Structures of Cys-containing Peptide Complexes of Pd(II) in Organic Solvents

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Cysteine-containing peptide (pep) complexes of Pd(II) have been synthesized and their structures in organic solvents such as dimethyl sulfoxide (Me_2SO) and *N,N*-dimethylformamide (DMF) were examined by use of ^1H-, ^{13}C-NMR, visible, and CD spectroscopies. In all cases of the Pd(II) complexes, only the thiolato group of the peptides coordinates to the metal. Upon mixing $[PdCl_4]^{2-}$ and the peptide, a *trans*(*S*) isomer $[PdCl_2(pep)_2]^{2-}$ immediately forms, which gradually isomerizes to the *cis*(*S*) isomer at 20 °C in DMF. The reactivity of Pd(II)–S bonds in these complexes was examined by addition of 2,2′-bipyridyl, 2-mercaptoethanol, or 3,4-toluenedithiol.

Transition metal complexes of amino acids, and oligo- and polypeptides have been investigated on the basis of their importan e for providing fundamental chemical information about the coordination environment of metal ions in metalloenzymes and metalloproteins. The thiolato group of cysteine has been found to coordinate to bioactive metal ions such as Fe, Cu, Mo, Zn *etc.*, for example, in ferredoxin,[1] molybdoenzymes,[2] and blue copper proteins.[3] Recently, Ni(II)–cysteine thiolato bonding has been verified in urease.[4]

We have focussed our research efforts upon establishing the coordination behavior of a variety of cysteine-containing oligopeptides to d-block transition metal ions. Cysteine-containing peptides are generally air sensitive and are not easily prepared or purified because of the reactive thiol groups. The heavy metal ions usually form polynuclear complexes with alkanethiols through thiolato bridges (μ-thiolato groups),[5] and these complexes defy attempts to elucidate their structures by conventional chemical techniques.

We have chosen Pd(II) as the metal ion for the present investigation because a) it forms complexes which are usually square planar and diamagnetic, b) it possesses a high affinity for thiolato ligands and suitable rates in ligand exchange reactions, c) fairly intense electronic absorptions in the visible region are expected, and d) it resists electron-transfer reduction by the thiolato ligand, which helps the characterization process.

The general importance of aqueous media in the study of peptide/metal ion systems has provided an impetus for investigation of structures in aqueous solutions.[6,7] However, the active sites of many metalloenzymes or metalloproteins are found to be in hydrophobic parts of the proteins. These findings prompted us to study aprotic solutions of metal/peptide systems. The behaviour and structures of metal peptide complexes in such solutions were found to be totally different in some cases.

In this paper, Pd(II) complexes of Cys-containing peptides such as *N*-acetyl-L-cysteine(Ac-Cys), *N*-benzyloxycarbonyl-L-alanyl-L-cysteine methyl ester (Z-Ala-Cys-OMe) and *N*-benzyloxycarbonyl-L-prolyl-L-cysteine methyl ester (Z-Pro-Cys-OMe) were investigated in aprotic solvents such as *N,N*-dimethylformamide (DMF) and dimethyl sulfoxide (Me_2SO). For comparison, $Na_2[PdCl_2(Z\text{-}Ala\text{-}cys\text{-}OMe)_2]$ and $Na_2[PdCl_2(Z\text{-}Pro\text{-}cys\text{-}OMe)_2]$ were synthesized by the direct reactions of Na_2PdCl_4 with the corresponding Cys(Acm)-peptides (Acm=acetamidomethyl). In order to help to identify the part of amino acid residues coordinating to a metal, we used lower case symbols, *e.g.* cys, in the chemical formulas.

Experimental

Starting Materials. All solvents were distilled by standard procedures and stored under nitrogen. 2,2′-Bipyridyl, 3,4-toluenedithiol, and 2-mercaptoethanol used were of reagent grade quality. Ac-Cys was gifted by Sun-Orient Chemical Co. The preparations of Z-Ala-Cys(Acm)-OMe and Z-Pro-Cys(Acm)-OMe and the deprotections of their Cys(Acm) residue will be metioned elsewhere.[8]

Preparations. *1) $Na_2[PdCl_2(Ac\text{-}cys)_2]\cdot 1/2(DMF)$*: To a solution of Ac-Cys (0.4 g, 2.5×10^{-3} mol) in DMF (5 cm^3), 7 cm^3 of a Na_2PdCl_4 (0.36 g, 1.25×10^{-3} mol) solution in DMF was added, followed by the addition of ethyl acetate (50 cm^3). An orange powder precipitated immediately, which was collected, washed with ethyl acetate, and dried over silica gel. Found: C, 23.86; H, 3.81; N, 5.62%. Calcd for $C_{11.5}H_{19.5}N_{2.5}O_{6.5}S_2Cl_2PdNa_2$: C, 23.64; H, 3.36; N, 5.99%. ^1H NMR signals were observed at 2.9, 3.0, and 8.0 ppm assignable to DMF.

