^{199}Hg NMR investigation on the solution structure of Hg(II) complexes of oligopeptides containing cysteine and histidine residues

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Abstract

The Hg(II) complexes of cysteine, histidine-containing oligopeptides, Hg_2Cl_3(Z–**cis**–**his**–OMe) (**1**), Hg_2Cl_3(Z–**cys**–Ala–Ala–**his**–OMe) (**2**), Hg_2Cl_3(Z–**cys**–Ala–Pro–**his**–OMe) (**3**) and Hg_2Cl_3(Z–**cys**–Pro–Val–**his**–OMe) (**4**), were synthesized from $HgCl_2$ and the corresponding *S*-acetamidomethyl-protected peptides. ^{199}Hg NMR studies suggested that Hg(II) ions rapidly exchange even at −55 °C between cysteine thiolate and histidine imidazole groups in solution. The ^{199}Hg NMR signals are observed at higher field from Me_2Hg in the order of chemical shift values; **1** > **4** > **2** > **3**. Self exchange of two Hg(II) occurs through an intermediate of peptide chelating coordination to one of the two Hg(II) ions. The energy-minimum calculations (Biograf) of the peptide complexes support the structure of the proposed intermediate. The observed ^{199}Hg NMR chemical shifts are correlated with the (S,N)-chelating ability of the oligopeptides at the cysteine and histidine residues.

Introduction

Chelating coordination of cysteine (Cys) thiolate and histidine (His) imidazole to metal ion has recently been found in many metalloproteins. For example, plastocyanin (blue copper protein) [1] has an invariant amino acid sequence, Cys–X–Pro–His (X = Ser, Ala or Gln) [2, 3], and the metal center has a distorted tetrahedral Cu(II) ion. In *Saccharomyces* Rieske iron–sulfur protein [4], a His(161)–Leu–Gly–Cys(164)---Cys(178)–Pro–Cys–His(181) fragment chelates to a 2Fe–2S cluster. *Xenopus laevis* zinc finger protein known for its DNA-binding ability also has a –Cys---His–amino acid fragment in the Zn(II)-binding site to sustain the unique protein structure [5].

A few model studies have been reported for the cooperative coordination of cysteine thiolate and histidine imidazole. Although studies on Hg(II)-substituted plastocyanin have revealed thiolate coordination to Hg(II), similar coordination of histidine imidazole to Hg(II) was not found [6]. Ghadiri and Choi have reported that α-helix conformation of a polypeptide, acetyl–Ala–Gly–Ala_3–Lys–Glu–Ala_3–Lys–Cys–Ala_3–His–Ala–NH_2, containing cysteine and histidine residues at the i and $i+4$ positions is stabilized by its coordination to transition metal ions [7]. Summers and co-workers have investigated the Cd(II) complex of an [18]-peptide consisting of three cysteine residues and one histidine residue using the ^{113}Cd NMR method [8]. Chelation with cysteine thiolate and histidine imidazole in this complex has been proposed.

Recently, cysteine coordination to Hg(II) in a mercury metalloregulatory protein, mercuric reductase, has also been investigated [9–12]. For Hg(II) complexes of cysteine-containing oligopeptides, two types of complexes, Hg_2Cl_2(Z–**cys**–X–Y–**cys**–OMe)** and Hg(Z–**cys**–X–Y–**cys**–OMe) (X–Y = Ala–Ala or Val–Val) have been characterized previously [13].

In order to evaluate the influences of X–Y residues in Cys–X–Y–His peptide ligands, we synthesized the four cysteine–histidine containing oligopeptides, Z–Cys–His–OMe, Z–Cys–X–Y–His–OMe (X, Y = Ala; X = Ala and Y = Pro; X = Pro and Y = Val), and the corresponding Hg(II) complexes. We employed the ^{199}Hg NMR technique to analyze the coordination mode of the peptide ligand to Hg(II) ion and demonstrated a correlation of the ^{199}Hg NMR chemical shift with peptide ligand conformational preference at the Cys–X–Y–His moiety at various temperatures.

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**cys and his represent the Cys and His residues coordinating to Hg(II). Z refers to benzyloxycarbonyl.

0020-1693/92/$5.00

Experimental

Materials

L-Alanine, L-valine, L-proline, L-cysteine hydrochloride, L-histidine dihydrochloride, benzyloxycarbonyl chloride (Z-Cl), and 2-tert-butoxycarbonyl oxyimino-2-phenylacetonitrile (Boc-on) were purchased from the Protein Research Foundation. Tetrahydrofuran (THF) and *N,N*-dimethylformamide (DMF) were purified by distillation after refluxing over calcium hydride. All other reagents used were of commercial grade.

