Mössbauer Spectra of Photosystem-I Reaction Centres from the Blue-Green Alga *Chlorogloea fritschii*

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Substantial amounts of iron have been shown by Mössbauer spectroscopy to be present in Photosystem-I preparations from the blue-green alga *Chlorogloea fritschii*. Changes in the spectra on chemical reduction provide evidence that some of this iron is very similar to that found in the 4Fe-4S centres of ferredoxins. Such reduced samples also show e.p.r. signals consistent with maximum reduction of iron-sulphur centres A and B of Photosystem I. An unchanged component in the spectra indicates, assuming all centres A and B are reduced, the presence of another iron-containing species.

In a previous study (Evans *et al.*, 1977) a form of iron similar to that found in non-magnetic Fe-S centres of ferredoxins was detected by Mössbauer spectroscopy of crude membrane preparations from the blue-green alga *Chlorogloea fritschii*. A second iron component, possibly associated with iron storage or transport was also found, and its amount decreased when the iron in the algal growth medium was lowered, being barely detectable when the algae were grown on 10% of the normal iron concentration.

It has been suggested, on the basis of measurements by e.p.r. spectroscopy (Malkin & Bearden, 1971; Cammack & Evans, 1975), that Fe-S centres are associated with the primary electron-acceptor complex of Photosystem I. Three distinct signals (A, B and X) have been shown to be present at various stages of light-induced and chemical reduction, of which signals A and B have been unambiguously associated with iron-containing centres (Evans *et al.*, 1976), their e.p.r. characteristics being similar to those of ferredoxins.

In the present paper, studies on Photosystem-I particles prepared from the crude membranes of *C. fritschii* are described. The Mössbauer signal of the non-magnetic Fe-S centre previously detected in the crude membrane preparations was observed only from the fraction purified with respect to Photosystem-I activity. Changes in the Mössbauer spectrum on chemical reduction of the Photosystem-I preparations are described and compared with the results obtained from e.p.r. spectroscopy of the same samples.

Experimental

Chlorogloea fritschii (Culture Collection of Algae and Protozoa, no. 1411/1; Department of Botany, University of Cambridge, Downing Street, Cambridge, U.K.) was grown as previously described on 10% of standard iron concentration (Evans *et al.*, 1977) with iron enriched in ^{57}Fe . Fractions enriched in Photosystem I, as measured by pigment P700 content, were isolated by digitonin and Triton extraction of the crude membranes by the method of Bengis & Nelson (1975).

Pigment P700 was measured as the difference between a sample oxidized by potassium ferricyanide and one reduced by sodium dithionite at 704 nm and by using an absorption coefficient of $64000\text{ M}^{-1}\cdot\text{cm}^{-1}$ (Ke, 1973), the spectra being measured by a Pye-Unicam SP.1800 spectrophotometer. Chlorophyll *a* was measured by the method of Strain *et al.* (1971).

The Photosystem-I preparations used had a pigment P700/chlorophyll *a* ratio of approx. 1:35 and retained the light-induced e.p.r. signals described by Malkin & Bearden (1971) and attributed to the Fe-S centre.

Reduced samples were prepared in the Perspex Mössbauer sample holder (1.5 ml total vol.) by reduction in the light (5 min illumination by a 1000 W tungsten-iodine lamp) with 0.04% sodium dithionite in the presence of Methyl Viologen ($5\text{ }\mu\text{M}$). A portion (0.2 ml) for e.p.r. measurements was removed to a silica tube (3 mm int. diam.). Both samples were frozen rapidly in liquid nitrogen in total darkness.

The e.p.r. characteristics of samples reduced in this fashion have been described (Heathcote *et al.*, 1978). Oxidized samples were prepared by freezing the sample rapidly, without addition of reductant, in liquid nitrogen in a Perspex (Lucite) sample holder. Mössbauer spectroscopy was performed as previously described (Evans *et al.*, 1977), as was e.p.r. spectroscopy (Evans *et al.*, 1976).

Results and Discussion

Fig. 1 shows the comparison between the Mössbauer spectra obtained at 77K from (a) the crude membrane fraction and (b) the purified Photosystem-I fraction. Neither of these spectra may be satisfactorily computer-fitted on the assumption of only one iron site being present, but the great similarity between the spectra suggests that the same form of iron is present in both fractions, i.e., that ferredoxin-like Fe-S centres are present in both the crude membrane (Evans *et al.*, 1977) and the Photosystem-I fractions.

The changes of the Mössbauer spectra on reduction are shown in Fig. 2(a) at 195 K, 2(b) at 77 K and 2(c) at 4.2 K. Clearly these changes are complex, but they are nevertheless reproducible from sample to sample. The state of reduction, as monitored by e.p.r. spectroscopy, is that signals A and B are observed with approximately maximum intensity, i.e., all reducible centres A and B are reduced, whereas signal X is not observed, indicating that component X is still oxidized.

