Bocarsly for assistance with the electrochemical measurements. We are grateful to Dr. B. C. UnniNair who kindly checked our Mn analytical data. This work has been supported by a grant from the Department of Energy/Solar Energy Research Institute (DE-FG02-80CS84003) and by an award from the Chicago Community Trust/Searle Scholars Program (G.C.D.).

Registry No. $Mn_2(AQL)_4$, 87556-35-2; $Mn_2(AQL)_4L_2$ (L = CH_3CN), 87556-36-3; $Mn_2(AQL)_4L_2$ (L = DME), 87556-37-4; 2-acetyl-1,4-benzoquinone, 1125-55-9; dimanganese decacarbonyl, 10170-69-1.

Positive Shift of Redox Potential of $[Fe_4S_4(Z\text{-cys-Gly-Ala-OMe})_4]^{2-}$ in Dichloromethane

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Abstract: $[Fe_4S_4(Z\text{-cys-Gly-OMe})_4][NMe_4]_2$ (**1**) and $[Fe_4S_4(Z\text{-cys-Gly-Ala-OMe})_4][NMe_4]_2$ (**2**) were prepared from $[Fe_4S_4(S\text{-}t\text{-Bu})_4][NMe_4]_2$ and the corresponding peptides as a model of *P. aerogenes* Fd. A positive shift in redox potential (120 mV) of **2** was observed in dichloromethane at 233 K. No shift was detected in the case of **2** in the dimethylformamide or **1** in CH_2Cl_2 even at low temperatures. The results strongly suggest that the shift of redox potential is caused by the NH---S hydrogen bond between Ala NH and Cys S in **2**. The CD spectra and IR spectra of **2** also support a peptide folding induced by the NH---S bond.

Introduction

The importance of redox potentials in biochemical electron transfer is well known, but factors controlling the redox potentials of native ferredoxins have to be elucidated. A synthetic model of 4Fe ferredoxin, e.g., $[Fe_4S_4(SCH_2Ph)_4]^{2-/3-}$, has a redox potential at −1.25 V (SCE) in DMF which is to be compared with those (e.g., −0.93 V (SCE) in Me_2SO, −0.67 V in aqueous solution for *C. pasteurianum*, −0.57 V in aqueous solution for *D. desulfuricans*) of native ferredoxin.[1-3] Holm and his co-workers[1] inferred that the value of 2−/3− couples of native ferredoxins such as *C. pasteurianum* Fd,[2] *D. desulfuricans* Fd,[3] and *C. acidiurici* Fd[3] arises principally from extrinsic protein structural effects. Their efforts in the synthesis of the peptide analogues have realized the positive shifts to some extent in $[Fe_4S_4(Ac\text{-cys-NHMe})_4]^{2-}$ [1] and $[Fe_4S_4(t\text{-Boc(-Gly-cys-Gly-)}_4NH_2)]^{2-}$,[4] which have redox potential at −0.98 V (SCE) in Me_2SO and −0.91 V (SCE) in Me_2SO, respectively. They reported that the redox potentials of those peptide complexes in Me_2SO approach that of denatured *C. pasteurianum* ferredoxin in Me_2SO. However, a redox potential difference of ca. 0.12 V between the *native* ferredoxins and the synthetic peptide analogues still exists.[1] In this paper, we show that the redox potential of native protein could successfully be reproduced in 4Fe-model complexes of Cys-containing tripeptide, Z-Cys-Gly-Ala-OMe.

There have been some speculations[5,6] on the difference of redox potentials, invoking formation of NH---S hydrogen bonds on the basis of the X-ray analysis of *P. aerogenes*[7] which has an homologous sequence to *Clostridium pasteurianum*.[8] However, Sweeny's group[9] has disputed this by the experimental facts that deuteration of the *exchangeable* amide protons involving the hydrogen bonds yields essentially no shift in the reduction potential.

We have examined the contribution of NH---S bonding to the redox potential of the synthetic 4Fe4S–peptide complexes with a *characteristic sequence* -Cys-Gly-Ala-. The sequence prefers special conformation in the protein circumstance. In this paper, we report the electrochemical behaviors of the 4Fe4S ferredoxin model complexes containing Z-L-Cys-Gly-OMe (Z = carbobenzoxy) or Z-L-Cys-Gly-L-Ala-OMe. Such a tripeptide containing a sequence Cys-Gly-Ala was selected as the characteristic sequence of *P. aerogenes* which causes the formation of the intramolecular NH---S bond[7] between the S atom of the Cys residue and to amide NH of the Ala residue. A 4Fe4S–dipeptide complex containing Cys-Gly was also examined because of its inability in intramolecular NH---S bonding as shown in Figure 1.

Experimental Section.

