Synthetic Analogues of the Active Sites of Iron–Sulfur Proteins. 15.[1] Comparative Polarographic Potentials of the $[Fe_4S_4(SR)_4]^{2-,3-}$ and *Clostridium pasteurianum* Ferredoxin Redox Couples

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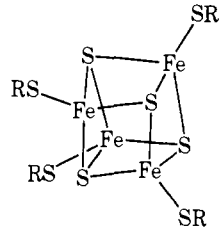
Abstract: Previous comparisons of redox potentials of iron–sulfur proteins in aqueous solution and their 4-Fe active site synthetic analogues in nonaqueous media show an apparent substantial negative shift of the latter compared to the former. This matter has been investigated by dc polarographic measurement of the half-wave potentials of the isoelectronic couples of *C. pasteurianum* ferredoxin, Fd_{ox}/Fd_{red}, and $[Fe_4S_4(SR)_4]^{2-,3-}$ at the dropping mercury electrode in the same solvent media ranging from 80% Me_2SO/H_2O v/v to pure water (pH 8.4). The protein in most solvent media exhibits a single diffusion-controlled cathodic wave for the Fd_{ox}/Fd_{red} process with slopes near −59 mV, consistent with a reversible reaction and a Nernst n value of 1. For the analogue couples $E_{1/2}$ (SCE) values increase and spectral band maxima (dianion) decrease from 80% Me_2SO to water: R = CH_2CH_2OH, −1.05 to −0.75 V, 409–374 nm; R = (*RS*)-Cys(Ac)NHMe, −0.91 to −0.73 V, 412–375 nm. For Fd_{ox}/Fd_{red} in the 80 to ca. 40% Me_2SO range these properties also monotonically change (−0.93 to −0.70 V, 407–386 nm (Fd_{ox})), but then remain essentially invariant to the pure aqueous limit. This behavior is interpreted as progressive solvation by water in the analogue systems as the aqueous content is increased, with similar behavior holding for the protein in solvents down to ca. 40% Me_2SO, under which conditions it exists largely in an unfolded state. At $\lesssim$40% Me_2SO content it is proposed that the protein reverts to its normal aqueous solution structure, thereby shielding the two 4-Fe active sites from solvent effects. Factors intrinsic and extrinsic to the protein sites which might cause the potential differences $\Delta E_{1/2} = E_{1/2}$ (analogue) $- E_{1/2}$ (Fd) in the same medium are discussed. Using potential data for the more realistic analogue (R = (*RS*)-Cys(Ac)NHMe) it is concluded that intrinsic effects on potentials are slight ($\lesssim$+0.03 V) and that $\Delta E_{1/2} = -0.06$ to −0.12 V observed in the 0–40% Me_2SO interval arises mainly from factors extrinsic to the active sites in the protein tertiary structure. In all media $\Delta E_{1/2}$ values are substantially smaller than those resulting from the previous comparisons under nonstandardized conditions, thus allowing designation of $[Fe_4S_4(SR)_4]^{2-,3-}$ complexes as active site analogues to be widened to include the property of redox potentials, at least for those proteins with $E \lesssim -0.40$ V vs. SHE.

Ferredoxins and related non-heme iron–sulfur proteins[3] function as electron carriers in a myriad of metabolic reactions. Previous reports in this series[1,4,5] have detailed the synthesis, structural and electronic characterization, and reactivity properties of the complexes $[Fe(SR)_4]^{-,2-}$, $[Fe_2S_2(SR)_4]^{2-}$, and $[Fe_4S_4(SR)_4]^{2-}$ which are related, respectively, to the active sites [Fe(S-Cys)$_4$] (Rd), [Fe_2S_2(S-Cys)$_4$] (Fd), and [Fe_4S_4(S-Cys)$_4$] (Fd, HP) in the indicated types of proteins.[6] Comparison of magnetic and spectroscopic properties of complexes and proteins has led to the establishment of certain isoelectronic relationships, which for proteins containing 4-Fe sites are arranged vertically in the electron transfer series (eq. 1).[5,7]

$$\begin{array}{ccccc} [Fe_4S_4(SR)_4]^{3-} & \rightleftharpoons & [Fe_4S_4(SR)_4]^{2-} & \leftrightarrow & [Fe_4S_4(SR)_4]^{1-} \\ Fd_{red} & & Fd_{ox} & & Fd_{s-ox} \\ HP_{s-red} & & HP_{red} & & HP_{ox} \\ C^- & & C & & C^+ \end{array} \quad (1)$$

This scheme is entirely equivalent to the "three-state" hypothesis of Carter et al.,[8] whose oxidation state symbols are included in the last row of the series. The synthetic analogue–protein relationships unambiguously define the total oxidation levels of active sites in the latter and eliminate any prior necessity to hypothesize their existence. Oxidation levels are specified by the net charge per tetramer unit, which in analogues and proteins consists of essentially congruent Fe_4S_4* cores of D_{2d} symmetry with each iron atom terminally coordinated by one thiolate ligand[5,9,10] (structure I).



I

In addition to the high degrees of similarity between given physical properties of proteins and complexes in fixed oxidation states, on which basis the term synthetic analogue was introduced,[11] the three types of complexes have been considered as redox analogues in the sense that their electron transfer series encompass all of the oxidation levels currently known for the corresponding proteins. This point is borne out by the series in eq 1 which includes, in addition to oxidation levels known to be physiologically significant, the "superreduced" form of *Chromatium* HP[12] and a "superoxidized" form of *C. acidiurici* Fd claimed to be formed in small amounts by ferricyanide oxidation of Fd_{ox}.[13] However, as emphasized on earlier occasions, the analogy does not appear to extend to actual potential values of isoelectronic redox couples. Analogue potentials ($E_{1/2}$ vs. SCE), determined polarographically in nonaqueous or 80% nonaqueous–20% aqueous media (v/v),[1,4b,7,14,15] are thus far substantially more negative than protein potentials (E_0', E_m vs. she), determined by various equilibrium techniques in 100% aqueous media,[16] 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 $\gtrsim$0.2 V (2−/3−) for the indicated couples of 1-Fe,[1,4b] 2-Fe,[15] and 4-Fe[7,14] analogues and protein sites, respectively.

Because of variations in experimental parameters (solution composition, measuring techniques, uncertainty in liquid junction potential corrections for measurements in nonaqueous media vs. aqueous reference electrodes), the foregoing potential differences cannot be accorded quantitative significance. At best they are suggestive of positive potential shifts when an iron atom or the core structures Fe_2S_2* and Fe_4S_4* are incorporated within a protein milieu. Even within classes of proteins

Table I. Selected Redox Potentials of 4-Fe Sites in Proteins

Protein	Couple	E, mV (25 °C)[c]	Conditions[d]	Ref
Bacillus stearothermophilus Fd[a]	2−/3−	−280	pH 8.0	17
Desulfovibrio desulfuricans Fd[a]	2−/3−	−330	pH 7.0	18
Bacillus polymyxa Fd I,II[a]	2−/3−	−380, −429	pH 7.2	19
Clostridium pasteurianum Fd[b]	2−/3−	−405, −408	pH 7.0, 7.2	20, 19
Clostridium acidi-urici Fd[b,e]	2−/3−	−434	pH 7.0, $\Delta E/\Delta$pH ~ −13 mV	20
		−414 to −475	pH 7.5, μ 0.01–1.0	
		−452	pH 7.5, 3 M urea	
		−441 to −448	pH 7.5, residue 1 variant	
		−423 to −472	pH 7.5–8.1, residue 2 variant	
Chromatium Fd[b]	2−/3−	−490, −488	pH 7, 8.5	21, 19
Azotobacter vinelandii Fd I[b]	1−/2−	−420, 350	pH ~8	22
Chromatium HP[a]	1−/2−	350, 356	pH 7.0, 7–11	23, 24

