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## Proton Magnetic Resonance Properties of the Tetranuclear Clusters $[\text{Fe}_4\text{S}_4(\text{SR})_4]^{3-}$ , Analogues of the 4-Fe Sites of Reduced Ferredoxins

John G. Reynolds, Edward J. Laskowski, and R. H. Holm\*

Contribution from the Department of Chemistry, Stanford University, Stanford, California 94305. Received January 16, 1978

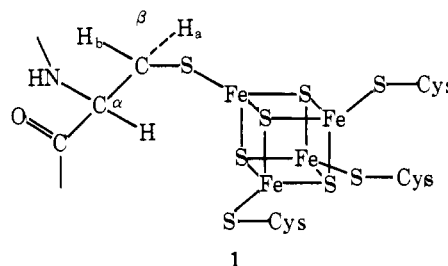
**Abstract:** The recent synthesis of the reduced tetranuclear clusters  $[\text{Fe}_4\text{S}_4(\text{SR})_4]^{3-}$  has permitted a detailed examination of structural and electronic properties, demonstrating that these trianions are analogues of the 4-Fe sites of reduced ferredoxin proteins ( $\text{Fd}_{\text{red}}$ ). Reported here are 100-MHz FT  $^1\text{H}$  magnetic resonance spectra of the series  $\text{R} = \text{CH}_2\text{Ph}$ ,  $\text{Ph}$ ,  $o\text{-C}_6\text{H}_4\text{CH}_3$ ,  $m\text{-C}_6\text{H}_4\text{CH}_3$ ,  $p\text{-C}_6\text{H}_4\text{CH}_3$  in  $\text{CD}_3\text{CN}$  solution over the temperature range of ca.  $-40$  to  $70$   $^\circ\text{C}$ . Also included are  $^1\text{H}$  NMR data not reported previously for the corresponding  $\text{Fd}_{\text{ox}}$  analogues  $[\text{Fe}_4\text{S}_4(\text{SR})_4]^{2-}$ . Trianions exhibit rather well-resolved spectra with large isotropic shifts. The temperature dependencies of methylene proton isotropic shifts and solution susceptibilities of  $[\text{Fe}_4\text{S}_4(\text{SCH}_2\text{Ph})_4]^{3-}$  parallel each other, indicating dominant contact interactions. Further evidence that isotropic shifts are principally contact in origin is obtained from the signs and temperature dependencies of shifts of  $\text{R} = \text{Ph}$  and tolyl complexes. In all cases the contact interactions appear to arise from ligand  $\rightarrow$  Fe(core) antiparallel spin transfer. The same properties follow from the  $^1\text{H}$  NMR spectra of the  $[\text{Fe}_4\text{S}_4(\text{SR})_4]^{2-}$  series, whose smaller isotropic shifts at ambient temperature are primarily due to smaller magnetic susceptibilities rather than large differences in contact interaction constants. All results support previous proposals that signal multiplicities and chemical shift ranges for the  $\beta\text{-H}$  protons of cysteinate residues bound to Fe in  $\text{Fd}_{\text{ox,red}}$  proteins arise from angularly dependent contact interactions within rigid protein structure. The analogue  $[\text{Fe}_4\text{S}_4(\text{SCH}_2\text{Ph})_4]^{3-}$  does not exhibit the positive and negative temperature coefficients of shifts observed in all  $\text{Fd}_{\text{red}}$  spectra, suggesting that the protein structure freezes an unsymmetrical electron distribution in the  $\text{Fe}_4\text{S}_4$  unit for a time long on the  $^1\text{H}$  NMR time scale.

### Introduction

NMR spectroscopy has proven to be a valuable component of the armamentarium of physicochemical techniques which have been applied to an elucidation of the properties of the redox sites of ferredoxin (Fd) proteins.<sup>1-3</sup> Spectroscopic studies in this field were pioneered by Phillips and co-workers, and include  $^1\text{H}$  spectral examinations of  $\text{Fd}_{\text{ox}}$  and  $\text{Fd}_{\text{red}}$  proteins having 2-Fe<sup>4-7</sup> ( $[\text{Fe}_2\text{S}_2(\text{S-Cys})_4]$ ) and one<sup>8-11</sup> and two<sup>12-16</sup> 4-Fe ( $[\text{Fe}_4\text{S}_4(\text{S-Cys})_4]$ ) sites per molecule. In addition  $^{13}\text{C}$  NMR studies of proteins of the last type have been reported.<sup>17,18</sup> Much of the work executed in the 1970-1973 period has been reviewed.<sup>14,15</sup>

Among the protein site properties which have been usefully probed by NMR spectroscopy are the nature of magnetic coupling within  $\text{Fe}_2\text{S}_2$  and  $\text{Fe}_4\text{S}_4$  core substructural units of the sites, electron distribution in these cores, similarities and differences in molecular environments of cysteinate and aromatic residues in related proteins, and redox potentials.<sup>18</sup> Such information is often accessible from NMR observations because 2-Fe and 4-Fe sites in all protein oxidation states obtainable in vitro are paramagnetic. This property generates

sizeable isotropic components (contact, dipolar) to the total chemical shifts of  $\alpha$  and  $\beta$  protons of cysteinate residues directly bonded to iron atoms, as for the cubane-type 4-Fe sites 1 established in several proteins by x-ray crystallography.<sup>19</sup>



The sign and magnitudes of the isotropic interactions are such as usually to shift  $\alpha$  and  $\beta$  resonances downfield of the complex absorption pattern at ca. 0-10 ppm arising from nonexchangeable protons of the polypeptide chain, thereby permitting their observation in a region uncomplicated by other signals.

The following isoelectronic relationships have been established between proteins with 4-Fe sites and synthetic complexes:  $Fd_{ox} \equiv [Fe_4S_4(SR)_4]^{2-}$ ,<sup>3</sup>  $Fd_{red} \equiv [Fe_4S_4(SR)_4]^{3-}$ .<sup>3,20-22</sup> The first of these relationships, coupled with the synthetic ease of R substituent variations, has been utilized in establishing certain <sup>1</sup>H NMR spectral properties inherent to clusters with the 2- oxidation level.<sup>23,24</sup> These include isotropic shifts dominantly contact in origin, positive temperature dependencies of  $CH_2S$  isotropic shifts which derive from interaction with the antiferromagnetically coupled electron spins of the  $[Fe_4S_4]^{2+}$  core, and resolvable chemical shift differences between  $\beta$  protons rendered diastereotopic by an  $\alpha$ -C chiral center. The recent isolation<sup>21,25</sup> of the  $Fd_{red}$  analogues  $[Fe_4S_4(SR)_4]^{3-}$  has led to a detailed study of their structural<sup>22,26</sup> and electronic<sup>21,22</sup> properties, including <sup>1</sup>H NMR spectra. The <sup>1</sup>H NMR spectra of  $Fd_{red}$  proteins containing 4-Fe sites<sup>9,10,12,14,15</sup> are considerably less well understood than those of the corresponding  $Fd_{ox}$  species. Assignments of multiple, isotropically shifted signals are incomplete, the extent of dipolar vs. contact interactions is unknown, and the source of opposite temperature dependencies of isotropically shifted resonances within the same molecule is unclear. In an attempt to provide at least partial clarification of these matters, <sup>1</sup>H NMR spectral data and their interpretation for a series of  $[Fe_4S_4(SR)_4]^{3-}$  analogue complexes are reported herein.

