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Metal Complexes of Peptides. I. The Cobalt(III) Complexes Containing Dipeptidate and *N*-Methyliminodiacetate as Mixed Ligands

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The mixed-ligand complexes, $Ba[Co(mida)(dipept)]_2$ (where mida is *N*-methyliminodiacetate and dipept denotes dianions of glycyl-L-alanine, L-alanylglycine, glycyl-L-leucine, L-leucylglycine, glycyl-β-alanine, β-alanylglycine, L-alanyl-β-alanine, and β-alanyl-L-alanine) have been prepared. The structure and properties of these complexes have been characterized by their electronic absorption, CD, and NMR spectral data.

Most studies on the metal complexes with peptides have attempted to understand the behavior of metal ions in living systems.[1] However, there are not enough data on the structures and properties of the cobalt(III) complexes with peptides.

The crystal structure of the cobalt(III) complex with glycylglycinate has been determined by the X-ray method.[2] In this complex the dipeptide binds to cobalt in terdentate fashion (meridional form) through the nitrogens of amino and amide groups and the oxygen of carboxyl group. The preparation and properties of the bis(dipeptidato) complex, $[Co(\alpha_1\text{-}\alpha_2)_2]^-$ ($\alpha_1\text{-}\alpha_2$ represents the dianion of the dipeptide $H_2\alpha_1\text{-}\alpha_2$, where α_1 is the *N*-terminal residue) have been studied in detail by Boas *et al.*[3] On the other hand, little work has been done on the mixed-ligand complexes containing dipeptide and one other ligand, though the complexes $[Co(NH_3)_3(\alpha_1\text{-}\alpha_2)]^+$ and $[Co(dien)(\alpha_1\text{-}\alpha_2)]^+$ have been prepared and characterized by Browning *et al.*[4]

Recently we found that the complexes of $[Co(ida)(\alpha_1\text{-}\alpha_2)]^-$ exhibit novel spectral behavior in the region of the d-d transition band,[5] compared with that for the typical cobalt(III) chromophore of the type $[Co(N)_3(O)_3]$.[6] In the present paper we report the preparation of mono(dipeptidato) complexes of the type $[Co(mida)(\alpha_1\text{-}\alpha_2)]^-$, where mida represents the dianion of *N*-methyliminodiacetic acid, H_2mida. These complexes have been characterized by elemental analysis, ^1H-NMR, absorption, and CD spectroscopy. In order to study the influence of the chelate-ring size of dipeptide on the properties of the $[Co(mida)(\alpha_1\text{-}\alpha_2)]^-$ complexes, especially on the absorption and CD spectra, the complexes of the dipeptides containing β-alanine have been studied in detail. The effects of the pH on the absorption and CD spectra have been also examined.

Experimental

Ligands. *N*-Methyliminodiacetic acid (H_2mida) was prepared according to the literature.[7]

Glycyl-β-alanine was prepared by a combination of conventional techniques, using *N*-(benzyloxycarbonyl)glycine (z-gly) and β-alanine ethyl ester (β-ala-OEt) as the starting materials.[8] Dicyclohexylcarbodiimine coupling was used to form the dipeptide derivative from these precursors. The peptide hydrobromide (H_2gly-β-ala·HBr) was formed by the reaction of z-gly-β-ala-OEt with aqueous NaOH solution and then with HBr-acetic acid. The HBr salts of β-alanylglycine (H_2β-ala-gly), L-alanyl-β-alanine (H_2L-ala-β-ala), and β-alanyl-L-alanine (H_2β-ala-L-ala) were also prepared by the same method as that of H_2gly-β-ala·HBr. These dipeptides were used for the syntheses of the complexes without removing HBr. The other dipeptides, glycylglycine (H_2gly-gly), L-alanylglycine (H_2L-ala-gly), glycyl-L-alanine (H_2gly-L-ala), L-leucylglycine (H_2L-leu-gly), and glycyl-L-leucine (H_2gly-L-leu), were obtained commercially from Sigma Chemical Company and Protein Research Foundation. These dipeptides were used without further purification.

