Metal Complexes of Peptides. V. Cobalt(III) Complexes with *N*-β-Alanyl-(*S*)-aspartate

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Three cobalt(III) complexes containing *N*-β-alanyl-(*S*)-aspartic acid (H_3L), [Co(L)(en)] (en: ethylenediamine), [Co(L)(dien)] (dien: diethylenetriamine), and $[Co(HL)(dien)]^+$, were newly prepared and characterized on the basis of their ^{13}C NMR, UV-visible absorption, and circular dichroism spectra. The dipeptide L functions as a quadridentate ligand in [Co(L)(en)] and as terdentate ligands in [Co(L)(dien)] and $[Co(HL)(dien)]^+$. The structures of these complexes are discussed in connection with those of $[Co((S)\text{-asp})(dien)]^+$ (H_2(*S*)-asp: (*S*)-aspartic acid) and $[Co(edma)(dien)]^{2+}$ (Hedma: ethylenediamine-*N*-acetic acid).

Many reports on the preparation and characterization of the cobalt(III) complexes containing dipeptides have been published.[1—17] In most of these complexes, the coordinated dipeptides function as terdentates in a meridional form with respect to the peptide backbones. In the previous paper,[1,2] we reported the novel coordination mode of dipeptide in $[Co(Hcar)(en)(OH_2)]^{2+}$ (H_2car: $H_2\beta$-ala-(*S*)-his, carnosine, or *N*-β-alanyl-(*S*)-histidine; en: ethylenediamine), where the carnosine coordinates to cobalt(III) as a facial terdentate with an uncoordinated amino group. This complex was prepared from $[Co(CO_3)_2(en)]^-$ and H_2car in an acidic aqueous solution. On the other hand, $[Co(car)(en)]^+$ complex containing a quadridentate dipeptide was prepared from the same materials in a basic aqueous solution.[1]

Replacement of the imidazolyl group in carnosine with a carboxyl group gives *N*-β-alanyl-(*S*)-aspartic acid (H_3L or $H_3\beta$-ala-(*S*)-asp), and it is expected that the latter exhibits similar coordination behavior to that of the former. In the present paper, the preparations of some cobalt(III) complexes with the ligand L will be described, and the coordination modes of the peptide will be discussed in detail on the basis of spectroscopic data of the complexes.

Experimental

Preparation of the Complexes. [Co(L)(en)] (Abbreviation: AA1): $K[Co(CO_3)_2(en)]\cdot H_2O$[18] (1.3 g) was dissolved in 30 cm^3 of water, and the pH of the solution was adjusted to 3 with 1 M ($M = mol\,dm^{-3}$) $HClO_4$. After the solution was stirred at room temperature for about 30 min, a solution containing 1.0 g of H_3L in 20 cm^3 of water was added to the solution. The pH of the combined solution was adjusted to 9 with 1 M NaOH, and then the solution was stirred at 65 °C for 8 h. The resultant solution was filtered and poured on an SP-Sephadex column (K^+ form, 4.7 cm×90 cm), and the adsorbed band was developed with water. The red band separated was eluted out, and the solution was concentrated to about 10 cm^3. The concentrated solution was again chromatographed on a QAE-Sephadex column (Cl^- form, 4.7 cm×90 cm). The adsorbed band was separated into pink and red parts by recycle-development with water on the same column. The solution eluted from the red band was concentrated under reduced pressure to a few milliliters, and methanol was added to the concentrated solution to obtain a crude complex. The crude complex was recrystallized from water by addition of methanol. Found: C, 32.94; H, 5.70; N, 16.94%. Calcd for $[Co(L)(en)]\cdot 0.5H_2O = C_9H_{18}N_4O_{5.5}Co$: C, 32.84; H, 5.51; N, 17.02%.

