

## Synthetic Approaches to the Active Sites of Iron-Sulfur Proteins

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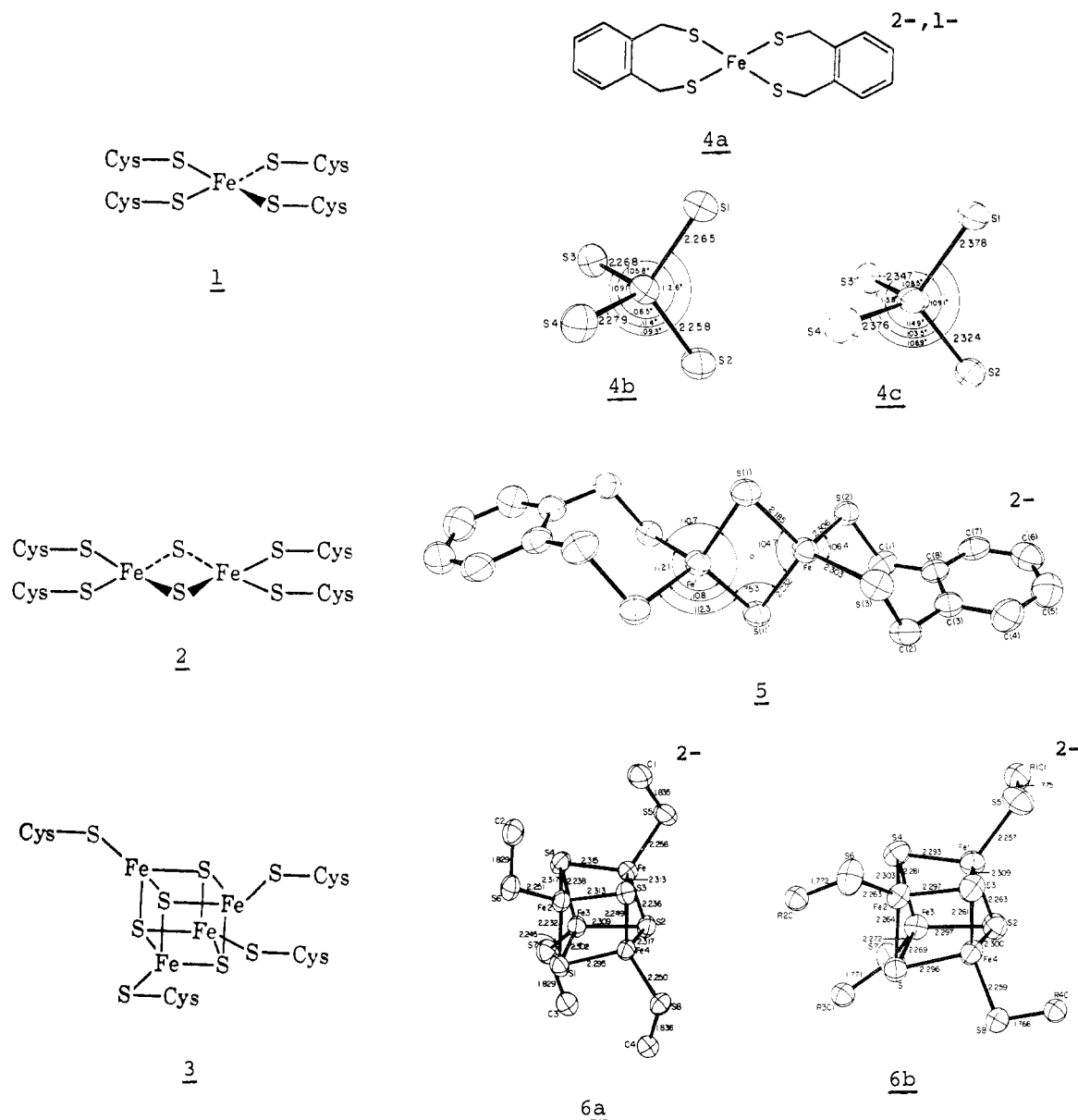
In a significant fraction of contemporary bioinorganic research by chemists, the starting point is an attempt to prepare a credible low molecular weight representation of a biological redox or catalytic metal coordination site, followed by detailed characterization of structural, physicochemical, and reactivity properties of the synthetic species. Those species which reproduce ligation modes and approach or achieve the stereochemistry of metalloprotein or metalloenzyme sites (to the extent that these sites are defined at near-atomic resolution) are termed synthetic analogues.<sup>1</sup> The synthetic-analogue approach has two particularly evident potential capabilities: (i) deduction of minimal biological-site structure based on coincident analogue-biomolecule properties; (ii) detection of the influence of the biological matrix on the intrinsic properties of the coordination unit as reflected by the analogue. The premise underlying (ii), noted by Hill<sup>2</sup> in similar context, is that the chemistry of a given metal ion may differ in degree but not in kind from that of the ion in abiological surroundings. At parity of metal ligation such differences arise from whatever environmental effects and precise stereochemical constraints are imposed by the normal protein structure. This Account offers a brief description of the synthetic analogue approach<sup>3</sup> to an elucidation of the active sites of iron-sulfur proteins and enzymes as conducted in the author's laboratory, together with other observations pertinent to modeling structure and function of biological coordination units.

**Types of Biological Coordination Sites.** Protein crystallographic results<sup>4-19</sup> allow recognition of four structural types of coordination units, specified in Table I. These serve as synthetic objectives for structure-function studies of sites that already enjoy a modicum of structural definition, but of course lack the precise metrical description of metal binding common in small molecule structures.<sup>20</sup> Coordination units a and c exhibit (apart from aqua binding) ligation by protein side chains only, and thus differ from units b and d

which contain integral substructures (heme groups, Fe<sub>4</sub>S<sub>4</sub>\* cores) around which further side-chain binding

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**Figure 1.** Schematic representations of the 1-Fe (1), 2-Fe (2), and 4-Fe (3) active sites of Fe-S proteins and their structurally characterized synthetic analogues 4-6: chelate structure of Rd analogues (4a); coordination units of  $[\text{Fe}(\text{S}_2\text{-}o\text{-xyl})_2]^{2-34}$  (4b) and  $[\text{Fe}(\text{S}_2\text{-}o\text{-xyl})_2]^{2-34}$  (4c);  $[\text{Fe}_2\text{S}_2(\text{S}_2\text{-}o\text{-xyl})_2]^{2-33}$  (5);  $[\text{Fe}_4\text{S}_4(\text{SCH}_2\text{Ph})_4]^{2-31}$  (6a);  $[\text{Fe}_4\text{S}_4(\text{SPh})_4]^{2-36}$  (6b). Phenyl rings are omitted in 6a,b.

