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COMPARISON OF THE EPR PROPERTIES OF PHOTOSYTEM I IRON-SULPHUR CENTRES A AND B IN SPINACH AND BARLEY

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Summary

The properties of Photosystem I iron-sulphur centres A and B from spinach and barley chloroplasts were investigated by electron paramagnetic resonance spectroscopy (EPR). Barley chloroplasts were shown to photoreduce significant amounts of centre B at cryogenic temperatures unlike those from spinach which only photoreduced centre A. Centre B in barley chloroplasts was also reduced by dithionite before centre A and the EPR spectrum of reduced centre B was obtained. Illumination of barley chloroplasts at 15 K where centre B was chemically reduced resulted in the reduction of centre A and the appearance of spectral features indicating interaction between the two reduced centres. The variation in behaviours of iron-sulphur centres A and B between species favours a scheme of electron flow for Photosystem I where either centre A or centre B act as parallel electron acceptors from the earlier acceptor X.

Introduction

Photosystem I of higher plants, green and blue-green algae (cyanobacteria) contains two membrane bound iron-sulphur centres, A and B, which function as electron acceptors [1]. These have been studied using low temperature electron paramagnetic resonance spectroscopy (EPR) where it was found that in spinach, centre A was reduced before centre B either chemically or photochemically at cryogenic temperatures [2,3]. Centre A has a midpoint redox

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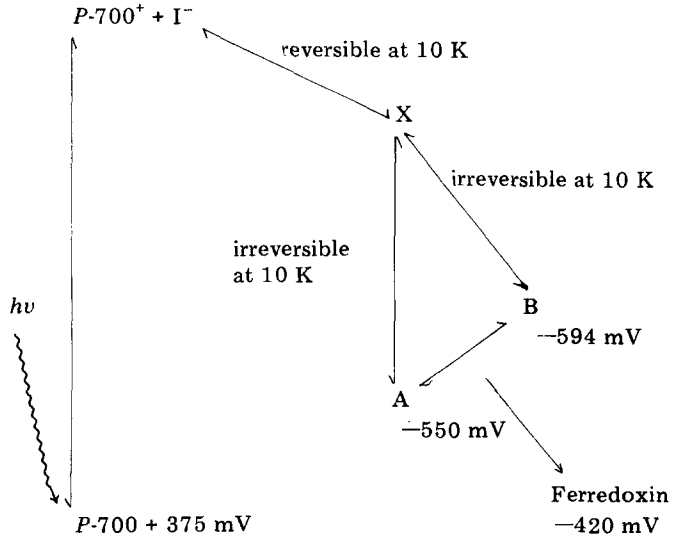
potential (E_m) of −550 mV and a spectrum with anisotropic g-values at g = 2.05, 1.94, 1.86 [4,5]. On further chemical reduction the spectrum becomes more complex with features at g = 2.05, 1.94, 1.92, 1.89 and reflects the reduction of the second iron-sulphur centre B (E_m = −585 mV) together with interaction between the two centres [4,6]. It has also been demonstrated that *P*-700, centre A and centre B are present in equivalent amounts [7] although their EPR properties result in apparently unequal signal intensities. The incorporation of glycerol into Photosystem I preparations was found to change the midpoint redox potentials of centre A to −510 mV and centre B to −545 mV and result in extensive reduction of centre B as well as centre A upon illumination at cryogenic temperatures [8]. This result partly clarified the dispute over the role of centres A and B caused by experiments incorporating glycerol and Evans and Heathcote [8] concluded that centre B was a component between X (A_2) and centre A.

Recently a variation to this situation was found in membrane preparations from the thermophilic cyanobacterium *Phormidium laminosum* where centre B was chemically reduced before centre A [9]. This occurred even in the absence of glycerol and indicated that centre B had a more positive redox potential than centre A. However, illumination at cryogenic temperatures resulted in the reduction of centre A. The observation of the spectrum of centre B at g = 2.065, 1.935 and 1.882 in chemically reduced samples confirmed the features of the fully reduced A and B spectrum as being due to spin-spin exchange interaction between the two centres. The authors indicated that electrons from *P*-700 at room temperature would equilibrate between centres A and B in accordance with their redox potentials but when frozen, centre A may be better placed to accept electrons than centre B (Scheme I).