Peptide synthesis

tert-Butoxycarbonyl derivatives of L-alanine, L-valine and L-proline were prepared by the procedure in the literature [13]. Histidine methylester dihydrochloride, HCl·H–Cys(Acm)–OH, and Z–Cys(Acm)–OH were also prepared according to the literature methods [13–15].

Synthesis of Z–Cys(Acm)–His–OMe

To a solution of Z–Cys(Acm)–OH (4.0 g, 12 mmol) and triethylamine (1.7 cm^3, 12 mmol) in THF (60 cm^3) was added isobutyl chloroformate (1.6 cm^3, 12 mmol) at −15 °C. After 10 min a solution of 2HCl·H–His–OMe (2.9 g, 12 mmol) and triethylamine (3.4 cm^3, 24 mmol) in DMF (40 cm^3) was added with stirring at −15 °C. The reaction mixture was stirred overnight. The solution was concentrated under reduced pressure. The addition of 4% $NaHCO_3$ aqueous solution (100 cm^3) to the residue resulted in separation of an oily material, which was extracted with ethyl acetate. The organic layer was washed subsequently with sat. NaCl aq., 4% $NaHCO_3$ solution, sat. NaCl aq. solution, water and dried over anhydrous Na_2SO_4. After the desiccant was filtered off, the solvent was removed under reduced pressure. The crude product was reprecipitated from methanol/diethyl ether (58% yield); m.p. 133–135 °C; $[\alpha]_D^{24}$ −13.6 (*c* 2.21, MeOH). *Anal.* Calc. for $C_{21}H_{28}N_5O_6S$: C, 52.71; H, 5.90; N, 14.63. Found: C, 52.12; H, 5.75; N, 14.42%.

Synthesis of Z–Cys(Acm)–Ala–Ala–His–OMe

Boc–Ala–OPac

A solution of Boc–Ala–OH (38.9 g, 210 mmol) and triethylamine (35.2 cm^3, 250 mmol) in THF (200 cm^3) was cooled to 0 °C. To this solution was added phenacyl bromide (50 g, 250 mmol). The reaction mixture was stirred for 8 h at room temperature. The solution was concentrated under reduced pressure. Ethyl acetate (800 cm^3) was added to the residue which was washed with water (500 cm^3). The crude product was recrystallized from ethyl acetate/petroleum ether (88% yield); m.p. 127–129 °C; $[\alpha]_D^{24}$ −60.5 (*c* 2.68, MeOH). *Anal.* Calc. for $C_{16}H_{21}NO_5$: C, 62.53; H, 6.89; N, 4.56. Found: C, 62.44; H, 6.87; N, 4.57%.

Z–Cys(Acm)–Ala–OPac

Hydrogen chloride gas was introduced into a solution of Boc–Ala–OPac (7.5 g, 0.24 mmol) in ethyl acetate. The solution was allowed to stand for 1 h at room temperature. White precipitates of HCl·H–Ala–OPac were formed gradually. The resulting white precipitates were collected on a glass filter. The hydrogen chloride salt obtained was dried over NaOH. Z–Cys(Acm)–Ala–OPac was prepared from Z–Cys(Acm)–OH and HCl·H–Ala–OPac by the same procedure as mentioned for Z–Cys(Acm)–His–OMe (63% yield); m.p. 178–179 °C; $[\alpha]_D^{24}$ −47.2 (*c* 1.85, DMF). *Anal.* Calc. for $C_{25}H_{29}N_3O_7S$: C, 58.24; H, 5.67; N, 8.15. Found: C, 57.95; H, 5.66; N, 8.00%.

Boc–Ala–His–OMe

The peptide was synthesized by the same procedure as for Z–Cys(Acm)–His–OMe. The crude product was recrystallized from ethyl acetate/diethyl ether (42% yield); m.p. 155–156 °C; $[\alpha]_D^{24}$ −6.7 (*c* 2.51, MeOH). *Anal.* Calc. for $C_{15}H_{24}N_4O_5$: C, 52.93; H, 7.11; N, 16.46. Found: C, 52.35; H, 7.10; N, 16.13%.

