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Synthetic Analogues of the Active Sites of Iron–Sulfur Proteins. XIII.[1] Further Electronic Structural Relationships between the Analogues $[Fe_2S_2(SR)_4]^{2-}$ and the Active Sites of Oxidized 2Fe–2S* Proteins

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In order to examine further the electronic relationships between the active sites of oxidized 2Fe ferredoxin proteins (Fd_{ox}) and the synthetic dimer bis[*o*-xylyl-α,α'-dithiolato-μ-sulfido-ferrate(III)] dianion ($[Fe_2S_2(S_2\text{-}o\text{-xyl})_2]^{2-}$), previously proposed as an active-site analogue, Mössbauer, magnetic susceptibility, and ^1H NMR properties of the latter have been determined and compared with those of spinach Fd_{ox}. Mössbauer results suggest a substantial degree of electronic similarity, imply a slightly lower metal site symmetry in the protein, and confirm a magnetic singlet ground state. A detailed series of measurements of the temperature-dependent magnetic properties of two salts of $[Fe_2S_2(S_2\text{-}o\text{-xyl})_2]^{2-}$ reveals antiferromagnetic behavior with $-J = 148 \pm 8$ cm^{-1}. The temperature dependencies of the isotropic methylene proton shifts and magnetic susceptibilities of the analogue parallel each other, indicating that the shifts are dominantly contact in nature. Values of the coupling constant A_{CH_2} are virtually identical in the protein and analogue. These results in conjunction with others reported earlier indicate that $[Fe_2S_2(S_2\text{-}o\text{-xyl})_2]^{2-}$ is a suitable minimal structural and electronic representation of 2Fe Fd_{ox} sites, and tend to confirm the μ_2-sulfido structure $[Fe_2S_2(S\text{-Cys})_4]$ proposed for the protein active sites. Certain matters pertinent to the measurement of magnetic properties of weakly magnetic materials by the vibrating-sample magnetometer employed here are briefly discussed.

Introduction

The three currently recognized types of active sites in nonheme iron–sulfur redox proteins,[3] specified in terms of minimal composition, are $[Fe(S\text{–}Cys)_4]$ (Rd), $[Fe_4S^*_4(S\text{–}Cys)_4]$ (4-, 8-Fe Fd; HP), and $[Fe_2S^*_2(S\text{–}Cys)_4]$ (2Fe Fd[4]). The structures of Rd_{ox}[5,6] and 8-Fe Fd_{ox}[5,7] and $HP_{red,ox}$[8] have been established by x-ray diffraction and contain sites of distorted tetrahedral and cubane-type stereochemistries, respectively. The structure of no 2Fe–2S* protein has yet been established by x-ray methods. However, an impressive array of physical data has been interpreted as fully consistent with the binuclear structure **1**,[9–11] which in oxidized proteins

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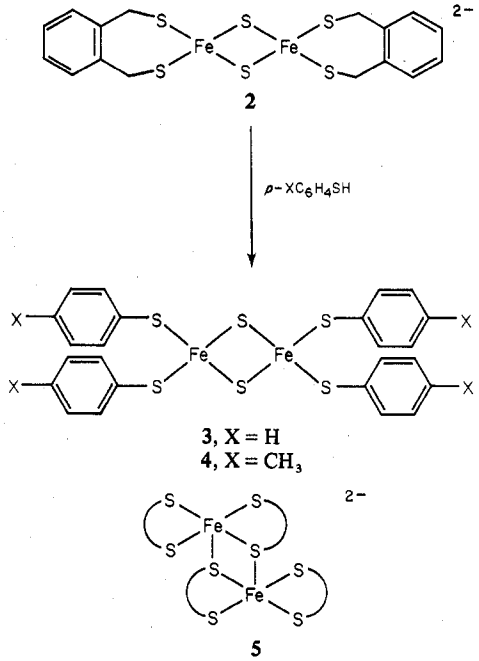
contains two tetrahedrally coordinated, high-spin, antiferromagnetically coupled[12] Fe(III) ions.

In an attempt to afford a further clarification of the nature of the active site(s) in the 2Fe–2S* class of proteins, the synthetic analogue approach[13] has been applied to the preparation and structural and electronic characterization of bis[*o*-xylyl-α,α'-dithiolato-μ-sulfido-ferrate(III)] dianion[14,15] ($[Fe_2S_2(S_2\text{-}o\text{-xyl})_2]^{2-}$, **2**) and its arylthiolate substitution products, among which are $[Fe_2S_2(SPh)_4]^{2-}$ (**3**) and $[Fe_2S_2(S\text{-}p\text{-tol})_4]^{2-}$ (**4**). X-ray determinations have shown that **2** and **4** are centrosymmetric dimers and conform to the overall stereochemistry proposed for the protein site **1**. Both contain planar, essentially isostructural $Fe_2S^*_2$ cores with the following dimensions for **2** and **4**, respectively: Fe–S*, 2.209, 2.201 Å; Fe–S*–Fe, 75.3, 75.4°; Fe···Fe, 2.698, 2.691 Å. Both of these complexes are considered to serve as minimal structural representations of 2Fe Fd_{ox} active sites. Together with the nonanalogue species bis[bis(1,2-ethanedithiolato)ferrate(III)] dianion[16,17] ($[Fe_2(edt)_4]^{2-}$, **5**, Fe···Fe = 3.410 Å), bridged by alkylthiolato groups, these are the only structurally defined dimeric iron complexes ligated exclusively by sulfur.[18]

