Synthetic Analogs of the Active Sites of Iron-Sulfur Proteins. V.[1] Proton Resonance Properties of the Tetranuclear Clusters $[Fe_4S_4(SR)_4]^{2-}$. Evidence for Dominant Contact Interactions

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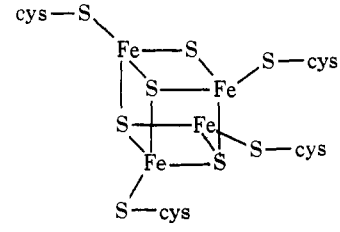
Abstract: Previous work has shown that the tetranuclear clusters $[Fe_4S_4(SR)_4]^{2-}$ are structural and electronic analogs of the active sites of oxidized ferredoxin and reduced "high-potential" iron-sulfur proteins. The proton magnetic resonance properties of an extensive series of synthetic analogs with R = alkyl (Me, Et, *n*-Pr, *i*-Pr, CH_2-C_6H_{11}, CH_2Ph, *m*-$C_6H_4(CH_2)_2$, *t*-Bu) and aryl (Ph, *p*-tolyl, *p*-$C_6H_4NO_2$) have been investigated in order to probe the electronic properties of the Fe_4S_4* core as manifested in pmr spectra. All tetramers exhibit isotropically shifted resonances. The magnitudes and temperature dependencies of CH_2–S chemical shifts are very similar to those of the downfield shifted resonances of the proteins and substantiate previous assignments of these resonances to methylene protons of cysteinyl residues bound to iron. The temperature dependencies of methylene isotropic shifts and solid state susceptibility of $[Fe_4S_4(SCH_2Ph)_4]^{2-}$ parallel each other, indicating that such shifts in the analogs and proteins are directly related to the antiferromagnetic properties of the clusters and are dominantly contact in origin. Further evidence for significant contact interactions is obtained from the signs and magnitudes of isotropic shifts in R = aryl tetramers, which are consistent with delocalization of positive spin in the highest filled π MO of the phenyl rings. Isotropic shifts of alkyl protons are negative and attenuate with increasing distance from the cluster, a behavior suggestive of a σ delocalization mechanism. The complex prepared from *m*-xylene-α,α'-dithiol has a proposed structure (**6**) in which the two phenyl rings are held approximately parallel to the cluster faces, similar to the orientation of aromatic residues in 8-Fe ferredoxins. Ring proton isotropic shifts, while small, differ from those in the R = CH_2Ph tetramer where the phenyl groups are not subject to any definite spatial orientation with respect to the cluster.

Recent investigations in these laboratories have resulted in the synthesis and structural and partial electronic characterization of low molecular weight iron–sulfur complexes which are close representations of the active sites of certain classes of iron–sulfur proteins.[4] Bis[*o*-xylyl-α,α'-dithiolato-μ_2-sulfido-ferrate(III)], $[FeS(SCH_2)_2C_6H_4]_2^{2-}$, has been shown to be an active site analog of the 2Fe–2S* proteins (S* is acid-labile or "inorganic" sulfur) and its structure has been proposed to correspond to that of the active site of the oxidized proteins.[5] Similarly, the tetranuclear clusters $[Fe_4S_4(SR)_4]^{2-}$ are structurally and electronically related to the bacterial 4Fe–4S* and 8Fe–8S* proteins.[4] Results presented elsewhere[6–8] demonstrate that the dianions possess the same total oxidation level as the active sites of the reduced 4-Fe "high-potential" protein (HP_{red}) from *Chromatium* and the oxidized 8-Fe ferredoxins (Fd_{ox}). The structure of the prototype complex $[Fe_4S_4(SCH_2Ph)_4]^{2-}$ is essentially congruent with those of the active sites of HP_{red}[9,10] and Fd_{ox} from *Peptococcus aerogenes*,[10,11] which have the basic cubane stereochemistry, **1**. In addition, the electronic properties[12] of $[Fe_4S_4(SR)_4]^{3-}$, which include "g = 1.94 type" epr spectra, are unquestionably closely similar to those of Fd_{red}. Hence, the collective body of data for the synthetic complexes suffices to show that many of the essential features of 2Fe–2S* and 4Fe–4S* active sites can be reproduced or closely approached outside of a protein environment.



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(2) (a) Massachusetts Institute of Technology. (b) National Science Foundation Predoctoral Fellow, 1969–1972.

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Establishment of the foregoing synthetic complexes as active site analogs provides a means for a more detailed examination of certain electronic properties of the iron–sulfur units comprising these sites than may be possible with the proteins themselves. The $[Fe_4S_4(SR)_4]^{2-}$ complexes, electronically equivalent to HP_{red} and Fd_{ox}, are particularly suitable for this purpose because of the variety of aliphatic and aromatic R substituents which are readily incorporated into the cluster structure by direct synthesis[7] or by ligand exchange reactions.[1] The proton resonance spectra of oxidized and reduced plant (2-Fe) ferredoxins,[13-15] $HP_{red,ox}$ from *Chromatium*[16,17] and *Rhodopseudomonas gelatinosa*,[17] the 4-Fe $Fd_{ox,red}$ from *Bacillus polymyxa*,[18] and 8-Fe $Fd_{ox,red}$ from *Clostridium pasteurianum*[19,20] and *Clostridium acidi-urici*[21] have been determined. Additionally, the ^{13}C shifts of tyrosyl residues located near the active sites of *C. acidi-urici* $Fd_{ox,red}$ have been reported.[22] In the pmr spectra of HP_{red} and 4-Fe and 8-Fe Fd_{ox} proteins in aqueous solution there appear, in addition to the complex absorption pattern at *ca.* 0–10 ppm due to nonexchangeable C*H* protons of the polypeptide chain, broadened resonances which at ambient temperatures occur at *ca.* 10–17 ppm downfield of DSS internal reference. These resonances have been assigned[16-21] to the intrinsically inequivalent CH_2–S protons of four cysteinyl residues, which are now known to be directly bonded to iron[9-11] in the active site structure **1**. The progressive downfield shifts of these resonances and the small increases in the average magnetic moment per iron (μ_{Fe}) with increasing temperature have been interpreted in terms of antiferromagnetic spin exchange coupling among the component iron centers in the active site. It has been tentatively proposed that the large downfield displacements of the methylene proton chemical shifts arise from contact interactions afforded by the electronic properties of the Fe_4S_4* clusters.[16-21] In view of the high degree of structural similarity between these clusters in proteins and in the synthetic analog,[7] it follows that the inherent electronic features of such a cluster unit in a given oxidation level may be little affected by the presence or absence of a protein environment.[23] Here we report the results of an investigation of the proton resonance properties of an extensive series of $[Fe_4S_4(SR)_4]^{2-}$ complexes containing alkyl and aryl substituents. This work has been undertaken in order to probe the electronic properties of the Fe_4S_4* cluster as manifested in proton resonance spectra, with emphasis on evaluation of signs and magnitudes of aliphatic proton shifts as dependent upon the extent of separation from magnetic centers and determination of the dominant component (contact or dipolar) of the total isotropic shifts. As will be shown, the results of this study provide strong support for the validity of the interpretation of HP_{red} and Fd_{ox} pmr spectra.

Experimental Section

Preparation of Compounds. A number of the compounds employed in this investigation were available from previous work.[6,7] The new complexes $[Fe_4S_4(SR)_4]^{2-}$, R = *n*-Pr, *i*-Pr, and *p*-tolyl, were prepared by the published procedure[7] as was the known R = *t*-Bu anion, which here was isolated as its tetramethylammonium salt. The new anions were obtained as quaternary cation salts which were purified by recrystallization from warm acetonitrile–methanol. *m*-Xylene-α,α'-dithiol was prepared from the corresponding dibromide by the thiourea method[25] and was purified by distillation, bp 185° (~28 mm) (lit.[26] bp 168–170° (25 mm)). Bis(*m*-xylene-α,α'-dithiolato)tetra(μ_3-sulfido-iron) anion, $[Fe_4S_4(m\text{-xyl-}S_2)_2]^{2-}$, was synthesized by the usual procedure and isolated as the tetra-*n*-butylammonium salt. This material was recrystallized several times from warm (~60°) DMF–methanol and obtained as very small crystals. Characterization data are given below; melting points were determined in evacuated tubes.

