An Iron(II) Complex with a Tetradentate Peptide Ligand, *cis*-C_6H_{10}(CO-Cys-Pro-Leu-Cys-OMe)$_2$-1,2, as a Model of Reduced Rubredoxin

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The complexes $[NEt_4]_2[Fe\{cis\text{-}C_6H_{10}(CO\text{-}Cys\text{-}OMe)_2\text{-}1,2\}_2]$ **1** and $[NEt_4]_2[Fe\{cis\text{-}C_6H_{10}(CO\text{-}Cys\text{-}Pro\text{-}Leu\text{-}Cys\text{-}OMe)_2\text{-}1,2\}]$ **2** were synthesised by ligand-exchange reaction of $[NEt_4]_2[Fe(SBu^t)_4]$ with the corresponding free peptide. In the ^1H NMR spectra the Cys $C_\beta H_2$ protons of **1** and **2** were observed between δ 200 and 270 in CD_3CN at 30 °C. The temperature dependence of these signals indicate that high-spin mononuclear iron(II) complexes are formed. Complexes **1** and **2** exhibit redox potentials at −0.60 and −0.78 V respectively *vs.* saturated calomel electrode in dimethyl sulfoxide. These negatively shifted redox potentials are explained by the difficulty in forming an intramolecular NH···S hydrogen bond involving the Leu residue in **2**, confirmed by the ^2H NMR spectrum.

It is believed that rubredoxin (Rd) is a kind of protein involved in electron transfer in biological systems.[1] The active site has a simple structure with an iron ion surrounded by four cysteine thiolate ligands involved in two invariant Cys-X-Y-Cys sequences (X, Y = amino acid residues). For example, *Clostridium pasteurianum* Rd has Cys(6)-Thr-Val-Cys(9) and Cys(39)-Pro-Leu-Cys(42) sequences around the active site.[2]

We have reported the iron(II) complex of $PhCH_2OCO$-Cys-Pro-Leu-Cys-OMe as a model of reduced Rd, which exhibited similar spectral properties (absorption, CD and ^1H NMR) to those of native Rd and a positively shifted Fe^{III}–Fe^{II} redox potential compared with simple alkane- or arene-thiolate iron(II) complexes.[3] However, it exhibits two isomers with different chelate orientations of the ligand to the tetrahedral iron(II) ion. Similar isomers have been reported for the complex formed by co-ordination of two $PhCH_2OCO$-Cys-Pro-Leu-Cys-OMe ligands to $[Mo^VO]^{3+}$.[4]

The partial structure of the active site of native *C. pasteurianum* Rd from X-ray analysis,[5] $[Fe^{II}\{CO\text{-}Cys(6)\text{-}Thr\text{-}Val\text{-}Cys(9)\text{-}NH\}\{CO\text{-}Cys(39)\text{-}Pro\text{-}Leu\text{-}Cys(42)\text{-}NH\}]^{2-}$, and the proposed structures of two isomers (Δ and Λ) of $[Fe^{II}\{CO\text{-}Cys(1)\text{-}Pro\text{-}Leu\text{-}Cys(2)\text{-}NH\}_2]^{2-}$ obtained by BIOGRAF[3] calculations (see Experimental section) are shown in Fig. 1. The Δ isomer has two Cys-Pro-Leu-Cys units in the same sequence as that of native Rd. On the other hand, in the Λ isomer one of them is in the opposite sequence. From the X-ray analysis the distances $N_{Cys(6)}$–$N_{Cys(39)}$ and $C(=O)_{Cys(9)}$–$C(=O)_{Cys(42)}$ are 8.2 and 8.4 Å, respectively. In the case of the Δ isomer, the distance $N_{Cys(1)}$–$N_{Cys(1')}$ obtained by using the BIOGRAF method is 8.7 Å while that in the Λ isomer is 11.1 Å. Therefore, the former isomer more closely mimics the structure of native Rd. This is supported by spectroscopic data for the iron(II) complex of a tetradentate chelating peptide, the synthesis of which is described in this paper. The ligand consists of two Cys-Pro-Leu-Cys tetrapeptide fragments linked at the nitrogen terminals by a *cis*-1,2-cyclohexylenedicarbonyl group.