Preparation of Peptides. **(a) *N*-Carbobenzoxy-L-*S*-(acetamidomethyl)cysteinylglycine methyl ester (Z-Cys(Acm)-Gly-OMe)** was prepared from *N*-carbobenzoxy-L-*S*-(acetamidomethyl)cysteine and glycine methyl ester by using dicyclohexylcarbodiimide. Recrystallization of the crude compounds was carried out from methanol/ether: mp 135.6–137.0 °C (dec; $[\alpha]^{25}_D$ −22.5° (*c* 0.111, DMF). Anal. Calcd for $C_{17}H_{23}N_3O_6S$: C, 51.37; H, 5.83; N, 10.57. Found: C, 51.15; H, 5.79; N, 10.41. **(b) *N*-Carbobenzoxyl-L-*S*-(acetamidomethyl)cysteinylglycyl-L-alanine methyl ester (Z-Cys(Acm)-Gly-Ala-OMe)** was prepared from glycyl-L-alanine methyl ester and *N*-carbobenzoxy-L-*S*-(acetamidomethyl)cysteine by following the mixed anhydride method: mp 180.2–181.6 °C dec; $[\alpha]^{25}_D$ −21.6° (*c* 0.111, DMF). Anal. Calcd for $C_{20}H_{28}N_4O_7S_4$: C, 51.27; H, 6.02; N, 11.96. Found: C, 51.57; H, 6.09; N, 11.25. **(c) Z-Cys-Gly-OMe.** Cleavage of S-blocking groups was performed by the following method. To the solution of Z-Cys(Acm)-Gly-OMe (0.84 g, 2.1×10^{-3} mol) in Me_2SO were added mercury(II) chloride (1.15 g, 0.42×10^{-3} mol) and 5 mL of water at room temperature. The white precipitates obtained were collected and washed with water. The mercury(II) complex of Z-Cys-Gly-OMe was dispersed in methanol, followed by bubbling gaseous hydrogen sulfide for 15 min. After black solids were filtered off, concentration of the methanol solution under reduced pressure gave white crystals that were washed with degassed ether. **(d) Z-Cys-Gly-Ala-OMe.** The Acm group of Z-Cys(Acm)-Gly-Ala-OMe was removed by the same procedure mentioned above.

Preparation of 4Fe–4S Complexes. All 4Fe–4S complexes of Cys-containing peptides were prepared from $[Me_4N]_2[Fe_4S_4(S\text{-}t\text{-Bu})_4]$ with the ligand exchange method reported by Holm.[1,4,10] $[Et_4N]_2[Fe_4S_4(SCH_2Ph)_4]$ was prepared by the method reported by Averill et al.[11]

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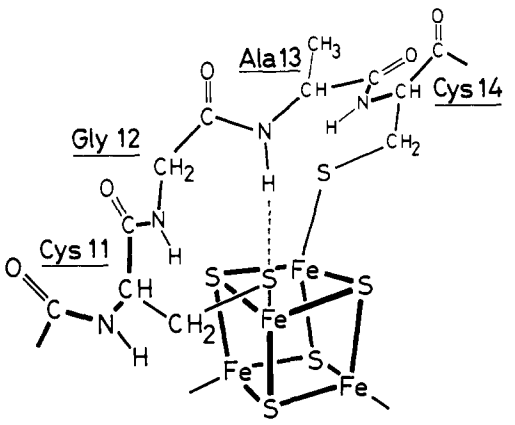

Figure 1. Structure of Cys-Gly-Ala around the active site of *P. aerogenes* illustrated on the basis of the X-ray analysis by Adman et al.[7]

which was used as a standard for electrochemical measurements in CH_2Cl_2.

(a) [Me_4N]$_2$[Fe_4S_4(Z-cys-Gly-OMe)$_4$].[12] To a solution of [Me_4N]$_2$[Fe_4S_4(S-*t*-Bu)$_4$] (50 mg, 5.57×10^{-5} mol) in DMF was added a solution of Z-Cys-Gly-OMe (74 mg, 2.23×10^{-4} mol) in DMF at room temperature. After the solution was warmed at 40 °C for 30 min, DMF and *tert*-butanethiol were removed under reduced pressure and the black solid was obtained and washed with degassed ether. No ^{1}H NMR signal of *tert*-butyl protons indicative of coordinated *tert*-butanethiolate was observed at 2.4 ppm in Me_2SO-d_6. The concentration of the synthetic complexes containing $Fe_4S_4^{2+}$ cores was determined with the method reported by Gillum et al.[13]

(b) [Me_4N]$_2$[Fe_4S_4(Z-cys-Gly-Ala-OMe)$_4$]. To a solution of [Me_4N]$_2$[Fe_4S_4(S-*t*-Bu)$_4$] (38 mg, 4.39×10^{-5} mol) in DMF was added a solution of Z-Cys-Gly-Ala-OMe (65 mg, 1.76×10^{-4} mol) in DMF at ambient temperature. The solution was warmed at 40 °C for 30 min and was concentrated under reduced pressure to remove the solvent and *tert*-butanethiol. An attempt to crystallize the solid was unsuccessful. The concentration of the complex was determined by the same method mentioned above.

Physical Measurements. All operations of physical measurements were performed under an argon atmosphere. ^{1}H NMR spectra were recorded on a Varian XL-100 spectrometer with Me_4Si as internal standard. Visible spectra and CD spectra were obtained by using a cell of 1 mm path length on JASCO UVDEC-5A and J-40A spectrometers, respectively. Infrared spectra were recorded on a JASCO DS-402G spectrophotometer with 0.1 mm cell path of KBr. Electrochemical measurements were carried out on a YANACO P8-CV with a three-electrode system consisting of a glassy carbon working electrode, a platinum-wire auxiliary electrode, and a saturated calomel compartment with a nonaqueous salt bridge connected by glass frits. Solutions were ca. 2×10^{-3} M in metal complex, and 0.1 M tetra-*n*-butylammonium perchlorate was used as a supporting electrolyte. DMF and CH_2Cl_2 were purified by distillation under an argon atmosphere before use.

