PROPERTIES OF THE EPR SPECTRUM OF THE INTERMEDIARY ELECTRON ACCEPTOR (A_1) IN SEVERAL DIFFERENT PHOTOSYSTEM I PARTICLE PREPARATIONS

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1. Introduction

The photosystem I reaction centre contains two bound iron–sulphur centres A and B which can be reduced by sodium dithionite in the dark as their mid-point redox potentials are −550 mV (centre A) and −590 mV (centre B) [1,2]. When centres A and B are reduced, illumination of photosystem I particles at cryogenic temperatures will reversibly photooxidise P700 [3] and reversibly photoreduce X [2,4,5], an iron-containing compound [6]. Recent optical studies [7–10] have suggested the presence of an intermediary electron acceptor, designated A_1, operating between P700 and X. Monitoring the time course of re-reduction of P700 after a saturating flash, the return of electrons from P430 (equated with centres A and B), from an acceptor A_2 (thought to be an iron–sulphur protein and equated with X), and the fast return of electrons from an unidentified electron acceptor (A_1) nearer to P700 than X was observed.

In [11] we reported the observation of an electron paramagnetic resonance (EPR) spectrum associated with the reduction of A_1. This spectrum, a symmetrical 14 g wide radical signal centred in the $g = 2.00$ region was 'frozen in' in photosystem I particles prepared using Triton X-100 by illumination of the particles at room temperature in the presence of sodium dithionite before and during freezing to 77 K. The electron acceptor X cannot be reduced in the dark by sodium dithionite as it has an estimated mid-point redox potential of −730 mV [12]. A_1 must have a more reducing potential, illuminating samples in the presence of dithionite is the only method of preparation that will yield an EPR spectrum of A_1 alone. Flash-induced kinetic EPR spectra of A_1 will contain a contribution from oxidised P700, and may contain artefacts due to the magnetic field modulation.

Shuvalov et al. [13] carried out kinetic, optical and EPR studies on photosystem I particles prepared using Triton X-100 and concluded that A_1 might be a chlorophyll *a* dimer. They obtained a kinetic EPR spectrum of A_1 plus P700 from flash-induced changes decaying with a lifetime of 1.3 ms at 5 K, a $\simeq$ 10 g wide asymmetrical radical signal centred at $g = 2.004 \pm 0.0005$.

Flash-induced EPR signals in the $g = 2.0$ region decaying rapidly (<1 ms) have been shown to exhibit the characteristics of chemically-induced dynamic electron polarisation (CIDEP) [14–16], and are believed to arise from the $P700^+$ cation radical formed when electrons move to A_1 [15]. Friesner et al. have suggested on the basis of these observations that A_1 is a small organic molecule [15], whilst McIntosh et al [16] have suggested the involvement of an organic radical other than chlorophyll because the observed kinetic EPR signals originate from a region between $g = 2.0040$ and $g = 2.0055$.

Here an EPR spectrum thought to be associated with the reduction of A_1 is described in photosystem I particles from spinach (*Spinacea oleracea*) prepared without using Triton X-100 and in particles from *Chlorogloea fritschii*. This EPR spectrum differs considerably from that observed for A, in particles prepared from spinach using Triton X-100 [11], and it is concluded that this detergent is altering the environment of A_1 and its EPR spectrum. The precise *g*-values for both types of EPR spectra are presented.

Evidence for a magnetic interaction between reduced A_1 and reduced X is also presented.

2. Materials and methods

Triton photosystem I particles were prepared from

spinach by the method in [17] and had a P700 : chlorophyll ratio of 1:30 to 1:50. Digitonin photosystem I particles were the 'stromal lamellae vesicle fraction' prepared from spinach as in [18], and had a P700 : chlorophyll ratio of 1:200. French press photosystem I particles were prepared from washed broken spinach chloroplasts as in [19] and had a P700 : chlorophyll ratio of 1:300. Photosystem I particles from *Chlorogloea fritschii* were a gift from Dr E. H. Evans, and were prepared as in [20] with the following modifications: 1.5% digitonin replaced 1% digitonin; 1.5% Triton X-100 replaced 4%. After Triton X-100 treatment the preparation was spun for 4 h at 100 000 × *g*. The supernatant was retained and spun overnight at 100 000 × *g*. The pellets were resuspended in buffer without the addition of Triton X-100, and had a P700 : chlorophyll ratio of 1:35 to 1:50.

EPR measurements were carried out using a Jeol FE-1X spectrometer and the sample temperature was maintained by an Oxford Instr. liquid helium cryostat or a Scanlon liquid nitrogen finger dewar. A 1000 W projector was used for illumination during the preparation of samples. Digitonin was purchased from Sigma Chem. Co. and sodium dithionite from BDH.