[a] 4-Fe protein. [b] 8-Fe protein. [c] E_0', E_m values relative to SHE. [d] Salt (buffer) concentration 25–100 mM in most cases. [e] See also ref 19.

containing the same type of active site, substantial potential variations occur. Selected aqueous solution data for 2−/3−[17-21] and 1−/2−[22-24] couples of proteins containing $[Fe_4S_4(S\text{-}Cys)_4]$ sites are collected in Table I. While not rigorously comparable owing to effects of pH and ionic strength on potentials,[20] the data nonetheless indicate that a variation as large as ca. 0.2 V for the 2−/3− couple is afforded by different protein structures. Among soluble proteins the largest apparent influence of this sort is that on the two 1−/2− couples in the same protein, *A. vinelandii* 8-Fe Fd I, for which the remarkable difference of 0.75 V has been reported.[22]

These variable effects of protein structure and environment make highly desirable a knowledge of potentials for redox couples in the series in eq 1 which are not subject to specific and unpredictable protein influences. In this investigation 2−/3− potential differences between 4-Fe analogues and protein sites have been examined by measurements of the couples

$$C.\ pasteurianum\ Fd_{ox} + e^- \rightleftharpoons Fd_{red} \quad (2)$$

$$[Fe_4S_4(SR)_4]^{2-} + e^- \rightleftharpoons [Fe_4S_4(SR)_4]^{3-} \quad (3)$$

under the same experimental conditions (80% Me_2SO to 100% aqueous media) using polarographic techniques. Underlying this work is the premise, considered more fully later, that at nominal parity of terminal thiolate ligands differences between analogue and protein potentials can be ascribed to the combined effects of protein structure and environment.

Experimental Section

Preparation of Compounds. (a) ***N*-Acetyl-(*R,S*)-cysteine-*N*-methylamide (Ac-(*RS*)-Cys-NHMe).** *p*-Nitrophenyl *N*-acetyl-*S*-benzyl-(*R,S*)-cysteinate[25] (49 g, 0.13 mol) was added to 350 mL of 40% aqueous methylamine. The yellow solution was stirred at room temperature for 4 h, cooled in ice, and adjusted to pH 4 with 8 M HCl. The crude amide was collected by filtration, washed with water, and dissolved in 500 mL of ethyl acetate. This solution was extracted with 1 M HCl (500 mL), and the aqueous phase was extracted with ethyl acetate (two 250-mL portions). The combined organic phases were washed with water (500 mL), 0.5 M $NaHCO_3$ (500 mL), 1 M HCl (100 mL), saturated NaCl (500 mL), and dried over $MgSO_4$. Removal of solvent and recrystallization of the residue gave 22 g (63%) of Ac-(*RS*)-Cys(Bzl)NHMe, mp 124–125 °C. This compound was converted to Ac-(*RS*)-Cys-NHMe with sodium in liquid ammonia by the method reported previously.[7] The crude yellowish solid was recrystallized from ethyl acetate (charcoal) under a dinitrogen atmosphere, yield 64%, mp 163–167 °C. Anal. Calcd for $C_6H_{12}N_2O_2S$: C, 40.89; H, 6.86; N, 15.90. Found: C, 40.66; H, 6.83; N, 15.66. Ac-(*S*)-Cys(Bzl)NHMe and Ac-(*S*)-Cys-NHMe were prepared earlier by different procedures.[7] The racemic compounds were further characterized by the identity of their ^1H NMR spectra with those of the (*S*) isomers.

Preparations b and c were carried out under anaerobic conditions.

(b) $(Me_4N)_2[Fe_4S_4(SCH_2CH_2OH)_4]$. This compound was prepared by thiolate ligand substitution.[14,26] To 100 mL of a stirred solution of 1.8 g (2.1 mmol) of $(Me_4N)_2[Fe_4S_4(S\text{-}t\text{-}Bu)_4]$[27] in DMF at ambient temperature was added 0.82 g (11 mmol) of 2-mercaptoethanol in 10 mL of DMF. The solution was warmed to 60–80 °C and solvent, *tert*-butylthiol, and excess 2-mercaptoethanol were removed in vacuo, and the black amorphous residue was maintained at 60–80 °C (0.05 Torr) for 2 h. This material was once recrystallized from 200 mL of hot acetonitrile, and the collected crystals were washed with 50 mL of acetonitrile at 5 °C. A second recrystallization from 100–150 mL of hot acetonitrile afforded upon slow cooling of the solution black, needle-like crystals which were washed as previously and dried in vacuo; dec ~145 °C (sealed tube). Anal. Calcd for $C_{16}H_{44}Fe_4N_2O_4S_8$: C, 23.77; H, 5.49; Fe, 27.63; N, 3.47; S, 31.73. Found: C, 23.80; H, 5.49; Fe, 27.90; N, 3.47; S, 31.84.

(c) $(Me_4N)_2[Fe_4S_4(S\text{-}(RS)\text{-}Cys(Ac)NHMe)_4]$. This compound was also prepared by ligand substitution in a manner similar to that for its (*S*) isomer.[7,14] To a solution of 0.94 g (1.1 mmol) of $(Me_4N)_2[Fe_4S_4(S\text{-}t\text{-}Bu)_4]$[27] in 150 mL of acetonitrile at ambient temperature was added 0.74 g (42 mmol) of Ac-(*RS*)-Cys-NHMe in 150 mL of acetonitrile. The solution was maintained under a partial vacuum for 30 min and then all volatile materials were removed in vacuo until a dry red-brown solid resulted. This product contains a mixture of diastereoisomeric tetramer dianions and, as with salts of $[Fe_4S_4(S\text{-}(S)\text{-}Cys(Ac)NHMe)_4]^{2-}$, it has proven difficult to crystallize. Its ^1H NMR spectrum ($Me_2SO\text{-}d_6$) was essentially identical with that of the tetramer with only (*S*) ligands[14] and contained no feature at ca. −2.8 ppm indicative of coordinated *tert*-butylthiolate.[14,28] The purity of the product in terms of Fe_4S_4* core content was assayed by ligand exchange with benzenethiol in 80% hexamethylphosphoramide–20% H_2O solutions using the procedures described elsewhere.[29] The amount of $[Fe_4S_4(SPh)_4]^{2-}$ found spectrophotometrically was 0.93 of that for an analytically pure compound. This factor was applied to the calculation of molarities of solutions prepared from this sample.

(d) *Clostridium pasteurianum* Fd_{ox}. This protein (A_{390}/A_{280} 0.77–0.79) was isolated by the procedure of Mortenson.[30]

Physical Measurements. All manipulations and measurements were carried out under an atmosphere of dinitrogen which was passed through a heated tower of BASF catalyst to remove dioxygen. Electronic spectra were obtained using a Cary 14 or 17 spectrophotometer and quartz cells. ^1H NMR spectra were measured on a Varian T-60 or XL-100 spectrometer with Me_4Si as the internal standard.

