

# Iron-Sulfur Coordination Compounds and Proteins

Stephen J. Lippard

Department of Chemistry, Columbia University, New York, New York 10027

Received August 28, 1972

Although powerful oxidizing and reducing agents exist among transition metal-aquo complexes (Fe(III)|Fe(II), 0.77 V; Cr(III)|Cr(II), -0.41 V), these simple ions are unavailable as biological redox catalysts because of extensive hydrolysis in the physiological pH range. Transition metal ions are found in the active sites of many redox metalloproteins, however.<sup>1</sup> Here the metal is generally coordinated to donor atoms supplied by the rich variety of amino acid functional groups. Occasionally a special ligand such as the heme group serves to bind the metal to the protein.

The isolation of highly purified bacterial and plant ferredoxins nearly a decade ago<sup>2</sup> led to intensive research activity on these and related iron-sulfur proteins (Table I).<sup>3</sup> X-Ray structural studies of rubredoxin,<sup>4</sup> a bacterial ferredoxin,<sup>5</sup> and the high-potential iron protein (HiPIP) from *Chromatium*<sup>6</sup> (Figure 1) established that iron is coordinated to the sulfur atoms of cysteine and, except for rubredoxin, to a biologically unique form of sulfur that can be released as hydrogen sulfide upon mild acidification. The geometry of the iron-sulfur core in the plant ferredoxins, adrenodoxin, and related Fe<sub>2</sub>S<sub>2</sub> proteins has not yet been directly established, but a host of indirect studies<sup>7</sup> have converged on the structure shown in Figure 2a as the most likely candidate. An alternative structure has been proposed<sup>8</sup> for adrenodoxin (Figure 2b) and will be discussed below.

Biological interest in the iron-sulfur proteins has centered around their widespread occurrence in redox systems, fulfilling such diverse functions as nitrogen fixation, steroid hydroxylation, and photosynthesis. The proteins appear to function as high- or low-potential "wires" in electron-transport chains. Perhaps the most interesting are the ferredoxins, which have some of the lowest reduction potentials (Table I) in biology.<sup>2</sup> The low value for the ferredoxin reduction potential compared to the Fe(III)|Fe(II) couple is not simply the result of sulfur *vs.* oxygen coordination, since rubredoxin has a potential of -57 mV. Even more striking is the difference of 730 mV between the values for the HiPIP and bacterial ferredoxins (Table I) despite the fact that the Fe<sub>4</sub>S<sub>4</sub> cores in both proteins are geometrically quite similar.<sup>9</sup>

Apart from the biological interest in these proteins, they have attracted the attention of the coordination

chemist. One reason is a purely synthetic one, for the preparation and characterization of iron-sulfur coordination compounds using only biologically significant ligands<sup>10</sup> have, until recently, remained elusive. The chief difficulties are the oxidation of sulfide or mercaptide ligands by iron(III) salts, the tendency for iron(II) complexes to form as insoluble, intractable polymers, and the oxygen sensitivity of the iron-sulfur compounds obtained.<sup>11</sup> The fact that iron-sulfur chromophores (also oxygen sensitive<sup>12</sup>) of this kind are found in the proteins<sup>13</sup> has thus stimulated considerable synthetic activity by coordination chemists.

Coordination chemists have also been interested in the low reduction potentials exhibited by certain classes of iron-sulfur proteins and the dependence of the reduction potential on the degree of polymerization and the local protein environment (Table I). Thus a second objective has been to examine the ef-

(1) For a comprehensive review see L. E. Bennett, *Progr. Inorg. Chem.*, **18**, 1 (1973).

(2) (a) L. E. Mortenson, R. C. Valentine, and J. E. Carnahan, *Biochem. Biophys. Res. Commun.*, **7**, 448 (1962); (b) K. Tagawa and D. I. Arnon, *Nature (London)*, **195**, 537 (1962).

(3) For reviews, see (a) R. Malkin and J. C. Rabinowitz, *Annu. Rev. Biochem.*, **36**, 113 (1967); (b) T. Kimura, *Struct. Bonding (Berlin)*, **5**, 1 (1968); (c) D. O. Hall and M. C. W. Evans, *Nature (London)*, **223**, 1342 (1969); (d) J. C. M. Tsibris and R. W. Woody, *Coord. Chem. Rev.*, **5**, 417 (1970).

(4) K. D. Watenpaugh, L. C. Sieker, J. R. Herriott, and L. H. Jensen: (a) Abstracts, American Crystallographic Association Meeting, Iowa State University, Ames, Iowa, 1971, p 52; (b) *Cold Spring Harbor Symp. Quant. Biol.*, **36**, 359 (1971).

(5) L. C. Sieker, E. Adman, and L. H. Jensen, *Nature (London)*, **235**, 40 (1972).

(6) (a) G. Strahs and J. Kraut, *J. Mol. Biol.*, **35**, 503 (1968); (b) C. W. Carter, Jr., S. T. Freer, Ng. H. Xuong, R. A. Alden, and J. Kraut, *Cold Spring Harbor Symp. Quant. Biol.*, **36**, 381 (1971).

(7) (a) W. R. Dunham, G. Palmer, R. H. Sands, and A. J. Bearden, *Biochim. Biophys. Acta*, **253**, 373 (1971), and references cited therein; (b) M. Poe, W. D. Phillips, J. D. Glickson, C. C. McDonald, and A. San Pietro, *Proc. Nat. Acad. Sci. U. S.*, **68**, 68 (1971); (c) C. E. Johnson, R. Commack, K. K. Rao, and D. O. Hall, *Biochem. Biophys. Res. Commun.*, **43**, 564 (1971).

(8) T. Kimura, Y. Nagata, and J. Tsurugi, *J. Biol. Chem.*, **246**, 5140 (1971).

(9) C. W. Carter, Jr., J. Kraut, S. T. Freer, R. A. Alden, L. C. Sieker, E. Adman, and L. H. Jensen, *Proc. Nat. Acad. Sci. U. S.*, **69**, 3526 (1972).

(10) The term "biologically significant ligands" will be used in the present context to mean, broadly, ligands with donor functions similar to those found in the naturally occurring amino acids and, in a more restricted sense, ligands containing the -CH<sub>2</sub>S- group. The sulfide ion, S<sup>2-</sup> (*cf.* Table I and Figures 1 and 2a) and the Cys-S-S- moiety (Figure 2b) are also included.

