Synthetic Analogs of the Active Sites of Iron–Sulfur Proteins. Structure and Properties of Bis[*o*-xylyldithiolato-μ_2-sulfidoferrate(III)], an Analog of the 2Fe–2S Proteins*

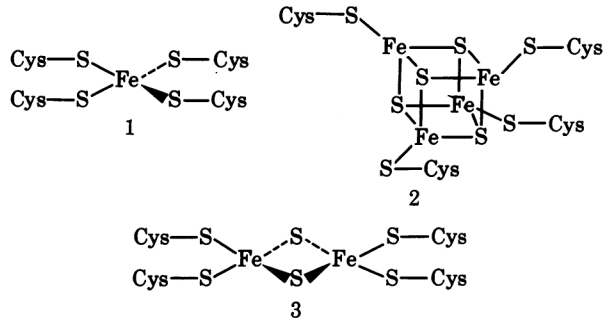
(Fe_2S_2 core/iron–sulfur complexes/x-ray diffraction)

J. J. MAYERLE†, R. B. FRANKEL‡, R. H. HOLM†, JAMES A. IBERS¶, W. D. PHILLIPS‖, AND J. F. WEIHER‖

† Department of Chemistry and ‡ Francis Bitter National Magnetic Laboratory, Massachusetts Institute of Technology, Cambridge, Mass. 02139; ¶ Department of Chemistry, Northwestern University, Evanston, Illinois 60201; and ‖ Central Research Department, E. I. du Pont de Nemours and Company, Wilmington, Delaware 19898

ABSTRACT **The synthetic analog approach has been applied to a clarification of the active sites of 2Fe–2S* proteins. The compound $(Et_4N)_2[FeS(SCH_2)_2C_6H_4]_2$, derived from *o*-xylyl-α,α'-dithiol, has been prepared and its structure has been determined by x-ray diffraction. The centrosymmetric anion contains two tetrahedrally coordinated ferric ions bridged by two sulfide ions and separated by 2.70 Å. Comparison of electronic, Mössbauer, and proton magnetic resonance spectra and magnetic susceptibility of the anion with the corresponding properties of the oxidized forms of the proteins reveals significant degrees of similarity. The anion also exhibits the essential redox capacity of the proteins. We conclude that $[FeS(SCH_2)_2C_6H_4]_2^{2-}$ possesses the same total oxidation level and electronic configuration as the active sites of the oxidized proteins, and that its structure provides a feasible representation of the minimal structure of the active site. $[FeS(SCH_2)_2C_6H_4]_2^{2-}$ is thus the first well-defined synthetic analog of the active sites of two-iron ferredoxins.**

X-ray diffraction and extensive physicochemical investigations have conclusively established that nonheme iron–sulfur proteins (1, 2), essential to many metabolic electron transport processes in bacteria, plants, and mammals (3), have at least three fundamental types of active sites. These possess the minimal compositions $Fe(S\text{-}Cys)_4$ (rubredoxins), $Fe_2S_2^*(S\text{-}Cys)_4$ (plant, mammalian, and certain bacterial proteins), and $Fe_4S_4^*(S\text{-}Cys)_4$ [4-Fe("high-potential" proteins, or HP) and 8-Fe bacterial proteins], in which S* is acid-labile or "inorganic" sulfur. X-ray studies have demonstrated the (distorted) tetrahedral structure **1** for the rubredoxin from *Clostridium pasteurianum* (4) and the "cubane" stereochemistry **2** for reduced and oxidized HP from *Chromatium* (5, 6) and the oxidized ferredoxin (Fd) from *Peptococcus aerogenes* (6, 7). The active-site structures of the 2Fe–2S* proteins, which appear to be the most widespread of the three types and are exemplified by spinach Fd, adrenodoxin, and putidaredoxin, have not been unequivocally defined by x-ray methods. However, a large body of spectroscopic and magnetic data is entirely consistent with the essential formulation **3** (1, 2, 8–10), which as **1** and **2** contains tetrahedrally coordinated iron. The oxidized forms of these proteins are characterized by the presence of two antiferromagnetically coupled high-spin Fe(III)