3. Results

Fig.1a shows that when digitonin photosystem I particles were illuminated at room temperature in the presence of dithionite and illuminated during freezing to 77 K, the EPR spectrum contains a large radical signal at $g = 2.00$ in addition to the signals attributed to A, B and X. If the light is turned off as the sample is frozen this signal has decayed considerably, although the $g = 1.76$ signal of X has not decayed and indeed appears larger (fig.1b). If the sample is left in the dark for 10 s before freezing the radical signal at $g = 2.00$ has almost completely decayed. These results parallel and confirm those obtained with Triton X-100 photosystem I particles [11]. If the spectrum of the signal at $g = 2.00$ in these samples is recorded at non-saturating microwave powers at 77 K (fig.2a), it differs considerably from that seen in Triton X-100 photosystem I particles. Instead of a symmetrical radical 14 g wide, the radical signal in digitonin particles shows several distinct features. These features could either reflect the true EPR spectrum of reduced A_1, or could be due to a contribution to the spectrum by

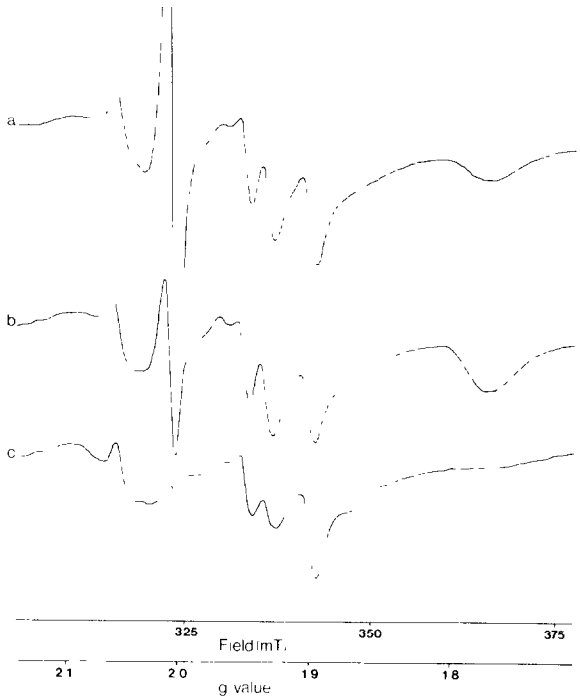

Fig.1. EPR spectra at 10 K of photosystem I particles prepared using digitonin (1.3 mg chl./ml) frozen during and after illumination at room temperature. Samples were illuminated for 2 min in the presence of sodium dithionite (0.2%, w/v) under anaerobic conditions and frozen: (a) under illumination; (b) as the light was turned off; (c) after 10 s dark period. The spectra were recorded using the following instrument settings: frequency, 9.0875 GHz; microwave power, 20 mW; modulation amplitude 1 mT; scan rate, 50 mT/min; instrument gain, 2.5×10^2.

other reduced electron acceptor(s) in the preparation. However all the features of the spectrum decay at the same rate supporting the view that the entire signal arises from a single electron transport component, A_1.

To establish whether Triton X-100 was affecting the EPR spectrum of A_1, varying concentrations of this detergent were added to digitonin photosystem I samples immediately prior to illumination in the presence of dithionite and freezing to 77 K. Fig.3 shows that Triton X-100 at 1% removes all the features from the EPR spectrum of reduced A_1 and produces a symmetrical signal 14 g wide. This concentration of Triton X-100 did not affect the EPR signals attributed to A, B and X.

The spectrum of A_1 was observed in French press photosystem I particles and particles from the blue-

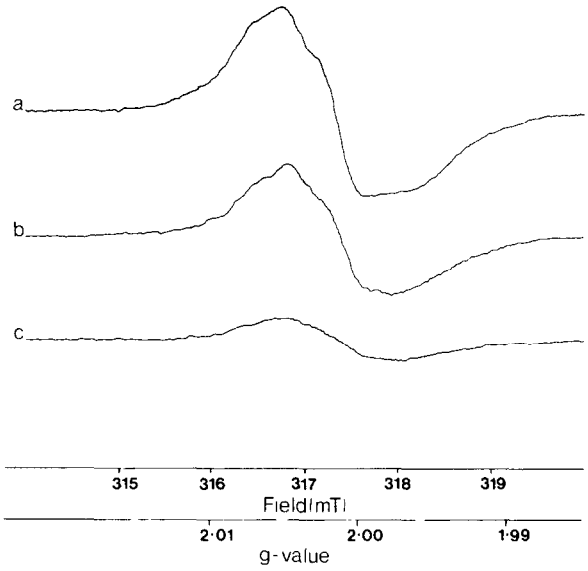

Fig.2. EPR spectra at 77 K of photosystem I particles prepared using digitonin (1.3 mg chl/ml) frozen during and after illumination at room temperature. Spectra are from the samples presented in fig.1. The spectra were recorded using the following instrument settings: frequency, 8.905 GHz; microwave power, 5×10^{-3} mW; modulation amplitude, 0.2 mT; scan rate, 2.5 mT/min; instrument gain, 2×10^3.