All electrochemical measurements were performed using a Princeton Applied Research Model 170 Electrochemical System. Preliminary experiments showed that Fd gave extremely poorly developed cyclic voltammograms. Consequently, the protein and two synthetic analogues were examined in the conventional dc polarographic mode of operation. The polarographic cell has been described previously.[7] Final conditions for measurement were influenced by the desirability of attaining simultaneously a combination of working electrode, supporting electrolyte and its concentration, and aqueous component buffer system, concentration, and pH which promoted Nernstian current–voltage characteristics in solutions ranging from 0 to 80% Me_2SO/H_2O content. Measurements in various Me_2SO/H_2O mixtures were desired in order to examine potentials under

Table II. Spectral Data for $[Fe_4S_4(SR)_4]^{2-}$ and Polarographic Data for $[Fe_4S_4(SR)_4]^{2-,3-}$ Redox Couples in Me_2SO/H_2O Solutions (0.1 M $(Et_4N)(BF_4)$, 50 mM TrisCl, aqueous component pH 8.4,[a] 25 °C)

Couple	Me_2SO, vol %	$[Fe_4S_4(SR)_4]^{2-}$, λ_{max}, nm (ϵ_M)[b]	$E_{1/2}$, V[c] SCE	SHE	−Slope,[d] mV	$\Delta\nu_{1/2}$, DPP, mV
$[Fe_4S_4(SCH_2CH_2OH)_4]^{2-,3-}$ [e]	100	414 (16 200); 297 (22 000)	−1.17		56	98
	80	409 (16 100); 294 (21 900)	−1.05		59	96
	60	404 (15 600); 294 (21 300)	−0.99		56	90
	40	395 (15 600); 294 (21 200)	−0.89		60	84
	20	382 (15 800); 296 (21 100)	−0.81		53	83
	0	374 (15 400); 296 (20 800)	−0.75	−0.51[g]	52	86
$[Fe_4S_4(S\text{-}(RS)\text{-}Cys(Ac)NHMe)_4]^{2-,3-}$ [f]	100	418 (17 200); 293 (23 200)	−0.98		62	96
	80	412 (16 900); 293 (21 700)	−0.91		59	84
	60	405 (16 200); 293 (21 700)	−0.85		59	90
	40	397 (16 500); 295 (22 000)	−0.82		51	85
	20	390 (15 400); 298 (21 000)	−0.79		60	90
	0	375 (14 900); 300 (22 300)	−0.73	−0.49[g]	60	98
$[Fe_4S_4(SEt)_4]^{2-,3-}$ [h]	80[i]	414 (15 700); 298 (21 400)	−1.16		59	
$[Fe_4S_4(S\text{-}(S)\text{-}Cys(Ac)NHMe)_4]^{2-,3-}$ [h]	100	407 (17 300); 294 (23 400)	−0.91		58	
$[Fe_4S_4(12\text{-peptide})]^{2-,3-}$ [h]	80[i]	406 (17 100); 290 (25 500)	−0.80			
$[Fe_4S_4(SCH_2CH_2CO_2)_4]^{6-,7-}$ [j]	0	400 (16 300); 300 (19 800)	−0.83	−0.59[e]	57	

[a] Before thiol addition. [b] In units of M^{-1} cm^{-1}. [c] ±5–10 mV. [d] ±5 mV. [e] 2.0–2.9 mM, 40 mM thiol. [f] 1.7–2.3 mM, 50 mM thiol. [g] Experimental value corrected to SHE. [h] Data from ref 14. [i] Unbuffered solution, no added thiol. [j] Data from ref 31, pH 10.1, thiol/tetramer mol ratio = 16/1.

Table III. Polarographic Data for $[Fe_4S_4(SR)_4]^{2-,3-}$ Redox Couples in Aqueous Solution (0.1 M $(Et_4N)(BF_4)$, 50 mM TrisCl, 25 °C)

Couple	Mol ratio $RSH/[Fe_4S_4(SR)_4]^{2-}$	pH	$E_{1/2}$, V (SCE)	λ_{max}, nm
$[Fe_4S_4(SCH_2CH_2OH)_4]^{2-,3-}$	3.8–38[a]	8.90–9.07	−0.75 to −0.77	
	3.6, 14[b]	9.45[e]	−0.75	
$[Fe_4S_4(S\text{-}(RS)\text{-}Cys(Ac)NHMe)_4]^{2-,3-}$	12–75[c]	8.05–8.25	−0.73 to −0.75	374–375
	4.9[d]	9.20[f]	−0.73	

[a–d] $[Fe_4S_4(SR)_4]^{2-}$ concentration (mM): [a] 1.59; [b] 2.79–2.84; [c] 0.66–1.56; [d] 4.11. [e] pH = pK_a of $HOCH_2CH_2SH$: D. M. E. Reuben and T. C. Bruice, *J. Am. Chem. Soc.*, **98**, 114 (1976). [f] pK_a of HS-(*RS*)-Cys(Ac)NHMe = 9.1 determined by titration with 0.1 M NaOH in 0.1 M $LiClO_4$.

Table IV. Spectral Data for *C. pasteurianum* Fd_{ox} and Polarographic Half-Wave Potentials for the Fd_{ox}/Fd_{red} Couple in Me_2SO/H_2O Solutions (0.1 M $(Et_4N)(BF_4)$, 50 mM TrisCl, aqueous component pH 8.4, 25 °C)

Me_2SO, vol %	Fd_{ox}[a] λ_{max}, nm[d]	$E_{1/2}$,[a,b] V SCE	SHE	−Slope,[c] mV	$\Delta E_{1/2}(V) = E_{1/2}([Fe_4S_4(SR)_4]^{2-,3-}) - E_{1/2}(Fd_{ox,red})$ R = CH_2CH_2OH	(*RS*)-Cys(Ac)NHMe
80	407, 287[e]	−0.93		63	−0.12	0.02
60	399, 286	−0.85		58	−0.14	0
47	390, 285	−0.74		60		
40	386, 285	−0.70		57	−0.19	−0.12
30	387, 286	−0.69		49		
20	386, 285	−0.70		58	−0.11	−0.09
0	388, 279	−0.67	−0.43[f]	57	−0.08	−0.06

[a] Fd concentration 0.20–0.35 mM. [b] ±10 mV. [c] ±7 mV. [d] ϵ_m 30 700–32 800 M^{-1} cm^{-1}. [e] Band maximum; low energy shoulder remains nearly constant with changes in solvent (cf. Figure 1). [f] Calculated value.

conditions where the solvent composition effects disruption of protein secondary and tertiary structure (see text). Under nominally identical conditions in solutions of the same solvent composition $E_{1/2}$ values for Fd and synthetic analogues are considered to be internally comparable. While the analogues were adequately responsive under most conditions tested, considerable difficulty was encountered in establishing a common set of conditions under which *C. pasteurianum* Fd was stable and afforded well-formed reproducible cathodic waves in purely aqueous *and* Me_2SO/H_2O solutions. In this respect TrisCl when used alone was unsatisfactory as the supporting electrolyte, giving little or no current response in certain Me_2SO/H_2O solutions, and ill-defined waves were obtained with rotating platinum and gold electrodes in aqueous and Me_2SO/H_2O solutions with several different supporting electrolytes. After substantial variation of experimental parameters the following conditions were selected for all analogue and protein conventional and differential pulse polarographic (DPP) measurements carried out in this investigation and reported in Tables II and IV: temperature, 25 ± 1 °C; working electrode, DME; reference electrode, KCl-saturated calomel electrode (for aqueous solutions $E_{SHE} = E_{SCE} + 0.242$ V at 25 °C); Hg column height, 62 cm; drop time, 0.5 s (regulated with a PAR Model 172 A drop timer); scan rate, 2–5 mV/s (Hg drop potential 1–2.5 mV); supporting electrolyte, 0.1 M $(Et_4N)(BF_4)$; concentrations, 0.20–0.35 mM Fd_{ox} and 1.7–2.3 mM $[Fe_4S_4(SR)_4]^{2-}$ (R = CH_2CH_2OH, (*RS*)-Cys(Ac)NHMe); aqueous phase, 50 mM TrisCl buffer, pH 8.4. Protein solutions were freshly prepared before use. All analogue solutions contained 40–50 mM added thiol to prevent solvolysis[31] which, however, was observed to occur at appreciable rates only in solutions

containing ≲60% Me_2SO and no thiol. In pure aqueous solutions containing thiol the final pH was 8.1–8.3, except for certain of the systems summarized in Table III. Background polarograms of all systems with and without added thiol were recorded to ensure proper identification of analogue and protein reduction waves. Reagent grade Me_2SO was fractionally distilled from sodium under reduced pressure and stored under a dinitrogen atmosphere. The aqueous phase containing 50 mM TrisCl, pH 8.4, was prepared by neutralizing an aqueous solution of Trizma Base (Sigma Chemical Co.) with aqueous hydrochloric acid. Controlled potential electrolytic reduction of Fd_{ox} was carried out at a Hg pool electrode at potentials of −0.90 (aqueous solution and −1.2 V (80% Me_2SO solution). Half-wave potentials in Tables II–IV were evaluated from plots of log $(i/(i_d - i))$ vs. E at $i = i_d/2$. Data were taken from current-sampled polarograms recorded at the above scan rates with capacitance compensation sufficient to give approximately zero slopes of the zero faradaic current plateau in background polarograms (solvent and electrolyte). Values of $E_{1/2}$ were unaltered at scan rates of 10 mV/s.

