Preparation and some properties of cobalt(III) complexes with a dipeptide containing L-methionine residue

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Abstract

Cobalt(III) complexes with a dipeptide containing L-methionine residue, $[Co(dipeptidato\text{-}N,N,O)_2]^-$, $[Co(dipeptidato)(diamine)]^+$ and *cis*-$[Co(dipeptidato)(NH_3)_2]^+$, have been prepared, where dipeptidate is L-methionyl-glycinate, glycyl-L-methioninate, L-methionyl-L-alaninate or L-alanyl-L-methioninate, and diamine is 1,2-ethanediamine or 1,3-propanediamine. All the complexes were characterized by electronic absorption, ^1H NMR and ^{13}C NMR and circular dichroism (CD) spectra. In the diamine and diammine complexes, the dipeptide coordinates to the cobalt(III) ion as the quadridentate through the nitrogen atoms of NH_2 and peptide N, the oxygen atom of COO^- and the sulfur atom of SCH_3. The 500 MHz ^1H NMR spectra indicate that the N–S chelate rings of the L-met residue take the chair conformation and the S-methyl groups take the *S*(*S*) configuration for the C-terminal L-met.. The CD spectra of the complexes were discussed with respect to their configurations.

Introduction

Cobalt(III) complexes with various dipeptides have been investigated for stereochemical and spectrochemical interests, and their important biochemical function [1–6]. However, the properties of cobalt(III) complexes with quadridentate dipeptides, which have four different donor sites, have been little investigated [5, 6]. A few complexes with such dipeptides containing the L-histidine residue have been isolated as a mononuclear complex, in which the dipeptide acts as the terdentate ligand, and a binuclear complex with the bridging group of the histidine residue [5]. Recently, the stereochemical and spectrochemical properties of the cobalt(III) complex with carnosine (*β*-alanyl-L-histidine) were reported, but the relationship between the geometrical configuration and the CD spectrum remains uncertain because of the peculiarity of the configurational chirality [6]. Hence, in order to clarify their stereochemical properties, we have attempted to investigate cobalt(III) complexes with quadridentate dipeptides containing the L-methionine residue which show characteristic absorption bands and an important biochemical function.

In the present work, the [Co(dipeptidato)(diamine)] type complexes were prepared and chromatographically purified, where dipeptidate is L-methionyl-glycinate (L-met-gly), glycyl-L-methioninate (gly-L-met), L-methionyl-L-alaninate (L-met-L-ala) or L-alanyl-L-methioninate (L-ala-L-met), and diamine is 1,2-ethanediamine (en) or 1,3-propanediamine (tn). The *cis*-$[Co(L\text{-}met\text{-}gly)(NH_3)_2]^+$ and *cis*-$[Co(gly\text{-}L\text{-}met)(NH_3)_2]^+$ complexes were also prepared in order to compare them with the corresponding diamine complexes. All the complexes were characterized by their electronic absorption, ^1H NMR and ^{13}C NMR and circular dichroism (CD) spectra. The CD spectra of the complexes were also discussed with respect to their configurations.

Experimental

Materials

Glycyl-L-methionine, L-methionyl-glycine, L-alanyl-L-methionine and L-methionyl-L-alanine were purchased from Sigma Chemical Co. Ltd., and used without further purification. The other reagents were obtained from Wako Pure Chemicals Ind. Co. Ltd., and also used without further purification.

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Preparation of complexes

(i) Sodium bis(glycyl-L-methioninato)-cobaltate(III), Na[Co(gly-L-met)$_2$]

After a solution containing equimolecular amounts of *trans*-[Co(Cl)$_2$(en)$_2$]Cl [7] and H$_2$(gly-L-met) was adjusted to pH *c.* 9, a small amount of activated charcoal was added to it. The mixture was mechanically stirred at 50–60 °C for 2 h. The reaction solution was left standing at room temperature for 2 days and then filtered to remove the insoluble materials. The filtrate was poured onto a column of SP-Sephadex C-25 (Na$^+$ form, 3.6 cm × 40 cm). Violet complexes were eluted by flushing the column with water. The elute was poured onto an anion exchange column (QAE-Sephadex A-25, Cl$^-$ form, 3.6 cm × 85 cm). After sweeping the column with water, the adsorbed band was eluted with a 0.025 mol dm^{-3} NaCl aqueous solution. Three small amounts of violet bands (A-1, A-2 and A-3) and a large amount of violet band (A-4) were eluted in this order. The A-2 and A-3 eluates were partially overlapped. The formation ratio was about A-1:(A-2 + A-3):A-4 = 2:1:20. It was found from the absorption and CD spectral measurements that the A-1 and A-4 eluates each contained an isomer of [Co(gly-L-met)$_2$]$^-$ and the A-2 and A-3 eluates were impurities. The A-1 isomer could not be isolated as a solid state because of its very poor yield. Its concentration was evaluated by the plasma emission spectral analysis. The A-4 eluate was concentrated to a small volume with a rotary evaporator at 25–30 °C. The deposited sodium chloride was filtered off. To the filtrate was added a large amount of ethanol and the deposited sodium chloride was filtered off again. When the filtrate was concentrated to a small volume, the crystals appeared. They were collected by filtration and recrystallized from as little water as possible by addition of ethanol. The pure A-4 complex was washed with ethanol and ether, and dried in a vacuum desiccator. It was found from the ^{1}H NMR spectral measurement that this A-4 isomer contained ethanol in its crystals. *Anal.* Found: C, 28.29; H, 4.72; N, 8.97. Calc. for Na-$CoC_{14}H_{24}N_4O_6S_2 \cdot 1/3C_2H_5OH \cdot 3/2NaCl$: C, 28.39; H, 4.71; N, 9.03%.

