An Electronic Influence of a Distant Aromatic Ring in Reduced Rubredoxin Models. Iron(II) Complexes with Z-Cys-Pro-Leu-Cys-Gly-X (X = $NHCH_2C_6H_4$-*p*-F, $NHCH_2CH_2C_6H_4$-*p*-F, and Phe-OMe)

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Cysteine-containing oligopeptides with a distal aromatic ring, Z-Cys-Pro-Leu-Cys-Gly-X (X = $NHCH_2C_6H_4$-*p*-F, $NHCH_2CH_2C_6H_4$-*p*-F and Phe-OMe), and their Fe(II) complexes were synthesized. The absorption, CD and ^{1}H-NMR spectral data indicate that mononuclear Fe(II) complexes were formed through the chelate coordination of the four cysteine thiolate residues. In particular, the complex with a phenylalanine residue, $[Fe(Z\text{-cys-Pro-Leu-cys-Gly-Phe-OMe})_2]^{2-}$, exhibits absorption maxima at 312 (8240) and 332 nm (6750) and CD transitions at 316 (–27.0) and 334 nm (16.1) in acetonitrile solution which are very similar to those of reduced rubredoxin in aqueous solution. The observation of isotropically shifted ^{19}F-NMR signals in both $[Fe(Z\text{-cys-Pro-Leu-cys-Gly-NHCH}_2C_6H_4\text{-}p\text{-F})_2]^{2-}$ and $[Fe(Z\text{-cys-Pro-Leu-cys-Gly-NHCH}_2CH_2C_6H_4\text{-}p\text{-F})_2]^{2-}$ indicates that there are some π–π interactions between the aromatic ring and the sulfur atom of the cysteine residue. Such interactions have already been presumed in native rubredoxin.

Introduction

Rubredoxin is a simplest iron–sulfur protein and has a function of electron transfer in the biological systems.[1] The redox potential of native rubredoxin is –0.31 V vs SCE in aqueous solution.[2] However, the reported monounclear simple Fe(II) tetrathiolate complexes, e.g. $[Fe(SR)_4]^{2-}$, exhibited redox potentials ranging from –0.5 to –1.1 V vs SCE, which were much more negative than that of rubredoxin.[3-5] We have found that the cysteine-containing peptide Fe(II) complexes show positively shifted redox potentials, for example, $[Fe(Z\text{-cys-Pro-Leu-cys-Gly-Val-OMe})_2]^{2-}$ = –0.46 V vs SCE and $[Fe(Z\text{-cys-Pro-Leu-cys-Gly-NH-C}_6H_5)_2]^{2-}$ = –0.38 V vs SCE in acetonitrile.[6,7] The difference of redox potentials between the simple thiolate complexes and these peptide complexes was explained by the contributions of specific NH---S hydrogen bonds.[6-8] Such a difference was also suggested to be due to the measurements in different solvent systems for 4Fe4S clusters.[9]

On the other hand, there is a 80-mV difference between the redox potentials of Fe(II) complexes of Z-Cys-Pro-Leu-Cys-Gly-Val-OMe and of Z-Cys-Pro-Leu-Cys-Gly-NHC_6H_5 in the same solvent as mentioned above, although the numbers for the NH---S hydrogen bonds are considered to be the same for both compounds. In addition, the latter one exhibited a shoulder at 330 nm (4400) in the absorption spectrum which is similar to that of reduced rubredoxin, but the former one lacks such a shoulder as reported in the previous papers.[6,7] These differences were considered to be the presence of the aromatic ring of anilide in $[Fe(Z\text{-cys-Pro-Leu-cys-Gly-NHC}_6H_5)_2]^{2-}$. In order to clarify the role of an aromatic ring in cysteine peptide Fe(II) model complexes, we synthesized $[Fe(Z\text{-cys-Pro-Leu-cys-Gly-NHCH}_2C_6H_4\text{-}p\text{-F})_2]^{2-}$ (**1**), $[Fe(Z\text{-cys-Pro-Leu-cys-Gly-NHCH}_2CH_2C_6H_4\text{-}p\text{-F})_2]^{2-}$ (**2**) and $[Fe(Z\text{-cys-Pro-Leu-cys-Gly-Phe-OMe})_2]^{2-}$ (**3**). Para F-substituted benzylamide and phenethylamide were introduced in **1** and **2**, respectively, for unique ^{19}F-NMR probes to investigate the interactions of benzene ring with the metal site.[7] Instead of a tyrosine residue in the sequence Cys(6)-X-Y-Cys(9)-Gly-Tyr found in the active site of native rubredoxin,[10,11] a peptide with a phenylalanine residue, Z-Cys-Pro-Leu-Cys-Gly-Phe-OMe, was selected since the hydroxyl group of the tyrosine may influence the thermal stability of cysteine oligopeptide Fe(II) complexes. A phenylalanine residue has been found to be near the FeS_4 core from an X-ray analysis of native rubredoxin, although the position of this residue (Phe49) is not adjacent to the coordinated cysteine residue (Cys9) in the amino acid sequence.[11]

In all of the native rubredoxins reported to date, the amino acid residues with aromatic groups, for example, Tyr11, Trp37 and Phe49, are preserved.[11] It has been proposed by Frey and Adman et al. that there is a possible role of these aromatic groups in mediating the electron transfer,[11,12] but the detail of the mechanism has been unknown and a model study is required.

Experimental Section

Material. Benzyloxycarbonyl chloride (Z-Cl), 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide (WSCD, water-soluble carbodiimide), 1-hydroxybenzotriazole (HOBt), *iso*-Boc-ON (Boc = *t*-butyloxycarbonyl), L-cysteine and all other L-amino acids were purchased from Protein Research Foundation, Osaka, Japan, and used without further purification. 2-Bromoacetophenone (phenacyl bromide) was from Nakarai Tesque. 4-Fluorobenzylamine and 4-fluorophenethylamine were obtained from Tokyo Kasei Co. Ltd. and Aldrich Chemical Co., Inc., respectively, and purified by distillation. Triton X-100 (polyethylene glycol mono-*p*-octylphenyl ether, n = 10) was also purchased from Tokyo Kasei Co. Ltd.