The results presented here arise from an EPR study of chloroplasts from



Scheme I. Photosystem I in spinach chloroplasts.

barley (*Hordeum vulgare*), a monocotyledonous plant. Barley was chosen because a large number of mutants have been selected from this plant. Several of these mutants have been photochemically characterised [10] and among these a few deficient in either Photosystem I [11,12] or Photosystem II [10,13] have been found. These mutants should be ideal for EPR studies of each of the two photosystems without the interference of signals from the other. Secondly, the thylakoid polypeptide composition of barley has been extensively studied [13,14] and this combined with the use of mutants should eventually allow the assignment of the iron-sulphur centres A and B to individual polypeptides. As a basis for such studies, we report here on a comparison of the EPR properties of centres A and B in spinach (*Spinacea oleracea*) and wild-type barley chloroplasts.

Materials and Methods

Spinach (*Spinacea oleracea* cv Dynamo) was grown in hydroponic culture at 22°C under artificial lighting using a regime of 12 h day/12 h night to suppress flowering. Leaves from young plants (5—12 weeks) were taken and chloroplasts were prepared as described in Ref. 15.

Barley (*Hordeum vulgare* cv Svalöfs Bonus or cv Mazurka) was grown either as above or under similar conditions as described in Ref. 13. The barley grew more rapidly than spinach and 7-day-old plants were used to make chloroplasts as described in Ref. 15 but required centrifugation at 8000 × *g* for 5 min to pellet the washed broken chloroplasts. Chlorophyll was assayed as described in Ref. 16.

Chloroplasts from pea (*Pisum sativum*) and lettuce (*Lactuca sativa*) were prepared as above. Membrane fragments from *Scenedesmus obliquus* prepared by centrifugation of French press broken cells were prepared by Dr. P. Heathcote. *Phormidium laminosum* particles were a gift from Dr. A.C. Stewart.

EPR measurements were made using a Jeol Felx spectrometer and the sample temperature was maintained using an Oxford Instruments liquid helium cryostat. A 1000-W projector was used for illumination of samples. Spectra were recorded into a Tektronix 4051 computer which was used to plot the figures shown. *g*-values were calculated using an external standard of Mn^{2+} in MgO and were checked internally using the *g*-value 2.0048 for Signal II. *g*-value and field scales used in the figures are approximate.

Results

Spinach chloroplasts reduced with ascorbate in the dark and then frozen have an EPR spectrum near $g = 2.0$ at 15 K as shown in Fig. 1(a). There is a signal near $g = 1.90$ which has been attributed to the reduced Rieske iron-sulphur centre of the chloroplast electron transport chain [17] together with a radical due to some Signal II (which is not fully shown). After illumination of the sample at 15 K signals due to reduced iron-sulphur centres appear irreversibly, Fig. 1(b). The difference spectrum obtained by subtraction of the two spectra reveals the spectrum of reduced centre A with *g*-values at 2.048, 1.947

