# Metal Complexes of Peptides. III. The Preparation and Properties of Diammine(oligopeptidato)cobalt(III) Complexes

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(Received March 26, 1982)

Complexes of the [Co(tripeptidato)$(NH_3)_2$] and $NH_4$[Co(tetrapeptidato)$(NH_3)_2$] types (tripeptidato and tetrapeptidato denote the tri- and tetraanions of the coordinating peptides respectively) have been prepared and characterized by means of their electronic, $^1$H NMR, and circular dichroism (CD) spectral data. In the $NH_4$-[Co(tetrapeptidato)$(NH_3)_2$] complex, the peptide coordinates to cobalt(III) as a quadridentate ligand through the nitrogens of an amino and three amide groups. The additivity of the vicinal CD spectra has been found to hold for the complexes of a series of tripeptides containing L-alanine and/or glycine residue. These tri- and tetrapeptidato complexes in water easily undergo aquation reaction, releasing the ammonia molecule.

A number of oligopeptide complexes of metals, such as Cu(II, III), Ni(II, III), and Pd(II), have been reported in which the peptides coordinate as quadridentate chelates.[1-16] Although the preparations of the cobalt(III) complexes with tri- or tetrapeptides have been reported in the literature,[17-22] there have been only two reports of the cobalt(III) complexes with a peptide coordinated as a quadridentate chelate.[21,22] Manyak *et al.* reported on the [Co(gly–gly–gly)$(NH_3)_2$] and Na[Co(gly–gly–gly)(gly)] complexes (gly–gly–gly denotes the trianion of glycylglycylglycine), in which the three chlate rings of the peptide were noncoplanar.[21] As X-ray structural studies have shown that the peptide group retains its rigid planar structure upon coordination,[23-27] the structure postulated by Manyak *et al.* is thought to be very unlikely. The complex [Co(gly–gly–gly)$(NH_3)_2$] described by them was rose in color and so different from the present complex (brown in color) with the same chemical formula. Evans *et al.* have prepared diammine(tripeptidato)cobalt(III) complexes in which the peptides coordinate as a coplanar quadridentate chelate and discussed in detail the effects of chelation on the $^{13}$C and $^1$H NMR resonances of peptides.[22]

Our recent works concerning dipeptidato complexes have shown that the [Co(*N*-methyliminodiacetato)-(dipeptidato)]$^-$ type of complex exhibits novel spectral behavior in the d-d transition region,[28] and that the absolute configurations of bis(dipeptidato)cobaltate(III) complexes can be determined on the basis of their CD patterns in the range of 40000 to 50000 cm$^{-1}$.[29] In the present paper, we wish to report the preparation and spectroscopic properties of the cobalt(III) complexes with a series of tri- and tetrapeptides in which the peptides coordinate as quadridentate chelates.

## Experimental

*Ligands.* Glycylglycylglycine ($H_3$gly–gly–gly) was synthesized according to the literature.[30a]

L-Alanyl-L-alanyl-L-alanine ($H_3$L-ala–L-ala–L-ala) was prepared by a combination of conventional techniques, starting with *N*-benzyloxycarbonyl-L-alanine (Z-L-ala)[30b] and L-alanine ethyl ester hydrochloride (L-ala–OEt·HCl).[30c] Dicyclohexylcarbodiimide (DCC) coupling was used to form a dipeptide derivative from these precursors.[30d] The dipeptide ethyl ester was formed by the reaction of Z-L-ala–L-ala–OEt with HBr–acetic acid (25%). It was then coupled with another equivalent of Z-L-ala, again using DCC. The tripeptide hydrobromide was formed by the reaction of Z-L-ala–L-ala–L-ala–OEt with an aqueous NaOH solution and then with HBr–acetic acid. The free peptide was obtained by the action of aqueous $NH_3$ on the tripeptide hydrobromide and was then recrystallized from the EtOH–water system.

Other tripeptides, L-alanyl-L-alanylglycine ($H_3$L-ala–L-ala–gly), L-alanylglycyl-L-alanine ($H_3$L-ala–gly–L-ala), glycyl-L-(alanyl-L-alanine ($H_3$gly–L-ala–L-ala), L-alanylglycylglycine ($H_3$L-ala–gly–gly), glycyl-L-alanylglycine ($H_3$gly–L-ala–gly), glycylglycyl-L-alanine ($H_3$gly–gly–L-ala), β-alanylglycylglycine ($H_3$β-ala–gly–gly), glycyl-β-alanylglycine ($H_3$gly–β-ala–gly), and glycylglycyl-β-alanine ($H_3$gly–gly–β-ala), were also prepared by the same procedure as that used for $H_3$L-ala–L-ala–L-ala.

The same DCC coupling method was used for the syntheses of tetrapeptides. In the case of glycyl-L-alanylglycylglycine ($H_4$gly–L-ala–gly–gly), the tetrapeptide derivative, Z–gly–L-ala–gly–gly–OEt, was prepared by the action of Z–gly with L-ala–gly–gly–OEt, but for the other tetrapeptides, L-alanylglycylglycylglycine ($H_4$L-ala–gly–gly–gly), glycylglycyl-L-alanylglycine ($H_4$gly–gly–L-ala–gly), glycylglycylglycyl-L-alanine ($H_4$gly–gly–gly–L-ala), β-alanylglycylglycylglycine ($H_4$β-ala–gly–gly–gly), glycyl-β-alanylglycylglycine ($H_4$gly–β-ala–gly–gly), glycylglycyl-β-alanylglycine ($H_4$gly–gly–β-ala–gly), and glycylglycylglycyl-β-alanine ($H_4$gly–gly–gly–β-ala), the corresponding peptide derivatives were prepared by coupling Z–dipeptide with dipeptide–OEt.

