Biochimica et Biophysica Acta, 459 (1977) 278–289

BBA 47230

NMR CHARACTERIZATION OF THREE FORMS OF FERREDOXIN FROM *DESULPHOVIBRIO GIGAS*, A SULPHATE REDUCER

JOSÉ J. G. MOURA[a], ANTÓNIO V. XAVIER[a], MIREILLE BRUSCHI[b] and JEAN LE GALL[b]

[a]*Centro de Química Estrutural da Universidade de Lisboa, I.S.T., Lisboa-1 (Portugal) and* [b]*Laboratoire de Chimie Bactérienne C.N.R.S., 13274, Marseille, Cedex 2 (France)*

(Received June 9th, 1976)

SUMMARY

A NMR and magnetic susceptibility study of the oxidized and reduced states of three different oligomers (forms) of a [4Fe-4S] ferredoxin protein from *Desulphovibrio gigas*, FdI, FdI', and FdII was carried out. FdI and FdI' are different trimers and FdII a tetramer of the same basic subunit. A probable assignment of the contact shifted resonances is indicated. Since the temperature dependences of the contact shifted resonances associated with each [4Fe-4S] are not all similar a delocalized model for the spin densities on the 4Fe does not apply. The exchange rate between oxidized and reduced states is slow on the NMR time scale. The three oligomers are not magnetically equivalent. Using the "three state hypothesis" terminology it is shown that FdI_{ox} is predominantly in the C^{2-} state and changes upon reduction into the C^{3-} state, while $FdII_{ox}$ is in the C^{-} state and changes into the C^{2-} state. FdI' does not easily fit into this classification. This study shows a similarity of magnetic behaviour between FdI and bacterial ferredoxins (e.g. *Bacillus polymyxa*) and between FdII and HiPIP from *Chromatium* sp.. The influence of the quaternary structure on the stabilization of the different oxidation states of ferredoxins as well as on their redox potentials is discussed.

INTRODUCTION

Low potential bacterial ferredoxins containing only a single cluster of four iron and four labile sulphur per molecule have been reported [1]. Another class of four-iron-four-sulphur proteins includes those with positive redox potentials known as high potential iron proteins (HiPIP) [1]. Three members of the first class FdI, FDI', and FdII have been isolated by us from *Desulphovibrio gigas*, an anaerobic sulphate reducer.

A previous characterization (amino acid composition, thermal stability, molecular weight, optical and biological activity data) has been presented [2]. The same subunit molecular weight of 6456 was obtained for the three forms which are isolated in different aggregation states, FdI and FdI' are trimers with different iso-

Abbreviation: Me_2SO, dimethylsulphoxide.

electric point and FdII is a tetramer. The basic monomer contains six cysteines, two methionines and a low content of aromatic residues (one phenylalanine) [2, 3]. The nature of the Fe/S centre is likely to be the same as the cubane arrangement of the four-iron-four-sulphur atoms of *Chromatium* HiPIP which has been shown by X-Ray diffraction to be identical with each of the clusters of eight-iron ferredoxin from *Peptococcus aerogenes* [4–7].

Important differences between the redox behaviour of the two classes of proteins, low and high potential, have been described. The reduced form of HiPIP, which is diamagnetic, changes upon oxidation into a paramagnetic state while ferredoxin upon reduction changes from a diamagnetic state into a paramagnetic state [6]. A difference of approx. 0.75 V is observed between their redox potentials. In order to explain these differences in behaviour it was postulated [6] that the [4Fe-4S] cluster can be in three different oxidation states C^- (paramagnetic), C^{2-} (diamagnetic), and C^{3-} paramagnetic ("three state" hypothesis). A convincing support of this hypothesis comes from the fact that a further reduction of the C^{2-} state of *Chromatium* HiPIP in Me_2SO (80 %) [8] as well as a further oxidation of the C^{2-} state of *Clostridium pasteurianum* ferredoxin were observed [9].

In this paper the magnetic susceptibility and PMR studies of both oxidized and reduced states of the three forms of *D. gigas* ferredoxin will be described demonstrating the similarity of the geometrical arrangement of the four iron cluster with other bacterial ferredoxins reported and allowing the classification of the states of the proteins according to the "three state" hypothesis.

METHODS AND MATERIALS

The three forms of ferredoxin were purified from *D. gigas* strain NCIB 9332 as previously described [1]. The protein solutions were dialyzed and concentrated simultaneously at 4 °C against ^2H_2O, 99,8 %, in a Diaflo ultrafilter, Amicon, membrane UM 10, under a nitrogen pressure of 2 bar/cm^2. The purity indexes were: $A_{280}/A_{405} = 1.26$ for FdI, $A_{280}/A_{415} = 1.05$ for FdI' and $A_{280}/A_{415} = 1.38$ for FdII.