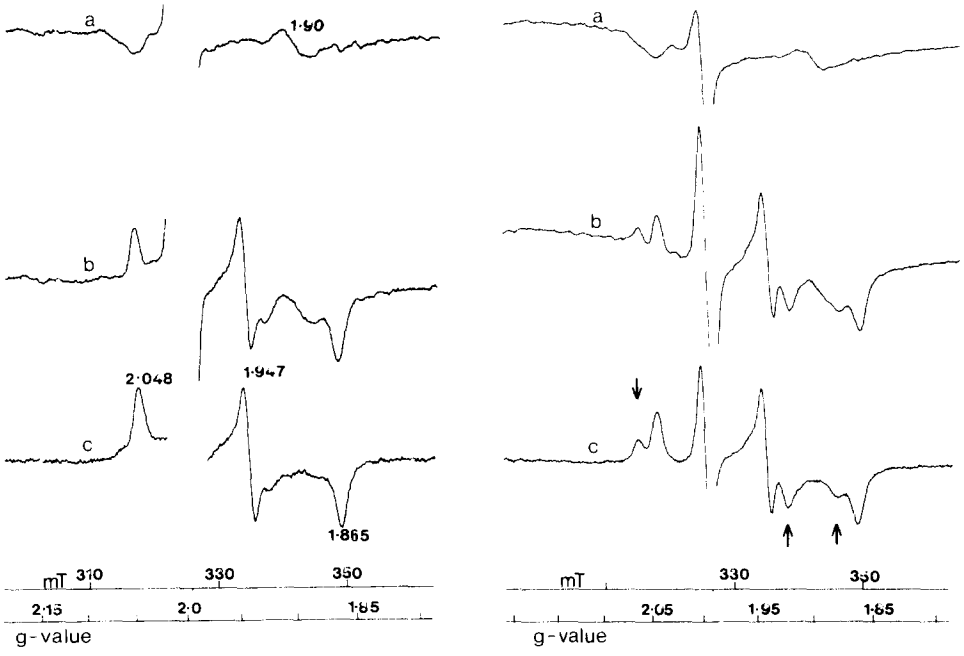


Fig. 1. EPR spectra of dark adapted spinach chloroplasts frozen 15 min after the addition of 10 mM sodium ascorbate (a) before and (b) after illumination at 15 K; (c) difference spectrum (b) — (a). Microwave power, 10 mW; time constant, 0.1 s; scan rate, 25 mT/min; modulation amplitude, 1 mT; instrument gain 10^3; temperature, 15 K; frequency, 9.117 GHz. Chlorophyll concentration 3 mg/ml.

Fig. 2. EPR spectra of dark adapted barley (cv Svalöfs Bonus) chloroplasts frozen 15 min after the addition of 10 mM sodium ascorbate (a) before and (b) after illumination at 15 K; (c) difference spectrum (b) — (a). Conditions as Fig. 1.

and 1.865. The radical region at $g = 2.0$ showing the corresponding increase in $P\text{-}700^+$ upon illumination is omitted.

Fig. 2 demonstrates the same experiment as Fig. 1 but using barley chloroplasts (cv Svalöfs Bonus). Fig. 2(a) shows the spectrum of barley chloroplasts reduced by ascorbate in the dark before freezing and is very similar to Fig. 1(a). However after illumination of the sample at 15 K, Fig. 2(b), as well as the appearance of centre A and a $P\text{-}700^+$ radical, additional features are seen at g-values 2.065, 1.925 and 1.88. These are arrowed in the difference spectrum Fig. 2(c) which shows the irreversible changes induced by illumination. The additional features have g-values corresponding to those observed by Cammack and coworkers [9] for centre B in *P. laminosum* and indicate that in a proportion of Photosystem I reaction centres, centre B has been photoreduced at 15 K.

The sample giving rise to the spectra in Fig. 2 was stored at 77 K for a few days and then then the EPR spectrum at 15 K was redrawn. It was found that some $P\text{-}700^+$ and reduced iron-sulphur centres A and B had recombined resulting in smaller EPR signals. Comparison of spectra revealed that in the dark at 77 K most of the centre A became oxidised but most of the centre B remained reduced. After illumination at 15 K more $P\text{-}700^+$ and reduced centres A and B reappeared but the relative proportion of centre B had increased over that seen

