DETECTION BY EPR SPECTROMETRY OF A NEW INTERMEDIATE IN THE PRIMARY PHOTOCHEMISTRY OF PHOTOSYSTEM I PARTICLES ISOLATED USING TRITON X-100

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1. Introduction

Electron paramagnetic resonance (EPR) spectrometry has been used to identify four different components involved in electron transport in the photosystem I reaction centre. A free radical EPR signal (signal I) [1] has been equated [2] with the electron donor P700, a chlorophyll dimer detected by optical spectroscopy [3]. Low temperature (<30 K) EPR spectrometry has demonstrated that there are three associated electron acceptors, the two membrane-bound iron–sulphur centres A and B and the unidentified compound X. Illumination of photosystem I particles frozen in the dark results in the irreversible photo-oxidation of P700 (appearance of signal I) and the irreversible photoreduction of centre A [4,5]. If centre A is reduced in a frozen sample, illumination at cryogenic temperatures will irreversibly photoreduce centre B [6,7]. Thus at cryogenic temperatures centre B would seem to precede centre A, although redox titrations at room temperature [7,8] show that the mid-point redox potentials (E_m) of A and B (E_m −550 mV and E_m −585 mV, respectively) are close enough together to result in a mixture of the two centres being reduced in a dark titration. In parallel with the reduction of centre B in photosystem I particles during a titration in the dark, illumination of the resulting samples at cryogenic temperatures results in the reversible photo-oxidation of P700 [6] and reversible photoreduction of a compound named X [9,10]. The E_m of X is too low to enable its reduction in a dark titration using dithionite, but an E_m −730 mV has been estimated from the extent of reversible P700 in a series of electrochemical titrations [11]. Doubts about the extent to which X participated in electron transport in photosystem I [12,13] have been resolved by quantitative work that demonstrated that P700, A and X, are present in photosystem I particles in stoichiometric amounts [14,15].

Recent work [16–18] has suggested the presence of another intermediate electron acceptor in the primary photochemistry of photosystem I, which acts closer to P700 than X. By observing the time course of the re-reduction of P700 after a saturating flash, under conditions where P700 is reduced by the electron tunnelling back from the eventual electron acceptor, they have concluded that the photosystem I reaction centre contains four possible electron acceptors. Electron acceptors, which require two electron equivalents to reduce them, are shown to resemble P430 [19] and thus presumably the bound iron–sulphur centres A and B. At room temperature in Triton photosystem I particles an electron takes 30 ms to return to P700 from these electron acceptors. If these acceptors are reduced by the addition of dithionite or pre-illuminating flashes, P700 is re-reduced in 250 μs at room temperature in these particles. The electron acceptor from which this electron is tunnelling back is designated A_2, and equated with X. If X is reduced in the sample prior to a saturating single flash the back-reaction rate increases to 3 μs. This acceptor, presumably closer to P700 than X, is designated A_1 and has been detected in SDS and digitonin photosystem I particles and broken chloroplasts as well as Triton photosystem I particles.

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We report here the observation of an EPR signal which may correspond to the electron acceptor A_1.

2. Materials and methods

Photosystem I particles were prepared from spinach (*Spinaciea oleracea*) using the non-ionic detergent Triton X-100 as in [14], with a P700 : chlorophyll ratio of 1:30 – 1:50. EPR measurements were made using a Jeol FE1-X spectrometer, interfaced with a Nicolet 1020 A signal averager. Sample temperature was controlled by an Oxford Instruments liquid helium cryostat or a liquid nitrogen finger dewar. EPR samples were prepared as in [5]. Light intensities were measured with a Lambda Quantum, Radiometer, Photometer system (T and J Crump Sci. Instr., Rayleigh, Essex). A 1000 W projector was used for illumination during the preparation of samples, and of samples in the cavity, with the addition of a 1 cm water plus copper sulphate filter and two Schott KG3 heat filters in the latter case. Sodium dithionite was from BDH Chemicals.