*Preparations of Complexes.* [*Co(tripeptidato)*$(NH_3)_2$]·x$H_2O$: This type of complex was prepared by two different methods.

*(1)*: A solution of tripeptide (5 mmol) in aqueous 1 M† NaOH (5 cm$^3$) was added to a DMSO solution (50 cm$^3$) containing[Co$(NH_3)_5(H_2O)$]$(ClO_4)_3\cdot H_2O$[31] (2.48 g, 5 mmol); the mixed solution was heated at *ca.* 50 °C with constant stirring. Ammonia was evolved, and after *ca.* 1 h brown precipitates started to separated out. After additional heating for a few hours, the reaction mixture was allowed to stand in a refrigerator overnight. The brown precipitates obtained by filtration were dissolved again in a minimum amount of water, and then the aqueous solution was chromatographed on a Sephadex G-10 column (3.5 cm × 80 cm), with water as the eluant, giving three bands. The first (red-brown) and third (pink) descended bands were rejected. The desired complex was isolated as crystalline powder from the eluate of the second band on the addition of a large amount of acetone; it was then dried over $CaCl_2$. The diammine(tripeptidato)cobalt(III) complexes with the following peptides: $H_3$gly–gly–gly, $H_3$L-

† 1 M = 1 mol dm$^{-3}$.

Table 1. Analytical data of the complexes

| Complex | C(%) Found (Calcd) | H(%) Found (Calcd) | N(%) Found (Calcd) |
|---|---|---|---|
| $[Co(gly\text{-}gly\text{-}gly)(NH_3)_2]\cdot 3H_2O$ | 21.02(21.63) | 5.77(6.05) | 21.02(21.02) |
| $[Co(L\text{-}ala\text{-}gly\text{-}gly)(NH_3)_2]\cdot 2H_2O$ | 25.35(25.54) | 5.93(6.12) | 21.54(21.27) |
| $[Co(gly\text{-}L\text{-}ala\text{-}gly)(NH_3)_2]\cdot 2H_2O$ | 26.00(25.54) | 5.85(6.12) | 20.91(21.27) |
| $[Co(gly\text{-}gly\text{-}L\text{-}ala)(NH_3)_2]\cdot 2H_2O$ | 25.77(25.54) | 5.66(6.12) | 20.82(21.27) |
| $[Co(\beta\text{-}ala\text{-}gly\text{-}gly)(NH_3)_2]\cdot 0.5H_2O$ | 27.78(27.82) | 5.47(5.67) | 23.20(23.18) |
| $[Co(gly\text{-}\beta\text{-}ala\text{-}gly)(NH_3)_2]\cdot H_2O$ | 26.87(27.02) | 5.81(5.83) | 21.91(22.50) |
| $[Co(gly\text{-}gly\text{-}\beta\text{-}ala)(NH_3)_2]\cdot 2.5H_2O$ | 24.71(24.86) | 6.14(6.26) | 20.58(20.07) |
| $[Co(L\text{-}ala\text{-}L\text{-}ala\text{-}gly)(NH_3)_2]\cdot 1.5H_2O$ | 28.52(28.75) | 6.47(6.33) | 21.16(20.95) |
| $[Co(L\text{-}ala\text{-}gly\text{-}L\text{-}ala)(NH_3)_2]\cdot 3H_2O$ | 26.62(26.60) | 6.41(6.70) | 19.20(19.39) |
| $[Co(gly\text{-}L\text{-}ala\text{-}L\text{-}ala)(NH_3)_2]\cdot 3H_2O$ | 26.77(26.60) | 6.31(6.70) | 19.44(19.39) |
| $[Co(L\text{-}ala\text{-}L\text{-}ala\text{-}L\text{-}ala)(NH_3)_2]\cdot H_2O$ | 31.78(31.86) | 6.54(6.54) | 20.33(20.64) |
| $NH_4[Co(gly\text{-}gly\text{-}gly\text{-}gly)(NH_3)_2]\cdot 3H_2O$ | 23.72(23.59) | 6.14(6.43) | 23.45(24.07) |
| $NH_4[Co(L\text{-}ala\text{-}gly\text{-}gly\text{-}gly)(NH_3)_2]\cdot 3H_2O$ | 25.57(25.65) | 6.63(6.70) | 23.45(23.07) |
| $NH_4[Co(gly\text{-}L\text{-}ala\text{-}gly\text{-}gly)(NH_3)_2]\cdot 1.5H_2O$ | 27.39(27.42) | 6.36(6.39) | 24.99(24.87) |
| $NH_4[Co(gly\text{-}gly\text{-}gly\text{-}L\text{-}ala)(NH_3)_2]\cdot 2.5H_2O$ | 26.02(26.22) | 6.52(6.60) | 23.42(23.78) |
| $NH_4[Co(\beta\text{-}ala\text{-}gly\text{-}gly\text{-}gly)(NH_3)_2]\cdot 3.5H_2O$ | 25.62(25.12) | 6.29(6.79) | 22.28(22.79) |
| $NH_4[Co(gly\text{-}\beta\text{-}ala\text{-}gly\text{-}gly)(NH_3)_2]\cdot 3.5H_2O$ | 25.78(25.12) | 6.61(6.79) | 22.53(22.79) |

ala–gly–gly, $H_3$gly–L-ala–gly, $H_3$gly–gly–L-ala, $H_3\beta$-ala–gly–gly, $H_3$gly–$\beta$-ala–gly, and $H_3$gly–gly–$\beta$-ala, were prepared by this method.