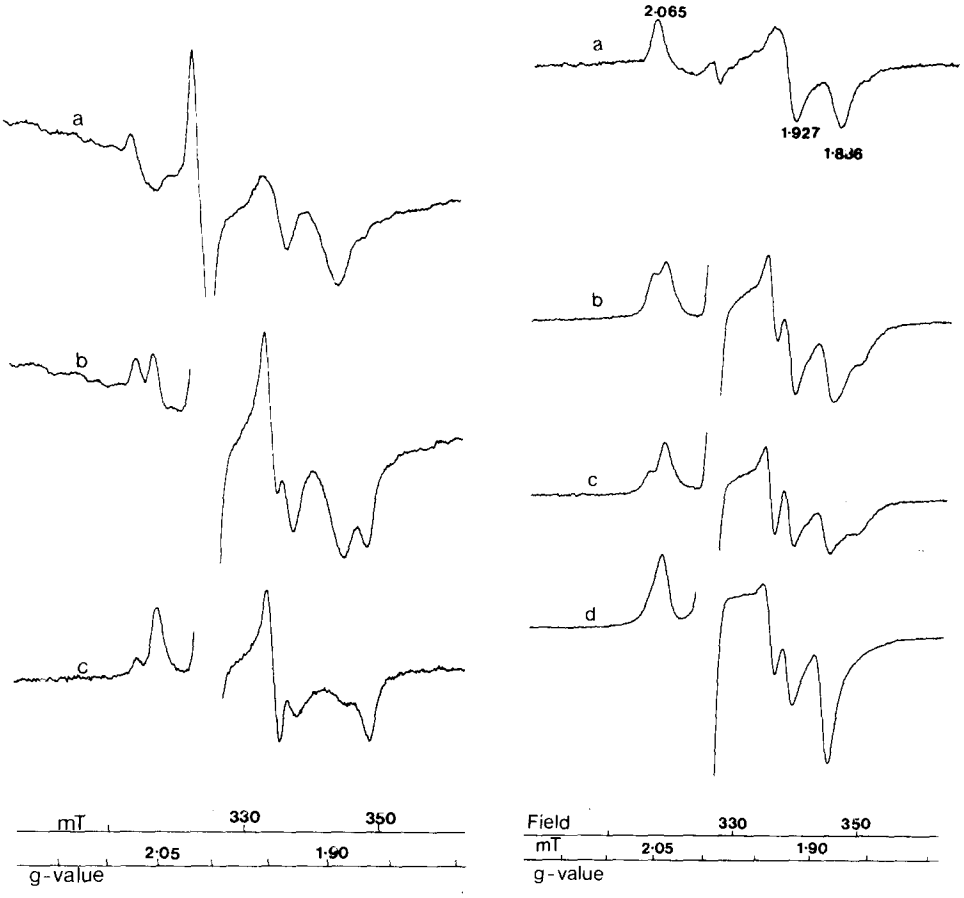


Fig. 3. EPR spectra of barley (cv Svalöfs Bonus) chloroplasts dark adapted and frozen 15 min after the addition of 10 mM sodium ascorbate. Illuminated at 15 K on two separate days: (a) spectrum after dark storage at 77 K; (b) after illumination at 15 K; (c) difference spectrum (b) — (a). Conditions as Fig. 1.

Fig. 4. EPR spectra of barley (cv Svalöfs Bonus) Photosystem I particles: (a) reduced for 2 min at pH 10 with dithionite; (b) illuminated at 15 K; (c) difference spectrum showing light induced signal; (d) barley chloroplasts reduced with dithionite for 30 min at pH 10. Conditions as Fig. 1 except instrument gain: (a) $2 \cdot 10^3$; (b) and (c) 10^3; (d) $5 \cdot 10^2$.

following the initial illumination.

Fig. 3 shows the spectrum of a sample of barley chloroplasts originally reduced with ascorbate in the dark before freezing and then illuminated on two separate days at 15 K before storage at 77 K in the dark for several days. Fig. 3(a) shows the spectrum at 15 K which has a radical mainly due to $P\text{-}700^+$ but an unusual reduced iron-sulphur spectrum which is clearly different from centre A (Fig. 1(c)). The absence of centre A is confirmed by the lack of a peak at g-value 2.05 with the main g-values of 2.065, 1.93, 1.88 having similar values to those of reduced centre B [9], but the presence of the reduced Rieske iron-sulphur centre (Fig. 1(a)) distors the region near $g = 1.9$. After illumination at 15 K centre A plus some centre B is reduced giving the spectrum shown in Fig. 3(b). The difference spectrum showing the effect of illumination is shown in Fig. 3(c). By comparing these spectra to Fig. 2 it can be seen that more total

centre B is present but that smaller increases in centre B occur on subsequent illuminations than after the initial one. The gradual increase in the amount of reduced centre B can be demonstrated more rapidly by warming samples illuminated at 15 K to 125 K for a few minutes and then recooling to 15 K. This has a similar effect to storage at 77 K in that almost all the centre A signal but only some of the centre B decays, leaving a spectrum similar to Fig. 3(a). After illumination at 15 K, when the warming and cooling cycle is repeated a gradual accumulation of centre B is seen. No transfer of electrons between centre A and centre B was demonstrated.