may be elaborated. Units a and c, replete with dissimilar side-chain binding, delicately distorted stereochemistries, and substitutionally labile metal ions, should prove to be the more difficult to model. Indeed, an analogue of only the  $\text{Rd}_{\text{ox}}$  site,<sup>21</sup> advantageous because of four identical ligands, has been achieved (vide infra). The advantage of substructures in analogue synthesis is effectively illustrated by clever substituent modification of porphyrin rings, leading to heme- $\text{O}_2$

binding sites reversibly functional under ambient conditions<sup>22-24</sup> and a crystalline dioxygen complex whose essential structural features have been determined by x-ray diffraction.<sup>23b</sup>

**Iron-Sulfur Proteins.** With the possible exception of heme proteins no class of metalloproteins has been as thoroughly investigated in the last decade as the nonheme iron-sulfur proteins, whose history and properties have been exhaustively reviewed.<sup>25</sup> The

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(20) Of the structures in Table I only those of the three nonheme iron-sulfur proteins approach this description.

(21) Abbreviations: Fd, ferredoxin; HMPA, hexamethylphosphoramide; HP, "high-potential" protein; Rd, rubredoxin; SCE, saturated calomel electrode; SHE, standard hydrogen electrode; s-ox, superoxidized; s-red, super-reduced; S\*, sulfide (where useful for clarity);  $\text{S}_2\text{-}o\text{-xyl}$ ,  $o\text{-xyl}$ - $\alpha,\alpha'$ -dithiolate.

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Table I  
Metal Coordination Units in Proteins and Enzymes Derived from X-Ray Investigations<sup>a</sup>

Molecule	Mol wt (subunits)	Coordination unit <sup>b</sup>	Ref
(a) Mononuclear, Side-Chain Binding			
Rubredoxin (Rd <sub>ox</sub> )	6 000	[Fe(S-Cys) <sub>4</sub> ] <sup>f</sup>	4
Carboxypeptidase A, B	34 500	[Zn(N-His) <sub>2</sub> (O-Glu)(OH <sub>2</sub> )] <sup>f</sup>	5a,b
Thermolysin	34 600	[Zn(N-His) <sub>2</sub> (O-Glu)(OH <sub>2</sub> )] <sup>f</sup> , 4 [Ca-O <sub>6,7</sub> ] <sup>g,h</sup>	6
Concanavalin A	102 000 (4)	[Mn(N-His)(O-Asp) <sub>2</sub> (O-Glu)(OH <sub>2</sub> ) <sub>2</sub> ], [Ca-O <sub>6</sub> ] <sup>g,h</sup>	7
Carbonic anhydrase B, C	~29 000	[Zn(N-His) <sub>3</sub> (OH <sub>2</sub> )] <sup>f</sup>	8
Liver alcohol dehydrogenase	80 000 (2)	[Zn(S-Cys) <sub>2</sub> (N-His)(OH <sub>2</sub> )] <sup>f</sup> , [Zn(S-Cys) <sub>4</sub> ] <sup>f</sup>	9
(b) Mononuclear, Porphyrin + Side-Chain Axial Binding			
b-type cytochromes	~11 000	[Fe(N <sub>4</sub> -porphyrin)(N-His) <sub>2</sub> ]	10
c-type cytochromes	~12 000	[Fe(N <sub>4</sub> -porphyrin)(S-Met)(N-His)]	11
Myoglobin, hemoglobin (deoxy, met)	17 800, 64 500 (4)	[Fe(N <sub>4</sub> -porphyrin)(N-His)]	12
(c) Binuclear, Side-Chain Bridging and Terminal Binding			
Superoxide dismutase <sup>c</sup>	32 000 (2)	[(His-N) <sub>3</sub> Cu-μ(N-His)-Zn(N-His) <sub>2</sub> (O-Asp)]	13
Hemerythrin <sup>d</sup>	108 000 (8)	[(His-N) <sub>2</sub> (Tyr-O)Fe-μ(Asn, Glu, OH <sub>2</sub> )-Fe(N-His) <sub>3</sub> ] <sup>g</sup>	14
(d) Bi-, Polynuclear, Inorganic Bridging + Terminal Side-Chain Binding			
Myohemerythrin (metazide) <sup>e</sup>	13 900	[(His-N) <sub>2</sub> (Tyr-O)Fe-O-Fe(O-Tyr)(N-His) <sub>2</sub> ]	15
Ferredoxin (8-Fe-Fd <sub>ox</sub> )	6 000	2[Fe <sub>4</sub> S <sub>4</sub> (S-Cys) <sub>4</sub> ]	16, 17a
"High-potential" protein (HP <sub>red,ox</sub> )	9 300	[Fe <sub>4</sub> S <sub>4</sub> (S-Cys) <sub>4</sub> ]	17

<sup>a</sup> Excluding siderophores,<sup>19a</sup> vitamin B<sub>12</sub> coenzymes,<sup>19b</sup> bacteriochlorophyll,<sup>19c</sup> calcium-binding proteins.<sup>19d</sup> <sup>b</sup> Per subunit. <sup>c</sup> Eukaryotic (bovine erythrocyte). <sup>d</sup> *Thermiste dyscritum*. <sup>e</sup> *Thermiste pyroides*, ~5-Å resolution. <sup>f</sup> Distorted tetrahedral. <sup>g</sup> Distorted octahedral. <sup>h</sup> Side-chain + H<sub>2</sub>O ligation. <sup>i</sup> Distorted planar.

biological function of the smaller soluble proteins (molecular weight ca. 6 000–20 000), where known, is redox coupling to enzymatic systems which catalyze a myriad of reactions, of which the currently most celebrated is the N<sub>2</sub> → NH<sub>3</sub> reduction by nitrogenases. The ubiquitous participation of these proteins in metabolic processes in all forms of life led Lovenberg<sup>26</sup> in 1974 to observe: "In retrospect, we wonder how we were able to feel that we had a reasonable understanding of biology a decade ago before iron-sulfur electron carriers had been discovered". The significance of this class of proteins was clearly evident earlier and stimulated my co-workers and me to commence a chemical attack on iron-sulfur active sites and their properties. The approach productive of useful results was initiated in mid-1971, at which time the distorted tetrahedral Rd site 1 and the cubane-type HP<sup>27</sup> site 3 (Figure 1) had emerged from x-ray studies. Proof of the existence of two sites 3 in a bacterial 8-Fe Fd by similar means became available shortly thereafter.<sup>29</sup> The 2-Fe site structure 2 with tetrahedral coordination was proposed as early as 1966, and is convincingly supported by a huge body of physicochemical data<sup>25,30</sup> as well as by the synthetic analogue approach itself (vide infra).