Z–Cys(Acm)–Ala–OH

To a solution of Z–Cys(Acm)–Ala–OPac (14.5 g, 28 mmol) in acetic acid (1000 cm^3) was added Zn powder (45.7 g, 0.7 mol). The reaction mixture was stirred overnight and filtered off; the solvent was removed under reduced pressure. Resulting crude material was washed with 10% citric acid aqueous solution and water. The product was dried over P_2O_5 *in vacuo* (22% yield) and used for the successive coupling reaction.

Z–Cys(Acm)–Ala–Ala–His–OMe

Hydrogen chloride gas was introduced into a solution of Boc–Ala–His–OMe (3.9 g, 12 mmol) in ethyl acetate. The solution was allowed to stand for 1 h at room temperature. White precipitates of 2HCl·H–Ala–His–OMe formed as the reaction proceeded. The resulting white precipitates were collected on a glass filter. The hydrogen chloride salt obtained was dried over NaOH. To a solution of Z–Cys(Acm)–Ala–OH (4.6 g, 12 mmol) and triethylamine (1.6 cm^3, 12 mmol) in DMF (50 cm^3) and THF (140 cm^3) was added isobutyl chloroformate (1.5 cm^3, 11.5 mmol) at −15 °C. After 10 min a solution of 2HCl·H–Ala–His–OMe and triethylamine (3.5 cm^3, 25.3 mmol) in DMF (60 cm^3) was added with stirring at −15 °C. The reaction mixture was stirred for 1 h at −15 °C and overnight at room temperature. The solution was concentrated under reduced pressure. The addition of 4% $NaHCO_3$ aqueous solution (120 cm^3) to the residual caused the separation

of an oily material, which was extracted with ethyl acetate. The organic layer was washed with sat. NaCl aq., 4% $NaHCO_3$ aq. solution, sat. NaCl aq. solution and dried over anhydrous Na_2SO_4. After the desiccant was filtered off, the filtrate was concentrated under reduced pressure. The crude product obtained was reprecipitated from methanol/diethyl ether (22% yield); m.p. 155–158 °C; $[\alpha]_D^{24}$ −14.7 (*c* 1.36, DMF). *Anal.* Calc. for $C_{27}H_{37}N_7O_8S$: C, 52.33; H, 6.02; N, 15.82. Found: C, 52.17; H, 6.26; N, 15.09%.

Synthesis of Z–Cys(Acm)–Ala–Pro–His–OMe, Z–Cys(Acm)–Pro–Val–His–OMe and Z–Cys(Acm)–Ala–OMe

The peptide, Z–Cys(Acm)–Ala–Pro–His–OMe, was prepared from Z–Cys(Acm)–Ala–OH and 2HCl·H–Pro–His–OMe fragment by the same procedure as mentioned for Z–Cys(Acm)–Ala–Ala–His–OMe. The peptide Z–Cys(Acm)–Pro–Val–His–OMe was prepared from Z–Cys(Acm)–Pro–Val–OH and 2HCl·H–His–OMe fragment by the same method as described for Z–Cys(Acm)–Ala–Ala–His–OMe. The peptide Z–Cys-(Acm)–Ala–OMe was prepared form Z–Cys(Acm)–OH and HCl·H–Ala–OMe by the same procedure as mentioned for Z–Cys(Acm)–Ala–OPac.

Synthesis of Hg(II)/Cys,His-containing peptide complexes

Hg_2Cl_3(Z–cys–his–OMe) (1)

To a solution of Z–Cys(Acm)–His–OMe (300 mg, 0.62 mmol) in MeOH (30 cm^3) was added a solution of $HgCl_2$ (1.7 g, 6.2 mmol) in MeOH (15 cm^3) with stirring at 40 °C. Then NaCl-saturated water (2 cm^3) was added. After 12 h another addition of water (200 cm^3) resulted in precipitation of white solids, which were collected with filtration, washed with water and dried over P_2O_5 *in vacuo* (60% yield); m.p. 127–130 °C. *Anal.* Calc. for $C_{18}H_{21}N_4O_5SHg_2Cl_3$: C, 52.33; H, 6.02; N, 15.82. Found: C, 52.17; H, 6.26; N, 15.08%.

Hg_2Cl_3(Z–cys–Ala–Ala–his–OMe) (2)

The complex was synthesized by the same method as described for **1**. As the reaction proceeded white solids were gradually precipitated, which were collected by filtration, washed with water and methanol and dried over P_2O_5 *in vacuo* (62% yield); m.p. 149–151 °C. *Anal.* Calc. for $C_{24}H_{31}N_6O_7Hg_2Cl_3S$: C, 27.23; H, 2.96; N, 7.96. Found: C, 26.99; H, 3.41; N, 7.73%.