(11) (a) D. Coucouvanis, S. J. Lippard, B. G. Segal, and J. A. Zubieta, *Proc. Int. Conf. Coord. Chem.*, **12th**, 190 (1969); (b) D. Coucouvanis and S. J. Lippard, unpublished results.

(12) D. Petering, J. A. Fee, and G. Palmer, *J. Biol. Chem.*, **246**, 643 (1971).

(13) The existence of the iron-sulfur chromophores in the proteins has on occasion led to an advancement in coordination chemistry. For example, the first evaluation of the crystal field splitting parameter *Dq* for mercaptide sulfur came through the analysis of the optical spectrum of rubredoxin (W. A. Eaton and W. Lovenberg, *J. Amer. Chem. Soc.*, **92**, 7195 (1970)), there being no simple metal-sulfur complex from which this information could be extracted.

Professor Lippard's research activities include synthetic and physical studies of redox metalloproteins and related coordination compounds. Impetus for his recent slant to more biological areas was provided by a recent sabbatical leave spent as a Guggenheim Fellow in the laboratory of Professor Bo Malmström in Göteborg, Sweden. Professor Lippard's training was in inorganic chemistry at MIT, where he received his Ph.D. in 1965 with F. A. Cotton. He is now Professor of Chemistry at Columbia.

Table I  
Physical and Chemical Properties of Iron-Sulfur Proteins<sup>a</sup>

Protein	Source	$E_0'$ , mV	Moles/mole of protein			$n$	Mol wt
			Iron	Sulfide	Cysteine		
Ferredoxin <sup>b</sup>	Bacteria	-500 (-400)	4(8)	4(8)	4(8)	1(2)	6,000
Ferredoxin	Plants	-430	2	2	5	1	12,000
Adrenodoxin	Animals	-270 <sup>c</sup>	2	2	4	1	12,000
Putidaredoxin	Bacteria	-235	2	2	4	1	12,000
Rubredoxin	Bacteria	-57	1	0	4	1	6,000
High-potential iron proteins	Bacteria	+330	4	4	4	1	9,500

<sup>a</sup> Data are obtained from ref 2. Abbreviations:  $n$ , number of electrons involved in redox process. <sup>b</sup> Numbers in parentheses refer to the 8Fe-8S *Clostridium* and other proteins in which there are two Fe<sub>4</sub>S<sub>4</sub> units (see ref 9). <sup>c</sup> J. J. Huang and T. Kimura, *Biochemistry*, 12, 406 (1973).

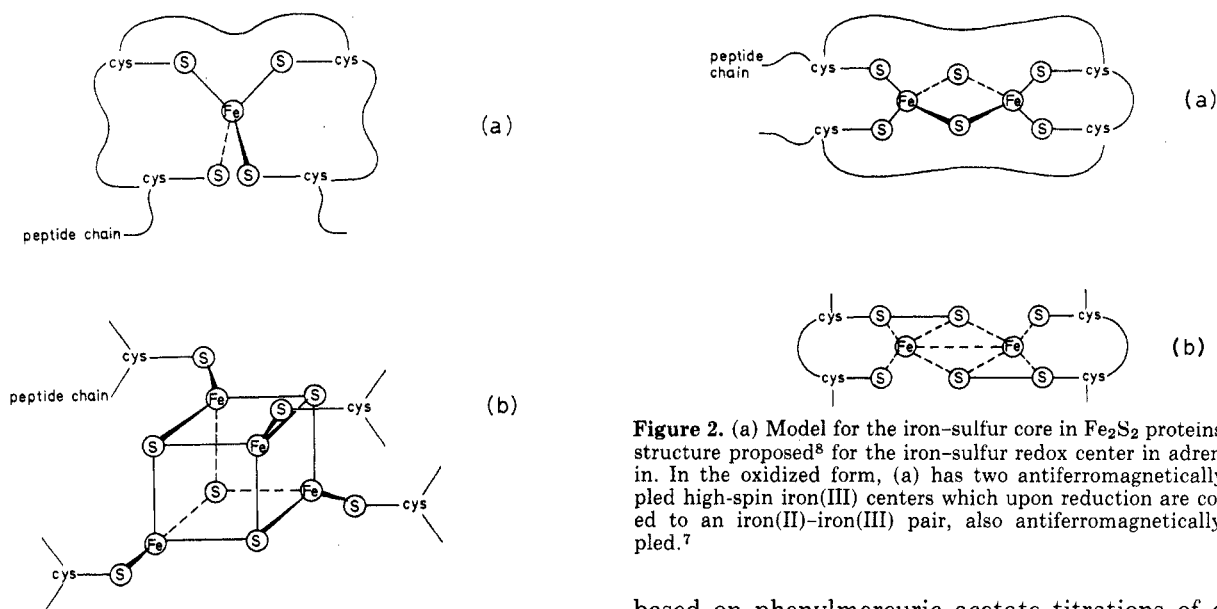


Figure 1. Structures of the iron-sulfur chromophores in (a) *C. pasteurianum* rubredoxin and (b) high-potential iron protein (HiPIP) from *Chromatium* and *C. pasteurianum* ferredoxin. The drawings are idealizations (see ref 4-6 and 9 for details).

fect of factors such as extent of polymerization, choice of ligand (e.g.,  $\text{RCH}_2\text{S}^-$  vs.  $\text{S}^{2-}$ ), solvent polarity, overall charge, and steric strain<sup>14</sup> on the redox properties of iron-sulfur complexes. Here again imaginative synthetic chemistry is required to reproduce the subtle environmental factors of a protein sheath using relatively simple ligand molecules.

In this Account, several experimental studies of iron-sulfur coordination compounds, mostly performed in this laboratory, are examined. These investigations have generated specific suggestions concerning the structural and redox properties of the proteins. We caution at the outset that only direct studies of the protein systems themselves can prove or disprove the validity of the ideas put forth. While such work is in progress both in our laboratory and elsewhere, it lies outside the scope of this Account.

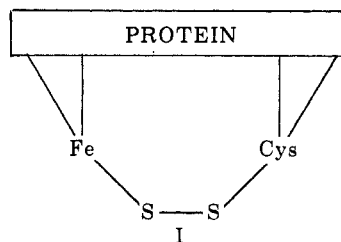
### The Fe-S-S-C Linkage

Before X-ray diffraction results were available for any of the iron-sulfur proteins, it was proposed,

(14) The "unusual" stereochemical properties of metal ion cores in redox proteins have long been recognized: B. G. Malmström, *Pure Appl. Chem.*, 24, 393 (1970), and references cited therein.