Accurate modeling of the active sites of iron-sulfur proteins offers a unique challenge. No other class of

metalloproteins presents three currently recognized types of sites, of which two (2, 3) contain unit d but with different substructures whereas the other (1) possesses mononuclear unit a. The order of structural complexity 3 > 2 > 1 suggests the same sequence of synthetic difficulty. In fact, analogues chronologically were obtained in the reverse order.

**Analogue Syntheses and Structures.** The basic premise underlying preparative experiments has been that structures 1–3 in at least some oxidation levels derive no special stability from a protein matrix and should be accessible as thermodynamically controlled, soluble reaction products derivable from labile Fe(II,III) salts, sulfide, and an appropriate thiol. Thus reaction 1 of Figure 2 was developed as a general route to the clusters [Fe<sub>4</sub>S<sub>4</sub>(SR)<sub>4</sub>]<sup>2-</sup> in 1971–1972,<sup>1,31</sup> and nearly contemporaneously reaction 2 was found to afford the 2-Fe site analogue [Fe<sub>2</sub>S<sub>2</sub>(S<sub>2</sub>-o-xyl)<sub>2</sub>]<sup>2-</sup>.<sup>32,33</sup> While seemingly trivial by comparison, analogues of the 1-Fe site 1 proved elusive for some time. Finally, suitable conditions were devised in 1974–1976 for reactions 3 and 4 which yielded the mononuclear species [Fe(S<sub>2</sub>-o-xyl)<sub>2</sub>]<sup>2-</sup>,<sup>1-34</sup> thereby completing the synthesis of the three types of protein sites. Lately an excellent synthesis of the reduced 4-Fe site analogues [Fe<sub>4</sub>S<sub>4</sub>(SR)<sub>4</sub>]<sup>3-</sup><sup>35</sup> has been achieved by reduction of [Fe<sub>4</sub>S<sub>4</sub>(SR)<sub>4</sub>]<sup>2-</sup> with acenaphthylenide ion, reaction 5. An elementary but key point is employment of o-xyl(SH)<sub>2</sub> in reactions 2 and 3. Its  $\overline{\text{S}} \cdot \overline{\text{S}}$  site distance disallows

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(27) This notation is retained for the *Chromatium* "high-potential" protein owing to its familiar prior usage. Because the [Fe<sub>4</sub>S<sub>4</sub>(S-Cys)<sub>4</sub>]<sup>1-</sup> site oxidation level is no longer unique to this protein<sup>28</sup> (although its positive potential remains so for soluble proteins), it is best considered a ferredoxin, as reflected in a nomenclature proposal submitted to the Commission on Biochemical Nomenclature under the guidance of Dr. H. Beinert, committee convener.

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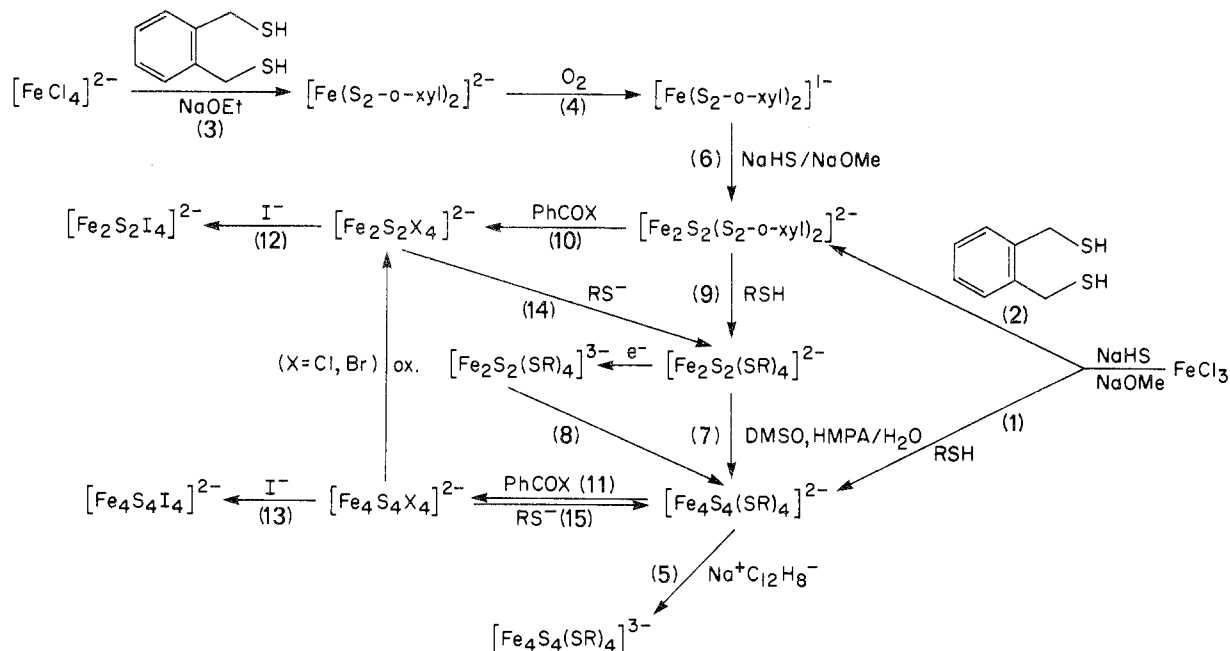


Figure 2. Syntheses and reactions of 1-Fe, 2-Fe, and 4-Fe active-site analogues.

chelation to one  $\text{Fe}_4\text{S}_4$  core, and the flexibility of its chelate ring permits near-tetrahedral angles around and normal bond distances to Fe(II,III) centers. When these reactions are correlated with the mononuclear-binuclear (reaction 6) and spontaneous binuclear-tetranuclear conversions<sup>35b</sup> (reactions 7 and 8) and the ligand exchange reaction 9,<sup>33</sup> the unifying scheme in Figure 2 emerges. Starting with a simple Fe(II) salt, 1-Fe, 2-Fe, and 4-Fe structures are progressively formed in the reaction sequence whose steps, except for (4), are executed under anaerobic conditions.