Protein solutions were reduced with crystalline dithionite, BDH, Analar, under nitrogen flux. PMR studies were performed on a JEOL (JNM 100 PFT) spectrometer equipped with a JEOL 980A computer. The solvent peak was suppressed by a second irradiation frequency f_2 applied to the sample in addition to the observation pulse frequency f_1. The frequency f_2 was chosen to coincide with the HDO resonance frequency and applied continously for about 1 s finishing 40 μs before the f_1 pulse [10, 11].

Positive values of chemical shifts correspond to down field shifts referred to 2,2,3,3-tetradeutero-3-(trimethylsilyl) propionic acid sodium salt (DTSS).

The temperature was determined from the separation of the methanol resonances [12].

The solutions for both high resolution and magnetic susceptibility studies were between 3.0 and 4.0 mM in protein, 0.125 M in Tris · HCl buffer and their nominal pH was 7.6. The ferredoxin concentrations were calculated using molar extinction coefficients previously reported [2]. The magnetic susceptibility measurements were performed on concentric cylindrical cells (Wilmad Glass Company, Inc.,

WGS-5BL) the internal capillary containing only the solvent and the marker. The methylproton resonances of DTSS were used as susceptibility markers.

RESULTS

Magnetic susceptibility

The paramagnetic contribution to the molar susceptibility ($\chi_M{}^P$) of both the oxidized and the reduced states of the three forms of ferredoxin from *D. gigas* were determined by a PMR method [13]. Longitudinal cylindric symmetry was postulated for the sample container (diamagnetization factor (α) = 2π). The differences between the resonance positions of the marker in the protein and in the capillary solutions Δf were measured and converted into magnetic susceptibilities using the relation

$$\chi_M = \frac{3}{2\pi}(\Delta f/fc) + \chi_0$$

where χ_M is the molar magnetic susceptibility, f is $100 \cdot 10^6$ Hz and c is the concentration of ferredoxin. The solvent and solution densities were assumed to be similar and no correction was made for the small diamagnetic contribution of the apoprotein ($\chi_M \simeq \chi_M{}^P$). In Table I we present the mean values for the temperature range studied. The temperature variation of $\chi_M{}^P$ values is almost negligible. However a small positive dependence of $\chi_M{}^P$ is observed for both redox states of FdI and FdI', and for $FdII_{ox}$. For $FdII_{red}$ a slightly negative dependence is observed but the addition of traces of *o*-phenanthroline decreases its magnetic susceptibility value suggesting that there is some contribution from free iron.

PMR

PMR spectra of oxidized and reduced states of the three oligomers of ferredoxin from *D. gigas* have been obtained in the 0–40 °C range. Spectra of reduced

TABLE I

MAGNETIC SUSCEPTIBILITY MEASUREMENTS OF THREE FORMS OF FERREDOXIN FROM *D. GIGAS*
(mean values in the 5–30 °C temperature range studied)

Ferredoxin *D. gigas*	Oxidized form		Reduced form		Temperature dependence of $\chi_M{}^P$
	$10^3 \cdot \chi_M{}^{P}$*	μ_{eff}**	$10^3 \cdot \chi_M{}^{P}$*	μ_{eff}**	
I	2.73	1.19	–	–	+
	–	–	6.56	1.99	+
I'	2.93	1.33	–	–	+
	–	–	6.02	1.91	+
II	6.46	1.97	–	–	+
	–	–	1.82	1.35 0.98***	–

* $cm^3 \cdot mol^{-1}$.
** Units of Bohr magneton.
*** In presence of traces of *o*-phenantroline.

