Synthesis of Hg(II) Complexes of Cysteine-Containing Oligopeptides

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Tetrapeptides, Z-Cys-Ala-Ala-Cys-OMe (Z=benzyloxycarbonyl), Z-Cys-Val-Val-Cys-OMe, and Z-Cys-Gly-Pro-Cys-OMe, having Cys-X-Y-Cys sequences were synthesized by using an acetamidomethyl (Acm) protecting group. The Hg(II) complexes of these peptides, Hg_2Cl_2(Z-Cys-Ala-Ala-Cys-OMe) **1**.[1] Hg(Z-Cys-Ala-Ala-Cys-OMe) **2**, Hg_2Cl_2(Z-Cys-Gly-Pro-Cys-OMe) **3**, Hg(Z-Cys-Val-Val-Cys-OMe) **4**, and Hg_2Cl_2(Z-Cys-Ala-Cys-OMe) **5** were synthesized by the reaction of the S(Acm) protecting peptides and $HgCl_2$ in *N,N*-dimethylformamide. Two Raman bands due to Cl-Hg-S of **1**, **3**, or **5** were observed at 314, 281, or 276 cm^{-1} in solid, whereas a single band due to S-Hg-S was found at 326 cm^{-1} for **2** or 327 cm^{-1} for **4**, in solid.

The tetrapeptide sequences of -Cys-X-Y-Cys- (X, Y=amino acid residues) serving as a macro-ring chelate with the cysteinyl thiolate coordinations are frequently found for metal binding sites of many of metal thiolate proteins: for example, Fe proteins; Cys(6)-Thr-Val-Cys(9) and Cys(39)-Pro-Leu-Cys(42) for *Clostridium pasteurianum* rubredoxin,[2] Cys(44)-Ser-Ser-Cys-(47) for *Spirulina plantesis* ferredoxin,[3] Cys(8)-Ile-Ala-Cys(11)- Gly-Ala-Cys(14) and Cys(35)-Ile-Asp-Cys(38)-Gly-Ala-Cys(41) for *Peptococcus aerogenes* ferredoxin:[4] Zn proteins; Cys(97)-Gly-Lys-Cys(100)-Arg-Val-Cys-(103) for horse liver alcohol dehydrogenase (E-chain),[5] Cys(137)-Lys-Tyr-Cys(140) for *Escherichia coli* aspartate carbamoyltransferase:[6] metallothioneins: Cys(21)-Lys-Gln-Cys(24), Cys(26)-Ala-Ser-Cys(29), and Cys(41)-Ala-Lys-Cys(44) for equine renal metallothionein 1B.[7] In such cases, conformational restriction due to the steric effects of the chelating tetrapeptides caused by side chains of the two amino acid residues, X and Y, interposed between the two cysteine residues definitively plays crucial roles on the stabilities of the chelate rings, the determination of geometries around a metal ion, and the revelation of enzymatic activities.

In order to elucidate the side chain conformational effects discussed above, we have been studying various metal complexes such as Pd(II)[8,9], Fe(III),[10] Co(II),[11] and Mo(IV, V)[12] of cysteine-containing tetrapeptide (Z-Cys-X-Y-Cys-OMe) in the relevance to the metal thiolate proteins. We were also interested in the formation of Hg(II) complexes of Z-Cys-Val-Val-Cys-OMe possessing more bulky side chains, Z-Cys-Gly-Pro-Cys-OMe having a restricted conformation with a Gly-Pro sequence,[13] and tripeptide, Z-Cys-Ala-Cys-OMe which contains only one amino acid residue between two cysteinyl residues. In biological systems with mercury, Hg(II) complexation with cysteine thiolate is involved in toxicity[14] and detoxicity. For example, mercuric reductase contains two active thiols which are proposed to bind Hg(II) ion to give RS-Hg-SR.[15] In this paper we describe detailed procedures for the syntheses of the cysteine-containing oligopeptides, Z-Cys-X-Y-Cys-OMe (X, Y=Ala; X, Y=Val; X=Gly and Y=Pro) and Z-Cys-Ala-Cys-OMe, and for the syntheses of Hg(II) complexes of the cysteine-containing peptides. We synthesized two types of Hg(II)/Cys-containing peptide complexes such as RS-Hg-SR and RS-Hg-Cl. The bulkiness and hydrophobicity around Cys residue may control the formation of either RS-Hg-SR or RS-Hg-Cl species.