Structures of the three types of analogues,<sup>31-34,36</sup> solved in each case by J. A. Ibers shortly after synthesis, are depicted in Figure 1. Comparisons of active-site and analogue structures with the same oxidation level are detailed elsewhere.<sup>3b,17a,34</sup> Structure **4b** is considered a stereochemically unconstrained version of the  $\text{Rd}_{\text{ox}}$  site, whose latest published x-ray structure<sup>4,37</sup> reflects a more distorted tetrahedral coordination unit. Structure **4c** and  $[\text{Fe}(\text{SPh})_4]^{2-}$ <sup>38</sup> are differently distorted from  $T_d$  symmetry; the detailed structure of the  $\text{Rd}_{\text{red}}$  site is unknown. The isoelectronic  $\text{Fe}_4\text{S}_4^*$  cores of  $\text{Fd}_{\text{ox}}$ ,  $\text{HP}_{\text{red}}$ , **6a**, **6b**, and the recently reported  $[\text{Fe}_4\text{S}_4(\text{SCH}_2\text{CH}_2\text{CO}_2)_4]^{6-}$ <sup>39</sup> are virtually congruent, with idealized  $D_{2d}$  symmetry evident in the more precise analogue structures. The  $\text{Fd}_{\text{red}}$  analogue  $[\text{Fe}_4\text{S}_4(\text{SPh})_4]^{3-}$  exhibits a slightly expanded core compared to **6b** and different form of distortion from cubic to  $D_{2d}$ .<sup>40</sup> There is no structural information for reduced 4-Fe or oxidized or reduced 2-Fe<sup>41</sup> protein sites. Recent analyses of EXAFS spectra of proteins with sites 1-3 and their

synthetic analogues indicate a convincing degree of correspondence (ca.  $\pm 0.03 \text{ \AA}$ ) in mean Fe-S distances.<sup>42</sup> All available information supports description of analogues as faithful structural representations of isoelectronic protein sites.

**Oxidation State Equivalencies and Physicochemical Properties.** In view of the electron-transfer function of iron-sulfur proteins, a property obligatory to designation of synthetic species as active-site analogues is a redox capacity which encompasses all known oxidation levels of the proteins. The electrochemical demonstration of the three electron-transfer series  $[\text{Fe}(\text{S}_2\text{-o-xy})_2]^{2-,1-}$ ,<sup>34</sup>  $[\text{Fe}_2\text{S}_2(\text{S}_2\text{-o-xy})_2]^{4-,3-,2-}$ ,<sup>33</sup> and  $[\text{Fe}_4\text{S}_4(\text{SR})_4]^{4-,3-,2-,1-}$ <sup>35b,43</sup> provides the required proof of this property. Because proteins and analogues have ground and/or excited paramagnetic states and contain a Mössbauer nucleus (<sup>57</sup>Fe), they are responsive to wide-ranging spectroscopic and magnetic examination. Extensive comparisons of physicochemical properties of proteins<sup>25,30,44</sup> and analogues,<sup>3b,34,35a</sup> much of which has been carried out in collaboration with R. B. Frankel, in conjunction with the one-electron redox couples of both has afforded the unambiguous oxidation level equivalencies displayed in Table II. These relationships and the data which support them are productive of three particularly noteworthy results. (i) Tetranuclear cluster dianions are electronically delocalized rather than trapped valence  $[2\text{Fe}(\text{II}) + 2\text{Fe}(\text{III})]$  species, and thus are the only recognized nonclassical coordination units in biology. (ii) Correlations between the  $[\text{Fe}_4\text{S}_4(\text{SR})_4]^{3-,2-,1-}$  oxidation levels and the indicated protein sites transpose from hypothesis to fact the

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Table II  
Protein Site-Analogue Oxidation Level Equivalences

Site	Analogue <sup>a</sup>
$[\text{Fe}(\text{S-Cys})_4]^{2-}$ (Rd <sub>red</sub> )	* $[\text{Fe}(\text{S}_2\text{-}o\text{-xyl})_2]^{2-}$ , * $[\text{Fe}(\text{SPh})_4]^{2-}$
$\Downarrow$ -0.04 to -0.06 V <sup>b</sup>	
$[\text{Fe}(\text{S-Cys})_4]^{-}$ (Rd <sub>ox</sub> )	* $[\text{Fe}(\text{S}_2\text{-}o\text{-xyl})_2]^{-}$
$[\text{Fe}_2\text{S}_2(\text{S-Cys})_4]^{3-}$ (2Fe-Fd <sub>red</sub> )	$[\text{Fe}_2\text{S}_2(\text{S}_2\text{-}o\text{-xyl})_2]^{3-c}$
$\Downarrow$ -0.24 to -0.43 V	
$[\text{Fe}_2\text{S}_2(\text{S-Cys})_4]^{2-}$ (2Fe-Fd <sub>ox</sub> )	* $[\text{Fe}_2\text{S}_2(\text{S}_2\text{-}o\text{-xyl})_2]^{2-}$
$[\text{Fe}_4\text{S}_4(\text{S-Cys})_4]^{3-}$ (4Fe-Fd <sub>red</sub> , HP <sub>s-red</sub> )	* $[\text{Fe}_4\text{S}_4(\text{SPh})_4]^{3-}$
$\Downarrow$ -0.28 to -0.49 V	
$[\text{Fe}_4\text{S}_4(\text{S-Cys})_4]^{2-}$ (4Fe-Fd <sub>ox</sub> , HP <sub>red</sub> )	* $[\text{Fe}_4\text{S}_4(\text{SR})_4]^{2-}$ (R = CH <sub>2</sub> Ph, Ph)
$\Downarrow$ -0.42, -0.05 to -0.13, +0.35 V	
$[\text{Fe}_4\text{S}_4(\text{S-Cys})_4]^{-}$ (4Fe-Fd <sub>s-ox</sub> , HP <sub>ox</sub> )	$[\text{Fe}_4\text{S}_4(\text{S-}t\text{Bu})_4]^{-c}$

<sup>a</sup> Those isolated and structurally characterized are indicated with an asterisk. <sup>b</sup>  $E'_o$ ,  $E_{in}$  vs. SHE. <sup>c</sup> Detected electrochemically, not isolated.