$(Me_4N)_2[Fe_4S_4(S\text{-}p\text{-tol})_4]$, mp 188–190° dec. *Anal.* Calcd for $C_{36}H_{52}N_2S_8Fe_4$: C, 43.56; H, 5.28; N, 2.82; S, 25.84; Fe, 22.50. Found: C, 43.51; H, 5.23; N, 2.91; S, 25.89; Fe, 22.40.

$(n\text{-}Bu_4N)_2[Fe_4S_4(m\text{-xyl-}S_2)_2]$, mp 193–194° dec. *Anal.* Calcd for $C_{48}H_{88}N_2S_8Fe_4$: C, 49.14; H, 7.56; N, 2.39; S, 21.87; Fe, 19.04. Found: C, 48.87; H, 7.39; N, 2.44; S, 21.88; Fe, 19.40.

$(Ph_4As)_2[Fe_4S_4(S\text{-}n\text{-Pr})_4]$, mp 171–173° dec. *Anal.* Calcd for $C_{60}H_{68}As_2S_8Fe_4$: C, 50.79; H, 4.83; As, 10.56; S, 18.08; Fe, 15.74. Found: C, 50.75; H, 4.90; As, 11.10; S, 18.17; Fe, 15.37.

$(Me_4N)_2[Fe_4S_4(S\text{-}t\text{-Bu})_4]$, mp 153° dec. *Anal.* Calcd for $C_{24}H_{60}N_2S_8Fe_4$: C, 33.65; H, 7.06; N, 3.27; S, 29.94; Fe, 26.08. Found: C, 33.72; H, 6.98; N, 3.53; S, 30.06; Fe, 25.88.

Proton Resonance Spectra. Spectra of $[Fe_4S_4(SR)_4]^{2-}$ salts were determined using a Varian HR-220 spectrometer operated in the CW or Fourier transform (FT) mode depending on the solubility properties of the sample. Solutions were prepared with exclusion of air using 99+% deuterium enriched acetonitrile, dimethyl sulfoxide, acetone, or methanol as solvents. All compounds were measured in CD_3CN, with the other solvents utilized primarily to extend the temperature range of measurement. 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 diamagnetic reference shifts were taken as those of the free thiols in the appropriate solvents at ambient temperature. In accord with the usual practice in dealing with spectra of paramagnetic species and their diamagnetic references, $(\Delta H/H_0)^{obsd}$ and $(\Delta H/H_0)^{dia}$ are taken as negative for chemical shifts downfield of TMS. An opposite sign convention has been employed in reporting protein pmr spectra using DSS internal reference.[13-21]

The following chemical shifts (ppm) have been obtained for the free thiols in the indicated solvents at ambient temperature (shifts of thiol SH protons are, in general, concentration and solvent dependent and have been studied extensively;[27] these shifts are not included here): MeSH −2.03 (d), CD_3CN; EtSH −2.51 (m, CH_2), −1.27 (t, CH_3), CD_3CN; −2.49 (q, CH_2), −1.24 (t, CH_3), DMSO-d_6; −2.52 (q, CH_2), −1.26 (t, CH_3), acetone-d_6; *n*-PrSH −2.47 (m, CH_2S), −1.57 (m, CH_2), −0.95 (t, CH_3), CD_3CN; −2.49 (t, CH_2S), −1.61 (q, CH_2), −0.97 (t, CH_3), CD_3OD; *i*-PrSH

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(23) This statement does not include redox potentials, which for the 2-/3- electron transfer are more negative than values for Fd_{ox}/Fd_{red} when compared on a common scale.[8] This property depends on molecular free energy changes associated with electron transfer and may be affected by alterations in protein structure, as shown by the detection of a "super-reduced" form of the HP protein in DMSO–H_2O solution by Cammack.[24]
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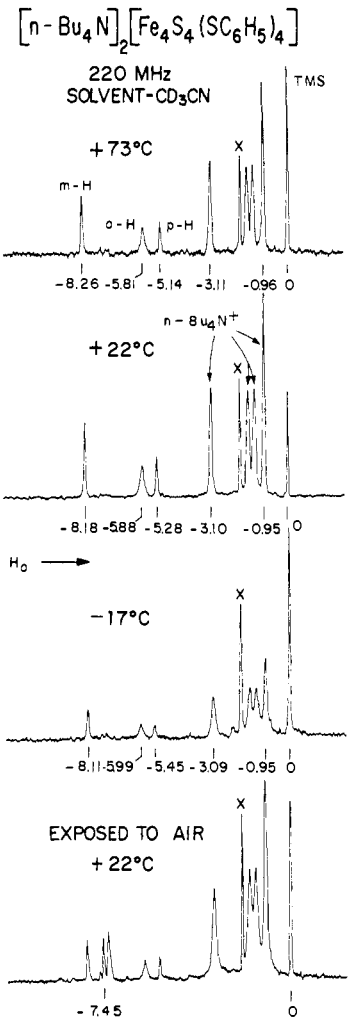

Figure 1. Pmr spectra (220 MHz) of $(n\text{-Bu}_4\text{N})_2[Fe_4S_4(SPh)_4]$ in CD_3CN at −17, 22, and 73° illustrating the downfield shift of *m*-H and upfield shifts of *o*- and *p*-H with increasing temperature. The spectrum at the bottom was recorded after the solution had been exposed to the air for *ca.* 5 min. Residual solvent proton resonance is marked with an ×. Chemical shifts are in ppm.

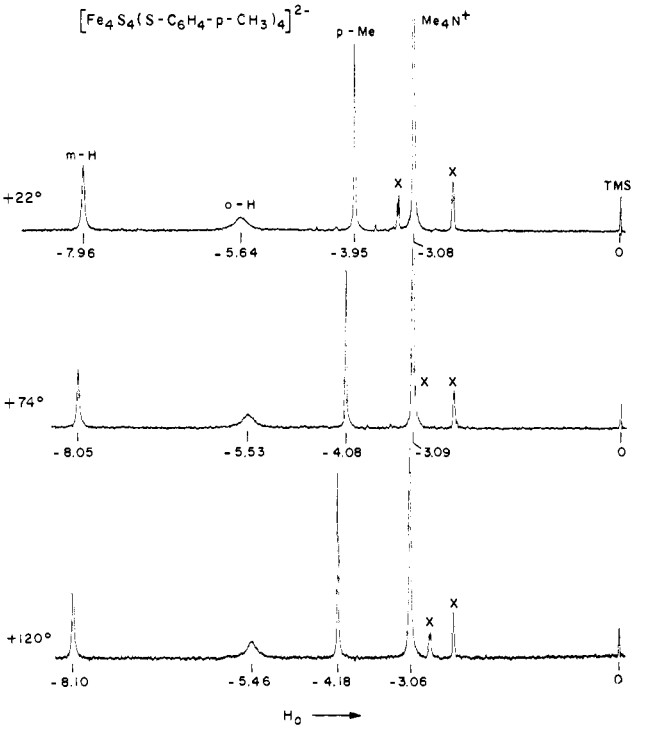

Figure 2. Pmr spectra (220 MHz) of $(Me_4N)_2[Fe_4S_4(S\text{-}p\text{-tol})_4]$ in DMSO-d_6 at 22, 74, and 120° illustrating the downfield shifts of *m*-H and *p*-Me and upfield shift of *o*-H with increasing temperature. The features marked with an × are due to water and residual solvent protons. Chemical shifts are in ppm.