Figure 2. (a) Model for the iron-sulfur core in Fe<sub>2</sub>S<sub>2</sub> proteins;<sup>7</sup> (b) structure proposed<sup>8</sup> for the iron-sulfur redox center in adrenodoxin. In the oxidized form, (a) has two antiferromagnetically coupled high-spin iron(III) centers which upon reduction are converted to an iron(II)-iron(III) pair, also antiferromagnetically coupled.<sup>7</sup>

based on phenylmercuric acetate titrations of dihydroorotate dehydrogenase,<sup>15</sup> that the labile sulfur (released as H<sub>2</sub>S upon acidification) might be in combination with cysteinyl sulfur in the form of a persulfide unit, I.



Thus an early objective was to prepare the Fe-S-S-R moiety in a nonbiological environment. Studies by Fackler and coworkers<sup>16</sup> had suggested a possible synthetic route, and the chemistry summarized by eq 1-3 was developed.<sup>17</sup> The compound Fe(TTD)(DTT)<sub>2</sub> was shown in an X-ray diffraction study to contain the Fe-S-S-C unit (Figure 3).<sup>17,18</sup> This structural and synthetic work supplied little bi-

(15) R. W. Miller and V. Massey, *J. Biol. Chem.*, 240, 1453 (1965).

(16) J. P. Fackler, D. Coucouvanis, J. A. Fetchin, and W. C. Seidel, *J. Amer. Chem. Soc.*, 90, 2784 (1968), and references cited therein.

(17) D. Coucouvanis and S. J. Lippard, *J. Amer. Chem. Soc.*, 90, 3281 (1968). Abbreviations: TTD = thio-*p*-toluoyl disulfide,  $\text{CH}_3\text{C}_6\text{H}_4\text{CS}_2^-$ ; DTT = dithio-*p*-toluate,  $\text{CH}_3\text{C}_6\text{H}_4\text{CS}_2^-$ .

(18) D. Coucouvanis and S. J. Lippard, *J. Amer. Chem. Soc.*, 91, 307 (1969).

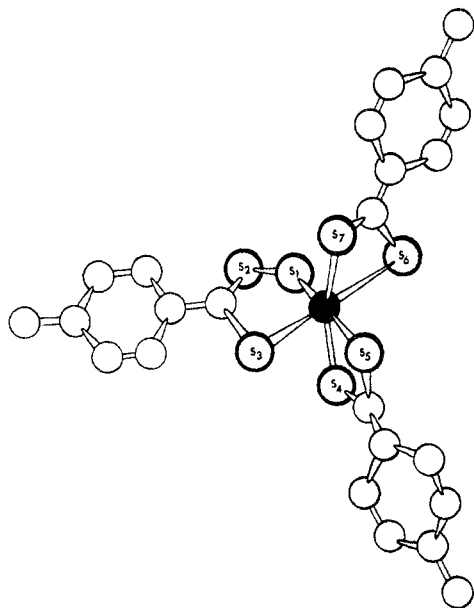
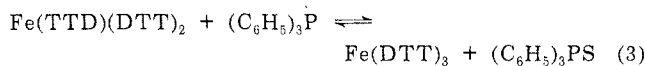
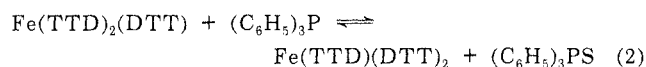
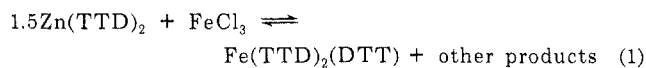


Figure 3. Molecular structure of  $\text{Fe}(\text{TTD})(\text{DTT})_2$ . The iron atom is at the center and carbon atoms are not labeled (reproduced from ref 18).



ological insight, however; it served merely to provide a small molecule prototype in the event that such a unit might be found in proteins.

Studies by Kimura, Nagata, and Tsurugi<sup>8</sup> revived interest in this chemistry and in the possible existence of an Fe-S-S-C linkage for at least some classes of iron-sulfur proteins. Extending reactions 2 and 3 to the protein adrenodoxin, these workers were able to titrate quantitatively the labile sulfur with triphenylphosphine to form triphenylphosphine sulfide. Neither  $\text{S}^{2-}$  nor  $\text{RS}^-$  will undergo such a redox reaction. Kimura, *et al.*, proposed the structure shown in Figure 2b for the iron-sulfur core in adrenodoxin.

This structure is not compatible with sulfhydryl titration data<sup>19</sup> on adrenodoxin, however (see ref 7a for discussion of related proteins). Moreover, since the work was performed in 33% ethanol, the protein active site may have been denatured, with oxidation of  $\text{S}^{2-}$  and  $\text{Cys-S}^-$  to form  $\text{Cys-S-S-S-Cys}$ . This species could then react with triphenylphosphine. The formation of trisulfides has been proposed to account for the oxygen sensitivity of spinach ferredoxin and putidaredoxin in the presence of denaturants.<sup>12</sup> On the other hand, organic solvents appear to have only minimal effect on the stability of oxidized adrenodoxin;<sup>20</sup> the abstraction of sulfur by triphenylphosphine also occurs under anaerobic conditions.<sup>8</sup> High-resolution X-ray diffraction studies of the protein would resolve this point. In the persulfide struc-

(19) T. Kimura, *Struct. Bonding (Berlin)*, **5**, 1 (1968).

(20) T. Kimura, *Biochem. Biophys. Res. Commun.*, **43**, 1145 (1971).

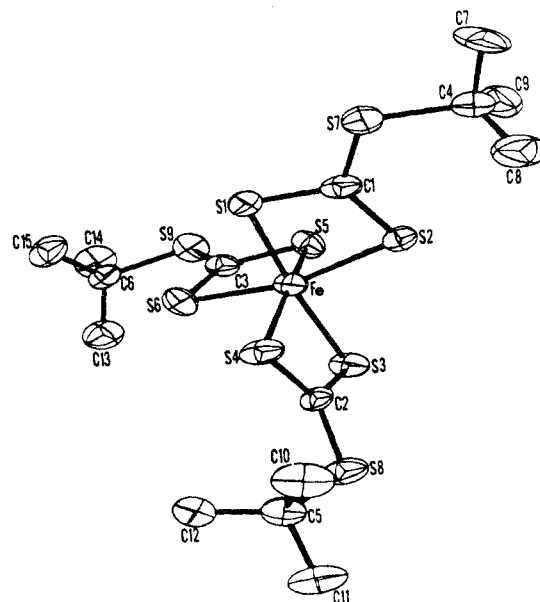


Figure 4. Molecular structure of  $\text{Fe}(\text{S}_2\text{CSR})_3$ ,  $\text{R} = t\text{-butyl}$ , showing the 50% probability thermal ellipsoids (reproduced from ref 23).