## Experimental Section

**Preparation of Compounds.**  $(Me_4N)_2[Fe_4S_4(S-p-tol)_4]^{23}$  (tol = tolyl) and  $(Et_4N)_2[Fe_4S_4(SCH_2Ph)_4]^{27}$  have been reported previously and were prepared by the method of direct tetramer synthesis.<sup>27</sup> The same procedure was used to prepare  $(Me_4N)_2[Fe_4S_4(SR)_4]$ , R = *o*-, *m*-tol, which were obtained as air-sensitive, red-black crystalline solids after recrystallization from acetonitrile/methanol. Anal. Calcd for  $C_{36}H_{52}Fe_4N_2S_8$ : C, 43.56; H, 5.28; Fe, 22.50; N, 2.82; S, 25.84. Found: R = *o*-tol, C, 43.75; H, 5.17; Fe, 22.60; N, 2.90; S, 25.90. R = *m*-tol, C, 43.64; H, 5.07; Fe, 22.69; N, 2.90; S, 25.67. The following properties were determined in DMF solutions at ambient temperature.  $(Me_4N)_2[Fe_4S_4(S-o-tol)_4]$ :  $\lambda_{max}$  460 nm ( $\epsilon_M$  18 300);  $E_{1/2} = -1.05$  (2-/3-),  $-1.74$  V (3-/4-).  $(Me_4N)_2[Fe_4S_4(S-m-tol)_4]$ :  $\lambda_{max}$  459 nm ( $\epsilon_M$  19 100);  $E_{1/2} = -1.07$  (2-/3-),  $-1.72$  V (3-/4-).  $(Me_4N)_2[Fe_4S_4(S-p-tol)_4]$ :  $\lambda_{max}$  461 nm ( $\epsilon_M$  18 600);<sup>28</sup>  $E_{1/2} = -1.09$  (2-/3-),  $-1.76$  V (3-/4-).<sup>29</sup>

The tetramer trianion salts  $(Me_4N)_3[Fe_4S_4(SR)_4]$ , R = *o*-, *m*-, *p*-tol, were prepared by reduction of the corresponding dianion salts with sodium acenaphthalenide in hexamethylphosphoramide<sup>25</sup> and obtained as extremely air-sensitive, black, crystalline solids after recrystallization from acetonitrile/THF. Tetraethylammonium salts of  $[Fe_4S_4(SCH_2Ph)_4]^{3-}$ <sup>25</sup> and  $[Fe_4S_4(SPh)_4]^{3-}$ <sup>25</sup> were synthesized and purified by the same method. Anal. Calcd for  $C_{40}H_{64}Fe_4N_3S_8$ : C, 45.03; H, 6.05; Fe, 20.94; N, 3.94; S, 24.04. Found: R = *o*-tol, C, 45.39; H, 5.89; Fe, 20.44; N, 3.83; S, 23.91. R = *m*-tol, C, 45.29; H, 5.84; Fe, 20.55; N, 3.95; S, 23.80. R = *p*-tol, C, 45.54; H, 6.01; Fe, 20.65; N, 3.91; S, 23.90.

**<sup>1</sup>H NMR Spectra.** <sup>1</sup>H NMR spectra of  $[Fe_4S_4(SR)_4]^{2-}$ -<sup>3-</sup> salts were determined on a Varian XL-100-15 spectrometer equipped with a deuterium lock and a Varian no. 4412 probe with a 5-mm insert. Solutions were prepared with exclusion of air from 99.0% acetonitrile-*d*<sub>3</sub> (Wilmad Glass Co.) subjected to freeze-pump-thaw-degas cycles and stored over Linde 4A molecular sieves. Data collection and processing was done by a Nicolet Model 1180 Fourier transform computer with a TT 1010A pulser unit and a Diablo Model 31 Magnetic Disc storage system. Typical spectra were made by approximately 500 acquisitions of 8132 bits on 5-10 mM solutions of tetramer salts contained in sealed NMR tubes containing 0.5 vol %  $Me_4Si$  as internal reference. Solution temperatures were regulated to  $\pm 0.5$  °C by a Varian V-6040 variable temperature controller. Isotropic shifts were calculated from the relation  $(\Delta H/H_0)_{iso} = (\Delta H/H_0)_{obsd} - (\Delta H/H_0)_{dia}$ , where diamagnetic reference shifts were taken as those of the free thiols in  $CD_3CN$  at ambient temperature. Thiol chemical shift data not reported previously<sup>23</sup> and required for this study are the following ( $CD_3CN$  solution): *o*- $CH_3C_6H_4SH$ ,  $-2.22$  ( $CH_3$ ),  $-7.07$  ppm (ring H); *m*- $CH_3C_6H_4SH$ ,  $-2.23$  ( $CH_3$ ),  $-7.05$  ppm (ring H). Following the usual convention for the spectra

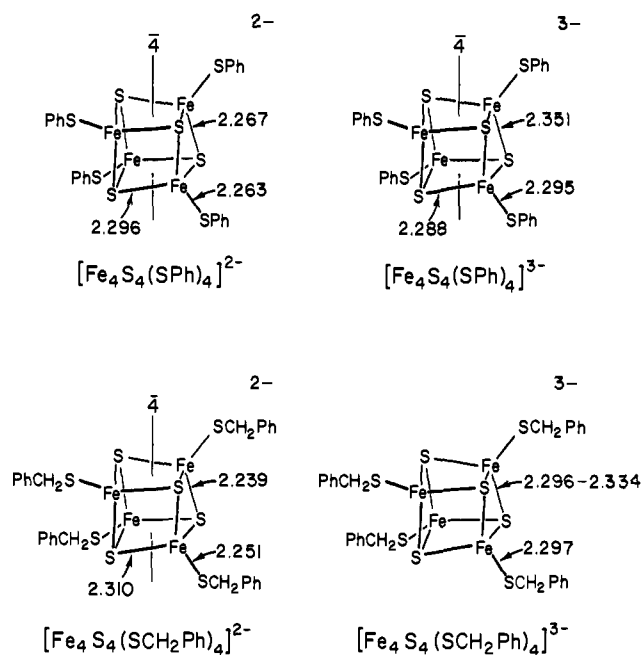
of paramagnetic molecules,  $(\Delta H/H_0)_{obsd}$  and  $(\Delta H/H_0)_{dia}$  are taken as negative for chemical shifts downfield of  $Me_4Si$ . For the majority of iron-sulfur protein <sup>1</sup>H NMR spectra, referenced to DSS internal standard, an opposite sign convention has been employed in literature reports.<sup>4-16</sup>

**Other Physical Measurements.** Magnetic susceptibilities of salts of  $[Fe_4S_4(SCH_2Ph)_4]^{3-}$  and  $[Fe_4S_4(SPh)_4]^{3-}$  were measured in ca. 9 mM  $CD_3CN$  solutions prepared as for <sup>1</sup>H NMR determinations but containing 2.0 vol %  $Me_4Si$ . The NMR susceptibility shift method<sup>30</sup> was employed using solutions sealed in Wilmad no. 536 coaxial tubes and  $Me_4Si$  as the reference signal. Solution densities were corrected for changes in temperature using density data for acetonitrile;<sup>31</sup> density differences between solution and pure solvent were neglected. The molar susceptibility of acetonitrile was taken as  $-27.6 \times 10^{-6}$  cgsu/mol.<sup>32</sup> Sixteen accumulations of  $Me_4Si$  signals gave a signal/noise ratio of 60/1 and an estimated chemical shift accuracy of  $\pm 0.025$  Hz. Electronic absorption spectra in anaerobic DMF solutions were recorded on a Cary Model 17 spectrophotometer. Cyclic voltammograms of DMF solutions were obtained at a Pt disk electrode recorded at 100 mV/s scan rate using equipment described elsewhere.<sup>25</sup>