Hg_2Cl_3(Z–cys–Ala–Pro–his–OMe) (3)

The complex was synthesized by the same method as described for **2**. As the reaction proceeded, white solids were gradually precipitated (54% yield); m.p. 148–149 °C. *Anal.* Calc. for $C_{26}H_{33}N_6O_7SHg_2Cl_3$: C, 28.88; H, 3.08; N, 7.77. Found: C, 29.45; H, 3.35; N, 7.55%.

Hg_2Cl_3(Z–cys–Pro–Val–his–OMe) (4)

To a solution of Z–Cys(Acm)–Pro–Val–His–OMe (250 mg, 0.37 mmol) in ethanol (10 cm^3) was added an ethanol solution (10 cm^3) of $HgCl_2$ (250 mg, 0.37 mmol) at room temperature. White solids were gradually precipitated, which were collected by filtration, washed with ethanol and dried over P_2O_5 *in vacuo* (65% yield); m.p. 140–143 °C. *Anal.* Calc. for $C_{28}H_{37}N_6O_7Hg_2Cl_3$: C, 30.32; H, 3.36; N, 7.58. Found: C, 30.21; H, 3.61; N, 7.47%.

HgCl(Z–cys–Ala–OMe) (5)

To a solution of Z–Cys(Acm)–Ala–OMe (500 mg, 1.21 mmol) in MeOH (20 cm^3) and DMF (5 cm^3) was added a NaCl-saturated aqueous solution (2 cm^3), H_2O (4 cm^3) and $HgCl_2$ (2.6 g, 9.6 mmol) at room temperature. White solids were gradually precipitated (90% yield); m.p. 186–188 °C. *Anal.* Calc. for $C_{15}H_{24}N_4O_5HgCl$: C, 31.31; H, 3.33; N, 4.87. Found: C, 31.21; H, 3.60; N, 5.24%.

$HgCl_2$(imidazole) (6)

To a solution of imidazole (200 mg, 2.94 mmol) in MeOH (10 cm^3) was added a methanol solution of $HgCl_2$ (798 mg, 2.94 mmol) at room temperature. White solids, which immediately precipitated, were collected by filtration, washed with methanol and dried over P_2O_5 *in vacuo* (90% yield); m.p. 190 °C. *Anal.* Calc. for $C_3H_4N_2HgCl_2$: C, 10.61; H, 1.19; N, 8.25. Found: C, 10.58; H, 1.20; N, 8.07%.

Physical measurements

Measurements of ^1H and ^{13}C NMR spectra were carried out on JEOL FX-90Q, GSX-270 and GSX-400 spectrometers at 30 °C. The ^{199}Hg NMR spectra were measured at 71.35 MHz with a JEOL GSX-400 spectrometer fitted with a 10-mm multinuclear probe locked to D_2O in an insert. The chemical shifts are reported in ppm relative to the peak of dimethylmercury in DMF or Me_2SO as external reference. All ^{199}Hg NMR spectra were recorded at an [Hg] = 24 mM solution of Hg(II) complex in DMF or Me_2SO at 30 °C. ^1H decoupling was employed to obtain better resolution.

Energy-minimum calculations were carried out using a Biograf molecular graphics/mechanics program (graphic analysis was performed with Biograf, version 1.40 (Biodesign Inc., Pasadena, CA)). Net charges for the metal and ligands were obtained by the extended Hückel molecular orbital (EHMO) calculations. EHMO calculations were performed using an NEC PC-98 EHMO program obtained from Kodansha Sci. Co. The bond parameters (Å) used in Hückel MO calculations

are: Hg–N, 2.34; Hg–S, 2.38; Hg–Cl, 2.28; C–S, 1.80; C–H, 1.09 Å. The bond lengths and bond angle values of imidazole ring part used were from the ones reported by Freeman [16]. As a model of HgCl–(S-**cys**)–C_β, HgCl(SCH_3) was used to obtain net charges on: Hg, 0.69; S, −0.44; Cl, −0.50; C, 0.07. The net charges for the HgCl–N(**his**) imidazole ring were obtained by the calculation on [HgCl(4-methylimidazole)]$^+$: net charge; Hg, 0.78; N(1) (coordinated to Hg), −0.26; C(2), 0.51; N(2), −0.13; C(4), 0.21; C(5), 0.04; $C(CH_3)$, 0.51. For other net charges on peptide ligands, default values in the program were used.

