Selective Activation of Methylene or Methine Groups in Coordinated Dipeptide Schiff Bases

Osamu UYAMA, Yasuo NAKAO, and Akitsugu NAKAHARA

Department of Chemistry, College of General Education, Osaka University, Toyonaka, Osaka

(Received July 18, 1972)

Nickel(II) and cobalt(III) chelates of the Schiff bases derived from salicylaldehyde and dipeptides such as glycylglycine, glycylalanine and alanylglycine have newly been prepared. The reactivity of methylene or methine groups in the dipeptide moieties of those Schiff base chelates has been investigated on the basis of their PMR spectra in D_2O. It has been confirmed that the methylene or methine protons of the *N*-terminal amino acid residue are more easily activated than those of the *C*-terminal amino acid residue.

It was demonstrated by Williams and Busch[1] that the methylene or methine protons of α-amino acids are activated on chelation with metal ions. Fore this, threonine had been synthesized from bis(glycinato)copper(II) and acetaldehyde in the presence of sodium carbonate.[2] It was reasoned that the amino group would be protected by coordination, and sufficient activation of the methylene group would result from the polarizing effect of the metal ion.[2-4]

Similar investigations have recently been extended to the case of coordinated dipeptides. For example, Noda *et al.*[5] reported that the main product out of the reaction between glycylglycine and formadehyde in the presence of copper(II) is serylglycine, not accompanied by glycylserine or serylserine at all. This suggests that the methylene group in the *N*-terminal amino acid residue is selectively activated on chelation with metal ion. On the other hand, Gillard *et al.*[6] reported that the methylene protons of the *C*-terminal amino acid residue in the cobalt(III) dipeptide chelates are replaced easily in alkaline solution by the deuterons of heavy water, but not those of the *N*-terminal amino acid residue.

In order to clarifying the two conflicting results, we have investigated the selective activation of methylene or methine groups in coordinated dipeptide Schiff bases.

This paper deals with the preparation of nickel(II) and cobalt(III) chelates of the Schiff bases derived from salicylaldehyde and dipeptides containing glycine and/or α-alanine, and the results of PMR study of those chelates in view of the selective activation of methylene or methine groups in coordinated dipeptide moieties. On the basis of our findings, the conclusions of Noda *et al.* and Gillard *et al.* have consistently been explained.

Experimental

Glycyl-α-alanine and *α-alanylglycine* were prepared according to the method of Hanson and Smith[7] and Miller *et al.*[8], respectively.

Na[Ni(Sal=Gly·Gly)]·H_2O was obtained in a similar way to that described in the preceding paper[9] employing 1 N sodium hydroxide solution. Found: C, 38.97; H, 3.29; N, 8.17%. Calcd for $Na[Ni(C_{11}H_9N_2O_4)]\cdot H_2O$: C, 39.67; H, 3.31; N, 8.42%.

K[Ni(Sal=L-Ala·Gly)]·H_2O: 1.5 g of L-alanylglycine and 3 g of bis(salicylaldehydato)nickel(II) were dissolved in 15 ml of water. The mixture was adjusted to pH 9—10 with 1 N potassium hydroxide solution, stirred at 25°C for an hour and filtered. To the filtrate was added 600 ml of a mixture of ether and ethanol (1 : 1 by volume) with constant stirring, orange crystals being deposited. These were dissolved in a small quantity of water, and then recrystallized by dropwise addition of a mixture of ethanol and ether (1 : 1 by volume). Found: C, 39.16; H, 3.22; N, 7.63%. Calcd for $K[Ni(C_{12}H_{11}N_2O_4)]\cdot H_2O$: C, 39.67; H, 3.58; N, 7.72%.

K[Ni(Sal=Gly-L-Ala)]·2H_2O was prepared by the same method as described above. Found: C, 37.73; H, 3.65; N, 7.42%. Calcd for $K[Ni(C_{12}H_{11}N_2O_4)]\cdot 2H_2O$: C, 37.82; H, 3.94; N, 7.35%.

Ba[Co(NO_2)$_2$(Sal=Gly·Gly)]·2H_2O: To a mixture of 4 g of $Na_3[Co(NO_2)_6]$ and 20 ml of water was added a solution of glycylglycine (1.3 g) and salicylaldehyde (1.2 g). The resulting mixture was adjusted to pH 9 with 2 N sodium hydroxide solution. After it had been stirred at 60°C for two hours, the reaction mixture was filtered. A hot saturated aqueous solution of $BaCl_2\cdot 2H_2O$(10 g) was then added to the filtrate. Upon cooling the solution, crystals were obtained and recrystallized from hot water. Found: C, 23.86; H, 2.36; N, 10.20%. Calcd for $Ba[Co(NO_2)_2(C_{11}H_9N_2O_4)]\cdot 2H_2O$: C, 23.68; H, 2.33; N, 10.05%.