Experimental

Materials. Glycine, L-alanine, L-valine, L-proline, L-cysteine hydrochloride, benzyloxycarbonyl chloride (Z-Cl), anhydrous hydrogen bromide in acetic acid (25%), DCC, and *N*-hydroxysuccinimide (HOSu) were purchased from Protein Research Foundation. Isobutyl chloroformate was purchased from Merck-Schuchardt and used without further purification. 2-(*t*-Butoxycarbonylthio)-4,6-dimethylpyrimidine (*t*-Boc-S) was obtained from Mitsubishi Chemical Industries Co., Ltd. Tetrahydrofuran (THF) and dioxane were distilled after refluxing over sodium. Acetonitrile was refluxed over calcium hydride and distilled. Purification of *N,N*-dimethylformamide (DMF) and triethylamine were purified by distillation. All other reagents used were of commercial grade.

Peptide Syntheses. Benzyloxycarbonyl derivatives of L-alanine, L-valine, and L-proline were prepared by the same procedures with Z-Cl cited in the literature.[16] *t*-Butoxycarbonyl derivative of L-alanine was prepared according to the literature method using *t*-Boc-S.[17] Glycine methyl ester hydrochloride was also prepared by the analogous method to a literature using thionyl chloride.[18] HCl·H-Cys(Acm)-OH was prepared by the modified method to a literature.[19]

1. Synthesis of Z-Cys(Acm)-Ala-Ala-Cys(Acm)-OMe Z-Cys(Acm)-OH. Benzyloxycarbonyl chloride (100 cm^3, 0.5 mol) and 4M NaOH solution (375 cm^3 (1 M=1 mol dm^{-3})) were added alternately to a solution of HCl·H-Cys(Acm)-OH (114.4 g, 0.5 mol) in 4 M NaOH solution (250 cm^3) over about 1.5 h period in an ice-cooled bath, and stirred for 3 h at room temperature. The product was obtained as an oil, which was crystallized on standing the chloroform solution, and recrystallized from ethyl acetate–ether: yield, 70 g (44%); mp 106—107 °C (dec); $[\alpha]_D$ −40.7° (*c* 0.905, methanol). Anal. Calcd for $C_{14}H_{18}N_2O_5S$: C, 51.52; H, 5.56; N, 8.58. Found: C, 51.50; H, 5.63; N, 8.40.

Z-Ala-Cys-(Acm)-OMe. A solution of Z-Ala-OH (45.0 g, 0.20 mol) and triethylamine (28.8 cm^3, 0.20 mol) in THF (200 cm^3) was cooled to −15 °C. To this solution was added iso-Boc-Cl (26.0 cm^3, 0.20 mol) with stirring at −15 °C. After 15 min, a solution of HCl·H-Cys(Acm)-OMe (60.0 g, 0.20 mol) and triethylamine (28.0 cm^3, 0.20 mol) in DMF (50 cm^3) was added with stirring at −15 °C. The reaction mixture was stirred for 1 h with cooling and then overnight at room temperature. The solution was concentrated under

reduced pressure. About 500 cm³ of water was added to the residue and the resulting white solid was collected on a glass filter, and washed sequentially with water, 4% $NaHCO_3$ aqueous solution, water, 2% HCl aqueous solution, and water. The product was dried over P_2O_5 *in vacuo*: yield, 50 g (61%); mp 143—145 °C (dec); $[\alpha]_D$ −21.7° (*c* 1.07, DMF). Anal. Calcd for $C_{18}H_{25}N_3O_6S$: C, 52.54; H, 6.12; N, 10.21. Found: C, 52.53; H, 6.12; N, 10.15.

Z-Ala-Ala-Cys(Acm)-OMe. To Z-Ala-Cys(Acm)-OMe (25 g, 0.06 mol) was added 25% HBr solution in acetic acid (50 cm³) at 0 °C. The solution was allowed to stand for 1.5 h with stirring at room temperature. Ether was added to this solution to precipitate HBr·H-Ala-Cys(Acm)-OMe. The supernatant was decanted off and the residue was washed with ether several times. The dipeptide hydrobromide salt obtained was dried over KOH *in vacuo*. To a solution of Z-Ala-OH (13.5 g, 0.06 mol) and triethylamine (8.3 cm³, 0.06 mol) in THF (200 cm³) was added iso-Boc-Cl (7.7 cm³, 0.06 mol) at −15 °C. After 15 min, a solution of HBr·H-Ala-Cys(Acm)-OMe and triethylamine (8.3 cm³, 0.06 mol) in DMF (30 cm³) was added with stirring at −15 °C. Following treatments were same as the syntheses of Z-Ala-Cys(Acm)-OMe: yield 10.5 g (36%); mp 183—186 °C (dec); $[\alpha]_D$ −32.9° (*c* 1.07, DMF). Anal. Calcd for $C_{21}H_{30}N_4O_7S$: C, 52.27; H, 6.27; N, 11.61. Found: C, 52.26; H, 6.32; N, 11.61.