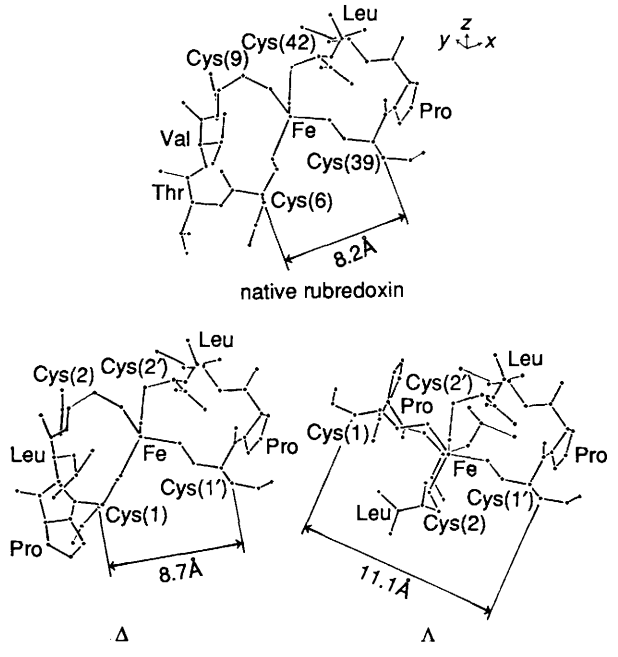

Fig. 1 Partial structure of native *C. pasteurianum* Rd,[5] $[Fe^{II}\{CO\text{-}Cys(6)\text{-}Thr\text{-}Val\text{-}Cys(9)\text{-}NH\}\{CO\text{-}Cys(39)\text{-}Pro\text{-}Leu\text{-}Cys(42)\text{-}NH\}]^{2-}$ and the proposed structures of two isomers (Δ and Λ) of $[Fe^{II}\{CO\text{-}Cys(1)\text{-}Pro\text{-}Leu\text{-}Cys(2)\text{-}NH\}_2]^{2-}$ obtained by using BIOGRAF energy-minimization calculations

Experimental

Materials.—All solvents were distilled before use. *cis*-Cyclohexane-1,2-dicarboxylic acid was obtained from Tokyo Kasei and used without further purification. The syntheses of *cis*-C_6H_{10}(CO-Cys-OMe)$_2$-1,2, *cis*-C_6H_{10}(CO-Cys-Pro-Leu-Cys-OMe)$_2$-1,2 and other cysteine-containing oligopeptides were reported elsewhere.[3,4] The *N*-deuteriated peptide was prepared by exchange reaction between the peptide and $[^2H_1]$-methanol.[3,6] The salt $[NEt_4]_2[Fe(SBu^t)_4]$ was synthesised by the method reported previously.[3] All reactions and manipulations were performed under argon.

Synthesis of $[NEt_4]_2[Fe\{cis\text{-}C_6H_{10}(CO\text{-}Cys\text{-}OMe)_2\text{-}1,2\}_2]$ **1**.—The complex was prepared by ligand-exchange reaction.[3,6] A solution of $[NEt_4]_2[Fe(SBu^t)_4]$ (5.4 mg, 8×10^{-6} mol) in acetonitrile (3 cm^3) and a solution of *cis*-C_6H_{10}(CO-Cys-OMe)$_2$-1,2 (7.4 mg, 1.8×10^{-5} mol) in tetrahydrofuran (3 cm^3) were mixed at room temperature and stirred for about 20 min. The reaction mixture was concentrated *in vacuo* and purified by the reported procedure.[3,6]