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Table I. UV–Vis, CD Spectral, and Electrochemical Data for Fe(II)/Cys–Peptide Complexes in Acetonitrile and DME and Reduced Rubredoxin in Aqueous Solution

complexes	UV–vis[b]	CD[c]	redox potential (V vs SCE) CH_3CN	redox potential (V vs SCE) DME	ΔE (mV)[d]
$(Et_4N)_2$[Fe(Z-cys-Pro-leu-cys-Gly-Val-OMe)$_2$][a]	312 (4500)	309 (–5.3) 338 (2.8)	–0.46	–0.35	110
$(Et_4N)_2$[Fe(Z-cys-Pro-Leu-cys-Gly-$NHCH_2C_6H_4$-*p*-F)$_2$] (**1**)	312 (6780) 331 (sh, 6030)	315 (–12.0) 335 (6.0)	–0.38	–0.35	30
$(Et_4N)_2$[Fe(Z-cys-Pro-Leu-cys-Gly-$NHCH_2CH_2C_6H_4$-*p*-F)$_2$] (**2**)	312 (7400) 330 (sh, 6200)	316 (–19.2) 335 (9.0)	–0.37	–0.35	20
$(Et_4N)_2$[Fe(Z-cys-Pro-Leu-cys-Gly-Phe-OMe)$_2$] (**3**)	312 (8240) 332 (6750)	316 (–27.0) 334 (16.1)	–0.35	–0.33	20
reduced rubredoxin in aqueous solution[e]	312 (10900) 333 (6000)	314 (–36) 334 (18)			

[a] Reference 7. [b] nm (ϵ, M^{-1} cm^{-1}) in acetonitrile. [c] nm ($\Delta\epsilon$, M^{-1} cm^{-1}) in acetonitrile. [d] $\Delta E = E_{DME} - E_{CH_3CN}$. [e] Reference 2.

$(Et_4N)_2$[Fe(S-*t*-Bu)$_4$] was prepared by a method described previously.[6] Acetonitrile, tetrahydrofuran (THF), *N*,*N*′-dimethylformamide (DMF), and 1,2-dimethoxyethane (DME) were distilled before use.

General Procedures for Preparation of Z-Cys(Acm)-Pro-Leu-Cys(Acm)-Gly-X (X = $NHCH_2C_6H_4$-*p*-F, $NHCH_2CH_2C_6H_4$-*p*-F, and Phe-OMe). Peptide fragments Z-Cys(Acm)-Pro-Leu-OPac (Acm = acetamidmethyl, Pac = phenacyl) and Boc-Cys(Acm)-Gly-X were prepared stepwise by the mixed anhydride (MA) method as reported in the literature.[7] The Pac and Boc groups were deprotected by usual procedures to afford peptides, Z-Cys(Acm)-Pro-Leu-OH and HCl·Cys(Acm)-Gly-X, respectively. These two fragments were coupled by the WSCD/HOBt method to give the desired peptides.[13]

A colorless oil, Z-Cys(Acm)-Pro-Leu-OH, (6 mmol) was dissolved in DMF (5 mL), and *N*-methylmorpholine (0.66 mL, 6 mmol) was added. The mixture was cooled to –15 °C. WSCD (1.08 mL, 6 mmol) and HOBt (1.13 g, 7.2 mmol) were added to the solution, before adding a mixture of HCl·Cys(Acm)-Gly-X (6 mmol) in DMF (5 mL) and *N*-methylmorpholine (0.66 mL, 6 mmol). It was stirred for 1 h at –15 °C and then overnight at room temperature and then purified by the reported procedures.[13] The residue was reprecipitated from chloroform and diethyl ether repeatedly to give a white powder in yields of 10–20%. The purity of these peptides was checked by ^{1}H-NMR spectra in chloroform-d_1 and elemental analyses.

Anal. Calcd for $C_{40}H_{57}N_8O_{10}S_2F$ [Z-Cys(Acm)-Pro-Leu-Cys(Acm)-Gly-$NHCH_2C_6H_4$-*p*-F][H_2O]: C, 53.80; H, 6.43; N, 12.55; S, 7.18. Found: C, 53.79; H, 6.18; N, 12.45; S, 7.08.

Anal. Calcd for $C_{41}H_{59}N_8O_{10}S_2F$[Z-Cys(Acm)-Pro-Leu-Cys(Acm)-Gly-$NHCH_2CH_2C_6H_4$-*p*-F][H_2O]: C, 54.29; H, 6.56; N, 12.35; S, 7.07. Found: C, 54.01; H, 6.28; N, 12.12; S, 7.10.

Anal. Calcd for $C_{43}H_{62}N_8O_{12}S_2$[Z-Cys(Acm)-Pro-Leu-Cys(Acm)-Gly-Phe-OMe][H_2O]: C, 54.53; H, 6.60; N, 11.83; S, 6.77. Found: C, 54.24; H, 6.40; N, 11.63; S, 6.75.

SH-Free-Peptides—Deprotection of S-Acm Groups. To a DMF solution (10 mL) of Z-Cys(Acm)-Pro-Leu-Cys(Acm)-Gly-X (0.1 mmol) were added $HgCl_2$ (136 mg, 0.5 mmol) and several drops of water. The mixture was stirred at ambient temperature overnight. A white precipitate was obtained by addition of water to the solution and removed by filtration and dried in vacuo.

Anal. Calcd for $C_{34}H_{43}N_6O_7S_2FHg_3Cl_4$[Z-Cys(HgCl)-Pro-Leu-Cys(HgCl)-Gly-$NHCH_2C_6H_4$-*p*-F][$HgCl_2$]: C, 27.70; H, 2.94; N, 5.70. Found: C, 27.53; H, 3.06; N, 5.52.

Anal. Calcd for $C_{70}H_{94}N_{12}O_{16}S_4F_2Hg_5Cl_6$[Z-Cys(HgCl)-Pro-Leu-Cys(HgCl)-Gly-$NHCH_2CH_2C_6H_4$-*p*-F]$_2$[$HgCl_2$]: C, 31.49; H, 3.40; N, 6.30. Found: C, 30.88; H, 3.50; N, 6.04.