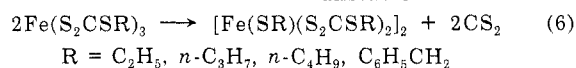
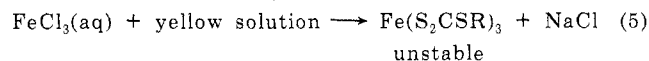
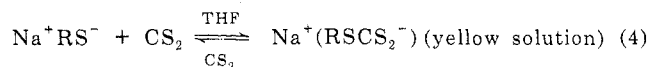
ture (Figure 2b), the closest S-S distance would be  $\sim 2.0\text{--}2.1 \text{ \AA}$ , compared to  $\sim 2.8 \text{ \AA}$  or more for the structure shown in Figure 2a.

Should a persulfide structure, or some variation thereof,<sup>21</sup> exist for adrenodoxin or any of the other iron-sulfur proteins, the electron-transport process would have to be reevaluated in terms of a non-metal-based redox reaction, *e.g.*, a sulfide  $\rightleftharpoons$  persulfide equilibrium. A persulfide structure may well occur in dihydroorotate dehydrogenase, being compatible with the sulfhydryl titer of the protein.<sup>15</sup> With the possible exception of adrenodoxin, however, there is no compelling reason to doubt the structure proposed<sup>7</sup> in Figure 2a for the  $\text{Fe}_2\text{S}_2$  proteins listed in Table I. This structure will be assumed in the ensuing discussion.

### Mercaptide-Bridged Thioxanthate Dimers of Iron(III)

As indicated in Table I, the reduction potentials of the  $\text{Fe}_2\text{S}_2$  proteins are  $\sim 0.2\text{--}0.4 \text{ V}$  lower than for rubredoxin. It was therefore of interest to obtain mono- and binuclear iron complexes with similar sulfur donor atom sets and to compare their redox properties.

Carbon disulfide elimination from the tris(*n*-alkyl thioxanthato)iron(III) complexes produced binuclear iron(III) complexes, eq 4-6.<sup>22</sup> In the case where  $\text{R} =$



(21) (a) E. Bayer, H. Eckstein, H. Hagenmaier, D. Josef, J. Koch, P. Krauss, A. Röder, and P. Schretzmann, *Eur. J. Biochem.*, **8**, 33 (1969); (b) G. T. Kubas, T. G. Spiro, and A. Terzis, *J. Amer. Chem. Soc.*, **95**, 273 (1973).

(22) D. Coucouvanis, S. J. Lippard, and J. A. Zubieta, *J. Amer. Chem. Soc.*, **91**, 761 (1969); **92**, 3342 (1970).

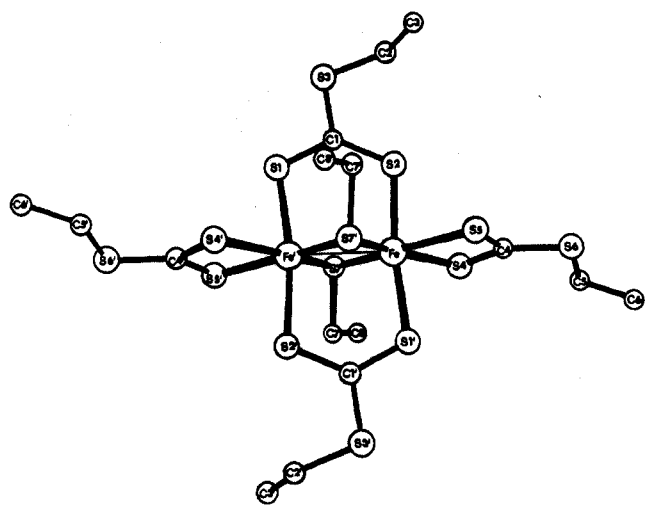


Figure 5. Molecular structure of  $[\text{Fe}(\text{SR})(\text{S}_2\text{CSR})_2]_2$ , R = ethyl (reproduced from ref 24).

$t\text{-C}_4\text{H}_9$ , monomeric  $\text{Fe}(\text{S}_2\text{CSR})_3$  was isolated under the conditions employed. The solid-state structures (the solution geometries are similar<sup>22</sup>) of both monomeric<sup>23</sup> and dimeric (R = ethyl)<sup>24</sup> products are shown in Figures 4 and 5, respectively. In both cases the coordination geometry of the iron(III) atom is a distorted octahedron. The dimer has an iron-iron bond as determined from geometric and magnetic criteria<sup>24</sup> and electronic structural considerations.<sup>22</sup> A qualitative bonding scheme is shown in Figure 6a.<sup>22,25</sup>

Although the  $\text{Fe}(\text{S}_2\text{CSR})_3$  and  $[\text{Fe}(\text{SR})(\text{S}_2\text{CSR})_2]_2$  complexes differ from rubredoxin and the  $\text{Fe}_2\text{S}_2$  proteins in several respects (the iron is six-coordinate and low-spin, the dimers do not contain labile sulfur, the thioxanthate ligands contain unsaturated  $\text{C}=\text{S}$  donor atoms), it was of interest to compare their electrochemical properties since they afforded an opportunity to study the redox potentials of mono- and binuclear iron(III) complexes with nearly identical sulfur donor ligands. Voltammetric studies in dichloromethane solution (Figure 7) established that the half-wave reduction potentials of the dimers (irreversible, two-electron reduction) were  $\sim 0.3$  V more negative than that of the monomer (reversible, one-electron process).<sup>22</sup> This result parallels the relative potentials of the mononuclear and binuclear iron-sulfur proteins (Table I), although the correlation may well be coincidental. As shown in Figure 7, electrons added to  $[\text{Fe}(\text{SR})(\text{S}_2\text{CSR})_2]_2$ , R =  $n$ -propyl, on the cathodic sweep are removed at a more positive potential, close to that observed for the reduction of the *tert*-butyl monomer. This behavior has been ascribed to a disruption of the metal-metal bonding interaction, possibly accompanied by a structural rearrangement.<sup>22,25</sup>

Since the hypothetical (short-lived?)  $[\text{Fe}(\text{SR})(\text{S}_2\text{CSR})_2]_2^{2-}$  dianions generated electrochemically were not isolated, synthetic and structural studies of the isoelectronic cobalt(III) compounds

(23) D. F. Lewis, S. J. Lippard, and J. A. Zubieta, *Inorg. Chem.*, **11**, 823 (1972).