centers (11), Mössbauer spectra containing one slightly broadened ^{57}Fe quadrupole doublet with a nearly invariant splitting of about 0.6 mm/sec (12–15), and four or five absorption features in the 280- to 700-nm region (1, 16–18). Plant ferredoxins (17), adrenodoxin (18), and all other 2Fe–2S* proteins thus far investigated (1, 2) undergo *in vitro* the one-electron transfer reactions indicated in Eq. **1**, which is considered to account

$$\underset{\mathbf{4}}{(\text{Cys-S})_2\text{Fe(III)S}_2^*\text{Fe(III)(S-Cys)}_2} \underset{-e^-}{\overset{+e^-}{\rightleftharpoons}} \underset{\mathbf{5}}{(\text{Cys-S})_2\text{Fe(III)S}_2^*\text{Fe(II)(S-Cys)}_2} \quad [1]$$

for the *in vivo* electron-carrying capacity of these proteins (12, 13). Physicochemical data summarized elsewhere (1, 2, 8) are concordant with formulation of the reduced proteins in terms of the mixed valence entity **5**. The coordination sites in this oxidation level are apparently sufficiently differentiated by the protein environment that electron transfer between them is slow compared with the time scales of the spectroscopic methods used.

In these laboratories we are engaged in a program whose purpose is the synthesis of iron–sulfur complexes which, on the basis of detailed structural, electronic, and reactivity characterization, can be shown to serve as analogs of the three recognized types of active sites in Fe–S proteins. Recently, we have reported the synthesis of the tetranuclear species $[Fe_4S_4(SR)_4]^{2-}$ whose structure (**2**) and other properties demonstrate it to be a close representation of the active sites of 4-Fe (HP_{red}) and 8-Fe (Fd_{ox}) proteins (19, 20). Because of the lack of definitive structural information, the active sites of 2-Fe proteins remain as attractive objects for clarification by the synthetic analog approach. In this approach mercaptides, and

Abbreviations: HP, high potential iron protein; Fd, ferredoxin.

* This is part III of the series; parts I and II are refs. 19 and 20, respectively.

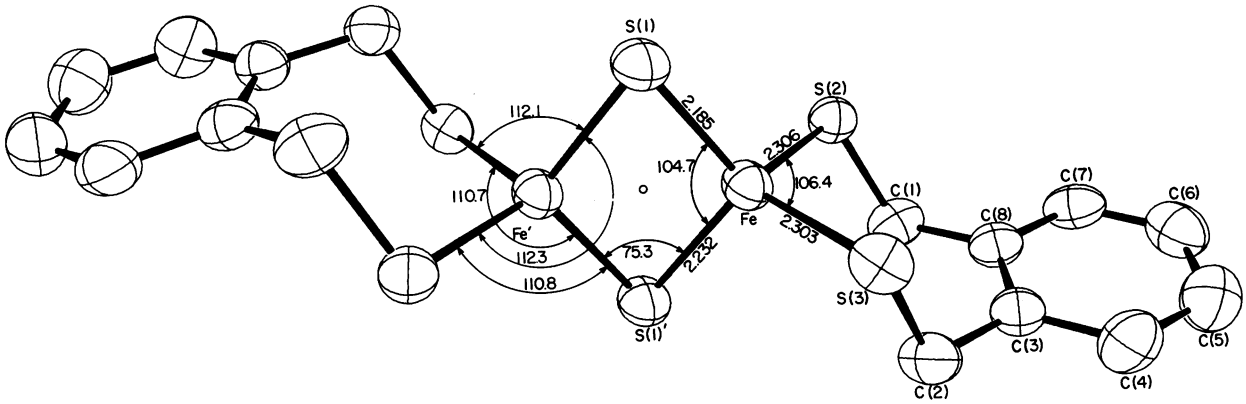


FIG. 1. The structure of the centrosymmetric $[FeS(SCH_2)_2C_6H_4]_2^{2-}$ anion; 50% probability ellipsoids of thermal vibration are shown; hydrogen atoms are omitted for the sake of clarity. Other important structural parameters: Fe...Fe', 2.698(1); S(1)...S(1)', 3.498(3), S(2)...S(3), 3.690(2); C(1)...C(2), 3.046(5) Å; dihedral angle between the planes FeS(1)S(1)' and FeS(2)S(3), 89.95(5)°.

no other type of sulfur ligand, are regarded as obligatory to simulation of the bonding function of cysteinyl residues. Using such ligands together with sulfide other workers (21, 22) have generated species in solution whose spectra are similar to those of certain 2-Fe proteins, However, these species were not isolated and their composition and structure have not been established. We report here the preparation, structure, and partial electronic characterization of a binuclear complex derived from Fe(III), sulfide, and *o*-xylyl-α,α'-dithiol, whose properties reveal it to be the first defined synthetic analog of oxidized form of 2Fe–2S* proteins.