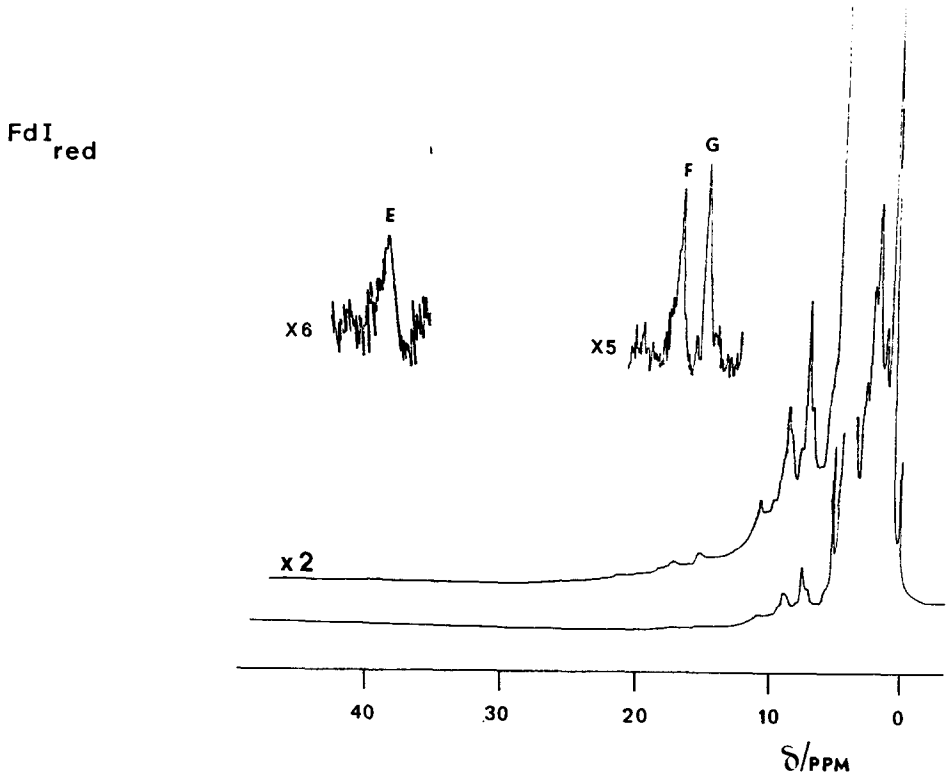

Fig. 1. 100 MHz PMR spectrum of *D. gigas* FdI_{red} at 20 °C. The protein concentration was 3.3 mM in ^2H_2O and the nominal pH 7.6.

FdI, reduced FdI′ and oxidized FdII are shown in Figs. 1, 2 and 3. Half-reduced samples have resonances of both redox states. Resonances A, B, C, and D were shown to correspond to two protons each by comparison with the resonances of the phenylalanine residue.

270 MHz spectra at 28 °C were also run to confirm that no peaks are lost at lower field strength. The 270 MHz spectra do not add more information about the contact shifted resonances which have identical line widths. The temperature dependence of the contact shifted low field resonances of FdI, FdI′, and FdII in both oxidation states are plotted in Fig. 4 together with some of the higher field resonances. No additional resonances were observed from −40 up to +100 ppm in FdI and FdI′ and from −40 up to 60 ppm in FdII. All the spectra display the usual strong absorption in the 0–8 ppm region characteristic of the diamagnetic protons of the protein amino acids. This region of the spectra is independent of the temperature as is expected in the absence of isotropic shifts and of conformational changes. The low intensity of the spectra in the 6–8 ppm range is in agreement with the low content in aromatic residues of these proteins [2, 3]. From their position, linewidth, independence of temperature and relative area the resonances at 6.87, 7.00 and 7.25 ppm in all the oligomers in all oxidation states were assigned to the aromatic protons of phenylalanine, the only aromatic residue in the sequence [3]. Other resonances present in the aromatic

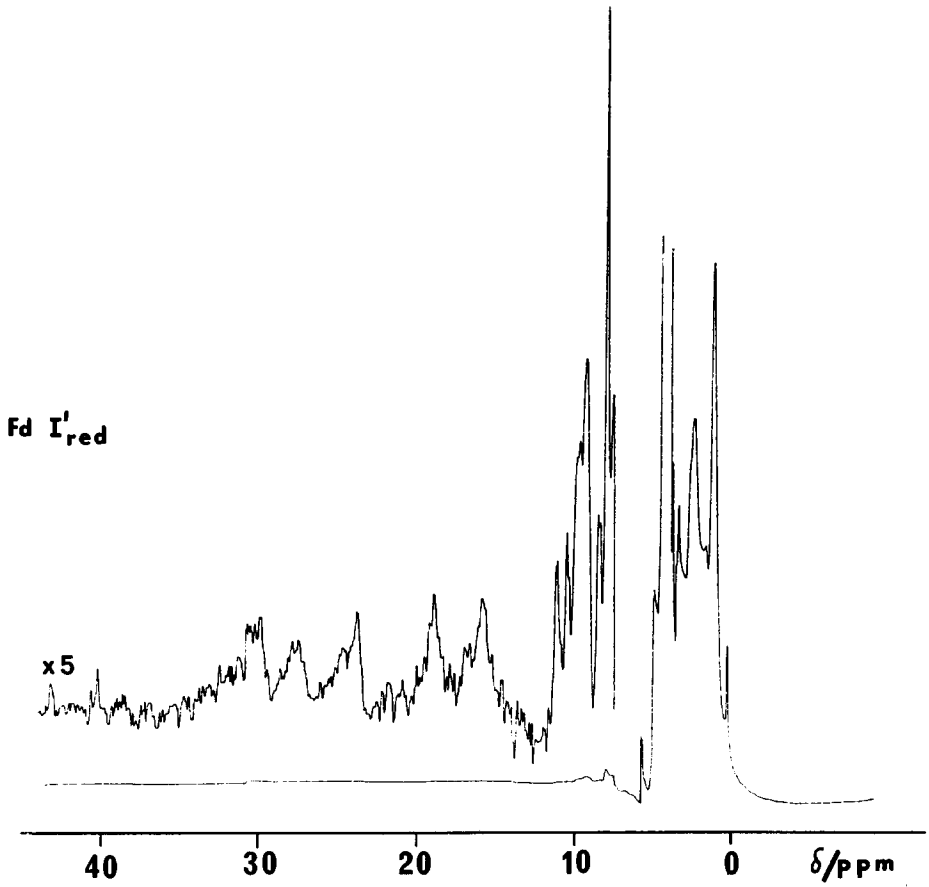

Fig. 2. 100 MHz PMR spectrum of *D. gigas* FdI'_{red} at 2 °C. The protein concentration was 3.4 mM in ^2H_2O and the nominal pH 7.6.