(24) D. Coucouvanis, S. J. Lippard, and J. A. Zubieta, *Inorg. Chem.*, **9**, 2775 (1970).

(25) D. F. Lewis, S. J. Lippard, and J. A. Zubieta, *J. Amer. Chem. Soc.*, **94**, 1563 (1972).

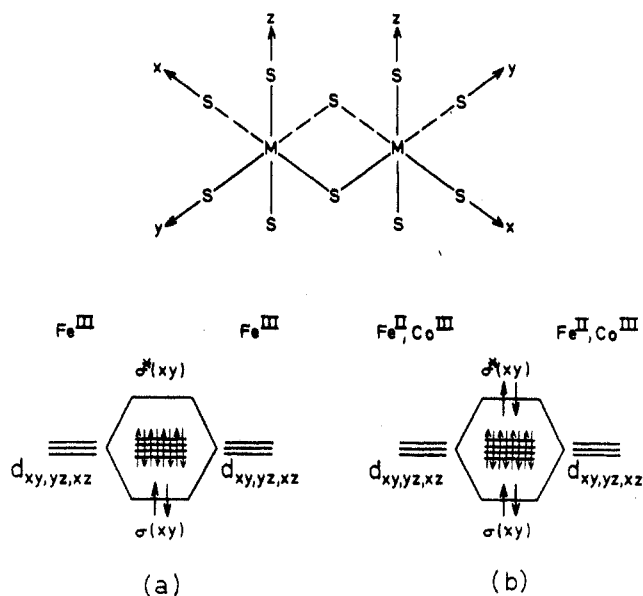


Figure 6. Qualitative bonding scheme for (a)  $[\text{Fe}(\text{SR})(\text{S}_2\text{CSR})_2]_2$  and (b)  $[\text{Co}(\text{SR})(\text{S}_2\text{CSR})_2]_2$  complexes showing the orbitals involved in the metal-metal interaction. For discussion see ref 22 and 25.

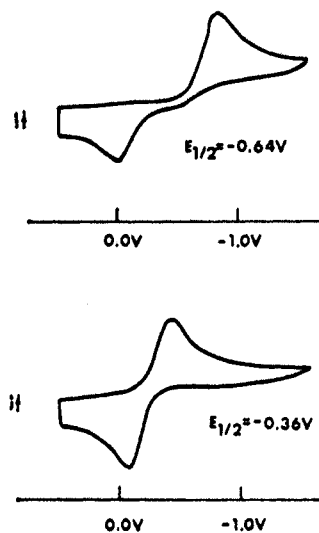
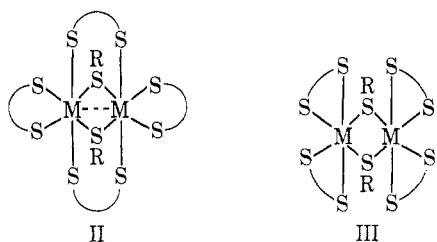


Figure 7. Reduction of  $[(n\text{-C}_3\text{H}_7\text{SCS}_2)_2(n\text{-C}_3\text{H}_7\text{S})\text{Fe}]_2$  (top) and  $[(t\text{-C}_4\text{H}_9\text{SCS}_2)_3\text{Fe}]$  (bottom) by cyclic voltammetry (reproduced from ref 22).

(Figure 6b) were carried out.<sup>25</sup> As with the iron system, both mononuclear and binuclear complexes were obtained, and the carbon disulfide elimination reaction (analog of eq 6) was directly verified. From X-ray diffraction and proton nmr data, the structure of  $[\text{Co}(\text{SC}_2\text{H}_5)(\text{S}_2\text{CSC}_2\text{H}_5)_2]_2$  was determined.<sup>25</sup> Whereas the iron analog adopts structure II, the cobalt derivative was found to have structure III.

From the X-ray results it was clear that the cobalt dimer has no metal-metal bond, as anticipated from electronic structural considerations (Figure 6).<sup>26</sup> Because of the long  $\text{Co}\cdots\text{Co}$  distance of 3.32 Å (compared to 2.62 Å for the iron dimer), the thioxanthate ligands are no longer able to bridge across the top and bottom of the  $\text{M}_2\text{S}_2$  rhombus (structure II).<sup>25</sup>

(26) See L. F. Dahl, E. R. de Gil, and R. D. Feltham, *J. Amer. Chem. Soc.*, **91**, 1653 (1969), for a general treatment of the stereochemical consequences of metal-metal bonding in ligand-bridged binuclear complexes.



The occurrence of structure III for  $[\text{Co}(\text{SC}_2\text{H}_5)(\text{S}_2\text{CSC}_2\text{H}_5)_2]_2$  provides some support, albeit indirect, for the idea that a structural rearrangement such as II  $\rightarrow$  III occurs in the electrochemical reduction of the iron(III) analog. The slowness of this rearrangement compared to the electron-transfer step could then account for the observed irreversibility of the electrode process. This analysis presumes the  $\sigma^*(xy)$  orbital (Figure 6) to be populated in the reduction of the  $[\text{Fe}(\text{SR})(\text{S}_2\text{CSR})_2]_2$  compounds. The high energy of this orbital and the presence of the bridging thioxanthate ligands render this an energetically unfavorable process, thus accounting for the low reduction potential of the dimers compared to monomeric  $\text{Fe}(\text{S}_2\text{CSR})_3$ .

#### A Rationale for the Low and Varying Reduction Potentials of the $\text{Fe}_2\text{S}_2$ Proteins

The results just discussed, together with studies carried out chiefly by Dahl and coworkers,<sup>26,27</sup> demonstrate that the metal-metal bond order in a bridged  $\text{M}_2\text{X}_2$  dimer can have a profound influence on its geometry. The redox behavior of the system also appears to be sensitive to geometric rearrangements or reactions<sup>28</sup> accompanying the transfer of electrons into or out of the orbitals involved in metal-metal bonding.