Fig. 1. Mössbauer spectra taken at 77K of (a) the crude membrane fraction (total chlorophyll *a*, 15mg; pigment P700/chlorophyll *a* ratio, 1:150) and (b) the Photosystem-I fraction (total chlorophyll *a*, 0.3mg; pigment P700/chlorophyll *a* ratio, 1:35)

Table 1. Mössbauer parameters of oxidized and reduced Photosystem-I samples at 77K

Spectra were analysed as described in the text. The chemical shift relative to pure iron at room temperature, δ , and quadrupole splitting, ΔE_Q , are given in mm/s with an accuracy of $\approx \pm 0.02$ mm/s. Also given for comparison purposes are parameters for the 4Fe-4S centre of *Bacillus stearothermophilus* (Middleton *et al.*, 1978).

	Component	δ	ΔE_Q
Photosystem-I samples			
Oxidized	{ O1	0.42	0.72
	{ O2	0.42	1.15
Reduced	{ R1	0.45	1.14
	{ R2	0.66	1.70
<i>Bacillus stearothermophilus</i>			
Oxidized		0.43*	0.97*
Reduced	{ 1	0.49	1.20
	{ 2	0.50	1.84

* Mean values of several closely similar components.

In the absence of an applied magnetic field, the most distinct differences between oxidized and reduced samples are observed at 77 K. The parameters obtained by computer analysis are given in Table 1 and represented by the 'stick' spectra in Fig. 2(b). The results of two component fits are given, as it was found that this was the minimum number of components required to reproduce the major features of the spectra. The actual situation may well be much more complicated, but this cannot be established from the present spectra. Under the present conditions of reduction the net changes in the spectra appear to indicate that most of the iron in the samples, represented by doublets O2 and R1, is unaffected by the reduction. An ill-defined minority component, represented by the doublet O1, present on the inside of doublet O2 in the oxidized sample, is not present in the reduced sample, which has instead a reasonably well-defined component, represented by the doublet R2, on the outside of doublet R1. The parameters of the 4Fe-4S ferredoxin from *Bacillus stearothermophilus* (Middleton *et al.*, 1978) are given in Table 1 for comparison purposes. The changes observed at 77 K on reduction of the Photosystem-I samples are consistent with the hypothesis that ferredoxin-like centres are involved; both the chemical shift and the quadrupole splitting increase, as expected from previous experience. From these spectra alone no reliable estimate of the fraction of iron that is changed on reduction can be made, as this is strongly dependent on the particular assumptions used in the analysis of the spectra, which have neither a sufficiently high signal-to-noise ratio nor sufficient resolution for a unique best fit to be found.

Fig. 2. Mössbauer spectra of oxidized (i) and reduced (ii) Photosystem-I fraction in zero applied magnetic field at (a) 195K, (b) 77K and (c) 4.2K

The 'stick' spectra in (b) are explained in the text.

The spectra at 195K (Fig. 2a) are not consistent with the behaviour of known 2Fe-2S ferredoxins on reduction, which show well-separated quadrupole doublets at this temperature, arising from distinct Fe^{2+} and Fe^{3+} atoms within the centre (see e.g. Rao *et al.*, 1971).

The spectra at 4.2K (Fig. 2c) show more complex behaviour than 77K. The reduced spectrum appears to be the superposition of the quadrupole doublet observed in the oxidized spectrum and a broad, ill-defined region of absorption over the range

-2.0 to +4.0 mm/s. Such broadening at 4.2K is relatively common in the Mössbauer spectroscopy of paramagnetic iron-containing proteins (e.g. Lang, 1970). In such cases it is often advantageous to apply an external magnetic field, which decouples the electronic and nuclear spins and sharpens the magnetic hyperfine spectrum. Fig. 3 shows spectra of the reduced sample at 4.2K with magnetic fields of (a) 0.5T and (b) 6.0T applied parallel to the γ -ray direction. A marked sharpening of the wings of the spectrum is observed (Fig. 3a), although detailed interpretation is hampered by the relatively poor signal-to-noise ratio. The spectrum obtained from the oxidized sample at 4.2K in small magnetic fields ($\lesssim 0.5$ T) is not noticeably different from the zero-field spectrum [Fig. 2(c)(i)] and hence a comparison of Figs. 2(c)(i) and 3(a) indicates the difference between the oxidized and reduced samples more clearly than spectra taken in zero field.