Results

The intrinsic properties of the 4Fe–4S center have been extensively investigated by Holm's group.[1] The main purpose of our study is to interpret the difference of the redox potentials between the *native* ferredoxins and the synthetic model complexes. The observed difference is thought to be attributable to the nature of the peptide ligands rather than the $Fe_4S_4^{2+}$ core. Two 4Fe–4S complexes containing peptide ligands, Cys-Gly or Cys-Gly-Ala, were prepared by the procedure established by Averill,[11] but crystallization of [Me_4N_2][Fe_4S_4(peptide)$_4$] (peptide = Z-cys-Gly-OMe, Z-cys-Gly-Ala-OMe) was unsuccessful.

Absorption and ^{1}H NMR Spectra. ^{1}H NMR spectra of these complexes showed the ratio of OMe protons to NMe_4 protons exactly corresponding to that of [Me_4N]$_2$[Fe_4S_4(peptide)$_4$]. The absorption spectra of [Fe_4S_4(Z-cys-Gly-OMe)$_4$]$^{2-}$ and [Fe_4S_4(Z-cys-Gly-Ala-OMe)$_4$]$^{2-}$ were measured to ensure the complete retention of the $Fe_4S_4^{2-}$ core. The visible absorption maxima for [Fe_4S_4(Z-cys-Gly-Ala-OMe)$_4$]$^{2-}$ were observed at 293 nm (ϵ 21 600) and 402 nm (ϵ 16 100) in DMF and at 288 nm (ϵ 21 500) and 402 nm (ϵ 15 300) in CH_2Cl_2, while maxima for [Fe_4S_4(Z-cys-Gly-OMe)$_4$]$^{2-}$ were observed at 289 nm (ϵ 21 800) and 406 nm (ϵ 14 800) in DMF and at 292 nm (ϵ 21 300) and 390 nm (ϵ 16 000) in CH_2Cl_2. Absorption maxima of [Fe_4S_4(Ac-cys-NHMe)$_4$]$^{2-}$ and [Fe_4S_4(12-peptide)]$^{2-}$ (12-peptide = *t*-Boc(-Gly-L-Cys-Gly-)$_4$$NH_2$) in Me_2SO are at 294 nm (ϵ 22 700) and 409 nm (ϵ 16 600)[4] and at 290 nm (ϵ 25 500) and 406 nm (ϵ 17 100 in 80% Me_2SO–water solution,[4] respectively. Therefore, our results indicate that the synthetic complexes have the $Fe_4S_4^{2+}$ core in either DMF or CH_2Cl_2.

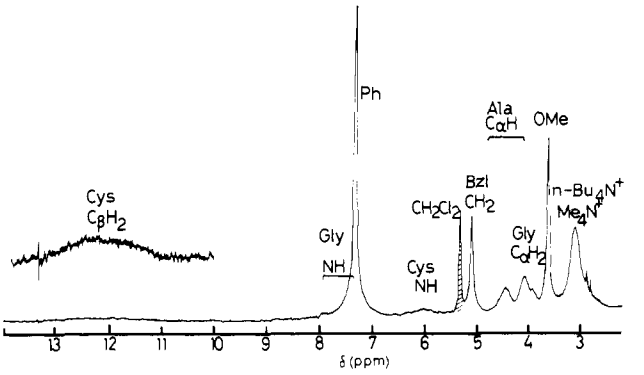

Figure 2. ^{1}H NMR spectra of [Fe_4S_4(Z-cys-Gly-Ala-OMe)$_4$][NMe_4]$_2$ in CD_2Cl_2. (n-Bu)$_4$$NClO_4$ was added to the solution to correspond to the electrochemical measurement.

Coordination of the cysteine thiolato to the $Fe_4S_4^{2+}$ core is also confirmed by the ^{1}H NMR contact shift of $C_\beta H_2$ protons. A peak was observed at 12.0 ppm for the $C_\beta H_2$ protons of [Fe_4S_4(Z-cys-Gly-Ala-OMe)$_4$]$^{2-}$ and at 13.2 ppm for those of [Fe_4S_4(Z-cys-Gly-OMe)$_4$]$^{2-}$ in CD_2Cl_2 as shown in Figure 2. Generally, $C_\beta H_2$ proton resonances of the Cys residue coordinated to a diamagnetic metal ion such as Pd(II) were observed at about 3 ppm.[15] The contact shifts of methylene or methyl protons adjacent to the coordinated thiolato ligand were reported to be observed at 10.1 ppm for [Fe_4S_4(SCH_2Ph)$_4$]$^{2-}$ in CD_3CN (295 K)[16] and at 12.8 ppm for [Fe_4S_4(SCH_3)$_4$]$^{2-}$ in CD_3CN (295 K),[16] at 10.1 ppm for [Fe_4S_4(SCH_2CH_3)$_4$]$^{2-}$ in CD_3CN (295 K),[16] and at 12.9 ppm for [Fe_4S_4(SCH_2CH_2OH)$_4$]$^{2-}$ in Me_2SO-d_6 (307 K).[17] On the other hand, the proton peaks of $C_\beta H_2$ analogous complexes containing Cys residues, such as [Fe_4S_4(Ac-cys-NHMe$_4$]$^{2-}$, [Fe_4S_4(9-peptide)(Ac-cys-NHMe)]$^{2-}$ (9-peptide = *t*-Boc(-Gly-Cys-Gly)$_3$$NH_2$), or [$Fe_4S_4$(12-peptide)]$^{2-}$, were reported to be found at 13.6 and 12.4 ppm, 13.0 ppm, or 13.0 ppm (304 K), respectively.[4] Broadened signals due to protons other than the $C_\beta H_2$ proton signals were observed without any contact shift. The complex of the dipeptide provided two clear NH signals, while [Fe_4S_4(Z-cys-Gly-Ala-OMe)$_4$]$^{2-}$ gave a sharp peak due to Cys NH at 6.0 ppm, but unfortunately NH protons of Gly and Ala residues were equivocal in CD_2Cl_2.

