LIGHT-INDUCED EPR SIGNALS AT CRYOGENIC TEMPERATURES IN SUBCHLOROPLAST PARTICLES ENRICHED IN PHOTOSYSTEM II

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1. Introduction

The study of electron paramagnetic resonance (EPR) signals appearing upon illumination of samples at cryogenic temperatures has provided much information about photosynthetic reaction centres. The exception has been in the study of photosystem II where only limited information has been obtained [1].

A free radical signal was shown [2] to be induced by flash illumination at 77 K in chloroplasts pre-oxidised by ferricyanide. The signal had similar EPR characteristics to those of oxidised P700 and was attributed to oxidised P680, the primary chlorophyll donor of photosystem II. Further studies [3] have given more detailed information on the behaviour of this signal.

The second EPR signal attributed to photosystem II is termed signal II. Two components of signal II, IIu and IIs, exhibited slow rise and decay kinetics at room temperatures. Further studies on the generation of the free radical signal II have revealed transient signals, IIf and IIvf, upon illumination of chloroplasts at physiological temperatures [4–11]. All these components of signal II have been assigned to the donor side of photosystem II. No light-induced changes in signal II have been observed at cryogenic temperatures.

Here we report the appearance of light-induced EPR signals near the g 2.00 region at cryogenic temperatures in subchloroplast particles enriched in photosystem II. One of these signals has characteristics of the free radical signal II.

2. Materials and methods

Chloroplasts were prepared from either spinach (*Spinacea oleracea*) or lettuce (*Lactuca sativa*). All operations were at 0–4°C. Chopped leaves were homogenised for 20 s in a Braun blender using fresh 0.33 M sorbitol, 16 mM Na pyrophosphate, 5 mM $MgCl_2$, 2 mM Na-ascorbate (pH 6.5) (125 g leaves to 300 ml buffer). The homogenate was strained twice through muslin and the chloroplast pellet collected by rapid centrifugation to 5000 × g. The chloroplasts were washed with 50 mM tricene, 0.2 M KCl, 0.5 mM dithiothreitol, 0.05% bovine serum albumin (pH 7.6) (buffer A) and collected by centrifugation at 5000 × g for 1 min. The pellet was resuspended in buffer A and twice passed through a French pressure cell at 5000 lb. in^{-2}. The suspension was centrifuged at 35 000 × g for 40 min and the pellet was resuspended in buffer A. Incubation with digitonin (Sigma) was as in [12] except that buffer A was used throughout. Both the 1 h and overnight digitonin treatments produced pellets after centrifugation which were enriched in photosystem II. These pellets (digitonin particles) were stored at 77 K until used. Chlorophyll in EPR samples was 1.5–2.0 mg/ml. EPR measurements were made using a Jeol JES Fel-X X band spectrometer incorporating the appropriate helium or nitrogen cryostat. Samples were illuminated by a 1 kW projector. Difference spectra were performed using a Nicolet 1020A or a Tektronix 4051 computer.

Photosystem I particles were prepared as in [13]. Chlorophyll was determined as in [14].

3. Results and discussion

When digitonin particles were illuminated for 1 min, frozen under illumination and stored overnight in the