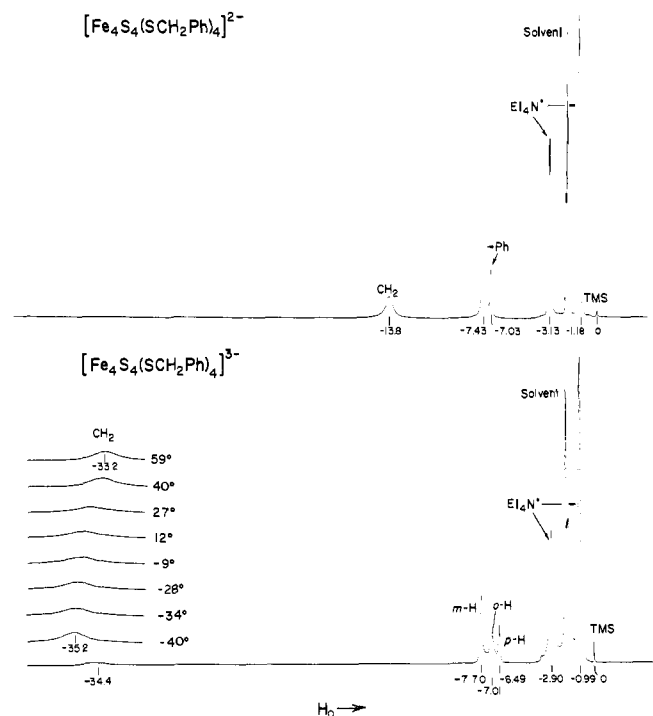
## Results and Discussion

The primary objects of the present <sup>1</sup>H NMR investigation are the  $Fd_{red}$  4-Fe site analogues  $[Fe_4S_4(SR)_4]^{3-}$  (R =  $CH_2Ph$ , Ph; *o*-, *m*-, *p*-tol). Also included for purposes of comparison are spectral data for the correspondingly substituted  $Fd_{ox}$  analogues  $[Fe_4S_4(SR)_4]^{2-}$ . The <sup>1</sup>H NMR properties of dianion complexes with R =  $CH_2Ph$ , alkyl, Ph, and *p*-tol were presented and analyzed in an earlier report.<sup>23</sup> Tetranuclear dianion salts were prepared by a standard method,<sup>27</sup> identified by their characteristic absorption spectral and electrochemical properties,<sup>28,29</sup> and chemically reduced with acenaphthalenide ion in hexamethylphosphoramide,<sup>25</sup> yielding analytically pure quaternary ammonium trianion salts. The cubane-type structures of the pairs  $[Fe_4S_4(SPh)_4]^{2-}$ ,<sup>3-22,33</sup> and  $[Fe_4S_4(SCH_2Ph)_4]^{2-}$ ,<sup>3-26,27</sup> are schematically illustrated in Figure 1. While no crystallographic symmetry is imposed in any case, the  $Fe_4S_4$  cores of all complexes except  $[Fe_4S_4(SCH_2Ph)_4]^{3-}$  are distorted from cubic toward idealized  $D_{2d}$  symmetry. Detailed structural comparisons<sup>3,19b</sup> show that the cores of tetramer dianions and  $Fd_{ox}$  sites are virtually congruent. No structural data are available for  $Fd_{red}$  proteins. Magnetic susceptibility studies of protein sites  $I^{34}$  in the reduced form of the "high-potential" protein ( $HP_{red}$ ) from *Chromatium*, isoelectronic with  $Fd_{ox}$  sites, and of the analogues  $[Fe_4S_4(SR)_4]^{2-}$ <sup>22</sup> have provided evidence for the existence of antiferromagnetic spin exchange leading to an  $S = 0$  ground state and thermal population of paramagnetic levels of the spin manifold at noncryogenic temperatures. EPR results for  $Fd_{red}$  proteins<sup>2,3</sup> and  $[Fe_4S_4(SR)_4]^{3-}$ <sup>20-22</sup> have established an  $S = 1/2$  ground state, and magnetic studies of the latter species in the crystalline state have demonstrated net antiferromagnetic coupling within the clusters and population of higher spin states down to 4.2 K.<sup>22</sup>

**General Spectral Features.** FT <sup>1</sup>H NMR spectra of  $[Fe_4S_4(SR)_4]^{2-}$ ,<sup>3-</sup> species (R =  $CH_2Ph$ , *o*-tol, *m*-tol, *p*-tol) recorded at 100 MHz and ambient temperature are directly compared in Figures 2-5. The spectra of  $[Fe_4S_4(SPh)_4]^{2-}$ , presented previously,<sup>23</sup> and  $[Fe_4S_4(SPh)_4]^{3-}$  (not shown) differ little from their tolyl derivatives, as may be seen from inspection of the ambient temperature isotropic shifts for all complexes collected in Table I. Temperature dependencies of selected isotropic shifts, referenced to the appropriate free thiol, are plotted in Figures 6 and 7. All trianions exhibit rather well-resolved spectra, although usually spin-doublet ground-state systems do not relax electron spin efficiently, thereby leading to broad lines. However, EPR spectra of solutions of trianions are not observable above ca. 60 K and are much enhanced in signal intensity and resolution below ca. 35 K, indicating an efficient mechanism for shortening  $T_{1e}$ . The failure to locate *o*-H resonances in trianion spectra is doubtless due

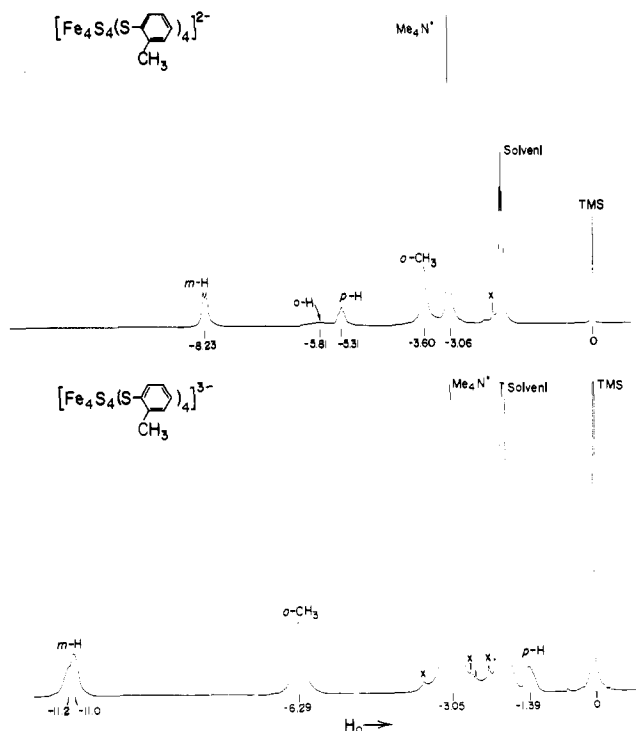


**Figure 1.** Schematic structures of  $[\text{Fe}_4\text{S}_4^*(\text{SPh})_4]^{2-}$ ,<sup>33</sup>  $[\text{Fe}_4\text{S}_4^*(\text{SPh})_4]^{3-}$ ,<sup>22</sup>  $[\text{Fe}_4\text{S}_4^*(\text{SCH}_2\text{Ph})_4]^{2-}$ ,<sup>27</sup> and  $[\text{Fe}_4\text{S}_4^*(\text{SCH}_2\text{Ph})_4]^{3-}$ .<sup>26</sup> The first three complexes have idealized  $\bar{4}$  symmetry axes and approach  $D_{2d}$  cluster symmetry; the first three complexes have approximately parallel and perpendicular to the  $\bar{4}$  axis and mean Fe-S\* distances are given. The last complex is of lower symmetry; the range of Fe-S\* and the mean of Fe-S distances are indicated in this case.

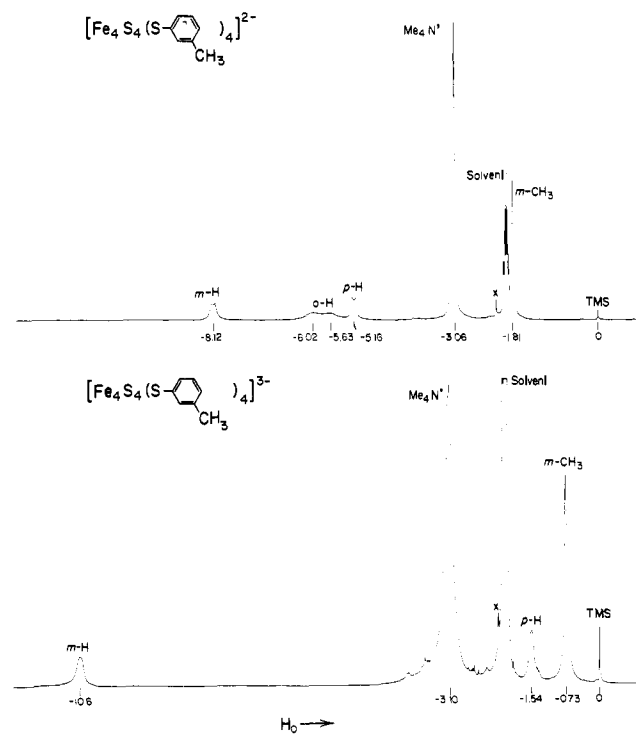


**Figure 2.** FT  $^1\text{H}$  NMR spectra (100 MHz) of  $[\text{Fe}_4\text{S}_4(\text{SCH}_2\text{Ph})_4]^{2-}$  (22 °C) and  $[\text{Fe}_4\text{S}_4(\text{SCH}_2\text{Ph})_4]^{3-}$  (27 °C) in  $\text{CD}_3\text{CN}$ . The insert shows the temperature dependence of the  $\text{CH}_2$  chemical shift of the trianion.

to paramagnetic relaxation effects, which are most pronounced for nuclei nearest the core ( $1/T_2 \propto r^{-6}$ ). However, these resonances could be located in the fast electron exchange spectra observed for the mixtures  $[\text{Fe}_4\text{S}_4(\text{S-}i\text{-}p\text{-tol})_4]^{2-}$ ,<sup>3-</sup> up to 0.5 mol fraction of trianion. The results in Figure 8 indicate that iso-



**Figure 3.** FT  $^1\text{H}$  NMR spectra (100 MHz) of  $[\text{Fe}_4\text{S}_4(\text{S-}o\text{-tol})_4]^{2-}$ ,<sup>3-</sup> (23 °C) in  $\text{CD}_3\text{CN}$ . Impurities are designated with an X.