3. Results

To obtain frozen EPR samples of photosystem I particles with A, B and X reduced we routinely illuminated the preparation with a 200 W slide projector (light intensity incident at sample = 1500 $\mu E.m^{-2}.s^{-1}$) under nitrogen gas in an EPR tube in the presence of excess dithionite, and continue to illuminate the sample as it is frozen in liquid nitrogen. This produces spectra similar to that shown in fig.1c. However, if a 1000 W projector (light intensity incident at the sample = 4750 $\mu E.m^{-2}.s^{-1}$) is used during this procedure a novel free radical signal appears in the g 2.00 region of the resulting EPR spectra, in addition to the signals attributed to A, B and X (fig.1a). If the light is turned off as the sample is frozen this novel free radical signal decreases considerably (fig.1b) and if the sample is left in the dark for 30 s before freezing this signal has completely disappeared (fig.1c). The signal at g 1.76 attributed to X has only partially decayed in this time.

It seems unlikely that the signal arises from oxidised P700, as the dithionite present should readily reduce this component. This conclusion was substantiated by scanning these samples at liquid nitrogen temperature, where the free radical signals normally seen in photosystem I preparations can be observed at non-saturating microwave powers [14]. At these temperatures the reduced centres A and B and X will not be contributing to the recorded EPR spectra, and a true representation of the line-shape of this free radical signal and its saturation properties can be obtained. Figure 2 confirms that a free radical signal is induced in these particles when they are frozen under illumination, and decays if a 30 s dark period precedes freezing. This free radical signal has a linewidth of

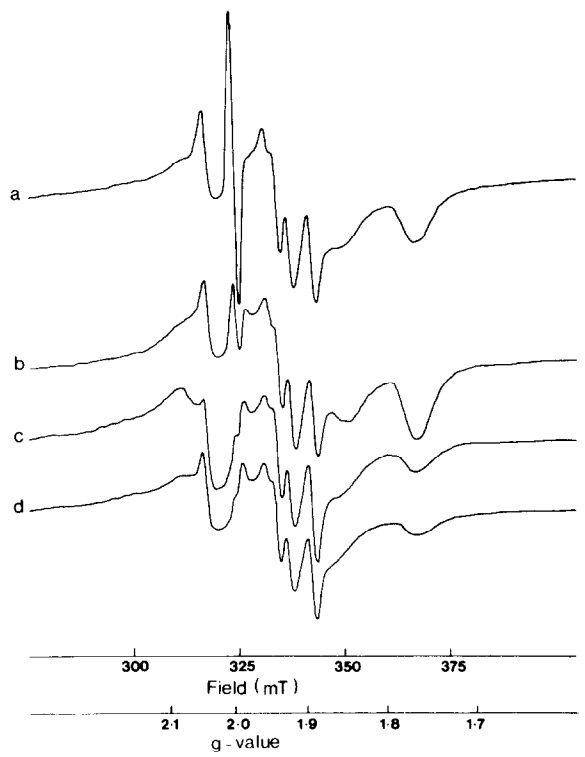

Fig.1. EPR spectra at 10 K of photosystem I particles prepared using Triton X-100 (1.0 mg chlorophyll/ml) during and after illumination at room temperature. Samples were illuminated for 2 min in the presence of sodium dithionite (0.2%, w/v) under anaerobic conditions and frozen: (a) under illumination; (b) as the light was turned off; (c) after 30 s dark period; (d) after 1 min dark period. The spectra were recorded using the following instrument settings: frequency, 9.075 GHz; microwave power, 20 mW; modulation amplitude, 1 mT; scan rate, 50 mT/min; instrument gain, 2.5×10^2.