2) $Na_2[PdCl_2(Z\text{-}Ala\text{-}cys\text{-}OMe)_2]$[8b]: To a solution of Z-Ala-Cys(Acm)-OMe (0.2 g, 5.9×10^{-4} mol) in DMF (3 cm^3), 7 cm^3 of a Na_2PdCl_4 (0.09 g, 3.0×10^{-4} mol) solution in DMF was added, and stirred overnight. Water (50 cm^3) saturated with NaCl was added, and the powder precipitated was collected, washed with water, and dried. Found: C, 38.92; H, 4.35; N, 6.34%. Calcd for $C_{30}H_{38}N_4O_{10}S_2Cl_2PdNa_2$: C, 39.94; H, 4.25; N, 6.21%. Molar conductivity of the complex in DMF was 19.7 S cm^2 equiv.$^{-1}$ (5 mmol/dm^3, 20 °C).

3) $Na_2[PdCl_2(Z\text{-}Pro\text{-}cys\text{-}OMe)_2]$: This complex was prepared by the same procedure used for the synthesis of *1*). Found: C, 43.95; H, 4.85; N, 6.61%.† Calcd for $C_{34}H_{42}N_4O_{10}S_2Cl_2PdNa_2$: C, 42.79; H, 4.44; N, 5.90%. The molar conductivity was 14 S cm^2 equiv.$^{-1}$ in DMF (7.8 mmol/dm^3, 20 °C).

Spectral Measurement. To a DMF solution of an oligopeptide (concentration: approximately 2×10^{-3} mol/dm^3), a solution of Na_2PdCl_4 in DMF was added in appropriate molar ratios at room temperature. A Varian XL-100 was used to obtain ^1H- and ^{13}C-NMR spectra. CD spectra were recorded on a JASCO J-40 spectropolarimeter. Visible spectra and IR spectra were obtained on JASCO UVIDEC 5A and Hitachi

† The Cl ligand of the Pd(II) complexes was found gradually substituted with water. Drying *in vacuo* results in slight change in the values of elemental analysis.

Table 1. Circular dichroism spectra of Pd(II)–oligopeptide complexes in DMF at room temperature

Complex	Molar ratio (metal : ligand)	λ/nm[a] ($\Delta\varepsilon$)				
Pd/Ac-Cys	(1 : 1)	540 (+0.04)	442 (+0.13)	376 (+0.17)	340 (+0.07)	312 (−0.97)
Pd/Ac-Cys	(1 : 2)	510 (−0.02)	470 (−0.34)	404 (−0.70)	351 (−0.60)	319 (−0.78)
Pd/Z-Ala-Cys-OMe	(1 : 1)		430 (+0.09)	399 (−0.06)	375 (+0.15)	309 (−0.88)
Pd/Z-Ala-Cys-OMe	(1 : 2)		485 (−0.28)	436 (+0.16)	396 (−0.57)	350 (+1.05)	318 (−0.76)
Pd/Z-Pro-Cys-OMe	(1 : 1)		428 (+0.18)	390 (−0.08)	360 (+0.03)		314 (−0.06)
Pd/Z-Pro-Cys-OMe	(1 : 2)		493 (−0.23)	438 (+0.17)	382 (−0.50)	343 (+0.16)	

a) These spectra were obtained after 24 h, and the $\Delta\varepsilon$ values were calculated on the basis of the concentration of Pd(II) ion.

FIR-3. CD spectra of the complexes on ligand substitution were also recorded after addition of 2,2′-bipyridyl, 3,4-toluenedithiol, and 2-mercaptoethanol to the DMF solution of these complexes with the corresponding molar ratios.