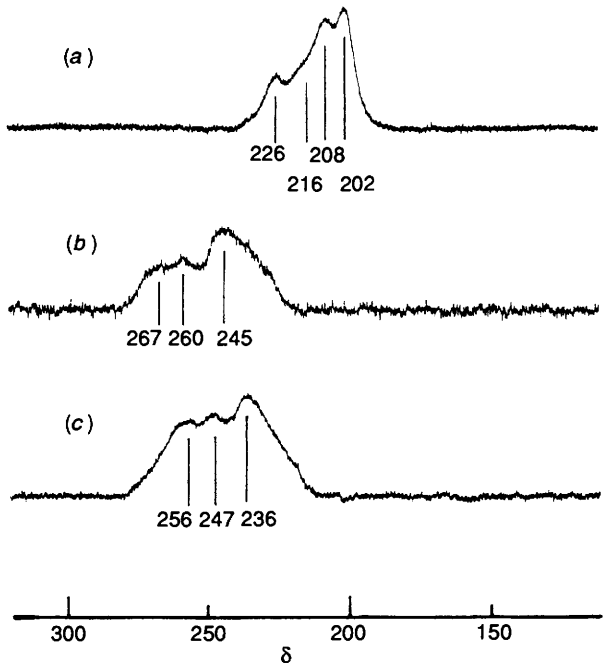

Fig. 2 The Cys $C_\beta H_2$ region of the ^1H NMR spectra in CD_3CN of complex **1** at 30 °C (*a*) and **2** at 30 (*b*) and 45 °C (*c*)

Table 1 Absorption and CD spectral data for complexes **1** and **2** in acetonitrile

Complex	$S^- \rightarrow Fe^{II}$ charge transfer		
1	UV/VIS[a]	316 (5200)	
	CD[b]	320 (−5.5)	345 (1.4)
2	UV/VIS[a]	316 (4600)	
	CD[b]	318 (−3.2)	351 (1.0)

[a] λ/nm (ε/dm^3 mol^{-1} cm^{-1}). [b] λ/nm (Δε/dm^3 mol^{-1} cm^{-1}).

Synthesis of $[NEt_4]_2[Fe\{cis\text{-}C_6H_{10}(CO\text{-}Cys\text{-}Pro\text{-}Leu\text{-}Cys\text{-}OMe)_2\text{-}1,2\}]$ **2**.—Reaction of $[NEt_4]_2[Fe(SBu^t)_4]$ (4.0 mg, 6 × 10^{-6} mol) and *cis*-$C_6H_{10}(CO\text{-}Cys\text{-}Pro\text{-}Leu\text{-}Cys\text{-}OMe)_2$-1,2 (6.2 mg, 6 × 10^{-6} mol) was carried out as described above.

The *N*-deuteriated peptide complex was also synthesised by the reaction of $[NEt_4]_2[Fe(SBu^t)_4]$ with the corresponding *N*-deuteriated peptide.

Physical Measurements.—Proton NMR spectra were measured on a JEOL JNM-GX500 FT spectrometer,[3] 61 MHz ^2H NMR spectra on a JEOL GSX 400 FT spectrometer,[6] and absorption spectra in the visible region on a JASCO Ubest-30 spectrophotometer. Circular dichroism (CD) spectral measurements were carried out on a JASCO J-40 spectropolarimeter. Cyclic voltammograms were recorded on a YANACO P-1100 with $NBu^n_4ClO_4$ (100 mmol dm^{-3}) as supporting electrolyte. Potentials were determined *vs.* the saturated calomel electrode (SCE) as a reference. The most stable conformation of iron(II) cysteine peptide complexes was calculated using a molecular model building program BIOGRAF as reported previously.[3]