Results and Discussion

The principal purpose of this investigation is to provide a meaningful measure of differences between protein and analogue redox potentials associated with the couples in eq 2 and 3. The ferredoxin selected for this purpose, that of *C. pasteurianum*, is a prototypic clostridial 8-Fe Fd whose electronic properties in both the Fd_{ox} and Fd_{red} states are also representative of a wider group of bacterial proteins containing $[Fe_4S_4(S\text{-}Cys)_4]$ sites. In particular, there can now be no question that this protein molecule contains two separate and essentially equivalent 4-Fe sites incorporated in a protein structure quite similar to that established for the 8-Fe Fd of *Peptococcus aerogenes*.[9,32] Its reduction potential is biased toward the more negative end of the range of Fd_{ox}/Fd_{red} potentials (Table I) and at pH ~7 is within 30 mV of the values for other clostridial Fds[19,20] and for *P. aerogenes* Fd.[19]

Protein and Analogue Redox Centers. The behavior of biological metalloredox centers may be considered in terms of two sets of factors which are expected to affect potentials. The first set involves interrelated properties *intrinsic* to the center in oxidation levels coupled by electron transfer [series 1] and includes detailed stereochemistry, nature of ligands, and charge distribution within the center. A structural comparison of *P. aerogenes* Fd_{ox}[9,32] and several $[Fe_4S_4(SR)_4]^{2-}$ analogues,[26,27] presented elsewhere,[5b] demonstrates that $Fe_4S_4{}^*S_4$ cluster geometries are nearly identical. Thus the structures of at least the oxidized protein sites and analogues[33] in couples 2 and 3 correspond satisfactorily. While R = alkyl substituents in structure I are adequate simulators of cysteinate binding when seeking to approach closely electronic properties of Fd_{ox} and Fd_{red},[5b,7,28,33] not all such substituents are necessarily satisfactory for meaningful comparison of potentials of these two couples. The potentials of couple 3 are strongly dependent on the electron releasing propensity of R as measured by the Taft constant σ^*, with the potential variation empirically represented by $E_{1/2}(V) = 0.411\sigma^* - 1.30$ in DMF solution.[7] In order to provide a simple but credible representation of cysteinate binding at each Fe site without recourse to more difficultly accessible cysteinyl peptides,[14] the ligand acetyl-(*R,S*)-cysteine-*N*-methylamide was employed. It is readily attached to the $Fe_4S_4{}^*$ core by ligand substitution,[14,26] yielding $[Fe_4S_4(S\text{-}(RS)\text{-}Cys(Ac)NHMe)_4]^{2-}$. With those similarities in structure and, presumably, terminal cysteinate ligation, a similarly close degree of charge distribution within analogues and protein centers seems assured. The electrostatic potential model of Kassner and Wang[34] implies that without equivalent charge distributions, potentials may differ on this basis alone.

The second set of properties consists of those *extrinsic* to the redox centers and includes microscopic dielectric (local polarity) and noncovalent interactions with the center, principally solvation and hydrogen bonding. These properties are expected to be set by native protein structure at all levels. One possible role of extrinsic factors has been proposed on the basis of x-ray data to account for the relative stability of lower oxidation levels in Fd vs. Hp; viz., more extensive cluster–protein (S··· H–N) hydrogen bonding in the former produces a more polar local environment capable of stabilizing a larger net negative cluster charge[32b] (series 1). Disruption of the HP structure in 80% Me_2SO/H_2O, and therewith the native hydrogen-bonding interactions, allows the nonphysiological $HP_{red} \rightarrow HP_{s\text{-}red}$ reduction.[12] In addition, Kassner has provided experimental evidence[35a] and a theoretical model[35b] for the effects of local medium dielectric on the redox potentials of cytochromes and heme complexes.

Because synthetic analogues are unencumbered by protein structural and environmental effects, they are inherently incapable of manifesting in their redox potentials whatever specific extrinsic effects contribute to values of the potentials of proteins in their normal (aqueous solution) configurations. Provided intrinsic properties are nearly constant, as is highly likely here, differences in analogue and protein potentials under nominally similar or identical conditions afford the closest approach to a measure of the perturbation of intrinsic potentials of the redox center by the protein milieu. In addition to measurements in purely aqueous media *C. pasteurianum* Fd and analogues have been examined in various Me_2SO/H_2O mixtures up to 80% v/v in Me_2SO in order to assess the effects of disruption of protein secondary and tertiary structure on potentials. Evidence that disruption or unfolding of protein structure occurs with this Fd is afforded by broadened low-field contact-shifted ^1H NMR resonances and changes in the high-field region suggestive of random coil arrangements[36] (≳50% Me_2SO), removal of EPR fine structure in Fd_{red}[37a] indicative of spin–spin interaction[37b] between two doublet-state sites (≳70% Me_2SO), red shifts of the 390-nm Fd_{ox} chromophore band[38] (80% Me_2SO, vide infra), and $Fe_4S_4{}^*$ active site core extrusion with thiols[38] (80% Me_2SO, negligible rate in pure water).

Analogue Potentials and Spectra. In addition to $[Fe_4S_4(S\text{-}(RS)\text{-}Cys(Ac)NHMe)_4]^{2-}$, the properties of the previously unreported analogue $[Fe_4S_4(SCH_2CH_2OH)_4]^{2-}$ were examined in solvent media ranging from 100% Me_2SO to 100% H_2O in order to inspect in more than one case solvent effects on analogous potentials and spectra. Tetramethylammonium salts of both analogues, readily obtainable by ligand substitution reactions,[14,26] are freely soluble in water but require the presence of added thiol to suppress solvolysis.[31] These and all other $[Fe_4S_4(SR)_4]^{2-}$ species examined to date are stable in anaerobic aprotic solvents such as Me_2SO in the absence of excess thiol. Spectral and polarographic data in solvents ranging in composition from pure Me_2SO to pure water are presented in Table II. To provide evidence that the observed solvent-dependent trends in potentials and visible band maxima of the two analogues do not arise from partial solvolysis or degradation of structure I, a series of measurements was performed in pure aqueous solutions (Table III). These solutions contained mole ratios of added thiol to tetramer dianion both larger and smaller than those for solutions of Table II. In several cases pH ≃ pK_a(thiol), under which condition data for the only water-soluble analogue previously reported, $[Fe_4S_4(SCH_2CH_2CO_2)_4]^{6-}$,[31] were obtained. The 2−/3− potentials of the two analogues examined in this work and the 6−/7− potential of this complex are similar (Table II). The initial solvolysis product of $[Fe_4S_4(SCH_2CH_2CO_2)_4]^{6-}$ reduces a potential 0.17 V more negative than that of the intact cluster.[31] From these observations and the data of Table III it is concluded that the standardized conditions of Table II are sufficient to maintain intact $[Fe_4S_4(SR)_4]^{2-}$ species in the various Me_2SO/H_2O mixtures.