Z-Cys(Acm)-Ala-Ala-Cys(Acm)-OMe. In order to remove the Z-group, Z-Ala-Ala-Cys(Acm)-OMe (2.3 g, 5 mmol) was treated with about 40 cm³ of 25% HBr/acetic acid. The resulting tripeptide hydrobromide salt, HBr·H-Ala-Ala-Cys(Acm)-OMe, was dried over KOH *in vacuo*. To a solution of Z-Cys(Acm)-OH (1.5 g, 5 mmol) and triethylamine (0.7 cm³, 5 mmol) in THF (100 cm³) was added iso-Boc-Cl (0.6 cm³, 5 mmol) at −15 °C. After 15 min, a solution of HBr·H-Ala-Ala-Cys(Acm)-OMe and triethylamine (0.7 cm³, 5 mmol) in DMF (20 cm³) was added with stirring at −15 °C. The reaction mixture was stirred for 1 h with cooling and stood overnight at room temperature. Subsequent work-up was done in the same manner as described for Z-Ala-Cys(Acm)-OMe: yield 1.2 g (36%); mp 210—213 °C (dec); $[\alpha]_D$ −33.6° (*c* 0.667, DMF). Anal. Calcd for $C_{27}H_{40}N_6O_9S_2$: C, 49.38; H, 6.14; N, 12.80. Found: C, 49.17; H, 6.15; N, 12.09.

t-Boc-Ala-Cys(Acm)-OMe. To a solution of *t*-Boc-Ala-OH (34.1 g, 0.18 mol) and triethylamine (24.8 cm³, 0.18 mol) in THF (200 cm³) was added iso-Boc-Cl (23.1 cm³, 0.18 mol) with stirring at −15 °C. After 15 min, an ice-cooled solution of HCl·H-Cys(Acm)-OMe (43.7 g, 0.18 mol) and triethylamine (24.8 cm³, 0.18 mol) in DMF (40 cm³) and chloroform (100 cm³) was added to the mixed anhydride solution with stirring at −15 °C. The reaction mixture was stirred for 1 h at 15 °C and overnight at room temperature. After evaporation of the solvent under reduced pressure, the addition of about 500 ml of saturated NaCl aqueous solution to the residue caused the separation of an oily material, which was extracted with ethyl acetate. The organic layer was washed sequentially with sat. NaCl aq, 4% $NaHCO_3$ aq solution saturated with NaCl, sat. NaCl aq, 10% citric acid aq solution saturated with NaCl, and sat. NaCl aq solution and dried over anhydrous Na_2SO_4. After the desiccant was filtered off, the solvent was removed under reduced pressure and ether was added to the residue to result in crystallization. The product was collected on a glass filter, washed with ether, and dried over silica gel *in vacuo*: yield, 32 g (47%); mp 85—86 °C (dec); $[\alpha]_D$ −48.7° (*c* 0.371, DMF). Anal. Calcd for $C_{15}H_{27}N_3O_6S$: C, 47.73; H, 7.21; N, 11.13. Found: C, 47.79; H, 7.20; N, 10.16.

t-Boc-Ala-Ala-Cys(Acm)-OMe. Hydrogen chloride gas was introduced into an ice-cooled solution of *t*-Boc-Ala-Cys(Acm)-OMe (20.4 g, 0.054 mol) in dioxane (150 cm³) for about 20 min to result in a white precipitate of HCl·H-Ala-Cys(Acm)-OMe. The reaction mixture was allowed to stand for about 30 min. After an addition of ether, the supernatant was decanted off. The dipeptide hydrochloride salt was washed with ether thoroughly and dried over KOH *in vacuo*. To a solution of *t*-Boc-Ala-OH (10.2 g, 0.054 mol) and triethylamine (7.5 cm³, 0.054 mol) with stirring at −15 °C for about 15 min. To this solution, an ice-cooled solution of the HCl·H-Ala-Cys(Acm)-OMe prepared previously and triethylamine (7.5 cm³, 0.054 mol) in DMF (10 cm³) and chloroform (100 cm³) was added with stirring under the continuous cooling. The reaction mixture was stirred at −15 °C for 1 h and at room temperature overnight. The solvent was removed under reduced pressure and about 500 cm³ of sat. NaCl aq solution was added to the residue. An oily material which was separated on the addition of the NaCl solution was extracted with ethyl acetate. Subsequent procedures were the same as described for *t*-Boc-Ala-Cys(Acm)-OMe: yield 4.2 g (17%); mp 144—145 °C (dec); $[\alpha]_D$ −34.1° (*c* 0.113, DMF). Anal. Calcd. for $C_{18}H_{32}N_4O_7S$: C, 48.20; H, 7.19; N, 12.49. Found: C, 47.23; H, 7.08; N, 12.07.

