^{2}H NMR Investigation of $[Fe_3S_4]^0$ Cluster in 7Fe8S Ferredoxin from *Bacillus schlegelii*

Ivano Bertini,*,† Claudio Luchinat,‡ Giovanna Mincione,‡ and Aileen Soriano†

Department of Chemistry, University of Florence, Via Gino Capponi 7, 50121 Florence, Italy, and Department of Soil Science and Plant Nutrition, University of Florence, P.le delle Cascine 28, 50144 Florence Italy

Received July 24, 1997

A ^{2}H NMR study has been performed on a 7Fe8S ferredoxin from *Bacillus schlegelii* which has been overexpressed in *Escherichia coli*. The protein cysteines have been deuterated at the β position by incorporating labeled cysteines into the growth media. The protein contains an Fe_3S_4 and an Fe_4S_4 cluster. The former has been investigated in the $[Fe_3S_4]^0$ and in the $[Fe_3S_4]^+$ states. Whereas the $[Fe_3S_4]^+$-containing species provides sharp ^{1}H and ^{2}H NMR spectra for the signals of the cysteine ligands, no corresponding ^{1}H or ^{2}H signals have been detected from the $[Fe_3S_4]^0$-containing species. Theoretical considerations predict observability of these signals unless a chemical equilibrium is operative. It is proposed therefore that the Fe^{3+} ion and the two $Fe^{2.5+}$ ions constituting the cluster exchange their valency with a rate in the order of 10^6 s^{-1}, which would cause coalescence of the signals.

Introduction

^{1}H NMR spectroscopy has provided deep insight into the electronic structure of FeS polymetallic centers in proteins.[1] In $[Fe_2S_2]^+$, it has been shown that the single unpaired electron is localized on one iron[2] which was found to be the one closer to the protein surface.[3] In $[Fe_4S_4]^{3+}$, it was found that there is a pair of Fe^{3+} ions and a pair of $Fe^{2.5+}$ ions that are antiferromagnetically coupled.[4] The pair of $Fe^{2.5+}$ ions originate from sharing one electron between Fe^{2+} and Fe^{3+}. In $[Fe_4S_4]^+$ there is a pair of Fe^{2+} ions antiferromagnetically coupled to a pair of $Fe^{2.5+}$ ions.[5,6] In both cases the ground state is characterized by $S' = {}^1/_2$ (S' is used throughout the text to denote a spin state of a magnetic-coupled system).

In all the above systems, the electronic relaxation time is equal to that for a single tetrahedral Fe^{2+} ($\sim 5 \times 10^{-11}$ s) or shorter. This is because the large J value ensures that the whole polymetallic center has a single effective electronic relaxation time, the value of which is close to that of the fastest relaxing metal ion in the cluster.[7] Furthermore, other electronic relaxation mechanisms may arise from magnetic coupling, which can make the effective electronic relaxation time even shorter than the above value. This value, on the other hand, already allows detection of ^{1}H NMR signals from β-CH_2 protons of the coordinated cysteines.[8] With this in mind, it is surprising that no signal from cysteine β-CH_2 protons has ever been reported for $[Fe_3S_4]^0$ proteins[9−18] or has been detected when specifically looked for.[12,16−18] In such clusters a pair of $Fe^{2.5+}$ ions is antiferromagnetically coupled to Fe^{3+} and the resulting ground state has $S' = 2$.[19] The conclusion is that the NMR line widths are too broad, which is either due to an abnormally long electronic relaxation time or to exchange broadening. We try here to address this problem by preparing a sample of a one-electron reduced 7Fe8S ferredoxin from *Bacillus schlegelii*[17] with cysteines deuterated at the β position. The labeled protein has been overexpressed in *E. coli*. The 7Fe8S protein contains one Fe_3S_4 cluster and one Fe_4S_4 cluster. Any dipolar line broadening is expected to be 42 times narrower for ^{2}H signals relative to ^{1}H signals because the magnetic moment of ^{2}H is 6.5 times smaller than that of ^{1}H and every dipolar mechanism of nuclear relaxation depends on the square of the magnetic moment.[7] In the case of $[Fe_3S_4]^+$, the single electron in the

* Corresponding author. Fax: +39-55-2757 555. E-mail: bertini@lrm.fi.cnr.it.

