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# EFFECTS OF GLYCEROL ON THE REDOX PROPERTIES OF THE ELECTRON ACCEPTOR COMPLEX IN SPINACH PHOTOSYSTEM I PARTICLES

M.C.W. EVANS and P. HEATHCOTE

*Department of Botany and Microbiology, University College London, Gower Street, London WC1E 6BT (U.K.)*

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## Summary

The effects of glycerol, ethylene glycol and sucrose on the redox properties of spinach Photosystem I particles prepared with Triton X-100 were investigated. It was found that in 50% glycerol low temperature illumination of ascorbate reduced particles results in extensive reduction of iron-sulphur Centre B as well as of iron-sulphur Centre A. Only Centre A is seen in aqueous samples. The oxidation reduction potentials of Centres A and B were significantly altered in 50% glycerol. Centre A midpoint potential $E_{m_{10}} \simeq -510$ mV compared to −540 mV in aqueous medium and Centre B $E_{m_{10}} \simeq -545$ mV compared to −590 mV in aqueous medium.

When Centres A and B are reduced *P*-700 photo-oxidation with the component X as electron acceptor is reversible at low temperature. The kinetics of the back reaction are altered in glycerol ($t_{1/2} = 210$ ms in aqueous medium, $t_{1/2} = 110$ ms in 50% glycerol). Glycerol was also found to affect the saturation characteristics of the EPR signal of *P*-700 and probably Centres A and B. Ethylene glycol has similar effects.

It is concluded that variations in the redox potential at which *P*-700 photooxidation becomes reversible and of the kinetic behaviour of Photosystem I reported in the literature are the result of the use of glycerol media. These results confirm that Centre B is involved in electron transfer in Photosystem I, acting between X and Centre A.

## Introduction

The properties of the Photosystem I reaction centre have been extensively investigated by optical and electron paramagnetic resonance (EPR) measurements at low temperature (see Ref. 1 for recent review). While it is clear that the reaction centre chlorophyll (*P*-700) is the electron donor identification of the 'primary' electron acceptor has proved more difficult, the electron acceptor complex containing three or four components which function even at low temperature

$$P\text{-}700 \xrightarrow{\text{Light}} [\text{I}] \rightarrow \text{X} \rightarrow \text{B} \rightarrow \text{A}$$

Electron transfer to centres A and B (four-iron four-sulphur centres) is essentially irreversible at low temperatures [2,3,8], but when Centres A and B are reduced the electron transfer from *P*-700 to X is rapidly reversible even at 6 K [3–5]. There is good agreement [6,7] on the redox potentials of A ($E_{m_{10.0}}$ = −540 mV) and B ($E_{m_{10.0}}$ = −590 mV) but there is considerable disagreement about the role of centre B in Photosystem I electron transport, and the potential at which the photo-oxidation of *P*-700 becomes reversible rather than irreversible. We have shown [8], using EPR measurements that centre B can be photoreduced at cryogenic temperatures when centre A has already been reduced, that centres A and B are present in Photosystem I particles in roughly equivalent amounts, and that *P*-700 photo-oxidation becomes reversible with $E_{m_{10.0}}$ = −590 mV in parallel with the reduction of centre B. This has led us to conclude that centre B is a component of the electron transfer chain in Photosystem I, and that it acts between X and centre A.

However other workers have had difficulty preparing samples in which B can be photoreduced [1]. A number of workers have used optical techniques to measure the potential at which irreversible *P*-700 oxidation is lost and have obtained a value of −530 mV [9,10], a similar value was also obtained using EPR measurements of signal 1 [11]. These results have led some authors to conclude that only Centre A can act as acceptor and that Centre B is not involved in contrast to our conclusion that Centre B is a component of the chain. The main experimental factor affecting the two groups of results is that our experiments were performed in aqueous media while the other workers have used glycerol media. It has recently been reported that organic media, such as ethylene glycol, can produce large effects on the redox properties of proteins [12]. We have therefore investigated the effect of glycerol on the properties of the electron acceptor complex in Photosystem I particles prepared with Triton X-100.