Anal. Calcd for $C_{74}H_{96}N_{12}O_{18}S_4Hg_5Cl_6$[Z-Cys(HgCl)-Pro-Leu-Cys(HgCl)-Gly-Phe-OMe]$_2$[$HgCl_2$]: C, 31.91; H, 3.47; N, 6.03. Found: C, 31.96; H, 3.66; N, 5.95.

The Hg(II)–peptide complex obtained was suspended in methanol and bubbled with hydrogen sulfide for about 20 min and then stirred for 2 h at room temperature. The black precipitates (HgS) were filtered off under argon atmosphere, and the filtrate was concentrated under reduced pressure. After being washed with diethyl ether to afford a colorless powder, the peptide was stored under an argon atmosphere.

The N-deuterated peptides were prepared by proton–deuteron exchange reaction of SH-free peptides with methanol-d_1 as described in the previous papers.[6,7]

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Syntheses of Cysteine Peptide Fe(II) Complexes. All the procedures were carried out under an argon atmosphere. The Fe(II) complexes of cysteine-peptide were synthesized by ligand-exchange reaction.[7] An acetonitrile solution (5 mL) of $(Et_4N)_2$[Fe(S-*t*-Bu)$_4$] (16.8 mg, 2.5 × 10^{-5} mol) was added to a tetrahydrofuran solution (5 mL) of Z-Cys(SH)-Pro-Leu-Cys(SH)-Gly-X (5 × 10^{-5} mol). The mixture was stirred for 20 min and then concentrated in vacuo. The complexes were purified by methods described previously.[6] For ^{19}F-NMR spectral measurements, an excess of the SH-free (about a 4-fold excess of the starting [Fe(S-*t*-Bu)$_4$]$^{2-}$) peptide ligand was used as an interal reference.

The complexes with N-deuterated peptides were also synthesized by the reactions of $(Et_4N)_2$[Fe(S-*t*-Bu)$_4$] with the corresponding N-deuterated SH-free peptides and used for ^{2}H-NMR spectral measurements.

Physical Measurements. Absorption spectra were recorded on a JASCO Ubest-30 spectrophotometer in visible region. Circular dichroism (CD) spectra measurements were carried out on a JASCO J-40 spectropolarimeter. Sample concentrations were 1.0 mM. Absorbance (ϵ) and CD transition ($\Delta\epsilon$) values were given in units of M^{-1} cm^{-1}. The 500-MHz ^{1}H-NMR and 470-MHz ^{19}F-NMR spectra were measured on a JEOL JNM-GX 500 FT NMR spectrometer. Tetramethylsilane (TMS) and $CFCl_3$ were used as external references in the ^{1}H- and ^{19}F-NMR, respectively. The 61-MHz ^{2}H NMR spectra measurements were performed on a JEOL GSX 400 NMR spectrometer.[7]

Cyclic voltammograms were recorded on a YANACO P-1100 instrument with a three-electrode system using a glassy-carbon working electrode, a Pt-wire auxiliary electrode, and a saturated calomel electrode (SCE). The scan rate was 100 mV/s. The concentration of the sample was about 2 mM, containing 100 mM of [(*n*-Bu)$_4$N][ClO_4] (in CH_3CN and DME solution) or Et_4NCl (in aqueous Triton X-100 micelle solution) as a supporting electrolyte. Potentials were determined at room temperature vs a saturated calomel electrode as a reference.

Results

Spectral Properties. The UV–vis and CD spectral data of [Fe(Z-cys-Pro-Leu-cys-Gly-$NHCH_2C_6H_4$-*p*-F)$_2$]$^{2-}$ (**1**), [Fe(Z-cys-Pro-Leu-cys-Gly-$NHCH_2CH_2C_6H_4$-*p*-F)$_2$]$^{2-}$ (**2**) and [Fe(Z-cys-Pro-Leu-cys-Gly-Phe-OMe)$_2$]$^{2-}$ (**3**) in acetonitrile are shown in Figure 1 and Table I. All of these complexes exhibited an absorption maximum at 312 nm just as other reported Fe(II) complexes of cysteine–peptide, for example, [Fe(Z-cys-Pro-Leu-cys-Gly-Val-OMe)$_2$]$^{2-}$ and [Fe(Z-cys-Pro-Leu-cys-Gly-NHC_6H_4-*p*-Y)$_2$]$^{2-}$ (Y = OMe, H, F, and CN).[6,7] However, only **3** gave a distinct absorption maximum at 332 nm (6750) which is very similar to that of reduced rubredoxin in aqueous solution (333 nm (6000)), while **1** and **2** exhibited shoulders at 331 (sh, 6030) and 330 nm (sh, 6200), respectively. On the other hand, the strength of CD transitions of **1**–**3** was in a trend of **1** < **2** < **3**, as shown in the bottom of Figure 1, although all the patterns are similar. In particular, **3** gives CD extrema at 316 nm (–27.0) and 334 nm (16.1), which are the strongest CD transitions among the Fe(II) cysteine peptide model complexes reported so far and are the nearest values to those of native rubredoxin (314 (–36), 334 nm (18) in aqueous solution).[2] Furthermore, both the absorption and CD spectra of **1**–**3** were almost solvent independent, since no essential difference was observed in the UV–vis and CD spectra of **1**–**3** in solvents CH_3CN,