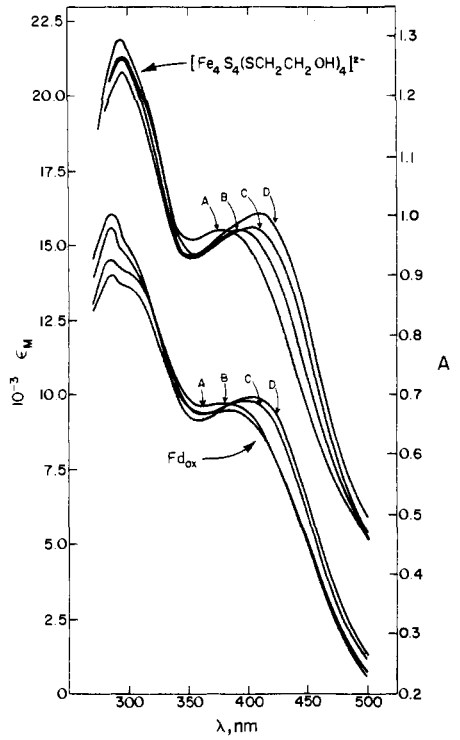


Figure 1. Dependence of the electronic spectra of $[Fe_4S_4(SCH_2CH_2OH)_4]^{2-}$ and *C. pasteurianum* Fd_{ox} on solvent composition in Me_2SO/H_2O solutions. The extinction coefficient and absorbance scales refer to analogue and protein, respectively. Solution conditions are given in Tables II and IV: A, 100% H_2O; B, 40% Me_2SO; C, 60% Me_2SO; D, 80% Me_2SO. In the lower set of spectra curve B is that immediately below curve A at ~350 nm.

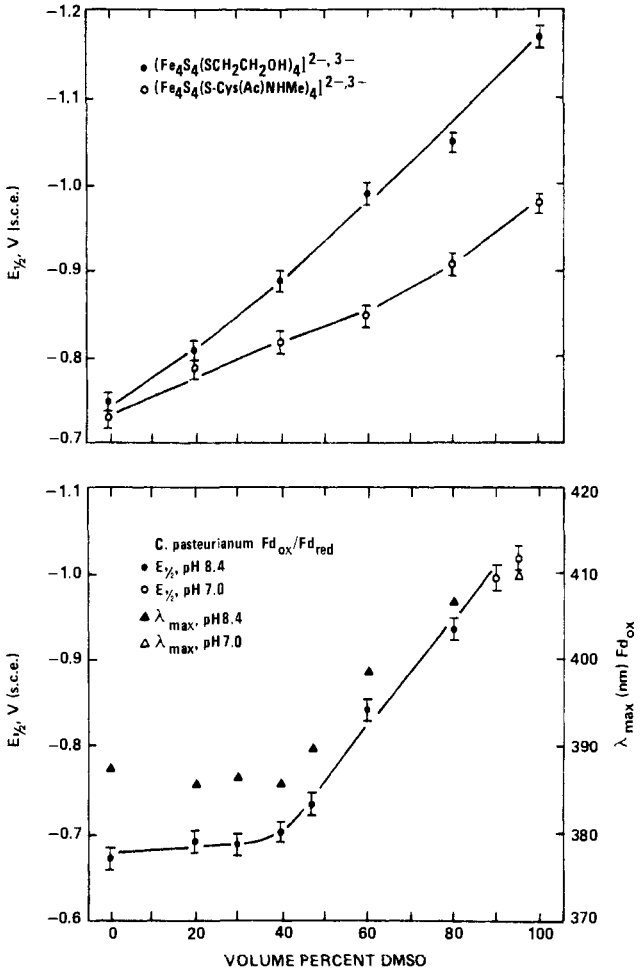


Figure 2. Dependence of half-wave potentials on solvent composition in Me_2SO/H_2O solutions. Upper: $[Fe_4S_4(SCH_2CH_2OH)_4]^{2-,3-}$ and $[Fe_4S_4(S\text{-}(RS)\text{-}Cys(Ac)NHMe)_4]^{2-,3-}$ couples. Lower: Fd_{ox}/Fd_{red} couple, with variation of visible absorption maximum also shown. Except for several Fd measurements of solutions whose aqueous component is pH 7.0 (50 mM TrisCl buffer), solution conditions are those defined in Tables II and IV.

Perturbation of analogue clusters by solvent changes is evidenced by the pronounced blue shifts of the Fe-S chromophore band in the visible upon passing from pure Me_2SO to pure aqueous solvent. These shifts are illustrated for $[Fe_4S_4(SCH_2CH_2OH)_4]^{2-}$ in Figure 1. The second Fe-S chromophore band is solvent insensitive, remaining virtually constant at 290-300 nm. Based on the current SCF-Xα-SW theoretical model of the electronic structure of $[Fe_4S_4(SR)_4]^{2-}$, these bands arise from (RS,S*) → Fe charge transfer excitations.[39] It is suggested that the solvent-sensitive band involves excitation from filled orbitals with dominant S* lone pair character inasmuch as models suggest that S* sites are less shielded from the environment by R substituents than are thiolate sulfur atoms.

The two analogues in the various solvent media exhibit well-defined 2−/3− dc polarographic or differential pulse polarographic (DPP) reduction waves for couples isoelectronic with Fd_{ox}/Fd_{red} and $HP_{red}/HP_{s\text{-}red}$ (series 1). In nearly all cases half-widths of pulse polarograms and plots of the polarographic Nernst equation

$$E = E_{1/2} - \frac{0.0592}{n} \log \frac{i}{i_d - i} \qquad (4)$$

are, or are within experimental uncertainty of, theoretical values of 90[40] and −59 mV, respectively, for a reversible process with $n = 1$ (vide infra). Examples of analogue polarograms[7,15,31] and demonstration that the product of the first polarographic reduction is $[Fe_4S_4(SR)_4]^{3-}$ [33] are given elsewhere. Concomitant with blue shifts of visible absorption bands are *positive* potential shifts of both analogues as the water content of solvent increases (Figure 2). Provided these shifts are not exclusively due to variations in liquid junction potential between analogue solutions of different solvent composition and the reference electrode solution, as seems unlikely,[41] tetramer trianions become more easily reduced as the hydrogen-bonding capability and dielectric constant increase. At constant solvent composition, where potential comparisons are more nearly exact, $E_{1/2}(R = CH_2CH_2OH) < E_{1/2}(R =$ (*RS*)-Cys(Ac)NHMe), an effect which diminishes with increasing water content and is only 0.02 V in pure aqueous solution compared to 0.19 V in pure Me_2SO. For the three analogues in pure water (Table II) the total potential spread is 0.10 V, or −0.49 to −0.59 V when converted to the SHE reference electrode.

Protein Potentials and Spectra. Data for *C. pasteurianum* Fd in 0-80% Me_2SO/H_2O solutions are collected in Table IV. The relationship between $[Fe_4S_4(SR)_4]^{2-}$, Fd_{ox}, and HP_{red} spectra has been discussed previously[5b,7,14] and for the first two species is apparent from Figure 1. Perturbation of the Fd_{ox} chromophore is evidenced by blue shifts of the visible band as the water content increases. Unlike the behavior of analogues, whose band shifts continue over the full range of solvent composition, Fd_{ox} band shifts remain virtually constant at ≲40% Me_2SO (Figure 2).