## Materials and Methods

Photosystem I particles with a *P*-700 : Chlorophyll ratio of 1 : 30 to 1 : 50 were prepared from spinach chloroplasts using the non-ionic detergent Triton X-100 as described previously [13]. Samples in 50% glycerol were prepared by suspending the particles at twice the required concentration in double-concentration reaction medium, adjusting the solution to the required pH and then diluting with an equal volume of glycerol. Samples for a small number of

experiments with higher (75%) or lower (25%) concentrations of glycerol, or with ethylene glycol or sucrose were prepared in a similar manner with appropriate adjustments of concentration.

Oxidation-reduction potentiometry was carried out by the method of Dutton [14] as described previously [15]. EPR spectra were recorded using a Jeol JES FE1X spectrometer, interfaced with a Tektronix 4051 microprocessor for calculation of theoretical curves for redox titration and production of light minus dark difference spectra, and a Nicolet 1020A signal averager for kinetic measurements. Samples were cooled to liquid helium temperatures using an Oxford Instruments cryostat and monitoring system. The computation involved in fitting the microwave power saturation data to theoretical curves was carried out on a Hewlett-Packard 9830A calculator, by kind permission of Dr. R. Cammack, King's College, University of London. Tetraquat, Triquat and Diquat were a gift provided by Imperial Chemical Industries Ltd. (Bracknell, Berkshire, U.K.). Tris, Triton X-100 (octylphenoxypolyethoxyethanol) were from Sigma (London) Chemical Co. (Kingston Upon Thames, U.K.). Other chemicals were from BDH Ltd. (Poole, Dorset, U.K.) using Analar-grade reagents where possible.

## Results

Fig. 1 shows the light minus dark difference EPR spectra for Photosystem I particles prepared and frozen in the dark in aqueous media and in 50% glycerol. Fig. 1a shows an EPR spectrum with peaks at $g = 2.05$, $g = 1.95$ and $g = 1.86$ characteristic of the reduced form of iron-sulphur centre A, which is normally observed to be irreversibly photoreduced on illumination of Photosystem I paticles at cryogenic temperatures, together with a signal at $g = 2.00$ due to the photo-oxidised electron donor *P*-700. The spectrum presented in Fig. 1b is clearly different with additional peaks at $g = 2.07$, $g = 1.93$ and $g = 1.89$. The signals at $g = 1.93$ and $g = 1.89$ arise from the photoreduction of centre B, which suggests that glycerol is affecting the relative redox potentials of centres A and B such that some reaction centres will now transfer an electron from *P*-700 to centre B upon illumination at cryogenic temperatures. The EPR spectrum of centre B is normally only observed in Photosystem I particles when centre A has already been reduced, and then has peaks at $g = 2.05$, $g = 1.93$ and $g = 1.89$. The reduced centre B spectrum seen in Fig. 1b probably has a low field peak at $g = 2.07$ rather than $g = 2.05$ because centre A is not already reduced, and therefore there is no magnetic interaction between the two iron-sulphur centres. It seems unlikely that glycerol is altering the interaction between the two centres, as in samples where both centres A and B are reduced there is a single low field peak at $g = 2.05$ and the high-field peak of centre A at $g = 1.86$ has shifted to $g = 1.89$ as previously described [6] (see Fig. 2 below). The apparent difference in size of the signals in Figs. 1a and 1b results from changes in saturation characteristics of the centres in glycerol, and may also reflect greater light penetration of the clear glycerol sample leading to more complete photoreduction of the bound iron-sulphur centres.

Fig. 2 shows the EPR spectra in the $g = 2.00$ region of Triton Photosystem I particles poised at different oxidation-reduction potentials in aqueous and