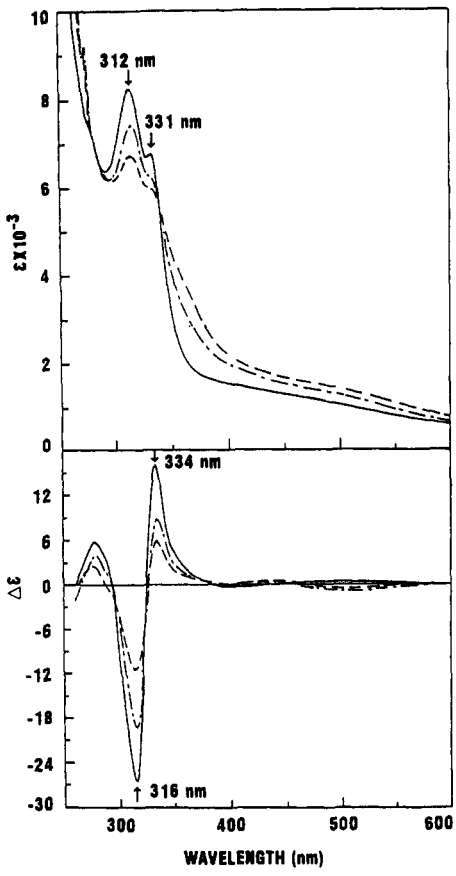

Figure 1. Absorption (top) and CD (bottom) spectra of (- - - -) [Fe(Z-cys-Pro-Leu-cys-Gly-$NHCH_2C_6H_4$-*p*-F)$_2$]$^{2-}$ (**1**), (- • -) [Fe(Z-cys-Pro-Leu-cys-Gly-$NHCH_2CH_2C_6H_4$-*p*-F)$_2$]$^{2-}$ (**2**) and (—) [Fe(Z-cys-Pro-Leu-cys-Gly-Phe-OMe)$_2$]$^{2-}$ (**3**) in acetonitrile.

DMF, or DME examined. From the characteristic absorption, CD and other spectral data, it is confirmed that the Fe(II) ion in **1**–**3** was coordinated by four sulfur atoms of cysteine residues with macro-ring chelating structure.

The ^{1}H-NMR spectroscopy can be used to confirm the formation of mononuclear high-spin tetrahedral Fe(II) complex.[4,14] The Cys $C_\beta H_2$ protons were isotropically shifted to lower fields through Fe–S–C bonds. As shown in Figure 2, the signals of Cys $C_\beta H_2$ of **1**–**3** were observed between 150 and 270 ppm as those of reduced rubredoxin[14] and other cysteine–peptide model Fe(II) complexes.[7]

The complex **3** exhibited six signals at 271, 262, 236, 223, 198, and 153 ppm in acetonitrile-d_3 at 30 °C. No significant difference was observed among the ^{1}H-NMR spectra of **1**–**3**. Similar ^{1}H-NMR spectra were also obtained for [Fe(Z-cys-Pro-Leu-cys-Gly-NHC_6H_4-*p*-Y)$_2$]$^{2-}$ (Y = OMe, H, F, and CN) in acetonitrile-d_3 at 30 °C. No signal was observed between 60 and 140 ppm where some polynuclear Fe(II) thiolate complexes exhibit signals as reported for [$Fe_2(SCH_2CH_3)_6$]$^{2-}$ and [$Fe_4(SCH_2CH_3)_{10}$]$^{2-}$.[15] The temperature-dependence of these isotropically shifted signals also indicate that **1**–**3** are mononuclear, high-spin Fe(II) complexes as discussed in the previous papers.[6,7]

The ^{2}H-NMR spectral measurements were very useful to examine the formation of NH---S hydrogen bonds.[6,7] The ^{2}H-NMR spectra of **1**–**3** are presented in Figure 3. Complex **3** gave isotropic shifted signals at 40.5 and 35.7, 18.8, –2.4, and –4.6 ppm in acetonitrile at 30 °C. **1** and **2** also presented similar signals around 40, 18, and –3 ppm. The other Fe(II) complexes with chelating peptides such as Z-Cys-Pro-Leu-Cys-OMe also show similar peaks. Hence, these signals were assigned to Cys(2) N^2H, Cys(1) N^2H, and Leu N^2H in the chelating ring of Cys(1)-Pro-Leu-Cys(2) in **1**–**3**, respectively.[6,7] As shown in parts a and b of Figure 3, the signals at –0.4 and –0.2 ppm observed for complexes **1** and **2** were thus reasonably assigned to N^2H-$CH_2C_6H_4$-*p*-F and $N^2HCH_2CH_2C_6H_4$-*p*-F, respectively, due to the formation of Cys(2)-S---$N^2HCH_2C_6H_4$-*p*-F and Cys(2)-S---$N^2HCH_2CH_2C_6H_4$-*p*-F hydrogen bonds. However, no corresponding signal was observed for complex **3**. When the ^{2}H-NMR spectrum of **3** was measured at –30 °C in acetonitrile, the signals shifted from 40.5, 35.7, 18.8, –2.4, and –4.6 ppm to 50.4, 41.9, 22.8, –6.9, and –9.4 ppm and a new signal at –1.2 ppm was detected (as shown in Figure 3d). This indicates that the signal of Phe N^2H was overlapped by the solvent signal at 30 °C and appeared at –1.2 ppm at –30 °C.

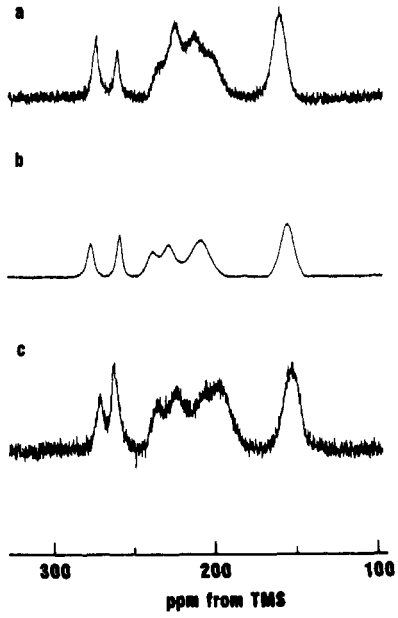

Figure 2. Cys $C_\beta H_2$ region of the ^{1}H-NMR spectra of Cys-containing peptide Fe(II) complexes in acetonitrile-d_3 at 30 °C: (a) [Fe(Z-cys-Pro-Leu-cys-Gly-$NHCH_2C_6H_4$-*p*-F)$_2$]$^{2-}$ (**1**); (b) [Fe(Z-cys-Pro-Leu-cys-Gly-$NHCH_2CH_2C_6H_4$-*p*-F)$_2$]$^{2-}$ (**2**); (c) [Fe(Z-cys-Pro-Leu-cys-Gly-Phe-OMe)$_2$]$^{2-}$ (**3**).