Under these preparative conditions, the formation ratio of [Co(gly-L-met)$_2$]$^-$ and [Co(gly-L-met)(en)]$^+$ (described in (ii)) was 1:1. When *trans*-[Co(Cl)$_2$(en)$_2$]$^+$ and H$_2$(gly-L-met) were reacted with a ratio of 1:2, the yield of [Co(gly-L-met)$_2$]$^-$ was unexpectedly very poor.

(ii) (1,2-Ethanediamine)(glycyl-L-methioninato)-cobalt(III) chloride, [Co(gly-L-met)(en)]Cl

A solution containing 0.50 g (1.8 mmol) of *trans*-[Co(Cl)$_2$(en)$_2$]Cl [7] in 3.5 cm^3 of water was added to a solution containing 0.30 g (1.5 mmol) of H$_2$(gly-L-met) in 3.5 cm^3 of water. The mixture was adjusted to pH *c.* 9 with a 1 mol dm^{-3} NaOH solution and mechanically stirred at 50–60 °C for 30 min. The color of the solution turned from dark green to reddish violet. After the solution was cooled to room temperature, it was poured onto a column of SP-Sephadex C-25 (Na$^+$ form, 3.6 cm × 40 cm) without filtration because there was no insoluble material. After sweeping the column with water, the adsorbed band was eluted with a 0.025 mol dm^{-3} NaCl aqueous solution. Only the reddish violet band was eluted and fractionated. It was found from the absorption and CD spectral measurements that each of the fractions contained only [Co(gly-L-met)(en)]$^+$. The crystals, which were obtained by a procedure similar to that used in (i), were collected by filtration, washed with ethanol and ether, and then dried in a vacuum desiccator. *Anal.* Found: C, 28.76; H, 5.56; N, 14.96. Calc. for $CoC_9H_{20}N_4O_3SCl \cdot 1/2H_2O \cdot 1/6NaCl$: C, 28.63, H, 5.60; N, 14.84%.

(iii) (1,2-Ethanediamine)(L-methionyl-glycinato)-cobalt(III) perchlorate, [Co(L-met-gly)(en)]ClO$_4$

This complex was prepared and chromatographed by a procedure similar to that used in (ii), using H$_2$(L-met-gly) instead of H$_2$(gly-L-met). The eluate was concentrated and the deposited sodium chloride was filtered off. The filtrate was passed through a column of Sephadex G-10 (3.6 cm × 85 cm) by eluting with water. The eluate was converted into the perchlorate salt using an anion exchange column (QAE-Sephadex A-25, ClO$_4^-$ form, 2.6 cm × 25 cm). When the eluate was concentrated to a small volume, the crystals appeared. They were collected by filtration, washed with ethanol and ether, and then dried in a vacuum desiccator. *Anal.* Found: C, 23.96; H, 4.69; N, 12.23. Calc. for $CoC_9H_{20}N_4O_7SCl \cdot 1/2H_2O \cdot 1/6NaClO_4$: C, 23.91; H, 4.67; N, 12.39%.

(iv) cis-Diammine(glycyl-L-methioninato)-cobalt(III) ion, cis-[Co(gly-L-met)(NH$_3$)$_2$]$^+$

A solution containing 0.52 g (1.8 mmol) of [Co(NO$_3$)$_3$(NH$_3$)$_3$] [8] in 15 cm^3 of water was added to a solution containing 0.50 g (1.5 mmol) of H$_2$(gly-L-met) in 5 cm^3 of water. The mixture was adjusted to pH *c.* 8.6 with a 1 mol dm^{-3} NaOH solution and mechanically stirred at *c.* 40 °C for 4 h. The dark reddish violet solution was cooled to room temperature and poured onto a column of SP-Sephadex C-25 (Na$^+$ form, 4.2 cm × 10 cm). After sweeping the column with water, the dark reddish violet adsorbed band was moved to the top of another column of SP-Sephadex C-25 (Na$^+$ form, 4.8 cm × 10 cm), because some species were decomposed and adsorbed

in the middle of the column. Only the reddish violet band was eluted with a 0.025 mol dm^{-3} NaCl solution. By comparing the absorption and CD spectra of this band with those of $[Co(gly\text{-}L\text{-}met)(en)]^+$, it was found that the reddish violet band contained only *cis*-$[Co(gly\text{-}L\text{-}met)(NH_3)_2]^+$. This complex could not be isolated because of the very small amount. Therefore, the concentration of the complex cation was evaluated by the plasma emission spectral analysis.

(v) cis-Diammine(L-methionyl-glycinato)cobalt(III) ion, cis-[Co(L-met-gly)(NH$_3$)$_2$]$^+$

This complex was prepared and purified by a procedure similar to that used in (iv), using H_2(L-met-gly) instead of H_2(gly-L-met), with a reaction time of 2 h. From the absorption and CD spectral measurements, it was found that the reddish violet band eluted with a 0.025 mol dm^{-3} NaCl solution contained only *cis*-$[Co(L\text{-}met\text{-}gly)(NH_3)_2]^+$. This complex could not be isolated therefore and the concentration of the complex cation was evaluated by the plasma emission spectral analysis.