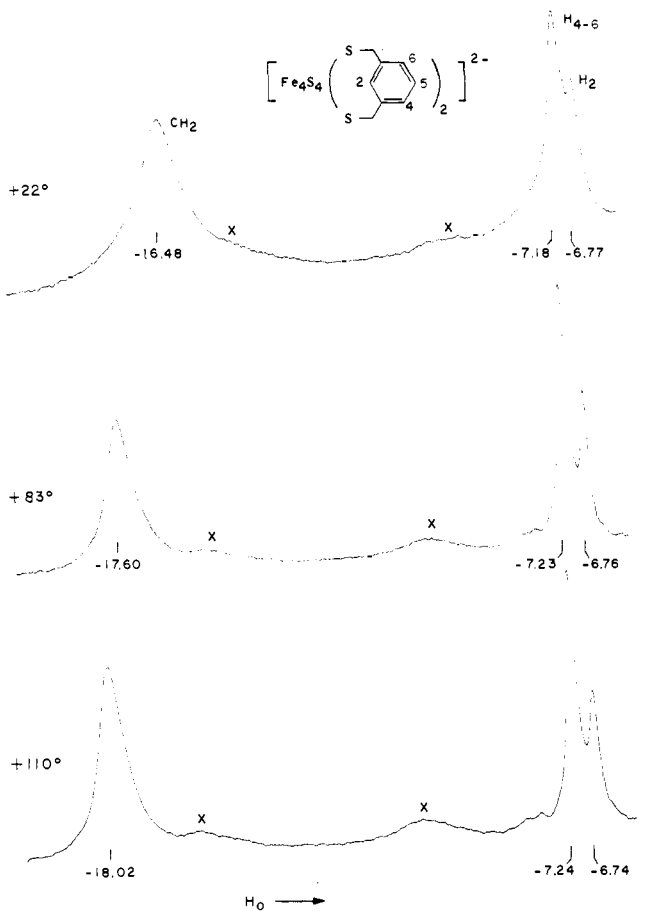

Figure 3. FT pmr spectra (220 MHz) of methylene and ring protons of $(n\text{-Bu}_4\text{N})_2[Fe_4S_4(m\text{-xyl-}S_2)_2]$ in DMSO-d_6 at 22, 83, and 110°. The broad features (×) are presumably due to impurities. Chemical shifts are in ppm downfield of TMS.

−3.10 (m, CH), −1.38 (d, CH_3), CD_3CN; $C_6H_{11}CH_2SH$ −2.34 (m, CH_2S), CD_3CN; $PhCH_2SH$ −3.65 (d, CH_2) −7.15 (s, Ph), CD_3CN; $1,3\text{-}C_6H_4(CH_2SH)_2$ −7.26 (m, H_2), −7.18 (m, H_{4-6}), −3.69 (d, CH_2), CD_3CN; −7.28 (m, H_2), −7.20 (m, H_{4-6}), −3.70 (d, CH_2), DMSO-d_6; *t*-BuSH −1.39 (s), CD_3CN; PhSH −7.20 (m), CD_3CN; −7.21 (m), acetone-d_6; −7.30 (m), DMSO-d_6; $p\text{-}CH_3C_6H_4SH$ (center of A_2B_2 spectrum, $|\Delta\nu_{AB}|$, CH_3) −7.10, 0.095, −2.28, CD_3CN; −7.08, 0.14, −2.20, acetone-d_6; −7.11, 0.14, −2.24, DMSO-d_6; $p\text{-}O_2NC_6H_4SH$ center of A_2B_2 spectrum, −7.68, $|\nu_{AB}|$ 0.61. In aliphatic thiols values of J_{CH-SH} and J_{CH-CH} are 7–8 and 6–7 Hz, respectively. Chemical shifts of protons ortho and meta to the S–H group in *p*-nitrothiophenol were taken as −7.36 and −7.97 ppm, respectively. Pmr spectra of thiophenol[28] and certain of its derivatives[29] have been analyzed. For thiophenol in cyclohexane the spread in ring proton shifts is 0.15 ppm, indicating that only a slight error is introduced by using the above multiplet center shifts in the calculation of isotropic shifts.

Results and Discussion

General Spectral Features. The $[Fe_4S_4(SR)_4]^{2-}$ complexes investigated contain terminal mercaptide ligands derived from a variety of aliphatic and aromatic thiols. The similarity between mercaptides containing

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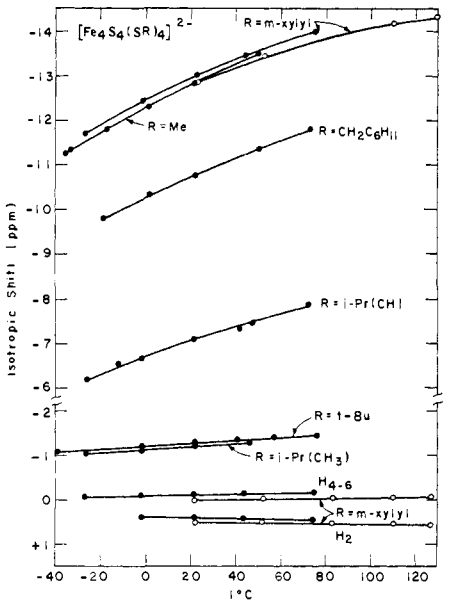


Figure 4. Temperature dependencies of the isotropic proton shifts of $[Fe_4S_4(SR)_4]^{2-}$ (R = Me, $CH_2C_6H_{11}$, *i*-Pr, *t*-Bu) and $[Fe_4S_4(m\text{-xyl-S}_4)_2]^{2-}$ in CD_3CN (●) and DMSO-d_6 (○) solutions.

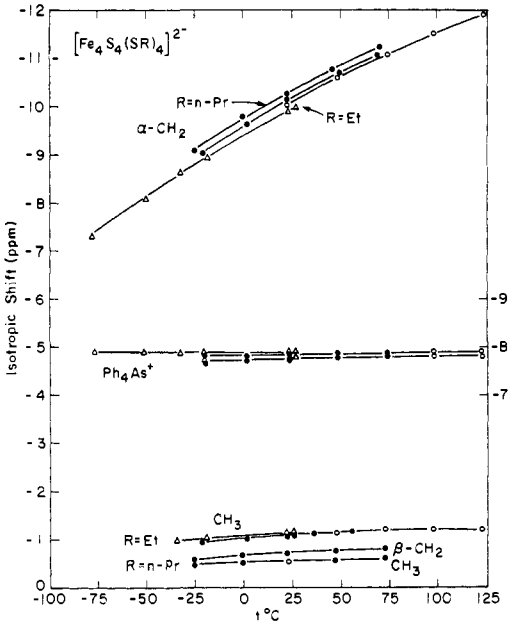


Figure 5. Temperature dependencies of the isotropic proton shifts of $(Ph_4As)_2[Fe_4S_4(SR)_4]$ (R = Et, *n*-Pr) in CD_3COCD_3 (Δ), CD_3CN (●), and DMSO-d_6 (○) solutions. The scale at the right refers to the chemical shifts of Ph_4As^+ relative to TMS.

an α-CH_2 group and cysteinyl residues in the protein active sites (**1**) is apparent. Pmr spectra of several complexes are shown in Figures 1–3. Temperature dependencies of mercaptide proton isotropic shifts, measured with respect to the appropriate thiol, are displayed in Figures 4–7 and isotropic shifts at 22° are collected in Table I. In most cases these shifts display a small solvent dependence. Resonances of the quaternary counterions were found to be slightly shifted and broadened compared to their simple diamagnetic halide salts, possibly indicating a small extent of ion clustering. As illustrated in Figures 1 and 5, cation shifts were observed to be virtually temperature inde-

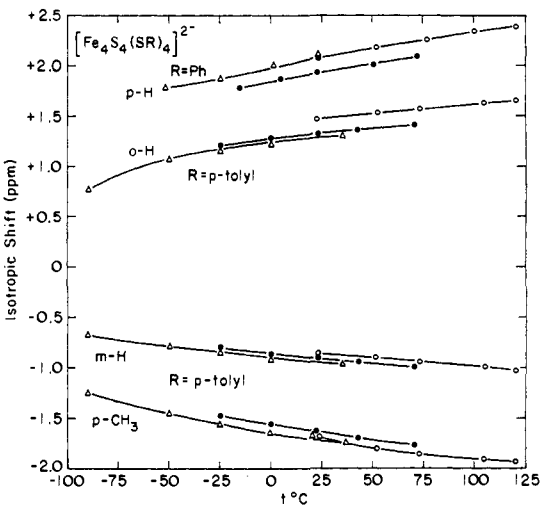


Figure 6. Temperature dependencies of the isotropic proton shifts of $[Fe_4S_4(SR)_4]^{2-}$ (R = Ph, *p*-tolyl) in CD_3COCD_3 (Δ), CD_3CN (●), and DMSO-d_6 (○) solutions. Shifts of the *o*-H and *m*-H resonances of the Ph tetramer (not shown) are very similar to those of the *p*-tolyl tetramer.