The ^{1}H NMR pattern due to cys $C_\beta H$ protons of [Fe_4S_4(Z-cys-Gly-Ala-OMe)$_4$]$^{2-}$ in CD_2Cl_2 was temperature dependent (12.0 ppm at 303 K to 9.5 ppm at 233 K). Observed temperature dependence is also reported for [Fe_4S_4(SCH_2Ph)$_4$]$^{2-}$ (8.55 ppm at 226 K to 11.3 ppm at 355 K).[16]

Electrochemical Properties. The redox potentials of the two model complexes were examined in DMF and CH_2Cl_2 by cyclic voltammetry. These solvents can be used without partial solvolyis or degradation of $Fe_4S_4^{2+}$ core because Hill et al. reported that the potentials of [Fe_4S_4(SCH_2CH_2OH)$_4$]$^{2-}$ or [Fe_4S_4(Ac-cys-NHMe)$_4$]$^{2-}$ were solvent dependent.[1] The 2-/3- redox potentials

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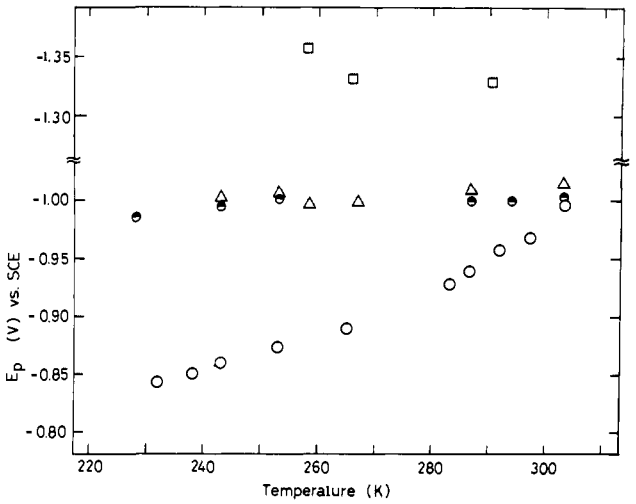

Figure 3. Temperature dependence of redox potentials (E_p vs. SCE) of $[NMe_4]_2[Fe_4S_4(Z\text{-cys-Gly-OMe})_4]$ (**1**) and $[NMe_4]_2[Fe_4S_4(Z\text{-cys-Gly-Ala-OMe})_4]$ (**2**). Sample codes: (a) ▲, **1** in CH_2Cl_2; (b) ○, **2** in CH_2Cl_2; and (c) ●, **2** in DMF; (d) □, $[Fe_4S_4(SCH_2Ph)_4][NMe_4]_2$ in CH_2Cl_2. Conditions: 0.1 M $(n\text{-Bu})_4NClO_4$.

Table I. Cyclic Voltammetric Data of 4Fe4S–Peptide Complexes

complexes	solvents	T, K	$E_{1/2}$	E_{pc}	E_{pa}	i_{pc}/i_{pa}
$[NMe_4)_2[Fe_4S_4$-(Z-cys-Gly-OMe)$_4]$	CH_2Cl_2	287	−1.01	−1.11	−0.91	0.83
		243	−1.00	−1.15	−0.84	1.14
$[NMe_4]_2[Fe_4S_4$-(Z-cys-Gly-Ala-OMe)$_4]$	DMF	287	−1.00	−0.86	−1.14	0.79
		243	−0.99	−0.78	−1.20	1.25
$[NMe_4]_2[Fe_4S_4$-(Z-cys-Gly-Ala-OMe)$_4]$	CH_2Cl_2	304	−0.99	−0.90	−1.08	0.70
		298	−0.97	−0.88	−1.05	0.75
		292	−0.96	−0.87	−1.04	0.76
		287	−0.94	−0.85	−1.03	0.75
		283	−0.93	−0.83	−1.03	0.85
		256	−0.89	−0.79	−0.99	1.14
		253	−0.87	−0.77	−0.97	1.14
		243	−0.86	−0.74	−0.98	1.40
		238	−0.85	−0.70	−1.00	1.09
		233	−0.84	−0.64	−1.04	1.08