These observations suggest<sup>25</sup> the following rationale for the low and varying reduction potentials of the  $\text{Fe}_2\text{S}_2$  proteins. If the geometries of the  $\text{Fe}_2\text{S}_2(\text{S-Cys})_4$  centers in these proteins are constrained by the surrounding polypeptide backbone to be the same in both oxidized and reduced forms, addition of an electron cannot be accompanied by a geometric adjustment. Assuming that the electronic structure of the  $\text{Fe}_2\text{S}_2(\text{S-Cys})_4$  center would require such an adjustment upon reduction (*vide infra*), but that the constraints of the polypeptide chain do not allow it, the protein in its reduced form would be a good electron donor. Changes in the amino acid composition among various classes of  $\text{Fe}_2\text{S}_2$  proteins could monitor the constraint on the iron-sulfur redox center, producing the observed variations in the reduction potentials (Table I). Besides the reduction potential, other properties of the reduced protein would be affected, for example, the unusual epr spectra observed for the reduced plant ferredoxins.<sup>2</sup> The "unique" properties of metalloproteins have previously been attributed to the presence of highly specific ligand geometries available in the macromolecule but not in simple ligand systems.<sup>29</sup> In a sense, the present hypothesis is an extension of these ideas.

(27) N. G. Connelly and L. F. Dahl, *J. Amer. Chem. Soc.*, **92**, 7472 (1970), and references cited therein.

(28) See, for example, J. A. Ferguson and T. J. Meyer, *Inorg. Chem.*, **11**, 631 (1972).

Before discussing possible experimental tests of the above rationale, let us consider in somewhat more detail exactly how the model might be applied to the  $\text{Fe}_2\text{S}_2$  proteins. To recapitulate, the major features are that (1) the electron enters a relatively high energy state and (2) the protein does not structurally rearrange to accommodate the extra electron. In the case of  $[\text{Fe}(\text{SR})(\text{S}_2\text{CSR})_2]_2$ , the high-energy state was the  $\sigma^*(xy)$  orbital (Figure 6). For the  $\text{Fe}_2\text{S}_2$  proteins, however, several lines of evidence indicate the presence of antiferromagnetically coupled, high-spin, tetrahedral iron(III) centers which upon reduction are converted to one iron(II) and one iron(III) center, also antiferromagnetically coupled.<sup>7</sup> The estimated  $J$  values<sup>7a</sup> show the interaction to be considerably weaker than expected for an iron-iron single bond. Bearden and Dunham assign<sup>30</sup> the electron in the reduced protein to the  $d_{z^2}$  orbital of the iron(II) ion. Since this orbital is directed toward the half-filled  $d_{z^2}$  orbital of the neighboring iron(III) center (the  $z$  axis is taken along the  $\text{Fe}\cdots\text{Fe}$  vector), a nonbonded electron repulsive term would render this a high-energy state compared to the oxidized protein.<sup>30</sup> Failure of the  $\text{Fe}_2\text{S}_2$  chromophore to relax this state energetically by making a geometric adjustment would result in a low reduction potential, as suggested above. The effect is like that of a metal-metal bonded system, only weaker. It is noteworthy that the reduction of the distorted tetrahedral iron(III) center to iron(II) in rubredoxin also involves the  $d_{z^2}$  orbital ( $e^2t_2^3 \rightarrow e^3t_2^3$ ). Here there is no neighboring iron atom, however, and the reduction potential is  $\sim 0.35$  V more positive. Since the electron does not enter a  $\sigma$ -antibonding ( $t_2$ ) orbital there is no reason to expect a gross geometric change, and none is experimentally observed.<sup>4</sup>

It is prudent to underscore the speculative nature of the foregoing analysis. There are, to be sure, alternative explanations for the various reduction potentials (see, for example, ref 1). Yet the rationale does present a working hypothesis which, like any other, must stand or fall based on experimental studies. The most revealing of these would be to obtain high-resolution X-ray data for both oxidized and reduced forms of an  $\text{Fe}_2\text{S}_2$  protein. Equally important would be to examine the effect on the reduction potential of chemical modifications of the redox centers of the proteins, and studies of this kind are in progress. Finally, investigating the effect of steric constraints on the electronic properties of simple iron-sulfur coordination compounds would provide further information on which to assess the rationale for the redox behavior of the proteins. Some preliminary work of this nature is outlined below.

#### Sterically Constrained Complexes with Biologically Significant Ligands

The dithio acid, persulfide, and thioxanthate complexes of iron(III) described above do not qualify as biologically significant in the sense defined here,<sup>10</sup>

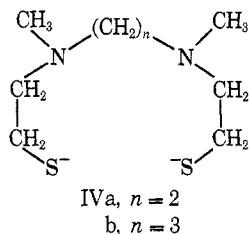
(29) (a) B. G. Malmström and T. Vänngård, *J. Mol. Biol.*, **2**, 118 (1960); (b) B. L. Vallee and R. J. P. Williams, *Proc. Nat. Acad. Sci. U. S.*, **59**, 498 (1968).

(30) A. J. Bearden and W. R. Dunham, *Struct. Bonding (Berlin)*, **8**, 1 (1970).

although they have influenced our thinking about the iron-sulfur proteins. A similar remark applies to iron-sulfur coordination compounds of a wide variety prepared and characterized in other laboratories. Two notable examples are  $(h^5-C_5H_5)_4Fe_4S_4$ <sup>31</sup> and  $Fe[S_2(PR_2)_2N]_2$ ,<sup>32</sup> the structures of which closely approximate the iron-sulfur cores in the bacterial ferredoxins (and HiPIP) and rubredoxin, respectively.

Lately, several iron-sulfur complexes with biologically significant ligands have been reported to be "identified" in solution by optical spectroscopy.<sup>33</sup> These compounds have varying degrees of stability and, in some cases, their electronic spectra bear a striking resemblance to those of certain iron-sulfur proteins. A major shortcoming of those studies, however, is their failure to produce crystalline products suitable for chemical and structural analysis. Since little is known about the geometries of the complexes formed in solution, new information concerning the structural possibilities for the iron-sulfur proteins has not been provided. This is not to minimize the value of the work in demonstrating that the optical properties of the proteins are congruent with data obtained on iron-sulfur chromophores constituted from biologically significant ligands.