Ba[Co(NO_2)$_2$(Sal=DL-Ala·Gly)]·3H_2O was obtained in the same way. Found: C, 24.22; H, 2.99; N, 9.69%. Calcd for $Ba[Co(NO_2)_2(C_{12}H_{11}N_2O_4)]\cdot 3H_2O$: C, 24.44; H, 2.89; N, 9.50%.

Ba[Co(NO_2)$_2$(Sal=Gly-L-Ala)]·2H_2O was obtained also in the same way. Found: C, 25.03; H, 2.65; N, 9.76%. Calcd for $Ba[Co(NO_2)_2(C_{12}H_{11}N_2O_4)]\cdot 2H_2O$: C, 25.21; H, 2.65; N, 9.80%.

PMR Measurement. The spectra were obtained by the use of a JEOL C-60HL Spectrometer for deuterium oxide solution containing about 200 mg of complex/0.8 ml D_2O. The chemical shift was measured relative to the sodium salt of trimethylsilylpropane sulfonic acid taken as an internal standard. The barium salts of the cobalt(III) complexes

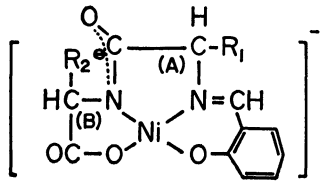
1) D. H. Williams and D. H. Busch, *J. Amer. Chem. Soc.*, **87**, 4644 (1965).
2) M. Sato, K. Okawa, and S. Akabori, This Bulletin, **30**, 937 (1957).
3) Y. Ikutani, T. Okuda, and S. Akabori, *ibid.*, **33**, 582 (1960).
4) T. T. Otani and M. Winitz, *Arch. Biochem. Biophys.*, **90**, 254 (1960).
5) K. Noda, M. Bessho, T. Kato, and N. Izumiya, This Bulletin, **43**, 1834 (1970).
6) R. D. Gillard, P. R. Mitchell, and N. C. Payne, *Chem. Commun.*, **1968**, 1150.
7) H. T. Hanson and E. L. Smith, *J. Biol. Chem.*, **175**, 833 (1948).
8) A. Miller, A. Neidle, and H. Waelsh, *Arch. Biochem. Biophys.*, **56**, 11 (1955).
9) Y. Nakao and A. Nakahara, This Bulletin, **46**, 187 (1973).

were converted into lithium salts using lithium sulfate, with which the barium ion was precipitated as $BaSO_4$. The pD-adjustment was performed by the addition of 2 N NaOD-heady water solution. A Yanagimoto pH-66A pH meter equipped with a MGR-11A combined electrode was used after standardization with a Nakarai standard buffer solution (pH 6.86 and 9.18 at 25°C).

Results and Discussion

Proton NMR spectroscopy is a convenient method for studying the behavior of hydrogen atoms in metal Schiff base complexes. We prepared a series of diamagnetic nickel(II) and cobalt(III) complexes of the Schiff bases derived from salicylaldehyde and dipeptides (glycylglycine, glycyl-α-alanine and α-alanylglycine) and investigated their PMR spectra at various pD-values.

Nickel(II) Complexes. The structures of nickel(II) complexes are given in Fig. 1, in which the methylene or methine group of the *N*-terminal amino acid residue is denoted by (A), and that of the *C*-terminal amino acid residue by (B).



R_1, R_2=H or CH_3

Fig. 1. General structure of nickel(II)-dipeptide-Schiff base complexes.

The PMR spectra for [Ni(Sal=Gly·Gly)]$^-$ are illustrated in Fig. 2; (a) is the spectrum recorded comparatively soon after the solution was prepared at room temperature and at pD 11.6. The resonance signals at 2.8 and 3.4 ppm are considered to be due to the methylene protons of (A) and (B), and those at 6.5—8 ppm the phenyl and the azomethine protons. Apparently the spectrum indicates that both the methylene protons of (A) and (B) are not replaced by deuterons. On the other hand, the spectrum shown in Fig. 2-(b), which was recorded after allowing the same solution to stand at 40°C for 27 days indicates that the signal at 3.4 ppm has decreased but not that at 2.8 ppm. This suggests that the only one methylene protons of either (A) or (B) are replaced by deuterons, but not those of the others. We see from Fig. 2-(c) that both the signals at 3.4 and 2.8 ppm decrease on allowing the solution to stand at 40°C and at pD 13.1 for 23 days. Under this condition the above trend is so remarkable as regards the signal at 3.4 ppm that even the mark of signal cannot be observed. It is considered that at this high pD-value both kinds of methylene protons are eventually activated, but the one is more easily activated than the other.