[Co(HL)(dien)]Br (AA2): The pH of a solution containing $[CoCl_3(dien)]$ (1.0 g)[19] (dien: diethylenetriamine) and H_3L (0.83 g) in 65 cm^3 of water was adjusted to 5 with 1 M NaOH. This solution was stirred for 48 h at 60 °C, keeping the pH of the solution to about 5. The reactant solution was cooled to room temperature, filtered, and then charged on an SP-Sephadex column (K^+ form). After the column was swept with 0.5 dm^3 of water, the adsorbed band was developed with 0.2 M KCl to separate a dark-orange band. The solution eluted from dark-orange band was concentrated to small volume under reduced pressure at 35—40 °C. Methanol was added to the concentrated solution, and potassium chloride deposited was removed by filtration. The filtrate was concentrated to about 10 cm^3 and treated with a QAE-Sephadex column (Br^- form). The treated solution was concentrated to small volume. A crude complex was obtained by addition of ethanol to the concentrate, which was recrystallized from water–ethanol solution. Found: C, 27.57; H, 5.88; N, 14.43%. Calcd for $[Co(HL)(dien)]Br\cdot 2H_2O = C_{11}H_{37}N_5O_7CoBr$: C, 27.51; H, 5.67; N, 14.58%.

[Co(L)(dien)] (AA3): A solution containing H_3L (0.83 g) in 15 cm^3 of water was added to the solution of $[CoCl_3(dien)]$ (1.0 g) in 50 cm^3 of warm water. After the pH of the solution was adjusted to 9 with 1 M NaOH, the solution was stirred for 24 h at 60 °C. The reactant solution was cooled to room temperature and filtered. The filtrate was poured onto an SP-Sephadex (K^+ form) column. The orange solution which was eluted with water was concentrated to ca. 20 cm^3 and loaded on a QAE-Sephadex column. Developing with water, the adsorbed band was separated into two parts; an earlier-eluted orange band and a later-eluted red band. The solution eluted from the later-eluted band was concentrated by using a vacuum evaporator and filtered. Crude complex obtained by addition of ethanol to the filtrate was recrystallized from water–ethanol solution. Found: C, 33.17; H, 6.64; N, 17.58%. Calcd for $[Co(L)(dien)]\cdot 2H_2O = C_{11}H_{26}N_5O_7Co$: C, 33.09; H, 6.56; N, 17.54%.

Isomers of [Co((*S*)-asp)(dien)]Cl and [Co(edma)(dien)]Cl$_2$: The geometrical isomers of [Co((*S*)-asp)(dien)]Cl (H_2(*S*)-asp: (*S*)-aspartic acid) and $[Co(edma)(dien)]Cl_2$ (Hedma: ethylenediamine-*N*-acetic acid) were obtained according to the methods described in the previous papers.[20–21]

Absorption and Circular Dichroism Spectral Changes with pH. **AA1** (100 mg) was dissolved in 2 cm^3 of water, and 2 cm^3 of the solution was placed in a measuring flask (10 cm^3). A prescribed volume of 2 M HCl was added to the solution. The volume of the acidic solution was adjusted to 10 cm^3 with water. By the same procedure, nine solutions were prepared at different pH's (0.5—6.0). Absorption and CD spectra and pH of each solution were recorded at room temperature. In the same manner, the spectral changes with pH's were examined for **AA2** and **AA3**.

Measurements. The absorption and CD spectra were measured by a Hitachi 557 spectrophotometer and a JASCO J-22 spectropolarimeter, respectively. The ^{13}C NMR spectra were recorded on a Hitachi R-90H spectrometer. The chemical shifts in ^{13}C NMR were measured relative to internal dioxane (δ=67.40).

Results and Discussion

Structural Assignments of the Complexes. [Co(L)(en)] (AA1): Figure 1 shows the ^{13}C NMR shift patterns of free (uncoordinated) L and its complexes, together with those of the related compounds. The numbering of the carbons in L is also given in Fig. 1.

The carbon resonances of the free ligand L show a considerable difference in chemical shift with the