(E_p) of the two complexes in DMF or CH_2Cl_2 were obtained at temperatures from 303 to 233 K. E_p is presented by $1/2(E_{p,c} + E_{p,a})$. The normal redox potential (E_p) obtained by cyclic voltammetry should be taken as approximately equal to the half-wave potential ($E_{1/2}$) generally used for native ferredoxins as reported by DePamphilis et al.[18] The redox potentials of $[Fe_4S_4(Z\text{-cys-Gly-Ala-OMe})_4]^{2-}$ shifted to the positive side at lower temperatures from −1.00 V (SCE) at 303 K to −0.85 V at 233 K in CH_2Cl_2 as shown in Figure 3. In DMF, the redox potential of the above complex remained unchanged even at 233 K. On the other hand, the same redox potential of $[Fe_4S_4(Z\text{-cys-Gly-OMe})_4]^{2-}$ exists at low temperature even in CH_2Cl_2. Table I lists the peak potentials ($E_{p,c}$ and $E_{p,a}$) and $i_{p,c}/i_{p,a}$ ratios of $[Fe_4S_4(Z\text{-cys-Gly-OMe})_4]^{2-}$ and $[Fe_4S_4(Z\text{-cys-Gly-Ala-OMe})_4]^{2-}$.

Stability of the $(Fe_4S_4)^{2+}$ in CH_2Cl_2 at low temperature was demonstrated by reversibility of the redox process of $[NMe_4]_2[Fe_4S_4(SCH_2Ph)_4]$.[18] A slight negative shift was observed with a value of −1.33 to −1.36 V (SCE) at temperatures of 291 K to 258 K. Unfortunately, below 258 K both cathodic and anodic peaks became broader because of the low solubility of the complex.

The results indicate that the different redox potentials of $[Fe_4S_4(Z\text{-cys-Gly-Ala-OMe})_4]^{2-}$ in DMF and in CH_2Cl_2 are attributed to solvent-dependent conformation at the peptide parts. Although a redox potential is influenced by the dielectric property of the solvent,[19] the redox potential of two model complexes in DMF or CH_2Cl_2 were approximately the same (ca. −1.00 V (SCE)). This behavior suggests that the reversibility and the positive shift are facilitated by the conformational freezing due to NH---S bonds in the sequence Cys-Gly-Ala in CH_2Cl_2 at the lower temperature.

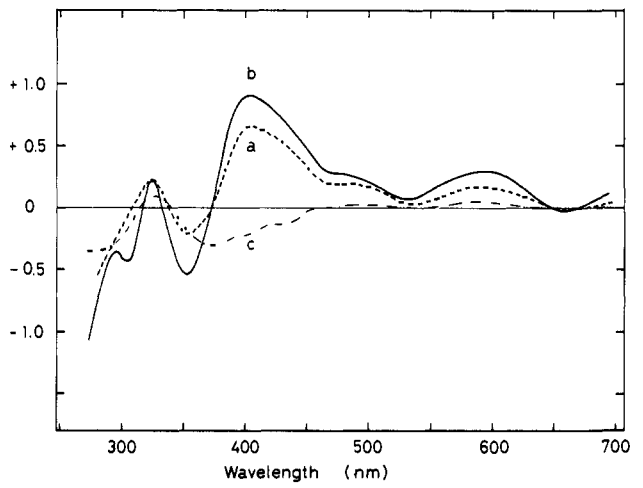

Figure 4. CD spectra of $[NMe_4]_2[Fe_4S_4(Z\text{-cys-Gly-Ala-OMe})_4]$ (**2**). Sample codes: (a) **2** at 303 K in CH_2Cl_2, (b) **2** at 275 K in CH_2Cl_2, and (c) **2** at 303 K in DMF.

Temperature Dependence of CD Spectra and ^1H NMR Spectra. When a peptide prefers a conformation with NH---S bonds at the lower temperature, a change of the CD spectra should be observed. The CD pattern of the complex $[Fe_4S_4(Z\text{-cys-Gly-Ala-OMe})_4]^{2-}$ in CH_2Cl_2 differs from that in DMF as shown in Figure 4a,b. The CD transitions of the tripeptide complex in CH_2Cl_2 were observed with higher ellipticities at low temperature such as 275 K. At 233–303 K, the presence of the $Fe_4S_4^{2+}$ core was confirmed by the visible spectra and the electrochemical method. Therefore, the temperature dependence of the CD spectra from 275 to 303 K reflects the change of probabilities of conformers in the peptide parts, and the stronger ellicipticities indicate freezing of a conformer having an NH---S bond. The similarity of CD transitions was found by comparison between $[Fe_4S_4(Z\text{-cys-Gly-Ala-OMe})_4]^{2-}$ in CH_2Cl_2 at 273 K and oxidized *Clostridium pasteurianum* ferredoxin. The CD extrema of the tripeptide complex in CH_2Cl_2 were observed at 352 nm ($\Delta\epsilon$ −0.54), 403 nm ($\Delta\epsilon$ −0.90), and 588 nm ($\Delta\epsilon$ +0.29) at 275 K, while the CD maxima of the native ferredoxin were reported at 374 nm ($\Delta\epsilon$ −1.96), 415 nm ($\Delta\epsilon$ +5.08), and 571 nm ($\Delta\epsilon$ +3.52) in aqueous solution at room temperature.[20] The difference of the ellipticities indicates that the Fe_4S_4 core of the native ferredoxin is fixed in space relative to four Cys thiolates in the peptide chain. Only weak CD extrema of $[Fe_4S_4(Z\text{-cys-Gly-OMe})_4]^{2-}$ in either DMF or CH_2Cl_2 were observed at 275 K.