Results and discussion

^1H and ^{13}C NMR spectra of Hg_2Cl_3(cys,his-peptide)

The coordination of the Cys,His-peptides to Hg(II) ions was investigated by means of $^{13}C\{^1H\}$ and ^1H NMR spectroscopy. Table 1 summarizes the $^{13}C\{^1H\}$ and ^1H chemical shift values of **1–6**.

A clear high-field shift (−2.2 to −2.5 ppm) of CysC_β carbon signals was observed for **1–4**. Such a shift is due to coordination of the cysteine thiolate group to Hg(II) [17].

Slight shifts (−0.5 to −0.9 ppm) of $^{13}C(2)_{im}$ signals of **1–4** from those of the corresponding free peptide ligand were observed upon coordination of peptide to Hg(II). These slight shifts are significant in comparison with the shift (−1.7 ppm; high-field shift) of the $^{13}C(6)$ signal observed for [MeHg(2-SC_5H_4N)]. Since the presence of Hg–N secondary interaction in [MeHg(2-SC_5H_4N)] has been proposed [18], **2–4** are considered to have a weak Hg–N interaction. Although the shift of the $^{13}C(2)_{im}$ signal of **1** from that of the free peptide ligand is smaller than that of **2–4**, ^1H NMR data indicate the weak Hg–N interaction of **1**.

Coordination of the histidine imidazole group to Hg(II) was also supported by ^1H NMR data. The $H(2)_{im}$ proton signals of **1–4** shift to lower field than that of the free peptide. These shifts (0.98–1.46) are due to coordination of the histidine imidazole group to Hg(II). ^1H NMR data for the NH_{im} proton also support this result. A similar shift (−2.0 ppm; high-field shift) of the $H(2)_{im}$ proton signal from that of the free peptide ligand is observed upon coordination of Ac–Ala–Glu–Ala_3–Lys–Glu–Ala_3–Lys–Cys–Ala_3–His–Ala–NH_2 to the Cd(II) ion [7]. **2–4** show a larger shift of NH_{im} and $H(2)_{im}$ signals from that of the free peptide ligand than that of **1**. This result indicates that the histidine imidazole group of **2–4** interacts with Hg(II) more strongly as compared to that of **1**. Thus, the coordination modes of the cysteine thiolate group of **1–4** are similar, whereas the histidine imidazole group weakly interacts with Hg(II) depending on the number of amino acid residues intervened between the Cys and His residues.

^{199}Hg NMR spectra of Cys,His-containing peptide complexes

The ^{199}Hg NMR spectra of the Hg_2Cl_3(**cys,his**-peptide) complexes show a single resonance in Me_2SO or DMF at 30 °C (Table 2). The ^{199}Hg NMR chemical shifts of **5** and **6** were also obtained for comparison with the above results. The ^{199}Hg NMR peaks shown by most common organomercury compounds fall into a range of +400 to −1600 ppm [19, 20]. Complex **1** exhibits a ^{199}Hg NMR signal at −1142 ppm in Me_2SO. The ^{199}Hg NMR signal of **1** exists at lower field than that of **6**. The low-field shift of the ^{199}Hg signal observed in **1–4** is in the order of **2** > **3** > **4** > **1** in DMF or Me_2SO.

Figure 1 shows the temperature dependency of the ^{199}Hg NMR signal of **3** in DMF. The broad signal does not separate into two peaks for the two Hg(II) ions in DMF even at −55 °C. At low temperature, a slight change of the ^{199}Hg NMR chemical shift was observed, which is the effect due to temperature variation, since the observed ^{199}Hg NMR chemical shifts are temperature dependent. A broad signal of **3** obtained at −55 °C suggests rapid exchange of the Hg(II) ion between the Cys thiolate group and His imidazole group in solution. The presence of such a rapid ligand exchange process has already been found by the ^1H NMR spectral data of a binary mixture of $Me_3CHgSCMe_3$ and 2,4,6-$Me_3C_6H_3HgSCMe_3$ in dichloromethane at −60 °C [21].

