Spectral and electrochemical properties of an iron(II) complex of Z–Cys–Ala–Pro–Cys–OMe and new synthesis of the corresponding Z–Cys–Ala–Ala–Cys–OMe analogue

Wei-Yin Sun, Norikazu Ueyama and Akira Nakamura*
Department of Macromolecular Science, Faculty of Science, Osaka University, Toyonaka, Osaka 560 (Japan)

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Abstract

Iron(II) complexes, $(Et_4N)_2[Fe(Z\text{–}\mathbf{cys}\text{–Ala–Ala–}\mathbf{cys}\text{–OMe})_2]$ (**1**) and $(Et_4N)_2[Fe(Z\text{–}\mathbf{cys}\text{–Ala–Pro–}\mathbf{cys}\text{–OMe})_2]$ (**2**), were synthesized by reaction of the corresponding SH-free peptides with $(Et_4N)_2[Fe(S\text{-t-Bu})_4]$. **1** and **2** give redox potentials at −0.49 and −0.58 V versus SCE in acetonitrile, respectively. The negatively shifted redox potential observed in **2** was explained by the lower contribution of NH---S hydrogen bonds. In the ^2H NMR spectra of **1** and **2** using N-deuterated cysteine peptide ligands, $[Fe(Z\text{–}\mathbf{cys}(1)\text{–Ala–Pro–}\mathbf{cys}(2)\text{–OMe})_2]^{2-}$ (**2**) exhibits isotropically shifted N^2H signals at 24.5 and 22.8 ppm in acetonitrile at 30 °C which were assigned to N^2H of the cys(2) residue due to the formation of a Cys(2)–N^2H---S–Cys(1) hydrogen bond while $[Fe(Z\text{–}\mathbf{cys}(1)\text{–Ala(1)–Ala(2)–}\mathbf{cys}(2)\text{–OMe})_2]^{2-}$ (**1**) gives signals at 33.3, 29.2 and −3.6 ppm due to the hydrogen bonds of Cys(2)–N^2H---S–Cys(1) and Ala(2)–N^2H---S–Cys(1), respectively.

Introduction

Many studies on NH---S hydrogen bonds have been carried out for both native iron–sulfur proteins and model complexes [1–8]. For example, the existence of NH---S hydrogen bonds in rubredoxin was suggested by X-ray analysis, namely three such bonds at Leu(41)–NH---S–Cys(39) (3.55 Å); Cys(42)–NH---S–Cys(39) (3.71 Å) and Val(44)–NH---S–Cys(42) (3.88 Å) in the Cys(39)–Pro–Leu–Cys(42)–Gly– Val chelating segment in *Clostridium pasteurianum* rubredoxin [2]. In the case of 2Fe2S ferredoxin, the nature of the NH---S hydrogen bonds was discussed by Raman spectroscopy [9]. On the other hand, the model studies on, for example, $[Fe(Z\text{–}\mathbf{cys}\text{–Pro–Leu–}\mathbf{cys}\text{–OMe})_2]^{2-}$ and $[Fe(Z\text{–}\mathbf{cys}\text{–Pro–Leu–}\mathbf{cys}\text{–Gly–Val–OMe})_2]^{2-}$, have revealed that such NH---S hydrogen bonds play important roles in regulating the redox potentials of the complexes [7, 8]. We systematically studied the Fe(II)–cysteine peptide complexes as models of reduced rubredoxin [7, 8, 10]. Such cysteine-containing oligopeptide Fe(II) complexes show positively shifted redox potentials compared to those of simple alkanethiolate Fe(II) complexes such as $[Fe(SEt)_4]^{2-}$ or $[Fe(S_2\text{-}o\text{-xyl})_2]^{2-}$, reported by Holm and co-workers [11, 12].