^{19}F-NMR Spectra of 1 and 2. The ^{19}F-NMR spectra of **1** and **2** and their SH-free peptides were measured in acetonitrile-d_3 at 30 °C and are shown in Figure 4. Both ^{19}F signals of **1** and **2** were isotropically shifted to the higher field compared to those of the corresponding SH-free peptide. Complex **2** exhibited two distinct signals at –119.8 and –120.3 ppm, while **1** presented two overlapped ones at –118.6 and –118.7 ppm. Signals at –118.1 and –119.3 ppm (Figure 4b,d) were from excess SH-free peptides (uncoordinated peptides) as shown in Figure 4a,c, respectively (see Experimental Section). The presence of two isotropically shifted signals for **1** and **2** indicates the existence of two coordination isomers for these complexes as in the cases of [Fe(Z-cys-Pro-Leu-cys-Gly-NHC_6H_4-*p*-F)$_2$]$^{2-}$ and [Fe(Z-cys-Pro-Leu-cys-Gly-NHC_6H_4-*m*-F)$_2$]$^{2-}$.[7]

The formation of two isomers was also supported by the ^{1}H-NMR and ^{2}H-NMR spectra.[6,7] The signals of ^{2}H-NMR spectra around 40 and –3 ppm split into two peaks not only for **1** and **2** but also for **3** as shown in Figure 3. The results show that the complex **3** is also composed of two isomers. Observations of six or seven Cys $C_\beta H_2$ signals in the ^{1}H-NMR spectra of **1**–**3** are also explained by the presence of two isomers.[6,7] Furthermore, the ratio of two isomers was almost 1:1 for **2** while in the cases of **1** and **3**, two isomers were found in a ratio of about 3:2 from ^{1}H-NMR, ^{2}H-NMR and ^{19}F-NMR spectra.

Electrochemical Properties. Cyclic voltammograms of **1**–**3** were recorded in acetonitrile and DME at 298 K. The redox potentials were summarized in Table I and Figure 5. **1**–**3** gave

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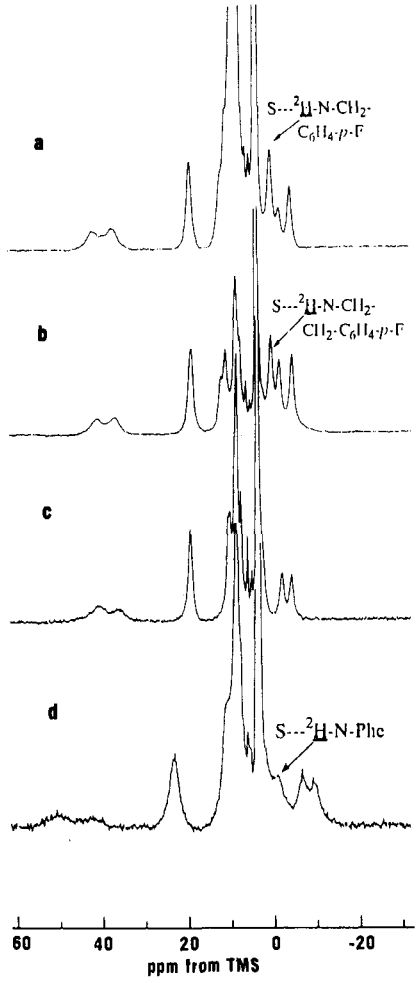

Figure 3. 61-MHz ^{2}H-NMR spectra of Cys-containing peptide Fe(II) complexes in acetonitrile: (a) $[Fe(Z\text{-cys-Pro-Leu-cys-Gly-NHCH}_2\text{-}C_6H_4\text{-}p\text{-}F)_2]^{2-}$ (**1**) at 30 °C; (b) $[Fe(Z\text{-cys-Pro-Leu-cys-Gly-NHCH}_2\text{-}CH_2C_6H_4\text{-}p\text{-}F)_2]^{2-}$ (**2**) at 30 °C; (c) $[Fe(Z\text{-cys-Pro-Leu-cys-Gly-Phe-OMe})_2]^{2-}$ (**3**) at 30 °C; (d) $[Fe(Z\text{-cys-Pro-Leu-cys-Gly-Phe-OMe})_2]^{2-}$ (**3**) at −30 °C.

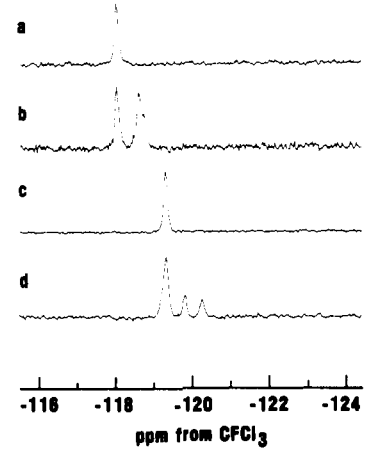

Figure 4. 470-MHz ^{19}F-NMR spectra: (a) Z-Cys(SH)-Pro-Leu-Cys(SH)-Gly-$NHCH_2C_6H_4$-*p*-F; (b) $[Fe(Z\text{-cys-Pro-Leu-cys-Gly-NHCH}_2\text{-}C_6H_4\text{-}p\text{-}F)_2]^{2-}$ (**1**); (c) Z-Cys(SH)-Pro-Leu-Cys(SH)-Gly-$NHCH_2CH_2$-C_6H_4-*p*-F; (d) $[Fe(Z\text{-cys-Pro-Leu-cys-Gly-NHCH}_2CH_2C_6H_4\text{-}p\text{-}F)_2]^{2-}$ (**2**) in acetonitrile-d_3 at 30 °C.

redox potentials at −0.35, −0.35, and −0.33 V vs SCE in DME, respectively. These values are very close to those of $[Fe(Z\text{-cys-Pro-Leu-cys-Gly-Val-OMe})_2]^{2-}$ and $[Fe(Z\text{-cys-Pro-Leu-cys-Gly-NHC}_6H_4\text{-}p\text{-}F)_2]^{2-}$ in the same solvent, DME.[7] However, in acetonitrile, complexes **1**–**3** exhibited redox potentials at −0.38, −0.37, and −0.35 V vs SCE, respectively, which were close to that of Fe(II) complex of Z-Cys-Pro-Leu-Cys-Gly-NHC_6H_4-*p*-F (−0.39 V vs SCE). The values were much more positively shifted compared to that of the Z-Cys-Pro-Leu-Cys-Gly-Val-OMe analogue in acetonitrile (−0.46 V vs SCE). A positively-shifted redox potential was observed for **1**, **2**, or **3** in DME, a low dielectric solvent, compared with that in CH_3CN. The difference in redox potential between these two solvents is very small for **1**–**3**, only 20 or 30 mV (Table I). The Fe(II) complex of a more hydrophobic peptide Z-Cys-Pro-Leu-Cys-Gly-Val-OMe exhibits a 110-mV more positively shifted redox potential in DME than that in acetonitrile.