Results and Discussion

Stoichiometry of Interaction of Pd(II) with Cys-containing Peptides. Reactions between Pd(II) and Cys-containing oligopeptides were investigated stoichiometrically. CD and visible spectra of the reaction products in DMF solutions were obtained with various molar ratios of Pd(II) to peptide. When a molar ratio of 1 : 2 was taken, a Pd(II)/peptide (1 : 2) complex was formed immediately. CD and visible spectra of the solution changed slowly on standing. After 24 h, a (1 : 1) and another isomeric (1 : 2) complex were found to be formed, as indicated by the CD spectra. The transitions and absorptions of the CD and visible spectra thus obtained are listed in Tables 1 and 2. The similarities among the CD spectra of the Pd(II) complexes of Ac-Cys, Z-Ala-Cys-OMe, and Z-Pro-Cys-OMe suggest coordination of the same portion of the peptide to the metal atom in each case.

Functional Groups Coordinating to Pd(II). Pd(II)/Ac-Cys Complexes: As the simplest member of Cys-containing peptides, complexes of Ac-Cys were examined. Ac-Cys has i) a thiolato group, ii) a nitrogen atom in the amide bond, iii) an oxygen atom in the amide bond, and iv) an oxygen atom in the carboxylic acid part as possible donor sites. The ^{1}H- and ^{13}C-NMR spectra of Pd(II)/Ac-Cys (1 : 1) and (1 : 2) complexes

Table 2. Visible spectra of Pd(II)–oligopeptide complexes in DMF at room temperature

Complex	Molar ratio (metal : ligand)	λ/nm[a] ($\varepsilon\times10^{-3}$)		
Pd/Ac-Cys	(1 : 1)	390 (1.6)		310 (5.5)
Pd/Ac-Cys	(1 : 2)	405 (2.8)	370 (3.6)	310 (6.8)
Pd/Z-Ala-Cys-OMe	(1 : 1)	386 (1.4)		305 (5.2)
Pd/Z-Ala-Cys-OMe	(1 : 2)	380 (2.2)		310 (6.6)
Pd/Z-Pro-Cys-OMe	(1 : 1)	386 (1.3)		307 (5.0)
Pd/Z-Pro-Cys-OMe	(1 : 2)	382 (1.8)		309 (5.7)

a) ε was calculated on the basis of the concentration of Pd(II) ion.

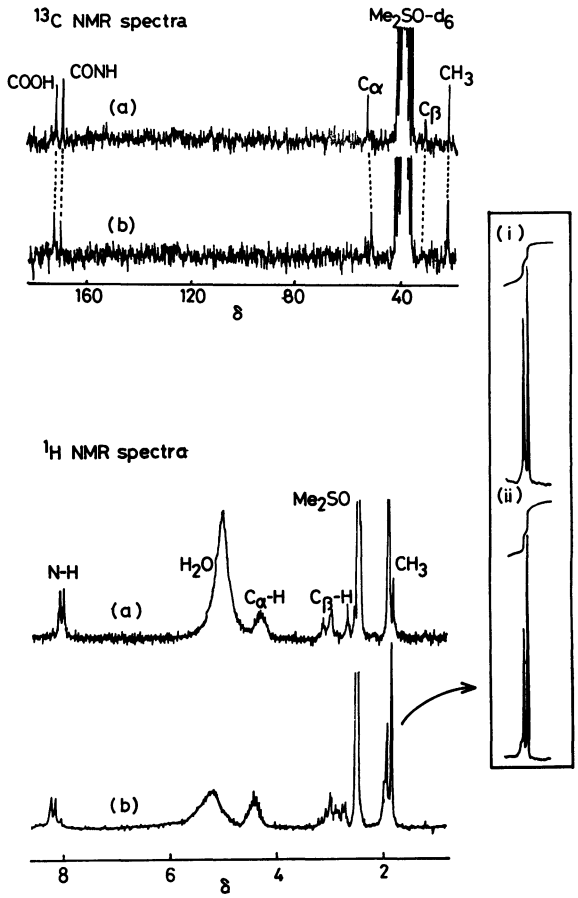
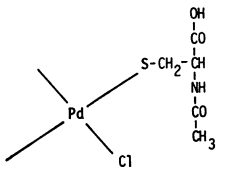


Fig. 1. ^{1}H- and ^{13}C-NMR spectra of Pd(II)/Ac-Cys in Me_2SO-d_6 at room temperature with a) the molar ratio of Pd(II)/Ac-Cys=1/1 and b)=1/2. The inset spectra show the time dependence of methyl signals in ^{1}H NMR spectra in Me_2SO-d_6 at 70 °C (Pd(II)/Ac-Cys=1/1). The spectra were taken i) after 1 h and ii) after 21.5 h.

in Me_2SO-d_6 are shown in Fig. 1. There were negligible differences between the spectra obtained in Me_2SO-d_6 and DMF, and similar spectra were obtained in each solvent. In the ^{13}C-NMR spectra, the resonance due to C_β of the cysteine residue was shifted downfield on complexation. The C_β of the (1 : 2) complex was observed with broadening at 33.73 ppm. A larger shift of Cys C_β (8.31 ppm from the C_β of the SH-free cysteine residue) for the (1 : 2) complex compared with that of the (1 : 1) complex (5.84 ppm from the Cys C_β of the SH-free cysteine) indicates the formation of the (1 : 2) complex with a stronger Pd(II)–S bond.