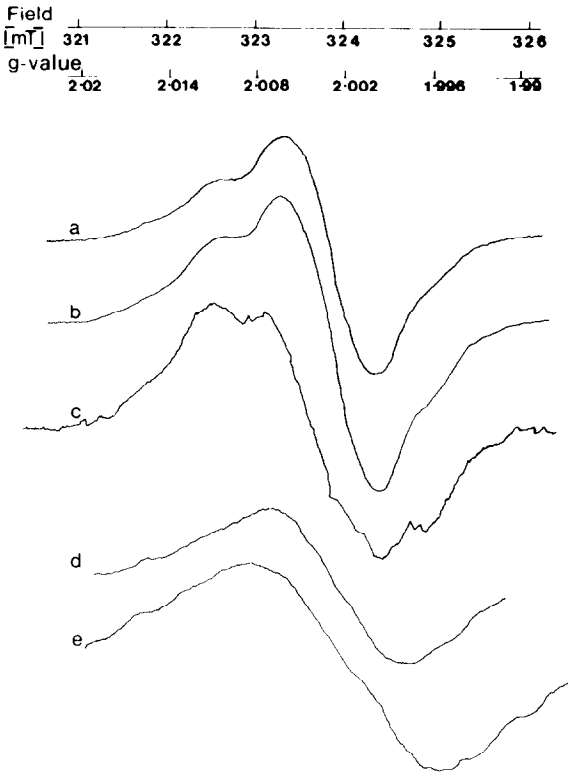


Fig.1. EPR spectra of digitonin particles frozen under illumination (a) before and (b) after illumination (c) difference spectrum (b) – (a) × 4. Broad band white light used for illumination. Microwave power, 1 mW; time constant, 0.1 s; scan rate, 5.0 mT/min; modulation amplitude, 0.2 mT; instrument gain 2:5 × 10^2; temperature 10 K, frequency, 9.078 GHz. (d) Dark after–dark before illumination difference spectrum, conditions as above except: temperature, 17 K; microwave power, 50 mW; instrument gain 1 × 10^3. (e) Dark after–dark before illumination difference spectrum, conditions as above except: temperature, 77 K; microwave power, 50 mW, instrument gain 2 × 10^3.

dark at 77 K, the EPR spectrum shown in fig.1a was observed at cryogenic temperatures. Illumination of this sample at 5–77 K produced the spectrum shown in fig.1b. The spectrum of the light-induced irreversible radical can be seen in the light–dark difference spectrum (fig.1c).

The spectrum has the characteristic shape of signal II [6] exhibiting partially resolved hyperfine splitting and a peak-to-peak linewidth of 1.9 mT (see also fig.2a). If the sample was now stored dark at 77 K overnight, the light-induced signal decayed completely but could be restored to its full extent by reillumination.

The EPR spectrum at higher microwave powers exhibited saturation and broadening effects. A considerable variability in the linewidth of the light-induced signal occurred, dependent on the temperature of measurement. At 77 K the spectrum of the light-induced signal at 50 mW had a linewidth of 2.0–2.5 mT but at 5–25 K a linewidth of 1.4–1.7 T was observed (fig.1d,e). These signals were wider than the signal in the sample under the same conditions before illumination, so that saturation effects do not fully explain the appearance of this broad signal at high microwave powers.

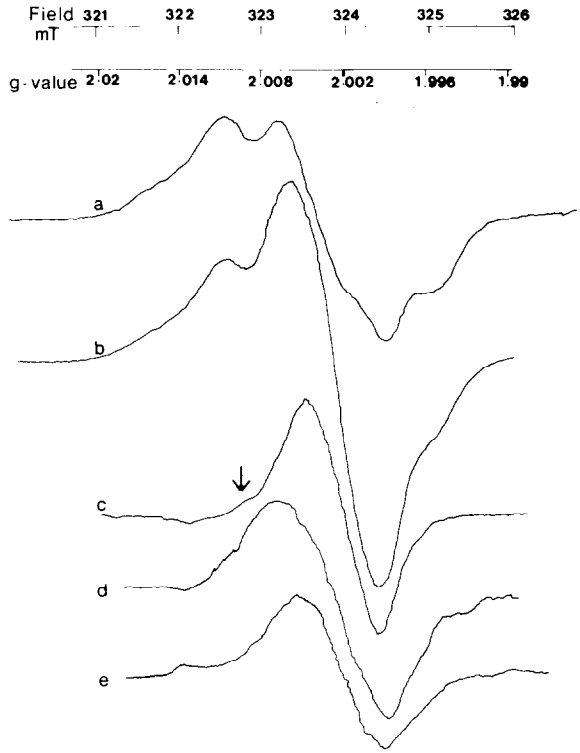


Fig.2. EPR spectra of frozen, dark-adapted digitonin particles (a) before and (b) after illumination (c) difference spectrum (b) – (a). Conditions as fig.1 except: instrument gain 1 × 10^3; temperature, 17 K. (d) Dark after–dark before illumination difference spectrum, conditions as above except: microwave power, 50 mW. (e) EPR spectra of frozen, dark-adapted photosystem I particles (1 mg/ml). Dark after–dark before illumination difference spectrum. Conditions as in (d).

The low temperature light-induced signal II showed a rise time of the same order as the time resolution of the EPR machine (1 ms). The signal was also fully induced by low light intensities. A 10 μs xenon flash induced > 50% of the total signal.

When digitonin particles were dark adapted for 30 min, then frozen, the 1st derivative EPR spectrum in the g 2.00 region at cryogenic temperatures was as shown in fig.2a. This signal again shows signal II characteristics and has been well characterised [6]. On illumination of this sample at 5–77 K a large irreversible radical was produced giving rise to the spectrum in fig.2b. Subtraction of the dark spectrum shows that the increase upon illumination was due to a radical of linewidth 0.8–0.9 mT (fig.2c). A small shoulder (arrowed) was usually evident on this peak. As the digitonin particles contained photosystem I the narrow radical in fig.2c was most probably due to P700 oxidation.