† Department of Chemistry.

‡ Department of Soil Science and Plant Nutrition.

(1) Bertini, I.; Ciurli, S.; Luchinat, C. In *Structure and Bonding*; Springer-Verlag: Berlin, 1995; Vol. 83, pp 1−54.
(2) Bertini, I.; Lanini, G.; Luchinat, C. *Inorg. Chem.* **1984**, *23*, 2729−2730.
(3) Dugad, L. B.; La Mar, G. N.; Banci, L.; Bertini, I. *Biochemistry* **1990**, *29*, 2263−2271.
(4) Bertini, I.; Capozzi, F.; Ciurli, S.; Luchinat, C.; Messori, L.; Piccioli, M. *J. Am. Chem. Soc.* **1992**, *114*, 3332−3340.
(5) Jordanov, J.; Roth, E. K. H.; Fries, P. H.; Noodleman, L. *Inorg. Chem.* **1990**, *29*, 4288−4292.
(6) Bertini, I.; Briganti, F.; Luchinat, C.; Messori, L.; Monnanni, R.; Scozzafava, A.; Vallini, G. *Eur. J. Biochem.* **1992**, *204*, 831−839.
(7) Banci, L.; Bertini, I.; Luchinat, C. *Nuclear and Electron Relaxation. The Magnetic Nucleus−Unpaired Electron Coupling in Solution*; VCH: Weinheim, 1991.
(8) Xia, B.; Westler, W. M.; Cheng, H.; Meyer, J.; Moulis, J.-M.; Markley, J. L. *J. Am. Chem. Soc.* **1995**, *117*, 5347−5350.
(9) Cheng, H.; Grohmann, K.; Sweeney, W. V. *J. Biol. Chem.* **1992**, *267*, 8073−8080.
(10) Cheng, H.; Grohmann, K.; Sweeney, W. V. *J. Biol. Chem.* **1990**, *265*, 12388−12392.
(11) Nagayama, K.; Ohmori, D.; Imai, Y.; Oshima, T. *FEBS Lett.* **1983**, *158*, 208−212.
(12) Macedo, A. L.; Moura, I.; Moura, J. J. G.; LeGall, J.; Huynh, B. H. *Inorg. Chem.* **1993**, *32*, 1101−1105.
(13) Busse, S. C.; La Mar, G. N.; Yu, L. P.; Howard, J. B.; Smith, E. T.; Zhou, Z. H.; Adams, M. W. W. *Biochemistry* **1992**, *31*, 11952−11962.
(14) Donaire, A.; Gorst, C. M.; Zhou, Z. H.; Adams, M. W. W.; La Mar, G. N. *J. Am. Chem. Soc.* **1994**, *116*, 6841−6849.
(15) Gorst, C. M.; Yeh, Y.-H.; Teng, Q.; Calzolai, L.; Zhou, Z.-H.; Adams, M. W. W.; La Mar, G. N. *Biochemistry* **1995**, *34*, 600−610.
(16) Bentrop, D.; Bertini, I.; Luchinat, C.; Mendes, J.; Piccioli, M.; Teixeira, M. *Eur. J. Biochem.* **1996**, *236*, 92−99.
(17) Aono, S.; Bertini, I.; Cowan, J. A.; Luchinat, C.; Rosato, A.; Viezzoli, M. S. *J. Biol. Inorg. Chem.* **1996**, *1*, 523−528.
(18) Bertini, I.; Dikiy, A.; Luchinat, C.; Macinai, R.; Viezzoli, M. S.; Vincenzini, M. *Biochemistry* **1997**, *36*, 3570−3579.
(19) Papaefthymiou, V.; Girerd, J.-J.; Moura, I.; Moura, J. J. G.; Münck, E. *J. Am. Chem. Soc.* **1987**, *109*, 4703−4710.