The formation of NH---S hydrogen bonds was investigated by ^2H NMR using N-deuterated peptide–Fe(II) complexes [7, 8]. Typical models, $[Fe(Z\text{–}\mathbf{cys}\text{–Pro–Val–}\mathbf{cys}\text{–OMe})_2]^{2-}$ and $[Fe(Z\text{–}\mathbf{cys}\text{–Pro–Leu–}\mathbf{cys}\text{–OMe})_2]^{2-}$, exhibited N^2H signals around 40, 20 and −5 ppm in acetonitrile at 30 °C, but the assignment of these signals was not straightforward [8].

The sequence of Cys–X–Pro (X = Ala, Ser, Glu etc.) exists in the active site of native metalloproteins. For example, *Solanum tuberosum* plastocyanin has the Cys(84)–Ala–Pro—His(87) sequence around the active site [13]. Instead of the histidine residue, Z–Cys–Ala–Pro–Cys–OMe and its Fe(II) complex were synthesized. Furthermore, the proline residue was considered to play important roles in determining the conformation of proteins since the side-chain of Pro is a five-membered ring and no hydrogen bond is involved in the amide part of Pro [14]. In this paper, we present new results on Fe(II) complexes of Z–Cys–Ala–Ala–Cys–OMe and Z–Cys–Ala–Pro–Cys–OMe. Assignment of the N^2H signals of ^2H NMR spectra was carried out and the influences of NH---S hydrogen bonds on the ^1H NMR spectra and the redox potential will be discussed.

*Author to whom correspondence should be addressed.

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Experimental

Solvents were purified by distillation and deoxygenated by purging with argon gas. All procedures were carried out under an argon atmosphere. Cysteine-containing peptides, Z–Cys–Ala–Ala–Cys–OMe and Z–Cys–Ala–Pro–Cys–OMe (Z = benzyloxycarbonyl), were prepared by the method reported elsewhere [10].

The Fe(II) complex of Z–Cys–Ala–Ala–Cys–OMe has been synthesized by reaction of $FeCl_2$ with an excess of the corresponding SH-free peptide [10]. Now, a new and more convenient ligand-exchange reaction method is employed for preparation of Fe(II)–cysteine peptide complexes as described in the previous paper [8].

$(Et_4N)_2$[Fe(Z–**cys**–Ala–Ala–**cys**–OMe)$_2$] (**1**) and $(Et_4N)_2$[Fe(Z–**cys**–Ala–Pro–**cys**–OMe)$_2$] (**2**) were prepared by ligand-exchange reactions between $(Et_4N)_2[Fe(S\text{-}t\text{-}Bu)_4]$ (15.13 mg, 2.2×10^{-5} mol) and the corresponding SH-free peptides (4.5×10^{-5} mol) as described in previous papers [7, 8]. The N-deuterated peptide ligands were obtained by proton–deuteron exchange of SH-free peptides with methanol-d_1.

Spectral measurements were carried out on the following instruments: UV–Vis, JASCO Ubest-30 spectrophotometer; circular dichroism (CD), JASCO J-40 spectropolarimeter; 400 MHz ^1H NMR and 61 MHz ^2H NMR, JEOL GSX 400 FT NMR spectrometer. Electrochemical measurements wre performed on a YANACO P-1100 with a three-electrode system using a glassy carbon working electrode, a Pt-wire auxiliary electrode and a standard calomel electrode. $[(n\text{-}Bu)_4N][(ClO_4]$ (100 mM) was used as a supporting electrolyte. The voltammograms were recorded at a scan rate of 100 mV/s. Potentials were determined versus saturated calomel electrode (SCE) as a reference.