Preparation of Complexes. *$Ba[Co(mida)(gly\text{-}L\text{-}ala)]_2\cdot 5H_2O$*: An aqueous solution (10 cm³) of cobalt(II) chloride hexahydrate (2.37 g, 0.01 mol) was added slowly to an aqueous solution (10 cm³) containing glycyl-L-alanine (1.46 g, 0.01 mol) and *N*-methyliminodiacetic acid (1.47 g, 0.01 mol), adjusting the pH of the mixed solution at *ca.* 9.5 with sodium hydroxide. The resulting solution was oxidized with lead dioxide (5 g) at 40 °C for about 1 h with stirring, and then it was cooled to room temperature. After removal of insoluble material by filtration, the filtrate, which contained $[Co(gly\text{-}L\text{-}ala)_2]^-$, $[Co(mida)_2]^-$, $[Co(mida)(gly\text{-}L\text{-}ala)]^-$, and some minor products, was chromatographed on a column (4.5 cm × 90 cm) of QAE-Sephadex A-25 using 0.05 M† KCl as an eluant. After the elution of bis(dipeptidato)cobaltate(III) complex, the objective dark-gray band ($[Co(mida)(gly\text{-}L\text{-}ala)]^-$) was separated completely from the overlapped brown-violet band (*fac*-$[Co(mida)_2]^-$) by further development of another column (4.5 cm × 90 cm) connected to the original column with a teflon tube (ϕ 1 mm). The eluted solution from the dark-gray band was concentrated to a small volume (20—30 cm³) using a rotary evaporator at *ca.* 35 °C. To the concentrated solution was added a large amount of methanol, and then potassium chloride which deposited was filtered off. The filtrate was concentrated again to a few milliliters, and the residual KCl in the solution was removed by using a Sephadex G-10 column (3 cm × 90 cm). The deliquescent potassium salt of the complex was converted to barium salt by chromatography on SP-Sephadex C-25 to give a crystalline product. The barium salt was recrystallized from an aqueous solution by addition of ethanol and acetone.

The preparations of the type $Ba[Co(mida)(\alpha_1\text{-}\alpha_2)]_2$ containing other dipeptide ligands were carried out by the same method as described above. Analytical data of the new complexes are listed in Table 1.

$[Co(NH_3)_3(gly\text{-}\beta\text{-}ala)]Cl\cdot H_2O$: Preparation of this compound was performed in the same way as that for the corresponding glycylglycinato complex.[4] Concentrated aqueous ammonia (15 cm³) was added to the solution containing glycyl-β-alanine hydrobromide (4.54 g, 0.02 mol) and cobalt(II) chloride hexahydrate (4.74 g, 0.02 mol) in water (10 cm³). Hydrogen peroxide (30%, 2 cm³) was added slowly to the above ammoniac solution with constant stirring and then the solution was gently warmed for 10 min. The complex, $[Co(NH_3)_3(gly\text{-}\beta\text{-}ala)]^+$, in the reactant solution

† 1 M = 1 mol dm^{-3}.

Table 1. Analytical data of the complexes

Complex	C, %		H, %		N, %	
	Found	(Calcd)	Found	(Calcd)	Found	(Calcd)
$Ba[Co(mida)(gly\text{-}gly)]_2\cdot 9H_2O$	22.73	(22.34)	4.73	(4.58)	8.24	(8.68)
$Ba[Co(mida)(gly\text{-}L\text{-}ala)]_2\cdot 5H_2O$	25.96	(26.00)	4.14	(4.36)	8.82	(9.10)
$Ba[Co(mida)(L\text{-}ala\text{-}gly)]_2\cdot 6H_2O$	25.79	(25.51)	4.18	(4.49)	8.62	(8.92)
$Ba[Co(mida)(gly\text{-}L\text{-}leu)]_2\cdot 6H_2O$	30.38	(30.36)	5.40	(5.29)	8.08	(8.17)
$Ba[Co(mida)(L\text{-}leu\text{-}gly)]_2\cdot 5H_2O$	30.70	(30.98)	4.99	(5.20)	8.14	(8.34)
$Ba[Co(mida)(gly\text{-}\beta\text{-}ala)]_2\cdot 4H_2O$	26.13	(26.52)	4.12	(4.23)	9.52	(9.28)
$Ba[Co(mida)(\beta\text{-}ala\text{-}gly)]_2\cdot 2H_2O$	27.53	(27.62)	3.81	(3.94)	10.13	(9.66)
$Ba[Co(mida)(L\text{-}ala\text{-}\beta\text{-}ala)]_2\cdot 6H_2O$	26.94	(27.25)	4.82	(4.78)	8.87	(8.68)
$Ba[Co(mida)(\beta\text{-}ala\text{-}L\text{-}ala)]_2\cdot 3H_2O$	28.81	(28.85)	4.07	(4.40)	8.87	(9.18)
$K[Co(\beta\text{-}ala\text{-}gly)_2]\cdot H_2O$	29.64	(29.71)	4.51	(4.49)	14.33	(13.83)
$[Co(NH_3)_3(gly\text{-}\beta\text{-}ala)]Cl\cdot H_2O$	19.68	(19.52)	6.30	(6.23)	23.11	(22.77)

was separated from by-products on a SP-Sephadex C-25 column (4.5 cm×50 cm) by elution with 0.05 M KCl solution. Potassium chloride was removed by the same method as that described above. The complex obtained was recrystallized from an aqueous solution by addition of ethanol.