S0020-1669(97)00920-8 CCC: $15.00
Published on Web 02/07/1998

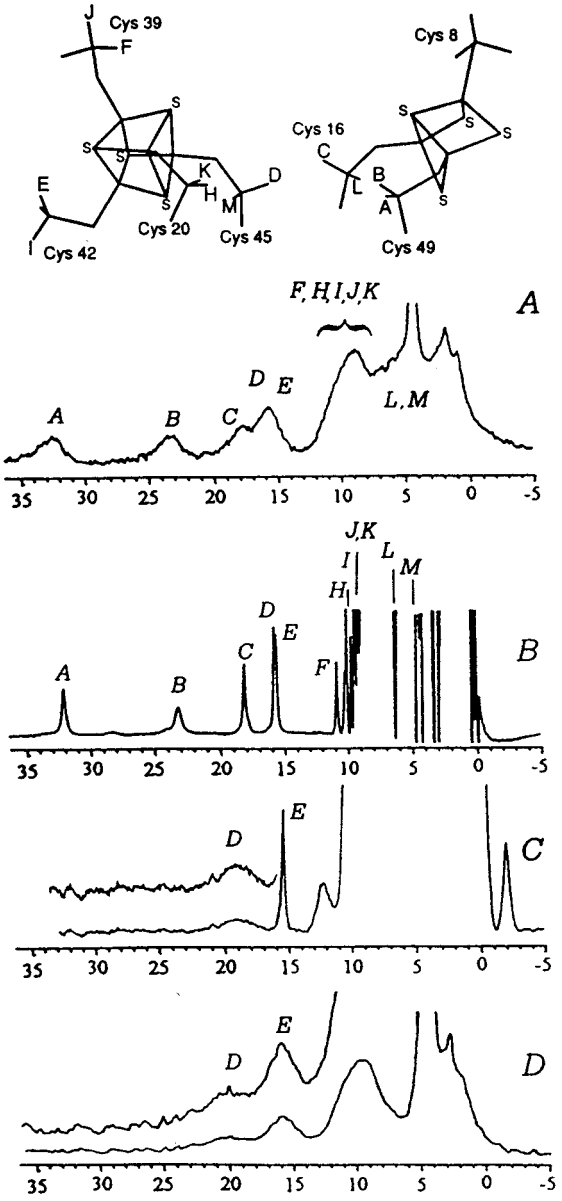

Figure 1. ^{2}H NMR spectrum of oxidized (A) and the corresponding ^{1}H NMR spectrum (B) of 7Fe8S ferredoxin showing the assignment of β-CH_2 cysteine signals.[17] The ^{1}H NMR spectrum of the one-electron reduced form is also shown (C) as well as the corresponding ^{2}H NMR spectrum (D). The inset shows a schematic diagram of the $[Fe_4S_4]$ cluster and the $[Fe_3S_4]$ cluster in 7Fe8S ferredoxin from *B. schlegelii* based on the structural information available on the homologous protein from *A. vinelandii*. The β-CH_2 of cysteines bound to the clusters are labeled according to the NMR assignment. Note the proximity of proton *D* with respect to the $[Fe_3S_4]$ cluster.

ground state is the result of antiferromagnetic coupling among three different iron ions[1] and the ^{1}H NMR spectra are relatively sharp.[9−18]

Experimental Methods

The protein sample (around 3 mM, pH 6.5) with cysteines specifically deuterated at the β position were prepared by growing the *E. coli* PKKFd54 expression host in M9 media containing deuterated cysteines (50 mg/L) (Cambridge Isotopes) and other unlabeled amino acids (100 mg/L) (Sigma). Growth conditions and purification of the protein were according to published procedures.[20] The protein sample was degassed by bubbling O_2-free argon for 15 min through the sample. One-electron reduction was achieved by adding a 5-fold excess of previously degassed dithionite stock solution, anaerobically, using a gastight Hamilton syringe. The sample was kept in an inert atmosphere throughout the experiment by using an NMR tube fitted with a screw cap and a septum (Wilmad).

^{2}H NMR spectra were recorded using MSL 200, AMX 600, and Avance 800 Bruker NMR machines. Recycle times using a simple 90° pulse program were 30 and 180 ms. ^{1}H NMR spectra were recorded as previously described.[17]

Table 1. Assignment of the β Protons (Deuterons) of Cysteines Attached to the Iron Sulfur Clusters in the Oxidized 7Fe8S Ferredoxin from *B. schlegelii*[17]

cysteine	cluster	β-CH_2 pair	chem. shift [ppm]
8	$[Fe_3S_4]^+$	−, −	<9, <9
16	$[Fe_3S_4]^+$	*C, L*	18.3, 6.6
20	$[Fe_4S_4]^{2+}$	*H, K*	10.35, 9.0
39	$[Fe_4S_4]^{2+}$	*F, J*	11.0, 9.0
42	$[Fe_4S_4]^{2+}$	*I, E*	9.7, 15.8
45	$[Fe_4S_4]^{2+}$	*D, M*	15.9, 5.2
49	$[Fe_3S_4]^+$	*B, A*	23.4, 32.2