Z-Cys(Acm)-Ala-Ala-Cys(Acm)-OMe. Isobutyl chloroformate (0.9 cm³, 7 mmol) was added to a solution of Z-Cys(Acm)-OH (2.2 g, 7 mmol) and triethylamine (1.0 cm³, 7 mmol) in THF (10 cm³) with stirring at −15 °C. After 15 min, a solution of HCl·H-Ala-Ala-Cys(Acm)-OMe, which was prepared by a treatment of *t*-Boc-Ala-Ala-Cys(Acm)-OMe (3.1 g, 7 mmol) with HCl gas in dioxane (30 cm³), and triethylamine (1.0 cm³, 7 mmol) in a mixed solvent of DMF (5 cm³) and chloroform (10 cm³) was added to the above mixed anhydride solution at −15 °C with stirring. Subsequent treatments were the same as described for Z-Ala-Ala-Cys-(Acm)-OMe: yield 2.9 g (23%); mp 205—208 °C (dec); $[\alpha]_D$ −30.7° (*c* 0.200, DMF). Anal. Calcd for $C_{27}H_{40}N_6O_9S_2$: C, 49.38; H, 6.14; N, 12.80. Found: C, 48.88; H, 6.07; N, 12.41.

2. Synthesis of Z-Cys(Acm)-Ala-Cys(Acm)-OMe. Dipeptide, Z-Ala-Cys(Acm)-OMe (3.8 g, 9 mmol), was treated with 25% HBr/acetic acid (40 cm³) to result in formation of HBr·H-Ala-Cys(Acm)-OMe. A DMF solution (30 cm³) of the free-base dipeptide prepared from the hydrogen bromide salt and triethylamine (1.3 cm³, 9 mmol) was added to a THF solution (100 cm³) of a mixed anhydride prepared from Z-Cys(Acm)-OH (3.0 g, 9 mmol), triethylamine (1.3 cm³, 9 mmol), and iso-Boc-Cl (1.2 cm³, 9 mmol) at −15 °C. Usual work-up as described for the synthesis of Z-Cys(Acm)-Ala-Ala-Cys(Acm)-OMe gave a tripeptide, Z-Cys(Acm)-Ala-Cys(Acm)-OMe: yield 1.0 g (19%); mp 209—212 °C (dec); $[\alpha]_D$ −34.8° (*c* 1.07, DMF). Anal. Calcd for $C_{24}H_{35}N_5O_8S_2$: C, 49.22; H, 6.02; N, 11.96. Found: C, 48.43; H, 5.92; N, 11.43.

iso-Butyl chloroformate (1.0 cm³, 8 mmol) was added to a solution of Z-Cys(Acm)-OH (2.6 g, 8 mmol) and triethylamine (1.1 cm³, 8 mmol) in THF (80 cm³) with stirring at −15 °C. After 15 min, a solution of HCl·H-Ala-Cys(Acm)-OMe, which was prepared by a HCl treatment of *t*-Boc-Ala-Cys(Acm)-OMe (3.0 g, 8 mmol) in dioxane (50 cm³) and triethylamine (1.1 cm³, 8 mmol) in DMF (10 cm³) and chloroform (20 cm³), was added to the mixed anhydride solu-

tion at −15 °C with stirring. After the same treatments as described above, Z-Cys(Acm)-Ala-Cys(Acm)-OMe was obtained: yield 4.0 g (85%); mp 211—212 °C (dec); $[\alpha]_D$ −30.6° (*c* 0.197, DMF). Anal. Calcd for $C_{24}H_{35}N_5O_8S_2$: C, 49.22; H, 6.02; N, 11.96. Found: C, 48.12; H, 5.86; N, 11.44.

3. Synthesis of Z-Cys(Acm)-Val-Val-Cys(Acm)-OMe. *Z-Val-Cys(Acm)-OMe*: The peptide was prepared from Z-Val-OH and H-Cys(Acm)-OMe by the same procedure as mentioned for Z-Ala-Cys(Acm)-OMe. The crude materials were recrystallized from methanol. Yield 45%; mp 164—166 °C; $[\alpha]_D$ −27.0° (*c* 0.97, DMF). Anal. Calcd for $C_{20}H_{29}N_3O_6S$: C, 54.65; H, 6.65; N, 9.56. Found: C, 54.58; H, 6.71; N, 9.53.