(vi) (Glycyl-L-methioninato)(1,3-propanediamine)-cobalt(III) tetraphenylborate, [Co(gly-L-met)(tn)]B(C$_6$H$_5$)$_4$.

To a solution containing 0.50 g (2.4 mmol) of H_2(gly-L-met) in 12 cm^3 of water was added 0.91 g (2.9 mmol) of *trans*-$[Co(Cl)_2(tn)_2]Cl$ [9]. The mixture was adjusted to pH *c.* 9 using a 1 mol dm^{-3} NaOH solution and mechanically stirred at *c.* 40 °C for 2 h. The dark reddish violet solution was cooled to room temperature and poured onto a column of SP-Sephadex C-25 (Na^+ form, 4.0 cm×85 cm). After sweeping the column with water, the adsorbed band was eluted with a 0.025 mol dm^{-3} NaCl aqueous solution. Only the reddish violet band was eluted and fractionated. It was found from the absorption and CD spectral measurements that each of the fractions contained only $[Co(gly\text{-}L\text{-}met)(tn)]^+$. The fractions were combined and concentrated to a small volume with a rotary evaporator. The deposited sodium chloride was filtered off. The filtrate was passed through a column of Sephadex G-10 (3.6 cm×90 cm) by eluting with water. The eluate was converted into the perchlorate salt using a procedure similar to that described in (iii). The eluate was concentrated to a small volume. To this was added a solution containing *c.* 0.4 g of sodium tetraphenylborate in a small amount of water, because this complex could not be isolated as the chloride or the perchlorate salt. The resulting precipitate was collected by filtration and dissolved in a small amount of acetone. After adding a small amount of water, the solution was kept in a refrigerator overnight. The crystals which appeared were collected by filtration, washed with water, ethanol and ether, and then dried in a vacuum desiccator. *Anal.* Found: C, 59.91; H, 6.47; N, 8.23. Calc. for $Co\text{-}C_{34}H_{42}N_4O_3SB\cdot H_2O$: C, 60.54; H, 6.57; N, 8.31%.

The crystals were kept in a vacuum desiccator because of their slight hygroscopic property.

(vii) (L-Methionyl-glycinato)(1,3-propanediamine)-cobalt(III) perchlorate, [Co(L-met-gly)(tn)]ClO$_4$

This complex was prepared and chromatographed by a procedure similar to that used in (vi), using H_2(L-met-gly) instead of H_2(gly-L-met), with a reaction time of 45 min. It was found from the absorption and CD spectral measurements that the reddish violet band eluted with a 0.025 mol dm^{-3} NaCl aqueous solution contained two kinds of complexes. After removing sodium chloride, the complex was poured onto a column of Sephadex G-10 (3.6 cm×80 cm). Two bands, orange and reddish violet, were eluted in this order by eluting with water. By comparing the absorption and CD spectra of these bands with those of $[Co(L\text{-}met\text{-}gly)(en)]^+$, it was found that the reddish violet band contained only $[Co(L\text{-}met\text{-}gly)(tn)]^+$. The eluate was converted into the perchlorate salt using a procedure similar to that described in (iii). When the eluate was concentrated to a small volume, crystals appeared which were collected by filtration, washed with ethanol and ether, and then dried in a vacuum desiccator. *Anal.* Found: C, 27.22; H, 5.01; N, 12.79. Calc. for $Co\text{-}C_{10}H_{22}N_4O_7SCl$: C, 27.50; H, 5.07; N, 12.82%.

A part of the reddish violet eluate, which was eluted from a column of Sephadex G-10, was mechanically stirred at *c.* 30 °C for 10 h. When this solution was passed through a column of Sephadex G-10 (3.0 cm×42 cm) again, only a reddish violet band was eluted and fractionated. From the absorption and CD spectra for each of the fractions, it was found that they contained only the identical species with the original reddish violet eluate, indicating that the reddish violet complex does not change to the orange one during concentration using a rotary evaporator. The orange isomer could not be identified in the present work.

(viii) (L-Alanyl-L-methioninato)(1,2-ethanediamine)-cobalt(III) chloride, [Co(L-ala-L-met)(en)]Cl

This complex was prepared and isolated by a procedure similar to that used in (ii), using H_2(L-ala-L-met) instead of H_2(gly-L-met). After the residual sodium chloride in the solution was removed completely by using a column of Sephadex G-10 (3.6 cm×90 cm), the desired complex was crystallized

by addition of ethanol to the concentrated solution. The crystals which appeared were collected by filtration, washed with cold ethanol and ether, and then dried in a vacuum desiccator. *Anal.* Found: C, 30.06; H, 6.43; N, 14.00. Calc. for Co-$C_{10}H_{22}N_4O_3SCl \cdot 3/2H_2O$: C, 30.04; H, 6.30; N, 14.01%.