$K[Co(\beta\text{-}ala\text{-}gly)_2]\cdot H_2O$: β-Alanylglycine hydrobromide (2.27 g, 0.01 mol) was dissolved in water (10 cm^3). The solution was adjusted to pH *ca.* 9.5 with sodium hydroxide. An aqueous solution containing $Co(NO_3)_2\cdot 6H_2O$ (1.46 g, 5 mmol) in water (10 cm^3) was added drop-by-drop to the above solution, adjusting the pH of the solution at *ca.* 9.5 with NaOH. After an addition of activated charcoal (0.5 g) the mixed solution was oxidized by an air stream for 12 h. The reactant solution was chromatographed on a QAE-Sephadex A-25 column (4.5 cm×90 cm). The objective complex was eluted with 0.05 M KCl, and KCl was removed by the same method as that for the $Ba[Co(mida)(gly\text{-}L\text{-}ala)]_2$ complex. The complex was recrystallized from water by addition of ethanol and acetone.

Measurements. The absorption spectra were measured by a Hitachi 557-type spectrophotometer and the CD spectra by a JASCO J-22 spectropolarimeter. The proton NMR spectra were recorded on a JEOL MH-100 spectrometer with DSS as an internal reference.

Results and Discussion

The mixed ligand complexes, $[Co(mida)(\alpha_1\text{-}\alpha_2)]^-$, were conveniently prepared by the oxidation of the aqueous solutions containing cobalt(II) chloride, dipeptide, and H_2mida with lead dioxide, but this preparative method gave also substantial quantities of by-products: $[Co(mida)_2]^-$, $[Co(\alpha_1\text{-}\alpha_2)_2]^-$, and others. The yields of the mixed ligand complexes depend very much on the pH of the reaction solutions; at low pH (*ca.* 7) a large amount of $[Co(mida)_2]^-$ and minor products were formed, and at high pH (*ca.* 11) the initially formed precipitate (presumably cobalt(II) hydroxide) prevented the formation of the mixed type complexes. The pH range of 9 to 10 was most suitable for the preparation of the mixed type complexes; the racemization of active peptides may also be ignored in this pH range.[3] With the dipeptides containing β-alanine, however, the reactions were carried out in the pH range of 8 to 9 to prevent the formation of precipitate.

The $[Co(mida)(\alpha_1\text{-}\alpha_2)]^-$ anion was separated from other uninegative complexes, $[Co(mida)_2]^-$ and $[Co(\alpha_1\text{-}\alpha_2)_2]^-$, on a QAE-Sephadex A-25 column by elution with 0.05 M KCl. The order of the elutions was: $[Co(\alpha_1\text{-}\alpha_2)_2]^-$, $[Co(mida)_2]^-$, and $[Co(mida)(\alpha_1\text{-}\alpha_2)]^-$. In the cases of the β-alanylglycine and β-alanyl-L-alanine, however, the $[Co(mida)(\beta\text{-}ala\text{-}\alpha_2)]^-$ was eluted faster than the $[Co(mida)_2]^-$ anion. For all the peptides used here, it was difficult to separate completely $[Co(mida)(\alpha_1\text{-}\alpha_2)]^-$ from $[Co(mida)_2]^-$ on a QAE-Sephadex column, but these complexes could be separated by the different solubility of their potassium salts in a mixed solvent of methanol–water. Partial separation of these complexes also occurs on a Sephadex G-10 column. The potassium salts of the $[Co(mida)(\alpha_1\text{-}\alpha_2)]^-$ could not be often obtained in crystal state, because they are very hygroscopic. Accordingly, the salts were converted to the barium salts to give crystalline products.

Proton NMR Spectra. The dipeptide containing only α-amino acids coordinates to cobalt(III) in meridional configuration, and the chelate rings of the dipeptide ligand take the coplanar form. This has been established for the bis(glycylglycinato) complex by two independent X-ray crystallographic studies.[2,9] The ^{1}H-NMR spectrum of $[Co(mida)(gly\text{-}gly)]^-$ in slightly basic D_2O shows only one AB pattern (δ_A=4.42, δ_B=4.02 ppm, J_{gem}=16.5 Hz) for the two CH_2 groups of mida, indicating that both gly-gly and mida adopt the *mer* disposition.[5] An additional support for the *mer* structure of $[Co(mida)(gly\text{-}gly)]^-$ is provided by ^{13}C-NMR data; the peaks at 66.1 and 183.1 ppm can be assigned to two methylene and two carboxyl carbons, respectively, indicating an equivalence of the two acetate groups in mida ligand. The ^{1}H-NMR data of other $[Co(mida)(\alpha_1\text{-}\alpha_2)]^-$ in slightly basic D_2O are listed in Table 2. With the dipeptides containing only α-amino acids, the structure of these complexes can be also concluded to be *mer* on the basis of the ^{1}H-NMR data of the mida ligand. In the spectra of $[Co(mida)(gly\text{-}L\text{-}ala)]^-$ and $[Co(mida)(gly\text{-}L\text{-}leu)]^-$, the signal of the C-terminal methine proton can not be assigned clearly because of the overlap of the methylene signals of mida. The same situation is also realized in $[Co(mida)(L\text{-}leu\text{-}gly)]^-$; the resonances due