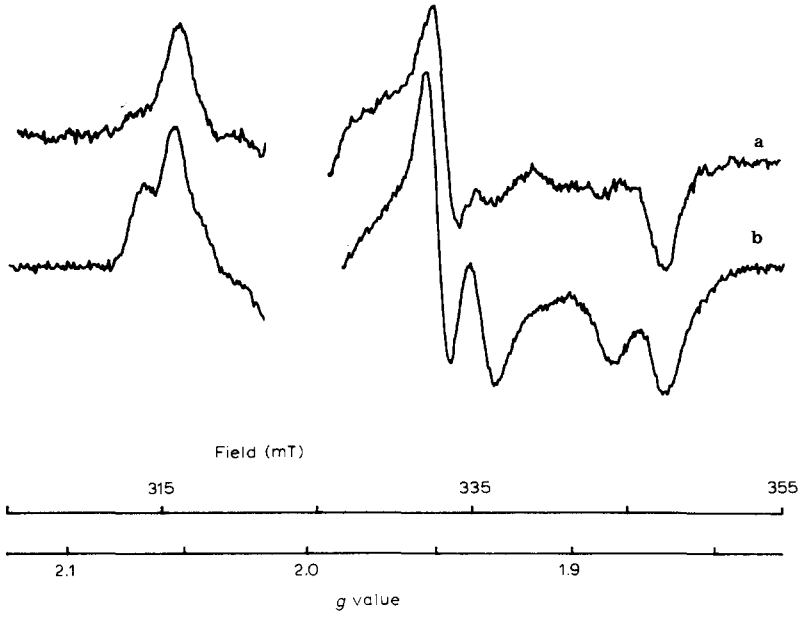


**Fig. 1. Light induced EPR spectra of Photosystem I particles in aqueous and glycerol media. Photosystem I particles (0.5 mg chlorophyll/ml) were suspended in 0.1 M Tris-HCl buffer, pH 8.0, 10 mM sodium ascorbate with (a) aqueous media or (b) 50% glycerol. The samples were frozen in the dark and illuminated at 20 K for 30 s. Spectra were recorded at 20 K with the following instrument settings: microwave power 5 mW; modulation amplitude 0.5 mT, instrument gain 250. Light minus dark difference spectra are shown.**

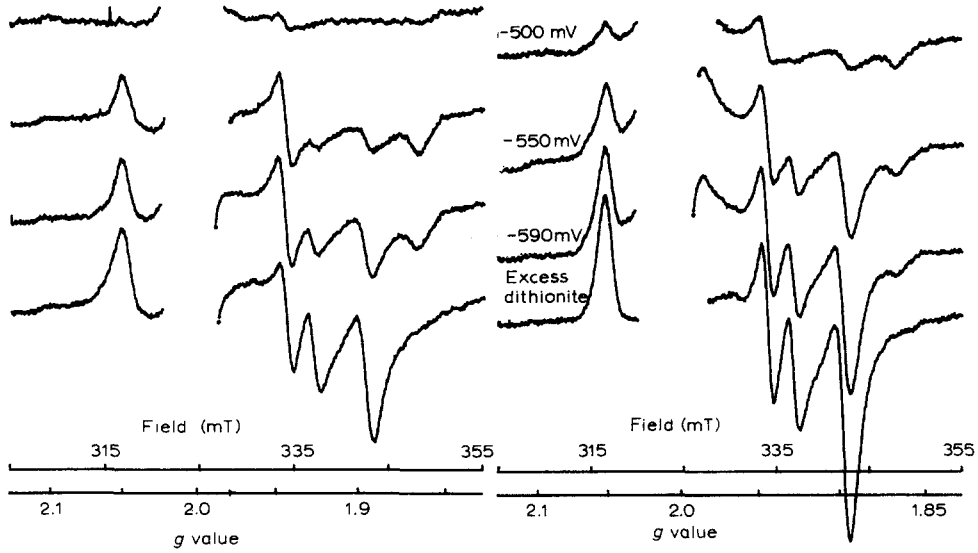


**Fig. 2. EPR spectra of Photosystem I particles prepared at different redox potentials. Left in aqueous medium, Right in 50% glycerol. Photosystem I particles were suspended in 0.1 M glycine-KOH buffer, pH 10, with 100 $\mu$M methyl viologen, 100 $\mu$M benzyl viologen, 100 $\mu$M Diquat, 100 $\mu$M Triquat and 100 $\mu$M Tetraquat as mediators and titrated to the required potential using 2% (w/v) sodium dithionite (in 0.1 M Tris-HCl buffer, pH 9.0) as reducing agent. EPR spectra were recorded as in Fig. 1.**