region in some oligomers must be assigned to contact shifted resonances. It should be noticed that in this spectral region several differences are observed between the three forms of ferredoxins and between the corresponding oxidation states of each oligomer. A comparison between the aromatic regions shown in Figs. 1, 2, and 3 and a detailed analysis of this region will be reported after a complete study at higher resolution. The observed differences are caused by differential conformational arrangements and/or magnetic interactions [15], in agreement with previous considerations [2].

Due to their large shifts, anomalously large line widths (see table II), and temperature dependence the resonances at low field should be assigned to protons subjected to contact shifts.

Distance estimation

In an attempt to confirm these preliminary assignements we have estimated the distances between the eight contact shifted protons and the para-magnetic center, inside the tetrahedron defined by the four iron atoms, using the values of $1/T_2$ shown in table II, and assuming that the electronic relaxation time τ_s is much shorter than the

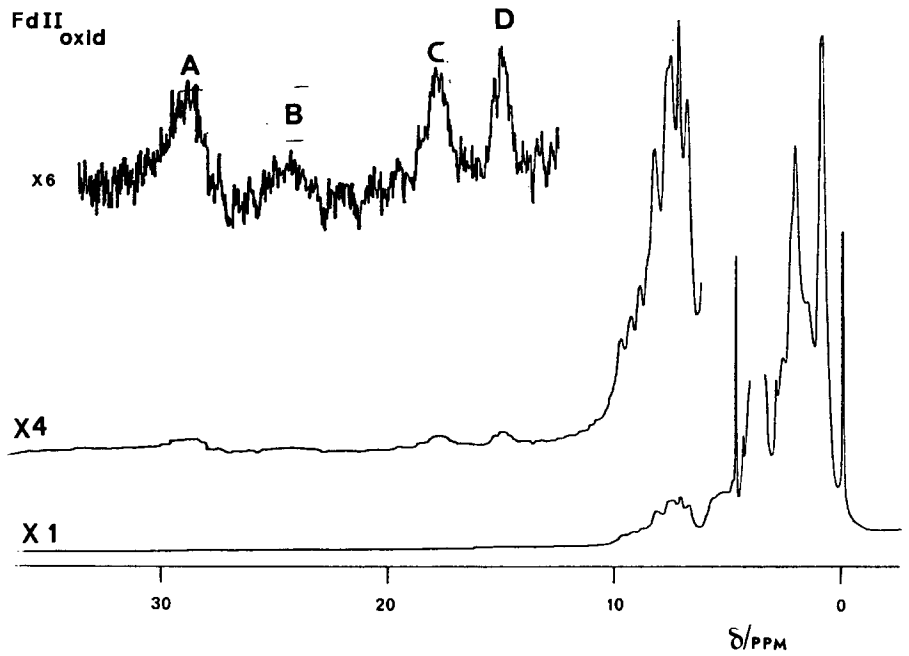

Fig. 3. 100 MHz PMR spectrum of *D. gigas* $FdII_{ox}$ at 28 °C. The protein concentration was 4 mM in ^2H_2O and the nominal pH 7.6.

rotational correlation time. The Solomon-Bloembergen equation [16] in this case is

$$\left(\frac{1}{T_2}\right)_{\text{observed}} = \frac{1}{15}\langle S_i^2\rangle \frac{g_e^2\beta_e^2 g_N^2\beta_N^2}{r^6}\left[7\tau_s + \frac{13\tau_s}{1+\omega_s^2\tau_s^2}\right] + \frac{1}{3}\left(\frac{A_i}{\hbar}\right)^2\langle S_i^2\rangle\left[\tau_s + \frac{\tau_s}{1+\omega_s^2\tau_s^2}\right] \quad (1)$$

where ω_s is the Larmor frequency, $\langle S_i^2\rangle$ is the thermal averaged spin distribution over all the spin states and all the other symbols have their usual meanings.