Infrared Spectra in CH_2Cl_2. In order for the NH--S bonds to be detected directly, we examined IR spectra of $[Fe_4S_4(Z\text{-cys-Gly-OMe})_2]^{2-}$, $[Fe_4S_4(Z\text{-cys-Gly-Ala-OMe})_2]^{2-}$, Z-Cys-Gly-OMe, and Z-Cys-Gly-Ala-OMe in CH_2Cl_2 at various temperatures. Figure 5 shows the IR spectra associated with vibrations of NH and CO groups of two Fe_4S_4/peptide complexes and Z-Cys-Gly-Ala-OMe in CH_2Cl_2. Strong NH bands at 3330 cm^{-1} indicate that most of the NH groups are involved in interaction with donor atoms forming the NH--O=C or NH---S bond. The NH bands of $[Fe_4S_4(Z\text{-cys-Gly-Ala-OMe})_4]^{2-}$ at 3330 cm^{-1} were strengthened at low temperature, whereas the CO bands in a region of 1670–1750 cm^{-1} were unchanged as shown in Figure 4.

Discussion

Detailed studies of 4-Fe ferredoxin model complexes by Holm's group[1] indicated positive potential shifts with introduction of

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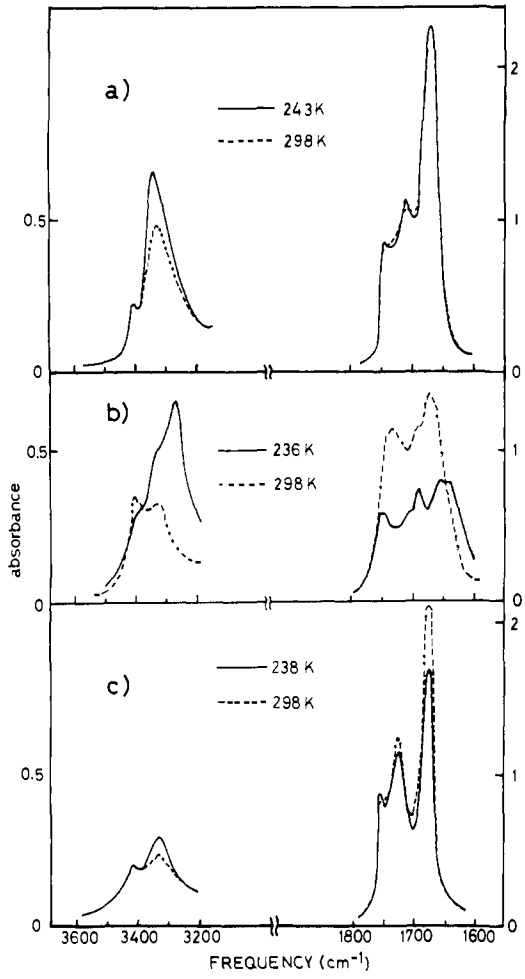

Figure 5. Temperature dependence of partial IR spectra of (a) $[Fe_4S_4(Z\text{-cys-Gly-Ala-OMe})_4][NMe_4]_2$, (b) Z-Cys-Gly-Ala-OMe, and (c) $[Fe_4S_4(Z\text{-cys-Gly-OMe})_4][NMe_4]_2$ in CH_2Cl_2.

electron-attracting groups to alkyl thiolate ligands. The value −1.33 V (SCE) for $[Fe_4S_4(SEt)_4]^{2-}$ may be compared with the values −1.17 V for $[Fe_4S_4(SCH_2\text{-}CH_2OH)_4]^{2-}$ or −0.98 V for $[Fe_4S_4(Ac\text{-cys-}NHMe)_4]^{2-}$ in 80% Me_2SO–water. They reported that not only a solvent of higher dielectric constant but also electron-attracting groups (e.g., –OH or –CONH–) of the ligands cause positive potential shifts and that the redox potential of $[Fe_4S_4(Ac\text{-Cys-}NHMe)_4]^{2-}$ or $[Fe_4S_4(t\text{-Boc-(-Gly-Cys-Gly-)}_nNH_2]^{2-}$ is fairly close to that of denatured *C. pasteurianum* ferredoxin in 80% Me_2SO–water.[4] They also suggested that the redox potentials of iron–sulfur proteins are higher than those of 4-Fe ferredoxin models by 60–120 mV due to the extrinsic effects of protein environments.

Our 4-Fe model complex of Z-Cys-Gly-Ala-OMe reproduced sufficiently the extrinsic effects that they suggested, though at a low temperature. A positive shift of the redox potential, namely −0.85 V (SCE) at 233 K from −1.00 V at 303 K, was obtained in our case in CH_2Cl_2. The redox potential (in CH_2Cl_2 at 233 K) corrected by difference in dielectric property between CH_2Cl_2 and water with the method reported by Kassner and Yang[19] was calculated to be −0.60 V at 233 K in water. This estimated value is quite close to the value −0.67 V (SCE) of *C. pasteurianum* ferredoxin or −0.57 V (SCE) of *D. desulfuricans* ferredoxin observed in aqueous solution at ambient temperature.