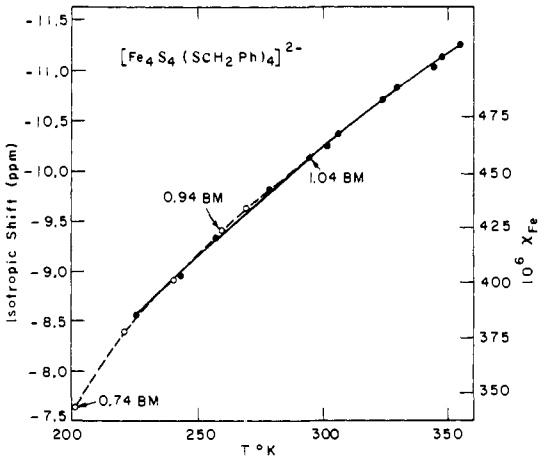


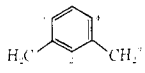
Figure 7. Temperature dependence of the methylene proton isotropic shifts of $(Et_4N)_2[Fe_4S_4(SCH_2Ph)_4]$ in CD_3CN (●) with the magnetic susceptibility per iron in the crystalline state (○). The scales were arbitrarily chosen such that $(\Delta H/H_0)^{iso}$ and χ_{Fe} coincide at 295°K.

pendent at the concentrations (*ca.* 0.01–0.05 *M*) employed in this work and are not considered further. All tetramers exhibited a single set of mercaptide resonances over the entire temperature interval of measurement, which for a number of complexes (Figures 5 and 6) approached 200°. This result is consistent with the solid-state structure of $[Fe_4S_4(SCH_2Ph)_4]^{2-}$[7] and a number of other spectroscopic measurements[30] with shorter time scales which have failed to differentiate the two Fe(II) and Fe(III) centers implied by formal oxidation-state considerations.[6,7]

Temperature Dependencies of Shifts. Magnetic data for several oxidized bacterial ferredoxins and one synthetic analog are summarized in Table II. Values of μ_{Fe} at or near ambient temperature fall in the 1.0–1.3

(30) The results of these measurements (Mössbauer, electronic, ESCA spectroscopy) indicate that $[Fe_4S_4(SR)_4]^{2-}$ complexes cannot be regarded as trapped valence (integral oxidation state) species and will be reported elsewhere: R. H. Holm, B. A. Averill, T. Herskovitz, R. B. Frankel, H. B. Gray, O. Siiman, and F. J. Grunthaner, *J. Amer. Chem. Soc.*, in press.

Table I. Isotropic Shifts of Mercaptide Protons in $(Q^+)_2[Fe_4S_4(SR)_4]^{2-}$ at 22°

R	Solvent	Isotropic shift, ppm
CH_3[a]	CD_3CN	−12.8
CH_2CH_3[b]	CD_3CN	−10.1 (CH_2), −1.09 (CH_3)
	DMSO-d_6	−10.0 (CH_2), −1.10 (CH_3)
	CD_3COCD_3	−9.91 (CH_2), −1.09 (CH_3)
$C(\alpha)H_2C(\beta)H_2CH_3$[b]	CD_3CN	−10.3 (α-CH_2), −0.668 (β-CH_2), −0.554 (CH_3)
	CD_3OD	−8.39 (α-CH_2), −0.686 (β-CH_2), −0.536 (CH_3)
$CH(CH_3)_2$[a]	CD_3CN	−7.01 (CH), −1.21 (CH_3)
$CH_2C_6H_{11}$[c]	CD_3CN	−10.7 (CH_2)
CH_2Ph[d]	CD_3CN	−10.1 (CH_2), −0.26, −0.42 (*o*-, *m*-H), ~0 (*p*-H)
	CD_3CN	−13.0 (CH_2), +0.40 (H_2),[g] −0.10 (H_{4-6})[g]
	DMSO-d_6	−12.8 (CH_2), +0.51 (H_2)[2], +0.02 (H_{4-6})[2]
$C(CH_3)_3$[e]	CD_3CN	−1.26
C_6H_5[a]	CD_3CN	+1.32 (*o*-H), −0.982 (*m*-H), +1.92 (*p*-H)
	DMSO-d_6	+1.56 (*o*-H), −0.891 (*m*-H), +2.09 (*p*-H)
	CD_3COCD_3	+1.40 (*o*-H), −0.923 (*m*-H), +2.10 (*p*-H)
p-$C_6H_4CH_3$[e]	CD_3CN	+1.30 (*o*-H), −0.900 (*m*-H), −1.64 (*p*-CH_3)
	DMSO-d_6	+1.47 (*o*-H), −0.855 (*m*-H), −1.70 (*p*-CH_3)
	CD_3COCD_3[f]	+1.34 (*o*-H), −0.938 (*m*-H), −1.74 (*p*-CH_3)
p-$C_6H_4NO_2$[e]	CD_3CN	+1.24 (*o*-H), −0.900 (*m*-H)

[a] *n*-Bu_4N^+ salt. [b] Ph_4As^+ salt. [c] *n*-Pr_4N^+ salt. [d] Et_4N^+ salt. [e] Me_4N^+ salt. [f] 35°. [g] Assignments tentative.

are always displaced downfield with increasing temperature. This behavior is also found with Fd_{ox}[18-21] and HP_{red}[16,17] proteins. Further, the *chemical* shifts of α-CH_2 groups of the analogs (in nonaqueous media) occur in the same range as those of the isotropically shifted protein resonances, as is shown by the data compared in Table II. With the possible exception of $[Fe_4S_4(m\text{-xyl-}S_2)_2]^{2-}$, whose pmr spectrum and structure are discussed below, only $[Fe_4S_4(Ac\text{-L-cys-NHMe})_4]^{2-}$ of the various analogs contains inequivalent CH_2–S protons, as do the proteins. The spectrum of, *e.g.*, *C. pasteurianum* Fd_{ox} at ambient temperature reveals eight resonances with a total chemical shift spread of *ca.* 6 ppm.[19,20] This effect may arise from the relatively rigid protein structure which affords a definite angular dependence to methylene proton contact shifts.[19] The pmr properties of the analogs provide convincing evidence for the validity of previous assignments of HP_{red} and Fd_{ox} protein signals in the *ca.* 8–20 ppm downfield region to the CH_2–S protons[16-21] associated with the active sites, **1**.