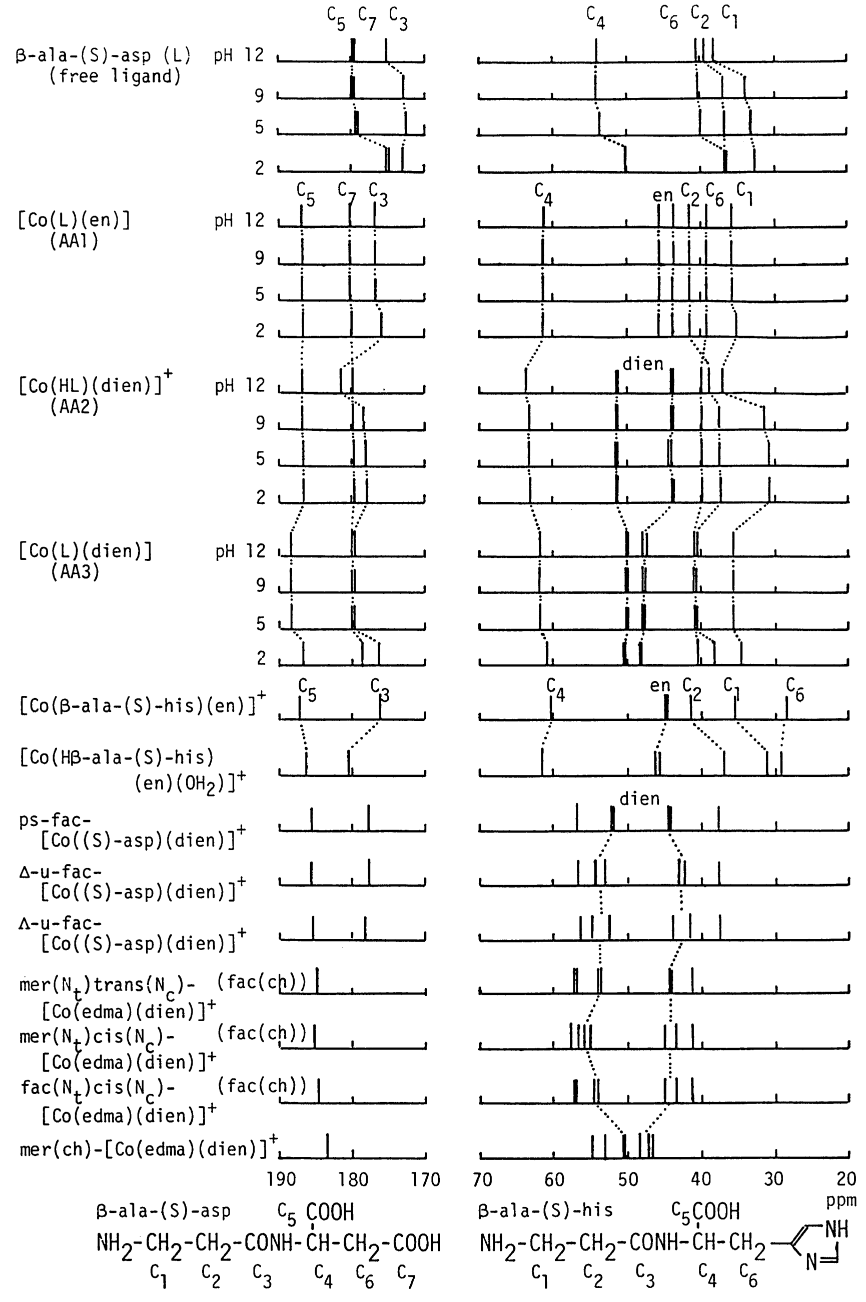
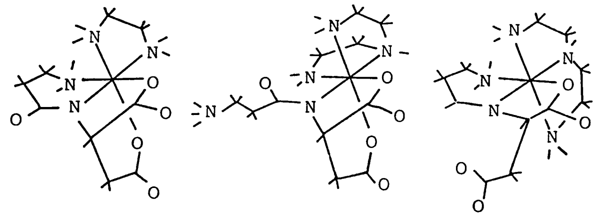


Fig. 1. ^{13}C NMR spectral patterns of β-ala-(S)-asp and the cobalt(III) complexes at various pH.

change of pH (12 to 2). The down-field shifts observed for the C_1, C_2, and C_3 carbons at pH 12 are due to the deprotonation of the terminal amino group ($-NH_3^+$). On the other hand, the up-field shifts of the C_4, C_5, C_6, and C_7 carbon resonances at pH 2 are due to the protonation to the α- and β-carboxylate anions. However, such the shift changes with pH as observed in the free ligand L are not observed in the ^{13}C shift pattern of **AA1**. This result suggests that the amino and two carboxyl groups of the peptide L in **AA1** coordinate to cobalt(III).