MATERIALS AND METHODS

o-Xylyl-α,α'-dithiol was prepared from the reaction of the corresponding dibromide with thiourea in aqueous ethanol followed by alkaline hydrolysis of the isothiuronium salt, neutralization, and distillation (melting point 44°C). Bis[*o*-xylyl-α,α'-dithiolato-μ_2-sulfidoferrate(III)], $[FeS(SCH_2)_2C_6H_4]_2^{2-}$, was afforded by reaction of 1 equivalent of ferric chloride and 2 equivalents of the dithiol and sodium methoxide in methanol, followed by addition of 1 equivalent of a methanolic solution of sodium methoxide/sodium hydrosulfide. The anion was isolated as its red–black tetraethylammonium and tetraphenylarsonium salts, which were recrystallized from *N,N*-dimethylformamide methanol. The structure of the former was determined by x-ray diffraction. The more soluble tetraphenylarsonium salt was used in most of the physical measurements in solution. Anal. Calcd. for $C_{32}H_{28}AsS_3Fe$: C, 60.10; H, 4.41; As, 11.71; S, 15.04; Fe, 8.73. Found: C, 60.61, H, 4.53; As, 11.69; S, 14.70; Fe, 8.37; decomposition point 73°C (evacuated tube). The compounds are soluble in polar organic solvents and are stable in solution and in the solid state in the absence of air.

X-Ray Data and Structural Solution. $(Et_4N)_2[FeS(SCH_2)_2C_6H_4]_2$ was obtained as red–black needle-shaped crystals of the monoclinic system with space group C_{2h}^5-$P2_1/n$. Cell dimensions are $a = 9.549(6)$, $b = 13.549(6)$, $c = 14.748(6)$ Å, $\beta = 95.42°(3)$, V = 1899 Å^3 [based on $\lambda(MoK_{\alpha 1} = 0.70930$ Å, t = 21.5°)]. $d_{calc} = 1.35$ g/cm^3 for Z = 4; the experimental density was not determined because of the air-sensitivity of the compound. Linear absorption coefficient (Mo radiation) = 11.05 cm^{-1}. Minimum and maximum transmission coefficients were 0.778 and 0.873. Data were collected on a Picker FACS-1 diffractometer, using MoK_α radiation monochromatized from the (002) face of a highly mosaic graphite crystal. The crystal used had approximate dimensions of 0.15 mm × 0.23 mm × 0.64 mm. A total of 2097 unique data having $F_o^2 > 3\sigma(F_o^2)$ were obtained and were processed in the usual manner (23). The structure was solved by symbolic addition with direct methods. The initial electron-density map yielded positions of all nonhydrogen atoms of the anion, which was clearly a centrosymmetric dimer. Isotropic refinement gave R = 0.237. The cation and hydrogen atoms were found on successive difference Fourier maps. Anisotropic refinement of all nonhydrogen atoms, with hydrogen atoms added as separate contributions, converged to final values of R = 0.033 and $R_W = 0.037$. The final error in an observation of unit weight was 1.38 e and maximum density on the final difference Fourier map was 0.23(5) e/Å^3, about 10% of the height of a carbon atom in the structure. No hydrogen-atom peaks in stereochemically reasonable positions were found near the S* atoms.