(ix) (1,2-Ethanediamine)(L-methionyl-L-alaninato)-cobalt(III) perchlorate, [Co(L-met-L-ala)(en)]ClO_4

This complex was prepared and isolated by the same procedure as described in (iii), using H_2(L-met-L-ala) instead of H_2(L-met-gly). *Anal.* Found: C, 27.49; H, 5.11; N, 12.79. Calc. for Co-$C_{10}H_{22}N_4O_7SCl$: C, 27.50; H, 5.07; N, 12.82%.

Measurements

The electronic absorption spectra of the complexes were recorded on a JASCO UVIDEC-610C spectrophotometer. The CD spectra were recorded on a JASCO J-20 spectropolarimeter. All the measurements were carried out in aqueous solution at room temperature. The 100 MHz ^{1}H NMR and ^{13}C NMR spectra were recorded on a JEOL JNM-FX-100 and JNM-FX-90Q NMR spectrometer in deuterium oxide at prove temperature, respectively. The 500 MHz ^{1}H NMR spectra were recorded with a Bruker AM-500 NMR spectrometer under the same conditions as the 100 MHz ^{1}H NMR ones. Sodium 4,4-dimethyl-4-silapentane-1-sulfonate was used as an internal reference. The pH of the aqueous solution was measured with a TOA pH meter, model HM-5ES. The plasma emission spectral analysis was recorded on a Jarrell-Ash model 975, emission spectrophotometer.

Results and discussion

Structural assignment

The absorption spectra of the A-1 and A-4 isomers for the bis(gly-L-met) complex coincide well with those of $S(C_2)$- and $R(C_2)$-[Co(dipeptidato-N,N,O)$_2$]$^-$ [2] over the whole region (Fig. 1 and Table 1). The two isomers show distinctly the first and second d–d absorption bands in the visible region ($14–30 \times 10^3$ cm^{-1}) and no characteristic intense band due to the sulfur-to-metal charge transfer (SMCT) transition is observed [10–13], although the present dipeptide ligand is potentially quadridentate-*N,N,O,S*. It has been suggested that for the diastereomeric complexes of [Co(dipeptidato-*N,N,O*)$_2$]$^-$, the intensity ratio of the first to second d–d absorption maxima (log ϵ_I/log ϵ_{II}) gives higher value for the earlier eluted isomer ($S(C_2)$ isomer) than for the later eluted one ($R(C_2)$ isomer) [2]. This trend in intensity is observed for the present isomers, namely, the intensity ratio of the first to second d–d absorption maxima is 1.32 for the earlier eluted A-1 isomer and 1.19 for the later eluted A-4 one (Table 1). Further, the ^{13}C NMR spectrum of the A-4 isomer shows seven resonance lines due to the fourteen carbon atoms for the two gly-L-met ligands (Table 2), indicating a C_2 symmetry. Taking these facts into consideration, it is probable that the A-1 isomer is $S(C_2)$-[Co(gly-L-met-N,N,O)$_2$]$^-$ and the A-4 isomer is the $R(C_2)$ one.

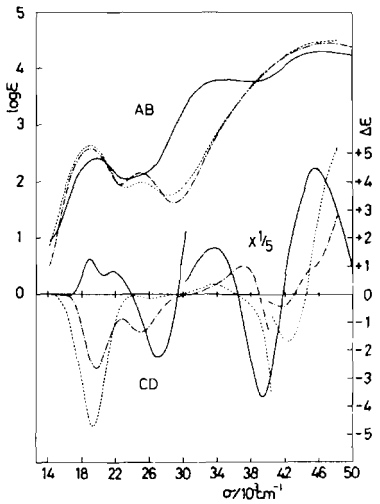

Fig. 1. Absorption and CD spectra of [Co(gly-L-met)(en)]$^+$ (——), S(C_2)-[Co(gly-L-met)$_2$]$^-$ (– – – –) and R(C_2)-[Co(gly-L-met)$_2$]$^-$ (–·–·–).

The absorption and CD spectral behavior for each of the diamine (en and tn) and diammine complexes with gly-L-met and L-ala-L-met are quite similar to one another over the whole region (Fig. 2 and Table 1), suggesting that these complexes take similar geometrical configurations. In contrast to [Co(gly-L-met-N,N,O)$_2$]$^-$ mentioned above, these complexes exhibit commonly the characteristic intense absorption bands (*c.* 35×10^3 cm^{-1}) which can be assigned as arising from the SMCT transition [10–13] (Fig. 1), and further, their first absorption maxima correspond well with those of the *cis*(SO)-[Co(S)(O)(N)$_4$]-type complexes (Fig. 2 and Table 1) [14, 15]. A similar behavior is also observed for the absorption spectra of the diamine and diammine complexes with L-met-gly and L-met-L-ala (Fig. 3 and 4, and Table 1). Here, it is to be noted that the gly(or L-ala)-L-met-*N,N,O,S* and L-met-gly(or L-ala)-*N,N,O,S* complexes are unable to be discriminated on the basis of their absorption spectra because of their quite similar behavior (Fig. 5 and Table 1). These results suggest that both the gly(or L-ala)-L-met and L-met-gly(or L-ala) ligands coordinated to cobalt(III) as quadridentate-*N,N,O,S* as

TABLE 1. Absorption and CD spectral data of $[Co(gly\text{-}L\text{-}met)_2]^-$, [Co(dipeptidato)(diamine)]$^+$ and $[Co(dipeptidato)(NH_3)_2]^+$