glycerol media. It is clear from these spectra that the reduction of both centres A and B begins at more oxidised potentials in the presence of glycerol. Fig. 3 shows the results of complete oxidation-reduction potential titrations of A and B in the presence and absence of glycerol. The midpoint potential ($E_m$) of both centres A and B is shifted to a more oxidised potential in the presence of glycerol, centre A from −540 mV to −510 mV and centre B from −590 mV to −545 mV. The effect is thus greater for centre B, as suggested by the results presented in Fig. 1. Titrations in glycerol are relatively difficult because of the high viscosity of the medium and variation in results was somewhat greater (±20 mV) than we usually obtain from titrations in aqueous media (±10 mV). The glycerol titrations showed slightly anomalous behaviour at very low potentials with a 20—30% increase in signal size occurring when excess dithionite was added to samples which had been equilibrated at the lowest measurable redox potential. This may indicate that not all of the iron-sulphur centres in the Photosystem I particles preparations are equally affected by glycerol.

There have been some discrepancies in the decay times reported for the back-reaction between $P\text{-}700^+$ and $X^-$ after illumination at cryogenic temperatures [4,16]. Fig. 4 shows the decay kinetics of signal I ($P\text{-}700^+$) following a 10 μs Xenon flash at 21 K in Photosystem I particles frozen with both centres A and B reduced in the presence and absence of glycerol. It is clear that the decay is considerably faster ($t_{1/2}$ = 110 ms) in 50% glycerol than in aqueous media ($t_{1/2}$ = 210 ms). The decay in the presence of glycerol is a mixture of a fast and slow component, probably because glycerol has not affected all of the Photosystem I reaction centres. The same results were obtained if the decay of $X^-$ was measured at 10 K.

Glycerol was also found to have an effect on the microwave power saturation characteristics of signal I ($P$-700), as shown in Fig. 5. The variation in

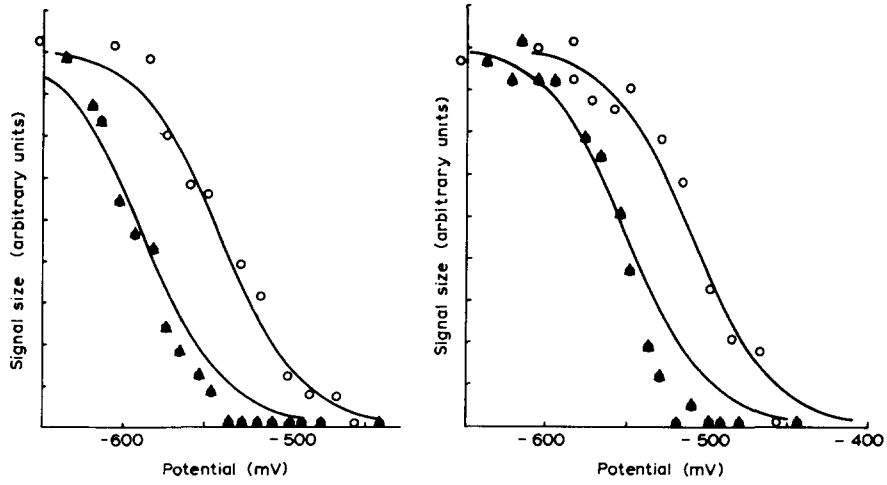


**Fig. 3. Oxidation-reduction potential titrations of centres A and B in aqueous medium and 50% glycerol. The titrations were carried out and spectra recorded as described in the methods and legend to Fig. 2. The curves drawn are the theoretical curves for a one electron transition giving the best fit to the data points. ○, 50% glycerol; ▲, aqueous medium. Left: Centre B, midpoints of the curves −590 mV and −545 mV. Right: Centre A, midpoints of the curves −540 mV and −510 mV.**