Furthermore, the Fe(II) complex of Z-Cys-Pro-Leu-Cys-Gly-Phe-OMe exhibited a redox potential at −0.26 V (E_{pa}, −0.20 V; E_{pc}, −0.32 V) vs SCE in an aqueous Triton X-100 micelle solution (10%) with a good reversibility (i_{pc}/i_{pa} = 0.93). The micelle seems to enhance the hydrophobicity at the Fe site to give a higher shift. The cysteine peptide Fe(II) complex without aromatic ring, Z-Cys-Pro-Leu-Cys-OMe, has been reported to show a redox potential at −0.37 V (E_{pa}, −0.25 V; E_{pc}, −0.49 V) vs SCE in an aqueous Triton X-100 solution (10%).[16]

Discussion

Interactions of an Aromatic Ring with the Sulfur Atom of the Cysteine Residue. The ^{19}F signals isotropically shifted from −119.9 ppm to −122.6 and −122.9 ppm by the coordination of Z-Cys(1)-Pro-Leu-Cys(2)-Gly-NHC_6H_4-*p*-F ligand to the Fe(II) ion in acetonitrile-d_3.[7] This was considered to be due to some electronic charge flow from sulfur atom of the coordinated cysteine residue to the benzene ring through the Cys(2)-S---NHC_6H_4-*p*-F hydrogen bond and to the para position through the π-spin transfer.[7,17] However, in the case of the complexes used here, there are one or two methylene groups, CH_2 or CH_2CH_2, between the amide NH and the benzene ring in the Fe(II) complex of Z-Cys-Pro-Leu-Cys-Gly-$NHCH_2C_6H_4$-*p*-F (**1**) or Z-Cys-Pro-Leu-Cys-Gly-$NHCH_2CH_2C_6H_4$-*p*-F (**2**) ligands, respectively. Thus it is not likely that the electronic charge flows from sulfur to the benzene ring through the NH---S hydrogen bond since the π-spin transfer would be disrupted by the methylene groups. The observation of isotropically shifted ^{19}F-NMR signals[18] in **1** and **2** indicates that there are some direct interactions between the aromatic ring and the sulfur atom of cysteine residue.

Similar spectral properties (see Figures 1–4) and almost the same electrochemical properties (see Figure 5) of **1** and **2** were found. This indicates that such spectral and electrochemical properties are independent on the numbers of methylene group between the amide NH and the aromatic ring of **1** and **2**. The result shows that the interactions are via an S → aromatic ring directly (through-space), not through Fe–S---H–N–C or Fe–S---H–N–C–C σ-bonds to the aromatic ring which are significantly dependent on the distance, i.e. the number of methylene groups. In the case of $[Fe(Z\text{-cys-Pro-Leu-cys-Gly-NHC}_6H_4\text{-}p\text{-}Y)_2]^{2-}$ (Y = OMe, H, F, and CN),[7] both through-space and through-bonds should be involved since there is nc

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(17) (a) La Mar, G. N. In *NMR of Paramagnetic Molecules*; La Mar, G. N., Horrocks, W. D., Jr., Holm, R. H., Eds.; Academic: New York, 1973; pp 86–123. (b) Horrocks, W. D., Jr. In *ESR and NMR of Paramagnetic Species in Biological and Related Systems*; Bertini, I., Drago, R., Eds.; D. Reidel: Boston, MA, 1979; pp 55–87.

(18) The mechanism of the isotropic shifts of ^{19}F-NMR signals, indicated by one reviewer, is a "contact" which was caused by the presence of net spin density in the aromatic ring via an S → aromatic interaction. On the other hand, the temperature dependence of isotropically shifted ^{19}F-NMR resonances was found not to conform to T^{-1} dependence. However, a linear relationship between $\Delta H/H_0$ and T^{-2} was obtained, and the extrapolations were found to be through the origin within experimental error. Thus it is also possible that the isotropic shifts in the ^{19}F-NMR of **1** and **2** consist of great part of dipolar contributions.

