THE EPR SPECTRUM OF IRON–SULPHUR CENTRE B IN PHOTOSYSTEM 1 OF *PHORMIDIUM LAMINOSUM*

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1. Introduction

The photosystem I complex of plant photosynthesis contains bound iron–sulphur centres which appear to act as terminal electron acceptors for electrons released from P700 in the primary photoreactions [1,2]. The centres can be observed in the reduced state by low temperature electron paramagnetic resonance (EPR) spectroscopy. In photosystem I preparations from chloroplasts, and green and blue–green algae, the centre which is first reduced, either photochemically by illumination at low temperature, or by chemical reduction, has a spectrum at $g = 2.05$, 1.94, 1.86. This was termed centre A [3]. On further chemical or photochemical reduction the spectrum changes to a more complex one with features at $g = 2.05$, 1.94, 1.92 and 1.89 [4]. This change was interpreted as due to reduction of a second centre, B [3]. The $g = 1.86$ feature was assumed to have shifted due to an interaction with centre A. Intensity measurements indicated that centres A and B and P700 were present in equivalent amounts [2]. However the spectrum of reduced centre B alone could not be extracted by subtraction of the spectrum of centre A from the fully reduced spectrum.

During EPR spectroscopic measurements of membrane preparations and photosystem I particles from the thermophilic blue–green alga *Phormidium laminosum*, we noted a signal at $g = 2.065$, 1.935 and 1.882 (±0.002) in samples reduced in the dark with dithionite. Chemical and photochemical reduction experiments indicated that it has the properties expected of centre B. The signal shows changes on reduction of centre A (including a shift of the $g = 2.065$ peak), analogous to the changes in the signal of centre A that occur on reduction of B in chloroplasts. It appears that on chemical reduction, centre B is preferentially reduced in these preparations because it has a more positive redox potential than centre A especially in the presence of glycerol. However on photochemical reduction at 20 K, centre A was reduced in preference to B. This indicates that electrons cannot readily be transferred between the centres at cryogenic temperatures. The spectrum of the fully reduced centres in *P. laminosum* is similar to that of chloroplasts, indicating that the spectrum of B is the same in all cases. It is concluded that the EPR spectrum of fully-reduced photosystem I reaction centres is caused by an exchange interaction between the spins on the iron–sulphur centres. Certain features of this spectrum, particularly the $g = 1.92$ feature, which were previously attributed to the spectrum of reduced centre B, are now seen as a consequence of the spin–spin interaction.

2. Materials and methods

Phormidium laminosum: (strain OH-1-p.Cl 1, [5]) was grown on medium D of [5] supplemented with 0.4 g $NaHCO_3$/l. Cultures were grown at 45°C in a Gallenkamp orbital incubator gassed with air enriched with 5% CO_2, and with illumination from four 30 W fluorescent tubes.

Membrane fragments were prepared from 5 l culture in late exponential phase (5–6 days' growth), by lysozyme treatment followed by osmotic shock to lyse the spheroplasts. Lysozyme treatment was for

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1 h at 37°C, in a medium containing 0.5 M sorbitol, 10 mM $MgCl_2$, 10 mM Hepes–NaOH and 5 mM phosphate buffer (pH 7.5) plus 12.5 mM EDTA and 0.1% solid lysozyme (BDH Chem., Poole, Dorset). The spheroplasts were collected by centrifuging (3000 × *g*, 7 min), then lysed by resuspension in a hypo-osmotic medium containing 30 mM Hepes–NaOH (pH 7.5) and 2 mM EDTA. Membrane fragments were collected by centrifugation (27 000 × *g*, 15 min), washed 3 times in the Hepes–EDTA medium, then finally resuspended in the minimum volume of the same medium. In some cases 25% (v/v) glycerol was added to the resuspending medium.

Chlorophyll *a* concentration was measured by the method in [6].

EPR spectra were recorded on a Varian E4 spectrometer with Oxford Instruments ESR9 liquid helium transfer system. Spectra were subtracted with a DL4000 signal processing system (Data Lab., Mitchem, Surrey).

The spectra shown were all run at 22 K, with the following instrument settings: microwave power 20 mW, frequency 9.28 GHz, modulation amplitude 0.5 mT, frequency 100 kHz.

g-Values were determined relative to a 1, 1-diphenyl-2-picrylhydrazyl standard, and were principal apparent *g*-values (derivative peaks or crossover positions) determined to ±0.002. However it should be noted that the exact *g*-values will vary somewhat depending on whether the samples were reduced chemically with $Na_2S_2O_4$, or photochemically at low temperature.