Z-Val-Val-Cys(Acm)-OMe: The peptide was prepared from Z-Val-OH and H-Val-Cys(Acm)-OMe by the same method mentioned above. The crude product was recrystallized from methanol. Yield 81%; mp 231—236 °C (dec); $[\alpha]_D$ −25.2° (*c* 0.95, DMF). Anal. Calcd for $C_{25}H_{38}N_4O_7S$: C, 55.74; H, 7.11; N, 10.40. Found: C, 55.36; H, 7.09; N,10.04.

Z-Cys(Acm)-Val-Val-Cys(Acm)-OMe: The peptide was synthesized from Z-Cys(Acm)-OH and H-Val-Val-Cys(Acm)-OMe by the same procedure for Z-Cys(Acm)-Ala-Ala-Cys(Acm)-OMe. The crude product was washed with ether and dried over P_2O_5 *in vacuo* and recrystallized from methanol. Yield 31%; mp 221—224 °C (dec); $[\alpha]_D$ −29.3° (*c* 0.98, DMF). Anal. Calcd for $C_{31}H_{48}N_6O_9S_2$: C, 52.22; H, 6.80; N, 11.79. Found: C, 51.45; H, 6.73; N, 11.21.

4. Synthesis of Z-Cys(Acm)-Gly-Pro-Cys(Acm)-OMe. Z-Cys(Acm)-Gly-OMe was prepared from Z-Cys(Acm)-OH and H-Gly-OMe with the mixed anhydride method mentioned above. Z-Cys(Acm)-Gly-OH was obtained by hydrolysis with NaOH in methanol/dioxane. Z-Pro-Cys(Acm)-OMe was prepared from Z-Pro-OH and H-Cys(Acm)-OMe by the same procedure for Z-Ala-Cys(Acm)-OMe. Synthesis of HBr·H-Pro-Cys(Acm)-OMe was done by to the same method described for HBr·H-Ala-Cys(Acm)-OMe.

To the reaction mixture containing 2.5 g of Z-Cys(Acm)-Gly-OH, HBr·H-Pro-Cys(Acm)-OMe, and 1.1 cm^3 of triethylamine in 20 cm^3 of DMF was added 0.8 g of HOSu and, then, 1.5 g of DCC at 0 °C. The reaction mixture was stirred for 1 h with cooling and overnight at room temperature. The solution was concentrated under reduced pressure. The residue was dissolved in chloroform and remaining *N,N'*-dicyclohexylurea was filtered off. The filtrate was washed sequentially with water, 2% HCl aq, water, 4% $NaHCO_3$ aq, and water, and dried over anhydrous Na_2SO_4. After removal of the solvent, Z-Cys(Acm)-Gly-Pro-Cys(Acm)-OMe was obtained as a white solid. Yield, 3.3 g (63%); mp 104 °C; $[\alpha]_D$ −93.6° (*c* 0.50, methanol). Anal. Calcd for $C_{28}H_{40}O_9N_6S_2$: C, 50.25; H, 6.03; N, 9.58. Found: C, 50.10; H, 6.07; N, 9.46.

5. Syntheses of Hg(II)/Cys-containing Peptide Complexes. *HgCl(Z-Ala-cys-OMe).* A solution containing 100 mg of Z-Ala-Cys(Acm)-OMe and 65 mg of $HgCl_2$ in 3 cm^3 of Me_2SO was stirred for several hours. A quick addition of 30 ml of water to the Me_2SO solution with cooling resulted in precipitation of a white powder, which was collected on a glass filter, washed with sat. NaCl aq and water, and dried over P_2O_5 *in vacuo*. Yield, 110 mg.

In order to secure a complete removal of Acm-group, it is preferable to use two equiv of $HgCl_2$ for an equiv of Acm-group. Mp 91—96 °C. Anal. Calcd for $C_{15}H_{10}N_2SHgCl$: C, 31.31; H, 3.33; N, 4.87. Found: C, 31.23; H, 3.40; N, 4.97.

Hg_2Cl_2(Z-cys-Ala-Ala-cys-OMe) 1. To a solution of Z-Cys(Acm)-Ala-Ala-Cys(Acm)-OMe (200 mg, 0.3 mmol) in 10 cm^3 of DMF was added 500 mg (1.0 mmol) of $HgCl_2$ during stirring at room temperature. After 5 h, 5 cm^3 of NaCl-saturated water and 5 cm^3 of methanol were added. Another addition of water (25 cm^3) resulted in precipitation of white solids, which were collected with filtration, washed with 10% NaCl aq solution and with two portions of methanol, and dried over P_2O_5 *in vacuo*. Yield 190 mg. Anal. Calcd for $C_{21}H_{28}N_4O_7S_2Hg_2Cl_2$: C, 25.53; H, 2.87; N, 5.72. Found: C, 24.38; H, 2.92; N, 5.51.