Possibility of the coordination of the other donor groups occurring in organic solvents was examined by recording the ^1H- and ^{13}C-NMR spectra. Especially, a deprotonated nitrogen atom in the amide bond is a potential candidate as a ligand group. For example, most of the known metal-peptide complexes have a metal-nitrogen bond formed by the deprotonated amide group[6,7] and a metal-oxygen bond with the carboxyl group[9] in aqueous solution.

Two carbon peaks around 170 ppm are assignable to the carbons of the amide and carboxylic acid groups. The shift of the signal of either the (1 : 1) or (1 : 2) complex from that of free Ac-Cys was observed to be less than 0.3 ppm. This fact suggests that only the thiolato group of Ac-Cys coordinates to the metal in Me_2SO-d_6 as illustrated in the following scheme. The H^+ released on the thiol of Ac-Cys should protonate the solvent such as DMF since the CD spectrum of the Pd(II)/Ac-Cys (1 : 2) complex with addition of the equimolar triethylamine remained the same. Fuhr and

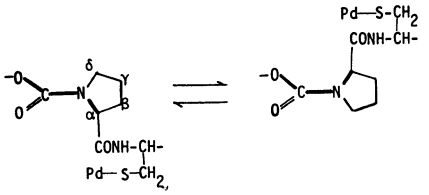


Rabenstein reported that Zn(II)/glutathione mixtures in aqueous solution provided a carbon signal due to Glu–CONH with a shift of 0.2 ppm from free glutathione.[10] Ionization of the peptide protons at pD 10.5 is followed by bonding of Zn(II) to the anionic N atom. Shifts of 0.8—1.0 ppm of the amide carbon and 1.0—3.0 ppm for the carboxyl carbon are observed, presumably due to their coordination to the Zn atom. The absence of deprotonation in the Pd(II)–peptide complex was also supported by the ^1H NMR spectra shown in Fig. 1. One NH proton was observed at 8.19 ppm for the (1 : 1) complex, and at 8.31 ppm for the (1 : 2) complex. The chemical shift values clearly show the absence of amide group coordination. Even if the amide NH is involved in coordination, a broad NH peak will be observed. As proton peak from contaminating water observed at 5.1—8.0 ppm (see Fig. 1) reveals that proton exchange between water and carboxylic acid occurs in Me_2SO-d_6. Usually, a peak assignable to water contaminated in the commercial Me_2SO-d_6 was observed at about 3 ppm, shifting to 5.1 ppm with an increase in the amount of water. The presence of the exchangeable proton assignable to the carboxyl group clearly indicates that the carboxylic acid part of the AcCys ligand does not coordinate to the metal.

TABLE 3. ^1H-NMR SPECTRA OF Cys-CONTAINING OLIGOPEPTIDES AND THE CORRESPONDING Pd(II) COMPLEXES IN Me_2SO-d_6 AT ROOM TEMPERATURE[11]

Compound	N–H region ppm	C_α–H region ppm
Ac-Cys	8.33	4.33
$Na_2[PdCl_2(Ac\text{-}cys)_2]$	8.31	4.43
Z-Ala-Cys-OMe	8.38(Cys)	4.50(Cys)
	7.3(Ala)	4.12(Ala)
$Na_2[PdCl_2(Z\text{-}Ala\text{-}cys\text{-}OMe)_2]$	8.32(cys)	4.70(cys)
	7.3(Ala)	4.14(Ala)
Z-Pro-Cys-OMe	8.38(Cys)	4.52(Cys)
		4.30(Pro)
$Na_2[PdCl_2(Z\text{-}Pro\text{-}cys\text{-}OMe)_2]$	8.24(cys)	4.70(cys)
		4.26(Pro)

Pd(II) Complexes of Dipeptides. The Pd(II) complexes of Z-Ala-Cys-OMe and Z-Pro-Cys-OMe, of which the amino- and carboxylic acid-ends are protected, were examined by CD and visible spectra. The



trans conformer *cis* conformer

same type of (1 : 2) complexes derived from the corresponding S-blocked peptides and Na_2PdCl_4 were isolated, which are air-stable and soluble in DMF, Me_2SO, methanol, or acetonitrile. The spectra of the isolated Pd(II) complexes were essentially the same as those of the reaction mixtures of $[PdCl_4]^{2-}$ and the SH peptides.