The redox potential of $[Fe_4S_4(Z\text{-cys-Gly-OMe})_4]^{2-}$ or $[Fe_4S_4(SCH_2Ph)_4]^{2-}$ used as an electrochemical standard exhibits no shift or slight negative shifts with a decrease in temperature. The value of $[Fe_4S_4(Z\text{-cys-Gly-OMe})_4]^{2-}$ in CH_2Cl_2 should correspond to that of $[Fe_4S_4(Ac\text{-cys-}NHMe)_4]^{2-}$ in CH_2Cl_2 although the potential of the latter has not been examined in such a solvent. Actually, Que et al.[4] reported a redox potential of −1.07 V (SCE) for $[Fe_4S_4(Ac\text{-cys-}NMe)_4]^{2-}$ in DMF (−0.91 V in 80% Me_2SO–water), whereas we obtained −1.00 V for $[Fe_4S_4(Z\text{-cys-Gly-OMe})_4]^{2-}$ in DMF. It may be concluded that all of the 4-Fe model complexes of oligopeptides containing one Cys residue exhibit almost the same redox potentials in DMF.

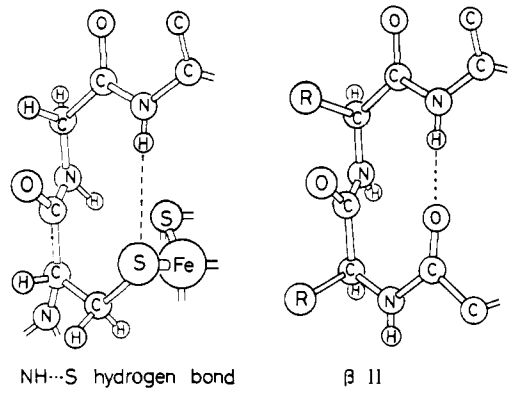

Figure 6. Conformation with the NH···S or NH···O=C hydrogen bond.

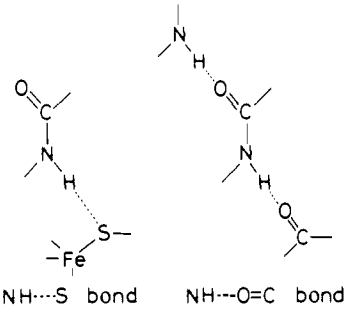

Figure 7. NH and C=O in the NH···S and NH···O=C hydrogen bonds of amide groups.

On the basis of the results described above, it is reasonable to expect that the difference between the redox potentials of the two model complexes in CH_2Cl_2 at 233 K is attributed to their peptide sequences. Cys-Gly-Ala can provide an NH···bond from NH of the Ala residue to S of the Cys residue as illustrated in Figure 6. The NH···S bond is impossible in the Fe_4S_4 complex of Z-Cys-Gly-OMe. Actually, the dipeptide does not possess a folding conformation induced by the hydrogen bond. The NH–S distance of Cys-Gly-Ala in the native ferredoxin is 360 pm,[7] while the van der Waals distance of normal NH···S bonds is known to be 340 pm.[22] Conformation of the tripeptide having an NH···S bond corresponds to the form with an NH···S bond in place of an NH···O=C bond as illustrated in Figure 6. This conformation has already been noted by Adman et al.[5] The change of the redox potentials in CH_2Cl_2 at 233 K is definitely a result of the NH···S bond which is considered *to be supported only in a solvent having a low dielectric constant such as* CH_2Cl_2. Our results are also consistent with the fact that, as with *P. aerogenes* ferredoxin, peptide sequences around the Fe_4S_4 core possess a number of hydrophobic side chains such as Cys, Ile, Ala, Pro, or Val and constitute a hydrophobic environment to stabilize NH···S bonds in a solvent having a low dielectric constant.

The conformational folding of the tripeptide with NH···S bonds was supported by the results of CD and IR spectra. The increased ellipticity of the CD spectra in CH_2Cl_2 indicates that the NH···S bonds are stabilized by the conformational freezing of Cys-Gly-Ala at the lower temperature whereas the complex containing Z-Cys-Gly-OMe does not show any increase even at low temperature. The similarity of CD transitions of $[Fe_4S_4(Z\text{-cys-Gly-Ala-OMe})_4]^{2-}$ to those of native ferredoxins suggests similar chiral environments around each $Fe_4S_4^{2+}$ core. A lower value of molar ellipticities of the model complex relative to native ferredoxins

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should be attributed to flexibility around the Cys residue.

The NH and C=O stretching regions of the IR spectra of peptides can be utilized to infer the state of hydrogen bonding at these functionalities. Bystrov et al. studied a terminally blocked dipeptide, Z-Ala-Ala-OMe, in dilute CCl_4 solution.[21] Out of two NH stretchings (3420 and 3340 cm^{-1}), the low-frequency band was assigned to the intra- or intermolecular hydrogen-bonded NH group. In the present case, the IR spectrum of Z-Cys-Gly-Ala-OMe shows NH bands at 3405 and 3325 cm^{-1} and CO bands at 1678 cm^{-1} at room temperature (Figure 5b). At 233 K, new bands appear at 3280 and 1661 cm^{-1}. These are thought to be due to intermolecular association through NH---O=C type hydrogen bonding.