Complex ion	Absorption σ (10^3 cm^{-1}) (log ϵ (mol^{-1} dm^3 cm^{-1}))	CD σ (10^3 cm^{-1}) ($\Delta\epsilon$ (mol^{-1} dm^3 cm^{-1}))
$S(C_2)$-$[Co(gly\text{-}L\text{-}met)_2]^-$	19.08 (2.62) 25.13 (1.99) 47.17 (4.49)	19.38 (−4.72) 26.11 (−0.16) 33.67 (+0.35) 42.37 (−8.43)
$R(C_2)$-$[Co(gly\text{-}L\text{-}met)_2]^-$	19.08 (2.57) 25.00 (2.16) 46.73 (4.44)	19.69 (−2.63) 25.00 (−1.36) 37.31 (+1.00) 41.67 (−2.18) 45.45 (+4.80 sh)[a]
[Co(gly-L-met)(en)]$^+$	19.92 (2.41) 34.97 (3.80) 46.51 (4.30)	16.53 (−0.05) 19.05 (+1.26) 21.79 (+0.82) 27.03 (−2.26) 33.78 (+8.18) 39.37 (−18.3) 45.45 (+22.4)
$[Co(gly\text{-}L\text{-}met)(NH_3)_2]^+$	20.08 (2.28) 36.2 (3.64 sh) 48.08 (4.26)	16.00 (−0.02) 18.66 (+0.79) 21.55 (+0.48) 26.60 (−2.23) 32.89 (+3.90) 39.04 (−14.7) 44.64 (+8.97)
[Co(gly-L-met)(tn)]$^+$	20.00 (2.29) 32.9 (3.67 sh) 36.8 (3.87 sh) 37.5 (3.93 sh) 45.0 (4.65 sh)	16.00 (−0.02) 18.62 (+1.13) 22.08 (+0.41) 26.46 (−2.91) 33.11 (+6.13) 39.06 (−14.8) 45.05 (+19.8)
[Co(L-ala-L-met)(en)]$^+$	19.96 (2.37) 34.97 (3.81) 46.51 (4.30)	16.39 (−0.07) 19.05 (+1.13) 22.22 (+0.66) 27.03 (−2.14) 33.33 (+9.14) 39.06 (−21.0) 45.87 (+20.2)
[Co(L-met-gly)(en)]$^+$	19.84 (2.37) 32.89 (3.70) 38.8 (3.82 sh) 45.87 (4.28)	19.46 (+1.04) 22.52 (−0.27) 27.32 (+2.04) 32.47 (−5.90) 38.17 (−2.13) 46.73 (+5.33)
$[Co(L\text{-}met\text{-}gly)(NH_3)_2]^+$	20.08 (2.26) 32.26 (3.58) 39.1 (3.74 sh) 47.62 (4.26)	19.61 (+0.71) 22.99 (−0.16) 26.88 (+1.80) 31.65 (−3.81) 35.46 (+0.78) 39.68 (−3.50) 44.64 (−7.00)
[Co(L-met-gly)(tn)]$^+$	19.84 (2.31) 31.75 (3.70) 37.9 (3.85 sh) 45.05 (4.33)	19.61 (+1.17) 22.88 (−0.22) 26.88 (+2.42) 31.45 (−4.72) 37.88 (−2.73) 45.45 (+5.32)
[Co(L-met-L-ala)(en)]$^+$	19.80 (2.31) 33.6 (3.69 sh) 38.02 (3.82) 46.30 (4.26)	18.87 (+0.16) 21.74 (−0.89) 27.47 (+1.54) 32.26 (−5.98) 35.71 (+0.52) 38.46 (−3.31) 44.05 (−5.70) 47.62 (+7.22)

[a]sh denotes a shoulder.

TABLE 2. ^{13}C NMR chemical shifts[a] of dipeptides and their complexes

Compound	C-1	C-2	M-1	A-1	G-1	M-2	M-3	M-4	A-2	Other (en or tn)
H_2(L-met-gly)	178.61	171.79	55.15		45.94	32.45	30.88	16.58		
H_2(L-met-L-ala)	181.97	170.92	54.93	53.96		32.61	30.77	16.63	19.72	
H_2(gly-L-met)	180.83	169.08	57.10		43.12	33.59	32.29	16.74		
H_2(L-ala-L-met)	180.56	172.71	57.26	51.68		33.48	32.39	16.74	19.02	
[Co(gly-L-met)$_2$]$^-$($R(C_2)$)	191.13	182.78	61.60		51.63	33.43	31.91	16.90		
[Co(L-met-gly)(en)]$^+$	188.20	179.75	60.24		50.98	32.83	28.93	19.12		47.24 47.57
[Co(L-met-gly)(tn)]$^+$	188.20	179.75	60.19		50.60	33.15	28.55	19.02		28.12 40.74 41.82
[Co(L-met-L-ala)(en)]$^+$	191.29	179.37	61.16	59.00		32.83	28.88	18.91	20.69	47.08 47.29
[Co(gly-L-met)(en)]$^+$	188.15	180.94	59.43		50.49	34.29	29.04	21.67		46.64 47.46
[Co(L-ala-L-met)(en)]$^+$	187.88	181.81	59.70	57.80		34.18	29.25	21.24	21.24	46.75 47.57

Numbering of the carbon atoms in the dipeptides

		L-met residue				gly residue	L-ala residue	
$-COO^-$ group	$-CO-NH-$	$-CH-CH_2-CH_2-S-CH_3$				$-CH_2-$	$-CH-CH_3$	
C-1	C-2	M-1	M-2	M-3	M-4	G-1	A-1	A-2

[a]In ppm from DSS.