Table 3 shows the chemical shifts of the N–H and C_α–H protons for the isolated Pd(II) complexes. The urethane N–H signal of $[PdCl_2(Z\text{-}Ala\text{-}cys\text{-}OMe)_2]^{2-}$, which was overlapped with that of the benzyl protons, was made observable by the upfield shift of the peak resulting from the addition of chloroform. This observation indicates that even the more basic urethane group than the amide group was not involved in coordination.

The simple monodentate coordination through the thiolato group was supported by the ^{13}C-NMR spectra. The *cis* and *trans* conformers resulting from the planarity of the urethane bond in the above equilibrium were detectable by using the ^{13}C-NMR spectra of $[PdCl_2(Z\text{-}Pro\text{-}cys\text{-}OMe)_2]^{2-}$ in Me_2SO-d_6. The *cis* and *trans* signals of Pro C_β and C_γ which present in the equilibrium were clearly observed at 30.5 and 23.5 ppm, respectively. Assignments of the *cis* and *trans* signals of Pro C_β or C_γ were carried out according to the chemical shifts of a number of Pro-containing peptides in Me_2SO-d_6 assigned by Dorman and Bovey.[12] The same probabilities of the *cis* and *trans* conformers as those of the S-blocked peptide as shown in Fig. 3 imply that only the thiolato group is involved in the coordination. If

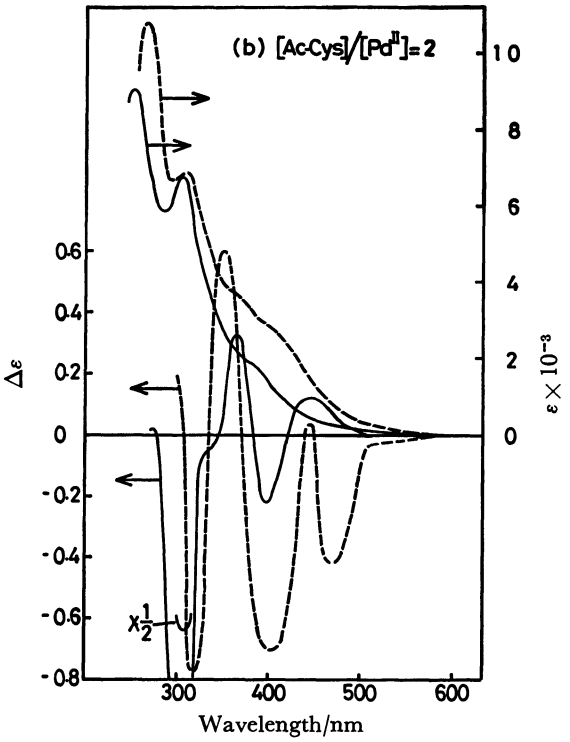


Fig. 2. Time dependence of the CD and visible spectra of Pd(II)/Ac-Cys= a) 1/1 and b) 1/2 in DMF at room temperature. The spectra after 10 min are represented by (——) and after 38 h by (------).

the N atom of the Pro residue coordinates to the metal, the probability of the *cis* or *trans* conformers will change with the coordination in the above equilibrium.

The presence of chloride ligands on the Pd(II) may not favor coordination of the amide nitrogen atoms of

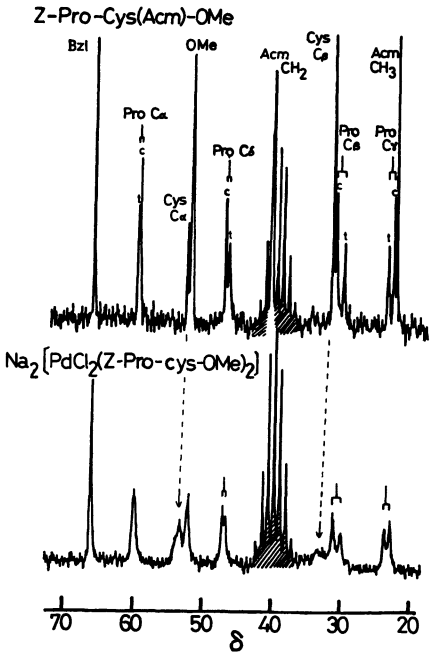


Fig. 3. ^{13}C-NMR spectra of Z-Pro-Cys(Acm)-OMe (Acm=acetamidomethyl) and $Na_2[PdCl_2(Z\text{-Pro-cys-OMe})_2]$ in Me_2SO-d_6 at room temperature.