3. Results

Spectra were recorded of unpurified membrane fragments from *P. laminosum* prepared by treatment with lysozyme and osmotic shock. The results obtained were therefore representative of the photosystem I in the intact membrane, without disruption by detergents. Most of the spectra recorded here were in 25% (v/v) glycerol. This had the effects of making the iron–sulphur centres more readily accessible to $Na_2S_2O_4$, and of making the midpoint reduction potential of centre B less negative relative to centre A [7]. However even in the absence of glycerol, centre B of *P. laminosum* appears to have a more positive midpoint reduction potential than in most photosynthetic organisms.

The addition of glycerol had little effect on the shape of the EPR spectra. Figure 1 shows the spectra of fully reduced centres A and B, in the absence and presence of glycerol, obtained by reducing samples with $Na_2S_2O_4$ and light before freezing. The spectra are similar to those seen in other blue–green algae and chloroplasts [1,2].

However spectra of samples at intermediate stages of reduction were different. After brief reduction with $Na_2S_2O_4$ a small signal at $g = 1.92$ was seen (fig. 2a). This signal has been observed in other blue–green algal preparations [8] and is presumably due to a non-photosynthetic component. After a few minutes' reduction with $Na_2S_2O_4$ a spectrum at $g = 2.065$, 1.935 and 1.882 appeared (fig.2b). This spectrum is

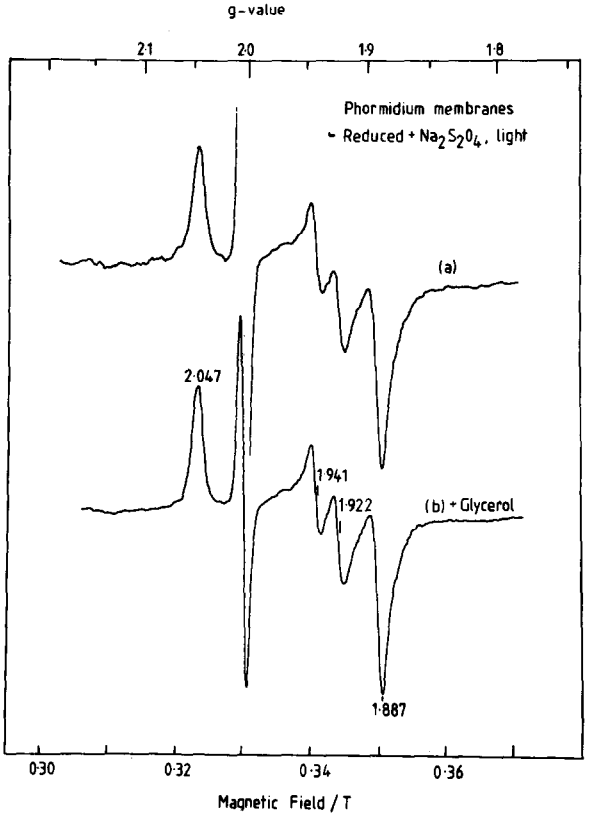

Fig.1. EPR spectra measured at 22 K of fully reduced centres A and B in membranes of *P. laminosum*: (a) without glycerol; (b) with 25% v/v glycerol in the resuspending medium. Samples, containing 0.88 mg chl./ml, were reduced with 3 mM $Na_2S_2O_4$ and illuminated with a 300 W projector with heat filter for 2 min then frozen under continuous illumination.