TABLE 2. ^{1}H-NMR DATA OF THE TYPE [Co(mida)(α_1-α_2)]$^-$ COMPLEXES

α_1-α_2	Mida(ppm)			N-Terminal (α_1) (ppm)		C-Terminal (α_2) (ppm)	
	-CH_2- (AB, J(Hz))		CH_3 (s)	-CH_2- (-CH-)	-CH_2-CH_2-	-CH_2- (-CH-)	-CH_2-CH_2-
gly-gly	4.42	4.02(16.5)	3.07	3.47(s)		4.35(s)	
gly-L-ala	4.42	3.99(17.0)	3.02	3.49(s)		a)	
L-ala-gly	4.42	4.00(16.5)	3.08	3.61(q, J=7.0)		4.36(s)	
gly-L-leu	4.41	3.93(16.5)	3.00	3.53, 3.38(AB, J=16.0)		a)	
L-leu-gly	4.39	3.97(16.5)	3.09	3.54(br)		{4.35, 4.38 a)	b)
gly-β-ala	4.53	3.75(16.5)	2.94	3.37(s)			2.47(br), 3.61(br)
β-ala-gly	4.50	3.70(17.0)	2.74		2.58(s)	4.20(s)	
L-ala-β-ala	4.55	3.80(16.5)	3.01	a)			2.50(br), 3.63(br)
β-ala-L-ala	4.51	3.76(16.5)	2.74		2.59(br, s)	a)	

AB: AB pattern resonance, s: singlet, q: quartet, br: broad.
a) Obscured by overlap of another resonance. b) Inside components of AB pattern.

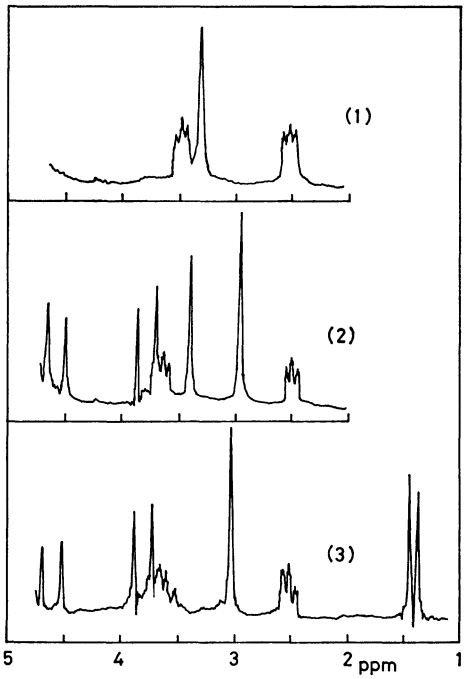

Fig. 1. The ^{1}H-NMR spectra of [Co(NH_3)$_3$(gly-β-ala)]$^+$ (1), [Co(mida)(gly-β-ala)]$^-$ (2), and [Co(mida)(L-ala-β-ala)]$^-$ (3) in alkaline D_2O.

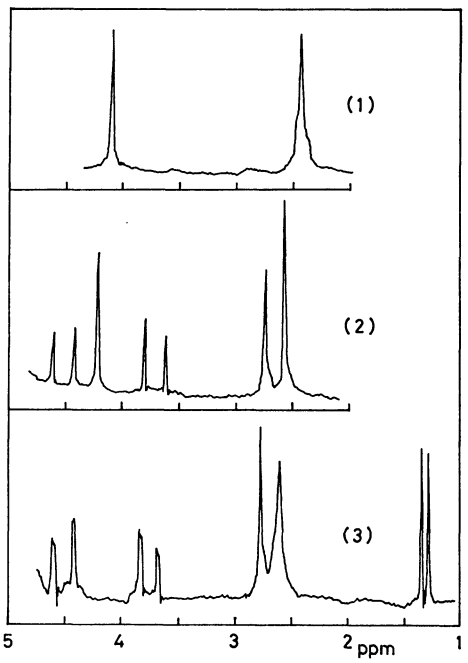

Fig. 2. The ^{1}H-NMR spectra of [Co(β-ala-gly)$_2$]$^-$ (1), [Co(mida)(β-ala-gly)]$^-$ (2), and [Co(mida)(β-ala-L-ala)]$^-$ (3) in alkaline D_2O.

to the C-terminal CH_2 which are only slightly inequivalent (4.35 and 4.38 ppm) are overlapped by one component of the resonances due to the CH_2 of mida. In the present study, the [Co(mida)(L-leu-gly)]$^-$ is the only example in which the C-terminal CH_2 protons are inequivalent at 100 MHz.