Hg(Z-cys-Ala-Ala-cys-OMe) 2. The complex was synthesized from $Hg(OCOCH_3)_2$ (320 mg, 1.0 mmol) and Z-Cys(Acm)-Ala-Ala-Cys(Acm)-OMe (200 mg, 0.3 mmol) in DMF (10 cm^3) by almost the same procedure employed for the synthesis of **1**. Anal. Calcd for $C_{21}H_{28}N_4O_7S_2Hg$: C, 35.23; H, 3.96; N, 7.90. Found: C, 37.11; H, 4.12; N, 7.95.

Hg_2Cl_2(Z-cys-Gly-Pro-cys-OMe) 3. The complex was synthesized by the same method described above. Yield 85%. Anal. Calcd for $C_{22}H_{28}N_4O_7S_2Hg_2Cl_2$: C, 26.50; H, 2.83; N, 5.65. Found: C, 24.13; H, 2.88; N, 5.26.

Hg(Z-Cys-Val-Val-Cys-OMe) 4. To a solution of Z-Cys(Acm)-Val-Val-Cys(Acm)-OMe (300 mg, 0.4 mmol) in 20 cm^3 of DMF, 300 mg (1.1 mmol) of $HgCl_2$ was added at room temperature. White solids precipitated gradually. After 8 h, the precipitate was collected with filtration, washed with DMF, NaCl-saturated aq solution, and two portions of methanol. The product was dried over P_2O_5 *in vacuo*. Yield 76%. Anal. Calcd for $C_{25}H_{36}N_4O_7S_2Hg$: C, 39.02; H, 4.72; N, 7.32. Found: C, 42.00; H, 5.28; N, 8.03.

Hg_2Cl_2(Z-cys-Ala-cys-OMe) 5. The complex was synthesized by the same method as described for **4**. Anal. Calcd for $C_{18}H_{23}O_6N_3S_2Hg_2Cl_2$: C, 23.66; H, 2.54; N, 4.62. Found: C, 24.61; H, 2.72; N, 4.11.

Physical Measurements. Raman spectra of Hg(II)/Cys-containing peptide complexes in solid were taken on a Jasco R-800 spectrometer with exciting lines at 488.0 and 514.5 nm. Optical rotations were measured with an automatic polarimeter JASCO model DIP-4. Measurements of ^{1}H-NMR spectra were carried out on a Varian XL-100 spectrometer at 100 MHz at *ca.* 30 °C. Purities of the synthetic peptides were checked by high-performance liquid chromatography on a WATERS model 6000 A and U6K equipped with WATERS μBondapak C18 column and detected at 254 nm with a Shimadzu spectrophotomeric detector SPD-1. An eluent system used was methanol/H_2O (2/1). The products were also characterized by ^{1}H-NMR spectra. Melting points were uncorrected.

Results and Discussion

Peptide Syntheses. The Acm-group has some distinctive advantages as the protecting group for cysteine thiolates especially in the construction of metallothiolate protein model complexes. It is cleaved in milder conditions than required for benzyl or *p*-methoxybenzyl derivative. Substitution of the Acm-group with Hg(II) ion and the subsequent removal of the Hg(II) ion with H_2S gas readily give SH-free peptides. Undesirable reducing reagents, such as 2-mercaptoethanol or dithiothreitol, are unnecessary

for the construction of metal complexes, *e.g.* iron-sulfur protein model complexes. An active site of metalloproteins has been known to be surrounded by hydrophobic environments particularly when they function as electron mediators.[20] Therefore, an investigation on the Hg(II)/Cys-containing peptide complexes should be carried out in various organic solvents because hydrophobic amino acid residues are involved.

In order to ensure the coordination of the cysteine thiolate to metal ion founds in metalloproteins, terminal amino and carboxyl groups must be protected by Z- or -OMe since Hg(II) readily coordinates to organic function groups such as COO^- and NH_2 groups as established by the X-ray analysis of $[MeHg]_2[SCMe_2CH(NH_2)CO_2]$.[21]