Figure 2d shows the spectrum of the light-induced radical at high microwave powers. Saturation leads to the broadening of the radical linewidth to 1.3–1.6 mT at 50 mW. This was wider than that of oxidised P700 in photosystem I particles prepared under the same conditions (fig.2e). It is possible that the shoulder seen in the light-induced signal at lower powers (arrowed, fig.2c) in digitonin particles frozen in the dark, has a greater effect on signal shape at higher powers or that a signal not seen at lower powers rises at higher microwave powers.

Figure 3 shows the microwave saturation profile at 9.5 K of the light-induced signal II as compared to that of the signal II already present in dark samples. Both signals were saturated at even the lowest microwave powers used (10^{-3} mW). At 77 K both signals were saturated above 10^{-2} mW. The light-induced signal II has a maximum signal size at higher microwave powers than the signal II present in the dark sample. At 77 K this effect was more marked and may be due to saturation increasing errors of measurement but probably results from the broader signal which appears at higher microwave powers. The origin of this signal is unclear.

Signal II components found previously have all been ascribed to the donor side of the photosystem II reaction centre. It is possible the new signal II component reported here arises from either donor or acceptor side:

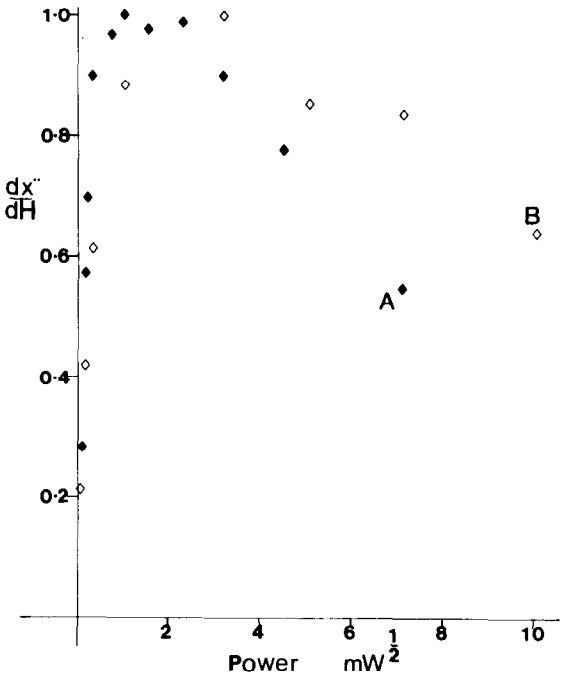
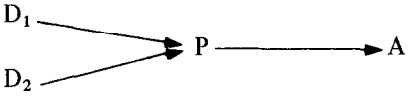


Fig.3. Microwave saturation curves of the signal II found in digitonin particles. (A) Digitonin particles frozen in the dark. Conditions as in fig.1 except: microwave powers as indicated; temperature, 8 K. (B) Digitonin particles frozen under illumination. Signal II induced by illumination at 9.5 K. Signal amplitude was measured between the low and high field maxima except at powers > 10 mW where the maximum amplitude was measured in the absence of resolved peaks. Amplitudes were normalised for comparison.



At cryogenic temperatures several donors (D) have been postulated to be capable of donation to the primary chlorophyll donor P680 (P). These include cytochrome b_{559}, an unknown donor termed D and at high redox potentials, chlorophyll [3]. Other results have indicated that plastoquinones possibly participate in both donor and acceptor sides of photosystem II [15]. The similarities between EPR signal II and the EPR signals of semiquinone or plastochromanoxyl radicals provide a possible chemical identification of signal II [16].

The low temperature light-induced signal II reported

here was only clearly seen when the particles were frozen under illumination. This procedure photo-oxidises photosystem I and eliminates most of the light-induced p700 signal at cryogenic temperatures. However, it must also alter the redox state of components of photosystem II as much lower amounts of light-induced signal II were seen in samples frozen in the dark. The illumination procedure may eliminate one or more donors or acceptors allowing the precursor of the signal II to participate in photoreactions.

Signal IIvf was concluded [10] to arise from a donor to P680 [10]. The present experiments do not allow us to decide whether the low temperature photo-induced signal II arises from a donor or acceptor. However the ability to observe this reaction at low temperatures indicates that this component is closely involved in the early photochemical events of photosystem II and together with the results in [10] indicate that it is a component of the primary donor complex.

Further characterisation of the signals reported here is difficult due to the presence of photosystem I components and the requirement for illumination of samples prior to freezing. Therefore further purification of the preparation is needed before an unequivocal assignment of the function of this component can be made.

Acknowledgements

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