E.P.R. SPECTRA OF IRON-SULPHUR PROTEINS IN DIMETHYLSULPHOXIDE SOLUTIONS: EVIDENCE THAT CHLOROPLAST PHOTOSYSTEM I PARTICLES CONTAIN 4Fe-4S CENTRES

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SUMMARY

A number of iron-sulphur proteins have been shown to undergo reversible changes in structure in 80% dimethylsulphoxide solution. EPR* spectra of the reduced proteins in this state show characteristic lineshapes and temperature dependence, according to whether the centres are of the 2Fe-2S or 4Fe-4S type. EPR spectra of Photosystem I particles from spinach chloroplasts, reduced in 80% dimethylsulphoxide, indicate the presence of 4Fe-4S centres. The integrated intensity of these signals is consistent with their having arisen from the membrane-bound iron-sulphur proteins of Photosystem I.

The iron-sulphur proteins are electron transfer agents found in many biological systems (1,2). At present two types of centre containing iron and acid-labile sulphur are known: a 2Fe-2S centre containing two spin-coupled iron atoms bridged by two labile sulphur ligands, and a 4Fe-4S centre in which the atoms are arranged in a box-like structure. Both types of centre in the reduced state can give rise to EPR* signals around g = 1.96. The EPR spectrum cannot be used as a diagnostic test for the type of

*Abbreviations: EPR, electron paramagnetic resonance; DMSO, dimethylsulphoxide; PSI, photosystem I.

centre, because the protein environment can alter the lineshape and temperature dependence of the signal.

Studies on a number of soluble iron-sulphur proteins have shown that, under anaerobic conditions, denaturing agents such as guanidine-HCl and DMSO* can cause the protein to unfold reversibly, leaving the iron-sulphur centre intact (3-5). The EPR spectra of the reduced proteins, unfolded by DMSO treatment, are characteristic of the type of centre present (6). 2Fe-2S centres give rise to a rather isotropic signal which is readily detected at temperatures up to 150 K; 4Fe-4S centres give an axial or near-axial signal which is only readily detected below 35 K, indicating a more rapid electron spin relaxation. The latter EPR signal resembles that of the reduced analogue compound, $[Fe_4S_4(RS)_4]^{3-}$ (7).

These observations suggested a method of distinguishing between the two types of iron-sulphur centres in complex proteins. We now report the application of this technique to the primary electron acceptor complex of Photosystem I in spinach chloroplasts.

Malkin and Bearden (8) detected an EPR signal in spinach chloroplast fragments due to a membrane-bound iron-sulphur centre which could be photo-reduced at low temperatures. The lineshape of this signal suggested at first that it was a plant-type (2Fe-2S) ferredoxin. Subsequent studies (9,10) indicated that two photoreducible iron-sulphur centres (Centres A and B) are present in the Photosystem I complex. The isolation from photosynthetic membranes of a protein with properties similar to those of bacterial four-iron ferredoxins (11) indicates that at least one of the centres may be of the 4Fe-4S type.

EXPERIMENTAL

Photosystem I particles: PSI* particles were prepared from broken, washed spinach chloroplasts by treatment with Triton X-100 as described by Vernon & Shaw (12). After centrifugation to remove PSII particles, the extract was diluted with three volumes of 0.02-M Tris-Cl buffer, pH 8.0, containing 0.2% Triton. The extract, equivalent to 200 mg chlorophyll in the original chloroplasts, was then applied to a 4.5 x 20cm column of DEAE-cellulose (Whatman DE23). The PS I particles became bound to the column, and, after washing with 1l of buffer, were eluted as a green band with 0.3-M NaCl in the same buffer. The preparation was concentrated by ultrafiltration on an Amicon Diaflo XM50 membrane. The P700:chlorophyll ratio was between 1:100 and 1:120. In some experiments, further chromatography on DEAE-cellulose was used to improve the ratio to 1:60.

P700 was measured by the oxidized minus reduced absorption difference at 700 nm using an Aminco-Chance DW2 spectrophotometer (13).

Preparation of EPR samples: In order to obtain a reproducible EPR signal from reduced DMSO-treated particles, it was found necessary to carry out the preparation at 0° C under oxygen-free conditions. The solution of particles was depleted of oxygen by blowing Ar gas over the surface for 30 min. Because of its high freezing point (18°C), DMSO (spectroscopic grade from BDH Chemicals Ltd., Poole, Dorset, U.K.) was kept at room temperature and bubbled with Ar before use. Sodium dithionite solution, 0.2-M, was prepared under Ar in 0.4-M Tris-Cl, pH 9.0.