To estimate τ_s the dipolar contribution to the total observed shift was assumed to be negligible as a first approximation ($\tau_s^{(1)}$), $A_i/\hbar$ was calculated from the slope of the temperature dependences of the shifts using the contact shift equation [17]

$$\left(\frac{\Delta H}{H}\right)_{\text{cont.}} = -\frac{g\beta S(S+1)}{3\hbar\gamma_N kT}A_i \quad (2)$$

and values of $g = 1.94$ for FdI_{red} and $g = 2.02$ for $FdII_{ox}$ which was obtained from EPR measurements [26] .The g value is very similar to the calculated value for the "superoxidized" form of *C. pasteurianum* ferredoxin [9]. As the line widths observed at 100 MHz are of the same order as those observed at 270 MHz, τ_s cannot be in the range $0.9 \cdot 10^{-11}$ to $2.4 \cdot 10^{-13}$ s for which $\omega_s\tau_s \approx 1$ and the line widths are field dependent. The breadth of the resonances exclude the range $\tau_s < 2.4 \cdot 10^{-13}$ s. Since we did not consider the dipolar contribution the values obtained for $\tau_s^{(1)}$ are upper limits and the actual values of τ_s cannot be longer than the shortest of the calculated

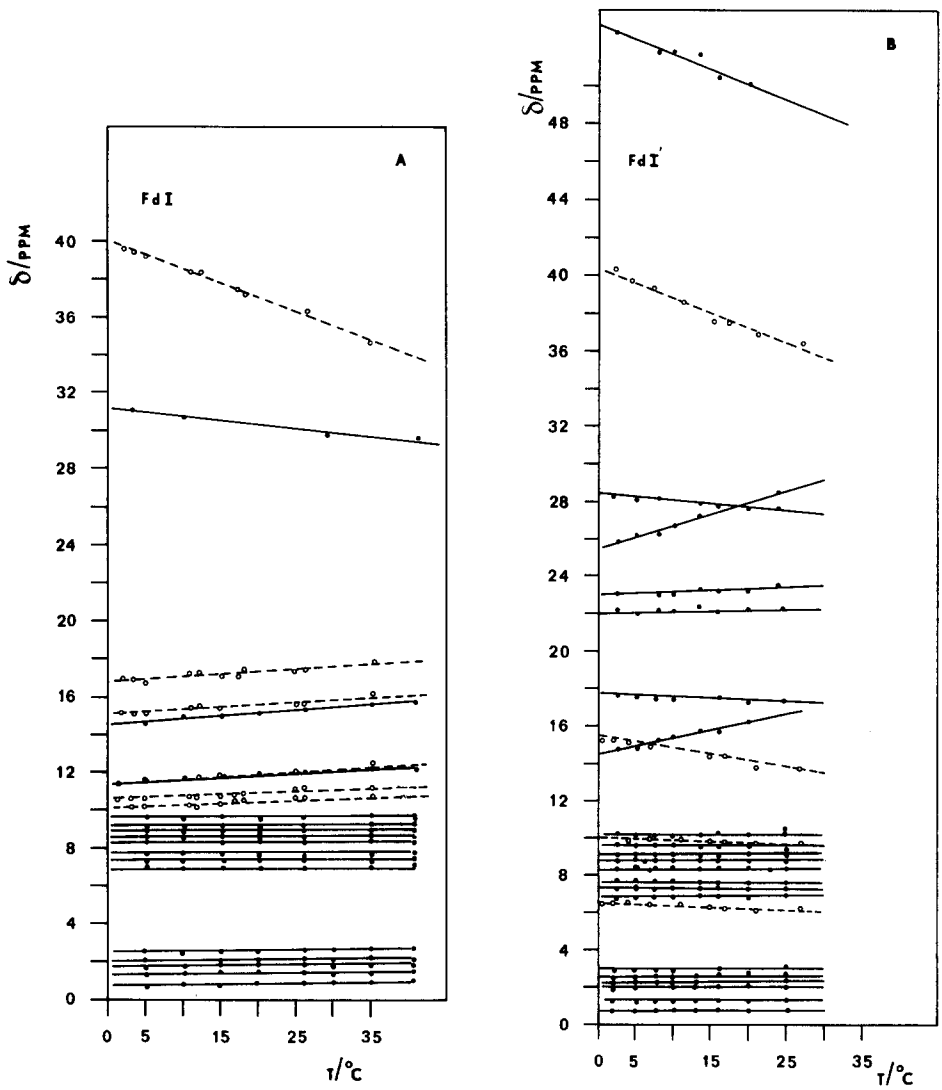

Fig. 4. See opposite page for legend.

$\tau_s^{(1)}$ values, $\tau_s^{(1)} = 2.0 \cdot 10^{-11}$ sec for FdII and $\tau_s^{(1)} = 2.5 \cdot 10^{-10}$ sec for FdI (see Table II). Using these values in equation 1 upper limits for the distances were obtained (see Table II).

The lower limit of $\tau_s = 0.9 \cdot 10^{-11}$ s, which is imposed by the condition $\omega_s \cdot \tau_s \approx 1$, was also used to calculate the lower values for these distances in order to obtain the total range of possible values for the distance parameters (see Table II).

