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MÖSSBAUER EFFECT STUDIES ON THE FOUR-IRON CENTRES OF TWO IRON-SULPHUR PROTEINS

D. P. E. DICKSON, C. E. JOHNSON, C. L. THOMPSON, R. CAMMACK (*)
M. C. W. EVANS (*), D. O. HALL (*), K. K. RAO (*) and U. WESER (*)

Oliver Lodge Laboratory, University of Liverpool, Liverpool L69 3 BX, U. K.

and (*) Department of Plant Sciences, King's College, 68, Half Moon Lane, London SE24 9JF, U. K.

Résumé. — L'effet Mössbauer de ^{57}Fe a été utilisé pour étudier les centres 4 Fe-4 S dans les ferredoxines de *Clostridium pasteurianum* et dans la protéine Fe-S à potentiel élevé (HiPIP) de *Chromatium*. Un examen des déplacements chimiques dans les différents états redox des protéines permet de caractériser les atomes de fer par des valences formelles. Les champs magnétiques hyperfins observés mettent en évidence un couplage antiferromagnétique des atomes de fer à l'intérieur de chaque centre. Les données de spectroscopie Mössbauer sont utilisées pour justifier un modèle concernant les atomes de fer dans ce centre.

Abstract. — ^{57}Fe Mössbauer effect studies have been carried out on the ferredoxin from *Clostridium pasteurianum* and on the high potential iron-sulphur protein (HiPIP) from *Chromatium* to investigate the 4 Fe-4 S centres found in these proteins. A consideration of the chemical shifts in the different redox states of the proteins enables a characterization of the iron atoms in terms of formal valences. The observed magnetic hyperfine fields give evidence for anti-ferromagnetic coupling of the iron atoms within each centre. The Mössbauer spectroscopy data is used to justify a model for the iron atoms in the centre.

1. Introduction. — The iron-sulphur proteins all contain non-haem iron in an active centre; the proteins so far isolated having one, two, four or eight iron atoms and (except for rubredoxins) an equivalent number of labile sulphur atoms. The purpose of the present work is to use Mössbauer spectroscopy to investigate the 4 Fe-4 S centres found in eight-iron bacterial ferredoxins (which have two such centres per molecule) and in four-iron high potential iron-sulphur proteins. The ^{57}Fe enriched Mössbauer samples of the ferredoxin from *C. pasteurianum* and HiPIP from *Chromatium* were prepared as described in references [1] and [2] where a more detailed discussion of the Mössbauer spectra of these proteins may also be found.

X-ray crystallographic analyses have shown that the four-iron centres of both proteins are identical to within the limits of the present structural determinations [3]. The centres consist of four iron atoms at four points of a cube with four labile sulphur atoms at the alternate points of a slightly larger cube, the iron atoms are coordinated along the diagonals of the cube to sulphur atoms in the cysteine residues of the amino acid chain of the protein. Thus each atom is in a near tetrahedral environment of four sulphur atoms as in the one and two-iron iron-sulphur proteins [4]. The biological role of the proteins is in electron transport processes; the ferredoxin transfers two electrons during the oxidation-reduction process with a redox

potential of -390 mV, while HiPIP transfers one electron with a redox potential of $+350$ mV [4]. The occurrence of identical active centres in two proteins with opposite redox properties has been rationalised by a proposal due to Carter and coworkers [3] that the centre can exist in three oxidation states: C (reduced HiPIP and oxidized ferredoxin), C^+ (oxidized HiPIP) and C^- (reduced ferredoxin). The C^- state of super-reduced HiPIP that would also be expected on the above scheme has recently been observed by Cammack [5].

2. Mössbauer spectra. — The Mössbauer spectra of the oxidized ferredoxin and the reduced HiPIP in a range of temperatures and applied magnetic fields are essentially identical, strong confirmatory evidence for them having the same active centre in the same oxidation state, C. Small differences in the size of the quadrupole splittings in the spectra of the two proteins in the C state are attributable to the protein environment of the active centres, which must indeed be different to account for the opposite redox properties of the proteins.