Results

The ^{2}H NMR spectra of oxidized and one-electron reduced 7Fe8S ferredoxin from *B. schlegelii* are shown in Figure 1 together with the corresponding ^{1}H NMR spectra. A schematic structure of the two clusters and the assignment of the deuteron (proton) signals in the β position of the coordinated cysteines is also shown, as published elsewhere.[17] The assignment is also summarized in Table 1 for the reader's convenience. The full deuteration of the protein cysteines at the β position is evidenced by the spectra of the oxidized form (Figure 1A,B) showing a clear correspondence between the ^{1}H and ^{2}H signals from the β position of the cysteines. The ^{2}H signals are broader because of quadrupolar line broadening which is enhanced by the long rotational correlation time.[7] In the ^{1}H NMR spectra, signals *A*−*D* were reported to disappear as a result of one-electron reduction.[17] Signals *A*−*C* belong to the oxidized $[Fe_3S_4]^{1+}$ cluster, while *D* belongs to the $[Fe_4S_4]^{2+}$ cluster.[17] Furthermore, the line widths of the ^{1}H signals *E*, *F*, and *H*, which also belong to the $[Fe_4S_4]^{2+}$ cluster, experience increases of 30, 90, and 8 Hz, respectively upon one-electron reduction of the protein.[17] The corresponding ^{2}H NMR spectrum for the one electron reduced protein shows evidence of signals *D* and *E* above 15 ppm. Several other ^{2}H signals are observed clustered around 10 ppm in a signal envelope that probably contains signals *F* and *H*−*K*. Signal *M* is presumably under the natural abundance ^{2}H signal from water. The ^{2}H signals upfield of the water signal presumably arise from the noncoordinated Cys 25. Reexamination of the ^{1}H NMR spectrum in the light of these results allowed us to detect signal *D* as a very broad signal at about 20 ppm, which was not previously detected (Figure 1C).

The ^{2}H signals of the cysteines coordinated to the $[Fe_3S_4]^0$ (signals *A*−*C* and *L*) cluster have been searched at 200, 600, and 800 MHz at sweep widths as wide as ±1000 ppm without success. At this point, it appears that even in the deuterated sample these signals are not detectable.

Discussion

The failure to observe the cysteine β-CH_2 signals in the NMR spectra (^{1}H and ^{2}H) of the protein containing reduced $[Fe_3S_4]^0$ cluster is a surprising result, in view of the fact that ^{2}H signals of coordinated cysteines have been observed in the reduced and oxidized rubredoxin.[8] The reduced rubredoxin has the same $S = 2$ ground state while the oxidized form has a ground state S

(20) Aono, S.; Nakamura, S.; Aono, R.; Okura, I. *Biochem. Biophys. Res. Commun.* **1994**, *201*, 938−942.