**Figure 4.** FT  $^1\text{H}$  NMR spectra (100 MHz) of  $[\text{Fe}_4\text{S}_4(\text{S-}m\text{-tol})_4]^{2-}$ ,<sup>3-</sup> (23 °C) in  $\text{CD}_3\text{CN}$ . Impurities are designated with an X.

ropic shifts vary linearly according to the equation

$$(\Delta H/H_0)_{\text{iso}^{2-,3-}} = X_{2-}(\Delta H/H_0)_{\text{iso}^{2-}} + (1 - X_{2-})(\Delta H/H_0)_{\text{iso}^{3-}} \quad (1)$$

Consequently, the  $o\text{-H}$  shift can be determined by extrapolation as +4.3 ppm at 23 °C. This value corresponds to a

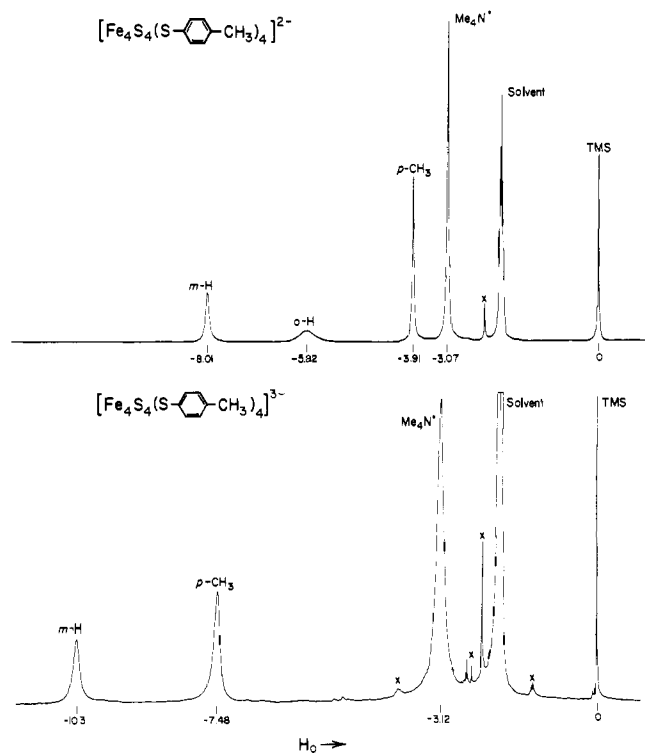


Figure 5. FT  $^1\text{H}$  NMR spectra (100 MHz) of  $[\text{Fe}_4\text{S}_4(\text{S-p-tol})_4]^{2-}$  (23  $^\circ\text{C}$ ) in  $\text{CD}_3\text{CN}$ . Impurities are designated with an X.

Table I.  $^1\text{H}$  Isotropic Shifts of  $[\text{Fe}_4\text{S}_4(\text{SR})_4]^{2-}$ ,  $^{3-}$  in  $\text{CD}_3\text{CN}$  at Ambient Temperature

R	position	$(\Delta H/H_0)_{\text{iso}}$ , ppm	
		$[\text{Fe}_4\text{S}_4(\text{SR})_4]^{2-}$	$[\text{Fe}_4\text{S}_4(\text{SR})_4]^{3-}$
	$\text{CH}_2$	-10.1 <sup>a,b</sup>	-30.8 <sup>d</sup>
	<i>o</i> -H	-0.26	+0.14
	<i>m</i> -H	-0.42	-0.55
	<i>p</i> -H	$\sim 0$	+0.66
	<i>o</i> -H	+1.32 <sup>a,b</sup>	<sup>e</sup>
	<i>m</i> -H	-0.98	-3.30 <sup>h</sup>
	<i>p</i> -H	+1.92	+5.55
	<i>o</i> -Me	-1.38 <sup>c</sup>	-4.07 <sup>c</sup>
	<i>o</i> -H	+1.26	<sup>e</sup>
	<i>m</i> -H	-1.16 <sup>f</sup>	-3.93, -4.13
	<i>p</i> -H	+1.76 <sup>g</sup>	+5.68
	<i>o</i> -H	+1.03, +1.42 <sup>c</sup>	<sup>e</sup>
	<i>m</i> -Me	+0.42	+1.50 <sup>c</sup>
	<i>m</i> -H	-1.07 <sup>f</sup>	-3.56
	<i>p</i> -H	+1.89 <sup>f</sup>	+5.51
	<i>o</i> -H	+1.28 <sup>c</sup>	+4.3 <sup>i</sup>
	<i>m</i> -H	-0.91	-3.25 <sup>c</sup>
	<i>p</i> -Me	-1.63	-5.20

<sup>a</sup> Data from ref 23. <sup>b</sup> 22  $^\circ\text{C}$ . <sup>c</sup> 23  $^\circ\text{C}$ . <sup>d</sup> 27  $^\circ\text{C}$ . <sup>e</sup> Not observed. <sup>f</sup> Center of doublet,  $J = 6-7$  Hz. <sup>g</sup> Center of triplet,  $J = 6-7$  Hz. <sup>h</sup> 29  $^\circ\text{C}$ . <sup>i</sup> Determined by the extrapolation in Figure 8.

chemical shift of  $-2.8$  ppm, placing this broadened signal in a region where it is obscured by cation and solvent resonances.

The following general spectral features have been observed at ca.  $-40$  to  $70$   $^\circ\text{C}$  in  $\text{CD}_3\text{CN}$  solutions and are evident in Figures 2-7 and from the data of Table I: (1) all  $[\text{Fe}_4\text{S}_4(\text{SR})_4]^{2-}$ ,  $^{3-}$  species display a single set of resonances; (2) protons and methyl groups at a given ring position in R = tol dianions and trianions have isotropic shifts of opposite sign; (3) given types of protons and methyl groups exhibit small but opposite temperature dependencies of isotropic shifts in di-

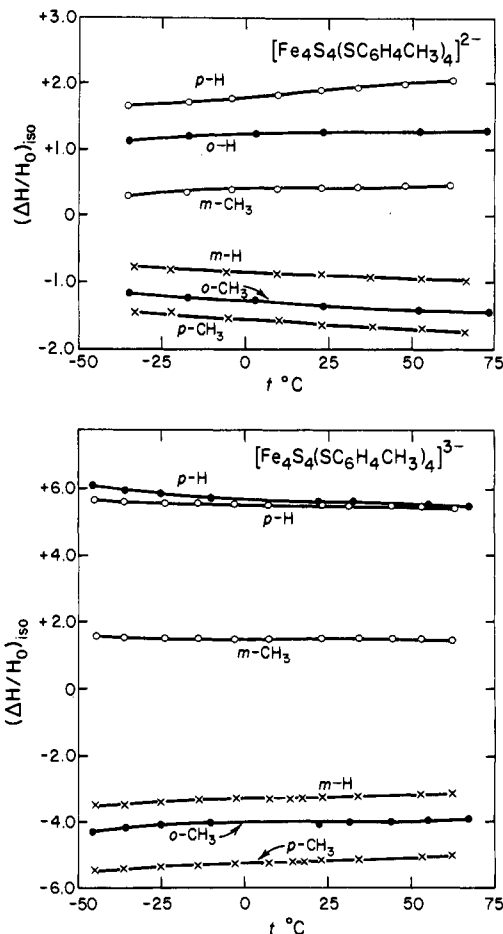


Figure 6. Temperature dependencies of the isotropic shifts (ppm) of  $[\text{Fe}_4\text{S}_4(\text{SR})_4]^{2-}$  (upper) and  $[\text{Fe}_4\text{S}_4(\text{SR})_4]^{3-}$  (lower) in  $\text{CD}_3\text{CN}$  solution:  $\bullet$ , R = *o*-tol;  $\circ$ , R = *m*-tol;  $\times$ , R = *p*-tol.