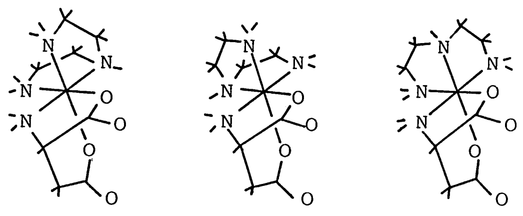
The chemical shifts of C_1, C_2, C_3, C_4, and C_5 in **AA1** are almost equal to those of the corresponding carbons of β-ala-(*S*)-his in $[Co(\beta\text{-ala-}(S)\text{-his})(en)]^+$ at pH 5, in which the β-ala-(*S*)-his coordinates to cobalt(III) as a quadridentate.[1] This result suggests that the coordination mode of L in **AA1** is similar to that of β-ala-(*S*)-his in $[Co(\beta\text{-ala-}(S)\text{-his})(en)]^+$. Accordingly, we propose that the ligand L in **AA1** coordinates to cobalt(III) in the quadridentate mode (Fig. 2(A)).

$[Co(HL)(dien)]^+$(AA2): The ^{13}C chemical shift behavior of the (*S*)-asp moiety (C_5, C_6, and C_7) in **AA2** resembles well to that in **AA1**. On the other hand, the shift behavior of the β-ala moiety (C_1 and C_2) in **AA2** is much different from that in **AA1**, but similar to that in $[Co(H\beta\text{-ala-}(S)\text{-his})(en)(OH_2)]^+$ where Hβ-ala-(*S*)-his coordinates to cobalt(III) as a facial terdentate with free $-NH_3^+$ group.[1,2] These results suggest that L in **AA2** coordinates to cobalt(III) in the coordination mode similar to that of β-ala-(*S*)-his in $[Co(H\beta\text{-ala-}(S)\text{-his})(en)(OH_2)]^+$; the ligand L coordinates with amide-N and two carboxyl-O's, and amino group is free. The down-field shifts of the β-ala moiety carbons (C_1 and C_2) at pH 12 compared with the shifts at pH 5 are due to the deprotonation from uncoordinated $-NH_3^+$.



(A) (AA1) (B) (AA2) (C) (AA3)

Fig. 2. Proposed structures of the cobalt(III) complexes containing β-ala-(*S*)-asp.



ps-fac Δ-u-fac Λ-u-fac

Fig. 3. Structures of the isomers of $[Co((S)\text{-asp})(dien)]^+$.

Although the coordination mode of the HL in **AA2** is clarified as mentioned above, three geometries are possible for the structures of **AA2** in the same manner as shown for $[Co((S)\text{-asp})(dien)]^+$ in Fig. 3. The geometrical structure of the isolated **AA2** can be assigned in comparison with its ^{13}C NMR spectrum with those of the $[Co((S)\text{-asp})(dien)]^+$ isomers; the ^{13}C NMR signals of dien in **AA2** appear in shift pattern similar to that in ps-*fac*-$[Co((S)\text{-asp})(dien)]^+$, but not in Δ-u-*fac* or Λ-u-*fac* (Fig. 3). On the basis of the above mentioned recognition, we propose the structure shown in Fig. 2 (B) for **AA2**.

Whereas the ps-*fac* type isomer of $[Co(HL)(dien)]^+$ was isolated in the preparation of **AA2**, the yields of Δ- and Λ-u-*fac* type isomers were so little that we did not succeeded to isolate them. The steric repulsion between β-ala moiety in HL and dien $-CH_2-$ may decrease the yields of the Δ- and Λ-u-*fac* type isomers.

[Co(L)(dien)] (AA3): The ^{13}C NMR signal of **AA3** shows the shift pattern characteristic to the complexes containing meridionally coordinated dien,[22,23] such as mer(ch)-$[Co(edma)(dien)]^{2+}$ (Fig. 1), and it obviously differs from those of the facial isomers of $[Co((S)\text{-asp})(dien)]^+$ and of $[Co(edma)(dien)]^{2+}$. This observation suggests that dien in **AA3** coordinates to cobalt(III) in meridional form as a terdentate, and that the ligand L occupies the remaining meridional coordination sites.

The shift behavior of the β-ala moiety (C_1 and C_2) in **AA3** is similar to those in **AA1** at pH 5—12. Such a shift behavior suggests that the $-NH_2$ group of the β-ala moiety in **AA3** coordinates to cobalt(III). In addition, the chemical shifts of β-carbon (C_6) and β-carboxyl-carbon (C_7) in the (*S*)-asp moiety are in up-field at pH 2 compared with its chemical shifts at pH 5. These up-field shifts arise from protonation on the free β-carboxyl group. From these results we propose the structure shown in Fig. 2(C) for **AA3**.