In addition to detailed structural results,[15] we have previously reported electronic spectral and redox properties of $[Fe_2S_2(S_2\text{-}o\text{-xyl})_2]^{2-}$ [14,15] and $[Fe_2S_2(SAr)_4]^{2-}$.[15] These properties, in conjunction with the limited magnetic susceptibility and Mossbauer spectral results then available,[14] were considered sufficient to establish **2**, whose alkylthiolate



groups simulate cysteinyl binding, as a minimal electronic representation of oxidized protein sites. By way of further assessing the suitability of this complex as an active-site analogue, we report here detailed magnetic susceptibility studies of two of its salts together with Mössbauer and ^1H NMR spectral results which augment those briefly described earlier.[14] The present work was in part motivated by several recent results. The nearly superimposable temperature dependence of susceptibility of $(Et_4N)_2[Fe_4S_4(SCH_2Ph)_4]$[19] and *Chromatium* HP_{red}[20] indicates that, in at least one case, isoelectronic and nearly isostructural[8,21] analogue and protein site clusters exhibit very similar antiferromagnetic interactions. The magnitude of antiferromagnetic coupling between two high-spin Fe(III) centers, as reflected by the interaction constant J, is significantly dependent upon the combined effects of Fe···Fe separation and the nature of the S-bridging ligand(s).[17,22] Consequently, it is reasonable to assume that structural and electronic similarities between 2Fe Fd_{ox} sites and $[Fe_2S_2(S_2\text{-}o\text{-xyl})_2]^{2-}$ would generate similar temperature-dependent magnetic behaviors and J values.

Large, negative values of the latter ($-J > 100$ cm^{-1}) have been reported[12] for spinach Fd_{ox}.

Experimental Section

Preparation of Compounds. $(Et_4N)_2[Fe_2S_2(S_2\text{-}o\text{-}xyl)_2]$, $(Ph_4As)_2[Fe_2S_2(S_2\text{-}o\text{-}xyl)_2]$, and $(Et_4N)_2[Fe_2S_2(SPh)_4]$ were prepared as described elsewhere.[15] Although obtained as analytically pure salts[15] suitable for other types of physical measurements, they were found to contain significant amounts of paramagnetic contaminants, particularly evident below ca. 45 K in magnetic measurements. In order to reduce the level of contamination and therewith the corrections necessary to obtain the true magnetic properties of the dimers over as large a temperature interval as possible, a number of purification procedures were attempted. None completely removed paramagnetic impurities, but the following did reduce the impurity level appreciably. The three salts, in the order given above, were recrystallized from 1:1 v/v DMF–methanol, 1:(2–3) v/v DMSO–ethanol, and 1:2 v/v acetonitrile–THF. A nearly saturated solution was obtained in DMF, DMSO, or acetonitrile at 60–65 °C and filtered at this temperature. The second component of the solvent pair, also at 60–65 °C, was slowly added and the solution was allowed to cool to room temperature. Further cooling to −20 °C afforded a nearly quantitative recrystallization. All operations were performed under a nitrogen atmosphere. The crystalline products were collected, dried in vacuo, and used in the magnetic measurements.

Physical Measurements. All operations were performed with exclusion of oxygen. ^{1}H NMR spectra were recorded on a Varian HR-220 spectrometer equipped with a CAT. Solvents used were CD_3CN, CD_3OD, and DMSO-d_6. Chemical shifts were determined relative to TMS internal reference. Isotropic shifts were calculated from the relation $(\Delta H/H_0)_{iso} = (\Delta H/H_0)_{obsd} - (\Delta H/H_0)_{dia}$, where the diamagnetic reference shifts were taken as the methylene proton shifts of *o*-xylyl-α,α'-dithiol[15] in the appropriate solvents at ambient temperature. Negative shifts refer to a downfield displacement relative to TMS or $(\Delta H/H_0)_{dia}$, consistent with the previous practice for Fe–S analogues[23] but opposite to the convention usually employed for Fe–S proteins.[24,25] Mossbauer spectra were obtained for polycrystalline samples at 295, 77, and 4.2 K in zero applied magnetic field and at 4.2 K in external magnetic fields with longitudinal configuration up to 80 kG. The source (^{57}Co in Rh) was at the same temperature as the absorber.

Magnetic Measurements and Estimated Errors. Magnetic measurements were made using the prototype vibrating-sample magnetometer[26] (VSM) operated at a magnetic field $H = 16.8$ kG over the temperature range 4.2–287 K. For measurements on the weakly paramagnetic compounds encountered in this investigation the VSM[26,27] employed two sets of series-opposing coils (wound normal to the vibrating or z axis). These coils had an air gap of 1.8 cm in which the glass Dewar and sample were contained. Accuracy of the data was improved by recording the output of the magnetometer directly with a strip chart recorder concurrently with temperature measured with a copper–constantan thermocouple. A continuous-data set of both quantities was obtained during warm-up from 4.2 to 290 K over a period of several hours. Samples of dimer salts (30–40 mg) were measured in containers of uniform size (height and diameter 4.5 mm, wall thickness 0.1 mm) fabricated from nylon or delrin stock and thoroughly cleaned to remove contaminants. The gram-susceptibility of a dimer salt at a given temperature was calculated from eq 1, where σ_s is the saturation moment of the Ni standard (55.1

$$\chi^g_d = \frac{\sigma_s m_s}{H m_x d_s}\left[d_x - \frac{4.2f}{T} d'_x\right] \qquad (1)$$