TABLE 1. Selected ^1H and $^{13}C\{^1H\}$ NMR data of peptide Hg(II) complexes **1–5** and $HgCl_2$(imidazole) (**6**) in Me_2SO-d_6

Compound	NH_{im}[a]	$H(2)_{im}$[b,e]	$^{13}C(2)_{im}$[c,e]	cys$^{13}C_\beta$[d]
Hg_2Cl_3(Z–**cys**–**his**–OMe) (**1**)	13.6	8.48	134.2	30.1
Hg_2Cl_3(Z–**cys**–Ala–Ala–**his**–OMe) (**2**)	14.2	8.96	133.9	30.3
Hg_2Cl_3(Z–**cys**–Ala–Pro–**his**–OMe) (**3**)	13.9	8.94	133.9	30.4
Hg_2Cl_3(Z–**cys**–Pro–Val–**his**–OMe) (**4**)	13.9	8.95	133.8	30.1
HgCl(Z–**cys**–Ala–OMe) (**5**)				36.7
$HgCl_2$(imidazole) (**6**)	[f]	8.0	[f]	

[a]Free peptide ligand (11.7–11.9 ppm). [b]Free peptide ligand (7.50–7.53). [c]Free peptide ligand (134.6–134.8 ppm). [d]S-protected peptide (32.5–32.7 ppm). [e]The imidazole ring numbered according to the IUPAC convention. [f]Signal could not be detected due to the poor solubility.

TABLE 2. Solution ^{199}Hg (71.35 MHz) NMR data of Hg(II) complexes

Compound	^{199}Hg (ppm)[a] ($\Delta\nu_{1/2}$ (Hz))		$\nu_{1/2}(Me_2SO)$
	DMF	Me_2SO	$\nu_{1/2}$(DMF)
Hg_2Cl_3(Z-**cys**-**his**-OMe) (**1**)	−1146(470)	−1142(770)	1.6
Hg_2Cl_3(Z-**cys**-Ala-Ala-**his**-OMe) (**2**)	−1017(680)	−1053(2170)	3.2
Hg_2Cl_3(Z-**cys**-Ala-Pro-**his**-OMe) (**3**)	−1030(400)	−1056(1490)	3.7
Hg_2Cl_3(Z-**cys**-Pro-Val-**his**-OMe (**4**)	−1099(650)	−1102(1020)	1.6
HgCl(Z-**cys**-Ala-OMe) (**5**)	−1123(430)	−1107(1020)	2.4
$HgCl_2$(imidazole) (**6**)	[b]	−1487[c]	

[a]The chemical shifts are reported in ppm relative to dimethylmercury in DMF or Me_2SO. All measurements of $\delta(^{199}Hg)$ were made in [Hg(II)] = 24 mM solution of complex in DMF or Me_2SO. [b]Signal could not be detected due to the poor solubility. [c]Poor solubility (precipitation).

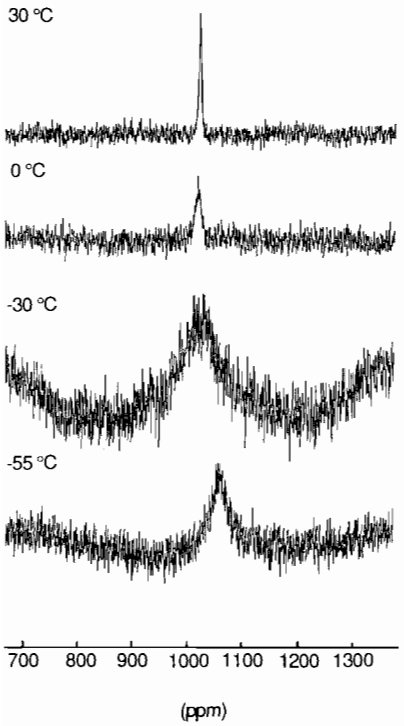


Fig. 1. Temperature dependence of ^{199}Hg NMR (71.35 MHz) spectra of Hg_2Cl_3(Z-**cys**-Ala-Pro-**his**-OMe) (**3**) in DMF at 30, 0, −30 and −55 °C, acquisition time = 0.164 s, pulse width = 20 μs, with applied line broadening of 20 Hz, 1024 scans.

Rabenstein and Fairhurst have reported the interaction of glutathione (γ-L-glutamyl-L-cysteinyl-glycine) with CH_3HgOH in equilibrium between N (amino group) and S (thiolate group) at *c.* pH 8 having a NH_2-glutathione-S$(HgMe)_2$ or (HgMe)NH_2-glutathione-S(HgMe) structure [22].

The ^{199}Hg NMR spectra of Hg_2Cl_3(**cys**,**his**-peptide) complexes showed a broad peak of ^{199}Hg in the range of −1017 to −1146 ppm in DMF with a wide half-width ($\Delta\nu_{1/2}$ = 400–680 Hz) and of −1053 to −1142 ppm in Me_2SO ($\Delta\nu_{1/2}$ = 770–2170 Hz). The half-widths of the ^{199}Hg signal for mercury compounds are known to be 50–60 Hz for $Hg(ClO_4)_2$ in 1.0 M $HClO_4$ solution [18], 16 Hz for $[Hg(SC_6H_4\text{-}2\text{-}SiMe_3)_2]$ and 27 Hz for $[Hg(2\text{-}SC_5H_3N\text{-}3\text{-}SiMe_3)_2]$ [23]. Thus, mercury compounds bound covalently by two thiolate ligands exhibit a narrow ^{199}Hg NMR signal.