The IR spectrum of $[Fe_4S_4(Z\text{-}Cys\text{-}Gly\text{-}Ala\text{-}OMe)_4]^{2-}$ exhibits NH and C=O stretchings at 3300 and 1660 cm^{-1} in CH_2Cl_2 at 298 K. On cooling at 243 K no new band appears (see Figure 5a). Thus, the above-mentioned intermolecular association does not take place. Then, two types of intramolecular hydrogen bonding are possible. The NH---O=C hydrogen bonding responsible for the β_{II} turn of the peptide is improbable due to steric reasons for this particular tripeptide. The NH---S type intramolecular hydrogen bonding is sterically best prefered and promoted by the anionic character of the thiolate group (see Figure 6). The IR data (Figure 5c) for $[Fe_4S_4(Z\text{-}cys\text{-}Gly\text{-}OMe)_4]^{2-}$ indicate the absence of intermolecular association. The coordinated peptide is too short to make the hydrogen bond to thiolate sulfur but is just right for forming an NH hydrogen bond to bridging inorganic sulfide.

Sweeny and Magliozzo have explained the positive shift of native ferredoxins on the basis of their deuteration study on oxidized *C. pasteurianum* ferredoxin, that is NH---S bonds are not important in modifying the redox potential of the $Fe_4S_4^{2+}$ cluster, contributing at best -0.2 ± 0.8 mV.[9] However, our results suggested that formation of the NH---S bonds stabilized in CH_2Cl_2 at low temperature contributes to the shift of the redox potentials (120 mV at 233 K) to the positive side. Two interpretations are possible: (1) charge on the thiolato ligand is delocalized though the NH---S bond or (2) conformational folding around the thiolato ligand brings about distortion of the Fe_4S_4 core.

Our observation of a slightly negative shift of the redox potential for $[Fe_4S_4(SCH_2Ph)_4]^{2-}$ in CH_2Cl_2 at low temperature indicates that the freezing of conformational rotation around the phenyl groups at low temperature probably contributes to a slight change of the redox potential. Actually, in the ^1H NMR spectra of $[Fe_4S_4(SCH_2Ph)_4]^{2-,3-}$,[23] the 2- complex exhibits an upfield shift of methylene protons, but those of the 3- complex show a downfield shift with a decrease in temperature. Such an effect of phenyl groups on the redox potentials of various types of ferredoxins has been noted.[24] More detailed investigations are required for further discussion on the effect of the phenyl groups in a solvent with a low dielectric constant, associated with interpretation of the role of Phe or Tyr residue located in the vicinity of the Fe_4S_4 core.

Summary

A positive shift in redox potential of $[Fe_4S_4(Z\text{-}cys\text{-}Gly\text{-}Ala\text{-}OMe)_4]^{2-}$ was observed in CH_2Cl_2 at 233 K. No shift was detected in the case of $[Fe_4S_4(Z\text{-}cys\text{-}Gly\text{-}Ala\text{-}OMe)_4]^{2-}$ in DMF or $[Fe_4S_4(Z\text{-}cys\text{-}Gly\text{-}OMe)_4]^{2-}$ in CH_2Cl_2 even at low temperature. Our observation strongly suggests that the positive shift of the redox potential is attributed to the NH---S bond between Ala NH and Cys S in the Z-Cys-Gly-Ala-OMe part of the complex. Also, our finding suggests that chemical function of the peptide chain around sites in iron-sulfur proteins could be realized by a special conformation which induces hydrophobic environments.

Registry No. 1, 87532-20-5; **2**, 87532-22-7; $[Fe_4S_4(S\text{-}t\text{-}Bu)_4][NMe_4]_2$, 52678-92-9; Z-Cys-Gly-OMe, 85134-27-6; Z-Cys-Gly-Ala-OMe, 87532-23-8; Z-Cys(ACM)-Gly-OMe, 78658-27-2; Z-Cys(ACM)-Gly-Ala-OMe, 87532-24-9; CH_2Cl_2, 75-09-2.

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Thermolysis of Azoalkanes Containing the 2,3-Diazabicyclo[2.2.2]oct-2-ene (DBO) Skeleton

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Abstract: The effect of one and two bridgehead substituents on the thermal stability of DBO is assessed by monitoring the disappearance of four new compounds (**8-11**). Two cyclopropyl groups are found to lower $\Delta G^{\ddagger}$ for thermolysis by twice the amount of one such group; however, a second bridgehead phenyl substituent is much less effective than the first. Comparison of these results with those of the previously studied methyl and vinyl DBO suggests two mechanisms that are indistinguishable on the basis of available data. In the first one, symmetrical azoalkanes decompose by simultaneous scission of both C-N bonds while unsymmetrical azoalkanes exhibit greater breaking of the weaker C-N bond at the transition state. This idea is described in a More O'Ferrall-Jencks-Thornton diagram. The second possible mechanism is reversible cleavage of the weaker C-N bond followed by loss of nitrogen from an intermediate diazenyl radical. Incorporation of endocyclic fused rings into the DBO skeleton generally slows down thermolysis, perhaps by inhibiting planarization of the carbon atoms α to the azo group.

The thermal stability of azoalkanes depends strongly upon their structure.[1-3] Among the most stable azoalkanes, one finds 2,3-diazabicyclo[2.2.2]oct-2-ene (DBO), which was studied over two decades ago by Cohen and Zand.[4] Since that time, thermolysis of several other DBO derivatives (e.g., **1-7**) has been reported. Bridgehead methyl groups were found to exert such a small effect on thermal stability that it was difficult to distinguish one bond from two bond scission.[5] Bridgehead vinyl groups, on the other hand, caused a large rate acceleration and led us to suggest

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