Table III  
Comparative Analogue-Protein Potentials<sup>a</sup>

Couple	$E_{1/2}$ , V (SCE)			Me <sub>2</sub> SO, vol. %
	80	40	0	
$[\text{Fe}_4\text{S}_4(\text{SCH}_2\text{CH}_2\text{OH})_4]^{2-3-}$	-1.05	-0.89	-0.75	(-0.51) <sup>b</sup>
$[\text{Fe}_4\text{S}_4(\text{S-RS-Cys}(\text{Ac})\text{NHMe})_4]^{2-3-}$	-0.91	-0.82	-0.73	(-0.49)
<i>C. pasteurianum</i> Fd <sub>ox</sub> /Fd <sub>red</sub>	-0.93	-0.70	-0.67	(-0.43)

<sup>a</sup> For experimental details cf. ref 48. <sup>b</sup> Vs. SHE.

"three-state" proposal of Carter et al.<sup>45</sup> invoked to rationalize the disparate potentials of the Fd<sub>ox</sub>/Fd<sub>red</sub> and HP<sub>ox</sub>/HP<sub>red</sub> couples. (iii) The near-coincidence of a host of spectroscopic and magnetic properties of the 2-Fe sites of Fd<sub>ox</sub> proteins and  $[\text{Fe}_2\text{S}_2(\text{S}_2\text{-}o\text{-xyl})_2]^{2-3,33,46}$  provides strong confirmatory evidence for structure 2, whose metrical details presumably closely approach the minimal model 5. While this case illustrates one of the capabilities of the synthetic analogue approach, the formulation of 2 (completed prior to availability of analogue data) stands as an example *par excellence* of structure deduction from analyses of physicochemical properties.<sup>30</sup>

**Comparative Analogue-Protein Potentials.** Although analogues exhibit all of the oxidation levels of proteins, potentials of isoelectronic synthetic couples in aprotic or aprotic-aqueous media and protein potentials in aqueous solution (Table II) have proven to be discrepant when referenced to a common potential scale without further correction. Differences amount to ca. 0.6–0.7 (1-/2-), 0.9–1.0 (2-/3-), and  $\geq 0.2$  V (2-/3-) for the indicated couples of 1-Fe,<sup>34</sup> 2-Fe,<sup>33</sup> and 4-Fe<sup>43,47</sup> analogues and protein sites. To ascertain for 4-Fe clusters the contributions to these differences from nonuniform experimental conditions and the effects of protein structure, potentials of two analogue couples and one protein couple were measured by dc polarography under the same experimental conditions

(0–80% v/v Me<sub>2</sub>SO/H<sub>2</sub>O).<sup>48</sup> Some comparative potentials are listed in Table III. Analogue potentials monotonically shift to less negative values with decreasing Me<sub>2</sub>SO content. Protein potentials behave similarly to ca. 40% Me<sub>2</sub>SO and then remain virtually constant to the pure aqueous limit, indicating transition from an unfolded to the normal protein configuration over a narrow interval of solvent composition. The protein potential in aqueous solution is within ca. 20 mV of more precise values determined by equilibrium techniques.<sup>49</sup> Adopting the premise that at nominal parity of terminal -CH<sub>2</sub>S ligands differences between analogue and protein potentials reflect the influence of the normally configured protein matrix, it is found that such influence amounts to 0.06–0.08 V.<sup>50</sup> These values are taken as the best available estimates of extrinsic protein structural and environmental effects on 4-Fe site potentials. Their determination is another example of the utility of the synthetic analogue approach; similar values are not yet available for 1-Fe and 2-Fe sites. While analogue-protein potential differences in aqueous solution are appreciable, they are substantially smaller than estimates based on aprotic solution analogue potentials and further enhance the credibility of  $[\text{Fe}_4\text{S}_4(\text{SR})_4]^{2-3-}$  species, in particular those with R/CH<sub>2</sub>S ligation, as analogues of 4-Fe sites.

(48) C. L. Hill, J. Renaud, R. H. Holm, and L. E. Mortenson, *J. Am. Chem. Soc.*, **99**, 2549 (1977).

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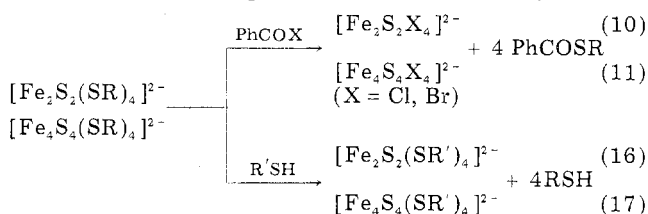
(50) A value of 0.16 V is obtained from Table II and the aqueous solution potential of  $[\text{Fe}_4\text{S}_4(\text{SCH}_2\text{CH}_2\text{CO}_2)_4]^{6-7-}$  (R. C. Job and T. C. Bruice, *Proc. Natl. Acad. Sci. U.S.A.*, **72**, 2478 (1975)).

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

(46) W. O. Gillum, R. B. Frankel, S. Foner, and R. H. Holm, *Inorg. Chem.*, **15**, 1095 (1976).

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**Reactions of Analogues.** The reaction chemistry of analogues is currently in a developmental stage. At present two types of anaerobic reactions may be recognized: those which proceed with retention or non-retention of  $\text{Fe}_2\text{S}_2^*$  and  $\text{Fe}_4\text{S}_4^*$  core structures.<sup>3,44</sup> Examples of the second type are binuclear-tetranuclear conversions, reactions 7 and 8 in Figure 2. Reactions proceeding with core retention necessarily involve alteration of terminal ligands, which are labile and have nucleophilic characteristics when coordinated.<sup>51,52a</sup> Demonstration of these properties is afforded by reactions 10 and 11 (Figure 2 and below) whereby thiolate