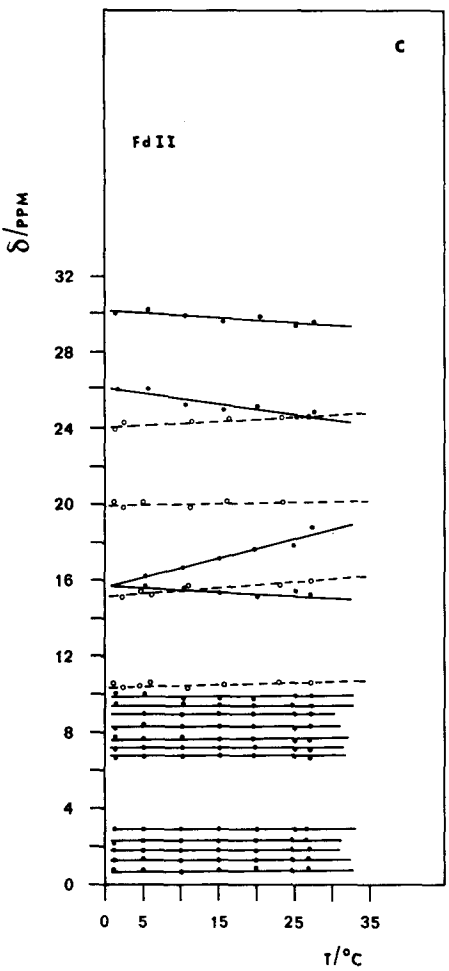

Fig. 4. Temperature dependence, showing contact-shifted resonances in the 100 MHz PMR spectra of *D. gigas* FdI (A), FdI′ (B) and FdII (C). Closed circles refer to the oxidized forms and open circles to the reduced forms. For the reduced forms only the temperature-dependent resonances are shown.

DISCUSSION

PMR data

Isotropic shifts have been reported for other ferredoxins in which the iron atom ligand field is largely dominated by the tetrahedral arrangement of the sulphur atoms [14]. Due to the high symmetry of this ligand field the contribution of pseudo-contact shift to the total isotropic shift is generally considered negligible particularly when compared to the large expected contact contribution [13].

Residual paramagnetism due to antiferromagnetic coupling between the iron atoms in the ground state has been observed for other ferredoxins in the temperature range studied [18, 19] and it was previously shown [20] that at the temperatures used

TABLE II

DISTANCES OF CONTACT SHIFTED PROTONS OF $FdII_{ox}$ AND FdI_{red} TO THE PARAMAGNETIC CENTRE

Proton resonance	$10^{-6} \times A_1/\hbar$ (rad · s^{-1})	$1/T_2$(obs.) (rad · s^{-1})	$\tau_s^{(1)}$ (s)	r(Å)	
				Minimum $\tau_s^{(1)}$	$\tau_s = 0.9 \cdot 10^{-11}$ s***
$FdII_{ox}$					
A	3.6	376.6	$1.16 \cdot 10^{-10}$	5.2*	4.5
B	4.1	534.7	$1.27 \cdot 10^{-10}$	4.9*	4.3
C	6.4	248.5	$2.40 \cdot 10^{-11}$	6.9*	5.2
D	1.8	165.7	$2.01 \cdot 10^{-11}$	–	5.0
FdI_{red}					
E	8.8	486.7	$2.5 \cdot 10^{-10}$	–	2.9
F	1.6	139.4	$2.17 \cdot 10^{-9}$	6.3**	3.6
G	1.2	122.0	$3.4 \cdot 10^{-9}$	6.1**	3.7

* Upper limit calculated using the minimum value of $\tau_s^{(1)}$ obtained for $FdII_{ox}$.
** Upper limit calculated using the minimum value of $\tau_s^{(1)}$ obtained for FdI_{red}.
*** Lower limit calculated from the condition $\omega_s \cdot \tau_s = 1$.

thermal energy is sufficient to decrease the degree of antiferromagnetic coupling and a small paramagnetism is observed. This type of paramagnetism is observed for $FdII_{red}$ and is the predominant one for FdI'_{ox} and in particular for FdI_{ox} as shown by their magnetic susceptibilities (see Table I) and by the shift of their contact shifted resonances. Some of these resonances are observed at very low field of these PMR spectra and are temperature dependent. This is in marked contrast with the resonances of the aromatic protons of phenylalanine which are relatively sharp and temperature independent implying that this residue is not in the proximity of the cluster.