Figure 3 shows the spectra of [Ni(Sal=L-Ala·Gly)]$^-$ under various conditions. Spectrum (a) recorded

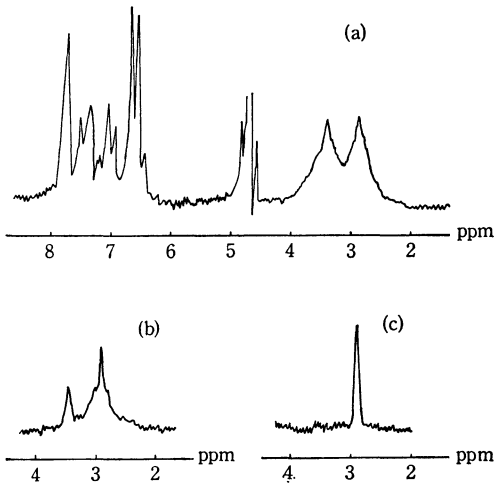


Fig. 2. PMR spectra of [Ni(Sal=Gly·Gly)]$^-$: (a) pD 11.6, room temp., after 2 hr; (b) pD 11.6, 40°C, after 27 days; (c) pD 13.1, 40°C, after 23 days.

comparatively soon after preparing the solution (room temperature, pD 10.2) indicates that neither the protons of the methylene group (B)(R_2=H) or of the methine group (A)(R_1=CH_3) are replaced by deuterons. The resonance signal of a doublet at 1.2 ppm arises from the methyl protons, that at 2.8 ppm from the methylene protons of (B) and that of a quartet at 3.4 ppm from the methine proton of (A). As is seen in Fig. 3-(b), the methyl doublet at 1.2 ppm partly collapses on allowing the solution to stand at 40°C and at pD 11, producing a singlet on account of the deuterization of the methine proton of (A), but the signal at 2.8 ppm due to the methylene protons of (B) does not decrease.

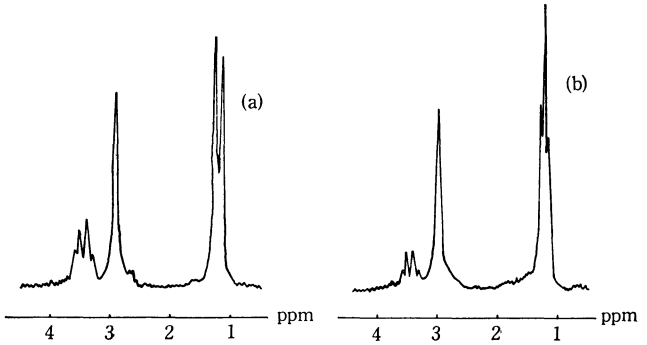


Fig. 3. PMR spectra of [Ni(Sal=L-Ala·Gly)]$^-$: (a) pD 10.2, room temp, after 2 hr; (b) pD 11, 40°C, after 27 days.

The spectra of [Ni(Sal=Gly–L-Ala)]$^-$ under various conditions are illustrated in Fig. 4. (a) is the spectrum obtained comparatively soon after preparing the solution (room temperature, pD 10.6), indicating that the protons of both the methylene group (A) (R_1=H) and the methine group (B)(R_2=CH_3) are not replaced by deuterons. The resonance signal of a doublet[10] at 1.3 ppm arises from the methyl protons

10) Careful inspection of the signal shows that it is composed of a couple of doublets. This may be because of the coexistence of two isomers relative to the different conformation of the methyl group, namely, pseudo-equatorial and pseudo-axial.

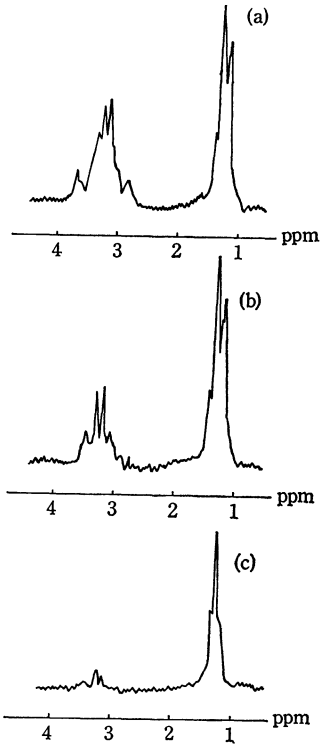


Fig. 4. PMR spectra of $[Ni(Sal=Gly\text{-}L\text{-}Ala)]^-$: (a) pD 10.6, room temp., after 2 hr; (b) pD 11, 40°C, after 27 days; (c) pD 14, 40°C, after 27 days.