*(2)*: In the present paper, a series of diammine(tripeptidato)cobalt(III) complexes were also prepared by modifying the method described in the literature.[22]

Tripeptide (3.4 mmol) was dissolved in 10 $cm^3$ of water, and the solution was adjusted to pH 9.0—9.5 with a 28% $NH_3$ solution. A fresh peroxo-dimer, $[(NH_3)_5CoO_2Co(NH_3)_5](NO_3)_4\cdot 2H_2O$, was added to the above peptide solution chilled in an ice bath with constant stirring. After stirring for *ca.* 2 h, the solution was allowed tostand in a refrigerator for one day. The resulting solution was filtered, and the red-brown filtrate was chromatographed on a Sephadex G-10 column (3.5 cm × 80 cm). The adsorbed band was separated into brown (main) and pink (a little) bands by elution with water. To the eluate from the brown band was added a large amount of acetone. The desired complex was then deposited by allowing the solution to stand overnight at room temperature; it was filtered, washed with acetone, and then dried over $CaCl_2$. This preparative method was used for all of the [Co(tripeptidato)$(NH_3)_2$] complexes presented here.

*$NH_4$[Co(tetrapeptidato)$(NH_3)_2]\cdot xH_2O$*: The preparation of this type of complex was carried out by the same method as (2) described above, except for the addition of a small amount of aqueous $NH_3$ (28%) prior to adding acetone. As the reaction of tetrapeptide with a peroxo-dimer was slower than that of tripeptide, the reaction solution was allowed to stand for two day in a refrigerator. Standing for more than two days gave a larger amount of another red-brown by-product (probably an aqua complex), which was eluted faster than the desired complex on a Sephadex G-10 column. The gly–gly–L-ala–gly, gly–gly–$\beta$-ala–gly, and gly–gly–gly–$\beta$-ala complexes were rechromatographed on a Bio-Gel P2 column (3.5 cm × 30 cm), with water as an eluant to ensure the purity. Despite such a procedure, these complexes separated out as syrup products for which the analytical data were unsatisfactory.

The analytical data for the [Co(tripeptidato)$(NH_3)_2$] and $NH_4$[Co(tetrapeptidato)$(NH_3)_2$] complexes are listed in Table 1.

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

## Results and Discussion

Although the reaction of tripeptide with an equimolar quantity of pentaammineaquacobalt(III) complex in DMSO yielded the desired product, the same reaction in water gave unsatisfactory results; many bands appeared when the reactant solution was chromatographed on a Sephadex G-10 column. As the [Co(tripeptidato)$(NH_3)_2$] type of complex is more labile in water than other ammine complexes such as [Co(dipeptidato)$(NH_3)_3]^+$, presumably the complicated consecutive reactions must occur in water. On the other hand, the [Co(tripeptidato)$(NH_3)_2$] complex is only slightly soluble in DMSO, being protected from the consecutive reactions. With $H_3$gly–gly–gly, $H_3$L-ala–gly–gly, $H_3$gly–L-ala–gly, $H_3$gly–gly–L-ala, $H_3\beta$-ala–gly–gly, $H_3$gly–$\beta$-ala–gly, and $H_3$gly–gly–$\beta$-ala, the preparative method in DMSO (Method (1)) was applied; however, the yields of the complexes depended on the sort of peptides: in the cases of the gly–gly–L-ala and gly–gly–$\beta$-ala complexes, the yields were below half of that for the gly–gly–gly complex. The application of this method to the preparation of the $NH_4$[Co(tetrapeptidato)$(NH_3)_2$] type of complex was unsuccessful.

The preparative method using the peroxo-dimer (Method (2)) gave constantly high yields of the desired products for all the tripeptides used here. When the reactant solution was chromatographed on a Sephadex G-10 column, two completely separated bands were usually observed, but in some cases, *e.g.*, in the [Co(gly–gly–$\beta$-ala)$(NH_2)_2$] complex, another red-brown band, which is presumably an aqua complex, was eluted earlier than the desired band. As the [Co(tripeptidato)$(NH_3)_2$] type of complex is relatively labile in water, as will be described below, rechromatography to ensure purity causes an aquation reaction and brings about a low yield, even though aqueous ammonia was used as an

TABLE 2. $^1$H NMR DATA OF THE TYPE [Co(tripeptidato)$(NH_3)_2$] COMPLEXES IN SLIGHTLY BASIC $D_2O$