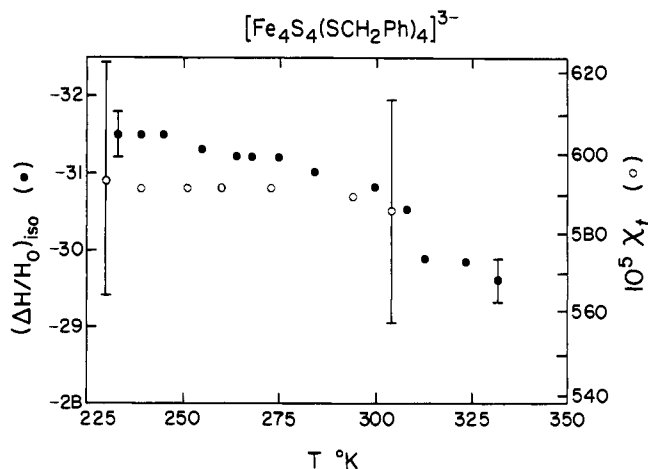


Figure 7. Temperature dependence of the methylene proton isotropic shifts ( $\bullet$ ) and of the magnetic susceptibility ( $\circ$ ) of  $[\text{Fe}_4\text{S}_4(\text{SCH}_2\text{Ph})_4]^{3-}$  in  $\text{CD}_3\text{CN}$  solution. The scales were arbitrarily chosen such that  $(\Delta H/H_0)_{\text{iso}}$  (ppm) and  $\chi_1$  coincide at 284 K.

anions vs. trianions; (4) at a fixed temperature trianion resonances display larger isotropic shifts than corresponding resonances of dianions; (5) the larger isotropic components of trianion shifts enhance effective chemical shift resolution (ring H in Figure 2, *m*-H in Figure 3).<sup>35</sup> Feature (1) will be considered in the final section of this report. Features (2)-(4) bear on the electronic origin of the observed isotropic shifts. Feature (5), accentuated chemical shift differences, is common to the

**Table II.** Magnetic and  $^1\text{H}$  Chemical Shift Data for  $[\text{Fe}_4\text{S}_4(\text{SR})_4]^{2-,3-}$  Clusters in Proteins<sup>a</sup> and Analogues

species	$\mu_t$ , <sup>b</sup> $\mu_B$	$-(\Delta H/H_0)_{\text{obsd.}}$ , <sup>c</sup> ppm	ref
$[\text{Fe}_4\text{S}_4(\text{SR})_4]^{2-}$			
<i>B. polymyxa</i> Fd <sub>ox</sub>	1.7–1.8 (5–30 °C)	8–16 (4–69 °C)	9
<i>D. gigas</i> Fd <sub>ox</sub>	2.4 (5–30 °C)	8–17, ~32 (5–40 °C)	10
<i>C. acidi-urici</i> Fd <sub>ox</sub>	2.4–2.5 (5–30 °C)	8–17 (5–30 °C)	12, 16
<i>C. pasteurianum</i> Fd <sub>ox</sub>	2.0–2.1 (5–30 °C)	8–19 (–20 to 90 °C) <sup>d</sup>	11, 13, 16
$[\text{Fe}_4\text{S}_4(\text{SCH}_2\text{Ph})_4]^{2-}$	2.2 (Me <sub>2</sub> SO, 26 °C)	12.2–14.9 (–47 to 82 °C)	23
	0.40–2.32 (–223 to 65 °C) <sup>h</sup>		22
$[\text{Fe}_4\text{S}_4(\text{SR})_4]^{3-}$			
<i>B. polymyxa</i> Fd <sub>red</sub>	3.4–3.2 (3–22 °C)	12–48 (5–50 °C)	9
<i>D. gigas</i> Fd <sub>red</sub>	4.0 (5–30 °C)	10–40 (5–35 °C)	10
<i>C. acidi-urici</i> Fd <sub>red</sub>	<i>e</i>	13–59 (5–40 °C)	12, 14
<i>C. pasteurianum</i> Fd <sub>red</sub>	<i>e</i>	16–64 (5–30 °C)	15
$[\text{Fe}_4\text{S}_4(\text{SCH}_2\text{Ph})_4]^{3-}$	3.30–3.77 (–43 to 31 °C) <sup>f</sup>	35.2–33.2 (–40 to 59 °C) <sup>f</sup>	<i>g</i>
	3.50–5.28 (–223 to 65 °C) <sup>h</sup>		22

<sup>a</sup> Data refer to aqueous solutions unless noted. <sup>b</sup>  $\mu_t = 2.828(\chi_{\text{cor}}^1 T)^{1/2} = 2\mu_{\text{Fe}}$ . <sup>c</sup> SCH<sub>2</sub> shifts; protein ranges are approximate. <sup>d</sup> D<sub>2</sub>O, CD<sub>3</sub>OD/D<sub>2</sub>O solutions. <sup>e</sup> Not reported. <sup>f</sup> Acetonitrile solution. <sup>g</sup> This work. <sup>h</sup> Crystalline Et<sub>4</sub>N<sup>+</sup> salt.

spectra of many paramagnetic complexes and examples have been considered in some detail elsewhere.<sup>36</sup>

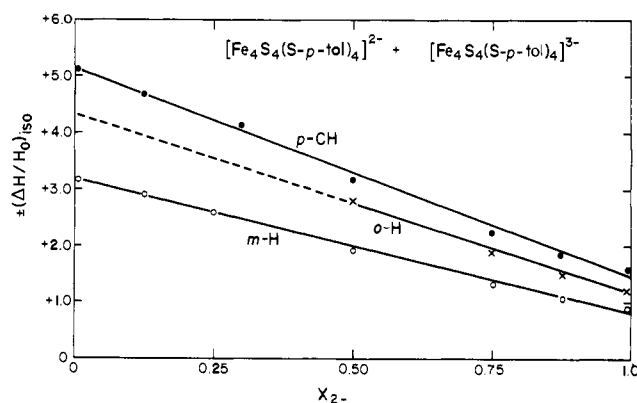
**Spectra of  $[\text{Fe}_4\text{S}_4(\text{SCH}_2\text{Ph})_4]^{2-,3-}$ .** Of the complexes in Table I these species are the closest analogues to site 1 owing to the presence of  $\beta$ -H methylene protons. Chemical shift and magnetic moment data for Fd<sub>ox,red</sub> proteins with 4-Fe sites are collected in Table II. Spectra of  $[\text{Fe}_4\text{S}_4(\text{SCH}_2\text{Ph})_4]^{2-,3-}$  are presented in Figure 2. It was shown earlier<sup>23</sup> that  $\beta$ -H signals of various  $[\text{Fe}_4\text{S}_4(\text{SCH}_2\text{R}')_4]^{2-}$  species occur within the chemical shift ranges containing resonances originally assigned to Fd<sub>ox</sub>  $\beta$ -H protons.<sup>8–15</sup> The same point is made with regard to Fd<sub>red</sub> and  $[\text{Fe}_4\text{S}_4(\text{SCH}_2\text{Ph})_4]^{3-}$  spectra. In the latter the equivalent  $\beta$ -H protons occur as broadened signals at –33 to –35 ppm over the –40 to 59 °C temperature interval. Protein shift ranges encompass these values. These observations improve the probability that protein signals in these ranges arise from  $\beta$ -H protons but do not necessarily provide unambiguous assignments, as may be seen from the recent elegant work of Packer et al.<sup>16</sup> on clostridial Fd<sub>ox</sub> spectra. Here it was shown that within the ranges in Table II certain  $\alpha$ -H and  $\beta$ -H resonances of site 1 are found, and that some of the  $\beta$ -H signals may occur in the complex upfield region of peptide absorptions. Assignments of comparable validity are not yet available for any Fd<sub>red</sub> case.