emu/g at 300 K), x is the sample, m is the mass, and d and d' are the VSM output in divisions relative to a common zero; $d_x = d_{c+x} - d_c$ with c being the container and primed values obtained at 4.2 K. Correction for paramagnetic impurities in all samples was made by assuming that at 4.2 K $\chi^g_x = \chi^g_i$, the impurity susceptibility per gram of sample, and that at higher temperatures χ^g_i followed the Curie law. The field dependence of $d'_x = d'_{c+x} - d'_c$ up to 16.8 kG revealed that for all samples at 4.2 K the moment was not a linear function of applied field. The low-field limit of the initial susceptibility was used to determine the impurity contribution. The initial slope of a plot of d'_x vs. H yielded the quantity fd'_x, proportional to impurity susceptibility. Equation 1 is then $\chi^g_d = \chi^g_x - \chi^g_i$. Duplicate and triplicate runs, involving 55 measurements over the entire temperature range for each run, were made for the Et_4N^+ and Ph_4As^+ salts of $[Fe_2S_2(S_2\text{-}o\text{-}xyl)_2]^{2-}$ taken from different preparations. The 22 data points below 55 K were rejected in each case due to the large impurity background and the resultant scatter in χ^g_d values. Reproducibilities of χ^g_d values at a given temperature are typified by the results for the Ph_4As^+ salt and, in terms of standard deviation from the mean compared to mean values, ranged from 21% at 103.5 K to 3.8% at 287.0 K.

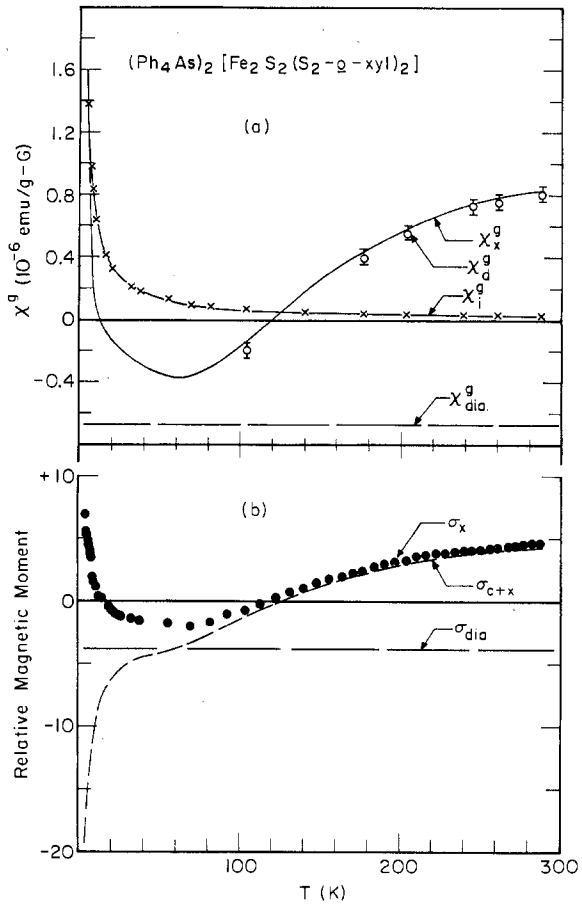


Figure 1. Results used for the determination of the temperature dependence of the molar susceptibility per iron χ^M_{Fe} of $(Ph_4As)_2[Fe_2S_2(S_2\text{-}o\text{-}xyl)_2]$. Data are from run 1 (*cf.* Experimental Section). (a) Gram-susceptibilities: total sample, χ^g_x; dimer, χ^g_d; paramagnetic impurities, χ^g_i; diamagnetic susceptibility correction, χ^g_{dia}. The data were taken at $H = 16.8$ kG and χ^g values were calculated assuming a linear magnetic moment vs. field for χ^g_x, χ^g_d, and χ^g_{dia}. The plot of χ^g_i is based on initial susceptibility data at 4.2 K and low applied fields and the assumption of a Curie law dependence of χ^g_i. Selected values of χ^g_d with estimated errors are shown. (b) Relative magnetic moment data vs. temperature: container plus sample, σ_{c+x} (- - -); solid points, selected data for sample, σ_x, when container contribution is subtracted; σ_{dia}, calculated diamagnetic contribution.

The measured magnetic moments in the present experiments are relatively small so that various background contributions to the total moment are significant. These contributions are common to all static magnetic measurements of dilute paramagnetic ions in large molecules and become dominant particularly for systems with a singlet ground state at low temperatures (such as the dimers examined in this work). In order to indicate clearly the present limitations we summarize the experimental results and assumptions involved in analysis of the data. The detailed features of the various contributions for a typical measurement are shown in Figure 1a and b.

The quantity actually measured is the total magnetic moment σ and hence the magnetic moment per gram σ^g. If σ is a linear function of applied field H, then $\chi^g = \sigma^g/H$. For the particular parameters of the VSM, both the resolution and sensitivity of the *magnetic moment* measurements was $\sim 5 \times 10^{-6}$ emu. For a 1-g sample and 10 kG, this sensitivity corresponds to a susceptibility of $\chi^g = 5 \times 10^{-10}$ emu/(g