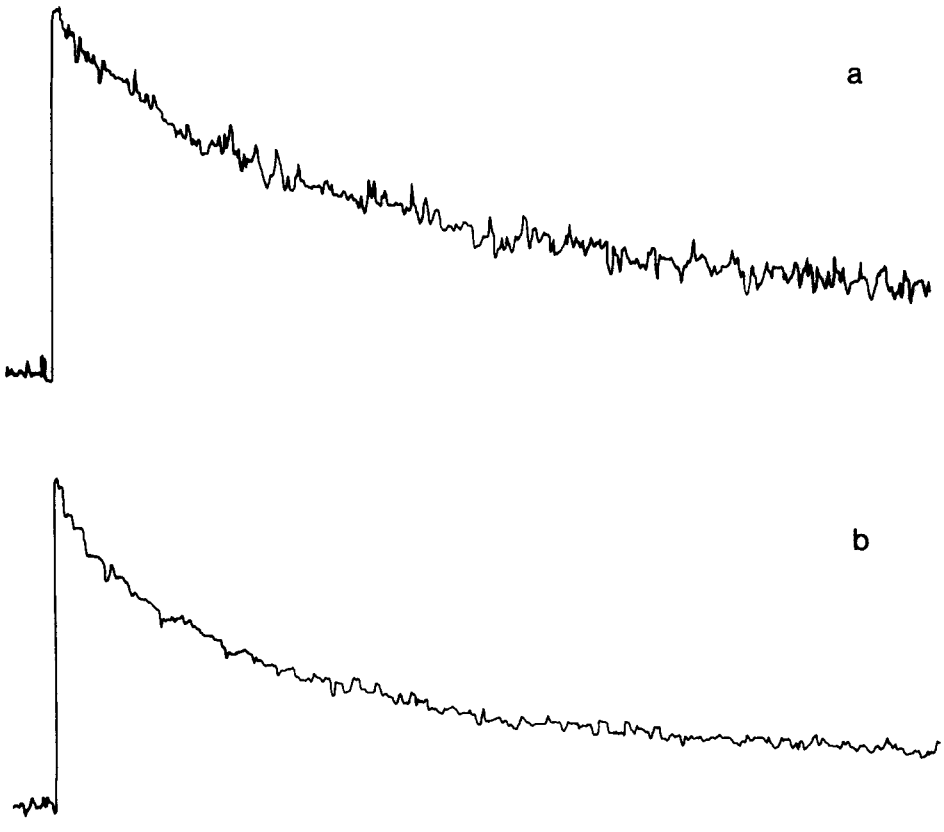


Fig. 4. Kinetics of the back-reaction between $P\text{-}700^{+}$ and $X^{-}$ at 21 K in Photosystem I particles in the presence and absence of glycerol. Photosystem I particles (0.2 mg chlorophyll/ml) in aqueous media (a) or 50% glycerol (b) were illuminated for 2 min at room temperature with the addition of dithionite (0.1% w/v), the light turned off for 30 s, and then frozen in the dark. The change in *P*-700 following flash illumination was measured at 21 K. Instrument settings: microwave power, 0.1 mW; modulation amplitude, 0.5 mT; instrument gain, 500. The field was set at the positive peak of signal I in the first derivative spectrum. The traces presented are the average of 128 10 μs xenon flashes, spaced apart by 10 s.

signal size of signal I with microwave power at 77 K has been plotted as log (signal size/(power)$^{1/2}$) vs. log power. In such a plot the line of points will be horizontal at microwave powers that are non-saturating. Using the method described by Rupp et al. [17] a curve based on the equation $S \propto P^{1/2}/(1 + P/P_{1/2})^{b/2}$ has been fitted to these points by a weighted least-squares procedure, which allows a quantitative determination of change in saturation characteristics in terms of $P_{1/2}$ (power at which the signal is half the size it would have been if not saturated) and $b$ (a inhomogeneity parameter which is 1.0 for a homogeneously broadened signal to 4.0 for a inhomogeneously broadened signal). This is necessary since a perturbation of a paramagnetic centre may change its saturation behaviour both by changing the half-saturation power $P_{1/2}$ or by changing the degree of homogeneous/inhomogeneous broadening. The curves fitted to the points displayed in Fig. 5 suggest that the presence of 50% glycerol alters the $P_{1/2}$ for signal I at 77 K in these particles from