Examination of molecular models indicates that the dipeptides containing β-alanine can coordinate to cobalt(III) in both *mer* and *fac* configurations. On the other hand, the mida ligand adopts preferably the *fac* disposition, although the *mer*-K[Co(mida)$_2$] has been stably isolated.[10] Figure 1 shows the comparison among the ^{1}H-NMR spectra of [Co(NH_3)$_3$(gly-β-ala)]$^+$, [Co(mida)(gly-β-ala)]$^-$, and [Co(mida)(L-ala-β-ala)]$^-$ ions in slightly basic D_2O. The spectrum of [Co(NH_3)$_3$(gly-β-ala)]$^+$ exhibits three resonance peaks: a singlet signal due to the N-terminal CH_2 at 3.28 and two broad triplet-like peaks due to the C-terminal -CH_2-CH_2- at 2.50 and 3.45 ppm. In the [Co(mida)(gly-β-ala)]$^-$ complex, the resonances of the gly-β-ala ligand are analogous to those of [Co(NH_3)$_3$(gly-β-ala)]$^+$, and an AB pattern in the range of 3.66 to 4.62 ppm can be assigned to the two CH_2 groups of mida, giving a hint of the equivalence of the two methylenes. This spectrum supports the assertion that the [Co(mida)(gly-β-ala)]$^-$ complex has the *mer* configuration. The same situation is realized in the [Co(mida)(L-ala-β-ala)]$^-$ complex, in which only one AB pattern resonance due to the two CH_2 of mida indicates that the *mer* configuration is taken.

Table 3. Absorption and CD spectra of the type [Co(mida)(α_1-α_2)]$^-$ complexes in the d-d transition region measured in water

α_1-α_2	AB_{max} $\sigma/10^3$ cm^{-1} (log ε)			CD_{ext} $\sigma/10^3$ cm^{-1} ($\Delta\varepsilon$)		
gly-gly	17.30(2.18)	21.05(2.27)	25.97(2.12)			
gly-L-ala	17.04(2.24)	20.83(2.32)	25.06(2.19)	16.81(−0.62)	20.75(−0.59)	25.19(−0.68)
L-ala-gly	17.09(2.19)	20.88(2.29)	25.13(2.15)	16.31(−0.37)	20.75(−0.19)	25.71(−0.25)
gly-L-leu	17.12(2.21)	21.19(2.30)	25.51(2.19)	16.39(−1.22)	21.28(−1.06)	*ca.* 24.50 sh (−0.90)
L-leu-gly	17.09(2.22)	20.83(2.34)	25.32(2.17)	16.37(−0.51)	*ca.* 19.50 sh (−0.26)	25.77(−0.29)
gly-β-ala	16.84(2.20)	20.24(2.19)	25.32(2.13)			
β-ala-gly	16.75(2.21)	20.41(1.85)	25.38(2.31)			
L-ala-β-ala	16.72(2.23)	20.41(2.21)	25.19(2.17)	16.39(−0.60)	20.75(+0.08)	24.69(−0.51)
β-ala-L-ala	16.67(2.20)	20.41(1.85)	25.45(2.33)	16.08(−1.13)	20.53(+1.83)	25.00(−3.71)

sh: Shoulder.

The complex, [Co(β-ala-gly)$_2$]$^-$, shows a simple NMR spectrum (Fig. 2). The two peaks with the relative intensity of 2:1 at 2.43 and 4.12 ppm can be assigned to the N-terminal $-CH_2-CH_2-$ and C-terminal CH_2 protons, respectively. In the spectrum of [Co(mida)(β-ala-gly)]$^-$, the two singlets at 2.58 and 4.20 ppm can be assigned to the N-terminal $-CH_2-CH_2-$ and C-terminal CH_2 protons, respectively, and the AB pattern (δ_A=4.50, δ_B=3.70 ppm, J_{gem}=17.0 Hz) to the two CH_2 groups of mida. It is also suggested that the configuration of [Co(mida)(β-ala-gly)]$^-$ is *mer*. A singlet resonance which arises from the $-CH_2-CH_2-$ group of the β-ala-gly in these complexes is in accordance with the data observed for the *cis*(N)*trans*-(O_g,N)-[Co(β-ala)(gly)(ox)]$^-$ complex.[11]