G). The above results do not represent the limits of sensitivity[27] of a VSM but were adequate for the present measurements with ~30-mg samples. At high temperature (287 K) the dimer contribution to the moment is at its largest value, yet the background contributions from the sample container, paramagnetic impurities, and diamagnetic matrix are significant. At low temperatures the background contributions dominate. Despite its small mass, the container contribution is appreciable even at high temperature ($\chi_c/\chi_d \approx 0.07$ at 287 K). If more sample is available, the relative contribution of the container can be reduced approximately inversely proportional to a container dimension. The impurity susceptibility χ^g_i is relatively small at high temperature for this sample ($\chi^g_i/\chi^g_d \approx 0.028$ at 287 K). Because the VSM method uses a uniform field which can be varied at will, the 4.2 K data were examined as a function of field. The lowest field data ($H \leq 1.5$ kG) permitted calculation of accurate initial susceptibility. (The average χ^g_i at 4.2 K and 1.5 kG is about 1.3 times the value of χ^g_i at 16.8 kG.) These low-field initial susceptibility data were used to calculate χ^g_i vs. T assuming a Curie law. Assuming $g = 2$ and only Fe^{3+} impurities, the data in Figure 1 yield about 1.5×10^{-3} Fe^{3+} free ions/dimer. (The impurity initial susceptibility χ_i would overcorrect the data at lowest temperature because the measured *average* value of the impurity susceptibility at 16.8 kG is smaller than χ_i. At temperatures above ~50 K this field dependence of the impurity susceptibility is negligible.) At all temperatures any error in the diamagnetic matrix susceptibility χ_{dia} would make a large error in the derived value of χ^g_d ($\chi^g_{dia}/\chi^g_d \approx 0.7$ at 287°K). This problem would be more severe for larger molecules (e.g., Fd_{ox} proteins). The assumption that χ^g_{dia} can be calculated accurately from Pascal's constants and that χ^g_{dia} is independent of temperature was not tested; any error in the absolute value or temperature dependence of χ^g_{dia} would have a first-order effect on the value of χ^g_d. As is evident in Figure 1 relatively larger effects of χ^g_{dia} are expected at low temperatures where χ^g_d becomes small.

An important objective of the magnetic studies has been to place uncertainty limits on the experimentally derived parameter of ultimate interest, J, in order to allow as meaningful a comparison as possible between J values of $[Fe_2S_2(S_2\text{-}o\text{-}xyl)_2]^{2-}$ and such values for other synthetic Fe–S dimers and 2Fe Fd_{ox} proteins as are presently known or estimated or may be determined in future work. For this reason, and because of the fact that the total magnetic moments of the dimer salts are very small, a detailed analysis of errors affecting χ^g_d values has been carried out. All quantities in the multiplicative factor of eq 1 and the temperature when $T > 100$ K are known to better than 1%. Based on experiments with standard and samples the error in any measurement of d is estimated as $\sim 0.05/d$ for the experimental range $30 < d < 0.3$ division; the maximum error in the factor f is estimated as 25%. For a typical measurement (run 1) of $(Ph_4As)_2[Fe_2S_2(S_2\text{-}o\text{-}xyl)_2]$ the experimental quantities σ_{c+x} and σ_x (the σ_c curve is omitted) together with calculated values of χ^g_x, χ^g_i, and χ^g_d are plotted in Figure 1 over the range 4.2–287 K. Standard error propagation treatment led to the limits of error λ in the result χ^g_d. To these limits was added an estimate of the errors associated with lack of reproducibility in positioning container and container plus sample in the magnetometer, taken as $(0.1d_c/d_{c+s})\chi^g_d$ for any run. (There is a small error which can be introduced if the sample position between the detection coils is changed. At 287 K this contribution to χ^g_d was <1%.) For selected temperatures final values of $\lambda(\chi^g_d)/\chi^g_d$ for run 1 and average values for the three runs are the following: 103.5 K, 0.21, 0.31; 177.2 K, 0.12, 0.14; 203.6 K, 0.082, 0.098; 244.9 K, 0.059, 0.078; 261.2 K, 0.055, 0.073; 287.0 K, 0.051, 0.067. Estimated errors in χ^g_d for run 1 are indicated in Figure 1. To fit the data to the antiferromagnetic coupling model (vide infra) the molar susceptibility per iron $\chi^M_{Fe} = (M\chi^g_d - \chi^M_{dia})/2$, calculated from averaged χ^g_d data for each compound, was employed. Taking the diamagnetic correction as a constant ($\chi^M_{dia} = -677 \times 10^{-6}$ from Pascal's constants[28]), the following uncertainties in χ^M_{Fe} expressed as $\Delta\chi^M_{Fe}/\chi^M_{Fe}$ are obtained for the Ph_4As^+ salt: 103.5 K, 0.14; 177.2 K, 0.063; 203.6 K, 0.051; 244.9 K, 0.046; 261.2 K, 0.043; 287.0 K, 0.041. The temperature dependence of χ^M_{Fe} for this compound is plotted in Figure 2; at the specified temperatures $\Delta\chi^M_{Fe}$ is a nearly constant 36×10^{-6} emu/(mol G). Similarly estimated values of $\Delta\chi^M_{Fe}/\chi^M_{Fe}$ for $(Et_4N)_2[Fe_2S_2(S_2\text{-}o\text{-}xyl)_2]$ and $(Et_4N)_2[Fe_2S_2(SPh)_4]$ are entirely comparable.

Results and Discussion

Spectroscopic and magnetic properties of the dimers

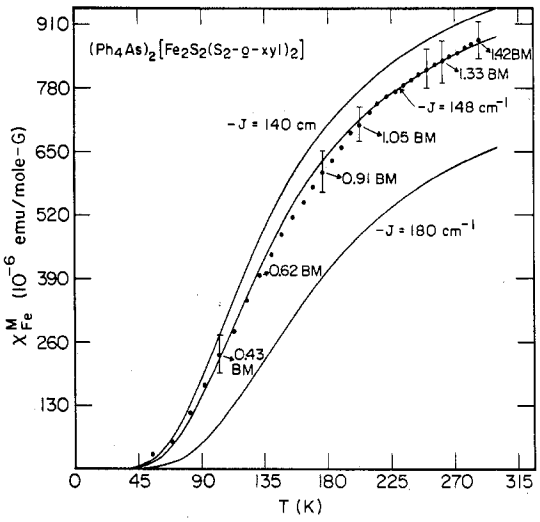


Figure 2. Temperature dependence of the molar susceptibility per iron χ^M_{Fe} of $(Ph_4As)_2[Fe_2S_2(S_2\text{-}o\text{-}xyl)_2]$ and calculated curves for different values of J. The data points are averages of three runs. Effective magnetic moments per iron at various temperatures were calculated from the Curie law, $\mu_{Fe} = 2.829(\chi^M_{Fe}T)^{1/2}$.