Although there have been numerous polarographic studies of small molecules as representations of protein prosthetic groups,[43] polarographic investigations of metalloproteins themselves are decidedly limited and for Fe-S proteins consist of two previous reports. For *Micrococcus lactilyticus* 8-Fe Fd Dalton and Zubieta[44] give $E_0 = -0.41$ V at pH 7 and a log plot

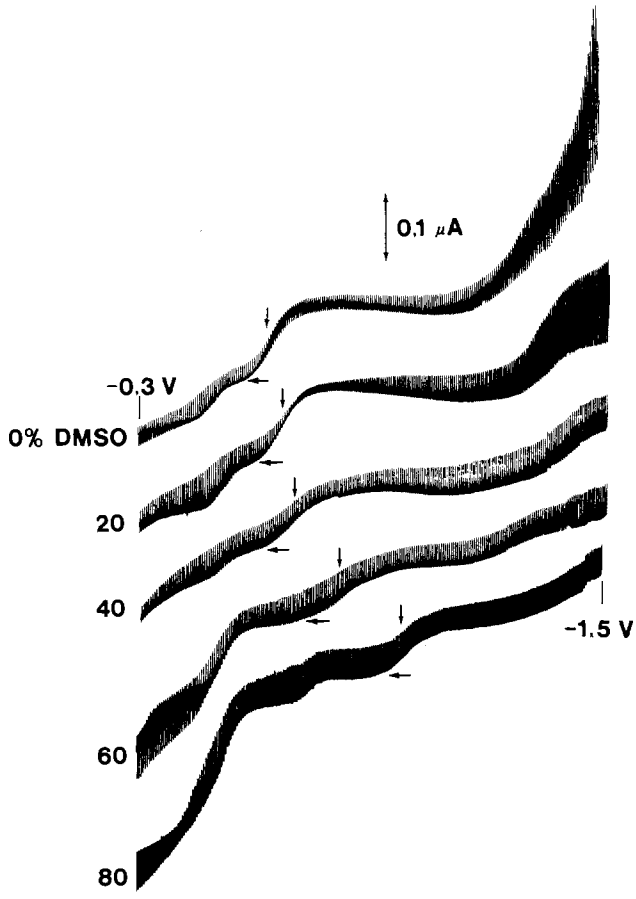


Figure 3. Survey polarograms of the reduction of *C. pasteurianum* Fd_{ox} as a function of solvent composition recorded at a scan rate of 10 mV/s over the potential range −0.3 to −1.5 V vs. SCE. $E_{1/2}$ positions are marked with vertical arrows and positions of zero faradaic current with horizontal arrows.

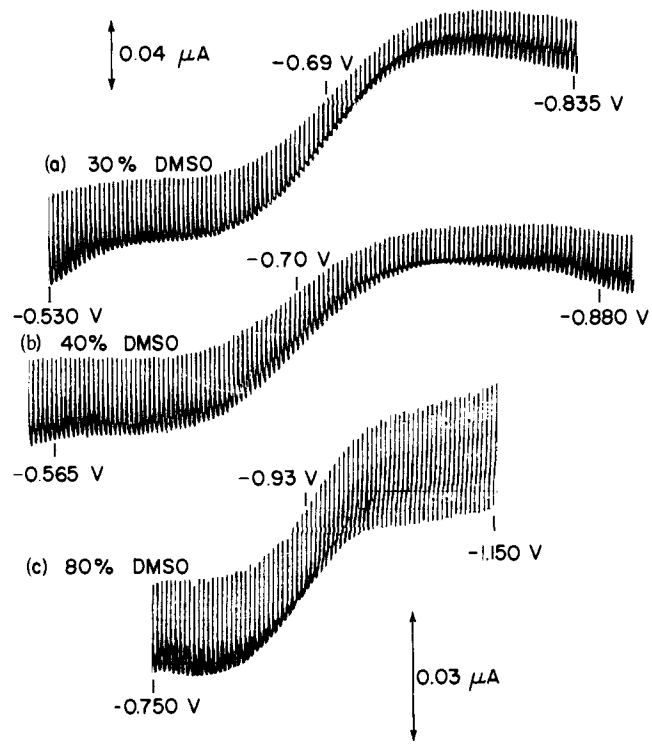


Figure 4. Polarograms of the Fd_{ox}/Fd_{red} reduction recorded in three different solvent media at scan rates of 5 (a, b) and 10 mV/s (c) and more sensitive current ranges than in Figure 3. The upper current scale refers to (a) and (b) and the lower to (c). $E_{1/2}$ values are given.

slope of 29 mV consistent with $n = 2$ in eq 4; experimental details were not included. Weitzman et al.[45] report for *C. pasteurianum* Fd $E_{1/2}$(SHE) = −0.325 (pH 7) and −0.340 (pH 8.5) in 0.1 M phosphate buffer. From their published polarographic waves we roughly estimate $|E_{3/4} - E_{1/4}| \sim 30$ mV for the reduction at pH 7 and ~75 and ~130 mV for Fd_{red} oxidation and Fd_{ox} reduction, respectively, in 0.1 M borate buffer, pH 9.2. Potentials are incompatible with those determined by other means (Table I) and variability in $|E_{3/4} - E_{1/4}|$ ($56/n$ mV for a reversible reaction) suggests irreversibility in the latter medium.

Clostridial ferredoxins have been established to be two-electron carriers.[3,46,47] Under our experimental conditions a single polarographic wave for the Fd_{ox}/Fd_{red} couple was observed, a finding not inconsistent with the small ($\lesssim$10 mV) difference in potentials determined for the two 4-Fe sites in *C. acidi-urici* and *C. pasteurianum* Fd by an NMR method.[48] Wide-scan polarograms of *C. pasteurianum* Fd in Me_2SO/H_2O solutions are presented in Figure 3. Waves assigned to the Fd_{ox}/Fd_{red} couple are marked with vertical arrows and several such waves recorded at increased current sensitivity are displayed in Figure 4. Demonstration that these waves are correctly assigned was obtained from spectral observations of aqueous and 80% Me_2SO solutions on which bulk electrolysis was performed at potentials −0.2 to −0.3 V more negative than $E_{1/2}$ values. In the experiments in Figure 5 solutions of Fd_{ox} first were reduced. Elimination of the visible absorption band and a decrease in intensity at wavelengths longer than ca. 350 nm are characteristic of the Fd_{red} chromophore.[3,46] These solutions were then reoxidized with air. Absorbance values at the visible band maxima were 0.95 (water) and 0.90 (80% Me_2SO) of the values prior to electrolysis.

The $E_{1/2}$ values for the Fd_{ox}/Fd_{red} couple in Table IV are plotted against solvent composition in Figure 2. The parallel response of visible absorption maxima and potential values is apparent, with both essentially invariant in solutions of $\lesssim$40% Me_2SO. The value of $E_{1/2}$ = −0.67 V vs. SCE or −0.43 V vs. SHE in aqueous solution departs substantially from the results of Weitzman et al.,[45] but is within 25 mV of the precisely determined values in Table I. In view of differences in measuring techniques and solution conditions the agreement may be considered satisfactory.

The present work is concerned with the reduction reactions 2 and 3. Inspection of the protein polarograms in Figure 3 reveals the presence of oxidation and, in several cases, additional reduction processes. While neither type has been examined in detail it is noted that the analogue couples $[Fe_4S_4(SR)_4]^{3-,4-}$ in aprotic solvents[7,15,49] and $[Fe_4S_4(SCH_2CH_2CO_2)_4]^{7-,8-}$ in water[31] have been observed with potential separations $\Delta E_{1/2}$ for the two reduction steps of ca. 0.70 and 0.45 V, respectively. In 20–47% Me_2SO solutions second cathodic protein waves with $E_{1/2}$ = −1.37 to −1.43 V ($\Delta E_{1/2} \sim 0.7$ V), slopes of 50–60 mV, and limiting current ratios with the first reduction of approximately unity have been detected. In the polarograms of Figure 3 a second wave is most evident in the 20% Me_2SO solution, but can be observed in other cases at higher current sensitivity or protein concentration. Above ca. 50% Me_2SO content waves in this potential region are distorted or obscured by background current. We conjecture that these waves correspond to reduction of Fd_{red} to a "superreduced" form, $Fd_{s\text{-}red}$, whose active sites are isoelectronic with $[Fe_4S_4(SR)_4]^{4-}$. As yet there is no evidence supporting a physiological role for this oxidation level.