| Tripeptidato | C-terminal (ppm) $-CH_2-$ $>CH-$ $-CH_2-CH_2-$ | Central (ppm) $-CH_2-$ $>CH-$ $-CH_2-CH_2-$ | N-terminal (ppm) $-CH_2-$ $>CH-$ $-CH_2-CH_2-$ | $-CH_3$ (ppm) C-terminal central N-terminal |
|---|---|---|---|---|
| gly-gly-gly | 4.18(s) | 3.93(s) | 3.72(s) | |
| L-ala-gly-gly | 4.11(s) | 3.88(s) | 3.75(q,$J$=7.0) | 1.45(d,$J$=7.0) |
| gly-L-ala-gly | 4.08(s) | 4.00(q,$J$=7.0) | 3.64(s) | 1.37(d,$J$=7.0) |
| gly-gly-L-ala | 4.30(q,$J$=7.0) | 3.87(s) | 3.62(s) | 1.56(d,$J$=7.0) |
| $\beta$-ala-gly-gly | 4.24(s) | 3.90(s) | 3.10[a],2.52[a] | |
| gly-$\beta$-ala-gly | 4.06(s) | 3.18[a],2.47[a] | 3.56(s) | |
| gly-gly-$\beta$-ala | 3.52[a],2.52[a] | 3.74(s) | 3.63(s) | |
| L-ala-L-ala-gly | 4.08(s) | 4.06(q,$J$=7.0) | 3.76(q,$J$=7.0) | 1.36(d,$J$=7.0)<br>1.46(d,$J$=7.0) |
| L-ala-gly-L-ala | 4.29(q,$J$=8.0) | 3.90(s,br) | 3.71(q,$J$=8.0) | 1.57(d,$J$=8.0)<br>1.45(d,$J$=8.0) |
| gly-L-ala-L-ala | 4.26(q,$J$=7.0) | 3.99(q,$J$=7.0) | 3.67,3.55 (AB,$J$=17.0) | 1.55(d,$J$=7.0)<br>1.40(d,$J$=7.0) |
| L-ala-L-ala-L-ala | 4.28(q,$J$=7.0) | 4.04(q,$J$=7.0) | 3.68(q,$J$=7.0) | 1.58(d,$J$=7.0)<br>1.40(d,$J$=7.0)<br>1.47(d,$J$=7.0) |

AB: AB pattern resonance, s: singlet, d: doublet, q: quartet, br: slightly broad. a) Observed as a triplet-like broad peak.

eluant. The major disadvantage of this method is that the yield of the desired complex significantly depends on the purity of the peroxo-dimer used as a starting material.

The reaction of the peroxo-dimer with $H_4$gly-gly-gly-gly afforded a high yield of the desired complex, $NH_4$[Co(gly-gly-gly-gly)$(NH_3)_2$], as needle crystals. The same reactions with $H_4$gly-L-ala-gly-gly, $H_4$gly-gly-gly-L-ala, $H_4\beta$-ala-gly-gly-gly, and $H_4$gly-$\beta$-ala-gly-gly also produced considerable quantities of the desired complexes, which were liable to separate out as syrup products upon the addition of acetone to the eluates from Sephadex G-10 columns. Fortunately, we could isolate these complexes as crystalline products by allowing the acetonic suspensions to stand at room temperature for several days. In the cases of $H_4$L-ala-gly-gly-gly, $H_4$gly-gly-L-ala-gly, $H_4$gly-gly-$\beta$-ala-gly, and $H_4$gly-gly-gly-$\beta$-ala, the reaction of these peptides with the peroxo-dimer gave a large amount of by-products. The precipitation of the diammine(tetrapeptidato)cobaltate(III) complex by adding acetone under weak acidic conditions (pH 3—4) gave the protonated complex, H[Co(tetrapeptidato)$(NH_3)_2$]·$x$H$_2$O.

*$^1$H NMR.* Evans *et al.* prepared seven kinds of the [Co(tripeptidato)$(NH_3)_2$] type of complexes in which the peptides coordinate through the terminal $NH_2$, two amide N, and terminal $CO_2^-$ groups and reported $^1$H and $^{13}$C NMR data in $D_2O$ for the complexes.[22] Figure 1 shows a comparison among the $^1$H NMR spectra of [Co(gly-gly-gly)$(NH_3)_2$], [Co(L-ala-gly-gly)$(NH_3)_2$], [Co(gly-L-ala-gly)$(NH_3)_2$], and [Co(gly-gly-L-ala)$(NH_3)_2$] complexes in slightly basic $D_2O$. The spectrum of [Co(gly-gly-gly)$(NH_3)_2$] shows three singlet peaks due to three $CH_2$ groups at 3.72, 3.93, and 4.18 ppm, suggesting that the chelate rings of the peptide are coplanar. The singlet peaks at 3.88 and

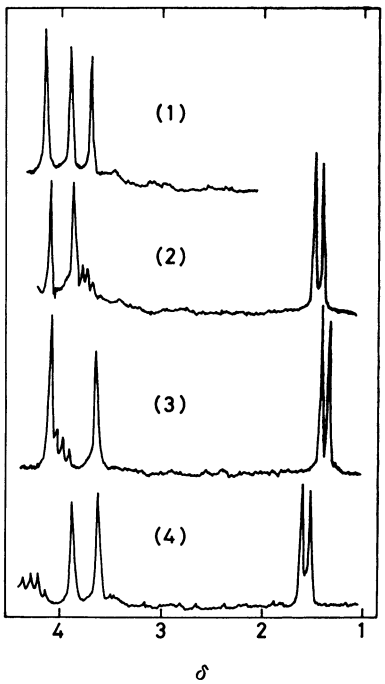


Fig. 1. The $^1$H NMR spectra of [Co(gly-gly-gly)$(NH_3)_2$] (1), [Co(L-ala-gly-gly)$(NH_3)_2$] (2), [Co(gly-L-ala-gly)$(NH_3)_2$] (3), and [Co(gly-gly-L-ala)$(NH_3)_2$] (4) in slightly basic $D_2O$.