Table I. Comparative Properties of Oxidized 2Fe–2S* Analogues and Proteins

Property	$[Fe_2S_2(S_2\text{-}o\text{-}xyl)_2]^{2-}$ Et_4N^+ salt	$[Fe_2S_2(S_2\text{-}o\text{-}xyl)_2]^{2-}$ Ph_4As^+ salt	$(Et_4N)_2$-$[Fe_2S_2$-$(SPh)_4]$	Spinach Fd_{ox}
δ,[a] mm/sec	+0.17 ± 0.01[b]	+0.17 ± 0.01[b]	+0.17 ± 0.01[b]	+0.22[c]
ΔE_Q, mm/sec	0.36 ± 0.01[b]	0.36 ± 0.02[b]	0.32 ± 0.02[b]	0.65[d]
$10^6\chi^M_{Fe}$, emu/(mol G)	857 ± 40[e,f]	877 ± 36[e,g]	853 ± 40[e]	960,[h] 628[e,i]
μ_{Fe}, BM	1.40 ± 0.03[c]	1.42 ± 0.02[d]	1.40 ± 0.03	1.51,[j] 1.20[e]
$-J$, cm^{-1}	149 ± 8[o]	148 ± 8[o]		143,[k] 183[k]
$(\Delta H/H_0)_{obsd}$, ppm	−39.5[l]			−37[m]
A_{CH_2}, G	~0.11			~0.10, ~0.14[n]

[a] Relative to Fe metal. [b] Temperature invariant, 4.2–295 K. [c] Reference 30e. [d] Reference 30c. [e] 287 K. [f] Average of two measurements. [g] Average of three measurements. [h] Aqueous solution room temperature datum of Ehrenberg quoted by others.[10,12] [i] Calculated using $-J = 183$ cm^{-1}. [j] Assuming 298 K. [k] Reference 12; uncertainties not specified. [l] 2:1 v/v CD_3OD–DMSO-d_6, 295 K. [m] D_2O, room temperature, ref 10 and 25. [n] See text. [o] Estimated errors obtained by fitting theoretical $\chi_{Fe}(T)$ curves to the limits of experimental uncertainty in χ_{Fe} at various temperatures.

$[Fe_2S_2(S_2\text{-}o\text{-}xyl)_2]^{2-}$ and $[Fe_2S_2(SPh)_4]^{2-}$ determined in this investigation together with corresponding values for spinach Fd_{ox} are collected in Table I.

Mossbauer Spectra. ^{57}Fe spectra were obtained for $(Ph_4As)_2[Fe_2S_2(S_2\text{-}o\text{-}xyl)_2]$ and $(Et_4N)_2[Fe_2S_2(SPh)_4]$ at 295, 77, and 4.2 K and in external fields up to 80 kG. Spectra of the latter compound are displayed in Figure 3; those of $(Et_4N)_2[Fe_2S_2(S_2\text{-}o\text{-}xyl)_2]$ have been described earlier.[14] The zero-field spectra of all three compounds consist of single, temperature-invariant, symmetric quadrupole doublets with identical isomer shifts (δ) and only slightly different quadrupole splittings (ΔE_Q) between **2** and **3**. Spectra in external magnetic fields are readily interpreted in terms of a single iron site with the magnetic field at the nucleus equal to the applied field, the sign of the principal component of the electric field gradient positive, and the orientation of the electric field

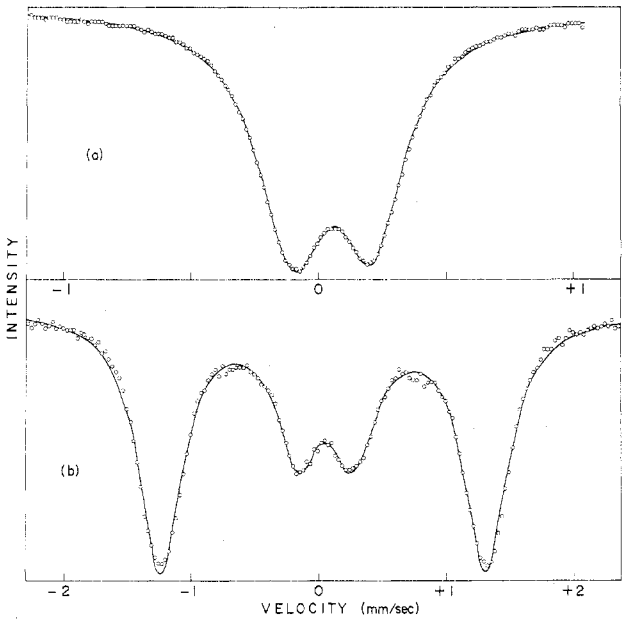


Figure 3. Mössbauer spectra of $(Et_4N)_2[Fe_2S_2(SPh)_4]$ at 4.2 K in (a) zero field and (b) a longitudinal magnetic field of 80 kG. The solid lines are theoretical least-squares fits assuming Lorentzian line shapes. The experimental line width is 0.31 mm/s. The excess line width is due to the strong (100 mCi) ^{57}Co in Rh source and the absorber thickness. The slight asymmetry of the lines is due to preferential orientation in the absorber. Using a narrow line source and thin absorber, a symmetric spectrum with a line width of 0.25 mm/s is observed at room temperature.

gradient in the polycrystalline materials randomized with respect to the applied field. These results indicate that the two iron atoms in each dimer are equivalent and are exchange coupled to produce a magnetic singlet ground state. At 4.2 K only the ground state is populated and no magnetic hyperfine interaction apart from that of the nucleus with the applied field is observed.