Proton magnetic resonance (PMR) measurements were made on a Varian HR-220 spectrometer operating in the Fourier transform mode, and are internally referenced to tetramethylsilane. Magnetic susceptibilities were determined by the Faraday method with $HgCo(NCS)_4$ calibrant. Electrochemical measurements were obtained with a PAR model 170 Electrochemistry System and potentials were measured at 25° against a saturated calomel electrode. Mössbauer measurements were made on powder samples with a constant acceleration spectrometer operating in the normalized mode and a ^{57}Co in Cu source held at the same temperature as the absorber. The measurements at 4.2°K in an external magnetic field were made with longitudinal geometry in a Nb_3Sn superconducting magnet operating in the persistent mode up to 85 kilo Oersteds (kOe).

RESULTS AND DISCUSSION

Description of the Structure. The crystal structure of $(Et_4N)_2[FeS(SCH_2)_2C_6H_4]_2$ consists of discrete anions and cations. The latter have the expected tetrahedral geometry and will not be discussed here. The anion is a centrosymmetric dimer (overall C_i symmetry) containing S_2FeS_2* coordination sites somewhat distorted from idealized tetrahedral stereochemistry. Essential details are summarized in Fig. 1 where three other structural features of prime importance are evi-

dent: (*i*) the occurrence of planar bridged FeS_2*Fe units; (*ii*) a nonbonded S*...S* distance of 3.498 Å; (*iii*) an Fe...Fe distance of 2.698 Å, indicative of some direct net metal–metal bonding. The majority of binuclear iron–sulfur complexes contain mercaptide bridges, and FeS_2*Fe units have previously been established only in $[FeS(CO)_3]_2$ (24) and $[(C_6H_5)FeS(SEt)]_2$ (25). However, both of these complexes lack feature (*ii*) inasmuch as their bridge sulfur–sulfur distances, as well as those of other complexes and salts containing S_2 units (26) (including Na_2S_2), do not exceed 2.15 Å. The S*...S* separation in $[FeS(SCH_2)_2C_6H_4]_2^{2-}$ indicates that the bridge atoms are best regarded as possessing the sulfide oxidation level. Hence, the anion contains two iron atoms in formal oxidation state (III), a description fully consistent with the physical properties described below. The relatively short Fe...Fe distance undoubtedly contributes to the antiferromagnetic exchange coupling between the metal centers (see below) and may be compared with analogous distances in the binuclear Fe(III) complexes $[Fe(SEt)(S_2CSEt)_2]_2$ (27) (diamagnetic, 2.618 Å) and $[Fe(SCH_2CH_2S)_2]_2^{2-}$ (28; unpublished data) (3.410 Å, J about -50 cm^{-1}).

The x-ray results for $[FeS(SCH_2)_2C_6H_4]_2^{2-}$ reveal that the basic structural features embodied in the proposed active-site model **2** are present in the anion. In the absence of structural data for the proteins themselves, demonstration that $[FeS(SCH_2)_2C_6H_4]_2^{2-}$ is a meaningful active-site analog requires establishment of adequate degrees of similarity between corresponding electronic properties of the proteins and the anion. Certain of these properties are considered next.

Electronic Spectra of $[FeS(SCH_2)_2C_6H_4]_2^{2-}$ in dimethylformamide and two oxidized 2-Fe proteins (16, 17) in aqueous solution are compared in the 300- to 600-nm interval in Fig. 2. Certain spectral similarities are evident, especially by the occurrence of well-defined maxima for all three species in the 320- to 340 and 410- to 420-nm regions. The anion has a shoulder at about 455 nm, while the proteins exhibit apparently corresponding features as maxima at 460–470 nm. The anion spectrum contains a definite maximum at 590 nm. The solution spectra of most 2-Fe proteins give evidence of one or more absorption features in the 550- to 650-nm region but these are nearly obscured by tailoff from more intense higher energy absorptions. However, *Azotobacter vinelandii* I shows a band near 560 nm as does adrenodoxin at low temperature (29). All 2-Fe proteins display absorption spectra sufficiently similar to demonstrate the presence of a common basic chromophore, which we conclude is closely related to that in $[FeS(SCH_2)_2C_6H_4]_2^{2-}$. Spectra of the anion in dimethylsulfoxide, benzonitrile, pyridine, and dichloromethane show only minor variations from that in Fig. 2, indicating little perturbation of the chromophore by potentially coordinating solvents. A small solvent dependence of the adrenodoxin spectrum has been reported (30).