The Absorption and CD Spectral Changes with pH: The absorption and CD spectra of **AA1**, **AA2**, and **AA3** in the neutral aqueous solutions are shown in Fig. 4. All of these complexes exhibited the absorption and CD spectral changes with pH in an aqueous solution. Figure 5 shows the CD spectral change of **AA2** over the pH range 0.63 to 6.08. The iso-circular dichroism points (iso-CD points) appear at 402, 498, and 529 nm, and the isosbestic points in the absorption spectra were observed at 429 and 494 nm. The pK_a value estimated from these changes was ca. 0.7. This value indicates that the spectral changes are related to the protonation on the coordinating amide group of L.[1,4,24] Similar spectral changes were also observed for **AA1** over the pH range 1.10 to 5.67 (iso-CD point: 414 nm; isosbestic point: 432 nm; pK_a value: ca. 0.9).

AA3, which has two proton acceptable groups

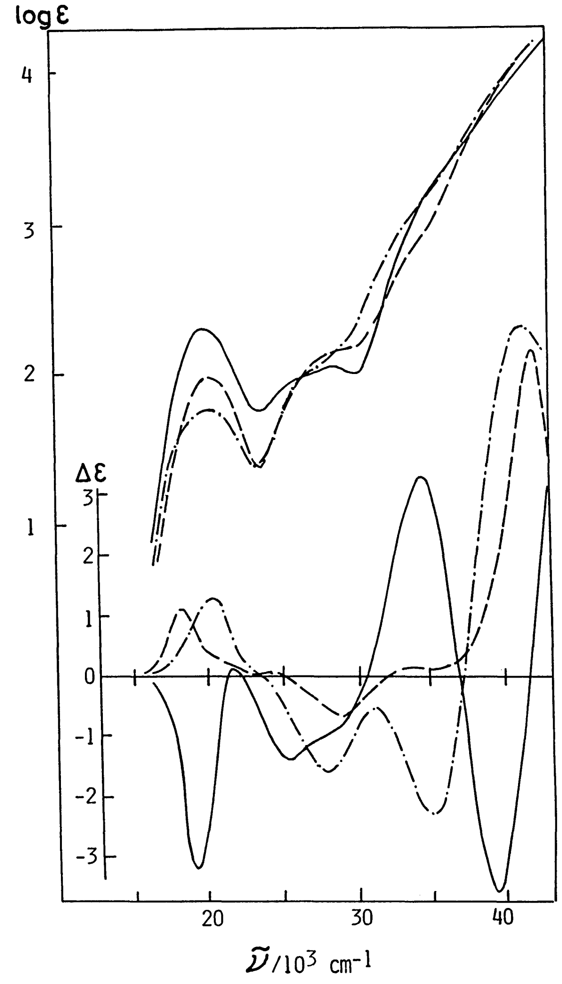


Fig. 4. Absorption and circular dichroism spectra of the complexes containing β-ala-(*S*)-asp: **AA1** (— - —), **AA2** (----), and **AA3** (——). ε and $\Delta\varepsilon$ values are given in $\mathrm{mol^{-1}\,dm^3\,cm^{-1}}$.

(amide and free β-carboxyl), exhibited more complicated CD spectral change in the pH range 6.48—0.88 compared with those of **AA1** and **AA2**. That is, there was no iso-CD point over the pH range 6.48 to 2.0. However, the pK_a value for the amide group was calculated to ca. 1.5 from the CD spectral change in the pH range 2.0—0.88, where iso-CD points appeared at 433 and 507 nm.

Comparing the pK_a values of the coordinating amide groups in **AA1**, **AA2**, and **AA3**, the following result is noteworthy; the pK_a value of **AA3**, in which the peptide backbone is flat and free from the strain caused by the coordination of the β-carboxyl group of L-asp moiety, is larger than those of **AA1** and **AA2**, in which the peptide backbones are bended and strained by the coordination of the β-carboxyl groups in (*S*)-asp moieties.

AA1 did not show obvious spectral changes with time in the pH range 6.0—1.0, but it showed slowly spectral change with time in a strong acidic (2 M HCl) solution, accompanying with lower energy shifts and intensity decreases of the first and second absorption bands. This spectral change with time may be attributed to the fission of the β-carboxyl-cobalt(III) bond.