In the case of the dipeptide complexes containing β-alanine, the chelate rings of the coordinated dipeptide are not coplanar. Consequently, the conformational isomerism due to the puckered β-alanine ring can be expected for the [Co(mida)(α_1-β-ala)]$^-$ or the [Co(mida)(β-ala-α_2)]$^-$. However, the interconversion between the ring conformations is presumed to be easy. The simple ^{1}H-NMR spectra of β-alanyl groups in their complexes may be attributed to the rapid interconversion. The [Co(mida)(α_1-α_2)]$^-$ complex provides another problem of the conformational isomerism arising from the choice of the conformation of mida ligands: which is the methyl group of mida directed to, the C- or the N-terminal residue of dipeptide ligand? In all the [Co(mida)(α_1-α_2)]$^-$ complexes isolated in this work, the signal assigned to the methyl protons of mida exhibited a sharp singlet, suggesting an existence of only one isomer. Our present data are not sufficient to decide the conformation of the mida ligand. The possibility of the rapid interconversion between the two conformational isomers due to the methyl moiety may be ignored, because such interconversion requires the rupture of the coordinate bond.

Absorption Spectra. Figure 3 shows the absorption spectra of the [Co(mida)(gly-L-ala)]$^-$ and [Co(mida)(L-ala-gly)]$^-$ complexes together with their CD spectra. The absorption spectra of both complexes are quite similar to each other and also to that of [Co(mida)(gly-gly)]$^-$ (the spectra are little affected by the α-substituents in the N- and C-terminal residues of dipeptides). Such similarities were also observed in the spectra of [Co(mida)(gly-L-leu)]$^-$ and [Co(mida)(L-leu-gly)]$^-$ complexes. The spectral data are summarized in Table 3. The typical cobalt(III) chromophore of the type *mer*-[Co(N)$_3$(O)$_3$] shows two d-d transition bands (the lower-energy one is slightly split) with nearly equal intensity in the visible region.[6] However, the [Co(mida)(α_1-α_2)]$^-$ complexes show three bands in the visible region; two components in the lower-energy band region have enhanced intensities and are markedly separated from each other.

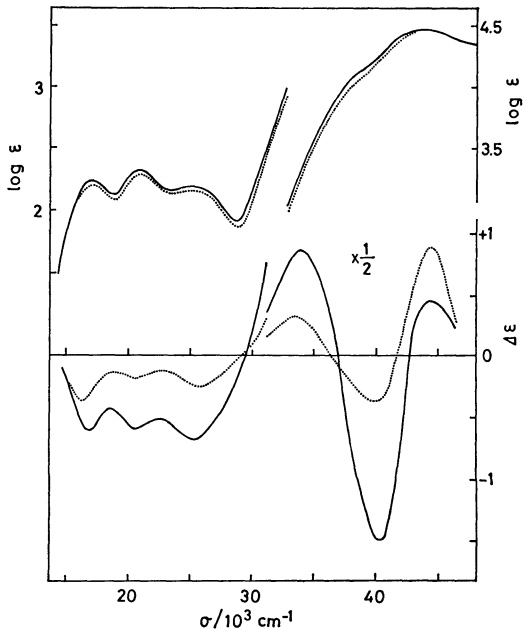

Fig. 3. The absorption and CD spectra of [Co(mida)(gly-L-ala)]$^-$ (——) and [Co(mida)(L-ala-gly)]$^-$ (·····) in water.

As shown in Fig. 4, the absorption spectrum of the [Co(mida)(L-ala-β-ala)]$^-$ complex is similar to that of the type [Co(mida)(α_1-α_2)]$^-$ complex in which both α_1 and α_2 are α-amino acid residues, though they differ from one another in the relative intensities of the two band components in the lower-energy band region. On the other hand, the [Co(mida)(β-ala-L-ala)]$^-$ (Fig. 4) shows the remarkably different absorption behavior in the d-d transition region, com-