4.11 ppm in the L-ala-gly-gly complex correspond to those at 3.93 and 4.18 ppm respectively in the gly-gly-gly complex. Therefore, these peaks can be assigned to either the C-terminal or central $CH_2$ of the tripeptide ligand. Similar relationships are also found between the gly-gly-gly complex and the gly-L-ala-gly or gly-gly-L-ala complex (Fig. 1 and Table 2). These results indicate that the N- and C-terminal $CH_2$ of the tripeptide

ligand resonate at the highest and lowest magnetic fields respectively; thus we are able to assign each peak. The same relationship is also realized for the complexes of the tripeptides containing the glycine and/or β-alanine residue (Table 2). Each spectrum of the β-ala–gly–gly, gly–β-ala–gly, and gly–gly–β-ala complexes exhibited two broad triplet-like peaks due to the $-CH_2-CH_2-$ group. The same pattern of the signals has been observed in the complexes of dipeptides containing the β-alanine residue.[28,29] An examination of the molecular models indicates that the tripeptides containing the β-alanine residue can coordinate to cobalt(III) in a noncoplanar configuration (*cis-α*); however, the possibility of the noncoplanar form can be rejected on the basis of the fact that the signals due to the $CH_2$ groups of glycine residues are singlets in these complexes. For the complexes of the tripeptides containing two or three L-alanine residues, the methine signals can be assigned on the basis of a comparison of the chemical shifts; in these complexes, the N- and C-terminal >CH– of the peptide ligands resonate at the highest and lowest magnetic fields respectively. The regular trend in chemical shifts is also observed for the $CH_3$ signals of the tripeptidato complexes containing the L-alanine residue, making it possible to assign those signals: the signals at 1.36—1.40, 1.45—1.47, and 1.55—1.58 ppm are assigned to central, N-terminal, and C-terminal $CH_3$ respectively. The $^1H$ NMR data of the [Co(tripeptidato)$(NH_3)_2$] complexes are also listed in Table 2.

In the present study, the [Co(gly–L-ala–L-ala)$(NH_3)_2$] complex is the only example in which the $CH_2$ protons are inequivalent at 100 MHz. The $CH_2$ signal of the L-ala–gly–L-ala complex is also a slightly broad singlet at 100 MHz, indicating a tendency toward splitting.

*Absorption Spectra.* Figure 2 shows the absorption spectra of the [Co(gly–gly–gly)$(NH_3)_2$] and [Co(gly–β-ala–gly)$(NH_3)_2$] complexes. The former complex

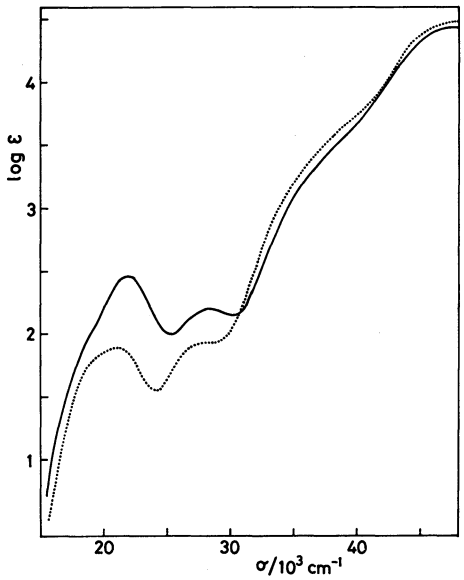


Fig. 2. The absorption spectra of [Co(gly–gly–gly)$(NH_3)_2$] (——) and [Co(gly–β-ala–gly)$(NH_3)_2$] (------) in water.

exhibits the characteristic spectrum for the complex containing chelated peptide: the first band at 21880 $cm^{-1}$ has an enhanced intensity ($\varepsilon$=292 $dm^3 \cdot mol^{-1} \cdot cm^{-1}$) in contrast with the second band. The ratio of the absorption coefficients for the first and second bands ($\varepsilon_1/\varepsilon_{11}$) of [Co(gly–gly–gly)$(NH_3)_2$] (2.14) is higher than that for [Co(gly–gly)$(NH_3)_3]^+$ (1.8),[32] but lower than that for $[Co(gly–gly)_2]^-$ (3.98).[29] A split component in the first band region for the [Co(gly–gly–gly)$(NH_3)_2$] complex is observed as a shoulder band at *ca.* 18200 $cm^{-1}$. The absorption spectra of other diammine(tripeptidato)cobalt(III) complexes, where the tripeptides are composed of only α-amino acid residues, are

Table 3. Absorption and CD spectra of the [Co(tripeptidato)$(NH_3)_2$] and [Co(tetrapeptidato)$(NH_3)_2$]-types of complexes in the d-d transition region measured in water

| Tripeptidato or tetrapeptidato | $\sigma_{max}/10^3$ $cm^{-1}$(log ε) | | $\sigma_{max}/10^3$ $cm^{-1}$(Δε) | | |
|---|---|---|---|---|---|
| | 1st band | 2nd band | 1st-band region | 2nd-band region | |
| gly–gly–gly | 21.88 (2.47) | 28.32 (2.14) | | | |
| L-ala–gly–gly | 22.05 (2.42) | 28.46 (2.20) | 18.87 (−0.47) | 22.37 (+0.04) | 28.57 (−0.28) |
| gly–L-ala–gly | 21.93 (2.41) | 28.01 (2.12) | 19.34 (−1.28) | | 27.91 (−0.31) |
| gly–gly–L-ala | 22.00 (2.51) | 28.02 (2.19) | 20.12 (−1.23) | 26.21 (+0.04) | 28.33 (−0.08) |
| L-ala–L-ala–gly | 22.15 (2.48) | 28.34 (2.24) | 19.16 (−1.78) | | 28.17 (−0.49) |
| L-ala–gly–L-ala | 22.17 (2.50) | 28.01 (2.22) | 19.42 (−1.59) | | 28.33 (−0.30) |
| gly–L-ala–L-ala | 22.10 (2.50) | 27.93 (2.19) | 19.57 (−2.42) | | 28.17 (−0.35) |
| L-ala–L-ala–L-ala | 22 25 (2.52) | 27.93 (2.21) | 19.31 (−2.52) | | 28.33 (−0.46) |
| β-ala–gly–gly | 21.65 (2 28) | 28.60sh(*ca.*2.16)[a] | | | |
| gly–β-ala–gly | 21.16 (1 89) | 28.40sh(*ca.*1.94)[a] | | | |
| gly–gly–β-ala | 21.61 (2 37) | 28.13 (2.11) | | | |
| gly–gly–gly–gly | 21.55 (2 24) | 28 82 (2 07) | | | |
| L-ala–gly–gly–gly | 21.74 (2 24) | 28.90 (2.14) | 19.92 (−0 59) | 22.42 (+0.18) | 28.82 (−0.32) |
| gly–L-ala–gly–gly | 21 65 (2 23) | 28.74 (2 06) | 19.84 (−2.01) | | 28.33 (−0.51) |
| gly–gly–gly–L-ala | 21.55 (2 22) | 29 24 (2 13) | 18.69 (−0.04) | 22.32 (+0.20) | *ca.*27.78sh(+0.04)[a] |
| β-ala–gly–gly–gly | 22 22 (2 20) | *ca.*28 57sh[a] | | | |
| gly–β-ala–glygly | 19 92 (1 86) | *ca.*28 00sh[a] | | | |