AA2 showed slowly spectral change with time in a strong acidic (2 M HCl) solution similarly to **AA1**. On the other hand, **AA3**, in which the β-carboxyl group of L does not coordinate to cobalt(III), did not show obvious spectral change with time in 2 M HCl solution. Such a fission of the β-carboxyl-cobalt bond occurring in **AA1** and **AA2** can be attributed to the strained coordination of the β-carboxyl group with six-membered chelate ring.[25]

The pH of the Reaction Solution Controlling the Coordination Mode of Peptide: The reaction between

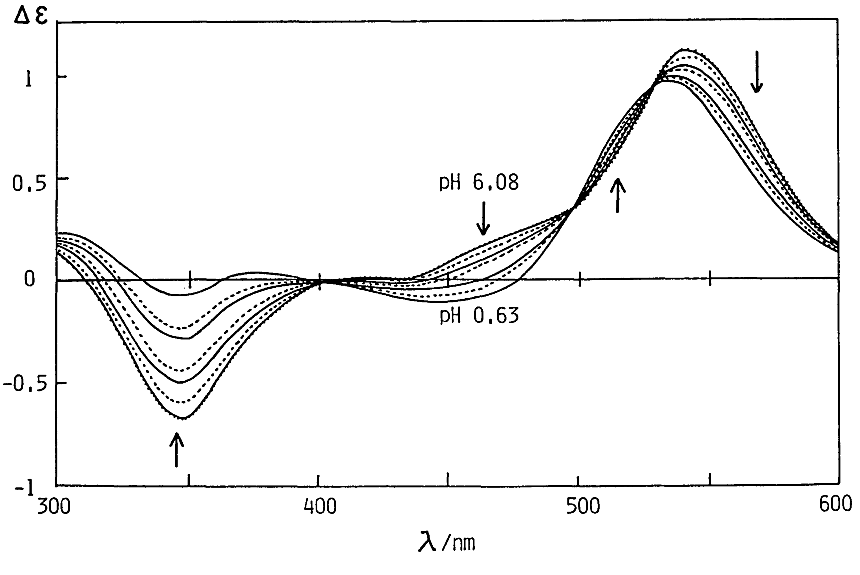


Fig. 5. CD spectral change with pH for the **AA2** complex. pH 6.08, 2.07, 1.60, 1.31, 1.18, 0.95, 0.85, and 0.63. $\Delta\varepsilon$ values are given in $\mathrm{mol^{-1}\,dm^3\,cm^{-1}}$.

[$CoCl_3$(dien)] and H_3L yielded mainly **AA3** (the peptide functions as a meridional terdentate with uncoordinated *β*-carboxyl group) in a weakly basic aqueous solution (pH 9) and **AA2** (the peptide functions as a facial terdentate with uncoordinated amino group) in a weakly acidic aqueous solution (pH 5). These results suggest that the coordination modes of peptides can be controlled by the pH of the reaction solution. That is, the amino group on the peptide can not easily coordinate to cobalt(III) in a weakly acidic solution because of the protonation on the amino group, but the carboxyl group can easily coordinate in the weakly acidic solution. Accordingly, the *β*-ala-(*S*)-asp ligand coordinates to cobalt(III) with the *α*-carboxyl O, *β*-carboxyl O, and amide N in the facial terdentate mode (**AA2**; the meridional terdentate mode with *α*-carboxyl O, *β*-carboxyl O, and amide N is sterically impossible). However, in a basic aqueous solution, the amino group on the *β*-ala-(*S*)-asp can be coordinated to cobalt(III) more easily than in an acidic solution. Consequently, when the *β*-ala-(*S*)-asp is forced into terdentates in the basic solution, the *β*-ala-(*S*)-asp ligand coordinates to cobalt(III) in the meridional terdentate mode with amino N, *α*-carboxyl O, and amide N (**AA3**), which is preferable to the facial mode for dipeptides.[1,4,5)

One of the important subjects in bioinorganic chemistry is to control the coordination modes of a peptide on a metal ion. The present results provide useful clues for this subject in the manner different from those obtaind for labile complexes such as copper(II) ones.[26,27) Though the coordination modes of a dipeptide in the labile complexes are controlled by the stability (thermodynamics) rather than by the reaction rates (kinetics) of the ligands in the labile complexes, those in the present cobalt(III) complexes are controlled by the reaction rate of the amino group on the dipeptide. The present cobalt(III) complexes are stable and do not show the structural changes in the skeltons in the wide pH range 1—11 for several hours, even though deprotonation or protonation on the coordinated dipeptide is observed.

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