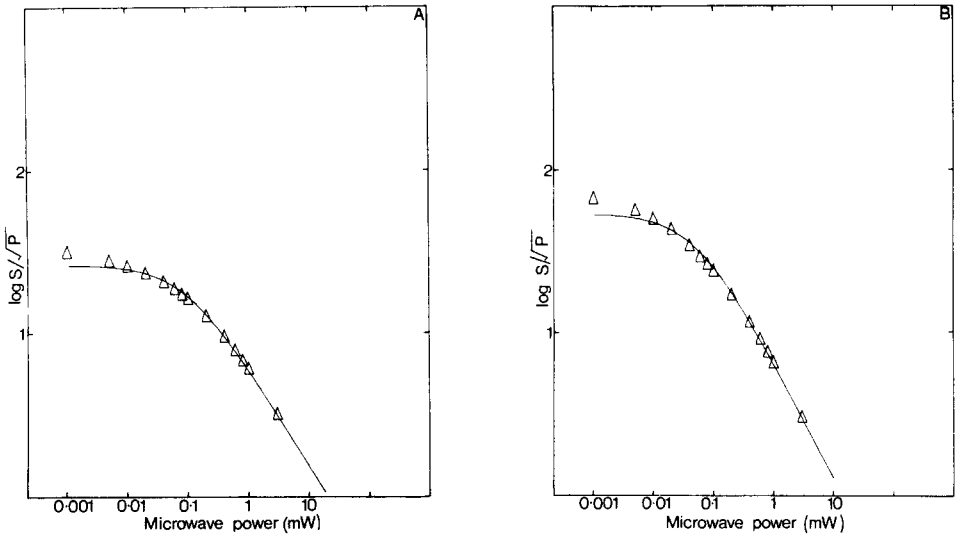


Fig. 5. The microwave power saturation characteristics of signal I at 77 K in Photosystem I particles in the presence and absence of glycerol. Photosystem I particles (0.6 mg chlorophyll/ml) were diluted with an equal volume of 50 mM Tris, pH 8.0 (A) or 50% glycerol (B), kept in the dark for 30 min and frozen in the dark. After illumination at 77 K the variation of the peak-to-peak signal intensity of signal I with microwave power was measured at 77 K with the following instrument settings: modulation amplitude, 0.2 mT; scan rate, 5 mT/min. The data was plotted as shown above, and the theoretical curves fitted to them as described in the text.

50 $\mu$W to 90 $\mu$W, and changes $b$ from 1.2 to 1.42. Care was taken to ensure that the samples for this comparison come from the same Photosystem I particle preparation, since we have recently found that the concentration of Triton X-100 present also affects the saturation characteristics of signal I. It is clear from Figs. 1 and 2 that glycerol is probably having an effect on the saturation characteristics of the bound iron-sulphur centres, since the signals from the Photosystem I particles prepared in glycerol are larger than those from the same particles in aqueous media. However it was not possible to quantify this effect, since attempts to fit curves to the saturation data gave inhomogeneity parameters of less than 1.0. This is due to the presence of centres with different saturation characteristics, and confirms that not all the bound iron-sulphur centres are equally affected by the presence of glycerol.

## Discussion

These experiments clearly show that glycerol has extensive effects on the environment and components of the Photosystem I reaction centre. It seems likely that the variation in potential of the 'primary electron acceptor' in Photosystem I measured by optical and EPR procedures arises form this effect. The results of Ke and coworkers [10,11] were obtained using glycerol solutions, Lozier and Butler [9] do not give details of sample preparation but presumably used the same technique as in a second paper published simultaneously [18] in which the samples contained 50% glycerol. The value of about −530

mV measured by optical techniques [9,10] probably reflects the potential of centre B in glycerol and these results can therefore be interpreted as confirming that centre B acts in the Photosystem I electron transfer chain between X and centre A as described in the introduction to this paper.

We have carried out preliminary investigations using ethylene glycol and sucrose which are also used to prepare samples for low temperature optical work. Ethylene glycol appears to have the same or greater effects than glycerol on the light-induced spectra and kinetics. Sucrose has little or not effect on the light-induced spectrum and a small effect on the kinetics.

Considerable caution should therefore be used in interpreting the results of experiments on chloroplast preparations in glycerol or ethylene glycol and the use of these results to predict the in vivo mechanism of photosynthesis.

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