Results and discussion

UV–Vis and CD spectral data of cysteine peptide–Fe(II) complexes are shown in Table 1. **1** and **2** exhibit absorption maxima at 314 (4850) nm and at 314 (4700) and 334 (sh, 4100) nm in acetonitrile, respectively. These absorptions were assigned to $S^- \rightarrow Fe(II)$ charge transfer (LMCT) since such absorptions were reported for reduced rubredoxin (313 (10900) and 333 (6000) nm in aqueous solution) and for model complexes, e.g. [Fe(Z–**cys**–Pro–Val–**cys**–OMe)$_2$]$^{2-}$ and [Fe(Z–**cys**–Pro–Leu–**cys**–OMe)$_2$]$^{2-}$ in acetonitrile [15, 8]. It is interesting to find that **2** has a shoulder at 334 (4100) nm while **1**, [Fe(Z–cys–Pro–Val–cys–OMe)$_2$]$^{2-}$ and [Fe(Z–cys–Pro–Leu–cys–OMe)$_2$]$^{2-}$ have no such absorption. Furthermore, in the CD spectrum **2** shows an additional through at 348 nm (−2.2) (Table 1). **1** and other cysteine peptide–Fe(II) complexes and reduced rubredoxin show consistently one peak and one trough in each CD spectrum. The results of UV–Vis and CD spectra indicate that **1** and **2** have a similar structure in solution to the reported Fe(II) complexes of Z–Cys–Pro–Val–Cys–OMe and Z–Cys–Pro–Leu–Cys–OMe.

The formation of mononuclear Fe(II) complexes of cysteine-containing peptide was detected by ^1H NMR spectra. The signals of the cys $C_\beta H_2$ protons were isotropically shifted to much lower field due to the paramagnetic properties of iron(II). *Desulfovibrio gigas* rubredoxin exhibits four signals at 236, 227, 192 and 150 ppm in D_2O at 55 °C which were assigned to cys $C_\beta H_2$ protons [16]. **1** gives four resonances at 258, 247, 174 and 166 ppm in acetonitrile-d_3 at 30 °C similar to those of native rubredoxin and [Fe(Z–**cys**–Pro–Val–**cys**–OMe)$_2$]$^{2-}$ as reported previously [8]. On the other hand, **2** shows cys $C_\beta H_2$ protons at 275, 262, 252 and 228 ppm in acetonitrile-d_3 at 30 °C. All these cys $C_\beta H_2$ signals shift downfield when the temperature is lowered. The plot of isotropic shifts ($\Delta H/H_0$) and reciprocal temperature (T^{-1}) was linear between −30 and 30 °C. Each extrapolation of the linear plots crossing at almost zero indicates that the isotropic shifts are due to contact contributions as discussed for Z–Cys–Pro–Val–Cys–OMe and Z–Cys–Pro–Leu–Cys–OMe analogues in the previous paper [8]. This indicates that the high spin mononuclear complexes were formed by coordination of Z–Cys–Ala–Ala–Cys–OMe and Z–Cys–Ala–Pro–Cys–OMe to the Fe(II) ion. In addition, no signals were observed between 60 and 120 ppm (in acetonitrile-d_3 at 30 °C). This ensures us of the absence of polynuclear species since the CH_2 protons of $[Fe_2(SCH_2CH_3)_6]^{2-}$ and $[Fe_4(SCH_2CH_3)_{10}]^{2-}$ give signals in this region [17].

The cys $C_\beta H_2$ signals of **2** appeared at lower field compared to those of **1**, [Fe(Z–**cys**–Pro–Val–**cys**–OMe)$_2$]$^{2-}$ or [Fe(Z–**cys**–Pro–Leu–**cys**–OMe)$_2$]$^{2-}$. Similar results were also found for the corresponding

TABLE 1. Absorption and CD spectral data of Fe(II)–cysteine peptide complexes in acetonitrile

Complexes	Absorption[a]	CD[b]
[Fe(Z–**cys**–Ala–Ala–**cys**–OMe)$_2$]$^{2-}$ (**1**)	314 (4850)	316 (−1.7), 347 (0.4)
[Fe(Z–**cys**–Ala–Pro–**cys**–OMe)$_2$]$^{2-}$ (**2**)	314 (4700), 334 (sh, 4100)	314 (−1.9), 328 (0.9), 348 (−2.2)

[a]In nm (ϵ, M^{-1} cm^{-1}). [b]In nm ($\Delta\epsilon$, M^{-1} cm^{-1}).