Mössbauer Spectra. The zero-field ^{57}Fe spectrum of $(Ph_4As)_2[FeS(SCH_2)_2C_6H_4]_2$ at ambient temperature is characterized by a single quadrupole doublet with splitting $\Delta E_Q = 0.360 \pm 0.005$ mm/sec and isomer shift $\delta = +0.17 \pm 0.01$ mm/sec (relative to iron metal). The linewidth $\Gamma = 0.26$ mm/sec and increases slightly to 0.30 mm/sec at 4.2°K; ΔE_Q and δ are almost temperature independent in this interval. Spectra obtained in the external field H_o at 4.2°K were analyzed by comparison with computer-generated spectra. For all values of the applied field, the spectra show a single iron site with the

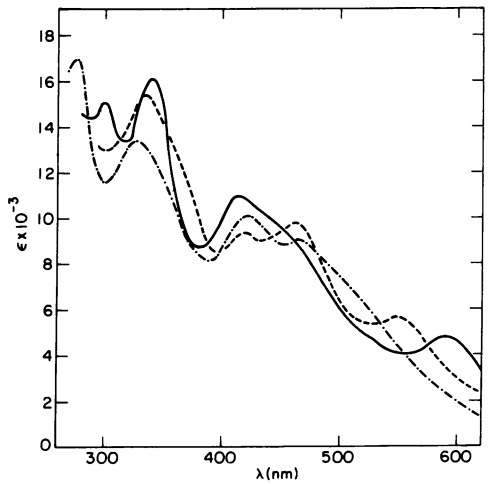


FIG. 2. Spectral comparison of $[FeS(SCH_2)_2C_6H_4]_2^{2-}$ (——) in dimethylformamide solution and two oxidized 2Fe–2S* proteins in aqueous solution. The spectra of the *A. vinelandii* protein I (– – –) and parsley ferredoxin (·— ·) were adapted from refs. 16 and 17, respectively.

magnetic field at the nucleus $H_n = H_o$ and the sign of the principal component of the electric field gradient positive. These results are interpreted as follows. The two iron atoms are equivalent and both ΔE_Q and δ are consistent with the presence of two high-spin ferric ions bonded to sulfur. The finding that $H_n = H_o$ at 4.2 °K implies the absence of magnetic hyperfine structure due to electron-nuclear interactions. As large hyperfine interactions are typically observed in paramagnetic ferric ions in large external magnetic fields, their absence here is interpreted in terms of antiparallel coupling of the spins at the two metal centers to give a singlet ground state. The coupling is strong enough that it is not perturbed by an applied field as high as 86 kOe. Recent data for the 2-Fe proteins (12–15) reveal that δ and ΔE_Q fall in the narrow ranges of +0.18 to +0.29 and 0.60 to 0.66 mm/sec, respectively, and $H_n = H_o$ in external magnetic fields up to 46 kOe. Comparison of the Mössbauer results for the anion and the oxidized ferredoxins indicates that both contain essentially the same binuclear magnetic unit, with a small inequivalence between the protein metal sites and a lower effective symmetry at each site revealed by slightly broadened quadrupole doublets and somewhat larger splittings. Parameters for $KFeS_2$ [$\delta = +0.20$ mm/sec, $\Delta E_Q = 0.50$ mm/sec (31)], which contains tetrahedral Fe(III) and which has recently been suggested as a model compound for exchange interactions in the proteins (32), are also quite similar to those of the proteins and the anion.

Magnetic Susceptibility. The temperature dependence of the magnetic susceptibility of $(Ph_4As)_2[FeS(SCH_2)_2C_6H_4]_2$ has been investigated in the range 77–296°K. The results, obtained at 17 kOe and corrected for molecular diamagnetism and a small amount of a ferromagnetic impurity, are indicative of antiferromagnetism arising from spin-coupling between the two metal centers. Both the magnetic susceptibility (χ_{Fe}) and magnetic moment (μ_{Fe}) per iron increase with increasing temperature. Some values of μ_{Fe}, calculated from the Curie law, are 0.28 BM (77°K), 0.99 BM (180°K), and 1.43 BM (296°K). These values are severely depressed from