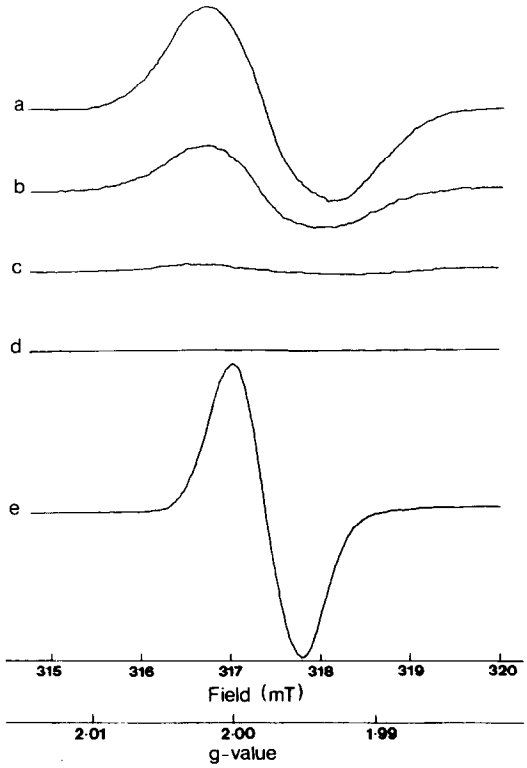

Fig.2. EPR spectra of photosystem I particles prepared using Triton X-100 (1.0 mg chlorophyll/ml) at 77 K. Spectra a–d are from the samples described in fig.1. Spectrum e is from particles pre-incubated in the dark for 30 min at room temperature, frozen in the dark, then illuminated at 77 K to irreversibly photo-oxidise P700. The spectra were recorded using the following instrument settings: frequency, 8.898 GHz; microwave power, 5×10^{-3} mW; modulation amplitude, 0.2 mT; scan rate, 2.5 mT/min; instrument gain 1.25×10^{3} (a–d) and 5×10^{2} (e).

14 G (fig.2a), unlike the P700 signal I free radical associated with the irreversible photoreduction of centre A at cryogenic temperature in these particles, which has a linewidth of only 7.5 G (fig.2e). The spectrum of this novel free radical seen in fig.2a contains a slight contribution from a narrow free radical which contributes <10% of the total signal size.

The saturation characteristics of this broad free radical differ from those of signal I. The curves shown in fig.3a,b demonstrate that under reducing conditions the relaxation time of signal I is increased, so that signal I decreases in size more rapidly at microwave power >1 mW, as in [14]. In contrast the broad free radical is less saturated than either signal I in the absence or presence of added sodium dithionite (fig.3c). This is in spite of the fact that the broad free radical is only observed under extreme reducing conditions, providing further evidence that this signal arises from a component in photosystem I electron transport distinct from the oxidised electron donor P700.

Illumination of the samples frozen whilst still being illuminated by the 1000 W projector (i.e., those with X reduced and a large broad free radical signal) at cryogenic temperatures did not photo-induce either an irreversible or reversible free radical signal with linewidth of 14 G. However, a reversible light-induced free radical of linewidth 7.5 G was observed (fig.4). This was shown to be associated with the light-