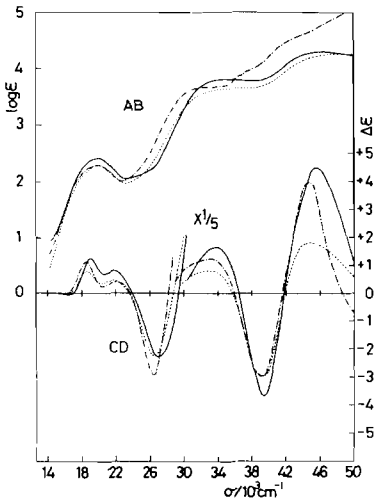

Fig. 2. Absorption and CD spectra of [Co(gly-L-met)$(N)_2$]$^+$: $(N)_2$; en (——), *cis*-$(NH_3)_2$ (– – – –) and tn (–·–·–). (The tn complex is $B(C_6H_5)_4$ salt).

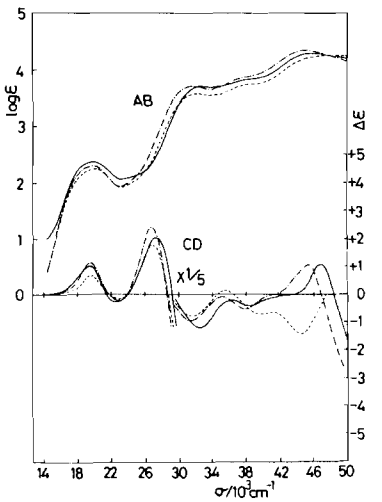

Fig. 3. Absorption and CD spectra of [Co(L-met-gly)$(N)_2$]$^+$: $(N)_2$; en (——), *cis*-$(NH_3)^2$ (– – – –) and tn (–·–·–).

shown in Fig. 6. These suggestions are consistent with the fact that each of the diamine and diammine complexes formed as only one species as described in 'Experimental', and are supported by their NMR and CD spectra (*vide infra*).

NMR spectra

The ^{13}C NMR chemical shift data for the free dipeptides and for their cobalt(III) complexes are summarized in Table 2. In the ^{13}C NMR spectrum of $R(C_2)$-[Co(gly-L-met)$_2$]$^-$, the resonance lines due to the G-1, C-1, C-2 and M-1 carbon atoms (refer to footnote in Table 2) shift to lower field than those of the free H_2(gly-L-met) (Table 2), as in the case of the cobalt(III) complexes with some origopeptides [16–18]. However, the resonance lines due to the M-2, M-3 and M-4 carbon atoms in the complex locate in a quite similar field to those of the free H_2(gly-L-met) (Table 2). These facts reflect that gly-L-met complexed to cobalt(III) functioned as a terdentate-*N,N,O*, that is, $R(C_2)$-[Co(gly-L-met-*N,N,O*)$_2$]$^-$. In contrast to the ^{13}C NMR spectral behavior of $R(C_2)$-[Co(gly-L-met-*N,N,O*)$_2$]$^-$, the amine and diammine complexes with L-met-gly(or L-ala) and gly(or L-ala)-L-met exhibit resonance lines due to the M-3 and M-4 carbon atoms shifted to extremely higher and lower field, respectively than those of the corresponding free dipeptides, while

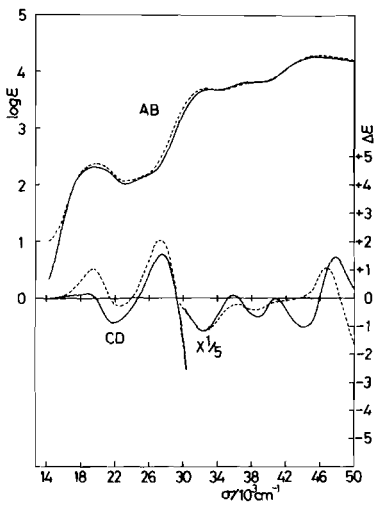

Fig. 4. Absorption and CD spectra of [Co(L-met-L-ala)(en)]$^+$ (——) and [Co(L-met-gly)(en)]$^+$ (– – – –).

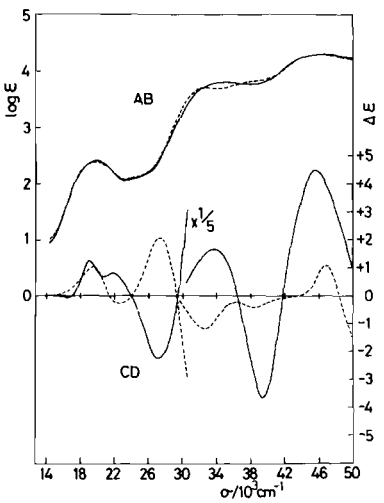

Fig. 5. Absorption and CD spectra of [Co(gly-L-met)(en)]$^+$ (——) and [Co(L-met-gly)(en)]$^+$ (– – – –).