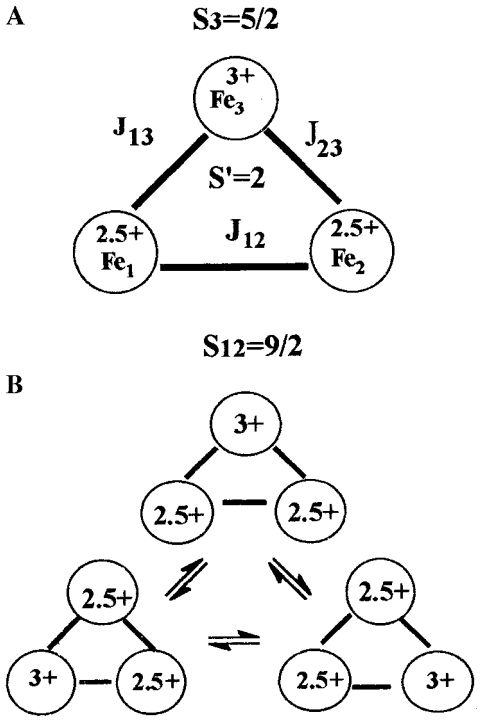

Figure 2. (A) Schematic diagram representation of the electronic ground state of reduced $[Fe_3S_4]^0$ cluster. The mixed-valence pair composed by Fe_1 and Fe_2 has $S_{12} = {}^9/_2$ subspin. This pair is coupled to an iron(II) ion (Fe_3) with $S_3 = {}^5/_2$. The total spin of the ground state is $S' = 2$. The Heisenberg exchange coupling constants J_{12}, J_{13}, and J_{23} are also shown. A double-exchange mechanism on the Fe_1-Fe_2 pair is also operative. (B) Schematic representation of the proposed equilibrium between species differing in the localization of the mixed-valence $S = {}^9/_2$ pair is shown.

$= {}^5/_2$, presumably with a long electron relaxation time. ^{2}H signals are also nicely observed in the oxidized form of the present system.

The problem is hereby addressed by discussing the probable reasons for the nonobservability of the β-CH_2 signals. The Mössbauer spectra have shown that the ground state is described by a mixed valence pair with $S_{12} = {}^9/_2$ subspin and an iron(III) $S_3 = {}^5/_2$ (Figure 2A).[19] The total spin in the ground state is $S' = 2$. One possible reason for ^{1}H NMR signals to escape detection is that the electron relaxation time is long and the paramagnetic contribution to the line width is very large. An upper limit estimate of the latter can be made from the broadening of signals *E*, *F*, and *D* when the protein is one-electron reduced (Table 2). If it is assumed that *E*, *F*, and *D* sense the closest iron of the $[Fe_3S_4]^0$ cluster, and that the ^{1}H NMR broadening is due only to dipolar coupling with the latter, an estimate of the electronic relaxation time in the $[Fe_3S_4]^0$ cluster can be made. The Fe−H distances have been estimated from the structure of the homologous 7Fe8S protein from *Azotobacter vinelandii*,[21] which appears to be similar on the grounds of the ^{1}H NMR spectra[17,22] and of the solution structure of the present protein recently refined.[23] In principle, the magnetic coupling scheme makes the three iron ions inequivalent as far as nuclear relaxation is concerned, although the effective electron relaxation time has only one value for the whole cluster.[7,24] A full treatment is reported in the Appendix, which shows that the neglect of this inequivalence does not affect the overall conclusion. If the broadening of signals *E*, *F*, and *D* depends on the sixth power of the distances from the iron ions (Table 2), i.e., $T_1^{-1} = Kr^{-6}$, then the β-CH_2 proton line widths of the $[Fe_3S_4]^0$ cluster are expected to range between 80 000 and 450 000 Hz depending on the Fe−S−C−H dihedral angle. It is clear that these values, being lower limits because any other relaxation mechanism would add up, make the ^{1}H NMR signals undetectable. However, the ^{2}H signals are predicted to be observable since the line widths are expected to be 42 times narrower (Table 2). Furthermore, with the experimental ^{1}H line width and from the Appendix, it also appears that if only the $S' = 2$ ground state were populated, the effective electronic relaxation time would be of the order of 10^{-9} s. This value is longer than that of Fe^{2+} with $S = 2$ and therefore we suspect that it is overestimated (see below). Even if it were the true electronic relaxation time, the ^{2}H signals should be detected because in oxidized rubredoxin with a similar τ_s value and larger *S* value, the ^{2}H signals of β-CH_2 protons have been detected.[8] Any other relaxation mechanism, like Curie relaxation,[25,26] does not improve the situation because the calculated contribution to line broadening would be much smaller on ^{2}H. Apparently, other reasons for the nonobservability of the ^{2}H signals have to be searched.