2Fe2S complexes. A peptide model of the 2Fe2S complex, $[Fe_2S_2(Z\text{–}\mathbf{cys}\text{–Ala–Ala–}\mathbf{cys}\text{–OMe})_2]^{2-}$, has been reported to show two distinct cys $C_\beta H_2$ signals at 30.7 and 22.9 ppm in DMSO-d_6 at 30 °C while $[Fe_2S_2(Z\text{–}\mathbf{cys}\text{–Ala–Pro–}\mathbf{cys}\text{–OMe})_2]^{2-}$ exhibits these peaks at 34.5 and 31.3 ppm [18, 19]. The latter one gives cys $C_\beta H_2$ signals at lower field than those of the former one. Thus, the Cys–X–Y–Cys–OMe peptide ligands were considered to chelate to Fe^{2+} and the $[Fe^{III}{}_2S_2]^{2+}$ core in a similar way [8].

The downfield-shifted cys $C_\beta H_2$ signals of **2** imply that fewer NH---S hydrogen bonds are formed (*vide infra*). The existence of NH---S hydrogen bonds was investigated by measurements of the ^2H NMR spectrum using N-deuterated cysteine peptide–Fe(II) complexes. 61 MHz ^2H NMR spectra of **1** and **2** are shown in Fig. 1. **2** presents isotropically shifted N^2H signals at 24.5 and 22.8 ppm in acetonitrile at 30 °C. These signals were assigned to cys(2) N^2H of $[Fe(Z\text{–}\mathbf{cys}(1)\text{–Ala–Pro–}\mathbf{cys}(2)\text{–OMe})_2]^{2-}$ (**2**) and were isotropically shifted to downfield through the Cys(2)–N^2H---S–Cys(1) hydrogen bond because of the absence of amide proton at the proline residue. Four isotropically shifted N^2H signals at 33.3, 29.2, 19.3 and −3.6 ppm were observed for complex **1** in acetonitrile at 30 °C. The signals at 33.3 and 29.2 ppm were suggested to correspond to the signals at 24.5 and 22.8 ppm in complex **2** and assigned to N^2H of the Cys(2) residue in $[Fe(Z\text{–}\mathbf{cys}(1)\text{–Ala}(1)\text{–Ala}(2)\text{–}\mathbf{cys}(2)\text{–OMe})_2]^{2-}$ (**1**). The signal at 19.3 ppm was assigned to cys(1) N^2H since no hydrogen bond was involved in cys(1) NH (or N^2H) as described previously [8]. Therefore, the peak at −3.6 ppm was left to Ala(2) N^2H due to the formation of the Ala(2)–N^2H---S–Cys(1) hydrogen bond. The observations of both up- and downfield shifted N^2H signals may be due to the opposite sign of spin density as mentioned previously [8]. Similarly, the N^2H signals around 40 and −5 ppm observed in $[Fe(Z\text{–}\mathbf{cys}(1)\text{–Pro–Val–}\mathbf{cys}(2)\text{–OMe})_2]^{2-}$ or $[Fe(Z\text{–}\mathbf{cys}(1)\text{–Pro–Leu–}\mathbf{cys}(2)\text{–OMe})_2]^{2-}$ were assignable to cys(2) N^2H and Val or Leu N^2H, respectively.