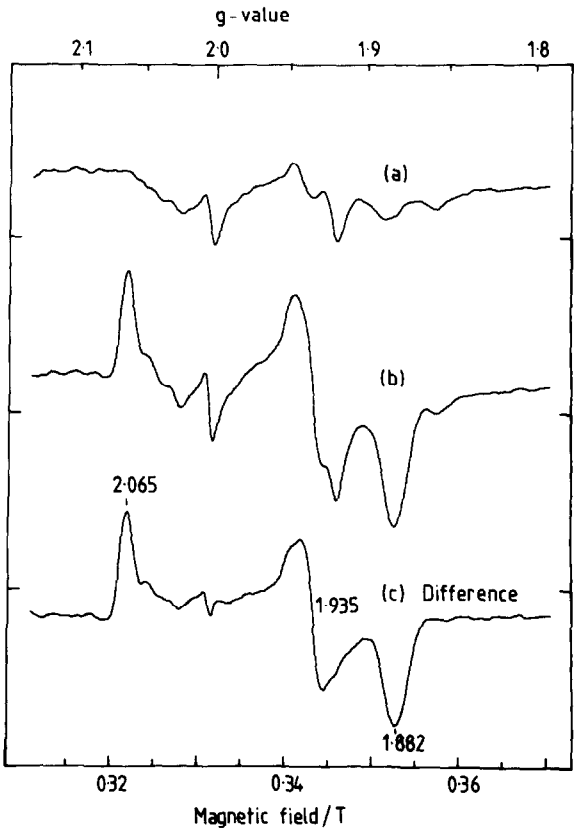

Fig.2. EPR spectrum attributed to reduced centre B obtained by chemical reduction of *P. laminosum* membranes: (a) membranes without glycerol, reduced with 3 mM $Na_2S_2O_4$ for 1 min at 20°C in the dark before freezing; (b) membranes in 25% glycerol, reduced with 3 mM $Na_2S_2O_4$ for 4 min; (c) subtracted spectrum, (b)–(a). Chlorophyll was 1.02 mg/ml.

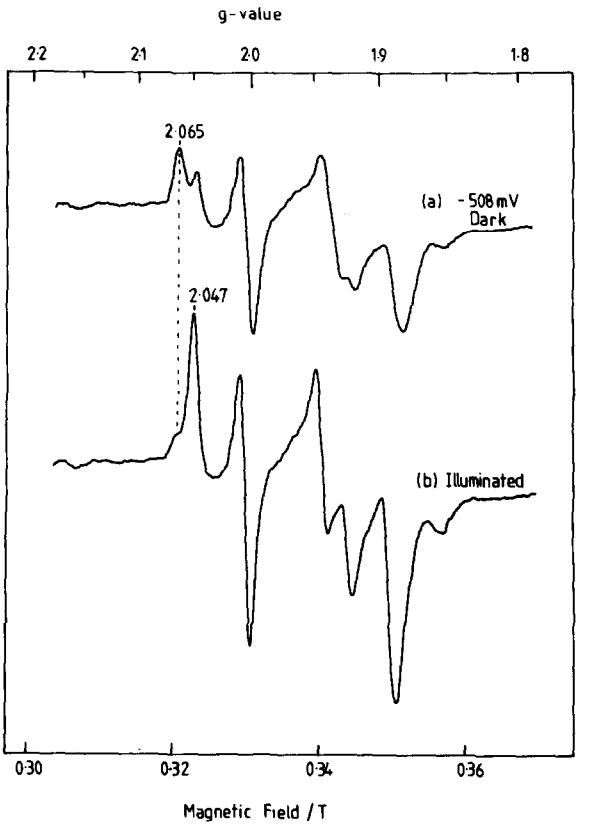

Fig.3. Effect of illumination at 22 K on the EPR spectrum of partially reduced membranes: (a) sample poised by reduction in the presence of viologen mediators [11,12] at a potential of −508 mV before freezing; (b) after illumination in the EPR cavity.

most clearly seen by subtracting spectrum 2a from 2b (fig.2c). It is unlike the spectrum due to centre A at $g = 2.05$, 1.94 and 1.86 seen in subchloroplast particles [1]. Moreover, like the $g = 1.86$ feature of centre A, the $g = 2.065$ feature is absent from the fully-reduced spectrum (fig.1).

The disappearance of the $g = 2.065$ feature on further reduction can be seen in the photochemical reduction at 20 K (fig.3). A sample partially reduced by poising at −508 mV (fig.3a) was illuminated in the cavity of the EPR spectrometer. There was an increase in the $g = 2.047$ feature, and a definite decrease in signal intensity at $g = 2.065$ (fig.3b). This observation indicates an intimate relationship between iron–sulphur centre A and the species giving rise to the new signal, and supports its assignment as centre B. Similar results were obtained by partial reduction of the membranes in the dark with $Na_2S_2O_4$.

It appears that in these particles, centre B has a more positive midpoint reduction potential than centre A which means that at equilibrium at room temperature, centre B will be reduced in preference to centre A. A detailed analysis of the redox behaviour of these iron–sulphur centres will be presented elsewhere.