The chloride ion of RHgCl mercury complexes can exchange with free chloride ion existing in solution [24, 25]. Godfrey *et al.* have observed a ^{199}Hg NMR signal in an LiCl-saturated solution of mercury(II) chloride and found Cl^- exchange as shown by a single resonance line with wide linewidth in the range of 1000 Hz [26].

The Hg_2Cl_3(**cys**,**his**-peptide) complexes show a single ^{199}Hg NMR signal at 30 °C due to a fast exchange of both mercury and chloride ions. The half-widths of the signals in Me_2SO are larger than those in DMF. Since the ^{199}Hg NMR signal of **2** shifts to the same extent even at double the concentration ([Hg] = 48 mM), only intramolecular interaction through bridging S(**cys**) between two Hg(II) ions can be considered. Intramolecular sulfur bridging should be involved in the interaction as shown in [HgCl(μ-S-**cys**)HgCl(N-**his**)] with a chelating structure of Z-Cys-X-Y-His-OMe (see Scheme 1 discussed later).

There are two groups of the half-width of the ^{199}Hg resonance line obtained for **1**–**4** (see Table 2). The first group contains **2** and **3** which exhibit a broad linewidth in Me_2SO. The second group includes **1** and **4** which show a narrow linewidth in Me_2SO. This result indicates that **2** and **3** have many conformational isomers in Me_2SO. The narrow linewidth of **1** and **4** in Me_2SO is due to a loss of conformational flexibility of the peptide ligand. The modes of conformational change of **1** with the dipeptide fragment are less than that of **2**–**4**. For **4** having a Val residue, the conformational change is limited due to the bulky side chain of the Val residue. Thus, the ^{199}Hg NMR chemical shift of **1**–**4** changes depending on the number and steric effects of amino acid residues intervened between the Cys and His residues.

Exchange of Hg(II) ions in Hg_2Cl_3(cys,his-peptide)

In order to elucidate the low-field shift of the ^{199}Hg signal of Hg_2Cl_3(**cys,his**-peptide) from that of $HgCl_2$(imidazole), three possible intermediates for the intramolecular Hg–Hg exchange in Hg_2Cl_3(**cys,his**-peptide) can be considered. Scheme 1 shows three possible intermediates upon self exchange of two Hg(II).

It is likely that a non-bridging form (A), a bridging form (B), and a μ-S form (C) are in equilibrium which results in mercury ion exchange between Hg(1) and Hg(2). Coordination of S(**cys**) to Hg(2) ion in structures (B) and (C) is expected to make Hg(2) deshield and shift the ^{199}Hg NMR signal to lower field.

The low-field shift is due mainly to the paramagnetic term of the nuclear magnetic shielding constant. The paramagnetic term is related to the electron density in the valence *n*p orbital (p mechanism) and of the holes in the valence (*n*-1)d orbital (d mechanism). The Hg(II) ion has electronic configuration d^{10} similar to Zn(II) and Cd(II) ions. For Zn(II) and Cd(II) ions the p mechanism is dominant compared with the d mechanism, so that the chemical shift increases with increasing electron-donating ability of the ligand [27]. Similarly for the ^{199}Hg ion the p mechanism is expected to be more important than the d mechanism. It is reported that the covalently bonded organomercury compound is the most deshielded [28].