($R_2=CH_3$). The signals at 3—3.5 ppm arise from the methylene and the methine protons of (A) and (B), respectively. On allowing the solution to stand at 40°C and at pD 11 for 27 days, the signals at 3—3.5 ppm turn out to be a quartet on account of the deuterization of the *N*-terminal methylene protons, whereas the doublet at 1.3 ppm remains unchanged (Fig. 4-(b)). However, at pD 14 the signal at 3—3.5 ppm completely disappears and even the doublet at 1.3 ppm completely collapses to give a singlet on allowing the solution to stand at 40°C for 27 days. This indicates that i) only the *N*-terminal methylene or methine group is activated at around pD 11, and ii) the protons of both (A)- and (B)-methylene or methine group are activated at around pD 13—14, the former being activated more easily.

The preferential activation of the methylene or methine group at the *N*-terminal amino acid residue appears to be different from the conclusion of Gillard *et al.*[6] In order to clarify whether this arises from the formation of chelates of dipeptide Schiff bases instead of simple dipeptide chelates or from the difference in the kind of central metal ion, we have further investigated the pmr spectra of cobalt(III) complexes of similar dipeptide Schiff bases.

Cobalt(III) Complexes. Three new dinitro-cobalt(III) complexes of the Schiff bases derived from salicylaldehyde and dipeptides (glycylglycine, α-alanylglycine and glycyl-α-alanine) were synthesized. It was concluded that their structures are *trans* form, since their infrared spectra are quite similar to the corresponding planar nickel(II)-dipeptide-Schiff base complexes. The general structural formula of these complexes is shown in Fig. 5, in which the cobalt(III) chelate ring containing the *N*-terminal amino acid residue is denoted by (C) and that containing *C*-terminal amino acid residue by (D).

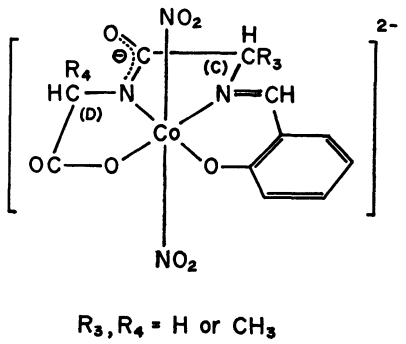


Fig. 5. General structure of cobalt(III)-dipeptide-Schiff base complexes.

The PMR spectra of $[Co(NO_2)_2(Sal=Gly\cdot Gly)]^{2-}$ in D_2O under various conditions are illustrated in Fig. 6. Spectrum (a), which was recorded comparatively soon after the preparation of the solution, shows that no protons of the methylene groups of (C) and (D) ($R_3=R_4=H$) are replaced by deuterons. The resonance signals at 4.1 and 4.5 ppm are due to the methylene protons of (C) and (D), and those at 6.5—8 ppm to the phenyl and the azomethine protons. After 3 days at 40°C (pD 10.6), the signal at 4.5 ppm decreased, but not that at 4.1 ppm (Fig. 6-(b)). This suggests that only one methylene group is activated at pD 10.6. Decomposition of the complex was observed in a stronger basic solution.

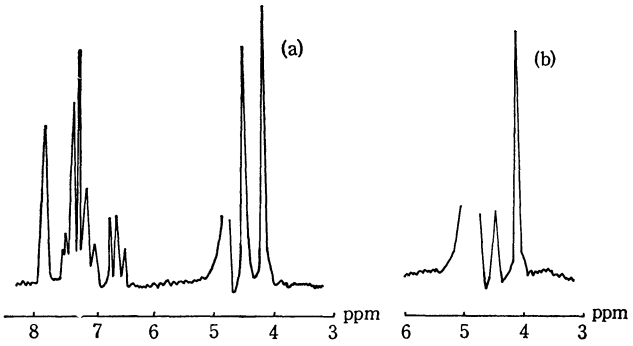


Fig. 6. PMR spectra of $[Co(NO_2)_2(Sal=Gly\cdot Gly)]^{2-}$: (a) pD 6.8, room temp., after 2 hr; (b) pD 10.6, 40°C, after 3 days.