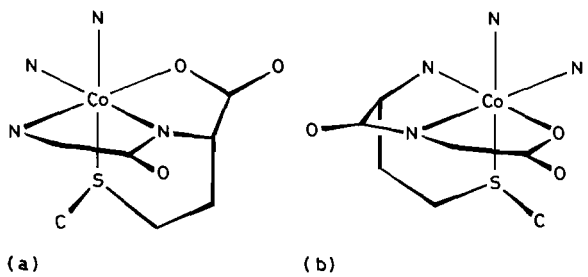

Fig. 6. Possible structures of [Co(gly-L-met)$(N)_2$]$^+$ (a) and [Co(L-met-gly)$(N)_2$]$^+$ (b).

the resonance lines due to the G-1, C-1, C-2 and M-1 carbon atoms show quite similar shifts to those of $R(C_2)$-[Co(gly-L-met-*N*,*N*,*O*)$_2$]$^-$ (Table 2). This suggests that the dipeptides functioned as a quadridentate-*N*,*N*,*O*,*S*.

It is possible for the N–S chelate ring in the L-met residue of the amine and diammine complexes to take a chair or skew conformation, although it has been estimated that it prefers to take a chair conformation in many cobalt(III) complexes with the L-met moiety [10, 11]. As shown in Fig. 7, the 500 MHz ^{1}H NMR spectra for both [Co(L-met-L-ala)(en)]$^+$ (4.408 ppm) and [Co(L-ala-L-met)(en)]$^+$ (3.369 ppm) exhibit one set of triplets due to the methine proton in the L-met residue, whose assignments were done by comparison with the result of the two-dimensional correlated shift (COSY) for [Co(gly-L-met)(en)]$^+$. The coupling constants for the adjacent methylene protons are 3.2 Hz (triplet) for both the L-met-L-ala and L-ala-L-met complexes. Similar trends were also observed for [Co(L-met-gly)(en)]$^+$ (3.452 ppm) and [Co(gly-L-met)(en)]$^+$ (4.359 ppm). These coupling constants suggest that the methine proton has equivalent dihedral angles (*c*. 60°) with the adjacent methylene protons [19]. Molecular model constructions reveal that both of the dihedral angles of a chair conformation are about 60°, but those of a skew boat one are 0 and 120°. Therefore, these facts seem to indicate that the N–S chelate ring for all of the complexes takes the chair conformation.

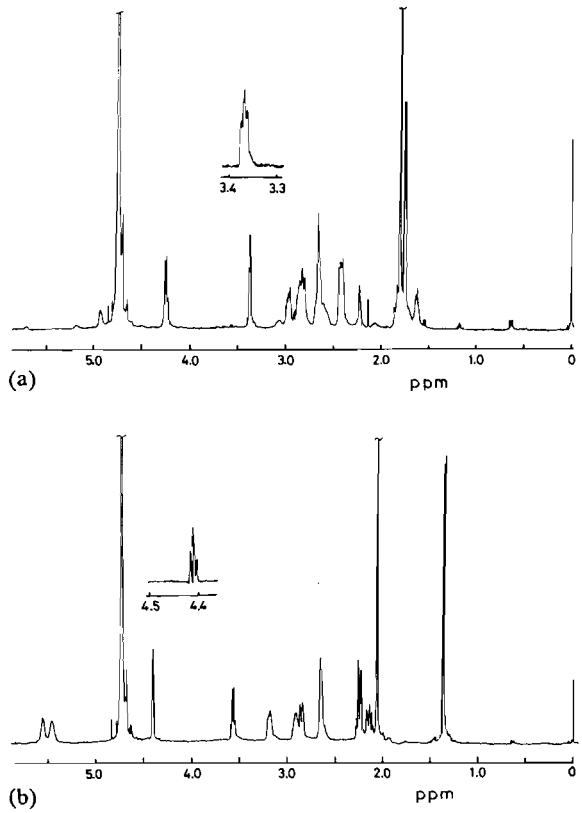

Fig. 7. 500 MHz ^{1}H NMR spectra of [Co(L-met-L-ala)(en)]$^+$ (a) and [Co(L-ala-L-met)(en)]$^+$ (b).

For the amine and diammine complexes, two isomers, *R*(*S*) and *S*(*S*), with respect to the absolute configuration of the chiral sulfur donor atom are expected. As shown in Table 2, these complexes exhibit only one ^{13}C NMR resonance line due to the S-methyl carbon atom (M-4). Similarly, these complexes also exhibit a singlet due to the S-methyl protons in the 100 and 500 MHz ^{1}H NMR spectra (Fig. 7). This suggests that the complexes in an aqueous solution take a selected absolute configuration for the sulfur donor atom; either the *R*(*S*) or the *S*(*S*) configuration. The complexes with the N-terminal L-met residue exhibit the ^{13}C NMR resonance line due to the S-methyl carbon more upfield than those with the C-terminal one and the ^{13}C NMR chemical shift differences for the former complexes are less than those for the latter ones (Table 2). A similar trend was also observed for the ^{1}H NMR behavior due to the S-methyl protons (1.77 ppm for [Co(L-met-gly)(en)]$^+$, 1.75 ppm for [Co(L-met-gly)(tn)]$^+$, 1.74 ppm for [Co(L-met-L-ala)(en)]$^+$, 2.12 ppm for [Co(gly-L-met)(en)]$^+$ and 2.07 ppm for [Co(L-ala-L-met)(en)]$^+$) (Fig. 7). When the N–S chelate ring takes a chair conformation, only the S-methyl group for the [Co{L-met-gly(or L-ala)}$(N)_2$]$^+$ type ($(N)_2$ = en, tn and *cis*-$(NH_3)_2$) complexes lies above the COO group in the *S*(*S*) configuration, suggesting the higher field shifts [20]. For the *R*(*S*) configuration, further, the S-methyl groups of these complexes have a seriously steric interaction with the diamine chelate ring. This suggests that the absolute configuration of the S-methyl group for the [Co{L-met-gly(or L-ala)}$(N)_2$]$^+$ type complexes is fixed in the *S*(*S*) configuration in an aqueous solution.