Four volumes of DMSO were added to one of PS I particles in a quartz EPR tube, purged with Ar, on ice, and stirred vigorously. After 2 min, 5 mM dithionite was added and stirred, and after a further 2 min the sample was frozen in liquid nitrogen.

RESULTS AND DISCUSSION

Typical examples of the effect of DMSO treatment on the EPR spectra of reduced iron-sulphur proteins are shown in Fig. 1 (a) to (d). The soluble ferredoxin from spinach chloroplasts, which has a 2Fe-2S centre, gives rise to a narrow signal around g=1.96

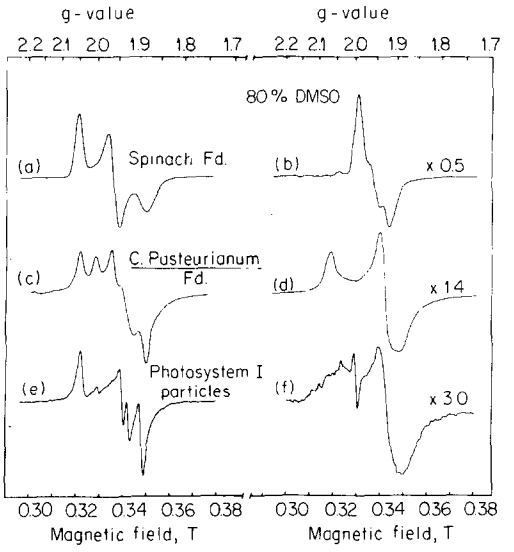

Fig. 1: EPR spectra of (a) & (b) spinach ferredoxin, (c) & (d) C. pasteurianum ferredoxin, and (e) and (f) PSI particles, all reduced with dithionite. The left-hand spectra were of samples in 0.8M NaCl, 20mM Tris-Cl, pH 8.0, and the right-hand spectra in the presence of 80% v/v DMSO. EPR spectra were recorded on a Varian E4 spectrometer with settings: microwave power, 20mW, frequency 9.20 GHz; modulation amplitude 1 mT, frequency 100 kHz; temperature, 20K.

(Fig. 1b). The ferredoxin from Clostridium pasteurianum, which has two 4Fe-4S centres per molecule, gives a near-axial signal at g=2.06, g=1.92.

Fig. 1(e) shows the spectrum of PSI particles in which the iron-sulphur centres were fully reduced by treatment with dithionite and illumination during freezing. After treatment of a sample of particles with 80% DMSO and reduction, the spectrum 1(f) was obtained. Double integration and correction for dilution gave a ratio of 1.0:1 for the intensities of the two signals. Because of the difficulty of reducing the particles completely and other errors, the uncertainty of this measurement is probably as much as $\pm$20%.

Similar spectra to Fig. 1(f) were obtained by treating PSI

particles prepared from spinach chloroplasts by French press treatment (9) and PSI particles from the blue-green alga Chlorogloea fritschii, with 80% DMSO. Dimethylformamide gave similar results to DMSO.

The shape of the signal 1(f) is clearly similar to that obtained with the bacterial ferredoxin, Fig. 1(d), and shows a similar temperature dependence, appearing only below 35K, and being readily saturated with microwave power at 10K. No signals characteristic of 2Fe-2S proteins in 80% DMSO (c.f. Fig. 1b) were detected at 77K.

Reduction of the iron-sulphur centres in the particle preparations is normally slow, and complete reduction is achieved only by prolonged incubation with dithionite, or by illumination during freezing. By contrast the centres were rapidly reduced by dithionite in the presence of 80% DMSO, maximal signal intensity being obtained after reduction for 2 min at 0 °C; illumination had no further effect. This difference is presumably due to disruption of the organization of the proteins in the particles, and exposure of the iron-sulphur centres to solvent. This effect has been observed with other proteins in which the centres are difficult or impossible to reduce in aqueous solution, e.g. Chromatium ferredoxin or Chromatium high-potential iron-sulphur protein (5,6).

A third EPR-detectable electron acceptor, of unknown chemical composition, has recently been observed in PSI particles (14,15). This component has spectral features at g=2.07, 1.86 and 1.76, and is photo-reduced reversibly by P700 at liquid helium temperatures when the iron-sulphur centres A and B are fully reduced. This component was therefore proposed to be the primary electron acceptor. It is possible that this component is a 4Fe-4S

centre, but in view of its highly unusual g-values it seems more likely to be a different species which, in the presence of 80% DMSO, is either denatured or converted to an EPR-silent form.

In conclusion, our results indicate that PSI particles contain 4Fe-4S centres, and it is probable that these are the membrane bound iron-sulphur centres A and B. There is no evidence for the presence of membrane-bound 2Fe-2S centres.

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