The structural features indicated by the Mössbauer results are consistent with crystallographic results for dimer salts.[15] The values of the parameters in Table I, presumably representative of a larger range of synthetic $[Fe_2S_2(SR)_4]^{2-}$ species than examined here, are comparable with those for other high-spin Fe^{III}–S_4 coordination units distorted from T_d symmetry.[1,29] The identity of δ and ΔE_Q for $[Fe_2S_2(S_2\text{-}o\text{-xyl})_2]^{2-}$ as its Et_4N^+ and Ph_4As^+ salts strongly suggests no important change in structure in the latter as compared to that determined in the former salt.[15] Values of δ lie on one end of the range (0.18–0.30 mm/s) found for 2Fe Fd_{ox} proteins whereas ΔE_Q values are smaller than those for the proteins (0.60–0.66 mm/s).[30] Other than observing that the differences in quadrupole splittings suggest a slightly higher site symmetry in the synthetic dimers than in the proteins, the Mössbauer data are indicative of appreciable structural and electronic similarity between the dimers and the oxidized protein sites. Moreover, the observation of a singlet ground state in the present compounds is entirely consistent with the results obtained by Mössbauer spectroscopy for oxidized proteins.[30a-d]

Magnetic Results. Below ca. 100 K plant,[31] bacterial,[32] and adrenal[32,33] 2Fe Fd_{ox} proteins have been reported to show either no or a trace paramagnetic susceptibility in contrast to the readily detectable paramagnetism of the reduced form with a spin-doublet ground state. Reexamination of lyophilized spinach Fd_{ox} at higher temperatures by Palmer et al.[12] has provided clear evidence for the antiferromagnetic nature of the active site of this protein. Under the usual Hamiltonian $\mathcal{H} = -2J\tilde{S}_1\cdot\tilde{S}_2$ the spins of the two metal centers are coupled to generate a manifold of states $S'_i = 0, 1, 2, ..., 5$ with energies 0, $2|J|$, $6|J|$, ..., $30|J|$. Equation 2 for the temperature de-

$$\chi^M{}_d = \frac{2g^2\beta^2N}{kT}Q \tag{2}$$

pendence of the susceptibility of two antiferromagnetically coupled high-spin Fe(III) ions ($S_1 = S_2 = {}^5/_2$) is readily obtained.[34] Q is given by eq 3, in which $x = -|J|/kT$, other

$$Q = \frac{e^{2x} + 5e^{6x} + 14e^{12x} + 30e^{20x} + 55e^{30x}}{1 + 3e^{2x} + 5e^{6x} + 7e^{12x} + 9e^{20x} + 11e^{30x}} \tag{3}$$

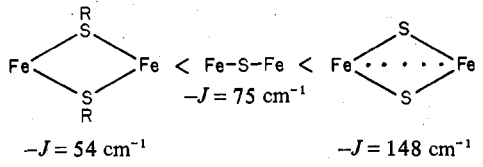
symbols have their usual meanings, and $\chi^M{}_d = 2\chi^M{}_{Fe}$. *Relative* protein susceptibilities were determined and the results[12] fit to eq 2 yielding $-J = 183$ cm^{-1}. This is the only J value currently available from magnetic measurements of 2Fe Fd_{ox} proteins over an appreciable temperature range above 100 K.[35]

Initial magnetic measurements of $[Fe_2S_2(S_2\text{-}o\text{-xyl})_2]^{2-}$ were confined to one salt (Ph_4As^+) and to the temperature range 77–296 K.[14] These results sufficed to demonstrate the antiferromagnetic nature of the dimer and indicated that J occurs in the interval −145 to −155 cm^{-1}. However, they were not reported in full due to the lack of low-temperature data required for impurity corrections and the desire to determine magnetic properties in more than one crystalline environment. More detailed studies of the Ph_4As^+ and Et_4N^+ salts at 4.2–287 K have been carried out in this investigation. The temperature dependence of $\chi^M{}_{Fe}$ for the former salt is given in Figure 2 together with the effective magnetic moments per iron (μ_{Fe}) calculated from the Curie law. Best fits of the data for this and the Et_4N^+ salt were calculated by assuming $g = 2.0$ and by giving greater weight to points at $T > 200$ K where $\chi^g{}_x$ is larger and $\chi^g{}_i$ substantially smaller than at lower temperatures. The essentially identical J values for the two salts are given in Table I together with other magnetic data. Duplicate measurements of $(Et_4N)_2[Fe_2S_2(SPh)_4]$ failed to afford as close a fit to theoretical susceptibility curves as those of the preceding two compounds, but the magnetic data at 287 K (Table I) are suggestive of a very similar J value.

The protein value[12] was obtained by positioning the relative susceptibility vs. temperature curve (analogous to curve σ_x in Figure 1b) on the ordinate until an acceptable fit was obtained with a calculated χ^M/T curve. Absolute susceptibilities were not obtained and no uncertainty range of any measured quantity or J was reported. As noted above, there are several large contributions to the magnetic susceptibility which must be measured in order to determine J; these contributions are larger in the protein measurements than in the present case. The only check currently available involves comparison of calculated and observed $\chi^M{}_{Fe}$ values for spinach Fd_{ox} given in Table I and by Palmer et al.[12] The computed susceptibility is 65% of the aqueous solution value obtained by Ehrenberg using the Gouy method. Susceptibilities of $[Fe_2S_2(S_2\text{-}o\text{-xyl})_2]^{2-}$ are in substantially better agreement with the solution result, for which, however, estimated errors and the dominant $\chi^M{}_{dia}$ contribution are not available. A change in J between the lyophilized sample and the protein in solution is possible, and Palmer et al.[12] reported the value $-J = 143$ cm^{-1}, very close to the dimer value (Figure 2), to be consistent with the solution susceptibility result of Ehrenberg. Because of the experimental uncertainties in ref 12, we are unable to assess whether J for $[Fe_2S_2(S_2\text{-}o\text{-xyl})_2]^{2-}$ differs significantly from the lyophilized protein value. We note that in the present case, the estimated uncertainties in $\chi^M{}_{Fe}$, shown for selected temperatures in Figure 2, are such as to preclude any fit to eq 2 with $-J \geq 160$ cm^{-1}.