A detailed general theory of the isotropic shifts in antiferromagnetic systems has not yet been developed.[33] However, a recent treatment[34] suffices to provide at least a semiquantitative description of the temperature dependence of such shifts provided they are wholly contact in origin. In this case the net contact shift of a proton in the tetramer is given by

$$\left(\frac{\Delta H}{H_0}\right)^{con} = \frac{P}{T}\frac{\sum_i A_i q_i S_i'(S_i'+1)e^{-E_i/kT}}{\sum_i q_i e^{-E_i/kT}} \qquad (1)$$

Table II. Magnetic and Chemical Shift Data for $Fe_4S_4(SR)_4$ Clusters

Compound	μ_{Fe}, BM	$-(\Delta H/H_0)^{obsd}$,[a] ppm	Ref
$[Fe_4S_4(SCH_2Ph)_4]^{2-}$[b]	0.23–1.04[c] (−103 to 23°)		6
	1.1 (DMSO, 26°)	12.2–14.9 (−47 to 82°)[d]	*g*
$[Fe_4S_4(SEt)_4]^{2-}$	*k*	9.80–14.4 (−78 to 118°)[e]	*g*
$[Fe_4S_4(m\text{-xyl-}S_2)_2]^{2-}$	*k*	15.3–18.3 (−27 to 128°)[f]	*g*
$[Fe_4S_4(Ac\text{-L-cys-NHMe})_4]^{2-}$	*k*	−12.4, −13.6 (~30°)[h]	1, *l*
Chromatium HP_{red}	*k*	11–18 (0–80°)[i]	16
C. pasteurianum Fd_{ox}	1.02–1.08 (5–30°)	10–19 (−20 to 90°)[i,j]	19, 20
C. acidi-urici Fd_{ox}	1.20–1.26 (5–30°)	10–17 (5–30°)[i]	21
B. polymyxa Fd_{ox}	0.86–0.91 (5–30°)	8–16 (4–69°)[i]	18

[a] SCH_2 shifts; ranges given for proteins are approximate. [b] Et_4N^+ salt. [c] Solid state. [d] CD_3CN solution. [e] CD_3COCD_3, CD_3CN, and DMSO-d_6 solutions. [f] CD_3CN and DMSO-d_6 solutions. [g] This work. [h] DMSO-d_6 solution. [i] D_2O solution, pH ~7. [j] CD_3OD–D_2O solution. [k] Not measured. [l] L. Que, Jr., M. A. Bobrik, and R. H. Holm, unpublished results.

BM range and may be compared with those for oxidized (Fe(III), 5.85 BM) and reduced (Fe(II), 5.05 BM) *C. pasteurianum* rubredoxin,[31] which contains an isolated distorted tetrahedral Fe–S_4 center.[32] These results indicate that the metal centers are weakly paramagnetic and, together with other spectral and magnetic data,[6,8,12] support the electronic similarity of the analogs and Fd_{ox}. That the small increases in μ_{Fe} for the proteins over limited temperature ranges in solution are due to antiferromagnetism has been confirmed by a detailed magnetic study of $(Et_4N)_2[Fe_4S_4(SCH_2Ph)_4]$ in the solid state.[6] The pmr spectra of all synthetic tetramers thus far examined are characterized by isotropically shifted and broadened resonances which, in the case of aliphatic mercaptide ligands (Figures 3–5, 7), with $P = -g\beta/12\gamma_H\hbar k$. The S_i' are the various spin levels, $S' = 0, 1, 2, \ldots$, with energies E_i under the spin Hamiltonian $\mathcal{H} = 2\Sigma J_{ij}\mathbf{S}_i\cdot\mathbf{S}_j$,[35] and the q_i are degeneracy factors appropriate to these levels. Equation 1 holds only if the individual spin states obey the Curie law, a usual assumption in the dipolar model of antiferromagnetism.[35] If the electron–nuclear hyperfine coupling constants A_i are independent of spin state, $A_i = A$ may be removed from the summation and, apart from a multiplicative constant, the quotient of

(31) W. D. Phillips, M. Poe, J. F. Weiher, C. C. McDonald, and W. Lovenberg, *Nature (London)*, 227, 574 (1970).

(32) K. D. Watenpaugh, L. C. Sieker, J. R. Herriott, and L. H. Jensen, *Acta Crystallogr., Sect. B*, **29**, 943 (1973).

(33) For an interpretation of the shifts in reduced 2Fe–2S* proteins, which is the most detailed yet attempted for any antiferromagnetic case, *cf.* W. R. Dunham, G. Palmer, R. H. Sands, and A. J. Bearden, *Biochim. Biophys. Acta*, **253**, 373 (1971).

(34) G. N. LaMar, G. R. Eaton, R. H. Holm, and F. A. Walker, *J. Amer. Chem. Soc.*, **95**, 63 (1973).

(35) R. L. Martin in "New Pathways in Inorganic Chemistry," E. A. V. Ebsworth, A. G. Maddock, and A. G. Sharpe, Ed., Cambridge University Press, London, 1968, pp 175–231; E. Sinn, *Coord. Chem. Rev.*, **5**, 313 (1970).

summed terms represents the temperature dependence of the magnetic susceptibility χ_{Fe}.[34-36] Thus at all temperatures[34]

$$\chi_{Fe} = R(\Delta H/H_0)^{con} \quad (2)$$

where the constant ($R = -g\beta N\gamma_H\hbar/A$) is independent of temperature. Contact shifts will then be directly proportional to magnetic susceptibility and their temperature dependencies will superimpose where scaled by R. This treatment has been applied to $[Fe_4S_4(SCH_2Ph)_4]^{2-}$ using CH_2 shifts in acetonitrile and solid-state susceptibility data.[38] The result, shown in Figure 7, reveals that the two quantities have closely similar temperature dependencies in the interval (225–295°K) common to both measurements. The applicability of eq 2 indicates that A does not depend strongly on spin state or that only the lowest paramagnetic level ($S' = 1$) is populated and implies that the isotropic methylene shifts of $[Fe_4S_4(SCH_2Ph)_4]^{2-}$ are dominantly contact in nature. The similarity of magnetic and pmr properties between the proteins and this and other analogs having α-CH_2 groups suggests that eq 2 is likely to be appropriate to HP_{red} and Fd_{ox} as well.

Evidence for Dominant Contact Interactions. Methods for analyzing the contact and dipolar contributions to total isotropic shifts ($(\Delta H/H_0)^{iso} = (\Delta H/H_0)^{con} + (\Delta H/H_0)^{dip}$) have recently been summarized by Horrocks.[39] In the absence of magnetic anisotropy data, which would allow at least a semiquantitative assessment of the magnitudes of dipolar shifts, less direct arguments must necessarily be used in establishing the nature of the isotropic shifts of $[Fe_4S_4(SR_4)_4]^{2-}$ (Table I). Characteristic features of the proton contact shifts of saturated ligands arising from a σ-delocalization mechanism are rapid attenuation of shifts as the number of bonds between the metal and the proton increases and nonalternation of the signs of the shifts. These features have been observed in a variety of octahedral[40] and tetrahedral[41] Ni(II) complexes containing saturated ligands or ligand fragments. In these complexes there can be little question that contact interactions are dominant,[39-41] particularly for the six-coordinate complexes with a $^3A_2$ ground state. Relative isotropic shifts are compared in Table III, from which it is evident that the shifts of the R = Et and *n*-Pr tetramers attenuate in a qualitatively similar fashion to those of the Ni(II) complexes. All alkyl tetramer shifts are downfield, a situation consistent with ligand → metal spin transfer which must involve antiparallel spin regardless of whether the tetrahedral metal centers are considered as Fe(II) ($e^3t_2^3$) or Fe(III) ($e^2t_2^3$). Delocalization of parallel (positive) spin would then afford negative contact shifts. Attenuation factors cannot be expected to be closely similar, even among the Ni(II) complexes, inasmuch as the distribution of spin will depend upon the forms of the ligand MO's[42] and ligand conformation.[40a,c]

Table III. Attenuation of Isotropic Shifts

Complex	Relative shifts[a] α-H	β-H	γ-H	Ref
$[Fe_4S_4(SEt)_4]^{2-}$	−1.00	−0.11		*b*
$[Fe_4S_4(S\text{-}n\text{-}Pr)_4]^{2-}$ [h]	−1.00	−0.065	−0.054	*b*
$[Fe_4S_4(SCH_2Ph)_4]^{2-}$	−1.00	−0.026, −0.042[d]	~0[e]	*b*
$[Ni(EtNH_2)_6]^{2+}$	−1.00	−0.35		40a
$[Ni(n\text{-}PrNH_2)_6]^{2+}$	−1.00	−0.20	−0.007	40a
Ni(acac)$_2$–piperidine	−1.00	−0.47	−0.11	40b
Ni(acac)$_2$-$C_6H_{11}NH_2$	−1.00	−0.06	−0.01	40c
Ni(Et$_2$-ati)$_2$[c]	−1.00	−0.066		41
Ni(*n*-Pr$_2$-ati)$_2$[c]	−1.00	−0.068	−0.036	41
Ni((PhCH$_2$)$_2$-ati)$_2$[c]	−1.00	−0.039,[f] −0.06[g]		41b