Results and Discussion

Proton NMR Spectra of Iron(II) Cysteine Peptide Complexes.—Owing to the paramagnetic properties of iron(II), the signals of the Cys $C_\beta H_2$ protons are isotropically shifted into the region δ 150–270.[3,6] The signal of the CH_2 protons of $[Fe(SEt)_4]^{2-}$ was observed at δ 196 in CD_3CN at 27 °C.[7] Reduced *Desulfovibro gigas* Rd has been reported to exhibit Cys $C_\beta H_2$ ^1H NMR signals at δ 236, 227, 192 and 150 in D_2O at 55 °C.[8] The Cys $C_\beta H_2$ signals of the present free peptide are observed at about δ 3. Proton NMR spectra of the Cys $C_\beta H_2$ region of complexes **1** and **2** are shown in Fig. 2. Complex **1** exhibited four isotropically shifted signals at δ 226, 216, 208 and 202 [Fig. 2(*a*)]. In the case of **2**, apparently three signals were observed at δ 267, 260 and 245 in CD_3CN at 30 °C [Fig. 2(*b*)]. The larger area of the signal at δ 245 as compared with that at δ 267 or 260 may indicate that the former signal is composed of two overlapping resonances; thus complex **2** is thought to give four isotropically shifted signals. Furthermore, these signals were not resolved even at 45 °C as shown in Fig. 2(*c*). They were assigned to Cys $C_\beta H_2$ protons according to previous work.[3,8]

An iron(II) complex of a non-chelate peptide such as $[Fe(PhCH_2OCO\text{-}Cys\text{-}Gly\text{-}Val\text{-}OMe)_4]^{2-}$ was found to give a single peak at δ 220 in CD_3CN at 30 °C.[3] This indicates that the four cysteine thiolate ligands are equivalent and the two protons of each Cys $C_\beta H_2$ give the same isotropically shifted signal without separation on the NMR time-scale. However, in the case of an iron(II) complex of a chelating peptide, *e.g.* $[Fe(PhCH_2OCO\text{-}Cys\text{-}Pro\text{-}Leu\text{-}Cys\text{-}OMe)_2]^{2-}$, multiple signals were found between δ 150 and 270.[3] Both complexes **1** and **2** presented four Cys $C_\beta H_2$ signals at lower field (δ 200–270), suggesting chelate co-ordination of the cysteine thiolates in solution. The temperature dependence of these Cys $C_\beta H_2$ signals at 30, 10, −10 and −30 °C showed a linear correlation between isotropic shifts ($\Delta H/H_0$) and reciprocal temperature ($1/T$) with near-zero intercepts. This indicates that high-spin mononuclear iron(II) complexes were formed for both **1** and **2** without significant magnetic interactions among the iron(II) ions.[3]

The complex $[Fe(PhCH_2OCO\text{-}Cys\text{-}Pro\text{-}Leu\text{-}Cys\text{-}OMe)_2]^{2-}$ shows Cys $C_\beta H_2$ proton signals at δ 257, 253, 244, 236, 190, 178 and 169 in CD_3CN at 30 °C.[3] These signals can be divided into two groups, one around δ 248 and the other at δ 179. Complex **2** also exhibits two groups of Cys $C_\beta H_2$ proton signals at about δ 264 and 245. However, the difference in chemical shifts of these two sets of signals was smaller than that of $[Fe(PhCH_2OCO\text{-}Cys\text{-}Pro\text{-}Leu\text{-}Cys\text{-}OMe)_2]^{2-}$.