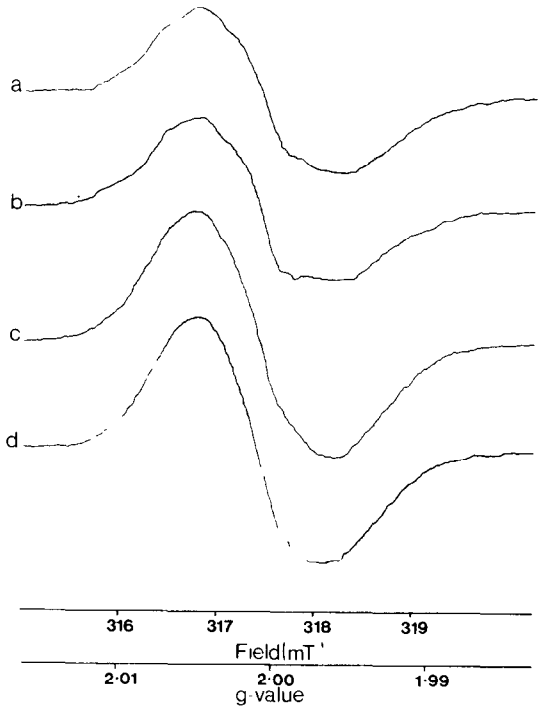

Fig.3.

green alga *Chlorogloea fritschii* (fig.4b) as well as in digitonin photosystem I samples (fig.4c). The lineshape of the spectrum is the same in all these preparations although the P700 : chlorophyll ratio is enriched from 1:300 (French press) or 1:200 (digitonin) to 1:40 (*Chlorogloea fritschii*). The amount of photosystem II present in these preparations is also considerably reduced, from the French press preparation to the particles from *Chlorogloea fritschii* which contain a negligible amount of photosystem II. This confirms that the spectrum is arising from reduced A_1, and does not contain any contribution either from the light-harvesting chlorophylls present in the preparation or from photosystem II.

The precise *g*-values of the features of these spectra were measured, using a powdered manganese oxide sample as a standard (fig.4a–c), as were the *g*-values for the spectrum of reduced A_1 (fig.4d) and P700 (fig.4c) in Triton photosystem I particles. The accuracy of this method of measurement was confirmed by measurement of a *g*-value of 2.0026 for the 7.5 g wide radical arising from oxidised P700. The 13.25 g wide radical arising from reduced A_1 in Triton photosystem I particles was centred around $g = 2.0037$, a value that corresponds to those obtained in [13,16].

4. Discussion

The EPR spectrum of A_1 observed in photosystem I particles from spinach prepared using French press fractionation or the detergent digitonin is asymmetrical. If Triton X-100 is added to digitonin photosystem I particles, or used in the preparation of the photosystem I particles from spinach, the spectrum of A_1 is altered to a symmetrical radical 13–14 g wide centred at $g = 2.0037$. Triton X-100 does not

Fig.3. EPR spectra at 77 K of digitonin photosystem I particles (1.3 mg chl/ml) frozen during illumination in the presence or absence of the detergent Triton X-100. Triton X-100 was added to the digitonin photosystem I particles in the EPR tube to the following concentration: (a) none; (b) 0.1%; (c) 1.0%; (d) 5%. The samples were then illuminated for 1 min in the presence of sodium dithionite and frozen under illumination. The spectra were recorded using the following instrument settings: frequency, 8.905 GHz, microwave power, 5×10^{-3} mW; modulation amplitude, 0.2 mT; scan rate, 2.5 mT/min; instrument gain 2×10^3.