a) sh: Shoulder.

quite similar to that of the [Co(gly–gly–gly)$(NH_3)_2$]; the spectral data are listed in Table 3. The [Co(β-ala–gly–gly)$(NH_3)_2$] and [Co(gly–gly–β-ala)$(NH_3)_2$] complexes also showed spectral patterns almost identical with that of the gly–gly–gly complex, indicating that the spectra are little affected by the exchanges of the $CH_2$ group with the $-CH_2-CH_2-$ group in the N- and C-terminal fragments of tripeptides (Table 3). As is shown in Fig. 2, however, the [Co(gly-β–ala–gly)-$(NH_3)_2$] complex shows remarkably different absorption behavior in the d-d transition region, compared with that of other [Co(tripeptidato)$(NH_3)_2$] complexes: The first band is broad (showing splitting) and has a relatively weak intensity ($\varepsilon=77$ dm$^3$ mol$^{-1}$ cm$^{-1}$).

In our previous works,[28,29] we reported that the complexes with dipeptides containing the N-terminal β-alanine residue were different in the spectral behavior of the d-d transition from the complexes with dipeptides containing the N-terminal α-amino acid residue. In the present case, the gly–β-ala–gly complex differs from the other tripeptidato complexes in spectral behavior. Such a difference in spectral behavior may arise from the differences in electronic delocalization around the peptide backbone and in the ring strain of the chelated peptide. The electronic delocalization and the ring strain must be affected by the position of the β-alanine residue in the peptide chain, even though simple molecular models provide no obvious reasons for this.

The first band maxima of the complexes with tripeptides containing the β-alanine residue shift to the lower-energy side compared with that of the gly–gly–gly complex. Such a blue shift is contrary to the red shifts observed for the copper(II) complexes of di- and tripeptides containing the β-alanine residue[33,34] and also for the cobalt(III) complexes of dipeptides containing the β-alanine residue.[29]

*CD Spectra.* In the case of the tetragonal coordination of tripeptides containing only α-amino acid residues to cobalt(III), the chelate rings of the peptide ligand are expected to take a coplanar form, ruling out any configurational and conformational contributions to optical activity. Therefore, the sole source of optical activity in the d-d transition region is the vicinal effect due to the asymmetric carbons.[35] As is shown in Fig. 3, the [Co(tripeptidato)$(NH_3)_2$] complexes exhibit two major negative CD bands associated with two d-d transitions in the visible region, while additional very weak positive CD components are observed at 22370 and 26210 cm$^{-1}$ for the L-ala–gly–gly and gly–gly–L-ala complexes respectively. The numerical CD data in the d-d transition region are summarized in Table 3.

For the [Co(gly–L-ala–gly)$(NH_3)_2$] and [Co(gly–gly–L-ala)$(NH_3)_2$] complexes, the magnitudes of $\Delta\varepsilon_{ext}$ in the first band region are nearly comparable to each other, but more than twice that for the [Co(L-ala–gly–gly)$(NH_3)_2$] complex. This result is consistent with those for [Co(*N*-methyliminodiacetato)(dipeptidato)]$^-$ complexes, in which the CD contribution from the C-terminal active source is larger than that from the N-terminal one.[28] The tripeptidato complexes containing two or three asymmetric carbons show enhanced negative CD bands associated with the first d-d bands