The multiplicity of isotropically shifted Fd<sub>ox</sub> signals (e.g., at least 16 are observed in the spectrum of the 8-Fe protein from *C. pasteurianum*<sup>11</sup>) arises from the intrinsic inequivalence of  $\alpha$ -H and diastereotopic  $\beta$ -H protons. Because  $[\text{Fe}_4\text{S}_4(\text{SR})_4]^{2-}$  analogues have been shown to generate isotropic shifts that are dominantly (and possibly exclusively) contact in origin,<sup>23</sup> the original proposal<sup>11</sup> that Fd<sub>ox</sub>  $\beta$ -H shifts at site 1 are influenced by angularly dependent contact interactions is supported. The simplest form of the interaction is given by  $A = A_0 \cos^2 \theta$  where  $A$  is a coupling constant and  $\theta$  a dihedral angle between the S–C–H<sub>a,b</sub> plane and a S p-orbital axis. Thus interpretation of multiple isotropically shifted proton resonances observed in Fd<sub>red</sub> spectra also requires information pertinent to contact vs. dipolar contributions to the total isotropic shifts. This matter has been examined by analysis of the spectra of the  $[\text{Fe}_4\text{S}_4(\text{SR})_4]^{3-}$  analogues in Table I using a procedure similar to that employed in an interpretation of dianion spectra.<sup>23</sup>

**Temperature Dependencies of Isotropic Shifts.** Isotropic shifts in paramagnetic molecules can arise from a combination of Fermi contact and dipolar (pseudocontact) interactions:<sup>37</sup>

$$(\Delta H/H_0)_{\text{iso}} = (\Delta H/H_0)_{\text{con}} + (\Delta H/H_0)_{\text{dip}} \quad (2)$$

In the absence of dipolar contributions isotropic shifts are



**Figure 8.** Isotropic shifts in CD<sub>3</sub>CN solutions (23 °C) containing different mole fractions of  $[\text{Fe}_4\text{S}_4(\text{S-p-tol})_4]^{2-}$  ( $X_{2-}$ ) and  $[\text{Fe}_4\text{S}_4(\text{S-p-tol})_4]^{3-}$ . Shifts (ppm) are negative for *p*-CH<sub>3</sub> and *m*-H and positive for *o*-H. The dashed line is the extrapolated portion of the *o*-H shift dependence on  $X_{2-}$ .

contact in origin and are proportional to magnetic susceptibilities at the same temperature. Following previous arguments<sup>23,38</sup> the net contact shift of a proton in an antiferromagnetically coupled tetramer with NMR-equivalent R groups may be represented by the equation

$$\left(\frac{\Delta H}{H_0}\right)_{\text{con}} = \frac{P}{T} \frac{\sum_i A_i q_i S'_i (S'_i + 1) e^{-E_i/kT}}{\sum_i q_i e^{-E_i/kT}} \quad (3)$$

in which  $P = -g\beta/12\gamma_H\hbar k$  and  $S'_i$  are the components of the spin manifold with degeneracies  $q_i$  and energies  $E_i$  under the appropriate spin Hamiltonian, whose simplest possible form is  $H = -2\sum_{i<j} J_{ij} S_i \cdot S_j$ .<sup>39</sup> Assuming  $A_i = A$ , independent of  $S'_i$ ,<sup>40</sup> the equation

$$\chi_t = Z(\Delta H/H_0)_{\text{con}} \quad (4)$$

for tetrameric units (t) follows for all temperatures with  $Z = -4g\beta N\gamma_H\hbar/A$  independent of temperature. Isotropic shifts of  $[\text{Fe}_4\text{S}_4(\text{SCH}_2\text{Ph})_4]^{2-}$ , which increase monotonically from –8.55 ppm at 226 K to –11.3 to 355 K, could be scaled to solid-state magnetic data for  $(\text{Et}_4\text{N})_2[\text{Fe}_4\text{S}_4(\text{SCH}_2\text{Ph})_4]$  according to eq 4,<sup>23</sup> leading to the conclusion that  $\beta$ -H shifts are primarily contact in origin and  $A_{\text{CH}_2}$  is positive.

Evidence that the  $[\text{Fe}_4\text{S}_4(\text{SR})_4]^{3-}$  species are net antiferromagnetically coupled based on magnetic measurements over the 4.2–338 K range will be presented elsewhere.<sup>22</sup> The mag-

**Table III.** Magnetic Susceptibility Data for  $[\text{Fe}_4\text{S}_4(\text{SR})_4]^{3-}$  Complexes in Acetonitrile Solution

$[\text{Fe}_4\text{S}_4(\text{SCH}_2\text{Ph})_4]^{3- a}$			$[\text{Fe}_4\text{S}_4(\text{SPh})_4]^{3- a}$		
$T, \text{K}$	$\chi_t \times 10^5 b$	$\mu_t, \mu_B$	$T, \text{K}$	$\chi_t \times 10^5 c$	$\mu_t, \mu_B$
230	594	3.30	230	565	3.22
239	592	3.36	239	565	3.29
251	592	3.46	250	565	3.36
260	591	3.50	260	564	3.42
273	592	3.60	274	564	3.52
283	594	3.67	287	562	3.59
294	589	3.72	304	562	3.70
304	585	3.77			

<sup>a</sup>  $\text{Et}_4\text{N}^+$  salts. Diamagnetic corrections: <sup>b</sup>  $-797 \times 10^{-6}$ , <sup>c</sup>  $-750 \times 10^{-6}$  cgsu/mol; calculated from data in P. W. Selwood, "Magnetochemistry", 2nd ed, Interscience, New York, N.Y., 1956, pp 78, 92, 93.

netic data in Table II for  $[\text{Fe}_4\text{S}_4(\text{SCH}_2\text{Ph})_4]^{3-}$  in crystalline and solution phases indicate thermal population of  $S'_i > 1/2$  levels. The  $S'_i = 1/2$  ground state, having  $g_{\perp} = 1.93$  and  $g_{\parallel} = 2.04$  in acetonitrile solution,<sup>22</sup> is readily detected by low-temperature EPR measurements. For occupation of this state alone  $\mu_t = 1.71 \mu_B$ . Unlike the case of  $[\text{Fe}_4\text{S}_4(\text{SCH}_2\text{Ph})_4]^{2-}$  the  $\beta$ -H isotropic shifts of  $[\text{Fe}_4\text{S}_4(\text{SCH}_2\text{Ph})_4]^{3-}$  are nearly temperature independent (Figure 2), varying from  $-31.5$  ( $-40^\circ\text{C}$ ) to  $-29.6$  ppm ( $59^\circ\text{C}$ ). Magnetic susceptibilities of the trianion, measured in the same solvent over a comparable temperature interval, are shown in Table III. Treatment of shift and susceptibility data in terms of eq 4 is depicted in Figure 7. Within experimental error of the nearly temperature-invariant quantities shifts and susceptibilities parallel each other, providing evidence that the isotropic shifts of  $\beta$ -H protons in  $[\text{Fe}_4\text{S}_4(\text{SCH}_2\text{Ph})_4]^{3-}$  are dominantly contact in origin and  $A_{\text{CH}_2}$  is positive. Dominant contact interactions in  $[\text{Fe}_4\text{S}_4(\text{SPh})_4]^{3-}$  are also concluded from its shifts and solution magnetic susceptibilities (Table III). This simple analysis does not require knowledge of an exact functional form of  $\chi_t(T)$ , which is currently under investigation for  $[\text{Fe}_4\text{S}_4(\text{SR})_4]^{3-}$ .

**Further Evidence for Contact Interactions.** Of the methods for analyzing contact and dipolar components in eq 2,<sup>41</sup> a convenient semiquantitative approach is inclusion of alternate hydrocarbon groups in ligand structure. Dominant contact interactions are manifested by alternating signs and, to a lesser extent, magnitudes of shifts arising from  $\pi$ -spin delocalization. Benzenethiolate and its methyl derivatives are of this type and several such ligands were employed in a previous study of the  $^1\text{H}$  NMR spectra of  $[\text{Fe}_4\text{S}_4(\text{SR})_4]^{2-}$ .<sup>23</sup> Use of R = *o*-, *m*-, *p*-tol trianion species (Figures 2-4) permits assignment of all signals (Table I), leading to the previously noted spectral feature (2).

The sign pattern of isotropic shifts which emerges from the spectra of  $[\text{Fe}_4\text{S}_4(\text{SR})_4]^{3-}$  with R = Ph and tol—*o*-H, *p*-H, *m*-CH<sub>3</sub>, positive; *m*-H, *o*-CH<sub>3</sub>, *p*-CH<sub>3</sub>, negative—is the same as that for corresponding  $[\text{Fe}_4\text{S}_4(\text{SR})_4]^{2-}$  complexes.<sup>23</sup> Shifts are not attenuated by distance, as would be expected for overriding dipolar interactions. Sign patterns for a given species and sign reversals consequent to H/CH<sub>3</sub> substitution at a given position are fully consistent with  $\pi$  delocalization of fractional unpaired electron spin and attendant contact interactions<sup>41</sup> as described by the McConnell equation  $A = Q\rho_C$  ( $Q_{\text{CH}} \sim -23$  G,  $Q_{\text{CCH}_3}$  positive but variable in magnitude). Hence, spectral feature (2) supports the dominance of contact interactions. The signs of  $A$  and  $\rho_C$  are easily obtained and are shown in Table IV together with a summary of shift and susceptibility behavior with temperature. Temperature dependencies of  $\chi_t$  for  $[\text{Fe}_4\text{S}_4(\text{SR})_4]^{2-}$  (R = CH<sub>2</sub>Ph, Ph) at 4.2-338 K from measurements of solids are clearly positive.<sup>22</sup> Those for corresponding trianions are negative but the differential