On photochemical reduction at 20 K however, reduction of centre A appeared to be favoured. Figure 4 shows a spectrum of particles frozen in the dark and illuminated at 20 K. The most prominent features

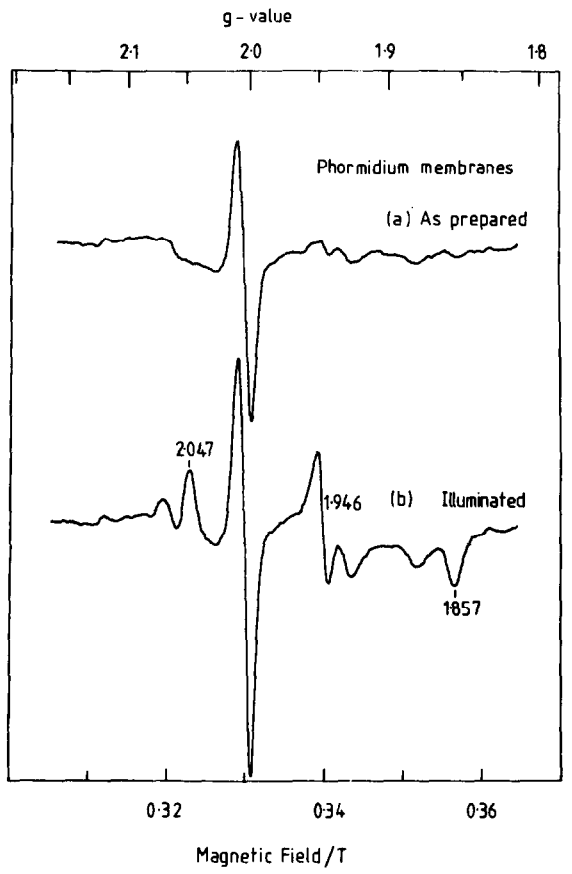

Fig.4. Illumination of unreduced particles at 22 K, showing the preferential reduction of centre A. Membranes in 25% glycerol: (a) dark; (b) after illumination.

are at $g = 2.047$, 1.946 and 1.857. Centre B was also partially reduced, but not to such a great extent. It seems therefore that centre A is more accessible to P700 in the frozen state.

An attempt was made to permit electron transfer to take place from centre A to B, by warming an illuminated sample as in fig.4 (b) to 120 K for a few minutes, then measuring again at 20 K. As a result, signal intensity due to centres A and B and the radical decreased, indicating electron transfer back to P700, but no transfer between the centres was detected.

4. Discussion

The spectrum of fully-reduced *P. laminosum* membranes (fig.1) is typical of fully-reduced plant photosystem I. Therefore it seems likely that the spectra of centre B in other plants, if they could be measured, would be similar to that of *P. laminosum*.

A comparison of the spectra of samples in which we see the reduced forms of centre A (fig.4b), centre B (fig.2c) and both centres (fig.1b) shows that they change in a complementary way when they interact. Just as the $g = 1.86$ feature of centre A disappears when centre B is reduced, [3], so the $g = 2.065$ feature of centre B in *P. laminosum* disappears when A is reduced. Moreover a feature at $g = 1.922$ appears which is present in neither of the component spectra. Since these changes can occur on illumination at 20 K (fig.3 and [9]) it is unlikely that they are due to a conformational change in a protein. It appears therefore that the spectrum of the fully-reduced system is a composite spectrum due to an electron exchange interaction between the spins on the two centres and could be described as a triplet ($S = 1$) state. As a result of this interaction the lines do not broaden, and no outer lines could be detected, which implies that the dipolar interaction between the centres is much weaker than the exchange interaction (cf. [13]). This may also explain why no half-field ($\Delta m_s = 2$) transition could be observed. An exchange interaction implies a pathway for electron transfer between the centres. However electron transfer may not take place at low temperatures because of an unfavourable energy of charge separation.

Because of the drastic changes in the EPR spectrum caused by interaction between the two spins, it is not possible to assign features of the fully-reduced spectrum (fig.1b) to g_x, g_y and g_z of the component spectra. To do so would require a complete simulation of the interacting system. For this reason in particular it is now obvious that the feature at $g = 1.92$, which we attributed to centre B [3] is in fact a consequence of the spin–spin interaction. This also means that studies of the orientation of the centres in oriented chloroplasts, in which the $g = 1.92$ feature showed a strong angular dependence [10] are considerably more difficult to interpret.

The observation that centre A appears to be preferentially photoreduced at low temperatures, argues against a linear sequence of electron transfer from P700, to B then to A. It seems likely that at room temperature, electrons would equilibrate between the

centres in accordance with their redox potentials. It may be that in the frozen state, a greater fraction of centres A than centres B are in a position to accept electrons more rapidly from X. It should be remembered that only a relatively small fraction (12% in fig.4) of the total centres was reduced on illumination at 22 K which indicates that the centres may be frozen in a variety of relative positions, only some of which are favourable for electron transfer.

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