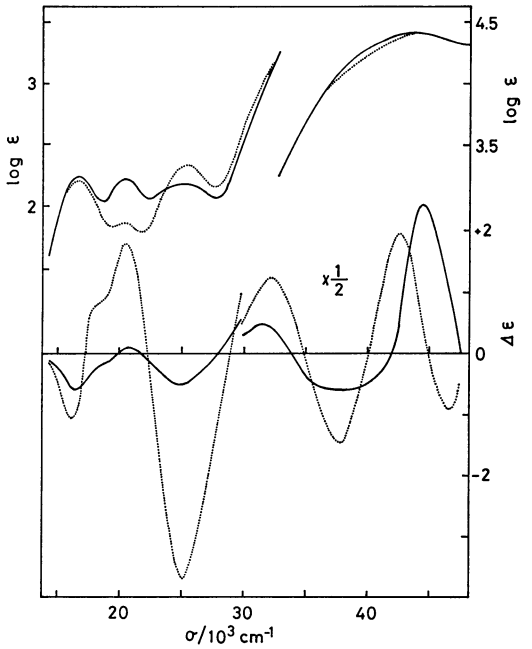

Fig. 4. The absorption and CD spectra of [Co(mida)(L-ala-β-ala)]⁻ (——) and [Co(mida)(β-ala-L-ala)]⁻ (·····) in water.

pared with those of the other [Co(mida)(α_1-α_2)]⁻ complexes; one of the split lower-energy bands has low intensity, but the higher-energy band has enhanced intensity. The spectrum of the [Co(mida)(β-ala-gly)]⁻ complex indicates quite similar behavior to that of the corresponding complex with β-ala-L-ala. Molecular models provide no obvious reasons for the difference between the spectral behaviors of the [Co(mida)(α_1-β-ala)]⁻ and [Co(mida)(β-ala-α_2)]⁻ complexes.

CD Spectra. Unlike the bis(dipeptidato)cobalt(III) complexes, the chelate rings forming the environment of the central cobalt are symmetrical in the [Co(mida)(α_1-α_2)]⁻ complex, ruling out any configurational contribution to the optical activity. Also, in the case of the dipeptide, α_1-α_2, where both α_1 and α_2 are α-amino acids, no conformational contribution to the optical activity can be invoked, because of the planarity of the entire dipeptide ligand. Therefore the sole source of optical activity in the d-d transition region is ascribed to the vicinal effect of the asymmetric carbon.[12]

Both the [Co(mida)(L-ala-gly)]⁻ and [Co(mida)(gly-L-ala)]⁻ complexes show three negative CD bands associated with three absorption bands in the visible region (Fig. 3 and Table 3). Similar CD spectra were obtained for the [Co(mida)(L-leu-gly)]⁻ and [Co(mida)(gly-L-leu)]⁻ complexes. It is remarkable that these complexes have a comparatively intense CD associated with the higher-energy d-d band. The asymmetry factor $g(=\Delta\varepsilon/\varepsilon)$ for the higher-energy d-d band is comparable to that for the lower-energy one, whereas in most optically active cobalt(III) complexes, the g value for the higher-energy band is much lower than that for the lower-energy band.[13] There is a large difference in the magnitude of $\Delta\varepsilon_{ext}$ between [Co(mida)(gly-L-ala)]⁻ and [Co(mida)(L-ala-gly)]⁻,

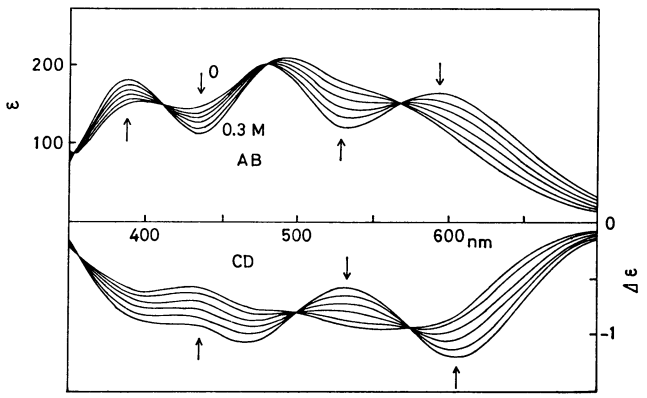

Fig. 5. The absorption (AB) and CD spectral changes of [Co(mida)(gly-L-leu)]⁻ in $HClO_4$ (0, 0.02, 0.05, 0.1, 0.2, 0.3 M).

which is not easy to explain, as the bis(dipeptidato)-complexes with these peptides have similar values of $\Delta\varepsilon_{ext}$. Also, this difference can not be attributed to the racemization of ligand during the synthesis of the complex, because in the weak alkaline solution only the C-terminal methylene or methine protons undergo the exchange reaction.[3]

As shown in Fig. 4, both the [Co(mida)(β-ala-L-ala)]⁻ and [Co(mida)(L-ala-β-ala)]⁻ complexes show the negative and positive CD bands associated with the lower-energy d-d transition and a strong negative CD band associated with higher-energy d-d transition. The CD data for these complexes also show that the magnitude of $\Delta\varepsilon_{ext}$ from the C-terminal active center is larger than that of $\Delta\varepsilon_{ext}$ from the N-terminal one. The [Co(mida)(β-ala-L-ala)]⁻ complex has extremely intense CD bands in the d-d transition region, especially in the higher-energy side. Though the puckering in the β-alanine-chelate ring of the β-ala-α_2 (or α_1-β-ala) ligand gives rise to some conformational contribution to the total CD, it is unlikely on the examination of molecular models that one conformation would be favored.