Reaction of Cys(Acm) Group with Hg^{2+}. Veber *et al.* reported that the Acm-group was deprotected in an aqueous solution at pH 4.0 by excess mercury(II) acetate.[22] A reaction between the (S-Acm)-peptide and $HgCl_2$ was performed in dimethyl sulfoxide (Me_2SO) because of insolubility of our fully protected peptides in water. An addition of water, which is crucial for the cleavage of the S-Acm bond with $HgCl_2$, resulted in precipitation of (S-$Hg^{II}Cl$)-peptide. Figure 1 shows the ^1H NMR of a mixture of Z-Ala-Cys(Acm)-OMe and $HgCl_2$ in Me_2SO. The spectrum (a) is ^1H-NMR spectrum of Z-Ala-Cys(Acm)-OMe in Me_2SO-d_6 showing cysteinyl C_β methylene protons and acetyl-(Acm) signals at 2.95 and 1.84 ppm, respectively. An addition of $HgCl_2$ to the Z-Ala-Cys(Acm)-OMe solution resulted in an appearance of new signals at 3.17 and 1.80 ppm as shown in spectrum (b). The results suggests a direct interaction between the Hg(II) ion and the S atom. A subsequent addition of D_2O to the reaction mixture containing Z-Ala-Cys(Acm)-OMe and $HgCl_2$ gave spectrum (c) with disappearance of signals of the cysteine C_β methylene at 2.95 ppm and Acm acetyl at 1.84 ppm. The signal at 3.26 ppm coincided with cysteine C_β methylene signals of HgCl-(Z-Ala-cys-OMe) at 3.20 ppm represented by spectrum (d). These facts indicate that the addition of water forces the quantitative cleavage of the S-Acm bond by Hg(II) ion.

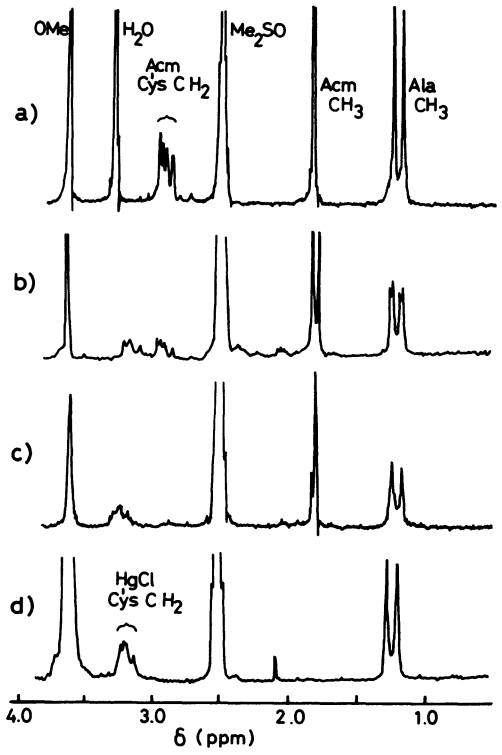

Fig. 1. a): H-NMR spectra of Z-Ala-Cys(Acm)-OMe (30 mg) in Me_2SO-d_6 (0.5 cm^3), b): (a) plus $HgCl_2$ (27 mg) in Me_2SO-d_6, c): (b) plus D_2O (0.2 cm^3), d): HgCl(Z-Ala-cys-OMe) (33 mg) in Me_2SO-d_6.

Two types of Hg(II) complexes were synthesized from the reaction between $HgCl_2$ and Cys-containing peptides. In the case of Z-Cys(Acm)-Val-Val-Cys(Acm)-OMe and Z-Cys(Acm)-Ala-Cys(Acm)-OMe in DMF, **4** and **5** having polymeric structures are obtainable without the addition of water. On the other hand, **1** and **3** were obtained with the addition of water to a DMF solution of $HgCl_2$ and the corresponding peptides. Such a mixed solvent system provides acidic conditions by the hydrolysis of $HgCl_2$ with the addition of water. The results indicate that under the acidic conditions the formation of HgCl(SR) (SR=Cys-containing peptide) predominates, whereas under neutral conditions, where competition of the formation between HgCl(SR) and $Hg(SR)_2$ occurs depending upon solubility. Actually, **2** was synthesized from the corresponding peptide and $Hg(OCOCH_3)_2$ in a weakly acidic aq DMF. The similar two types of the Hg(II)/cysteine complexes has been found to be formed in an aqueous solution by Hay and Porter.[23]

Raman Spectra of Hg(II)/Cys-containing Peptide Complexes. The two types of the complexes, *i.e.* Hg_2Cl_2(peptide)$_2$ and Hg(peptide)$_2$ was examined by their Raman spectra. Figure 2 shows the Raman spectra of Hg_2Cl_2(Z-cys-Ala-Ala-cys-OMe), Hg(Z-cys-Ala-Ala-cys-OMe), Hg_2Cl_2(Z-cys-Gly-Pro-cys-OMe), Hg(Z-cys-Val-Val-cys-OMe), and Hg_2Cl_2(Z-cys-Ala-cys-OMe) in solid state. The intensities of strong bands in the region of 280—320 cm^{-1} were proportional to that of a band at 1003 cm^{-1} due to phenyl of Z group.