At this point, we are proposing that a chemical equilibrium may be operative, as shown in Figure 2B, and that the equilibrium occurs on a time scale that approximately matches the separation in chemical shifts of the pair of species. In each form, the shifts of the β-CH_2 of the cysteines bound to the mixed-valence $S = {}^9/_2$ pair would be largely downfield, while those of the β-CH_2 of the cysteines bound to the $S = {}^5/_2$ ion would be largely upfield. These expectations derive from theory (see Appendix) and from the fact that behavior of this type is observed in oxidized $[Fe_4S_4]^{3+}$ systems where the protons of the cysteines sensing the mixed-valence $S = {}^9/_2$ pair are downfield while those sensing the $S = 4$ pair are upfield.[27] Note that the nuclei coupled with the larger spin are downfield and those coupled with the smaller spin are upfield. Similarly, the cysteines sensing the Fe^{3+} ($S = {}^5/_2$) in $[Fe_2S_2]^+$ systems are shifted downfield while those sensing Fe^{2+} ($S = 2$) tend to be shifted upfield.[2] In $[Fe_2S_2]^+$ systems there is no evidence of chemical equilibrium between two species differing in the valence distribution, whereas in the proteins containing the $[Fe_4S_4]^{3+}$ cluster an equilibrium has been proposed between two species differing in the localization of the mixed-valence $S = {}^9/_2$ pair.[28] The equilibrium is, however, fast on the shift time scale, and average shifts are observed. The difference in shift is of the order of 5×10^4 Hz at 600 MHz. Therefore, the exchange rate could be set to $\gg 10^5$ s^{-1}. With a total $S' = 2$, the present Fe_3S_4 system could have a difference in shift more than an order of magnitude larger (see Appendix). If the chemical exchange between two or more different locations of the mixed valence $S = {}^9/_2$ pair is of the order of 10^6 s^{-1}, no cysteine β-CH_2 signal is expected to be observable. If a modest pseudocontact shift is present, and/or this chemical equilibrium is also accompanied by a small rearrangement, then the

(21) Stout, C. D. *J. Mol. Biol.* **1989**, *205*, 545−555.
(22) Sweeney, W. V. *J. Biol. Chem.* **1981**, *256*, 12222−12227.
(23) Aono, S.; Bentrop, D.; Bertini, I.; Donaire, A.; Luchinat, C.; Niikura, Y.; Rosato, A., submitted.
(24) Bertini, I.; Luchinat, C. *NMR of Paramagnetic Substances*; Coordination Chemistry Reviews 150; Elsevier: Amsterdam, 1996; pp 1−300.
(25) Gueron, M. *J. Magn. Reson.* **1975**, *19*, 58−66.
(26) Vega, A. J.; Fiat, D. *Mol. Phys.* **1976**, *31*, 347−355.
(27) Banci, L.; Bertini, I.; Capozzi, F.; Carloni, P.; Ciurli, S.; Luchinat, C.; Piccioli, M. *J. Am. Chem. Soc.* **1993**, *115*, 3431−3440.
(28) Banci, L.; Bertini, I.; Ciurli, S.; Ferretti, S.; Luchinat, C.; Piccioli, M. *Biochemistry* **1993**, *32*, 9387−9397.

Table 2. Observed and Calculated Paramagnetic Line Broadening in the NMR Spectra of the One-Electron Reduced 7Fe8S Ferredoxin from *B. Schlegelii*

		^{1}H line broadening [Hz]		^{2}H line broadening [Hz]	
signal	r[Å][a]	obsd	calcd	obsd	calcd
E	15.0	20	29	0.8	
F	12.4	90	90		2.2
D	6.0	2 400	7 000	200	167
β-CH_2's of $[Fe_3S_4]$ cluster	≈4		80 000		1 900
	≈3		450 000		10 700

[a] Distance from the closest iron ion belonging to the $[Fe_3S_4]$ cluster estimated from the *A. vinelandii* 7Fe8S ferredoxin structure.[21]

broadening of signal *D* and *E* could also be accounted for. This would account also for the overestimate of upper limit of τ_s.

Concluding Remarks

In this study, the 7Fe8S ferredoxin from *B. schlegelii* has been expressed with the cysteines specifically deuterated at the β position. The ^{2}H signals of both $[Fe_3S_4]^+$ and $[Fe_4S_4]^{2+}$ clusters have been observed. Upon one-electron reduction, the signals from the $[Fe_3S_4]^0$ domain were not observable, similar to what has been found in the corresponding ^{1}H spectrum. Since deuterium has a magnetic moment 6.5 times smaller than that of proton, its paramagnetic line broadening should be 42 times smaller relative to that of the corresponding proton signal. The signals should therefore be observable for an upper limit of τ_s estimated for the broadening of nearby nuclei sensing the cluster ($\tau_s = 10^{-9}$ s) which, however, is unrealistic because τ_s is expected to be smaller than that of an isolated Fe^{2+} ion (5×10^{-11} s). The $[Fe_3S_4]^0$ cluster has the $2Fe^{2.5+}-1Fe^{3+}$ structure. The hyperfine shifts of the NMR signals of nuclei from the $2Fe^{2.5+}$ site are expected to be far downfield and those from the Fe^{3+} site to be far upfield. It is proposed that there are chemical equilibria among three species, each species differing in the localization of the 3+ oxidation state among the three iron ions, and that the exchange rates are about 10^5-10^6 s^{-1}. Such rates would cause coalescence of the lines and their disappearance.