Absorption and CD Spectra.—The UV/VIS and CD spectra of complexes **1** and **2** were measured and the results are summarized in Table 1. Both complexes exhibit a ligand-to-metal charge-transfer (l.m.c.t.) band at 316 nm in acetonitrile. Similar bands were found for other cysteine peptide model complexes, *e.g.* $[Fe(PhCH_2OCO\text{-}Cys\text{-}Pro\text{-}Leu\text{-}Cys\text{-}OMe)_2]^{2-}$ as reported previously[6] and reduced Rd which shows absorption maxima at 312 (10 900) and 333 nm (ε 6000 dm^3 mol^{-1} cm^{-1}) in aqueous solution.[3,9] This indicates that **1** and **2** have a FeS_4 core like native Rd in solution, which is also supported by the CD spectra. Complexes **1** and **2** gave CD extrema at 320, 345 and 318, 351 nm in acetonitrile, respectively. Reduced Rd and $[Fe(PhCH_2OCO\text{-}Cys\text{-}Pro\text{-}Leu\text{-}Cys\text{-}OMe)_2]^{2-}$ also show one trough and one peak in their CD spectra. The patterns of the CD spectra of all four species were almost the same, although the peak heights for **1** and **2** were smaller than those of native Rd. The spectral data (UV/VIS, CD and ^1H NMR) indicate that the peptides with nitrogen terminals linked by a *cis*-1,2-cyclohexylenedicarbonyl group can co-ordinate to iron(II) ion and form mononuclear complexes, $[Fe\{cis\text{-}C_6H_{10}(CO\text{-}Cys\text{-}OMe)_2\text{-}1,2\}_2]^{2-}$ and $[Fe\{cis\text{-}C_6H_{10}(CO\text{-}Cys\text{-}Pro\text{-}Leu\text{-}Cys\text{-}OMe)_2\text{-}1,2\}]^{2-}$, respectively.

The l.m.c.t. band of complex **2** at 316 nm is slightly red-shifted compared to that of $[Fe(PhCH_2OCO\text{-}Cys\text{-}Pro\text{-}Leu\text{-}Cys\text{-}OMe)_2]^{2-}$ [314 nm (ε 5900 dm^3 mol^{-1} cm^{-1}) in acetonitrile]. This reflects the difficulty in forming an intramolecular NH ··· S hydrogen bond involving the Leu residue in complex **2** as will be discussed later.

Electrochemical Properties.—Table 2 shows the redox potentials and the relevant parameters of complexes **1** and **2**. The redox potentials were −0.60 and −0.78 V *vs* SCE in

Table 2 Electrochemical properties of complexes **1** and **2** in dimethyl sulfoxide

Complex	E_{pa}*	E_{pc}*	$E_{\frac{1}{2}}$*	i_{pc}/i_{pa}
1	−0.45	−0.75	−0.60	1.10
2	−0.73	−0.83	−0.78	0.88

* In V *vs.* SCE.

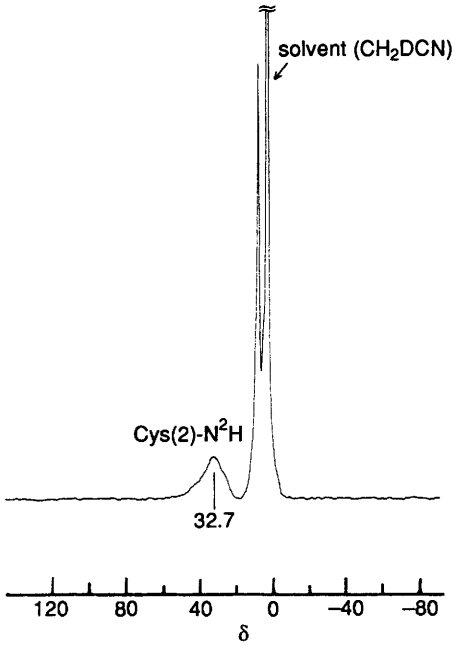

Fig. 3 The ^2H NMR spectrum of complex **2** in acetonitrile at 30 °C

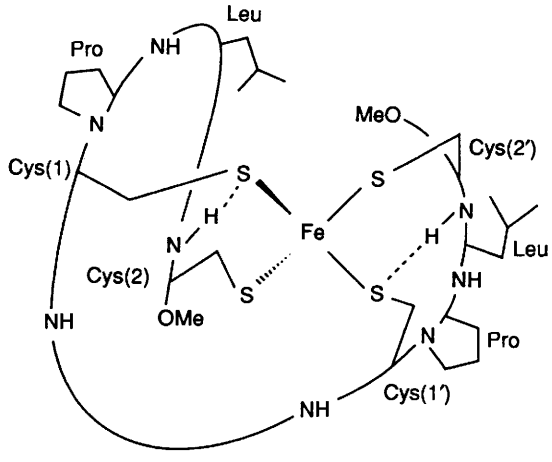

Fig. 4 Schematic representation of the proposed structure of complex **2** showing the Cys(2)-NH • • • S-Cys(1) hydrogen bond and the absence of a Leu-NH • • • S-Cys(1) hydrogen bond