Recently we have synthesized and characterized crystalline iron-sulfur complexes using ligands of type IV.<sup>34</sup> These ligands cannot afford an exact



match to the coordination environment of the iron-sulfur proteins since labile sulfur is not available and nitrogen donor atoms almost certainly will coordinate, an unlikely possibility, although not yet entirely eliminated, for the  $Fe_2S_2$  proteins. Nevertheless, the ligands were designed for several specific reasons. First, they do provide the desired  $-CH_2S^-$  function. Second, extensive polymerization was expected to be suppressed by the multidentate character of the ligand. Third, chemical control of steric strain is possible through variation of  $n$ .

In the case where  $n = 1$  or  $2$ , a study of models showed that a severely strained complex would result from coordinating IV to a single metal ion with a pseudotetrahedral geometry. For  $n = 3$ , the strain is relieved. Finally, since primary mercaptide sulfur

(31) (a) R. A. Schunn, C. J. Fritchie, and C. T. Prewitt, *Inorg. Chem.*, **5**, 892 (1966); (b) C. H. Wei, G. R. Wilkes, P. M. Treichel, and L. F. Dahl, *ibid.*, **5**, 900 (1966).

(32) (a) A. Davison and E. S. Switkes, *Inorg. Chem.*, **10**, 837 (1971); (b) A. Davison and D. L. Reger, *ibid.*, **10**, 1967 (1971); (c) M. R. Churchill and J. Wormald, *ibid.*, **10**, 1778 (1971).

(33) (a) A. Ali, F. Fahrenholz, J. C. Garing, and B. Weinstein, *J. Amer. Chem. Soc.*, **94**, 2556 (1972); (b) Y. Sugiura and H. Tanaka, *Biochem. Biophys. Res. Commun.*, **46**, 335 (1972); (c) L. G. Stadtherr and R. B. Martin, *Inorg. Chem.*, **11**, 92 (1972); (d) S. A. Grachev, L. I. Shchelkunova, Yu. A. Makashev, and F. Ya Kul'ba, *Zh. Neorg. Khim.*, **16**, 198 (1971); (e) C. S. Yang and F. M. Huennekens, *Biochemistry*, **9**, 2127 (1970); (f) A. Tomita, H. Hirai, and S. Makishima, *Inorg. Chem.*, **7**, 760 (1968); **6**, 1746 (1967).

(34) W. J. Hu, K. D. Karlin, D. Barton, and S. J. Lippard, *Proc. Int. Conf. Coord. Chem.*, **14th**, 598 (1972).

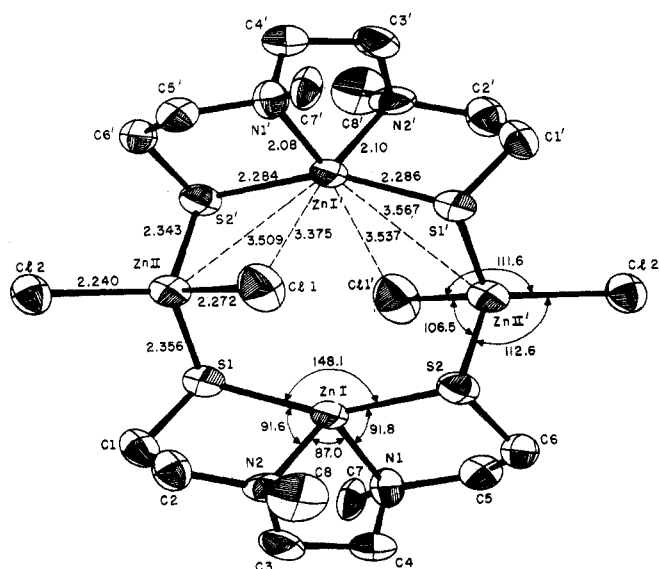
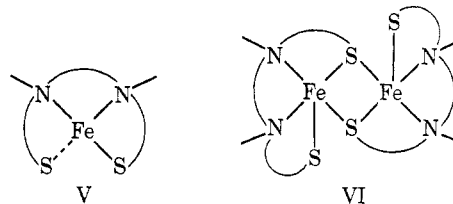


Figure 8. The tetranuclear cluster in  $[Zn_2Cl_2L]_2 \cdot 2H_2O$  (reproduced from ref 36).

atoms have a known tendency to form three bonds and bridge metal atoms,<sup>35</sup> the ligands were expected to provide low molecular weight oligomeric compounds. Since in the absence of additional ligands the coordination number of the metal would be limited to four (monomer, V) or five (dimer, VI), iron-



sulfur complexes with a resultant weak ligand field might obtain. These would be of obvious interest since high-spin iron(III) centers are known to occur in the proteins. In brief, then, the employment of multidentate ligands IV was based upon a desire for control and variation of the stereochemical properties of the donor atom set at the expense of exact duplication of the protein ligands.

To study its coordination properties, ligand IVa was allowed to react with zinc(II) chloride to form an air-stable, crystalline salt, the structure of which is shown in Figure 8.<sup>36</sup> Two relevant features of this structure are: (1) the tetrahedral geometry of type I zinc atoms is strongly distorted and (2) a tetranuclear array of metals with bridging mercaptide atoms is formed. Both of these results were anticipated for ligand IVa, as mentioned above.

The synthesis of the iron(II) complexes of IVa and IVb,  $FeL$  and  $FeL'$ , respectively, was achieved by reaction of the appropriate ligand in excess with ferric acetylacetonate.<sup>37</sup> Red-brown crystals of both compounds were obtained and shown by X-ray diffraction to have structures similar to VI.<sup>37</sup> The iron(II) atoms in the binuclear complexes are in a dis-

(35) S. E. Livingstone, *Quart. Rev., Chem. Soc.*, **19**, 386 (1965).

(36) W. J. Hu, D. Barton, and S. J. Lippard, *J. Amer. Chem. Soc.*, **95**, 1170 (1973).

(37) W. J. Hu, K. D. Karlin, and S. J. Lippard, unpublished results.

torted trigonal-bipyramidal environment. Although the bonded iron-donor atom distances remain essentially constant for the two molecules, there are severe angular distortions in  $(\text{FeL})_2$ . As a consequence, the nonbonded  $\text{Fe}\cdots\text{Fe}$  distance is reduced to 3.205 (7) Å from the value of 3.371 (2) Å in relatively unstrained  $(\text{FeL}')_2$ .