The use of the chemical shift in determining the valence state of iron in biological molecules has been discussed by Johnson [6]. In the case of the proteins under investigation we have a very useful calibration for the chemical shift from the Mössbauer data on the

one- and two-iron iron-sulphur proteins, which also contain iron in a tetrahedral environment of sulphur atoms. Table I shows the chemical shifts in *C. pasteurianum* ferredoxin and *Chromatium* HiPIP compared with the shifts for Fe^{3+} and Fe^{2+} atoms in other iron sulphur proteins. The proposed centres in terms of formal valences are shown in the final column. Recent work on analogue compounds [7] of the iron-sulphur proteins with 4 Fe-4 S centres also suggests that the normal C state of the centre consists formally of two Fe^{3+} and two Fe^{2+} atoms.

TABLE I

Chemical shifts (mm/s) at 77 K relative to pure iron, taken from Thompson et al. [1]

	Proposed Centre
Fe^{3+} in rubredoxin	0.25
Fe^{3+} in adrenodoxin	0.26
Fe^{3+} in spinach ferredoxin	0.22
Oxidized <i>Chromatium</i> HiPIP	0.32 C ⁺ 3 Fe^{3+} + 1 Fe^{2+}
Reduced <i>Chromatium</i> HiPIP	0.42
Oxidized <i>C. pasteurianum</i> ferredoxin	0.43 } C 2 Fe^{3+} + 2 Fe^{2+}
Reduced <i>C. pasteurianum</i> ferredoxin	0.57 C 1 Fe^{3+} + 3 Fe^{2+}
Fe^{2+} in rubredoxin	0.65
Fe^{2+} in spinach ferredoxin	0.56

Although it is useful to consider the centre in terms of formal valences for the iron atoms, the Mössbauer spectra do not show separate Fe^{3+} and Fe^{2+} spectra as are seen in the Mössbauer spectra of the two-iron plant-type ferredoxins, which are thought to contain a centre of two Fe^{3+} atoms in the oxidized state and one Fe^{3+} and one Fe^{2+} atom in the reduced state. This important difference is clearly seen in figure 1. This suggests that there is considerable delocalization of the *d*-electrons within the four-iron centre of the proteins.

The small line seen at 2.8 mm/s in the Mössbauer spectrum of reduced *C. pasteurianum* ferredoxin results from denatured material. Gersonde *et al.* [9] have also observed this and other denaturation products of the reduced ferredoxin. The absence of these spurious lines in the Mössbauer spectra is a good indication of the purity of the protein samples.

The C state proteins (reduced HiPIP and oxidized ferredoxin) do not exhibit any internal hyperfine field in their Mössbauer spectra in applied magnetic fields. This confirms the EPR and susceptibility evidence that the iron atoms within the centres are coupled together to give zero total spin.

The use of magnetic hyperfine interactions in investigating the centres of iron-sulphur proteins has been discussed by Johnson [6]. Figures 2 and 3 show the Mössbauer spectra of reduced ferredoxin and oxidized HiPIP at low temperatures in small and large applied