the peptides used in this study. Therefore $Pd(NO_3)_2$ was used to further examine Pd(II)/peptide interactions in DMF. The CD spectrum of $Pd(NO_3)_2$/Z-Ala-Cys-OMe indicated the presence of the same type of the (1 : 2) complex as that of $PdCl_4^{2-}$/Z-Ala-Cys-OMe. The solvent seems to occupy the remaining coordination sites on Pd(II) in the case of $Pd(NO_3)_2$. Addition of NR_4Cl to the solution gave the same $[PdCl_2(Z\text{-Ala-cys-OMe})_2]^{2-}$ anion as prepared from $PdCl_4^{2-}$. Thus, absence of the amide coordination is one of the consequences of solvent effect coupled with the influence of thiolato coordination on the metal.

Infrared absorptions of these complexes are listed in Table 4 in comparison with those of the peptides themselves. The ν(S–H) band in all the peptides examined at 2500—2600 cm^{-1} disappeared in the Pd(II) complexes because of the coordination of the thiolato group to the metal. No shift of the ν(C=O) band of the Pd/Ac-Cys complex from the band of the free carboxylic acid was observed. The coordination of carboxylate usually results in thift to the lower wave number (1600—1650 cm^{-1}).[6] The bands of ν(N–H) and ν(C–O) of the amide group for all complexes remained unchanged. Observation of the bands due to Pd(II)–S and Pd(II)–Cl in the far infrared region indicates that the complexes possess a $PdCl_2S_2$ core.

Therefore the visible absorption and CD transitions mentioned above (Tables 1 and 2) should be due to the chromophore of the $PdCl_2S_2$ core. A visible absorption at 370—405 nm in Table 2 corresponds mainly to the overlapped bands of the d-d transitions of the Pd(II) ion.[13,14] On the other hand, an absorption around 310 nm is assignable to a charge transfer band (Pd(II)–S).[13,14] These bands are similar to the charge transfer band of $Pd(cys\text{-}OEt)_2$ at 312 nm,[15] and a d-d transition

TABLE 4. SELECTED IR FREQUENCIES OF Cys-CONTAINING OLIGOPEPTIDES AND THE CORRESPONDINGPd(II) COMPLEXES IN SOLID STATE

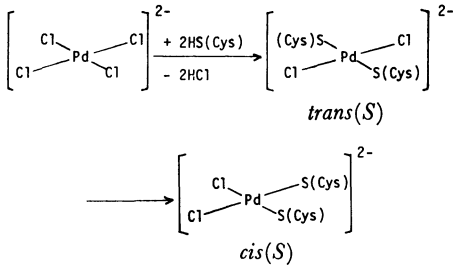
Compound	IR (ν/cm^{-1})				
	ν(N–H)	ν(S–H)	ν(C=O)	ν(Pd–S)	(Pd–Cl)
Ac-Cys	3360	2450	1710[a)] 1640[b)]		
$Na_2[PdCl_2(Ac\text{-}cys)_2]$	3250br	—	1730[a)] 1645[b)]	388	330
Z-Ala-Cys-OMe	3320	2570	1725[a)] 1680 1640[b)]		
$Na_2[PdCl_2(Z\text{-}Ala\text{-}cys\text{-}OMe)_2]$	3300br	—	1725[c)] 1660br[d)]	360	320
Z-Pro-Cys-OMe	3310	2590	1740[c)] 1690 1665[b)]		
$Na_2[PdCl_2(Z\text{-}Pro\text{-}cys\text{-}OMe)_2]$	3300	—	1740[c)] 1670br	362	310

a) Assignable to carbonyl group of free carboxylic acid. b) Carbonyl group of amide. c) Carbonyl group of ester. d) br refers to a broad band.

at 423 nm and a charge transfer band at 319 nm of $Pd(cys)_2$.[16] In the d-d transitions, the transitions of d_{xy}-$d_{x^2-y^2}$ and d_{xy},d_{yx}-$d_{x^2y^2}$ are magnetic dipole-allowed, while the transition of d_{xy}-$d_{x^2-y^2}$ is magnetic dipole-forbidden. The CD peak of the former transitions should be strongest and that of the latter transition should be weakest. In the CD spectra of the isolated (1 : 2) complexes in Table 1, the bands at about 480 nm are therefore assignable to d_{xy}-$d_{x^2-y^2}$ transition, and the positive peaks about 435 nm and the negative peaks about 390 nm to the d_{xy},d_{xy}-$d_{x^2-y^2}$ transitions. The strong peaks around 350 and 320 nm correspond to charge transfer bands (Pd(II)–S).