In the oxidized state of both 2Fe-2S and 4Fe-4S centres of ferredoxins the magnetic moments are spin-

Fig. 3. Mössbauer spectra of reduced Photosystem-I fraction at 4.2K in magnetic fields of (a) 0.5T and (b) 6.0T applied parallel to the γ -ray direction

The computer simulations described in the text are represented as follows: —. —. non-magnetic iron-sulphur centre component; ···· and ----, sub-spectra due to the reduced iron-sulphur centre component calculated by using the parameters of *Bacillus stearothermophilus*; —, total simulation (normalized to the experimental spectrum), with an oxidized-to-reduced ratio of 1:1 and the addition of a parabolic background.

coupled to give a non-magnetic ground state for the centres (i.e. the total net spin is zero, $S = 0$) (see, e.g., Cammack *et al.*, 1977). In these cases no paramagnetic hyperfine splitting is observed, and chemical-shift data are the only reliable method of distinguishing between 2Fe-2S and 4Fe-4S centres. In the 2Fe-2S centres the spin-coupled Fe atoms both have formal valence Fe^{3+} and the chemical shift is typical of Fe^{3+} in a tetrahedral sulphur environment ($\delta \sim 0.26 \text{ mm/s}$ at 77 K and below). In the 4Fe-4S centres the iron atoms have formal valence $2\text{Fe}^{3+} + 2\text{Fe}^{2+}$ (i.e. average valence $4\text{Fe}^{2.5+}$) and hence the chemical shift is different from the 2Fe-2S centres owing to the increased ferrous nature of the Fe atoms or equivalently the decreased average valence ($\delta \sim 0.42 \text{ mm/s}$ at 77 K and below). In general both the 2Fe-2S and the 4Fe-4S centres receive one electron per centre on reduction, leading to a paramagnetic ground state ($S = \frac{1}{2}$) and the observation of paramagnetic hyperfine splitting at 4.2 K. These paramagnetic hyperfine spectra are significantly different for 2Fe-2S and 4Fe-4S centres.

The consistency of the behaviour observed with that of known 4Fe-4S centres is demonstrated by the computer simulations shown in Fig. 3. These are constructed by the superposition of spectra from non-magnetic (oxidized) and paramagnetic (reduced) 4Fe-4S centres. The parameters of the oxidized component are taken to be those of the quadrupole doublet observed in the 4.2 K zero-field reduced-sample spectrum [Fig. 2(c)(ii)]. The parameters of the reduced component are taken to be those found for the reduced 4Fe-4S protein from *B. stearothermophilus* by Middleton *et al.* (1978), and the calculation is performed as described in that paper. The spectrum shown by a solid line is the result of such simulations with a ratio of oxidized to reduced components of 1:1 and with allowance made for the parabolic background that is a characteristic feature of the constant-acceleration Mössbauer spectrometers used in the present work. The individual component subspectra are also shown in Fig. 3; two subspectra are shown for the reduced component as in the model successfully used for the reduced 4Fe-4S centre by Middleton *et al.* (1978), the four iron atoms being treated pairwise, the atoms within a pair being effectively identical but distinct from those of the other pair. For both the low- and high-magnetic-field data the major features of the spectra, particularly those of the reduced component, are well reproduced by the simulations, which therefore provide very strong evidence that the changes in the Photosystem-I spectra observed on reduction of centres A and B are due to a significant fraction of the iron present being in a chemical form very similar to that of the reduced 4Fe-4S centres of ferredoxins. As would be expected from the conclusions of Evans *et al.* (1977), coupled with the results of purification of Photosystem-I described in the

present paper, the behaviour of the unchanged iron is consistent with the hypothesis that it is in a chemical form similar to that found in the oxidized non-magnetic Fe-S centres of ferredoxins. The chemical shift of this unchanged non-magnetic component is $0.44 \pm 0.02 \text{ mm/s}$ at 4.2 K, which suggests it is more similar to the 4Fe-4S ferredoxin centres than to the 2Fe-2S centres. Thus the chief difference between this iron species and centres A and B may be that the former is not reducible under the conditions of sample preparation as used in the present work.

The observation by e.p.r. spectroscopy of two distinct signals, A and B, raises the question of whether there are two different 4Fe-4S centres in the reaction centre of Photosystem I, and if so, whether they are close enough to influence each other. The observation of magnetic hyperfine splitting at 4.2 K in the absence of an applied magnetic field is more consistent with the behaviour of Fe-S proteins with a single (and hence isolated) 4Fe-4S centre than with the behaviour of proteins with two 4Fe-4S centres per molecule at a separation of about 1.2 nm (12 Å) (Cammack *et al.*, 1977). In the latter case an intercentre interaction of some kind seems to be responsible for fast electronic spin relaxation, even at 4.2 K, with the result that the magnetic hyperfine interactions are time-averaged almost completely to zero over the timescale of the Mössbauer-absorption process, and a somewhat broadened quadrupole doublet is observed experimentally.

Thus it has been demonstrated that, under the growth conditions used in the present work, the majority of the iron present in the crude membrane fraction of *Chlorogloea fritschii* is retained in the Photosystem-I fraction on subsequent purification and extraction. The assignment of the e.p.r. signals A and B from Photosystem-I to paramagnetic 4Fe-4S centres similar to those found in reduced ferredoxins, on the basis of the similarity of the spin states of the iron-containing centres in the two cases, has been confirmed by the more detailed valence-state and spin-state information inherent in the Mössbauer spectra. An additional feature in the Mössbauer results is that, when taken in conjunction with the e.p.r. evidence that in these samples centres A and B are completely reduced, they indicate that a substantial proportion of the iron in the Photosystem-I preparations is not in the form of reducible A and B centres.

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