Figure 7 shows the spectrum of $[Co(NO_2)_2(Sal=$ DL-Ala·Gly$)]^{2-}$. It is obvious that the protons of methylene or methine groups are not replaced by deuterons within two hours at room temperature and at pD 7. The signal at 1.4 ppm arises from the methyl protons ($R_3=CH_3$), that at 4.2 ppm from the methylene protons (D) ($R_4=H$), and that of a quartet at 4.4 ppm from the methine proton (C). On allowing the solution to stand at 40°C and at pD 10.6 for a day, the doublet signal for the methyl group at 1.4 ppm slightly collapses to give a singlet on account of the deuterization of the methine proton (C), the signal

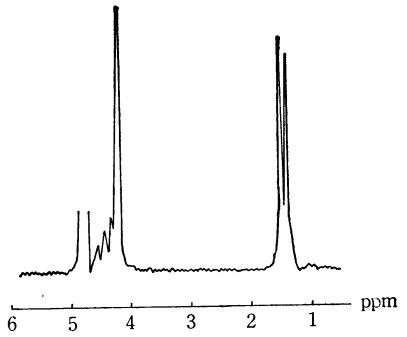


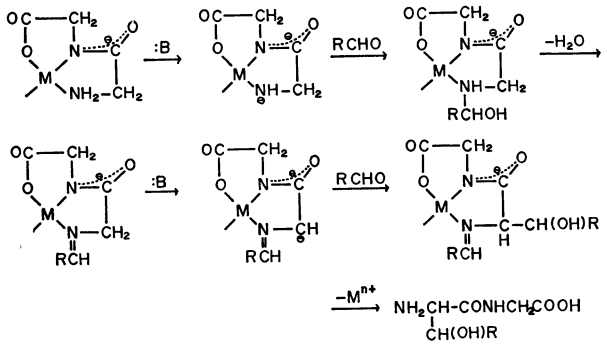
Fig. 7. PMR spectrum of $[Co(NO_2)_2(Sal=DL\text{-}Ala\cdot Gly)]^{2-}$: pD 7, room temp., after 2 hr.

for the methylene protons (D) remaining unchanged. Further deuterization expected to occur in the methylene group of (D) was not successfully observed on account of gradual decomposition of the complex. It can be said also for the cobalt(III)-dipeptide-Schiff base chelates that the methylene or methine group of (C) is activated more easily than that of (D). In other words, there is no essential difference between the nickel(II)- and cobalt(III)-chelates as far as the selective activation of the methylene or methine group in coordinated dipeptide Schiff bases is concerned.

Thus the confliction with the results of Gillard *et al.*,[6] who concluded the preferential activation of the methylene or methine group of (D), appears to arise from the difference in the structure of the terminal amino group (dipeptide Schiff base or simple dipeptide).

Reactions of Coordinated Dipeptide Schiff Bases with Formaldehyde. A preliminary experiment on the reactions of the above nickel(II) or cobalt(III) complexes with formaldehyde was carried out. In the case of the reaction of *N*-salicylideneglycylglycinatonickelate(II) in a slightly basic solution at 100°C the main products were serylglycine and α-hydroxymethylserylglycine. Apparently the result is consistent with the conclusion of the above described PMR-study and at the same time with the result reported by Noda *et al.*[5] Though the peptide they used was glycylglycine and not *N*-salicylideneglycylglycine, it might be considered that glycylglycine in the presence of copper(II) reacted with formaldehyde to form the Schiff base. If this is true, the selective activation of the methylene group at the *N*-terminal glycine moiety is understandable in the light of the above PMR-study. In the structure of the coordinated dipeptide Schiff bases, the electron-withdrawing effect of the metal ion can reach the α-carbon atom through azomethine nitrogen atom, whose electronic structure should differ entirely from that of the simple amino nitrogen in glycylglycine. This will probably enhance the reactivity of the *N*-terminal methylene group in glycylglycine moiety.

Thus, we proposed a reaction mechanism as illustrated in Scheme 1 for the reaction of Noda *et al.* The



Scheme 1.

mechanism may explain why the main products of the reaction were serylglycine and α-hydroxymethylserylglycine. Further it is also consistent with the mechanism presented by Ichikawa *et al.*[11] for the reaction of the bis(glycinato)- or pyruvylideneglycinatocopper(II) with formaldehyde. It does not contradict the conclusion on the selective activation of the methylene or methine group at the *C*-terminal amino acid moiety only in simple dipeptide chelates.[6]

The authors are grateful to Mr. H. Okuda for PMR measurements. Expenses for this work were defrayed in part by a grant from the Ministry of Education.

11) T. Ichikawa, S. Maeda, T. Okamoto, Y. Araki, and Y. Ishido, This Bulletin, **44**, 2779 (1971).