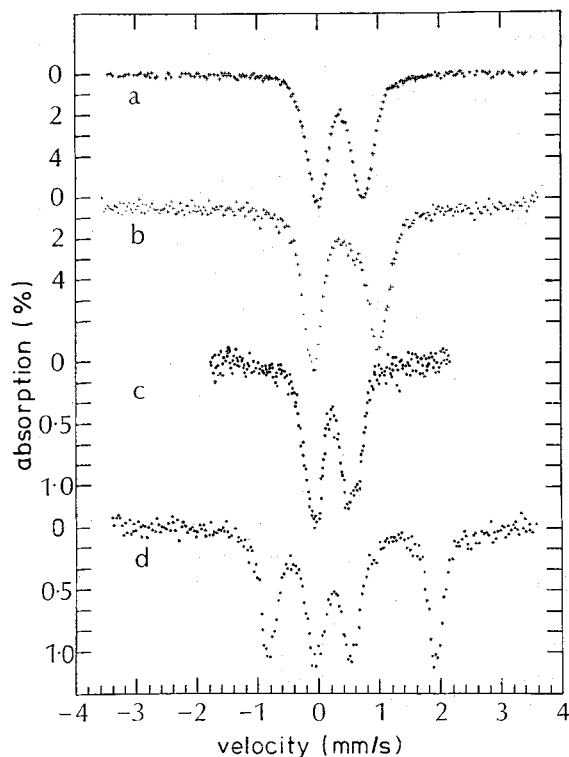


FIG. 1. — Mössbauer spectra at 195 K of: a) oxidized *C. pasteurianum* ferredoxin; b) reduced *C. pasteurianum* ferredoxin; c) oxidized *Scenedesmus* ferredoxin and d) reduced *Scenedesmus* ferredoxin.

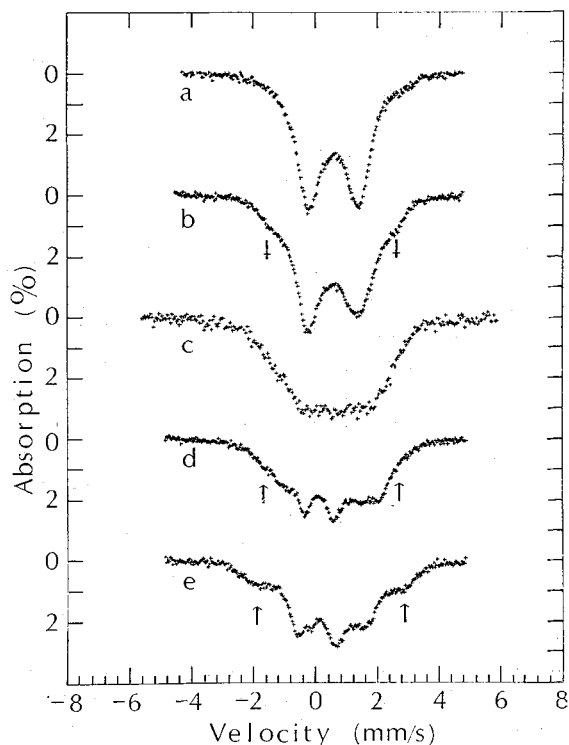


FIG. 2. — Mössbauer spectra of reduced *C. pasteurianum* ferredoxin at 4.2 K showing the effects of magnetic fields applied perpendicular to the γ -ray direction: a) zero field; b) 0.05 T; c) 1.5 T; d) 3.0 T; e) 6.0 T.

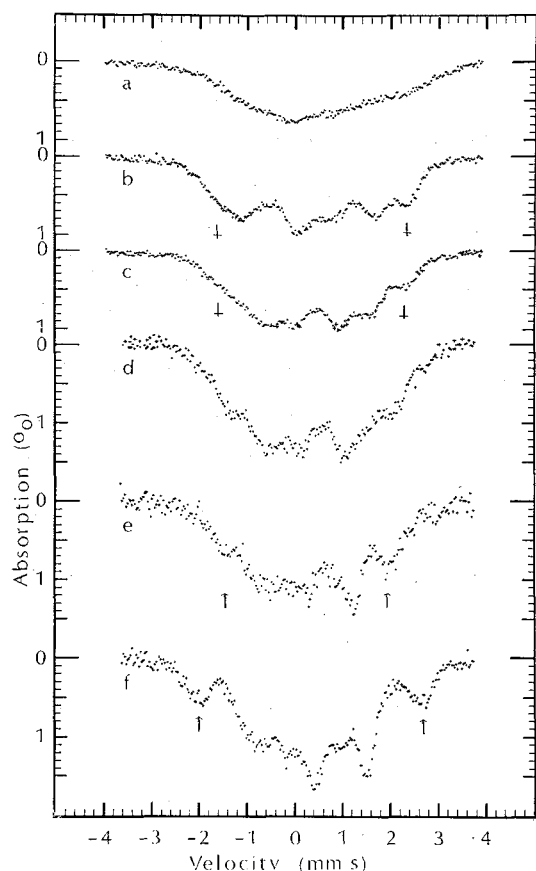


FIG. 3. — Mössbauer spectra of oxidized *Chromatium* HiPIP at 4.2 K showing the effect of applied magnetic fields: a) zero field; b) 0.05 T parallel to the X-ray direction, and fields perpendicular to the γ -ray direction of c) 0.05 T; d) 1.5 T; e) 3.0 T and f) 6.0 T.