Coordination Geometry of Pd(II)/Peptide Complexes. Two acetyl peaks in the ^{1}H-NMR spectrum of the 1 : 2 complex, as shown in Fig. 1, were observed at 1.94 and 1.86 ppm. The latter peak grew up gradually at 70 °C, as shown in the inset of Fig. 1. In addition, the peaks due to NH and contaminating water were observed intact even after the solution was kept overnight. The water peak at 5.4 ppm indicates the existence of a proton exchange between water and –COOH because a peak of usual water in Me_2SO-d_6 should be observed between 2.5 and 5.1 ppm. The results indicate the interchange between *trans*(*S*) and *cis*(*S*) isomers of the $[PdCl_2(Ac\text{-}cys)_2]^{2-}$ complex. This interconversion was also observed in the CD and visible spectra. The *cis*(*S*) (1 : 2) complex could be isolated from a mixture of DMF and ethyl acetate, and the *cis*(*S*) isomer is thus thermodynamically more stable. Pneumatikakis and Hadjiliadis[6] concluded that a medium intensity band at 330 cm^{-1} for $PdCl_2(cys\text{-}OMe)_2$ which was immediately isolated after mixing $PdCl_4^{2-}$ and Cys-OMe is due to ν(Pd–Cl) stretching of a *trans*(*S*) isomer. Similarly the $Na_2[PdCl_2(Ac\text{-}cys)_2]$ isolated immediately showed a band at 330 cm^{-1} due to ν(Pd–Cl) of a *trans*(*S*) isomer. The same (1 : 2) complex obtained after aging overnight in DMF at 70 °C showed a few broad bands at 320—340 cm^{-1} due to ν(Pd–Cl) of a *cis*(*S*) isomer.

Figure 2 shows the CD and visible spectra of the DMF solution of Pd(II)/Ac-Cys (1 : 1) and (1 : 2) after 10 min or after one day from complex formation. The CD patterns of the both complexes are similar immediately after reaction, indicating formation of a (1 : 2) complex. The spectrum of the (1 : 1) solution does not change with time significantly, but that of the (1 : 2) solution clearly changes at 470 and 404 nm in DMF. The same trend was observed in the visible spectra. The absorption coefficient of the (1 : 2) solution after 1 d was 1.7 times larger than that after 10 min. This slow interchange between the *trans*(*S*) and *cis*(*S*) isomers with time is illustrated in the following scheme. A kinetically-controlled formation of the

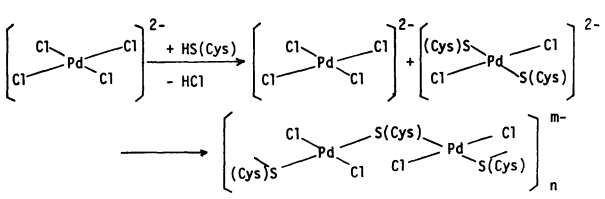


trans(*S*)

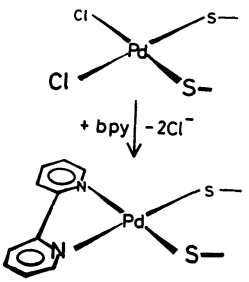
cis(*S*)

trans(*S*) isomer occurs immediately when $[PdCl_4]^{2-}$ and Ac-Cys are mixed. Then the complex isomerizes to a thermo-dynamically stabilized *cis*(*S*) form. A visible absorption of the *cis*(*S*) isomer (local C_{2v} symmetry) is known to be stronger than that for the *trans*(*S*) isomer (local D_{2h} symmetry).[9,17] Also, the stronger CD peak should be expected for the *cis*(*S*) isomer compared to that for the *trans*(*S*) isomer, in which the dipole moment is negligible. Figure 2(b) shows the peaks of d-d transitions at 472 nm in the CD spectra shifting to a longer wavelength. These results indicate that d electrons are delocalized over the PdS_2 core and the Pd(II)–S bond is stabilized in the *cis*(*S*) complexes.

In the case of the (1 : 1) solution, the *trans*(*S*) (1 : 2) isomer may form as an intermediate and is converted to polynuclear complexes. Similar complexes were formed by the corresponding dipeptides.