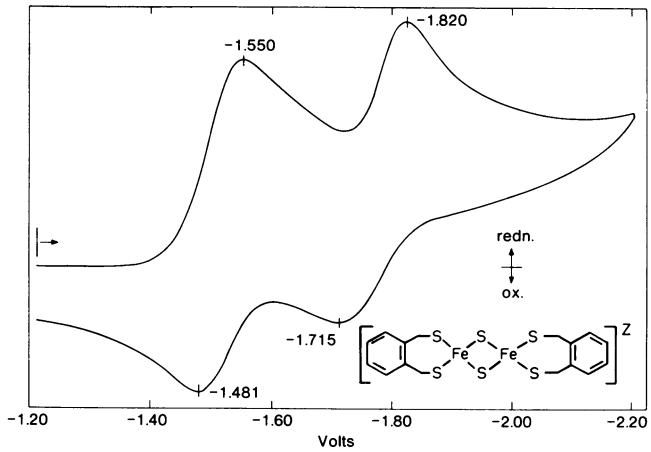


FIG. 3. Cyclic voltammogram of $(Et_4N)_2[FeS(SCH_2)_2C_6H_4]_2$ in dimethylformamide solution at 25° recorded at a scan rate of 50 mV/sec. Cathodic (E_p^c) and anodic (E_p^a) peak potentials are indicated.

μ_{Fe} about 5.9 BM expected for magnetically dilute high-spin Fe(III), and their approach to zero with decreasing temperature is consistent with a singlet ground state. Susceptibility data for the proteins reveal a definite magnetic similarity with $[FeS(SCH_2)_2C_6H_4]_2^{2-}$. Oxidized spinach and parsley Fd, adrenodoxin, and putidaredoxin are essentially diamagnetic below about 100°K (33). Spinach Fd has been investigated at higher temperatures by Palmer *et al.* (11). From their susceptibility data and that of Ehrenberg quoted by them we calculate μ_{Fe} about 1.25 BM (298°K) and about 1.51 BM (room temperature), respectively, in good agreement with the 1.43 BM value for the anion. Previous analysis of the spinach Fd data of Palmer *et al.* and Ehrenberg with the antiferromagnetic spin-coupling model with the Hamiltonian $H = -2J\mathbf{S}_1 \cdot \mathbf{S}_2$ ($S_1 = S_2 = 5/2$) had yielded $J = -183$ and -143 cm^{-1}, respectively. Our present data indicate that for the anion J occurs in the -145 to -155 cm^{-1} interval, but a detailed fit will not be attempted until other salts have been examined and the measurements have been extended to 4.2°K. Both the susceptibility and Mössbauer results presently at hand lead to the conclusion that the anion and the oxidized proteins contain the Fe(III)S_2*Fe(III) magnetic unit in common.

Proton Resonance Spectra. The PMR spectrum of $(Ph_4As)_2[FeS(SCH_2)_2C_6H_4]_2$ has been determined at 218–358°K in [*U*-^{2}H]dimethylsulfoxide/CD_3OD solution. Of principal importance are the methylene resonances, which occur as extremely broad signals (e.g., $\Delta\nu_{1/2}$ about 1830 Hz at 311°K) shifted far downfield of the free ligand position by isotropic magnetic interactions. The large linewidths would presumably obscure any shift differences that might arise from slow interconversion of chelate ring conformers or the small pairwise inequivalence of CH_2 groups found in the crystalline state (Fig. 1). Magnetic field dependencies of linewidths should be useful in sorting out these effects. Methylene chemical shifts display a positive temperature coefficient with values ranging from -32.6 to -42.1 ppm at the extremes of the temperature interval. The observed temperature dependence is qualitatively consistent with antiferromagnetic behavior (34). The PMR data for the anion afford a partial clarification of the spectra of the oxidized proteins, whose cysteinyl methylene resonances were originally attributed to broad features observed at -13 to -15 ppm at 278–303°K (35). A more recent study of spinach Fd has revealed an even broader, lower field signal at about -34 ppm (278°K), which has been assigned to the methylene groups (36). Inasmuch as the anion exhibits a resonance at -38.0 ppm (274°K), we conclude that this latter assignment of the protein signal is correct. Isotropically shifted protein signals at higher field may be due to methine protons of cysteinyl residues. It has also been proposed that these signals arise from protons of residues other than cysteinyl which are coordinated to Fe(III) (36).