magnetic fields. In both cases there are outer lines seen in the low field spectra (shown daggered) which move in as the applied field is increased, and outer lines seen in the high field spectra (shown arrowed) which move out as the applied field is increased. There is therefore direct evidence of both positive and negative hyperfine fields and thus there must be antiferromagnetic coupling between the iron atoms. In the case of oxidized HiPIP (Fig. 3f) the area of the spectrum due to the iron atoms with the positive hyperfine field (defined by the area of the outermost lines) is half the total spectral intensity, indicating that the iron atoms must be equally divided between those with positive and negative hyperfine fields. The spectra of the reduced ferredoxin are also consistent with half the iron atoms having a positive hyperfine field with the other half having a negative hyperfine field. The positive hyperfine field in both cases is approximately 9 T while the negative hyperfine field is approximately 12 T.

3. Discussion. — The Mössbauer effect data as outlined above appears to confirm the hypothesis of Carter *et al.* [3] for the three different states of the four-iron centres of these proteins. It is useful to consider whether the additional information obtained from the Mössbauer spectra, concerning valence states, hyperfine field, antiferromagnetic coupling and electron delocalization, can be incorporated into a more detailed model for the four-iron centre.

The direct evidence of iron atoms with both positive and negative hyperfine fields is incompatible with the 3d electrons being in molecular orbitals delocalized over all four iron atoms. Our preliminary proposal is for a model of the C state centre of oxidized ferredoxin and reduced HiPIP that is shown in figure 4.

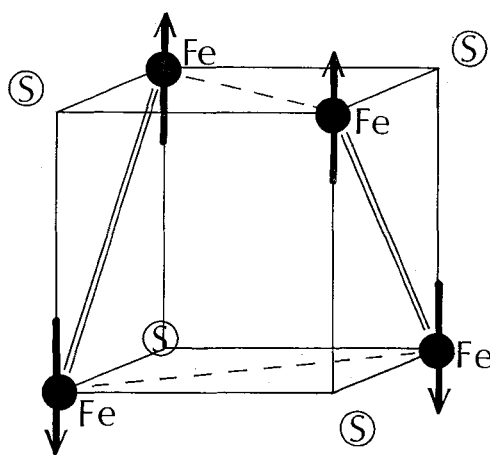


FIG. 4. — Proposed model for the 4 Fe-4 S centre (C state) of oxidized *C. pasteurianum* ferredoxin and reduced *Chromatium* HiPIP. The dashed lines represent electron hopping or delocalization, while the parallel lines indicate antiferromagnetic coupling.

The antiferromagnetic coupling between the iron atoms occurs via the sulphur atoms. There is fast hopping (relative to the lifetime of the ^{57}Fe excited state) or delocalization of the sixth 3d electrons between the two spin-up iron atoms and between the two spin-down iron atoms. Effectively each iron atom is $\text{Fe}^{2\frac{1}{2}+}$ giving the observed average chemical shift. The centre will have the required net spin of zero. Movement or delocalization of the sixth 3d electrons between spin-up and spin-down iron atoms is inhibited by the Pauli exclusion principle. (It is the operation of the exclusion principle that ensures the localization of the extra 3d electron in the case of the reduced two-iron plant-type ferredoxins.) The mechanism whereby the presence of one more or one less 3d electron when the centre is in the C^- or C^+ states still produces Mössbauer spectra characteristic of equivalent iron atoms is not fully understood.

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