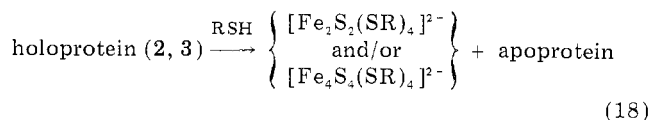


dimer and tetramer dianions are converted in good yield to chloride and bromide species; these may then be metathesized to iodo complexes (reactions 12 and 13) or converted to thiolate dimers and tetramers (reactions 14 and 15).  $[\text{Fe}_2\text{S}_2\text{X}_4]^{2-}$  and  $[\text{Fe}_4\text{S}_4\text{X}_4]^{2-}$  complexes constitute a heretofore unknown class of fundamental inorganic anions. While of interest in their own right, they are valuable as probes of core properties because they are the only species containing  $\text{Fe}_2\text{S}_2^*$  and  $\text{Fe}_4\text{S}_4^*$  cores yet isolated with terminal ligands other than thiolate. Overall structures of  $[\text{Fe}_2\text{S}_2\text{Cl}_4]^{2-}$  and  $[\text{Fe}_4\text{S}_4\text{Cl}_4]^{2-}$  correspond to those of **5** and **6a,b**, respectively, with nearly identical core dimensions.<sup>52b</sup> Spin-spin coupling within the cores, leading to anti-ferromagnetic intracluster behavior, are virtually independent of halide vs. thiolate ligation. Other properties such as 2-/3- redox potentials and absorption spectra are far more ligand dependent. Within the two halide series the multifaceted visible spectra are dominated by  $\text{L} \rightarrow \text{M}$  charge-transfer absorptions, whose progressive low-energy displacements ( $\text{Cl} < \text{Br} < \text{I}$ ) provide one of the more clear-cut examples of the ligand dependence of this type of excitation. Comparative properties of halide and thiolate dimers and tetramers are detailed elsewhere.<sup>52</sup>

A second process proceeding with core retention is thiolate ligand substitution by added thiol  $\text{R'SH}$ , reactions 16 and 17, initially reported in 1974-1975.<sup>33,53</sup> When  $\text{R} = \text{alkyl}$  and  $\text{R}' = \text{aryl}$  complete conversion to the  $\text{R'S}$ -substituted dimer or tetramer can be achieved in aprotic or aprotic-aqueous solvents at ambient temperature with only a small excess of thiol. These reactions, which may be monitored by spectrophotometry or  $^1\text{H}$  NMR,<sup>36,49,47,53</sup> have important chemical and biochemical ramifications. They are of definite synthetic utility, especially for production of complexes not readily accessible by the direct methods illustrated by reactions 1 and 2 (Figure 1). At present the most direct route to dimers other than  $[\text{Fe}_2\text{S}_2(\text{S}_2\text{-o-xyl})_2]^{2-}$  is reaction 9. An example of the utility of reaction 17 is incorporation of cysteinyl peptide structure around one

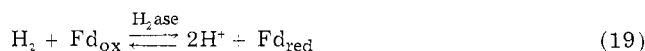
or more of the  $\text{Fe}_4\text{S}_4^*$  cores originally present in  $[\text{Fe}_4\text{S}_4(\text{S-}t\text{-Bu})_4]^{2-}$  followed by its removal with benzenethiol, yielding  $[\text{Fe}_4\text{S}_4(\text{SPh})_4]^{2-}$  as the sole product complex.<sup>47</sup>

**Core Extrusion Reactions.** The preceding reactions offered clear precedent for reaction 18, in which

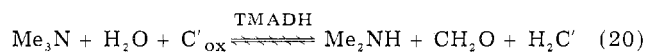


protein active-site cores are extruded (displaced) intact in the form of synthetic analogues that are identifiable by their absorption spectra or other characteristic properties. Evidence of the feasibility of extrusion of 2-Fe and 4-Fe cores from protein sites, using an 80%  $\text{Me}_2\text{SO}/\text{H}_2\text{O}$  solvent medium for protein structure unfolding and benzenethiol as the extruding reagent, was first published in 1975.<sup>54</sup> Disruption of protein structure is essential for these reactions to proceed at an appreciable rate. Subsequent studies have extended extrusion reactions to other Fd proteins (mol wt range 6 000-13 000) with known 2-Fe and 4-Fe sites<sup>55-58</sup> and have demonstrated by spectrophotometric assay that cores may be extruded quantitatively. As one example, extrusion of the 8-Fe Fd<sub>ox</sub> protein from *C. pasteurianum* in 80% HMPA/ $\text{H}_2\text{O}$  with benzenethiol yields  $2.0 \pm 0.1$   $\text{Fe}_4\text{S}_4^*$  cores as  $[\text{Fe}_4\text{S}_4(\text{SPh})_4]^{2-}$  ( $\lambda_{\text{max}}$  458 nm),<sup>56</sup> in accordance with the presence of two sites **3** established by other means for this protein.<sup>25</sup>

With the viability of core extrusion as applied to smaller proteins in hand, the principal current interest in this reaction resides in its potential to identify  $\text{Fe-S}^*$  sites which may not be conveniently or unambiguously identified by physicochemical measurements. Initial applications are encouraging. Work with L. E. Mortenson using benzenethiol and an 80% HMPA/ $\text{H}_2\text{O}$  reaction medium has established that the Chen-Mortenson preparation of the hydrogenase (mol wt 60 500) from *C. pasteurianum* most probably contains three 4-Fe sites **3**.<sup>56</sup> Similar experiments have shown that a hydrogenase from the same organism but with a lower Fe content also contains a 4-Fe site.<sup>55</sup> Thus the extrusion results provide information essential to mechanistic interpretation of reaction 19 and any de-



velopment of synthetic enzyme models. A somewhat more complex iron-sulfur enzyme is trimethylamine dehydrogenase<sup>59</sup> (mol wt 147 000) which catalyzes reaction 20 in the presence of an added electron carrier,



(54) (a) L. Que, Jr., R. H. Holm, and L. E. Mortenson, *J. Am. Chem. Soc.*, **97**, 463 (1975). Contemporaneous studies were carried out by Orme-Johnson and co-workers (W. H. Orme-Johnson, private communications, 1974-1975, and ref 58), but full details have not yet been published.

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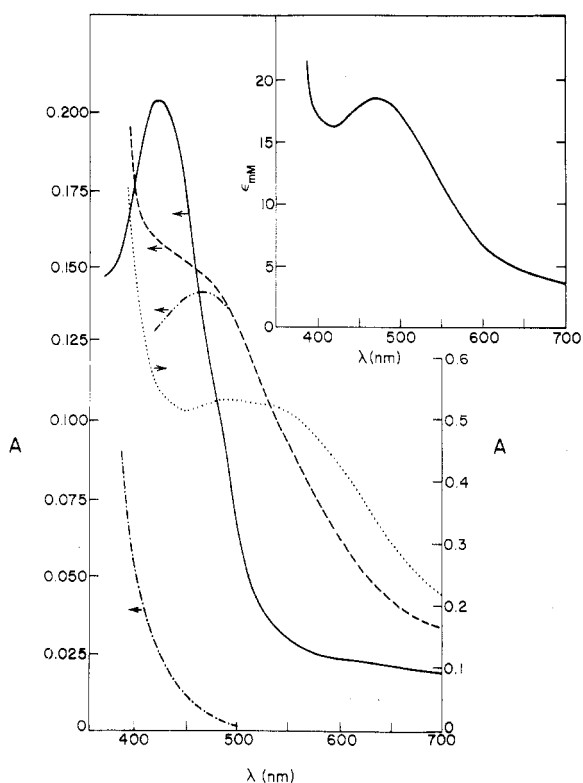
(58) W. H. Orme-Johnson and L. C. Davis, *Iron-Sulfur Proteins*, **3**, 15 (1977).