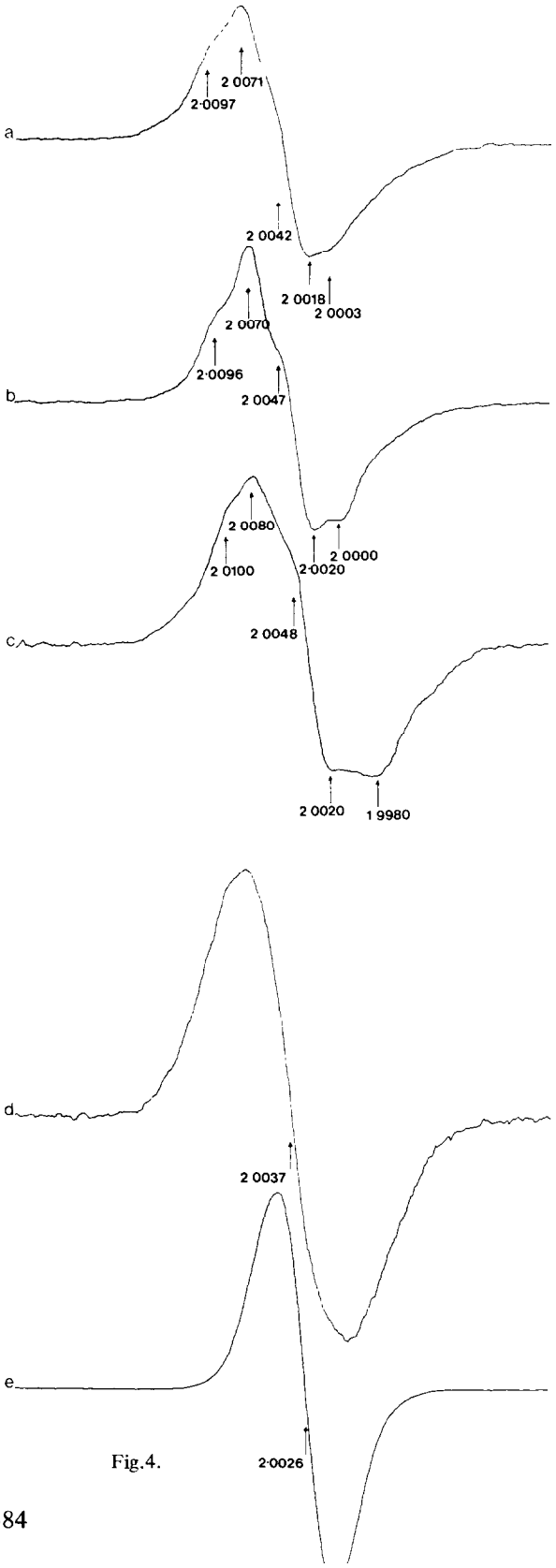

Fig.4.

interfere with the role of A_1 as an intermediary electron carrier, but it is acting upon the environment of the unpaired electron giving rise to the EPR spectrum. It is possible that the asymmetrical appearance of the true spectrum of A_1 in the absence of Triton X-100 is due to unresolved proton hyperfine splitting, as in the EPR spectrum of signal II.

Fig.1 provides clear evidence for an interaction between A_1 and X, which is altering the signal intensity of the $g = 1.76$ peak of the EPR spectrum of X. This peak is considerably smaller when A_1 is substantially reduced (fig.1a) than when A_1 has decayed (fig.1b). Triton X-100 does not affect this interaction, since the same effect was observed in Triton photosystem I particles [11].

In preliminary kinetic studies we have observed transient EPR signals in the $g = 2.00$ region in digitonin photosystem I particles that decay with lifetimes <0.5 ms. Although these changes appear to correspond with the EPR spectra of P700 and A_1; there are additional transient signals occurring faster than the resolution time of the EPR machine (200 μs) which resemble the transient EPR signals attributed to CIDEP in [16]. Photosystem I particles prepared using Triton X-100 show transient changes with lifetimes ~0.5 ms, but do not appear to exhibit these CIDEP-associated signals, again suggesting an alteration of the reaction centre environment by Triton X-100.

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Fig.4. Precise g-values for the measured EPR spectra of reduced A_1 and the EPR spectrum of oxidised P700. Samples were prepared by illumination for 1 min in the presence of sodium dithionite (0.2%, w/v) and frozen under illumination, and were: (a) French press particles from spinach (4 mg chl/ml); (b) photosystem I particles from *Chlorogloea fritschii* (1.1 mg chl/ml); (c) digitonin photosystem I particles from spinach (1.3 mg chl/ml); (d) Triton X-100 photosystem I particles from spinach (0.4 mg chl/ml). Spectrum (c) was obtained from Triton X-100 photosystem I particles (1 mg chl/ml) that were frozen after 30 min in the dark at room temperature, and illuminated for 1 min at 77 K. The spectra were recorded at 77 K and a microwave power of 5×10^{-3} mW.

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