dimethyl sulfoxide, respectively. The complex of the tetradentate ligand **2** showed a more negative redox potential than that of the complexes containing bidentate ligands, *e.g.* $[Fe(PhCH_2OCO\text{-}Cys\text{-}Pro\text{-}Leu\text{-}Cys\text{-}OMe)_2]^{2-}$, which exhibited a redox potential of −0.47 V in the same solvent.[6]

NH • • • S *Hydrogen Bonds.*—The existence of NH • • • S hydrogen bonds was investigated by IR or Raman spectroscopy indirectly.[10,11] Recently, we have found direct evidence for such bonds in iron(II) cysteine peptide complexes by observation of isotropically shifted N^2H signals using ^2H NMR spectroscopy.[6] The ^2H NMR spectrum of complex **2** was measured in acetonitrile at 30 °C (Fig. 3) and compared with that of $[Fe(PhCH_2OCO\text{-}Cys\text{-}Pro\text{-}Leu\text{-}Cys\text{-}OMe)_2]^{2-}$. Complex **2** exhibited an isotropically shifted N^2H signal at δ 32.7 in acetonitrile at 30 °C while $[Fe(PhCH_2OCO\text{-}Cys\text{-}Pro\text{-}Leu\text{-}Cys\text{-}OMe)_2]^{2-}$ gave ones at δ 40.1, 34.7 and −4.1. The signal at δ 32.7 of **2** definitely corresponds to those at δ 40.1 and 34.7 of the complex of $PhCH_2OCO\text{-}Cys\text{-}Pro\text{-}Leu\text{-}Cys\text{-}OMe$, and this single peak indicates that only one species is formed by co-ordination of *cis*-$C_6H_{10}(CO\text{-}Cys\text{-}Pro\text{-}Leu\text{-}Cys\text{-}OMe)_2$-1,2 to Fe^{II}.

As reported previously,[12] $[Fe\{PhCH_2OCO\text{-}Cys(1)\text{-}Ala\text{-}Pro\text{-}Cys(2)\text{-}OMe\}_2]^{2-}$ shows N^2H signals at δ 24.5 and 22.8 in acetonitrile at 30 °C which was assigned to a Cys(2)-N^2H • • • S-Cys(1) hydrogen bond. Therefore, we assigned the N^2H signal at δ 32.7 of complex **2** to Cys(2)-N^2H • • • S-Cys(1). The signals at δ 40.1, 34.7 and −4.1 of $[Fe\{PhCH_2OCO\text{-}Cys(1)\text{-}Pro\text{-}Leu\text{-}Cys(2)\text{-}OMe\}_2]^{2-}$ were assigned to Cys(2)-N^2H • • • S-Cys(1) and Leu-N^2H • • • S-Cys(1) hydrogen bonds. The absence of a N^2H signal at higher field in the ^2H NMR spectrum of complex **2** (see Fig. 3) suggests the absence of a Leu-N^2H • • • S-Cys(1) hydrogen bond. This result was supported by the slight red shift of l.m.c.t. absorption as mentioned above which is attributable to the formation of only one NH • • • S hydrogen bond; the formation of a second NH • • • S hydrogen bond was previously found to result in a blue shift of the l.m.c.t. band.[3,13]