Reversibility and Adsorption. The slope data for analogue and protein reductions in Tables II and IV are in most instances consistent with reversible processes and $n = 1$ despite the

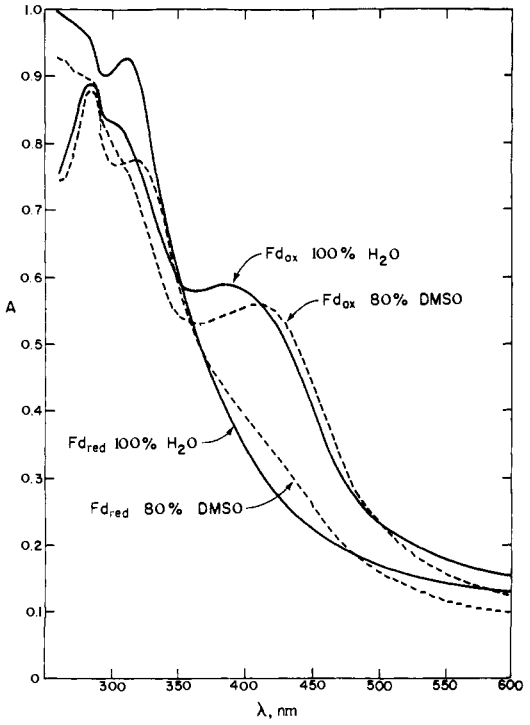


Figure 5. Spectra from controlled potential electrolysis of Fd_{ox} in pure aqueous and 80% Me_2SO solutions at −0.90 and −1.2 V vs. SCE, respectively. Fd_{ox} spectra were obtained by aerial oxidation of Fd_{red} solutions prepared electrolytically. (The protein used in this experiment had A_{390}/A_{280} 0.66.)

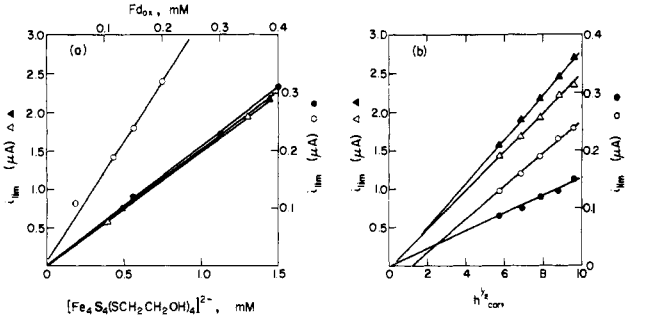


Figure 6. Tests for diffusion control in polarographic reductions of $[Fe_4S_4(SCH_2CH_2OH)_4]^{2-}$ (△,▲) and Fd_{ox} (○,●) in pure aqueous (△,○) and 80% Me_2SO (▲,●) solution at 25 °C: (a) concentration dependence of limiting current (h = 60 cm); (b) corrected merury column height dependence of limiting current: △, 1.3; ▲, 1.5; ○, 0.12; ●, 0.15 mM. Other solution conditions are the same as in Tables II and IV. Values of h_{corr} in 8% Me_2SO were approximated to be the same as those given for aqueous solution (ref 43a, p 132).

two-electron capacity of the protein. An elementary point is that adherence to eq 4 is a necessary but not sufficient criterion of reversibility because combinations of n and the transfer coefficient α in $0.0542/\alpha n$, the log term multiplier in the equation, otherwise identical with eq 4, for a totally irreversible process[50a] can afford a slope of −59 mV. An associated problem is intervention of non-diffusion-controlled polarographic currents with accompanying departures from the reversible behavior of eq 4.[50] One source of such behavior, particularly with proteins, is adsorption of electroactive species on the electrode surface. Adsorption effects have been encountered in polarographic studies of, e.g., cytochrome c[51] and *C. pasteurianum* Fd.[45]

To test for departures from purely diffusion-controlled polarographic waves, the behavior of one analogue, $[Fe_4S_4(SCH_2CH_2OH)_4]^{2-}$, and Fd_{ox} were examined in the limiting pure aqueous and 80% Me_2SO media of Tables II and IV over the concentration ranges in Figure 6a. The classical criteria employed here for diffusion control and adsorption effects are discussed elsewhere:[50] (i) dependence of $E_{1/2}$ on concentration and drop time (t = 0.5–12 s)—$E_{1/2}$ invariant (±0.01 V); (ii) dependence of limiting current on concentration—proportional to total concentration, Figure 6a; (iii) dependence of limiting current on corrected mercury column height—proportional to $h^{1/2}_{corr}$, Figure 6b; (iv) temperature coefficient of limiting current (4–26 °C, %/deg, calculated after Meites[52])—analogue, +1.1 (H_2O), +1.2 (80% Me_2SO); Fd, +1.2 (H_2O), +0.9 (80% Me_2SO); (v) wave form—single waves in all cases at concentrations of 0.1 mM or higher. Of the four systems examined in this phase of the work, Fd in 80% Me_2SO was the least satisfactory. In 0.15–0.40 mM concentration range *exact* wave shapes were somewhat irreproducible and not strictly Nernstian with a mean slope of −63 mV; $E_{1/2}$ = −0.93 ± 0.01 V for all waves. In addition, Fd_{ox} in this medium, unlike Fd_{ox} in aqueous solution, at concentrations below ca. 50 μM, displayed two closely spaced reduction waves indicative of adsorption.[50b,c] Owing to the limited quality of these small waves the functional dependence of limiting current and $E_{1/2}$ on h_{corr} and Fd concentration could not be accurately determined.

Results i-v tend to eliminate catalytic processes and kinetic current control (preceding chemical reaction) as significant contributors to the electrode process.[50] They do not necessarily eliminate any Fd-Hg electrode adsorption effects,[53] particularly the case of adsorption of both oxidized and reduced forms. The possibility cannot be dismissed that $E_{1/2}$ values may be perturbed by adsorption complications. However two results already noted, the adequate agreement between $E_{1/2}$ for the protein and the data in Table I, and the parallel behavior of $E_{1/2}$ and λ_{max} for the analogues and proteins with medium variation, constitute good evidence that adsorption effects on potentials are slight compared to other factors. Strict electrochemical reversibility of the Fd_{ox}/Fd_{red} process as described by eq 4 is more difficult to establish from the data at hand. The approach of the majority of log plot slopes in Table IV to the value of −59 mV is highly suggestive of reversible or near reversible electron transfers with n = 1, but could also arise from an irreversible process with $\alpha n \simeq 0.9$. There is no evidence under the conditions employed here for a reversible process with n = 2 (slope −30 mV). Recent precise studies of the reduction potentials of ferredoxins[19,20] by equilibrium techniques show that the experimental data are best described by n = 1 in the appropriate Nernst equation. We favor interpretation of our polarographic current-voltage curves with slopes near −59 mV as essentially reversible processes which conform to eq 4 with n = 1. In addition to the larger errors in analyzing current-voltage curves of protein compared to analogues owing to lower concentrations of the former (dictated in part by the supply of ferredoxin), deviations from the theoretical slope value could conceivably arise from several sources: (i) unresolved overlapping waves from the two 4-Fe centers which distort overall wave shape; (ii) rates of electron transfer from electrode to Fd_{ox} molecules insufficient to maintain strict Nernstian concentrations at the electrode surface during the time scale of measurement. In the latter case the observed $E_{1/2}$ will be cathodically shifted from the reversible $E_{1/2}$ and the reactions may be regarded as quasi-reversible.

Summary and Conclusions

In offering the following statements several points of emphasis are in order. We do not insist that all potentials in Tables II and IV refer to demonstrably reversible reductions totally unaffected by adsorption effects and thus are of strict thermodynamic significance in the sense that $E_{1/2} = E_0 - (0.0592/n)$ log $(D^{1/2}_{ox}/D^{1/2}_{red})$ where D is the diffusion

coefficient of the indicated species. Use of the same media in obtaining the data in Tables II and IV obviously cannot assure equivalent extrinsic perturbations of analogues and sites in unfolded proteins. Nonetheless, the set of potentials in these tables presents a greatly improved basis for assessment of relative protein-analogue values than that available prior to its acquisition. In this sense internal comparison of potentials is considered legitimate, especially in the same medium.