The Hg_2Cl_2(peptide) complexes exhibited two strong bands at 281 and 314 cm^{-1}, whereas Hg(peptide) type provided only one strong band at 313—327 cm^{-1}. One strong Raman band is expected for linear S-Hg-S bonding ($D_{\infty h}$ symmetry). Two Raman bands should be observed for linear S-Hg-Cl structure ($C_{\infty v}$ symmetry). Therefore, a strong band at 327 cm^{-1} for Hg(Z-cys-Ala-Ala-cys-OMe) or Hg(Z-cys-Val-Val-cys-OMe) is assignable to ν(Hg-S). The ν(Hg-Cl) band of $HgCl_2$ was observed at 312 cm^{-1} in solid state, although ν(Hg-Cl) was reported to be observed at 348 cm^{-1} in inert gas matrices.[24] Tentatively two bands at 318 and 281 cm^{-1} for Hg_2Cl_2(Z-cys-Ala-Ala-cys-

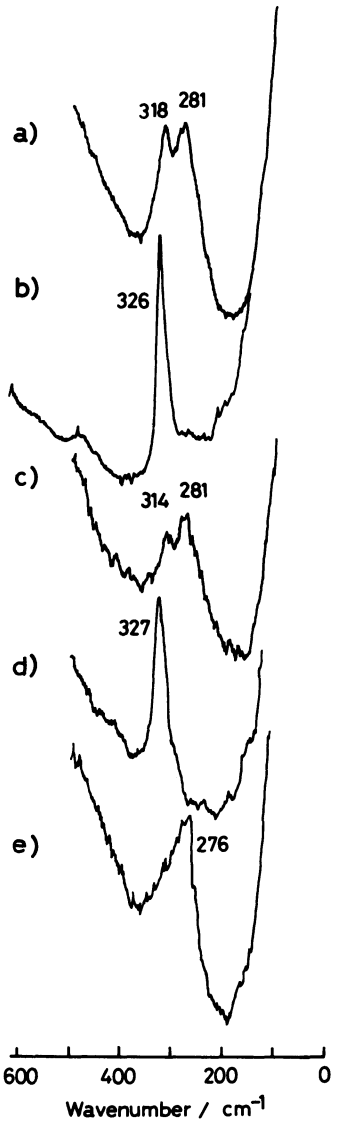

Fig. 2. Raman spectra of a): Hg_2Cl_2(Z-cys-Ala-Ala-cys-OMe), b): Hg(Z-cys-Ala-Ala-cys-OMe), c): Hg_2Cl_2(Z-cys-Gly-Pro-cys-OMe), d): Hg(Z-cys-Val-Val-cys-OMe), and e): Hg_2Cl_2(Z-cys-Ala-cys-OMe) in solid.

OMe) and at 314 and 281 cm^{-1} for Hg_2Cl_2(Z-cys-Gly-Pro-cys-OMe) are assigned to ν(Hg-S) and ν(Hg-Cl) by considering their masses. Two Raman bands have been observed at 349 and 325 cm^{-1} for PhHg(H_2-cys-OH)[25] and MeHg(H_2-cys-OH),[26] respectively.

It is interesting that one ν(Hg-S) band of Hg_2Cl_2-(Z-cys-Ala-cys-OMe) was observed at 276 cm^{-1}. The low frequency seemed unusual because the other S-Hg-Cl type complexes exhibit a band at 281 cm^{-1}. Similar low frequency bands were reported by Carty *et al.* for 3-coordinated Hg(II) in $[HgCl_2]_2[SCMe_2CH(NH_3)COOH]\cdot 2H_2O$.[27] The shift of ν(Hg-S) of Hg_2Cl_2(Z-cys-Ala-cys-OMe) may be attributed to the S-Hg-Cl bond weaken by the steric hindrance of two crowded Hg-S(cys) groups.

As described above, the Raman spectroscopy may be utilized as an important diagnostic tool for the characterization of cysteine-containing peptides. The present results will contribute to the investigation of Hg(II)-binding thiol sites surrounded by hydrophobic environments in proteins or burried in membrane. Reductive elimination of metallic mercury from Hg(Z-cys-X-Y-cys-OMe) complexes is important for a model reaction of mercuric reductase proposed to contain RS-Hg-SR in the active site. Further investigation on this point is in progress.

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1) Small letter cys refers to a cysteinyl residue coordinating to a metal ion.
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