For $FdII_{ox}$ three resonances A, B, and D (corresponding to six protons) move to higher field with the temperature (see Fig. 4) exhibiting a non-Curie law dependence and another resonance C (corresponding to two protons) follows a Curie law dependence. The contact shifted resonances of HiPIP have a similar shift and temperature dependences [18]. In this case they were assigned to the eight β-CH_2 cysteinyl protons [18]. In order to support the assignment of resonances A, B, C, D, E, F, and G to cysteinyl protons we compared their distances to the paramagnetic center obtained from T_2 measurements (see Table II) with those obtained from CPK molecular models [21] which indicate that β-CH_2 protons are within 2.6–4.3 Å and the α-CH_2 protons are within 2.3–5.5 Å of the metal ion. These ranges of distances compare well with distance ranges calculated for the resonances assigned to cysteinyl protons.

The shifts and temperature dependences of the contact shifted resonances of FdI_{ox} are analogous to those obtained for *B. polymyxa*, FdI_{ox} [19], but an additional resonance was observed at approx. 31 ppm. In the case of *B. polymyxa* these resonances were tentatively assigned to β-CH_2 cysteinyl protons based in the analysis of model compounds [19]. However the reduced forms present important differences. The observed *B. polymyxa* FdI_{red} contact shifted resonances were not assigned in particular to β-CH_2 cysteinyl protons. The calculated distances for *D. gigas* FdI_{red} shown in Table II confirm the assignment of these resonances to cysteinyl protons.

TABLE III

COMPARISON OF μ_{eff} VALUES OBTAINED FOR [4Fe-4S] PROTEINS

[4Fe-4S] proteins	μ_{eff}		Redox couple state
	oxid. form	red. form	
D. gigas FdI	1.19	1.99	$C^{2-}+C^{-}(?)/C^{3-}$
D. gigas FdI′	1.33	1.91	$(C^{-}+C^{2-})/C^{3-}$ (?)
D. gigas FdII	1.97	0.98	C^{-}/C^{2-}
B. polymyxa FdI	0.90	1.60	C^{2-}/C^{3-}
Chromatium HiPIP	1.84	0.48	C^{-}/C^{2-}
$[Fe_4S_4]$Synthetic analog.	1.04	–	–

The shift and temperature dependence of the resonances of FdI′ do not compare well with those of any other iron-sulphur protein previously reported.

Since the contact shifted resonances of all the three oligomers have a non-uniform temperature dependence, a completely delocalized model for the spin density does not apply in this case [18]. The fact that the equilibrium between the oxidized and the reduced forms of one 4Fe cluster ferredoxins (e.g. *B. polymyxa*) is slow and for two 4Fe cluster ferredoxins (e.g. *C. pasteurianum*) is fast was taken as an indication that the two clusters of 8 Fe ferredoxins interact [19]. It is interesting to point out that although the different forms of *D. gigas* ferredoxins have three and four 4Fe clusters per aggregate this equilibrium is still slow.

Magnetic susceptibility data

The magnetic susceptibility measurements show that the three forms of ferredoxin from *D. gigas* are paramagnetic in both redox states for the temperature range studied. However they are not magnetically equivalent (see Table I).

The non-Curie law dependence observed for all the solutions with the exception of $FdII_{red}$ (see Table I) indicates the presence of a high degree of antiferromagnetic exchange coupling. As was pointed out in the results section the slightly negative temperature dependence found for $FdII_{red}$ should be due to the presence of free iron, most probably originated from denatured protein. We cannot relate this effect to instability of the reduced form since it can be reoxidized and it has been shown that approx. 1 % of denatured protein can account for the difference in the residual paramagnetism observed [22].

FdI_{red}, FdI'_{red}, and $FdII_{ox}$ have similar μ_{eff} values which compare with those reported for the paramagnetic states of *B. polymyxa* FdI and *Chromatium* HiPIP. The value of the magnetic susceptibility of $FdII_{red}$ compares with the values obtained for cases where antiferromagnetic coupling is the only contributing mechanism [19]. However for FdI_{ox} and in particular for FdI'_{ox} the measured values of the magnetic susceptibilities are higher than those usually obtained in these cases. These results can be compared with those reported for *Chromatium* HiPIP and *B. polymyxa* ferredoxin respectively which represent two quite distinct cases of iron-sulphur proteins (see Table III). FdI′ has an intermediate magnetic behaviour and in the present study it is not possible to characterize it with confidence.

The optical spectra [2], the PMR and the magnetic susceptibility data, and in

particular a comparison with other four-iron-four-sulphur proteins enables us to describe the states at which the different forms are predominantly encountered and are shown in Table III. The deduced characterisation of FdII is of great interest. Although FdII uses the C^{-}/C^{2-} redox states it is not a high potential protein since it has negative redox potential [2]. This shows that the redox potential of the [4Fe-4S] clusters is not exclusively determined by its state of oxidation. An analogous case was recently reported for one of the clusters of *Azotobacter vinelandii* [23]. Thus the "three state" hypothesis cannot always be used to predict their redox potentials, as has been previously implied [6]. Some other factors than oxidation state must be responsible for the large range of redox potentials shown by the [4Fe-4S] proteins.