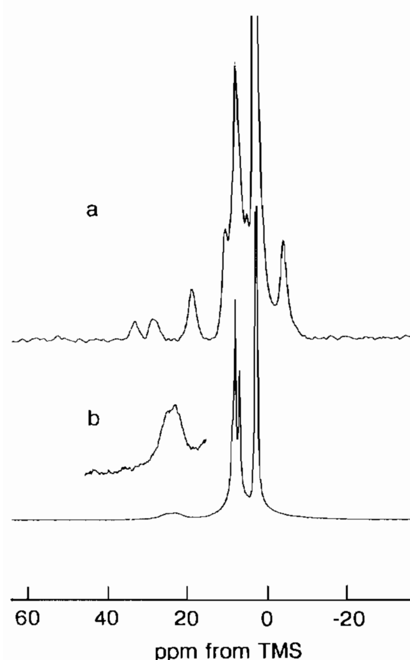

Fig. 1. ^2H NMR spectra of cysteine-containing peptide Fe(II) complexes in acetonitrile at 30 °C. (a) $[Fe(Z\text{–}\mathbf{cys}\text{–Ala–Ala–}\mathbf{cys}\text{–OMe})_2]^{2-}$ (**1**) and (b) $[Fe(Z\text{–}\mathbf{cys}\text{–Ala–Pro–}\mathbf{cys}\text{–OMe})_2]^{2-}$ (**2**).

The formation of NH---S hydrogen bonds has been shown to cause a positive shift of the redox potential [7, 8, 10]. Hence, the difference in number of NH---S hydrogen bonds between **1** and **2** was expected to cause a difference in the redox potentials of **1** and **2**. Thus, the electrochemical properties of cysteine peptide–Fe(II) complexes were examined by cyclic voltammograms and relevant parameters are presented in Table 2. **1** shows the Fe(II)/Fe(III) redox couple at −0.49 V versus SCE in acetonitrile similar to that of $[Fe(Z\text{–}\mathbf{cys}\text{–Pro–Leu–}\mathbf{cys}\text{–OMe})_2]^{2-}$ (−0.54 V versus SCE in acetonitrile). In the case of **2**, the redox potential obtained at −0.58 V versus SCE was negative shifted compared to those of **1** and $[Fe(Z\text{–}\mathbf{cys}\text{–Pro–Leu–}\mathbf{cys}\text{–OMe})_2]^{2-}$ in the same solvent. This was explained by the different number of NH---S hydrogen bonds. There is only one Cys(2)–NH---S–Cys(1) hydrogen bond in the Fe(II) complex of Z–Cys(1)–Ala–Pro–Cys(2)–OMe. There are two hydrogen bonds, i.e. Cys(2)–NH---S–Cys(1) and Ala(2)–NH---S–Cys(1), in complex **1** as mentioned above.

The other characteristic point in the electrochemical properties of **1** and **2** is the difference in reversibility of the cyclic voltammograms. **1** presents a quasireversible redox couple ($i_{pc}/i_{pa}=0.90$) while **2** exhibits one with poor reversibility ($i_{pc}/i_{pa}=0.34$). This was supported by the instability of **2** in air. During the spectral measurements, we found that **2** was much more air-sensitive than **1** and other cysteine peptide–Fe(II) complex such as $[Fe(Z\text{–}\mathbf{cys}\text{–Pro–Leu–}\mathbf{cys}\text{–OMe})_2]^{2-}$.

The formation of NH---S hydrogen bonds has been confirmed to decrease the electron density on the hydrogen-bonded S atom of the cysteine residue [7]. Therefore, the electron density of the S atom in **2** was higher than that in complex **1** and this caused the downfield shifted signals of the cys $C_\beta H_2$ group [20] and the negative shift of the redox potential of **2**.

In conclusion, this study provides further evidence of the role of NH---S hydrogen bonds in native iron–sulfur proteins as well as in model complexes.

TABLE 2. Electrochemical data of Fe(II) complexes of cysteine-containing peptides in acetonitrile

Complex	E_{pa}[a]	E_{pc}[a]	$E_{1/2}$[a]	i_{pc}/i_{pa}
$[Fe(Z–cys–Ala–Ala–cys–OMe)_2]^{2-}$ (**1**)	−0.40	−0.58	−0.49	0.90
$[Fe(Z–cys–Ala–Pro–cys–OMe)_2]^{2-}$ (**2**)	−0.35	−0.81	−0.58	0.34

[a]V vs. SCE.

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