**Table IV.** Summary of Isotropic Shift Properties of  $[\text{Fe}_4\text{S}_4(\text{SR})_4]^{2-,3-}$  Analogues

quantity/ position (R)	$[\text{Fe}_4\text{S}_4(\text{SR})_4]^{2- d}$	$[\text{Fe}_4\text{S}_4(\text{SR})_4]^{3-}$	sign <sup>c</sup> of	
			$A$	$\rho_C$
$\Delta\chi_t/\Delta T$	$+^a$	$\sim 0$ to $-^b$		
$\Delta(\Delta H/H_0)_{\text{iso}}/\Delta T$				
-SCH <sub>2</sub>	$+^a$	$-^b$	$+$	$+(\rho_H)^e$
<i>o</i> -H	$+$	$-$	$-$	$+$
<i>m</i> -H	$-$	$+$	$+$	$-$
<i>p</i> -H	$+$	$-$	$-$	$+$
<i>o</i> -CH <sub>3</sub>	$-$	$+$	$+$	$-$
<i>m</i> -CH <sub>3</sub>	$+$	$-$	$-$	$+$
<i>p</i> -CH <sub>3</sub>	$-$	$+$	$+$	$-$

<sup>a</sup> Increases with increasing temperature. <sup>b</sup> Decreases with increasing temperature. <sup>c</sup> Applicable to  $[\text{Fe}_4\text{S}_4(\text{SR})_4]^{2-,3-}$ . <sup>d</sup> Results from this work and ref 23. <sup>e</sup>  $A = Q_H\rho_H$ ,  $Q_H$  positive.

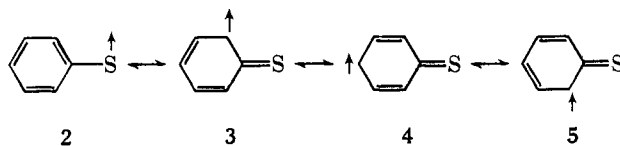
**Table V.** Relative Isotropic Shifts of  $[\text{Fe}_4\text{S}_4(\text{SR})_4]^{2-,3-}$  Analogues at Ambient Temperature

R	Position		
	<i>o</i>	<i>m</i>	<i>p</i>
2-, Ph	-1.35	1.00	-1.96
3-, Ph	<sup>a</sup>	1.00	-1.68
2-, <i>p</i> -tol	-1.32	1.00	-1.79
3-, <i>p</i> -tol	-1.32	1.00	-1.60
3-/2-, Ph	<sup>a</sup>	3.37	2.89
3-/2-, <i>p</i> -tol	3.36	3.57	3.19
3-/2-, CH <sub>2</sub> Ph		3.02 (CH <sub>2</sub> )	

<sup>a</sup> Signal not observed in trianion.

change in the solid state over the temperature interval of Figure 6 is quite small.<sup>42</sup> Solution values (Table III) are constant within experimental error. If a small negative value of  $\Delta\chi_t(\text{solution})/\Delta T$  is accepted, the opposite temperature dependencies of dianion and trianion shifts, spectral feature (3), are explicable in terms of the contact interaction model of eq 3.

The signs of coupling constants and spin densities at given positions (Table IV) are the same for dianions and trianions, and ratios of isotropic shifts (normalized to the meta position) shown for several cases in Table V are quite similar. These results indicate equivalent pathways of spin delocalization. As shown earlier<sup>23</sup> ligand  $\rightarrow$  Fe(core) antiparallel spin transfer results in positive (parallel) spin in S 3p-type orbitals whose nonorthogonality with phenyl ring  $\pi$  orbitals allows spin delocalization as described by the valence bond structures 2-5.



This mechanism affords positive spin density at *o*-C and *p*-C and negative density at *m*-C, in agreement with the experimental signs of  $A$ . The direction of spin transfer is consistent with the results of SCF-X $\alpha$ -SW calculations for  $[\text{Fe}_4\text{S}_4^*(\text{SR})_4]^{2-,3-}$ .<sup>43</sup> In idealized  $T_d$  symmetry the electronic configuration of the highest filled MO (76-80% Fe, 12-15% S, 7-8% S\* in character) are  $t_2^4$  and  $t_2^5$  for dianion and trianion, respectively, and Fe(3d)-S(3p-like) overlaps are evident from contour maps. Reduction to experimental  $D_{2d}$  symmetry (Figure 1) with the splitting  $t_2 \rightarrow e + b_2$  affords ground (e.g.,  $e^4b_2^1$ ) and excited configurations (e.g.,  $e^3b_2^2$ ) of trianion and dianion involving half-filled orbitals which must accept antiparallel spin in a RS  $\rightarrow$  Fe(core) spin transfer mode. The opposite delocalization pathway,<sup>44</sup> Fe  $\rightarrow$  ligand parallel spin

transfer involving the lowest vacant phenyl group MO, is predicted to lead to small or zero spin density at the para position,<sup>23</sup> contrary to experimental results.

The ratio of contact shifts for a given proton in  $[\text{Fe}_4\text{S}_4(\text{SR})_4]^{2-}$ ,<sup>3-</sup> at the same temperature is given by the equation

$$\frac{(\Delta H/H_0)_{\text{con}}^{3-}}{(\Delta H/H_0)_{\text{con}}^{2-}} = \frac{A^{3-}\chi_t^{3-}}{A^{2-}\chi_t^{2-}} \quad (5)$$

Shift ratios which include highest and lowest values in the data set are included in Table V. Using isotropic shift and susceptibility data at  $\sim 300$  K the following  $A^{3-}/A^{2-}$  ratios were obtained: R = CH<sub>2</sub>Ph, 1.04; R = Ph, 1.01 (*p*-H), 1.18 (*m*-H). Consequently, the larger isotropic shifts of the trianions (spectral feature (4)) appear to be governed primarily by susceptibility effects, i.e.,  $\chi_t^{3-} \sim 3\chi_t^{2-}$  at ambient temperature, rather than by differences in hyperfine coupling constants (whose ratio may fortuitously be near unity).

The foregoing results for  $[\text{Fe}_4\text{S}_4(\text{SR})_4]^{3-}$  complexes, as those presented previously for tetramer dianions,<sup>23</sup> demonstrate the dominance of contact interactions in effecting the sign and magnitude of observed isotropic shifts. They do not eliminate the possibility of dipolar contributions, a matter which can satisfactorily be settled only by determination of molecular magnetic anisotropies.<sup>41</sup> The occurrence of axially symmetric doublet ground states of trianions suggests nonvanishing dipolar shifts. Estimates of the latter based on *g*-tensor anisotropies alone are, however, not useful in the present cases owing to substantial thermal occupation of higher electronic states of unknown anisotropies.

**Comparative Fd<sub>red</sub>/Analogue Spectral Features.** The close correspondence in structural and electronic properties (absorption and Mössbauer spectra, magnetism) between the analogues  $[\text{Fe}_4\text{S}_4(\text{SR})_4]^{2-}$  and Fd<sub>ox</sub> (and HP<sub>red</sub>) proteins has been well documented.<sup>3</sup> Such correspondence can be said to extend to  $^1\text{H}$  NMR spectra in the sense that both proteins and analogues exhibit positive  $\Delta(\Delta H/H_0)_{\text{iso}}/\Delta T$  behavior and the latter show resolvable  $\beta\text{-H}_{a,b}$  signal pairs when four equivalent ligands, each with a chiral  $\alpha\text{-C}$  center, are present.<sup>24</sup> The spectral similarity does not extend to the multiplicities of isotropically shifted protein resonances.<sup>8-16</sup> Indeed, the simpler spectra of  $[\text{Fe}_4\text{S}_4(\text{SR})_4]^{2-}$  species, whose Fe sites and rapidly rotating R substituents are structurally<sup>3</sup> (Figure 1) and electronically<sup>45</sup> equivalent, offer substantial proof of the existence of angularly dependent contact interactions in rigid structures of Fd<sub>ox</sub> proteins.