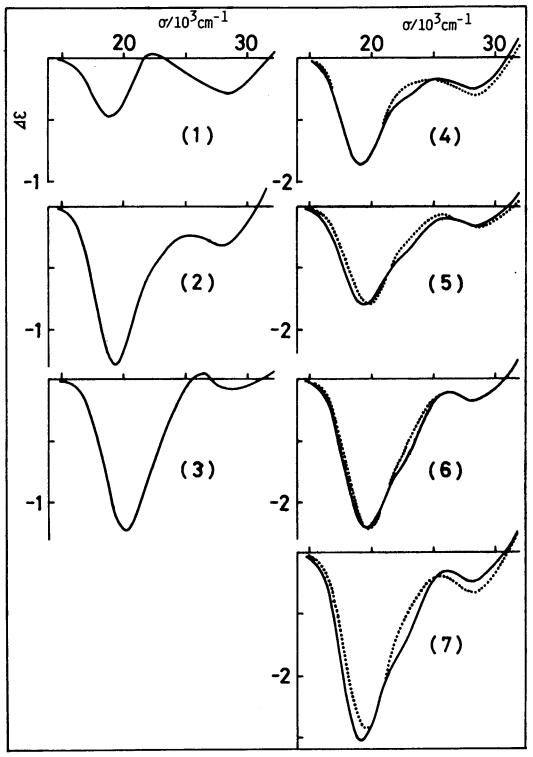


Fig. 3. The CD spectra of the type [Co(tripeptidato)-$(NH_3)_2$] in water (——). The dotted lines represent the CD curves calculated from the CD data of two (or three) tripeptidato complexes containing only one asymmetric carbon.
Tripeptidato: (1) L-ala–gly–gly, (2) gly–L-ala–gly, (3) gly–gly–L-ala, (4) L-ala–L-ala–gly, (5) L-ala–gly–L-ala, (6) gly–L-ala–L-ala, (7) L-ala–L-ala–L-ala.

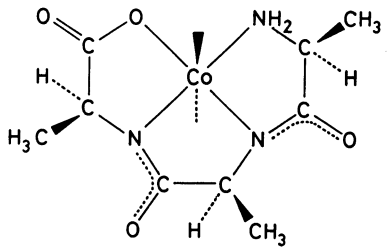


Fig. 4. The structure of [Co(L-ala–L-ala–L-ala)$(NH_3)_2$] drawn from the direction of the $NH_3$–Co–$NH_3$ axis.

(Fig. 3 and Table 3).

The dotted lines in Fig. 3 represent the CD curves calculated from the CD data of two (or three) tripeptidato complexes containing only one asymmetric carbon; *e.g.*, in the case of the L-ala–L-ala–gly complex, the dotted line represents the sum of the two CD curves observed for the L-ala–gly–gly and gly–L-ala–gly complexes. In each case, the good coincidence obtained between the observed (solid line) and calculated (dotted line) CD curves demonstrates the additivity of the vicinal contribution. Such direct comparison may be valid for the present series of complexes, because the spectral behaviors of these complexes are quite similar to each other in the d-d transition region.

Figure 4 shows the structure of the [Co(L-ala–L-ala–L-ala)$(NH_3)_2$] complex drawn from the direction of the

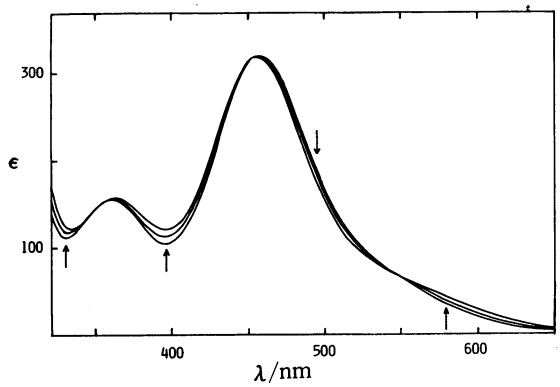


Fig. 5. Change of absorption spectrum with time for [Co(gly–gly–L-ala)$(NH_3)_2$] in water at 30.1 °C. The curves show the spectra measured at 1, 21, and 61 min, respectively.

$NH_3$–Co–$NH_3$ axis, in which two $NH_3$ are omitted and in which all methyl groups of the side chain are above the chelate plane. The octant rule has been proposed to account for the results of the CD signs in the visible absorption region of transition metal complexes;[36] however, the octant rule can not account for the additivity of the CD spectra of the tripeptidato complexes. Whenever the nodal surfaces are drawn, the octant rule predicts that the gly–L-ala–gly complex should exhibit signs opposite to those of the L-ala–gly–gly and gly–gly–L-ala complexes. The same situation has also been realized in the Cu(II) and Ni(II) complexes. The double octant rule has been proposed to account for the CD signs of those complexes in the d-d transition region.[2] The double octant rule is actually valid in the diammine(tripeptidato)cobalt(III) complexes.

*Chemical Properties.* The absorption and CD spectra of the diammine(tripeptidato)cobalt(III) complexes in an aqueous solution changed with the lapse of time. Figure 5 shows the time-course of the absorption curve for the gly–gly–L-ala complex in water at 30.1 °C. Over an initial period of *ca.* 1 h, the isosbestic points were observed, but not from that time on, indicating that the next reaction occurred. The same situation was also realized for other tripeptidato complexes: The changes in spectra for the complexes of tripeptides containing glycine and/or L-alanine residues, especially the L-ala–L-ala–L-ala and L-ala–L-ala–gly complexes, were fast; however, those for the tripeptidato complexes containing the β-alanine residue were slow and almost negligible over a period of one hour after dissolving. The spectral changes were accelerated in the acidic and basic aqueous solutions (even in an $NH_3$ aqueous solution); however, in the 1 M $NH_4Cl$ aqueous solution with its pH of the solution adjusted at 7 with $NH_3$, the spectral change was not observable for *ca.* 2 h. For the complex of tripeptide containing the L-alanine residue, the CD spectral change was the same as the absorption spectral change. These results suggest that the following reaction occurs initially:

$$[\text{Co(tripeptidato)}(NH_3)_2] + H_2O \longrightarrow [\text{Co(tripeptidato)}(NH_3)(H_2O)] + NH_3.$$

*Tetrapeptidato Complexes.* On the basis of the examination of molecular models and the fact that the $^1H$ NMR spectrum of the [Co(gly–gly–gly–gly)$(NH_3)_2$]$^-$ complex in a weak alkaline $D_2O$ shows four singlet peaks due to four $CH_2$ groups of the gly–gly–gly–gly ligand at 3.52, 3.72, 3.86, and 3.90 ppm, it can be concluded that the most probable structure of [Co(gly–gly–gly–gly)$(NH_3)_2$]$^-$ is the *trans*($NH_3$) form, in which the tetraglycine coordinates to cobalt(III) as a tetragonal quadridentate chelate. The other tetrapeptidato complexes prepared in this study also showed singlet peaks due to the $CH_2$ of the glycine-rings, indicating the same structure as that of the gly–gly–gly–gly complex.