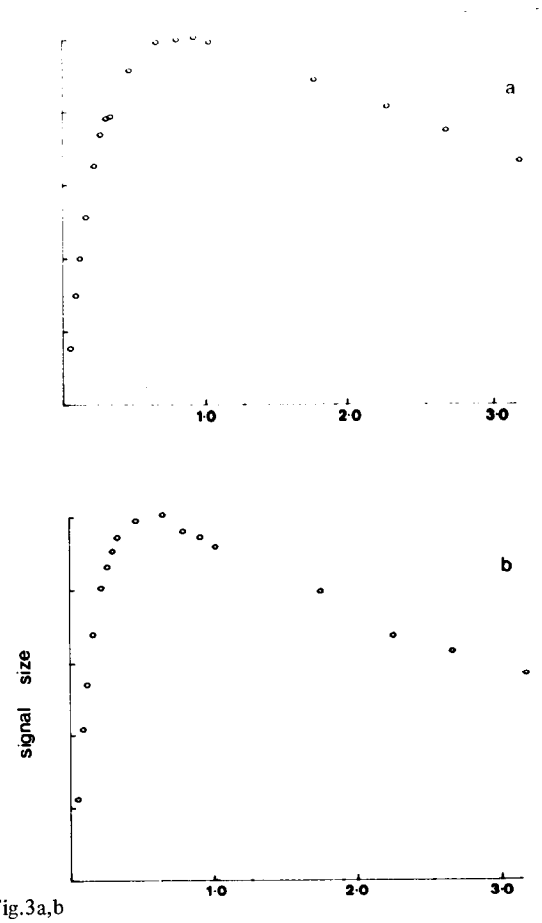

Fig.3a,b

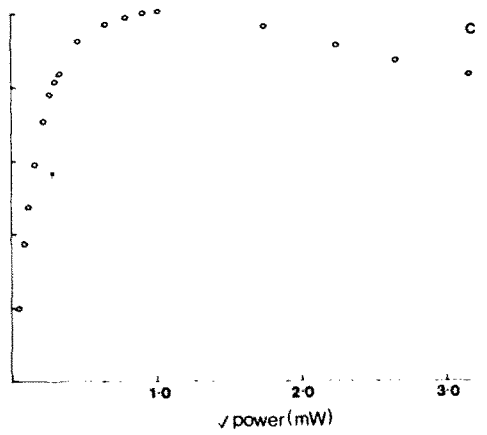

Fig.3. The effect of microwave power on the signal intensity of the $g = 2.00$ region signal from: (a) photosystem I particles pre-incubated and frozen in the dark, and illuminated at 77 K to irreversibly photo-oxidise P700; (b) photosystem I particles illuminated in the presence of dithionite and frozen after 1 min dark period and illuminated during measurement to reversibly photo-oxidise P700; (c) photosystem I particles illuminated in the presence of dithionite and frozen under illumination, freezing in the sample a broad free radical signal. Spectra were recorded at 77 K and the instrument settings described in fig.2.

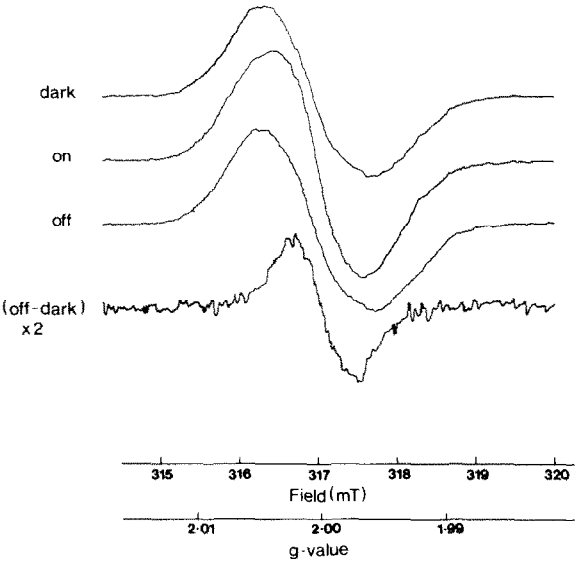

Fig.4. Effect of illumination at 77 K on the spectrum of photosystem I particles illuminated at room temperature in the presence of dithionite and frozen under illumination (see fig.1a,2a). Spectra were recorded at 77 K and the following instrument settings: frequency, 8.898 GHz; microwave power, 5×10^{-3} mW; modulation amplitude, 0.2 mT; scan rate, 2.5 mT/min; instrument gain, 1.25×10^3.

induced reversible reduction of X, a proportion of this acceptor having remained oxidised during the preparation of the sample. Attempts to completely reduce X by illumination of photosystem I particles in the presence of mediators as well as dithionite to improve the rate of donation of electrons to P700 were unsuccessful. Although the EPR spectra of such samples contained a larger contribution from a broad free radical and X, the mediators (reduced viologens and oxidised neutral red) were found to contribute to the free radical region of the spectrum.

4. Discussion

The EPR spectrometer has a limited time resolution ~1 ms, and as the $t_{1/2}$ for the back reaction between A_1 and P700 at 5 K is ~120 μs [18] (in broken chloroplasts), it was not possible to observe either the appearance or the decay of the broad free radical signal following saturating single flash illumination. However, as the broad free radical signal was only observed frozen in these photosystem I particles when X had already been reduced, it seems likely that it is a very low potential component which may function as an intermediate in electron transport acting between P700 and X.

As this free radical signal has a linewidth of 14 G, it does not appear to arise from the dimer of chlorophyll molecules (P700) acting as electron donor in photosystem I which have been shown to have a linewidth of 7.5 G. As it would appear to act in the sequence of photosystem I electron transport between P700 and I, it can be equated with the acceptor A_1 postulated [16–18]. They excluded the possibility that A_1 was a triplet, as the rate of back-reaction from A_1 to P700 was not affected by the presence of oxygen [17]. A linewidth of 14 G may indicate the reduction of a molecule of pheophytin or a chlorophyll monomer, acting as an intermediate in photosystem I electron transport in the same manner as the molecule of bacteriopheophytin in the reaction centres of photosynthetic bacteria.

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