Appendix

In exchange-coupled systems, the hyperfine shift experienced by a nucleus coupled to a metal ion *j* is given by

$$\delta_j = K_j \frac{\sum_i C_{ij} S'_i(S'_i+1)(2S'_i+1)e^{-E_i/kT}}{\sum_i (2S'_i+1)e^{-E_i/kT}} \quad \text{(A1)}$$

where S'_i and E_i are the spin states and energies of the coupled systems and K_j is a constant. Equation A1 is to be compared with the equivalent equation for the isolated metal ion *j*:

$$\delta_j = K_j S_j(S_j+1) \quad \text{(A2)}$$

Analogously, the enhancement of nuclear relaxation rates (R) in the coupled system is given by

$$R_j = K_j^* \frac{\sum_i C_{ij}^2 S'_i(S'_i+1)(2S'_i+1) f(\omega,\tau_{c_i}) e^{-E_i/kT}}{\sum_i (2S'_i+1)e^{-E_i/kT}} \quad \text{(A3)}$$

which compares with the equivalent equation for the isolated metal ion *j*:

$$R_j = K_j^* S_j(S_j+1) f(\omega,\tau_c) \quad \text{(A4)}$$

The C_{ij} coefficients in eqs A1 and A3 are related to the projection of the spin of metal *j* on the coupled level spin S_i. Note that the C_{ij} coefficients are squared in the relaxation equation.

In a trimetallic system, the C_{ij} coefficients are given by[24]

$$C_{i1} = \frac{S'_i(S'_i+1) + S_{12}(S_{12}+1) - S_3(S_3+1)}{2S'_i(S'_i+1)} \times \frac{S_{12}(S_{12}+1) + S_1(S_1+1) - S_2(S_2+1)}{2S_{12}(S_{12}+1)} \quad \text{(A5)}$$

$$C_{i2} = \frac{S'_i(S'_i+1) + S_{12}(S_{12}+1) - S_3(S_3+1)}{2S'_i(S'_i+1)} \times \frac{S_{12}(S_{12}+1) + S_2(S_2+1) - S_1(S_1+1)}{2S_{12}(S_{12}+1)} \quad \text{(A6)}$$

$$C_{i3} = \frac{S'_i(S'_i+1) + S_3(S_3+1) - S_{12}(S_{12}+1)}{2S'_i(S'_i+1)} \quad \text{(A7)}$$

where S_1, S_2, and S_3 are the individual spin quantum numbers and S_{12} is the subspin of the mixed valence pair. In the present case, a qualitative estimate of shift and relaxation properties can be made by assuming that only the $S = 2$ ground state is populated. In this approximation, eqs A1 and A3 become

$$\delta_j = K_j C_{1j} S'_1(S'_1+1) \quad \text{(A8)}$$

and

$$R_j = K_j^* C_{12}^2 S'_1(S'_1+1) f(\omega,\tau_{c_1}) \quad \text{(A9)}$$

respectively. The ground state values of the coefficients are $C_{11} = 55/54$, $C_{12} = 22/27$, and $C_{13} = -5/6$.

Comparison of eq A9 with eq A4 shows that the estimate of τ_s made using eq A4 for a monomeric ion should be corrected by a factor $S_j(S_j+1)/C_{1j}^2 S'_1(S'_1+1)$. This factor ranges approximately between 1 and 2, depending on which individual metal ion of the trimetallic system is taken into consideration. The δ_j values are predicted to be similar in magnitude (although opposite in sign for Fe^{3+}) to those of the monomeric system (ca. 800 ppm in oxidized rubredoxin). The overall spreading would thus be about 1600 ppm.

IC970920R