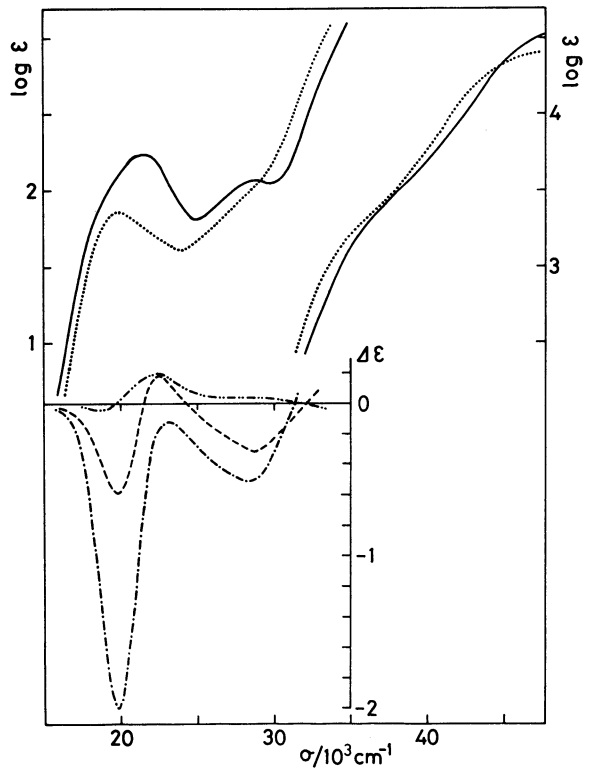


Fig. 6. The absorption spectra of [Co(gly–gly–gly–gly)$(NH_3)_2$]$^-$ (——) and [Co(gly–β-ala–gly–gly)$(NH_3)_2$]$^-$ (·····) in water, and the CD spectra of [Co(L-ala–gly–gly–gly)$(NH_3)_2$]$^-$ (------), [Co(gly–L-ala–gly–gly)$(NH_3)_2$]$^-$ (·–·–·), and [Co(gly–gly–gly–L-ala)$(NH_3)_2$]$^-$ (–···–) in water.

As is shown in Fig. 6 and Table 3, the absorption spectrum of the diammine(tetraglycinato)cobaltate(III) complex exhibits the first and second bands at 21550 and 28820 $cm^{-1}$ respectively. The first band has an enhanced intensity ($\varepsilon$=175 $dm^3\,mol^{-1}\,cm^{-1}$), following a tendency toward splitting (shoulder band at *ca.* 19000 $cm^{-1}$). This spectral behavior is similar to that of the diammine(tripeptidato)cobalt(III) complex and supports the assertion that the tetraglycinato complex has the *trans*($NH_3$) structure. The absorption spectra of the other tetrapeptidato complexes are similar to that of the tetraglycinato complex. The only exception is the gly–β-ala–gly–gly complex, which shows a lower intensity of the first band ($\varepsilon$=72 $dm^3\,mol^{-1}\,cm^{-1}$), just as in the gly–β-ala–gly complex (Table 3 and Fig. 6).

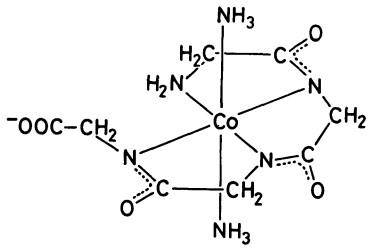

Fig. 7. The structure of $[Co(gly\text{-}gly\text{-}gly\text{-}gly)(NH_3)_2]^-$.

The CD spectra of the $[Co(L\text{-}ala\text{-}gly\text{-}gly\text{-}gly)(NH_3)_2]^-$ and $[Co(gly\text{-}L\text{-}ala\text{-}gly\text{-}gly)(NH_3)_2]^-$ complexes in the d-d transition region are similar in pattern to those of the $[Co(L\text{-}ala\text{-}gly\text{-}gly)(NH_3)_2]$ and $[Co(gly\text{-}L\text{-}ala\text{-}gly)(NH_3)_2]$ complexes respectively; however, the $[Co(gly\text{-}gly\text{-}gly\text{-}L\text{-}ala)(NH_3)_2]^-$ complex shows a remarkably different CD behavior in the d-d transition region compared with those of the L-ala-gly-gly-gly, gly-L-ala-gly-gly, and also tripeptidato complexes (Fig. 6 and Table 3). The $[Co(gly\text{-}gly\text{-}L\text{-}ala\text{-}gly)(NH_3)_2]^-$ complex, which was not isolated in the pure state, showed two negative signs of CD associated with the first and second bands. These results indicate that the tetrapeptides coordinate to cobalt(III) as quadridentate chelates through the terminal $NH_2$ and three amide N groups, as is shown in Fig. 7.

The diammine(tetrapeptidato)cobaltate(III) complexes also show the absorption and CD spectral changes in water with the same order of time-course as those of diammine(tripeptidato)cobalt(III) complexes; these changes are ascribed to an aquation reaction.

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