[a] Data at ambient temperature, α-C directly bonded to S or N, negative isotropic shift. [b] This work. [c] ati = *N*,*N*-disubstituted aminotroponeiminate. [d] *o*-, *m*-H. [e] *p*-H. [f] *o*-H. [g] *m*-H. [h] Relative shifts were incorrectly quoted elsewhere.[20]

Ligands which correspond to alternant hydrocarbons are frequently useful as detector groups for contact shifts inasmuch as the signs and, to a lesser extent, the magnitudes of their shifts are characteristic of π spin delocalization. Benzenethiolate is such a ligand, and, accordingly, tetramers with R = Ph, *p*-tolyl, and *p*-$C_6H_4NO_2$ have been synthesized and their pmr spectra investigated. Spectra of the first two complexes at several temperatures are shown in Figures 1 and 2. Ring proton assignments were made by para substitution and from relative line widths with the broadest resonance attributed to *o*-H, which is nearest the paramagnetic centers. In $[Fe_4S_4(SPh)_4]^{2-}$ high field shifts are observed for *o*-H and *p*-H whereas *m*-H is displaced to low field; the shifts are not attenuated by distance. In $[Fe_4S_4(S\text{-}p\text{-}tol)_4]^{2-}$ the signs of the ortho and para proton shifts are unaffected and their values only slightly changed by methyl substitution, but the shift at the para position is reversed in sign compared to the phenyl tetramer. These observations are diagnostic of dominant contact shifts,[39] and similar shift patterns have been found in other complexes with alternant ligand systems containing phenyl groups.[39,41,43] From the McConnell relation $A = Q\rho_C$ ($Q_{CH} \sim -23$ G, Q_{CCH_3} positive but variable in magnitude) and eq 1, it is seen that the signs of the observed shifts are consistent with positive spin density at the ortho and para carbons and negative spin density at the meta carbons. The low field displacement of the methyl resonance is compatible with π-spin density at the para position,[44] leading to a direct contact interaction with the methyl protons *via* hyperconjugation. The opposite temperature dependencies of *o*-, *p*-H, *vs.* *m*-H, *p*-Me (Figure 6) are also consistent with this model.

Because of the lack of orthogonality between ligand

(36) The exact form of the susceptibility expression is not required here. Magnetic properties of tetranuclear D_{2d} or T_d clusters have been treated elsewhere.[35,37]

(37) J. A. Bertrand, A. P. Ginsberg, R. I. Kaplan, C. E. Kirkwood, R. L. Martin, and R. C. Sherwood, *Inorg. Chem.*, **10**, 240 (1971).

(38) Solution magnetic data would be more appropriate for comparison with isotropic shifts, but these could not be obtained with sufficient accuracy due to inadequate solubility and low susceptibility.

(39) W. D. Horrocks, Jr., in "NMR of Paramagnetic Molecules: Principles and Applications," G. N. LaMar, W. D. Horrocks, and R. H. Holm, Ed., Academic Press, New York, N. Y., 1973, Chapter 4.

(40) (a) R. J. Fitzgerald and R. S. Drago, *J. Amer. Chem. Soc.*, **90**, 2523 (1968); (b) J. A. Happe and R. L. Ward, *J. Chem. Phys.*, **39**, 1211 (1963); (c) T. Yonezawa, I. Morishima, and Y. Ohmori, *J. Amer. Chem. Soc.*, **92**, 1267 (1970).

(41) (a) D. R. Eaton, A. D. Josey, W. D. Phillips, and R. E. Benson, *J. Chem. Phys.*, **37**, 347 (1962); (b) D. R. Eaton, A. D. Josey, and R. E. Benson, *J. Amer. Chem. Soc.*, **89**, 4040 (1967).

(42) W. D. Horrocks, Jr., and D. L. Johnston, *Inorg. Chem.*, **10**, 1835 (1971).

(43) (a) D. R. Eaton, A. D. Josey, R. E. Benson, and W. D. Phillips, *J. Amer. Chem. Soc.*, **84**, 4100 (1962); (b) J. E. Parks and R. H. Holm, *Inorg. Chem.*, 7, 1408 (1968).

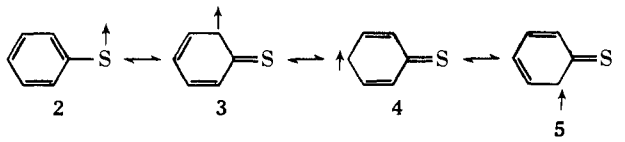
(44) H. M. McConnell, *J. Chem. Phys.*, **24**, 764 (1956); A. D. McLachlan, *Mol. Phys.*, **1**, 233 (1958).

Table IV. Calculated and Experimental Spin Distribution in Benzyl and C_6H_5X Radicals

Radical	ρ_X	ρ_{CX}	ρ_o	ρ_m	ρ_p	Ref
$C_6H_5CH_2\cdot$, VBT	+0.685	−0.321	+0.330	−0.177	+0.330	45, *b*
$C_6H_5X\cdot$, VBT[a]	+0.596	−0.318	+0.372	−0.200	+0.378	*b*
	Relative values		*o*	*m*	*p*	
$C_6H_5CH_2\cdot$ ρ_{calcd}			−1.86	1.00	−1.86	45, *b*
$\lvert A_{obsd}\rvert$			2.94	1.00	3.51	45
$C_6H_5X\cdot$ ρ_{calcd}			−1.86	1.00	−1.89	*b*
$C_6H_5O\cdot$ $\lvert A_{obsd}\rvert$			3.37	1.00	5.31	*c*
$C_6H_5SCH_2\cdot$ $\lvert A_{obsd}\rvert$			2.47	1.00	2.15	*d*
$[Fe_4S_4(SPh)_4]^{2-}$ $(\Delta H/H_0)^{iso}$			−1.34	1.00	−1.96	*b*
$[Fe_4S_4(SC_6H_4NO_2)_4]^{2-}$ $(\Delta H/H_0)^{iso}$			−1.24	1.00		*b*
p-$O_2NC_6H_4S\cdot^{2-}$ $\lvert A_{obsd}\rvert$			0.35	1.00		*e*

[a] $\gamma_{CX} = 1.2\gamma$. [b] This work. [c] T. J. Stone and W. A. Waters, *Proc. Chem. Soc., London*, 253 (1962). [d] A. Hudson and K. D. J. Root, *J. Chem. Soc. B*, 656 (1970). [e] R. D. Allendoerfer and P. H. Rieger, *J. Chem. Phys.*, **46**, 3266 (1967).