Although *D. gigas* FdI and FdII have similar amino acid composition, biological activity (FdII is actually more effective for concentrations lower than saturation), and redox potentials they are isolated in different oxidation states showing that the environment of the cluster is important for the stabilization of the different oxidation states. This supports previous indications that the oxidation states of proteins can be controled without changing their polypeptide sequence [2] and is a striking example of Vallee and Williams "entatic state" theory [24, 25]. The environmental modifications should be due to quaternary structure interactions caused by the different aggregation states in which these forms are isolated suggesting that the study of *D. gigas* ferredoxin may be useful for the understanding of more complex iron-sulphur proteins.

ACKNOWLEDGMENTS

We should like to thank the Oxford Enzyme Group for the utilization of their 270 MHz NMR spectrometer and Mr. K. Eguchi from JEOL (U.K.) Ltd. for designing and installing the circuits necessary to execute solvent suppression on our NMR spectrometer.

REFERENCES

1 Hall, D. O., Cammack, R. and Rao, K. K. (1974) Iron in Biochemistry and Medicine (Jacobs, A. and Woorwood, M., eds.), pp. 279–334, Academic Press, London, and reference therein
2 Bruschi, M., Hatchikian, E. C., Le Gall, J., Moura, J. J. G. and Xavier, A. V. (1976) Biochim. Biophys. Acta 449, 275–284
3 Travis, J., Newman, D. J., Le Gall, J. and Peck, Jr., H. D. (1971) Biochem. Biophys. Res. Commun. 45, 452–458
4 Freer, S. T., Alden, R. A., Carter, C. W., Jr. and Kraut, J. (1975) J. Biol. Chem. 250, 46–54
5 Carter, Jr., C. W., Kraut, J., Freer, S. T. and Alden, R. A. (1974) J. Biol. Chem. 249, 6339–6346
6 Carter, Jr. C. W., Kraut, J., Freer, S. T., Alden, R. A., Sieker, L. C., Adam, E. T. and Jensen, L. H. (1972) Proc. Natl. Acad. Sci. U.S. 69, 3526–3529
7 Sieker, L. C., Adam, E. and Jensen, L. H. (1972) Nature 235, 40–42
8 Cammack, R. (1973) Biochem. Biophys. Res. Commun. 54, 548–554
9 Sweeney, W. V., Bearden, A. J. and Rabinowitz, J. C. (1974) Biochem. Biophys. Res. Commun. 59, 188–194
10 Campbell, I. D., Dobson, C. M., Jeminet, G. J. and Williams, R. J. P. (1974) FEBS Lett 49, 115–118
11 Bleich, H. E. and Glasel, J. A. (1975) J. Mag. Res. 18, 401–405
12 Van Geet, A. L. (1968) Anal. Chem. 40, 2227–2229
13 Poe, M. and Phillips, W. D. (1972) Methods in Enzymology, Vol. 24, pp. 304–317, Academic Press, New York

14 Poe, M. and Phillips, W. D. (1973) in Iron-Sulphur Proteins, Vol. II, pp. 225–284, Academic Press, and references therein
15 Poe, M., Phillips, W. D., McDonald, C. C. and Lovenberg, W. (1970) Proc. Natl. Acad. Sci. U.S. 65, 797–804
16 Carrington, A. and McLachlan, A. D. (1967) in Introduction to Magnetic Resonance, p. 228
17 McConnell, H. M. and Chesnut, D. B. (1958) J. Chem. Phys. 28, 107–117
18 Phillips, W. D., Poe, M., McDonald, C. C. and Bartsch, R. G. (1970) Proc. Natl. Acad. Sci. U.S. 67, 682–687
19 Phillips, W. D., McDonald, C. C., Stombaugh, N. A. and Orme-Johnson, W. H. (1974) Proc. Natl. Sci. U.S. 71, 140–143
20 Dunham, W. R., Palmer, G., Sands, R. H. and Bearden, A. J. (1973) Biochim. Biophys. Acta 253, 373–384
21 Salmeen, I. and Palmer, G. (1972) Arch. Biochem. Biophys. 150, 767–773
22 Petering, T. H. D. and Palmer, G. (1969) J. Biol. Chem. 244, 2275–2277
23 Sweeney, W. V., Rabinowitz, J. C. and Yoch, D. C. (1975) J. Biol. Chem. 250, 7842–7847
24 Vallee, B. L. and Williams, R. J. P. (1968) Proc. Natl. Acad. Sci. U.S. 59, 498–507
25 Williams, R. J. P. (1971) Cold Spring Harbour Symp. Quant. Biol. 36, 53–62
26 Cammack, R., Moura, J. J. G., Xavier, A. V., Bruschi, M., Le Gall, J. and Gayda, J. P. (1977) Biochim. Biophys. Acta, in the press