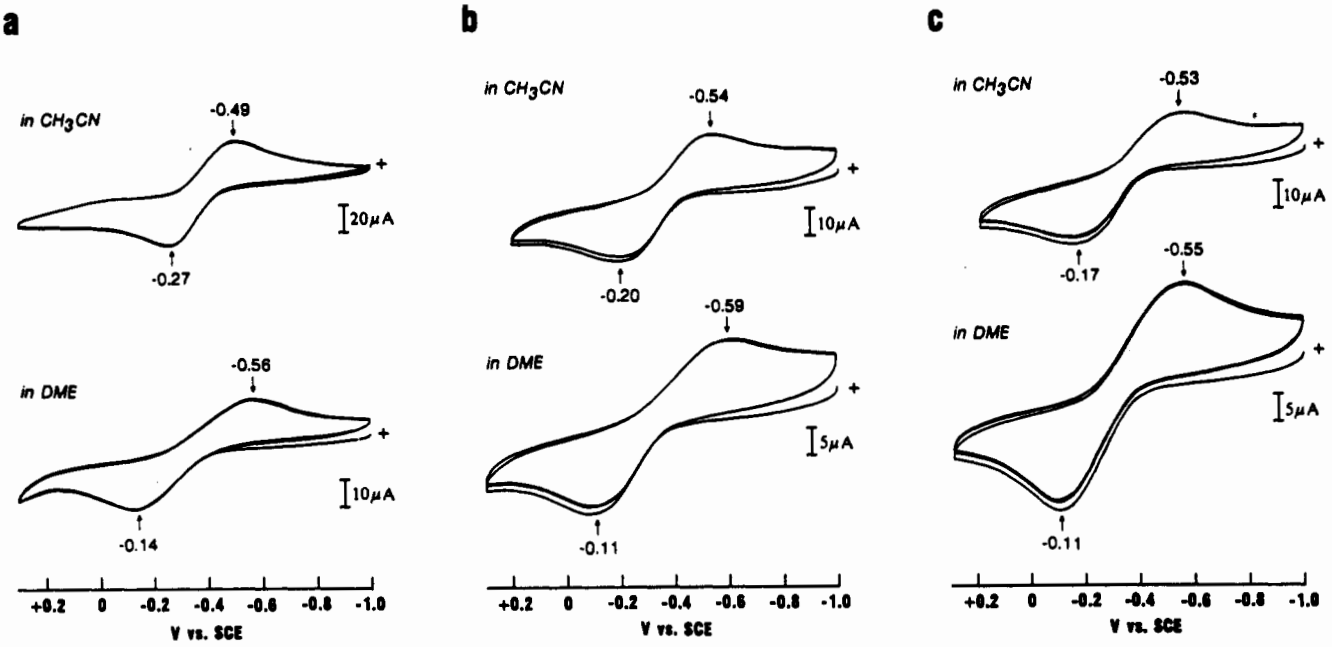

Figure 5. Cyclic voltammograms: (a) $[Fe(Z\text{-cys-Pro-Leu-cys-Gly-NHCH}_2C_6H_4\text{-}p\text{-F})_2]^{2-}$ (**1**); (b) $[Fe(Z\text{-cys-Pro-Leu-cys-Gly-NHCH}_2CH_2C_6H_4\text{-}p\text{-F})_2]^{2-}$ (**2**); (c) $[Fe(Z\text{-cys-Pro-Leu-cys-Gly-Phe-OMe})_2]^{2-}$ (**3**) in acetonitrile (top) and DME (bottom).

methylene group between the amide NH and the aromatic ring of anilide.

The existence of such interactions between the aromatic ring and sulfur has also been supported by the X-ray analyses of *Desulfovibrio digas* and *Desulfovibrio vulgaris* rubredoxins.[11,12,19] The effects of phenylalanine residue have also been examined by calculations for Pd(II) complex[20] and by chemically replacing the underlying aromatic groups by modified groups (Phe → Ala or Tyr) for protein.[21]

The Effects of Aromatic Ring in Absorption, CD, ^{1}H-NMR, and ^{2}H-NMR Spectra. The $RS^- \rightarrow Fe(II)$ charge transfer (LMCT) absorption maximum was observed at 312 nm for **1–3**. It is independent of the X residue in $[Fe(Z\text{-cys-Pro-Leu-cys-Gly-X})_2]^{2-}$ (X = $NHCH_2C_6H_4$-*p*-F, $NHCH_2CH_2C_6H_4$-*p*-F, and Phe-OMe). This feature has been reported for the complexes $[Fe(Z\text{-cys-Pro-Leu-cys-Gly-NHC}_6H_4\text{-}p\text{-Y})_2]^{2-}$ (Y = OMe, H, F, and CN), for which the absorption maximum also appeared at 312 nm independent of the para substituents.[7] On the other hand, the position of the absorption maximum around 330 nm was dependent on the identity of the amino acid residue or organic amide in the sequence Z-Cys-Pro-Leu-Cys-Gly-X. When the X is valine methyl ester (Val-OMe), there is no absorption maximum around 330 nm, while for X = NHC_6H_4-*p*-Y (Y = OMe, H, F, and CN), $NHCH_2C_6H_4$-*p*-F, or $NHCH_2CH_2C_6H_4$-*p*-F, a shoulder was observed around 330 nm. Only in complex **3**, i.e. X = Phe-OMe, was a clear absorption maximum observed at 332 nm (6750), as that of reduced Rd in aqueous solution. Therefore, the absorption maximum around 330 nm is strengthened by the optically active amino acid residue such as phenylalanine.

The large molar extinction coefficient (6000–7000 M^{-1} cm^{-1}) suggests the absorption maximum around 330 nm is also due to a charge transfer from S^- to the Fe(II) ion. The presence of two kinds of charge-transfer bands is considered to be due to the existence of two kinds of torsion angles, $Fe\text{–}S_{Cys(1)}$ and $Fe\text{–}S_{Cys(2)}$, which was caused by a C_2 distortion from the D_{2d} symmetry as discussed for the oxidized rubredoxin by theoretical calculations.[8b,10,14] The distorted geometry was also reported for native rubredoxin by the resonance Raman study.[22] In our model complexes, $[Fe(Z\text{-cys}(1)\text{-Pro-Leu-cys}(2)\text{-Gly-X})_2]^{2-}$ (X = $NHCH_2C_6H_4$-*p*-F (**1**), $NHCH_2CH_2C_6H_4$-*p*-F (**2**) and Phe-OMe (**3**)), the formation of an X-NH---S–Cys(2) hydrogen bond and the interactions of the aromatic ring with the sulfur atom of cysteine residue make a sufficient difference between the $Fe\text{–}S_{Cys(2)}$ torsion angle and the $Fe\text{–}S_{Cys(1)}$ one while in the case of the Fe(II) complex of Z-Cys(1)-Pro-Leu-Cys(2)-Gly-Val-OMe, only one absorption band at 312 nm indicates that the difference between the two Fe–S torsion angles was not enough through formation of a Val–NH---S–Cys(2) hydrogen bond.