The *para*-substituent effect in the complexes $[Fe(PhCH_2\text{-}OCO\text{-}Cys\text{-}Pro\text{-}Leu\text{-}Cys\text{-}Gly\text{-}NHC_6H_4X\text{-}p)_2]^{2-}$ (X = MeO, H, F or CN) indicates that some electronic charge flows from Fe^{II} to the anilide through the NH • • • S hydrogen bond.[6] Hence, the electron density on the S atom of the co-ordinated cysteine residue was decreased by formation of the NH • • • S hydrogen bond.[13] Therefore, the electron density on the S atom was deduced to be higher in complex **2** than that in $[Fe(PhCH_2OCO\text{-}Cys\text{-}Pro\text{-}Leu\text{-}Cys\text{-}OMe)_2]^{2-}$ because of the absence of a Leu-NH • • • S-Cys(1) hydrogen bond in **2**, resulting in a red shift of the l.m.c.t. band and a negative shift of the redox potential.

In general, the larger the spin density the larger are the Fermi contact shifts in the ^1H NMR spectrum.[14] The observation of Cys $C_\beta H_2$ ^1H NMR signals at much lower field for complex **2** than that for $[Fe(PhCH_2OCO\text{-}Cys\text{-}Pro\text{-}Leu\text{-}Cys\text{-}OMe)_2]^{2-}$ was explained by the higher spin density on the S atom due to the absence of a Leu-NH • • • S-Cys(1) hydrogen bond. Actually, $[Fe(PhCH_2OCO\text{-}Cys\text{-}Ala\text{-}Pro\text{-}Cys\text{-}OMe)_2]^{2-}$ shows four Cys $C_\beta H_2$ ^1H NMR signals between δ 228 and 275 in CD_3CN at 30 °C,[12] similar to those of complex **2**.

The absence of a Leu-NH • • • S-Cys(1) hydrogen bond in complex **2** is ascribed to steric congestion between the nitrogen terminal of the tetrapeptide fragments bridged by the cyclohexylene ring. A schematic structure of complex **2** is shown in Fig. 4. The distance $N_{Cys(1)}$–$N_{Cys(1')}$ in **2** is 4.7 Å estimated from BIOGRAF calculation, while those in the two isomers of $[Fe(PhCH_2OCO\text{-}Cys\text{-}Pro\text{-}Leu\text{-}Cys\text{-}OMe)_2]^{2-}$ were 8.7 and 11.1 Å, respectively. Thus, it is clear that this bond length has become shorter upon connection of the nitrogen terminals of the Cys-Pro-Leu-Cys fragments and this probably makes a large conformational difference between **2** and $[Fe(PhCH_2OCO\text{-}Cys\text{-}Pro\text{-}Leu\text{-}Cys\text{-}OMe)_2]^{2-}$. Similar results have been reported for $[Mo^VO]^{3+}$ complexes.[4] Thermally unstable $[Mo^VO\{cis\text{-}C_6H_{10}(CO\text{-}Cys\text{-}Pro\text{-}Leu\text{-}Cys\text{-}OMe)_2\text{-}1,2\}]^+$ was prepared by ligand-exchange reaction between the free peptide and $[Mo^VO(SPh)_4]^-$ in acetonitrile solution. However, the corresponding complex of $PhCH_2OCO\text{-}Cys\text{-}Pro\text{-}Leu\text{-}Cys\text{-}OMe$ is stable. The thermal instability of the former complex was believed to be due to the steric congestion of the two Cys-Pro-Leu-Cys peptide chains. In the case of iron(II) a thermally stable complex is formed by co-ordination of *cis*-$C_6H_{10}(CO\text{-}Cys\text{-}Pro\text{-}Leu\text{-}Cys\text{-}OMe)_2$-1,2. However, the spectral and electrochemical data indicate that the $C_6H_{10}(CONH)_2$ group is too short to serve as a suitable spacer for two peptide chains. The steric congestion at the spacer causes the disruption of the Leu-NH • • • S-Cys(1)

hydrogen bond. A further study of this point will involve finding a suitable spacer between the two chelating peptide chains.

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Received 26*th June* 1992; *Paper* 2/03379H