Similar comments, but with qualifications, apply to the properties of  $[\text{Fe}_4\text{S}_4(\text{SR})_4]^{3-}$ <sup>20-22</sup> analogues and Fd<sub>red</sub> proteins in the native state. No Fd<sub>red</sub> structures are available. Evidence has been collected which indicates that the elongated  $D_{2d}$  structure of  $[\text{Fe}_4\text{S}_4(\text{SPh})_4]^{3-}$ <sup>22</sup> rather than the irregular structure of  $[\text{Fe}_4\text{S}_4(\text{SCH}_2\text{Ph})_4]^{3-}$ <sup>26</sup> (found in the Et<sub>4</sub>N<sup>+</sup> salt, Figure 1) is the inherently stable configuration of the  $[\text{Fe}_4\text{S}_4]^{+}$  core of trianions. Magnetic (Table II) and, to a lesser extent, Mössbauer properties of the latter in the solid state differ to a greater degree from those of reduced proteins than is true for crystalline salts of  $[\text{Fe}_4\text{S}_4(\text{SPh})_4]^{3-}$ .<sup>22</sup> However, the magnetic and Mössbauer properties of  $[\text{Fe}_4\text{S}_4(\text{SCH}_2\text{Ph})_4]^{3-}$  when examined in frozen acetonitrile solutions (ca. 4.2–200 K) approach those of  $[\text{Fe}_4\text{S}_4(\text{SPh})_4]^{3-}$ , which are much less variant with change in phase.<sup>22</sup> Thus a structural change of  $[\text{Fe}_4\text{S}_4(\text{SCH}_2\text{Ph})_4]^{3-}$  toward elongated  $D_{2d}$  stereochemistry upon removal of crystalline constraints is probable. In this event the observed NMR equivalence of the four thiolate ligands is expected and is observed<sup>47</sup> as spectral feature (1) for all trianions. Owing to rigid protein structure multiple  $\beta\text{-H}$  and  $\alpha\text{-H}$  resonances are also found for Fd<sub>red</sub> proteins.<sup>9,10,12,14,15</sup> Because of dominant contact shifts in  $[\text{Fe}_4\text{S}_4(\text{SR})_4]^{3-}$  analogues it is probable that angularly dependent contact inter-

actions contribute significantly to signal multiplicities and the range of isotropically shifted signals (Table II<sup>46</sup>). However, proteins differ from analogues most conspicuously in the temperature dependencies of these signals. For example, of the 11 (*B. polymyxa* 4-Fe Fd<sub>red</sub><sup>9</sup>) and 16 (*C. aciduri* 8-Fe Fd<sub>red</sub><sup>12,14</sup>) resonances described as contact shifted in two proteins, the shifts of 5–6 of these decrease with increasing temperature (as for  $[\text{Fe}_4\text{S}_4(\text{SCH}_2\text{Ph})_4]^{3-}$ ) while the remaining increase (similar to Fd<sub>ox</sub> cases) or are little changed. This behavior suggests an inequivalence in unpaired electron distribution over the four Fe sites and attendant contact interactions of opposite sign, possibly due to RS  $\rightarrow$  Fe parallel and antiparallel spin transfer. Pairwise inequivalence of Fe atoms in  $[\text{Fe}_4\text{S}_4(\text{SR})_4]^{3-}$  (solid and solution)<sup>22</sup> and at least one Fd<sub>red</sub> protein<sup>48</sup> has been observed in low-temperature Mössbauer spectra (characteristic time constant  $\sim 10^{-7}$  s). The lack of opposite temperature dependencies of  $[\text{Fe}_4\text{S}_4(\text{SCH}_2\text{Ph})_4]^{3-}$   $\beta\text{-H}$  shifts suggests that the protein structure "freezes in" an asymmetric electronic distribution in Fd<sub>red</sub> site 1 at and above ambient temperature for a time long ( $\geq 10^{-3}$ – $10^{-4}$  s) on the  $^1\text{H}$  NMR time scale. This effect is absent in structurally unconstrained  $[\text{Fe}_4\text{S}_4(\text{SR})_4]^{3-}$  complexes, which appear electronically symmetrized when examined under the same conditions, and is one of the few properties of protein sites 1 not approached or duplicated by synthetic analogues.

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## Synthetic Analogues of the 4-Fe Active Sites of Reduced Ferredoxins. Electronic Properties of the Tetranuclear Trianions $[\text{Fe}_4\text{S}_4(\text{SR})_4]^{3-}$ and the Structure of $[(\text{C}_2\text{H}_5)_3(\text{CH}_3)\text{N}]_3[\text{Fe}_4\text{S}_4(\text{SC}_6\text{H}_5)_4]$

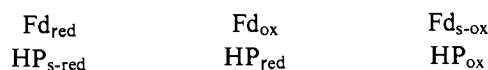
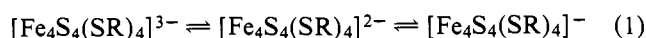
E. J. Laskowski,<sup>1a</sup> R. B. Frankel,<sup>\*1b</sup> W. O. Gillum,<sup>1a</sup> G. C. Papaefthymiou,<sup>1b</sup> J. Renaud,<sup>1ac</sup> James A. Ibers,<sup>\*1d</sup> and R. H. Holm<sup>\*1a</sup>

Contribution from the Departments of Chemistry, Stanford University, Stanford, California 94305, and Northwestern University, Evanston, Illinois 60201, and the Francis Bitter National Magnet Laboratory, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139. Received January 16, 1978

**Abstract:** The electronic properties (EPR and Mössbauer spectra, magnetic susceptibilities) of the recently synthesized, reduced tetranuclear cluster salts  $(\text{R}'_4\text{N})_3[\text{Fe}_4\text{S}_4(\text{SR})_4]$  have been investigated in detail. The collective results fully support a description of the trianions as analogues of the 4-Fe sites of reduced ferredoxin proteins ( $\text{Fd}_{\text{red}}$ ). The compound  $(\text{Et}_3\text{MeN})_3[\text{Fe}_4\text{S}_4(\text{SPh})_4]$  crystallizes in the monoclinic space group  $C_2^4-Cc$  with 8 formula units in a cell of dimensions  $a = 11.426$  (5),  $b = 24.806$  (7),  $c = 39.147$  (10) Å, and  $\beta = 90.75$  (2)°. The two crystallographically independent trianions have virtually identical structures which resemble that of  $[\text{Fe}_4\text{S}_4(\text{SPh})_4]^{2-}$  in having  $\text{Fe}_4\text{S}_4$  core configurations of idealized  $D_{2d}$  symmetry, but differ in that the cores are elongated rather than compressed along the 4 axis. Trianions exhibit axial EPR spectra and temperature and power saturation characteristics similar to those of  $\text{Fd}_{\text{red}}$  proteins. Zero-field Mössbauer spectra of polycrystalline samples and acetonitrile solutions at low temperatures consist of two overlapping quadrupole doublets with parameters very similar to those of *B. stearothermophilus*  $\text{Fd}_{\text{red}}$ . Magnetic properties of polycrystalline and solution samples of  $[\text{Fe}_4\text{S}_4(\text{SR})_4]^{3-}$  are consistent with a spin-doublet ground state and appreciable population of higher spin states, arising from net antiferromagnetic spin coupling, at temperatures down to 4.2 K. A significant observation is that the appreciable differences in Mössbauer and magnetic behavior of polycrystalline samples of  $[\text{Fe}_4\text{S}_4(\text{SPh})_4]^{3-}$  and  $[\text{Fe}_4\text{S}_4(\text{SCH}_2\text{Ph})_4]^{3-}$  (whose core structure is less regular than that of the former trianion) are much attenuated when examined in frozen acetonitrile solutions. Because the properties of  $[\text{Fe}_4\text{S}_4(\text{SPh})_4]^{3-}$  are much less sensitive to phase changes, we conclude that an elongated tetragonal geometry is the intrinsically stable core structure of  $[\text{Fe}_4\text{S}_4(\text{SR})_4]^{3-}$ . On this basis it is speculated that the native forms of "high-potential", but not  $\text{Fd}$ , proteins may have evolved to resist the ca. 0.08 Å axial core expansion found in passing from analogue dianion to trianion. The former proteins are reducible only in the unfolded state.

### Introduction

The existence of three physiologically significant total oxidation levels of the  $[\text{Fe}_4\text{S}_4(\text{S-Cys})_4]$  clusters present in non-heme iron-sulfur redox proteins<sup>2,3</sup> ( $\text{Fd}$ ,  $\text{HP}^4$ ) is now firmly established. Based on comparative physicochemical properties of the active sites of proteins and synthetic analogues<sup>7</sup> and their known one-electron redox reactions, the vertical isoelectronic relationships in series 1 have emerged. As yet no single protein



in aqueous solution has been found to undergo reversible electron transfer encompassing all three oxidation levels. Although evidence has been presented for the existence of the  $\text{Fd}_{\text{s-ox}}/\text{Fd}_{\text{ox}}$  (1 - /2 -) couples in proteins from *Azotobacter*