In contrast to those of the [Co{L-met-gly(or L-ala)}$(N)_2$] type complexes, the [Co{gly-(or L-ala)-L-met}$(N)_2$]$^+$ type complexes seem to show no serious interaction between the S-methyl group in the L-met residue and the COO or diamine group and the absolute configuration of the S-methyl groups could not be estimated by the molecular model examination. However, the nuclear overhauser enhanced correlated shift (NOESY) experiment for [Co(gly-L-met)(en)]$^+$ represents the correlation due to the long range coupling between the S-methyl protons (2.050 ppm) and one of the methylene protons (3.231 ppm (J = 17.06 Hz) of 3.329 ppm (quartet)) in the gly residue. This indicates that the S-methyl group lies over the N–N chelate ring of the gly residue, namely, the S-methyl group takes the *R*(*S*) configuration for [Co(gly-L-met)(en)]$^+$. This result also supports the chair conformation of the N–S chelate ring in the L-met residue, because the S-methyl group in the skew-boat conformation for both of the *R*(*S*) and *S*(*S*) configurations does not lie over the N–N chelate ring. From the similarity of the NMR spectral behavior for the diamine and diammine complexes with gly(or L-ala)-L-met, the S-methyl groups in these complexes seem also to take the *R*(*S*) configuration.

CD spectra

In the regions of $14–40 \times 10^3$ cm^{-1}, the CD spectra of [Co(gly-L-met)$(N)_2$]$^+$ ($(N)_2$ = en, tn and *cis*-$(NH_3)_2$) are quite similar to one another (Fig. 2 and Table 1). A similarity also exists with the CD spectra of [Co(L-met-gly)$(N)_2$]$^+$ (Fig. 3 and Table 1). These indicate that the CD spectra of the gly-L-met-*N*,*N*,*O*,*S* or L-met-gly-*N*,*N*,*O*,*S* complexes are little dependent on the two remaining coordination sites occupied by the diammine and five- or six-membered diamine chelate ring. Namely, their optical activities are mainly attributed to the configuration of the coordinated quadridentate dipeptide, as in the case of the cobalt(III) complexes with *S*-(2-aminoethyl)-L-homocysteinate (L-aehc), [Co(L-aehc)$(N)_2$] ($(N)_2$ = en and *cis*-$(NH_3)_2$) [10].

The CD spectrum of [Co(L-ala-L-met)(en)]$^+$ is quite similar to that of [Co(gly-L-met)(en)]$^+$, although they show a slight difference in the CD intensity which is comparable to the CD effect due to only the asymmetric carbon atom, [Co(L-ala)$(NH_3)_4$]$^{2+}$ [21]. However, [Co(L-met-L-ala)(en)]$^+$ shows a significant difference in the CD intensity for the d–d transition region from the corresponding [Co(L-met-gly)(en)]$^+$ (Fig. 4). The molecular model constructions reveal that the L-met-L-ala complex has a non-bonded interaction between the methyl group in the L-ala residue and the adjacent N–S chelate ring in the L-met residue, while the L-met-gly complex does not have such a steric interaction. This suggests that the strain for the conformation of the N-terminal chelate rings in the dipeptide ligand affects the CD intensity without the CD pattern.

The CD spectra of the gly-L-met-*N*,*N*,*O*,*S* and L-met-gly-*N*,*N*,*O*,*S* complexes reflect favorably their configurations in contrast to their absorption spectra. [Co(L-met-gly)$(N)_2$]$^+$ shows the CD spectrum broadly enantiomeric to [Co(gly-L-met)$(N)_2$]$^+$, especially, $22–36 \times 10^3$ cm^{-1} (Fig. 5). Similar relations are also found for the CD spectra of [Co(L-met-L-ala)(en)]$^+$ and [Co(L-ala-L-met)(en)]$^+$ which have an additional CD contribution due to L(C) of the L-ala residue. Namely, the enantiomeric CD spectral behavior between the L-met-gly(or L-ala)-*N*,*N*,*O*,*S* and gly(or L-ala)-L-met-*N*,*N*,*O*,*S* complexes reflects a quasi-antipodal relation in overall form as shown in Fig. 6,

although their absorption spectra are quite similar to one another (Fig. 5). In addition, for the present system, deviation from the enantiomeric CD patterns is observed in the d–d transition region. This deviation seems to be caused by the asymmetric carbon and the conformation of the chelate ring as mentioned above, which significantly contribute to the CD spectra in this wavelength region [12].

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