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(51) G. R. Dukes and R. H. Holm, *J. Am. Chem. Soc.*, **97**, 528 (1975).

(52) (a) G. B. Wong, M. A. Bobrik, and R. H. Holm, *Inorg. Chem.*, in press; (b) M. A. Bobrik, K. O. Hodgson, and R. H. Holm, *ibid.*, **16**, 1851 (1977).

(53) M. A. Bobrik, L. Que, Jr., and R. H. Holm, *J. Am. Chem. Soc.*, **96**, 285 (1974).



**Figure 3.** Active-site core extrusion of trimethylamine dehydrogenase in 80% HMPA/H<sub>2</sub>O:<sup>57</sup> the enzyme before (—) and after (---) addition of 200 equiv of *p*-MeOC<sub>6</sub>H<sub>4</sub>SH/equiv of Fe; (---) cofactor peptide in the presence of excess thiol; (.....) difference between preceding two spectra; (.....) extrusion of spinach Fd<sub>ox</sub> with *p*-MeOC<sub>6</sub>H<sub>4</sub>SH. Insert: spectrum of an isolated salt of [Fe<sub>4</sub>S<sub>4</sub>(SC<sub>6</sub>H<sub>4</sub>OMe)<sub>4</sub>]<sup>2-</sup>. Arrows designate absorbance scales.

C'. Initial attempts to develop a molecular picture of enzyme action require identification of two types of prosthetic groups, the Fe-S\* site(s) and an organic chromophore. The latter is apparently the catalytic center and may be removed by tryptic digest from the enzyme as a cofactor peptide; its structure is perhaps of a flavinoid type but has not been established. Core extrusion, illustrated in Figure 3, and related experiments conducted with D. J. Steenkamp and T. P. Singer<sup>57</sup> have demonstrated that TMADH contains one 4-Fe site, whose presumed role is electron-transfer coupling of the catalytic center and the external carrier. The extrusion technique has also proved of value for Fe-S\* site identification in the components of the nitrogenase enzyme complex. The electron-carrying Fe protein contains one 4-Fe site,<sup>56,58</sup> which may act as a structural bridge between its two subunits (mol wt 27 500). About 12 of the ~20 Fe atoms in the catalytic Fe-Mo protein (mol wt ≥200 000, 4 subunits) appear to be organized into 4-Fe units.<sup>60</sup>

Current prognosis for the extrusion technique as an independent or complementary means of 2-Fe and 4-Fe site identification is favorable. Further elaboration of the technique to circumvent interference in identification of extruded analogues by chromophoric and magnetic sites in the residual protein will be required to generalize the technique to other iron-sulfur proteins and enzymes, nearly all of which contain additional prosthetic groups. A further point of interest is the

capability of extension to membrane proteins, e.g., those in mitochondria and electron acceptors in plant and bacterial photosystems. Experimental approaches to meet these problems, certain of which are under study in this laboratory, are considered elsewhere together with a partial compilation of candidate proteins for site identification by the extrusion technique.<sup>44</sup> Lastly, it is emphasized that the technique at present identifies only the two known types of core structure and conveys no direct information as to terminal ligation or other structural and environmental site features, which require elucidation by other means.

**Unfulfilled Goals and Related Aspects of the Fe-S Analogue Approach.** A prime objective of this approach from its initiation has been the synthesis and detailed characterization of the three structural types of protein sites in all oxidation levels recognized *in vitro*. Inspection of Table II reveals that the structural part of this objective is complete and that five of the seven oxidation levels for the three structures have been stabilized in isolated compounds. Remaining are analogues for the HP<sub>ox</sub> (Fd<sub>s-ox</sub>) and 2-Fe-Fd<sub>red</sub> sites. Access to species of the type [Fe<sub>4</sub>S<sub>4</sub>(SR)<sub>4</sub>]<sup>-</sup> is imperative in view of the fact that the site in HP<sub>ox</sub> itself is the only structurally characterized example of this oxidation level. Frequent assignment of equivalent structures and oxidation levels in membrane-bound or otherwise difficultly characterized proteins, based on EPR spectral similarities with HP<sub>ox</sub>, remains tenuous until the inherent properties of a tetramer monoanion can be examined under a variety of conditions. Further, a SCF-SW-X $\alpha$  theoretical electronic model of Fe-S clusters<sup>61</sup> suggests that a monoanion in *T<sub>d</sub>* symmetry may not have an orbitally degenerate ground state and thus not be susceptible to first-order Jahn-Teller distortions. In this event an *S* = 3/2 ground state may follow, raising the possibility that the spin-quartet iron units believed to be present in the Fe-Mo protein of nitrogenase<sup>58,62</sup> may be relatively undistorted 4-Fe cluster monoanions. Synthesis of [Fe<sub>2</sub>S<sub>2</sub>(SR)<sub>4</sub>]<sup>3-</sup> will require elimination of spontaneous dimer-tetramer conversion (reaction 8, Figure 2). A leading question that may be pursued with such species in hand relates to electron distribution. All reduced 2-Fe sites which have been examined are trapped valence (Fe(II) + Fe(III)) in nature,<sup>30</sup> contrasting with delocalized 4-Fe sites and indicating that one iron atom is a unique redox center. In the absence of a protein x-ray structure, spectroscopic examination of dimer trianions constitutes the most effective means of ascertaining whether a trapped valence configuration is an intrinsic property of the binuclear site or an exigency of protein structure. Further, accurate structures of all seven site analogues in unconstrained forms will permit estimates of upper limits of structural reorganization in protein sites, assuming the protein matrix serves to attenuate structural differences between coupled redox states in order to diminish the Franck-Condon barrier. Available data for [Fe(S<sub>2</sub>-o-xy)<sub>2</sub>]<sup>2-,1-34</sup> and [Fe<sub>4</sub>S<sub>4</sub>(SPh)<sub>4</sub>]<sup>3-,2-36,40</sup> indicates that structural changes are small, especially for 4-Fe sites where electron distribution is not confined to a single metal atom.