With the availability of a firm value of J for $[Fe_2S_2(S_2\text{-}o\text{-xyl})_2]^{2-}$, the antiferromagnetic interaction sequence noted

earlier[17] can be specified more precisely for sulfur-bridged high-spin Fe(III) dimers. Assuming that J values are dominated by the nature of the bridging unit, the sequence



$-J = 54$ cm^{-1} $\qquad$ $-J = 148$ cm^{-1}

emerges in which data for the first two compounds are taken from measurements of $[Fe_2(edt)_4]^{2-}$ [17] and $[Fe(salen)]_2S$.[22] The structure of the latter complex has not been established, but, being a congener of structurally defined, antiferromagnetic $[Fe(salen)]_2O$,[36] the presence of the indicated bridge structure is probable. Exchange coupling increases as Fe···Fe distances decrease (terminal members) and thiolate bridges are replaced by sulfide. That the large interactions found for the FeS_2Fe unit may be of general occurrence is suggested by the extraction of $-J \approx 200$ cm^{-1} from an analysis of the linear-chain antiferromagnet $KFeS_2$ (Fe···Fe = 2.70 Å).[37] Thus, while we are unable to conclude that the J value for $[Fe_2S_2(S_2\text{-}o\text{-xyl})_2]^{2-}$ is experimentally indistinguishable from that of at least one 2Fe Fd_{ox} protein, the protein interaction constant accords better with the bridge unit in proposed structure **1** than with the other two types of units for which magnetic data are available. In this sense J values, after their accumulation in larger numbers of examples, may be diagnostic for the presence of FeS_2Fe units.[38]

^{1}H NMR Spectra. In the original 220-MHz ^{1}H NMR investigations of 2Fe Fd_{ox} proteins the broad feature observed at −13 to −15 ppm at 278–303 K was assigned to contact-shifted cysteinyl methylene protons.[42] Subsequent experiments employing a larger sweep range at 60 MHz resulted in the detection of a very broad downfield resonance for spinach Fd_{ox} which increases from *ca.* −34 to −37 ppm in D_2O solution as the temperature is raised from ca. 278 to 303 K.[10,25] The spectrum of *S. lividus* Fd_{ox} also reveals a broad resonance at −34 ppm (293 K).[35] As pointed out earlier[14] the occurrence of a broad signal of comparable chemical shift in the spectrum of $[Fe_2S_2(S_2\text{-}o\text{-xyl})_2]^{2-}$ tends to confirm assignment of the latter signals to cysteinyl $-CH_2S-$ protons. Observed methylene shifts at 295 K are the following: −40.3 ppm, CD_3CN; −39.6 ppm, DMSO; −39.5 ppm ($\Delta\nu_{1/2} \approx 1830$ Hz at 311 K), 2:1 v/v CD_3OD–DMSO-d_6. The largest temperature range was afforded by the mixed-solvent system, and the temperature dependence of *isotropic* shifts in the range 218–358 K is plotted in Figure 4. The dimer, as the protein in a narrower temperature interval, exhibits progressive downfield shifts as the temperature is raised. Magnetic results provide an adequate rationalization for the temperature dependence of the shifts of the oxidized protein and $[Fe_2S_2(S_2\text{-}o\text{-xyl})_2]^{2-}$. If the isotropic shifts are dominantly or entirely contact in origin, i.e., dipolar (pseudocontact) shifts are negligible, $(\Delta H/H_0)_{iso} \simeq (\Delta H/H_0)_{con}$ and the shifts will be directly proportional to magnetic susceptibility at a given temperature. In this case the net contact shift of a proton in the dimer is given by eq 4 provided the electron–nuclear

$$\left(\frac{\Delta H}{H_0}\right)_{con} = -\frac{g\beta A}{\gamma_H kT\hbar}Q \qquad (4)$$

coupling constant A is independent of spin state and/or only the lowest paramagnetic state ($S' = 1$) is appreciably populated.[43] From eq 4 it follows that $\chi^M_{Fe} = R(\Delta H/H_0)_{con}$ where the constant $R = -g\beta N\gamma_H\hbar/A$ is independent of temperature. The χ_{Fe} values for the Et_4N^+ and Ph_4As^+ salts

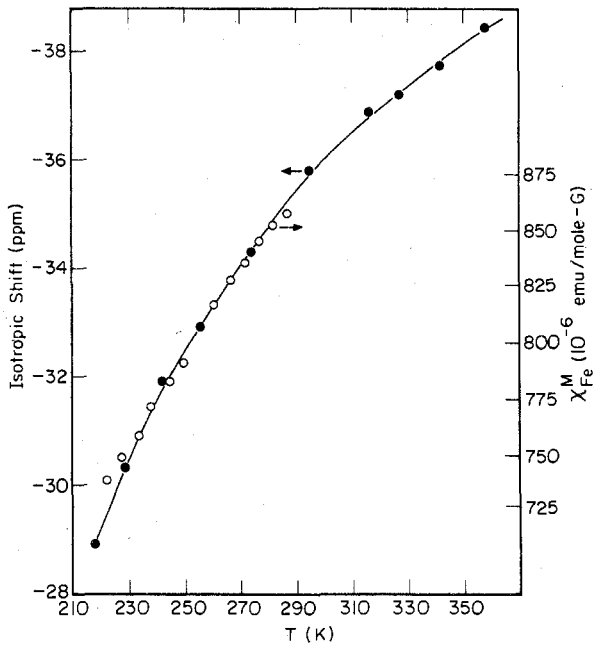


Figure 4. Relationship between the temperature dependence of methylene proton isotropic shifts of $[Fe_2S_2(S_2\text{-}o\text{-xyl})_2]^{2-}$ in 2:1 v/v CD_3OD–DMSO-d_6 (•) and χ^M_{Fe} in the solid state (○). The scales were arbitrarily chosen such that $(\Delta H/H_0)_{iso}$ and χ^M_{Fe} coincide at 256 K.