When the cysteine peptide ligand was extended from Z-Cys-Pro-Leu-Cys-OMe to Z-Cys-Pro-Leu-Cys-Gly-Val-OMe, the redox potentials of their Fe(II) complexes changed greatly, especially in a weak nonpolar solvent as DME: $[Fe(Z\text{-cys-Pro-Leu-cys-OMe})_2]^{2-}$, –0.59 V; $[Fe(Z\text{-cys-Pro-Leu-cys-Gly-Val-OMe})_2]^{2-}$, –0.35 V vs SCE.[7] However, there is no significant change in the CD spectra. The Fe(II) complex of Z-Cys-Pro-Leu-Cys-Gly-Val-OMe exhibits CD extrema at 309 (–5.3) and 338 nm (2.8) while the analogue of Z-Cys-Pro-Leu-Cys-OMe gives ones at 320 (–6.3) and 340 nm (3.3). Both of them are much weaker than those of native rubredoxin. Similar to the absorption spectra, only when there is an aromatic group at the end of peptide ligand, namely, Z-Cys-Pro-Leu-Cys-Gly-X (X = $NHCH_2C_6H_4$-*p*-F, $NHCH_2CH_2C_6H_4$-*p*-F and Phe-OMe), the CD transitions becomes stronger as in the cases of **1–3**. It is thus considered that the absorption maximum at 333 nm (6000) and the strong CD transitions of reduced rubredoxin in aqueous solution are caused by some contributions of aromatic groups such as tyrosine or/and phenylalanine etc. to the perturbation of the Fe–S LMCT transition.

In ^{2}H-NMR spectra, the ^{2}H signal of N^2H-Val was isotropically shifted to –20 ppm in $[Fe(Z\text{-cys}(1)\text{-Pro-Leu-cys}(2)\text{-Gly-Val-OMe})_2]^{2-}$ and the ^{2}H signal of $N^2HC_6H_4$-*p*-F in $[Fe(Z\text{-cys}(1)\text{-Pro-Leu-cys}(2)\text{-Gly-NHC}_6H_4\text{-}p\text{-F})_2]^{2-}$ was observed at 9.5 ppm. The corresponding signals of **1–3** were observed at about 0 ppm, which was the middle position between the above-mentioned two complexes. This is explicable by the contributions of aromatic ring of X (X = $NHCH_2C_6H_4$-*p*-F, $NHCH_2CH_2C_6H_4$-*p*-F, and Phe-OMe) in $[Fe(Z\text{-cys-Pro-Leu-cys-Gly-X})_2]^{2-}$ (**1**–

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3), since these aromatic rings have been determined to act as electron donors and the sulfur of cysteine residue to function as an electron acceptor.[23,24] Such electronic interactions will cause some spin density variation on the sulfur atom of Cys(2) in $[Fe(Z\text{-}cys(1)\text{-}Pro\text{-}Leu\text{-}cys(2)\text{-}Gly\text{-}X)_2]^{2-}$ and this spin density change was expected to effect the isotropic shifts in the ^1H- and ^2H-NMR spectra of $[Fe(Z\text{-}cys\text{-}Pro\text{-}Leu\text{-}cys\text{-}Gly\text{-}X)_2]^{2-}$.[25] Thus, the observation of X–N^{2}H signals at about 0 ppm was considered to be the algebraic sum of the X–N^{2}H---S–Cys(2) hydrogen bond contributions and the aromatic ring contributions. In ^1H-NMR spectra, the Cys $C_\beta H_2$ signals of **3** observed at 271, 262, 236, 223, 198, and 153 ppm, shifted relative downfield compared to those of $[Fe(Z\text{-}cys\text{-}Pro\text{-}Leu\text{-}cys\text{-}Gly\text{-}Val\text{-}OMe)_2]^{2-}$, which appeared at 257, 215, 200 and 147 ppm, indicate that the spin density in complex **3** is higher than that in the latter one,[25] which is due to the interactions of the sulfur atom of the cysteine residue with the aromatic ring of X.

Roles of the Aromatic Ring in the Chemical Stabilization of Fe(II) Cysteine Peptide Complexes. The simple thiolate Fe(II) complexes were reported to be extremely sensitive to air and water.[4] The cysteine peptide model complexes without any aromatic ring such as $[Fe(Z\text{-}cys\text{-}Gly\text{-}Val\text{-}OMe)_4]^{2-}$ and $[Fe(Z\text{-}cys\text{-}Pro\text{-}Val\text{-}cys\text{-}OMe)_2]^{2-}$ also react with air immediately to give the corresponding Fe(III) complexes, which decompose gradually.[26] However, the complexes with aromatic rings, e.g. **1–3**, were found to be relatively stable to air and water. For example, for the absorption spectrum of **3**, the absorption maxima at 312 and 332 nm were observed without significant change for about 30 min after addition of about 10 equiv of oxygen. A quasi-reversible cyclic voltammogram was obtained for complex **3** in an aqueous Triton X-100 micelle solution (10%) while the bis(tetrapeptide thiolate) Fe(II) complexes were reported to be unstable in an aqueous Triton X-100 solution (10%).[16] The higher stability against air and water of **3** can be explained by the contributions of the aromatic ring of the phenylalanine residue.[23] Further studies on the spectral and electrochemical properties of cysteine peptide complexes with aromatic rings in aqueous solution are going on and the results will be reported eleswhere.

The solvent-independence of absorption and CD spectra and the small positive shift of redox potentials in DME indicate that the conformation of Fe(II) cysteine peptide complexes with aromatic rings (**1–3**) is relatively fixed in solution and that the central Fe(II) ion is surrounded by the peptide chains and by the aromatic rings of the peptide ligands. Therefore, it is reasonable that the Fe(II) ion was protected by the peptide chains and the aromatic rings and became resistant to air or water. This seems to be one of the important roles of the aromatic rings near the iron site.

Another role of these aromatic rings is that the π–π interactions described above can provide a channel for electron transfer,[27-29] which also occurs by NH---S hydrogen bonds in rubredoxin and in other electron-transfer proteins.[30]

Conclusion

The results of this study indicate that the aromatic ring plays an important role through the π–π interactions with the sulfur atom of the cysteine residue in the Fe(II) cysteine-containing peptide model complexes. The amino acid residues with an aromatic ring, such as Phe and Tyr, which are located near the active site of native proteins, are also speculated to play important roles in a similar way. One of the roles of these aromatic rings is to stabilize cysteine-containing peptide model complexes as well as the proteins and protect them from air and water.[23,31]

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