To obtain the spectrum of barley centre B, Photosystem I particles were reduced for a few minutes with dithionite in the dark before freezing. The spectrum of barley centre B is shown in Fig. 4(a) and has peak *g*-values of 2.065, 1.927 and 1.886. After illumination of this sample at 15 K, the spectrum becomes more complex as more centre A and centre B are reduced, Fig. 4(b). The difference spectrum, Fig. 4(c) is complex due to interaction between centre A and centre B when reduced in the same Photosystem I reaction centre. The spectrum of Photosystem I with fully reduced centres A and B is shown in Fig. 4(d). This has peak *g*-values 2.05, 1.935, 1.918 and 1.885. Comparison of this spectrum with Fig. 4(b) and 4(c) reveals that illumination of the sample containing reduced centre B causes the reduction of centre A in the same Photosystem I reaction centre and therefore causes the interaction which changes the spectral shape of the individual centres. Note that the gain in Fig. 4(a) is four times that of Fig. 4(d) and twice that of Figs. 4(b) and 4(c).

Similar results to those shown for the barley variety Svalöfs Bonus were obtained with a second variety, Mazurka. Chloroplasts from pea and lettuce were also investigated and proved to have properties similar to spinach in the behaviours of their Photosystem I iron-sulphur centres in that only low amounts of centre B were photoreduced at cryogenic temperatures. However, membrane fractions from *P. laminosum* and *S. obliquus* photoreduced centre B in significant quantities at 15 K and showed similar properties to barley chloroplast Photosystem I. Membrane fractions from another cyanobacterium *Anacystis nidulans* behaved in a similar way to spinach chloroplasts.

Discussion

The spectrum of reduced centre B in barley, Fig. 4(a) confirms the work of Cammack and coworkers [9] using the thermophilic cyanobacterium *P. laminosum*. The spectra of reduced centre B in the two cases are almost identical in overall shape and *g*-values and strengthen the view that this is the characteristic spectrum. As shown in Ref. 9 the spectrum showing fully reduced centre A and centre B, Fig. 4(d), demonstrates the interaction between the two iron-sulphur proteins when they are both reduced in the same Photosystem I reaction centre. The $g = 2.065$ feature of centre B disappears as does the $g = 1.86$ feature of centre A and a $g = 1.92$ feature appears which is present in neither individual spectrum. Cammack et al. [9] concluded that this was due to electron exchange interaction between the spins of the two centres and implied a pathway for electron transfer between the centres which may not function at low temperatures.

Photoreduction of centre B at cryogenic temperatures is observed in Photosystem I particles when these are prepared in high concentrations of glycerol [1,8]. The reason for this is a glycerol mediated shift in the redox potentials of both iron-sulphur centres, A and B but having the effect of bringing their midpoint potentials closer together [8]. In the present study, the observations on the photoreduction of centres A and B were all performed in the absence of glycerol. The results obtained here must therefore not be confused with those from the previous study.

The results presented here show that there is considerable variation in the properties of centres A and B between species. In barley chloroplasts the photoreduction of centre B in reaction centres which have oxidised centre A is demonstrated by the appearance of a pure centre B spectrum with the $g = 2.065$ peak indicating a lack of the interaction described earlier. This observation taken together with the photoreduction of centre A in barley Photosystem I reaction centres which have chemically reduced centre B argues against a simple linear scheme X → centre B → centre A for Photosystem I electron flow. Although no transfer of electrons from either centre A to centre B or vice versa was found at cryogenic temperatures, it may occur at physiological temperatures, it may occur at physiological temperatures as suggested in Ref. 9. The results presented here therefore favour the theory that centre A or centre B accept electrons from X in a ratio determined by their midpoint redox potentials and that transfer between the two iron-sulphur centres may occur under physiological conditions. The electron transfer reactions related to Photosystem I, based on a similar scheme in Ref. 18 are depicted in Scheme I.

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