(i) The Me_4N^+ salts of $[Fe_4S_4(SCH_2CH_2OH)_4]^{2-}$ and $[Fe_4S_4(S\text{-}(RS)\text{-}Cys(Ac)NHMe)_4]^{2-}$ are soluble and stable in aqueous alkaline solution in the presence of added thiol. Together with the original contribution by Job and Bruice[31] such compounds permit extension of analogue chemistry, developed mainly in aprotic solvents,[5] to aqueous solution.

(ii) Half-wave potentials of the couples $[Fe_4S_4(SCH_2CH_2OH)_4]^{2-,3-}$ $[Fe_4S_4(S\text{-}(RS)\text{-}Cys(Ac)NHMe)_4]^{2-,3-}$ and visible absorption spectral maxima of the dianions undergo monotonic positive and high-energy shifts, respectively, as the solvent composition is varied from 80% Me_2SO/H_2O to pure water.

(iii) *C. pasteurianum* Fd_{ox} in 0–80% Me_2SO/H_2O solutions exhibits a single cathodic wave for the Fd_{ox}/Fd_{red} couple with $E_{1/2} = -0.67$ to -0.93 V and log plot slopes in nearly all cases within experimental error of -59 mV for a reversible process with $n = 1$. For this and other 8-Fe proteins[19,20] this n value implies reduction of two independent equivalent sites,[47c] a circumstance which, on the basis of protein structure[9,32] (site separation ca. 12 Å) and slight 4-Fe site potential differences,[48] would appear to be very closely approached.

(iv) Half-wave potentials of the Fd_{ox}/Fd_{red} couple and visible absorption spectral maxima undergo monotonic positive and high-energy shifts as the solvent composition is varied from 80 to ~40% Me_2SO/H_2O. At $\lesssim$40% Me_2SO to pure aqueous solution potentials and band maxima are nearly invariant. As in the case of the analogues in (ii) monotonic shifts arise from increasing solvation and hydrogen bonding of the $Fe_4S_4(S\text{-}Cys)_4$ sites by water. The invariant behavior is attributed to conversion of the protein from an unfolded structure with sites rather well exposed to solvent to its normal aqueous structure (or close thereto) within a narrow solvent composition range (~40–50% Me_2SO). In this structure the two sites are less accessible to the medium and reside in an environment provided by packing of hydrophobic residues and hydrogen bonding.[9,32,54] Previous ^1H NMR experiments have shown that this protein undergoes reversible denaturation in solutions containing ca. 50–60% Me_2SO,[36] a finding reasonably consistent with the present results.

(v) Potential differences between analogues and protein in the same medium vary somewhat irregularly (Table IV). For $[Fe_4S_4(S\text{-}(RS)\text{-}Cys(Ac)NHMe)_4]^{2-,3-}$, presumably the more realistic analogue couple, these differences do not exceed -0.12 V. In 80% Me_2SO any differences in intrinsic cluster properties affecting protein vs. analogue potentials should be largest owing to the (expected) maximally unfolded protein structure. The potential difference in this medium amounts to $+0.02$ V. This small difference reinforces an earlier conclusion[14] that protein denaturation affords the active sites in a condition such that features inherent to $[Fe_4S_4(S\text{-}Cys)_4]$ analogue clusters in the same medium are approached. In solutions with $\lesssim$40% Me_2SO content and particularly in pure water where the protein adopts its normal structure, the largest extrinsic effects on protein vs. analogue potentials are anticipated. Under these conditions potential differences are -0.06 to -0.12 V, which are considered to be the best available estimates of extrinsic protein structural effects on 4-Fe site potentials. Similar estimates may be obtained from the data in Table I using ca. -0.50 V as the inherent value of the $[Fe_4S_4(S\text{-}Cys)_4]^{2-,3-}$ couple in water unperturbed by influences of protein structure and environment. We refrain from numerical values because of the lack of standardized conditions of potential determination. However, within the body of data presented variations of residues 1 and 2 in *C. acidi-urici*, differences between clostridial and *Chromatium* proteins, and the unprecedented difference between $1-/2-$ potentials of *A. vinelandii* Fd I[55] represent clear examples of extrinsic effects.

(vi) From (v) it follows that designation of $[Fe_4S_4(SR)_4]^{2-,3-}$ species as synthetic analogues may be widened to include the property of redox potentials for at least those proteins with $E \lesssim -0.40$ V in Table I. If the potentials of *B. stearothermophilus* Fd and *D. desulfuricans* Fd are correct, we infer from the present results that their large positive shifts compared to the unperturbed potential of ca. -0.50 V arise principally from extrinsic protein structural effects.

Acknowledgment. This research was supported at Stanford University by National Institutes of Health Grants GM 22351-01 and GM 22352, and at Purdue University by National Institutes of Health Grant AI 04865 and National Science Foundation Grant GB-22629. We thank B. V. DePamphilis for certain preliminary experiments, Dr. N. A. Stombaugh for provision of protein samples used in those experiments, and Dr. R. W. Johnson for stimulating discussions.

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(6) Abbreviations used in this paper: Fd, ferredoxin; HP, high-potential protein; Rd, rubredoxin; S-Cys, cysteinate; S^*, sulfide (where useful for clarity); SHE standard hydrogen electrode; SCE, saturated calomel electrode.
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Stereochemistry of Manganese Porphyrins. 3. Molecular Stereochemistry of $\alpha,\beta,\gamma,\delta$-Tetraphenylporphinato-(1-methylimidazole)manganese(II)[1,2]

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Received November 8, 1976

Abstract: $\alpha,\beta,\gamma,\delta$-Tetraphenylporphinato(1-methylimidazole)manganese(II) crystallizes as the tetrahydrofuran solvate in the monoclinic system, space group $P2_1/n$. The unit cell has $a = 27.405$ (7) Å, $b = 9.645$ (5) Å, and $c = 17.768$ (9) Å, and $\beta = 112.45$ (2)°, and contains four molecules. The calculated and experimental densities are 1.258 and 1.27 g/cm^3, respectively, at 20 ± 1 °C. Measurement of diffracted intensities employed ω scans with graphite-monochromated Mo Kα radiation on a Syntex four-circle diffractometer. All independent reflections for $(\sin\theta)/\lambda \leq 0.626$ Å^{-1} were measured; 5276 reflections were retained as observed. These data were employed in the determination of structure using the heavy-atom method and block-diagonal least-squares refinement of the 540 structural parameters. The final conventional and weighted discrepancy factors are 0.052 and 0.061, respectively. The structural parameters of the square-pyramidal MnN_5 coordination group reflects the large size of the high-spin manganese(II) atom; the average equatorial Mn–N distance is 2.128 Å and the axial Mn–N bond distance is 2.192 Å. The manganese atom is displaced 0.56 Å from the mean skeletal plane. The relevance of this structure to deoxyhemoglobin and manganese-substituted deoxyhemoglobin is discussed.

The stereochemistry of high-spin manganese(II) porphyrins, the isoelectronic high-spin iron(III) and the high-spin iron(II) porphyrins are expected to be dominated by the large size of the metal atom. This large size should yield derivatives in which the metal atom is substantially out of the porphyrin plane. We report herein the molecular stereochemistry of a five-coordinate high-spin manganese(II) derivative, $\alpha,\beta,\gamma,\delta$-tetraphenylporphinato(1-methylimidazole)manganese(II), to be written as Mn(1-MeIm)(TPP).[4] We compare the molecular stereochemistry of Mn(1-MeIm)(TPP) with other five-coordinate metalloporphyrin derivatives including those which, like the high-spin manganese(II) derivative, contain spherically symmetric metal ions. We also compare the results for Mn(1-MeIm)(TPP) with those obtained for high-spin four-coordinate MnTPP[5] in which the out-of-plane displacement is considerably smaller.

The structure of Mn(1-MeIm)(TPP) is of considerable interest as a model for the coordination group in manganese-substituted heme proteins, and in particular for manganese-substituted hemoglobin, MnHb. MnHb has been shown to exhibit allosteric properties in its axial ligation and oxidation–reduction reactions.[6] In addition, x-ray studies have