntosh, L. (1988) *Proc. Natl. Acad. Sci. U.S.A.* **85**, 427–431
13. Ford, R. C. & Evans, M. C. W. (1983) *FEBS Lett.* **160**, 159–163
14. Bishop, N. I. (1971) *Methods Enzymol.* **23**, 372–408
15. Metz, J. G. & Siebert, M. (1984) *Plant Physiol.* **76**, 829–832
16. Rutherford, A. W., Siebert, M. & Metz, J. G. (1988) *Biochim. Biophys. Acta* **932**, 171–176
17. Thurnauer, M. C., Katz, J. J. & Norris, J. R. (1975) *Proc. Natl. Acad. Sci. U.S.A.* **72**, 3270–3274
18. Thurnauer, M. C. & Norris, J. R. (1977) *Chem. Phys. Lett.* **47**, 100–105
19. Rutherford, A. W. & Mullet, J. E. (1981) *Biochim. Biophys. Acta* **635**, 225–235
20. Frank, H. A., McLean, M. B. & Sauer, K. (1979) *Proc. Natl. Acad. Sci. U.S.A.* **76**, 5124–5128
21. Rutherford, A. W. & Thurnauer, M. C. (1982) *Proc. Natl. Acad. Sci. U.S.A.* **79**, 7283–7287

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