Voltammetry. Because the most important biophysical property of Fe–S proteins appears to be the ability to effect electron transfer, an obligatory feature of any proposed active-site analog is the existence of redox reactions connecting total oxidation levels equivalent to those in the proteins. Polarographic and cyclic voltammetric examination of $(Et_4N)_2[FeS(SCH_2)_2C_6H_4]_2$ in dimethylformamide solution reveals *two* redox processes, which are illustrated in Fig. 3. The cathodic polarogram consists of waves with $E_{1/2} = -1.51$ and -1.81 V, slopes of 61 and 64 mV, and a diffusion current ratio of 0.96. The slopes are consistent with one-electron processes and that of the first wave is very close to the theoretical value of 59 mV for a reversible reaction. Cyclic voltammetry at 50–500 mV/sec indicates that values of E_p and $E_p^c - E_p^a$ for the first process more closely approach the diagnostic criteria for reversible charge transfer (37) than do those of the second process, which on an electrochemical basis may be only quasireversible. The initial cathodic electron transfers are interpreted as stepwise reduction of the two metal centers. Because the results described above establish that the anion and oxidized active sites possess the same total oxidation level, the reduction at -1.51 V corresponds to the protein reduction described by Eq. **1**. A second reduction step has not been detected in the proteins (17, 18). Hence, $[FeS(SCH_2)_2C_6H_4]_2^{2-}$, as the Fd analogs $[Fe_4S_4(SR)_4]^{2-}$ (19), possesses the minimal redox capacity presently established for the proteins. However, the redox potentials of both types of synthetic complexes are decidedly more cathodic than those of the proteins, with this difference being about 1 V for the 2-Fe analog. These large disparities in potentials are not understood but may arise in part from specific environmental effects of the protein structure and from unknown structural differences.

The results presented here, when compared with the extensive physicochemical information for the proteins, establish that $[FeS(SCH_2)_2C_6H_4]_2^{2-}$ is a meaningful active-site analog, thereby demonstrating that the essential structural and electronic features of the 2-Fe active sites can be closely approached outside of a protein environment. The principal conclusions from this work are the following. (*i*) The *minimal* active-site structure is represented by formulation **3** containing tetrahedral S_2FeS_2* units; proposed structures involving perthiocysteinyl groups or persulfide (S_2^{2-}) bridges are unacceptable. (*ii*) The anion and the active sites possess the same total oxidation level (**4**). (*iii*) Both the anion and the oxidized proteins contain antiferromagnetically coupled high-spin Fe(III) centers. (*iv*) Cysteinyl CH_2 resonances of the oxidized proteins occur in the region of about -30 to -40 ppm downfield. (*v*) The anion possesses a redox capacity consistent with that of the proteins, and its two well-separated one-electron processes signify redox-coupled metal centers. Potentials of the anion are considerably more negative than those of the

proteins. The qualification of the active-site structure represented by **3** or Fig. 1 as minimal is made in order to emphasize that the present results do not permit definite exclusion of other ligands coordinated to iron. Further, the anion structure obviously does not incorporate any R–S....H or S*...H hydrogen-bonding interactions or nonbonded environmental effects such as may be present in the proteins. If any additional ligating interactions do exist in the oxidized proteins, we conclude that they effect only minor perturbations on the active-site electronic properties.

This investigation further emphasizes the utility of the synthetic analog approach, which here provides a feasible representation of the 2-Fe active-site structure of the oxidized proteins in the absence of structural data for the proteins themselves. Indeed, the precise 2Fe–2S* structure reported here might serve as a useful starting point for an attack on the solution of the protein structure by Patterson search methods. Lastly, if conclusion (*i*) is accepted, it is seen that the 2-Fe structure **3** may be regarded as a fragment of the 4-Fe structure **2** and is formally derivable from it by incorporation of a cysteinyl sulfur at each iron and rupture of four Fe–S* bonds. Inasmuch as the 2-Fe proteins appear to be descendants of the 4- and 8-Fe proteins of anaerobic and photosynthetic bacteria (38), this relationship between the two types of active sites may be relevant to the evolutionary development of Fe–S proteins.

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