of $[Fe_2S_2(S_2\text{-}o\text{-xyl})_2]^{2-}$ were averaged and scaled to the observed shifts by a factor R. The results are plotted in Figure 4 and show that the two quantities have closely similar temperature dependencies in the interval (228–287 K) common to both measurements. This relationship provides a reasonable demonstration that the isotropic methylene proton shifts of $[Fe_2S_2(S_2\text{-}o\text{-xyl})_2]^{2-}$ arise principally from contact interactions, a result also obtained by the same procedure for the 4Fe analogue $[Fe_4S_4(SCH_2Ph)_4]^{2-}$.[23]

In view of the absence of appreciable dipolar shifts in $[Fe_2S_2(S_2\text{-}o\text{-xyl})_2]^{2-}$, a similar situation appears probable for the 2Fe Fd_{ox} sites. Due to the lack of directly determined susceptibilities and the availability of isotropic shift data over only a very restricted temperature interval, the scaling procedure, which yields the coupling constant A, cannot be applied to the proteins. However, a value of A can be estimated from eq 4 using J values and cysteinyl methylene proton shifts. From published data[10,25] we take the *isotropic* shift to be −34 ppm at 295 K for spinach Fd_{ox}. With $-J = 183$ and 143 cm^{-1}, values of $A = 0.14$ and 0.10 G, respectively, are obtained (A (G) $= hA$ (Hz)$/g\beta$). From the R value for $[Fe_2S_2(S_2\text{-}o\text{-xyl})_2]^{2-}$ $A = 0.11$ G is found. This extremely close agreement is probably to an extent fortuitous in view of the large line widths of both protein and analogue resonances. Line broadening in the protein arises from the inequivalence of the eight cysteinyl methylene protons in site **1** and the associated angular dependence of coupling constants. In the analogue four methylene protons are inequivalent in the crystalline state[15] but this inequivalence could be essentially removed by rapid conformational changes of the chair-type chelate rings. Superimposed on any inequivalency effects in both cases is electron–nuclear dipolar line broadening due to the proximity of methylene protons to a paramagnetic center. Calculated Fe···H distances in $[Fe_2S_2(S_2\text{-}o\text{-xyl})_2]^{2-}$ are 2.9, 3.0, 3.9, and 4.0 Å, whereas Salmeen and Palmer[25] have estimated 3.4–5.3 Å from an analysis of line widths in the spinach Fd_{red} spectrum. Thus, A values obtained (preferably) from measured shifts and susceptibilities or indirectly from shifts and J values are in these cases composite or phenomenological parameters rather than precise measures of specific nucle-

ar–electron interactions. Although the close agreement between protein and analogue A values is not given the same weight of significance accorded to other comparative physical properties described here and elsewhere,[14,15] it is nonetheless additional evidence supporting a structural and electronic relationship between $[Fe_2S_2(S_2\text{-}o\text{-}xyl)_2]^{2-}$ and 2Fe Fd_{ox} sites. The positive values of A signify positive spin density at the methylene protons, which is most simply visualized as arising from σ-bond spin polarization and/or direct interaction of H 1s orbitals with the 3d orbitals of Fe(III).[44] In the latter case the half-filled nature of the metal orbitals requires antiparallel ligand → metal spin transfer, leaving a net positive spin at the hydrogens and producing a negative (downfield) contact shift.

Summary

The comparative spectroscopic and magnetic properties in Table I, in conjunction with the x-ray structural, electronic spectral, and redox properties presented earlier,[14,15] constitute an adequate body of evidence that the alkylthiolate dimer $[Fe_2S_2(S_2\text{-}o\text{-}xyl)_2]^{2-}$ is a minimal structural and electronic representation of the active sites of 2Fe Fd_{ox} proteins. These and other results for spinach Fd_{ox} and related proteins[3,11] confirm the proposed active-site structure **1**. The qualification of the synthetic analogue as a "minimal" representation is made in the sense of earlier discussions.[13,14] Because analogues are devoid of protein structure, they manifest unconstrained structures and hence serve as stereochemically and electronically symmetrized versions of active sites. As such they cannot necessarily reproduce fine structural and electronic details of a given oxidation level of a site in low-symmetry protein environments. Examples are found in the apparent slight inequivalence of iron atoms observed in certain protein Mossbauer spectra[11,30a–d] compared to rigorous equivalence in the analogue by the same technique, and the possible difference in J value for the analogue and spinach Fd_{ox}. As already stressed, it cannot be established from present information whether or not this difference is real. Indeed, it remains to be seen if J values for a variety of proteins occur in a fairly narrow interval, as would be expected for a common active-site structure.

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Registry No. $(Et_4N)_2[Fe_2S_2(S_2\text{-}o\text{-}xyl)_2]$, 56083-11-5; $(Ph_4As)_2[Fe_2S_2(S_2\text{-}o\text{-}xyl)_2]$, 58501-17-0; $(Et_4N)_2[Fe_2S_2(SPh)_4]$, 55939-70-3.

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