Reactivity of the Pd(II)–Dipeptide Complexes. The strength of the Pd(II)–thiolate bond was examined as follows. Addition of 3,4-toluenedithiol, 2-mercaptoethanol, or 2,2′-bipyridyl results in cleavage of the weaker bonds among the Pd(II)–S or Pd(II)–Cl bonds of the $PdCl_2S_2$ core. Figure 4 shows the spectral changes



that occurred on addition of the above ligands to $Na_2[PdCl_2(Z\text{-Ala-cys-OMe})_2]$. Addition of 3,4-toluenedithiol first diminished the $\Delta\varepsilon$ values of the CD peaks. Addition of two molar equivalents of 3,4-toluenedithiol then results in complete disappearance of the CD peaks, while in the visible spectra, a d-d band at 860 nm and a charge transfer band at 360 nm were observed due to the formation of $[Pd(dithiolato)_2]^{2-}$. The results indicate that the 3,4-toluenedithiol easily breaks Pd(II)–Cl and Pd(II)–S(Cys) bonds because of its *cis*(*S*) chelating effect and the formation of an electron-delocalized complex having strong Pd(II)–S(–Ph–) bonds.

On the other hand, addition of 2-mercaptoethanol, which is a primary thiol, as is the cysteine residue, induced the formation of a different complex with two moles of the thiol to one mol of the Pd(II). Addition

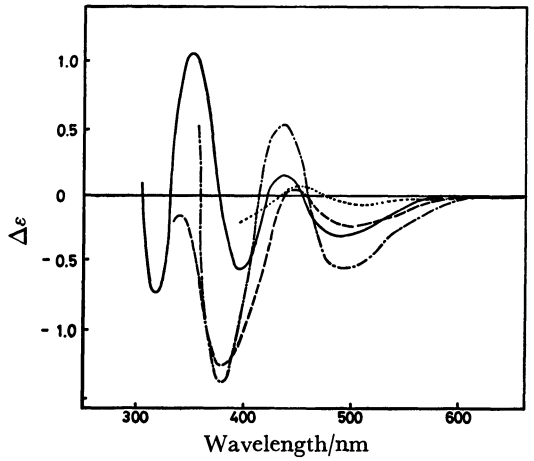


Fig. 4. CD-spectral change of $Na_2[PdCl_2(Z\text{-Ala-cys-OMe})_2]$ (——) in DMF upon addition of 2,2′-bipyridyl (– – –), 2-mercaptoethanol (–·–·–), and 3,4-toluenedithiol (------), the ratio, Pd(II)/additive, is 1/2.

of larger amounts of the thiol over the (1 : 2) molar ratio does not change the spectrum. These results clearly indicate the formation of $[Pd(SCH_2CH_2OH)_2\text{-}(Z\text{-Ala-cys-OMe})_2]^{2-}$ which contains a PdS_4 core. 2-Mercaptoethanol cleaves the Pd(II)–Cl bonds, but does not affect the original Pd(II)–S bonds. Although simple thiols such as CH_3SH, PhSH, *etc.*, usually give polynuclear complexes $[Pd(SR)_2]_n$, the Pd(II)/peptide complex system containing a cysteine residue does not form a $[Pd(S(cys))_4]^{2-}$ or polynuclear species because of the steric hindrance around the cysteine thiolato group.

The CD spectrum obtained by addition of 2,2′-bipyridyl at a (1 : 1) molar ratio does not change with further addition of 2,2′-bipyridyl. Two chloride ligands of the complex are easily replaced by one 2,2′-bipyridyl. However, the Pd(II)–S bonds are resistant to such a substitution with 2,2′-bipyridyl.

It is plausible that the entering ligand such as 2,2′-bipyridyl or 3,4-toluenedithiol would give a transient five-coordinate intermediate with a trigonal bipyramidal configuration. Then each of the *cis*(*S*) or *trans*(*S*) complex provides the same *cis*(*S*) complex having the chelating ligand. However, further information is necessary for discussing the mechanism of such ligand substitution reaction.

Conclusion. Cys-containing peptides coordinate to Pd(II) ions through only the thiolato group in organic solvents, giving (1 : 1) and (1 : 2) Pd(II)–peptide complexes. A (1 : 2) molar ratio of reagents gives the kinetically controlled *trans*(*S*) isomers immediately, which are subsequently converted to the *cis*(*S*)-isomers. The Pd(II)–S bonds of the *cis*(*S*) complexes are not cleavable even with the strong donor ligand such as 2,2′-bipyridyl.

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