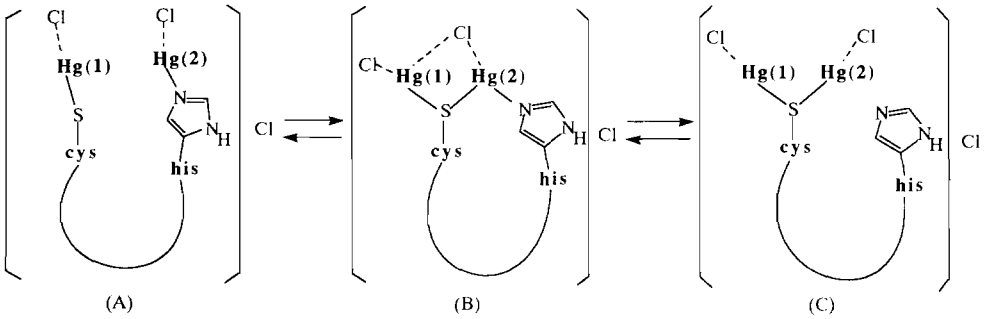
Since the covalently bonded sulfur is somewhat more electron donating than a similar coordination of nitrogen atom or chloride ion to mercury(II), the Hg(2) ion in structures (B) and (C) is deshielded and the ^{199}Hg NMR signal shifts to lower field. Therefore, the low-field shift of the ^{199}Hg NMR signals is correlated with ease of forming intramolecular μ-S(**cys**) bridging between two Hg(II) ions.

Conformational analysis of Cys,His-peptide ligand in Hg(II) complexes

In order to estimate the tendency to form the bridging structure in Hg_2Cl_3(**cys,his**-peptide) complexes, the conformational analysis of Hg_2Cl_3(**cys,his**-peptide) complexes was carried out using a molecular modeling program 'Biograf'. Energy-minimized conformational (local minimum) of both the non-bridging structure (A) and the bridging structure (B) were determined for the $[Hg_2Cl_2$(**cys,his**-peptide)$]^+$ complexes in which the counter anion (Cl^-) is excluded (Table 3).

The bridging structure (B) involves an imaginary bond of S(**cys**)–HgN(**his**) in the –Hg(1)Cl–S(**cys**)–Hg(2)Cl–N(**his**)– unit; the Hg(1) ion is in linear geometry and the Hg(2) ion is in trigonal geometry. The energy-minimization of the non-bridging structure (A) was started by severing the S(**cys**)–Hg(2) bond from the bridging structure (B). For all complexes, the bridging structure (B) has a higher conformational energy than that of the non-bridging structure (A).

The tendency to form the bridging structure is estimated by the ΔE value (Table 3). The ΔE value is



Scheme 1. Proposed Hg(II) exchange process for Hg_2Cl_3(**cys,his**-peptide).

TABLE 3. Total energy (kcal/mol) of the local minimum structures of bridging and non-bridging structure of Hg_2Cl_3(**cys,his**-peptide) (**1–4**)

Compound	Total energy (kcal/mol)		$\Delta E = E_2 - E_1$
	E_1 (non-bridging; (A))	E_2 (bridging; (B))	
Hg_2Cl_3(Z–**cys**–**his**–OMe) (**1**)	36	220	184
Hg_2Cl_3(Z–**cys**–Ala–Ala–**his**–OMe (**2**)	41	212	171
Hg_2Cl_3(Z–**cys**–Ala–Pro–**his**–OMe) (**3**)	53	223	170
Hg_2Cl_3(Z–**cys**–Pro–Val–**his**–OMe) (**4**)	70	239	169

the difference in the conformational energy between the non-bridging structure (A) and the bridging structure (B). The energy difference (ΔE) for **1** is 184 kcal/mol and **2**, **3** and **4** show a similar ΔE value ($\approx$170 kcal/mol). Since the complexes with smaller ΔE values tend to form the bridging chelate structure (B), the Hg_2Cl_3(Z–**cys**–X–Y–**his**-OMe) can form the bridging structure (B) more easily compared with Hg_2Cl_3-(Z–**cys**–**his**–OMe). Among complexes **1–4**, complex **4** has a higher conformational energy for both the bridging and non-bridging structure than **3** and **2**. The calculated larger conformational energy for the bridging and the non-bridging structure of **4** is due to the steric congestion by the side chains of Pro and Val residues. Therefore, **4** does not have any advantage of forming a bridging structure relative to **2** and **3**.

Conclusions

Thus, our ^{199}Hg NMR studies of Hg_2Cl_3(**cys,his**-peptide) and Biograf energy-minimum calculations reveal that the ^{199}Hg NMR chemical shift of Hg_2Cl_3(Z–**cys**–X–Y–**his**–OMe) in solution is correlated with the chelating ability of Cys–X–Y–His according to the characteristics of interposed amino acid residues, X–Y. The observed ability of the Cys–X–Y–His peptide fragments to vary the electronic character of the Hg(II) ion is important for the understanding of the coordination chemistry of the active site of metalloproteins containing cysteine and histidine residues.

Acknowledgement

The authors are grateful to Professor Yukiteru Katsube, Institute for Protein Research, Osaka University, for kind permission to use the Biograf molecular graphics/mechanics program.

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