(σ and π phenyl ring orbitals, sulfur lone pair orbitals) and spin-containing metal orbitals, there is in principle no unique spin delocalization pathway. However, a simple feasible mechanism leading to a spin distribution pattern consistent with the above observations is postulated to occur by mixing of the local metal d orbitals, all of which are at least half-filled, with the sulfur lone pair orbitals. Ligand → metal antiparallel spin transfer results in positive spin on sulfur which is principally delocalized as indicated by the VB structures **2**–**5**. Negative spin density arises at the meta



2 **3** **4** **5**

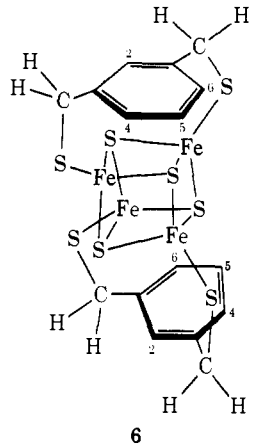
positions through spin correlation effects. This mechanism results in the creation of net positive spin in the highest filled MO of the phenyl ring and imparts to the ligand the character of a generalized C_6H_5X π-radical. The most thoroughly studied example of such a species is the benzyl radical, whose experimental[45] and theoretical[45-47] spin densities serve as a rough index of the expected spin distribution in the phenylsulfenyl radical.[48] While rather involved MO calculations[46] have produced spin densities for the benzyl radical in good agreement with experiment, the valence bond method[45,47] has been moderately successful in accounting for experimental spin densities in this species as well as in metal complexes containing alternant ligands.[39,41,43] The C_6H_5X radical has been treated by this method using a basis set of five Kekulé-type structures and variable exchange integrals. The results are not too sensitive to the choice of parameters or inclusion of additional (Dewar) structures in the basis set. Those shown in Table IV, together with data for related radicals, are typical. Simple HMO calculations were also performed using a range of h and k parameters ($\alpha_s = \alpha + h\beta$, $\beta_{CS} = k\beta$) considered appropriate to organosulfur radicals.[49] At, *e.g.*, $h = k = 1.0$, $\rho_0 = 0.150$, $\rho_m = 0.0125$, and $\rho_p = 0.197$ for the $3B_1$ MO.

Relative isotropic shifts should represent relative spin densities provided these shifts are contact in nature. While no precise agreement between shift ratios for $[Fe_4S_4(SPh)_4]^{2-}$ and calculated spin density ratios for $C_6H_5X\cdot$ is found (nor can such be expected in view of the approximate nature of the model), the results in Table IV do indicate that the relative shifts reasonably conform to the spin distribution for π-delocalization consequent to ligand → metal spin transfer. Further, spin is not appreciably delocalized into the lowest unoccupied phenyl group MO (metal → ligand parallel spin transfer). HMO calculations predict $\rho_o = \rho_m = 0.250$ and $\rho_p = 0$ (independent of h and k) for the $2A_2$ MO of $C_6H_5X\cdot^{2-}$. The relative shifts of $[Fe_4S_4(SC_6H_4NO_2)_4]^{2-}$ are in definite disagreement with the relative coupling constants of the p-nitrobenzenethiolate dianion radical (Table IV).

$[Fe_4S_4(m\text{-xyl-}S_2)_2]^{2-}$. A feature common to most bacterial 8-Fe ferredoxins is the conservation of aromatic residues (Phe, Tyr) at positions 2 and 28 or 30 in the polypeptide chain.[50] In *P. aerogenes* Fd_{ox} a Tyr residue is roughly parallel to one face of each of the two Fe_4S_4* units.[11] The possible role of these residues in mediating electron transfer in 8-Fe proteins has been considered recently.[22] In view of the potential influence on the electronic properties of the Fe_4S_4* clusters by such nearby residues, an analog has been sought in which phenyl rings are closely juxtaposed to the faces of the cluster. Inspection of a scale model constructed from the atomic coordinates of $[Fe_4S_4(SCH_2Ph)_4]^{2-}$ [7] indicated that the m-phenylenedimethylene group could strainlessly span the *ca.* 7 Å distance between mercaptide sulfurs. Use of m-xylyl-α,α'-dithiol in the tetramer synthesis[7] afforded a salt whose analysis is consistent with the formulation $[Fe_4S_4(m\text{-xyl-}S_2)_2]^{2-}$ for the anionic component. Electronic spectral and redox properties[8] are also consistent with a tetranuclear dianion complex. Structure **6** is tentatively proposed for this complex. From the model the phenyl rings are rather rigidly held approximately

(45) A. Carrington and I. C. P. Smith, *Mol. Phys.*, **9**, 137 (1965).
(46) J. Nowakowski, *Theor. Chim. Acta*, **18**, 133 (1970); N. Trinajstic, *Chem. Phys. Lett.*, **10**, 172 (1971).
(47) I. Vandoni and M. Simonetta, *Theor. Chim. Acta*, **14**, 17 (1969).
(48) This radical has been detected only in rigid media at low temperatures and no proton hyperfine splitting has been resolved. The high average g value and g tensor anisotropy indicate a large degree of spin localization on sulfur: U. Schmidt, A. Muller, and K. Markau, *Chem. Ber.*, **97**, 405 (1964).
(49) J. Fabian in "Sulfur in Organic and Inorganic Chemistry," A. Senning, Ed., Vol. 3, Marcel Dekker, New York, N. Y., 1972, Chapter 23.
(50) M. Tanaka, M. Haniu, G. Matsueda, K. T. Yasunobu, R. H. Himes, J. M. Akagi, E. M. Barnes, and T. Devanathan, *J. Biol. Chem.*, **246**, 3953 (1971); M. Tanaka, M. Haniu, K. T. Yasunobu, R. H. Himes, and J. M. Akagi, *ibid.*, **248**, 5215 (1973).

parallel to two of the faces of the Fe_4S_4* cluster, with the ring centers ~3 Å from the nearest S* atom. The C_2 atom is equidistant (~3.4 Å) from the closest two



6

iron atoms; C_{4-6} are considerably further removed from these atoms.

Pmr spectra of $[Fe_4S_4(m\text{-xyl-}S_2)_2]^{2-}$ are shown in Figure 3. Other properties will be reported elsewhere.[8] Isotropic shifts of the methylene protons show a temperature dependence (Figure 4) characteristic of the R = alkyl tetramers. Their values are somewhat larger than for other tetramers; chemical shifts fall in the range found for the proteins (Table II). Although these protons are inequivalent in structure **6**, only one methylene signal was observed at all temperatures. Two resonances at higher field occur in ~3/1 intensity ratio and are assigned to the ring protons. The less intense signal shows a larger isotropic shift, which is positive, with a small but definite temperature dependence (Figure 4). The other signal has a smaller shift with a slight temperature dependence. Comparison of these shifts with those of $[Fe_4S_4(SCH_2Ph)_4]^{2-}$, all of which are downfield, suggests that proton shifts can be perturbed as a consequence of a roughly parallel orientation of the phenyl ring with respect to the cluster. Substantial ^{13}C but approximately zero proton isotropic shifts have been found for the Tyr groups in *C. acidi-urici* $Fd_{ox,red}$, where the ring is 4–5 Å from the cluster.[22] As with the proteins, it is not yet possible to prove whether the shifts are mainly contact or dipolar in origin. In view of the evidence presented above dominant contact interactions are favored in both cases. If correct, the upfield proton shift could arise from spin polarization of the C_2–H_2 σ electrons under the influence of two metal centers. Other mechanisms can also be proposed. The upfield isotropically shifted signal is tentatively assigned to H_2.

Solution Stability of Tetramers. Like the proteins all $[Fe_4S_4(SR)_4]^{2-}$ species thus far prepared are sensitive to oxygen in solution, and the results reported herein were obtained from anaerobically prepared solutions. Nmr tubes containing solutions of the R = Et, *n*-Pr, and Ph tetramers were opened and exposed to the air for various times, and the pmr spectra were recorded. Tetramer signals decreased in intensity relative to cation resonances, an effect that was particularly evident after exposure times exceeding ~10 min. New signals appeared which could be assigned to the corresponding disulfide. The bottom spectrum in Figure 1 is illustrative, with the feature centered near −7.45 ppm due to PhSSPh. In addition, solutions prepared from analytically pure R = alkyl tetramers without exposure to air exhibited one or more signals due to minority paramagnetic species. The spectra of $[Fe_4S_4(m\text{-xyl-}S_2)_2]^{2-}$ (Figure 3) show two broadened signals outside the diamagnetic region which are attributed to such species. Spectra of $[Fe_4S_4(SCH_2C_6H_{11})_4]^{2-}$ in CD_3CN reveal a definite low field shoulder on the methylene signal. The two features parallel each other in temperature dependence, shifting from −14.1 ppm at 50° to −14.7 ppm at 73°. Related observations were made with solutions of the benzyl tetramer in CD_3CN. In this case a separate signal with a roughly parallel temperature dependence and about 7% of the intensity of the principal methylene signal was observed *ca.* 4 ppm downfield of the latter. The nature of these minority components is unknown. Their shifts are not indicative of an oxidized 2Fe–2S* species.[5] Inasmuch as their signal intensities appear to increase after heating or standing, they may result from partial degradation of the cluster structure, which is perhaps initiated by mercaptide ligand substitution[1] with solvent or solvent impurity. Minority paramagnetic components have been observed in the pmr spectrum of *C. pasteurianum* Fd_{ox}.[20]