(61) C. Y. Yang, K. H. Johnson, R. H. Holm, and J. G. Norman, Jr., *J. Am. Chem. Soc.*, **97**, 6569 (1975).

(62) E. Münck, H. Rhodes, W. H. Orme-Johnson, L. C. Davis, W. J. Brill, and V. K. Shah, *Biochim. Biophys. Acta*, **400**, 32 (1975).

(60) W. H. Orme-Johnson, M. T. Henzl, B. A. Averill, P. Wyeth, and E. Münck, *Fed. Proc., Fed. Am. Soc. Exp. Biol.*, **36**, 880 (1977).

While electron transfer appears to be the biological function of smaller proteins having sites 1-3, the possibility that these sites or variants thereof may be capable of catalytic transformations should be borne in mind. Hydrogenase is the best current example of an iron-sulfur enzyme containing no other metals or prosthetic groups. Given the numerous precedents in metal complex chemistry,<sup>63</sup> it is entirely probable that dihydrogen activation and heterolytic cleavage, evidenced by H/D exchange in the absence of an electron carrier,<sup>64</sup> occurs at a 4-Fe site. The terminal mono-oxygenase of the 4-methoxybenzoate O-demethylase system from *Pseudomonas putida* has been described as a nonheme protein whose catalytic site may be similar to the 2-Fe sites of Fd proteins.<sup>65</sup> The catalytic site(s) of the Fe-Mo protein of nitrogenase remains undefined despite massive research attention to this enzyme, rendering it perhaps the next most exhaustively examined metalloprotein complex after hemoglobin and cytochrome oxidase. Numerous model studies, which have afforded catalytic systems for the  $N_2 \rightarrow NH_3$  conversion<sup>66</sup> involving unidentified intermediates and direct ammonia formation from defined dinitrogen complexes,<sup>67</sup> have focussed heavily on molybdenum as the biocatalytic center. The large complement of Fe-S sites<sup>58,60,62</sup> demands consideration of 2 or 3 or a yet-unrecognized Fe<sup>58,62</sup> or Fe-Mo<sup>68</sup> coordination unit as a binding and activation center. Consequently, the analogues of Table II may be, or may serve as precursors to, complexes useful as test vehicles for stoichiometric or catalytic conversions of biological substrates.

(63) B. R. James, "Homogeneous Hydrogenation", Wiley-Interscience, New York, N.Y., 1973.

(64) L. E. Mortenson and J.-S. Chen, in "Microbial Iron Metabolism", J. B. Neilands, Ed., Academic Press, New York, N.Y., 1974, Chapter 11.

(65) F. H. Bernhardt, H. Packowsky, and H. Staudinger, *Eur. J. Biochem.*, **57**, 241 (1975).

(66) G. N. Schrauzer, *Angew. Chem., Int. Ed. Engl.*, **14**, 514 (1975).

(67) J. Chatt, A. J. Pearman, and R. L. Richards, *Nature (London)*, **253**, 39 (1975); C. R. Brület and E. E. van Tamelen, *J. Am. Chem. Soc.*, **97**, 911 (1975).

(68) For one proposal of this type of site, cf. *Scientia Sinica*, **19**, 460 (1976).

Lastly, consideration of a perplexing matter in iron-sulfur protein chemistry is solicited. How does an apoprotein containing at least four cysteinyl residues select, or at least preferentially stabilize, (i) a  $[Fe(S-Cys)_4]$  (1), (ii) a  $[Fe_2S_2(S-Cys)_4]$  (2), or (iii) a  $[Fe_4S_4(S-Cys)_4]$  (3) site with the latter capable of physiologically sustaining either the 2-/3- or 1-/2- redox couples.<sup>69</sup> Equivalently, one may ask, as has been done,<sup>70</sup> by what means and at what point in the biosynthesis of a polypeptide is the Fe or Fe-S\* constituent introduced? Preference but not specificity for a natural binding site is reflected in the formation of an unstable 1-Fe complex of the adrenodoxin peptide with properties similar to  $Rd_{ox}$ . It spontaneously converts to the native  $Fe_2S_2^*$  protein upon treatment with sulfide.<sup>71</sup> Possibly a combination of amino acid sequence and protein crystallographic data will ultimately delineate site stabilization factors. In the meantime synthetic elaboration of cysteinyl peptides (guided by sequence results<sup>72</sup>) followed by complexation with Fe(II, III), introduction of *preformed*  $Fe_2S_2^*$  and  $Fe_4S_4^*$  cores (reactions 16 and 17), and examination of the relative stabilities of these species could prove enlightening. One tetracysteinyl dodecapeptide has been shown to stabilize both 1-Fe<sup>73</sup> and 4-Fe<sup>47,74</sup> sites, but no attempt to convert the former to the latter or to a 2-Fe complex was attempted.

*The research described herein was supported by the National Institutes of Health. Special thanks are due to my enthusiastic collaborators whose names are to be found in the text and references.*

(69) For interpretations of the potential differences of these couples, cf. ref 17a and E. Adman, K. D. Watenpaugh, and L. H. Jensen, *Proc. Natl. Acad. Sci. U.S.A.*, **72**, 4854 (1975).

(70) J. W. Brodrick and J. C. Rabinowitz, *Iron-Sulfur Proteins*, **3**, 101 (1977).

(71) Y. Sugiura, K. Ishizu, and T. Kimura, *Biochem. Biophys. Res. Commun.*, **60**, 334 (1974).

(72) K. T. Yasunobu and M. Tanaka, *Iron-Sulfur Proteins*, **2**, 27 (1973).

(73) J. R. Anglin and A. Davison, *Inorg. Chem.*, **14**, 234 (1975).

(74) Note, however, that this and another cysteinyl peptide may not necessarily act as ligands to a single  $Fe_4S_4^*$  core; cf. footnote 30 of ref 47.

## Mechanisms of Oxidative Addition of Organic Halides to Group 8 Transition-Metal Complexes

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Most organic reactions that are catalyzed by transition metals occur by a series of steps, producing re-

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active intermediates, at least one of which contains a carbon-metal  $\sigma$  bond. One important reaction type by which intermediates containing carbon-metal  $\sigma$  bonds are formed is the oxidative addition of an organic substrate to the transition metal. In this reaction, the increase in the oxidation state of the metal is usually accompanied by an increase in the coordination number.<sup>1</sup> The propensity to undergo oxidative ad-

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