Protonation on [Co(mida)(α_1-α_2)]⁻. An example is described in detail of the protonation on the [Co(mida)(α_1-α_2)]⁻ species with glycyl-L-leucinate in acid solution. As shown in Fig. 5, on increasing the acid concentration, two components of the lower-energy d-d band approach each other and the higher-energy d-d band shifts to the higher-energy side with increasing of the intensity. The CD bands in the lower-energy d-d transition change in the same pattern as the absorption bands do, whereas the CD band in the higher-energy band region decreases in intensity. The changes of the absorption and CD spectra show isosbestic points and also are reversible, indicating that the changes are due to the protonation on the amide oxygen of the coordinated peptide,[14] and ruling out the successive protonation on the coordinated carboxyl group which has been proposed for the $[Co(NH_3)_3(\alpha_1\text{-}\alpha_2)]^+$ complexes.[4] The conjugate system of the amide group should be strikingly affected by the protonation following the spectral change. These acidic solutions were stable on standing at room temperature for several hours. The same situation was realized for

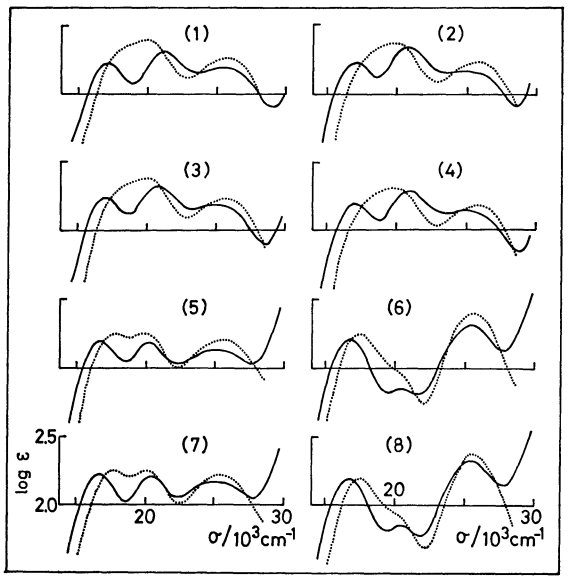

Fig. 6. The absorption spectra of the type [Co(mida)-$(\alpha_1\text{-}\alpha_2)$]$^-$ in water (——) and 60% $HClO_4$ (·····). α_1-α_2: (1) gly-L-leu, (2) L-leu-gly, (3) gly-L-ala, (4) L-ala-gly, (5) gly-β-ala, (6) β-ala-gly, (7) L-ala-β-ala, (8) β-ala-L-ala.

the other [Co(mida)$(\alpha_1\text{-}\alpha_2)$]$^-$ complexes examined. Figure 6 shows the comparison between the absorption spectra in water and in 60% $HClO_4$, *i.e.*, the spectra of the deprotonated and protonated type complexes. The spectral change caused by protonation is larger for the [Co(mida)$(\alpha_1\text{-}\alpha_2)$]$^-$ complex than for the [Co-$(\alpha_1\text{-}\alpha_2)_2$]$^-$ complex.

The protonation on the amide group intimately depends on the kind of dipeptide ligand; for the dipeptides containing β-alanine, especially N-terminal β-alanine, the protonation occurred in the weakly acidic solution, whereas for the dipeptides with N-terminal substituent, the protonation took place in the strongly acidic solution. However, it was impossible to calculate precisely the pK_a for the protonation on the amide group from the variation of ε or $\Delta\varepsilon$ with pH, because of the difficulty of the measurement of meaningful pH in strongly acidic solutions. The pK_a values estimated semi-quantitatively decreased in the following order:

[Co(mida)(β-ala-L-ala)]$^-$ $\simeq$ [Co(mida)(β-ala-gly)]$^-$ > [Co(mida)(gly-β-ala)]$^-$ > [Co(mida)(L-ala-β-ala)]$^-$ > [Co(mida)(gly-L-leu)]$^-$ $\simeq$ [Co(mida)(gly-L-ala)]$^-$ > [Co(mida)(gly-gly)]$^-$ > [Co(mida)(L-leu-gly)]$^-$ $\simeq$ [Co(mida)(L-ala-gly)]$^-$.

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