Summary

The pmr data for $[Fe_4S_4(SR)_4]^{2-}$ complexes lead to the following results and conclusions. (i) Chemical shifts of α-CH_2 protons occur in the same range as the downfield displaced resonances of Fd_{ox} and HP_{red} proteins, thereby confirming assignment of the latter to the CH_2 protons of cysteinyl residues bound to iron. (ii) Temperature dependencies of the shifts in (i) for the synthetic analogs and the proteins are quite similar and arise from a common electronic property which, because of the parallel temperature dependence of the CH_2 shifts and susceptibility of $[Fe_4S_4(SCH_2Ph)_4]^{2-}$, is the inherent antiferromagnetism of the Fe_4S_4* cluster. (iii) Isotropic shifts appear to be dominantly contact in origin in both the proteins and analogs as deduced from the shift-susceptibility behavior in (ii), ring proton shift patterns in the R = Ph, *p*-tolyl, and *p*-$C_6H_4NO_2$ tetramers, and (less significantly) rapid attenuation of shifts in R = alkyl tetramers. (iv) Proton shifts of a phenyl group held near a face of the Fe_4S_4* cluster ($[Fe_4S_4(m\text{-xyl-}S_2)_2]^{2-}$) are slightly perturbed compared to a spatially unconstrained group ($[Fe_4S_4(SCH_2Ph)_4]^{2-}$). It is emphasized that the results obtained in this work cannot demonstrate the absence of dipolar shifts in any case nor can they exclude the possibility of (minor) σ delocalization of spin in the phenyl-substituted tetramers. In the absence of magnetic data requisite to the calculation of dipolar shifts,[39,51] attempts have been made to estimate relative values of these shifts in the R = CH_2Ph and Ph cases using structural parameters deduced from a model of the benzyl tetramer. The estimated shifts are in definite disagreement with ratios of observed isotropic shifts. However, they cannot be accorded quantitative significance because of the simplifications made in the complicated averaging of

(51) R. J. Kurland and B. R. McGarvey, *J. Magn. Resonance*, 2, 286 (1970).

distance and angular parameters for any one proton with respect to all four iron centers. Further magnetic studies of $[Fe_4S_4(SR)_4]^{2-}$ complexes are in progress.

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Copper(II) Complex Catalysis of Amino Acid Ester Hydrolysis. A Correlation with Complex Stability

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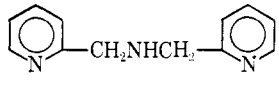
Abstract: The hydrolysis of glycine methyl ester (MeGly) is catalyzed by the Cu(II) complex, $Cu(DPA)^{2+}$, where DPA is bis(2-pyridylmethyl)amine. The kinetic and equilibrium results have been interpreted in terms of the mechanism $Cu(L)^{2+} + MeGly \rightleftharpoons Cu(L)(MeGly)^{2+}$, K_x; $Cu(L)(MeGly)^{2+} + OH^- \rightarrow Cu(L)(Gly)^+ + MeOH$, k_{OH}. Equilibrium constant determinations for the formation of $Cu(DPA)^{2+}$ (K_L) and for its binding of MeGly (K_x) indicate that $Cu(L)MeGly^{2+}$ is the predominant species in solution prior to rate-determining OH^- attack in the hydrolysis step. An examination of several $Cu(L)^{2+}$ complexes, where L is DPA, $HN(CH_2CH_2NH_2)_2$, $HN(CH_2CO_2^-)_2$, or $N(CH_2CO_2^-)_3$, indicates that strongly coordinating L ligands (*i.e.*, those with high K_L values) reduce the ability of $Cu(L)^{2+}$ to bind MeGly (low K_x values) and also reduce the rate (k_{OH}) of MeGly hydrolysis in $Cu(L)(MeGly)^{2+}$. These trends are understandable in terms of the reduced Lewis acidity of the metal ion in $Cu(L)^{2+}$ complexes bearing strong donor L ligands. These results also suggest that the activity of metalloenzymes will be influenced by the nature of the donor groups which bind the metal ion to the apoenzyme.

Certain metalloenzymes are known[2] to catalyze the hydrolysis of amino acid esters. While the metal ion is known to be at the active site in some of these enzymes, the protein groups binding and neighboring the metal ion also play a major role in determining the overall catalytic properties of the enzyme. In order to provide some basis for understanding the effect of the protein on the catalytic activity of the metal ion, we have examined the catalytic effect of a series of Cu(II) complexes, $Cu(L)^{2+}$, on the rates of methyl glycinate (MeGly) hydrolysis.[3]

In the present paper, we extend these studies to the Cu(II) complex, $Cu(DPA)^{2+}$, of bis(2-pyridylmethyl)-amine



DPA

and draw some general conclusions about the effect of the ligand L on the catalytic properties of $Cu(L)^{2+}$ complexes in the hydrolysis of amino acid esters.

Experimental Section

Reagents. Glycine (Mann Research Laboratories), MeGly·HCl (Aldrich Chemical Co.), and the trihydrochloride salt of 2,2′,2″-tris(aminoethyl)amine, tren·3HCl (Strem Chemical Co.), were of the highest purity available and were used without further purification. Bis(2-pyridylmethyl)amine, DPA, was prepared by the slow addition of 12.3 g of freshly distilled 2-chloromethylpyridine to a 50 ml CH_3OH solution of 2-aminomethylpyridine (30.0 g) according to the general procedure of Romary, *et al.*[4] Following reaction at 40–45° for 1 hr, the solution was evaporated under vacuum leaving an oil. This oil was dissolved in a minimum of H_2O; the resulting solution was made strongly alkaline with KOH. The organic product layer was separated and the aqueous layer was extracted with CCl_4. These organic phases were distilled giving DPA (bp 146–149° (0.5 mm), yield 56%). Anhydrous HCl was bubbled into an ethanol solution of DPA, whereupon DPA·3HCl precipitated. It was recrystallized by dissolving in CH_3OH and adding acetone at 0°.

Glycine solutions were standardized by pH titration. Solutions of MeGly·HCl, DPA·3HCl, and tren·3HCl were standardized by passing them through a Dowex 50W-X8 strongly acidic cation-exchange resin and titrating the acidic effluent solutions with standardized NaOH.[5] Metal ion solutions of $Cu(NO_3)_2 \cdot 3H_2O$ and $Ni(NO_3)_2 \cdot 6H_2O$ were standardized similarly.

Potentiometric Measurements. A Corning Digital 112 Research pH meter was calibrated in terms of hydrogen ion concentration, pH_c, according to the procedure of Rajan and Martell[6] using standard HCl, acetic acid, and NaOH solutions. Titrations were carried out in a doubled-walled titration cell of 50-ml capacity. The temperature of all solutions was maintained at 25.0 ± 0.1° by circulation of thermostated water through the outer jacket of the cell. The titration cell was fitted with Corning glass and calomel electrodes, a microburet delivery tube, and a nitrogen inlet tube. The solutions were stirred with a magnetic stirrer.

Protonation constants of DPA, Gly, and MeGly and the hydroxo formation constant of $Cu(DPA)^{2+}$

$$Cu(DPA)^{2+} + OH^- \overset{K_{OH}}{\rightleftharpoons} Cu(DPA)(OH)^+ \qquad (1)$$

were determined in this cell. Also the mixed ligand formation constants, K_x

$$Cu(DPA)^{2+} + X^{0,-} \overset{K_x}{\rightleftharpoons} Cu(DPA)(X)^{2,1+} \qquad (2)$$

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