The structural results demonstrate the effect of external ligand constraints (such as a protein might produce) on the geometry of the  $\text{Fe}_2\text{S}_2$  bridging system. If nonbonded electron interactions do affect the reduction potentials in such systems, as suggested above, then it would be interesting to examine the redox properties of  $(\text{FeL})_2$  and  $(\text{FeL}')_2$ . Unfortunately, the compounds decompose or are insoluble in most solvents tried to date. A study of the dependence of the magnetic susceptibilities of the solid complexes over the range  $80 < T < 400$  K, however, shows them to have measurably different electronic structures. Both contain antiferromagnetically coupled, high-spin iron(II) atoms, but the room temperature moment and Néel temperature for  $(\text{FeL}')_2$  are 4.2 BM and 160 K, respectively, while the corresponding values for  $(\text{FeL})_2$  are 3.4 BM and  $\sim 350$  K. Thus, the structural and magnetic properties of  $\text{Fe}_2\text{S}_2$  dimers in a weak field environment using biologically significant donor ligands are sensitive to steric strain supplied by the ligand. Further studies are in progress.

### Summary and Overview

In a review of the bioinorganic chemistry of vitamin  $\text{B}_{12}$  and related compounds, it was suggested that the biological studies inspired more advances in the coordination chemistry of cobalt than *vice versa*.<sup>38</sup> A similar comment is applicable, at least in part, to the iron-sulfur systems discussed here. The synthetic goal of producing crystalline and well-characterized iron-sulfur complexes with only biologically significant ligands has been realized, not only in the preparation of  $(\text{FeL})_2$  but also in recent work from the laboratory of Holm.<sup>39</sup> These latter compounds, which have been structurally characterized by Ibers,<sup>39,40</sup> provide the closest simulation yet to the actual iron-sulfur centers in both the  $\text{Fe}_4\text{S}_4$  and  $\text{Fe}_2\text{S}_2$  protein classes. The extent to which any of the above preparative achievements will enhance our understanding of how the proteins function remains an open question. It appears that a good beginning has been made, however.

Reasoning by admittedly speculative analogy to the iron(III) and cobalt(III) thioxanthate systems, a rationale for the low and varying reduction potentials of the  $\text{Fe}_2\text{S}_2$  proteins has been proposed. New iron-sulfur complexes with sterically constraining polydentate ligands have been synthesized to provide some experimental criteria on which to base further assessment of this rationale. Besides primary mer-

captides, the ligands chosen contain nitrogen donor atoms most likely not available to the iron in the proteins. Nevertheless, they may be more suited to the purpose of correlating redox behavior with steric strain than all sulfur donor ligands in which a strained configuration is lacking. There seems to be no compelling reason to strive for perfect duplication of the protein active site environment (*e.g.*, through the use of polypeptide ligands) to simulate or attempt to understand its properties. Indeed, no molecule smaller than the protein itself is likely to be capable of displaying *all* its relevant physical and chemical properties.

The discussion in this Account has focused primarily on rubredoxin and the  $\text{Fe}_2\text{S}_2$  proteins, in which the oxidation states of the metal and ligand atoms are known. An important piece of information which is lacking for the  $\text{Fe}_4\text{S}_4$  proteins is the overall charge on the tetranuclear cluster. Thus, differences in the average valence state of the iron atoms in the bacterial ferredoxins and the HiPIP molecules could account for their different reduction potentials.<sup>39a</sup> A further question is whether the electron in the reduced  $\text{Fe}_4\text{S}_4$  cluster is distributed over all four iron atoms or whether, as in the  $\text{Fe}_2\text{S}_2$  proteins, it is more highly localized.

In the  $\text{Fe}_4\text{S}_4$  proteins, the choice of  $\text{S}^{2-}$  has a natural explanation in its role as a triply bridging ligand, for which cysteine sulfur would be somewhat inferior.<sup>41</sup> In the  $\text{Fe}_2\text{S}_2$  proteins, however, it would appear that cysteine sulfur could replace the labile sulfide as the bridging atoms. It is therefore important that the possibility of a  $\text{Fe-S-S-C}$  linkage, discussed earlier, or  $\text{Fe-S-S-Fe}$  units<sup>21</sup> receive a critical evaluation. Again the difference in overall charge on the iron-sulfur core, for  $\text{S}^{2-}$  *vs.*  $\text{RSS}^-$  or  $\text{RCH}_2\text{S}^-$  donor ligands, may be an important factor.

Besides the relatively well-characterized iron-sulfur proteins listed in Table I, numerous others constitute essential parts of biological systems.<sup>1,3a</sup> For example, epr studies of mitochondrial and sub-mitochondrial particles provide strong evidence for iron-sulfur redox cores,<sup>42</sup> but little detailed information about purified protein materials is yet available. These proteins offer potential challenges for future work and will possibly reveal a new relevance of iron-sulfur coordination compounds already well understood.

*The experimental studies carried out in the author's laboratory were made possible by generous support from the National Institutes of Health under Grant GM-16449 and by the fruitful labors of the several coworkers cited in individual references. This article was written during a sabbatical leave, supported in part by the John Simon Guggenheim Foundation, at the Chalmers Institute of Technology in Göteborg, Sweden. The hospitality and stimulation of Professor Bo Malmström are gratefully acknowledged.*

(41) Primary mercaptides can utilize all valence electrons to coordinate three metal atoms (W. Harrison, W. C. Marsh, and J. Trotter, *J. Chem. Soc., Dalton Trans.*, 1009 (1972), and references cited therein), but this is rare.

(42) (a) N. R. Orme-Johnson, W. H. Orme-Johnson, R. E. Hansen, H. Beinert, and Y. Hatefi, *Biochem. Biophys. Res. Commun.*, **44**, 446 (1971); (b) T. Ohnishi, D. F. Wilson, T. Asakura, and B. Chance, *ibid.*, **46**, 1631 (1972).

(38) D. L. Brown, *Progr. Inorg. Chem.*, **18**, 177 (1973).

(39) (a) T. Herskovitz, B. A. Averill, R. H. Holm, and J. A. Ibers, *Proc. Int. Conf. Coord. Chem.*, **14th**, 1 (1972); (b) T. Herskovitz, B. A. Averill, R. H. Holm, J. A. Ibers, W. D. Phillips, and J. F. Weiher, *Proc. Nat. Acad. Sci. U. S.*, **69**, 2437 (1972); (c) R. H. Holm, private communication.

(40) M. R. Snow and J. A. Ibers, *Inorg. Chem.*, **12**, 249 (1973).