February, 1976] BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN, VOL. 49(2), 477—479 (1976) 477

Selective Activation of Methylene or Methine Groups of Tripeptide-Schiff Bases Coordinated around Nickel(II)

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(Received October 16, 1975)

Nickel(II) chelates of the Schiff bases derived from salicylaldehyde and tripeptides such as glycylglycylglycine, glycyl-DL-α-alanylglycine, glycylglycyl-DL-α-alanine, and DL-α-alanylglycylglycine have newly been prepared. The reactivity of methylene or methine groups in the tripeptide moieties of those Schiff base chelates has been studied by PMR spectra in D_2O. It has been found that the methylene or methine protons of the N-terminal amino acid residues are most effectively activated by the coordination around nickel(II) as compared with those of the C-terminal and central amino acid residues.

It is well known that the coordination of amino acids or oligopeptides to metal ions results in activations of their methylene or methine protons of the α-positions.[1,2] In a previous paper,[3] we reported that deuteration of the methylene or methine groups in tripeptides coordinated around nickel(II) occurred most easily for those of the C-terminal as compared with those of the N-terminal and central amino acid residues.

The present paper deals with the preparation of nickel(II) chelates of the Schiff bases derived from salicylaldehyde and tripeptides containing glycine and/or DL-α-alanine, and the results of PMR study of those chelates in view of the selective activation of methylene or methine protons in coordinated tripeptide moieties. The general discussions on the relative reactivity of the methylene or methine protons of oligopeptides and amino acids in coordination are also described on the basis of our recent findings as well as the present investigation.

Experimental

Glycylglycylglycine was purchased from the Protein Research Foundation. Tripeptides[3] containing DL-α-alanine residue and bis(salicylaldehydato)nickel(II)[4] were prepared according to the directions described previously.

Preparation of Nickel(II) Chelates of the Schiff Bases Derived from Salicylaldehyde and Tripeptides. To a mixture of 0.004 mol of each tripeptide and 20 ml of water was added 0.004 mol of bis(salicylaldehydato)nickel(II). The reaction mixture was adjusted to pH 8—10 with concentrated potassium hydroxide solution. After it had been stirred at 30—40 °C for about half an hour, the reaction mixture was filtered. The *N*-salicylidene-glycylglycylglycinato- and -DL-α-alanylglycylglycinato-nickelate(II) were deposited respectively as orange crystals from the filtrate upon the addition of a large quantity of ethanol with vigorous stirring, and were recrystallized from water-ethanol. The *N*-salicylidene-glycyl-DL-α-alanylglycinato- and -glycylglycyl-DL-α-alaninato-nickelate(II) were isolated as orange crystalline products by adding a mixture of ethanol and ether(1 : 1 by volume) with constant stirring. The products 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). For elemental analysis the products were dried at 70 °C over P_4O_{10}. The results of elemental analysis are shown in Table 1.

Measurements. The visible and ultraviolet absorption spectra were obtained with a Union Giken SM-401 high-sensitivity recording spectrophotometer. The PMR spectra were recorded with a JEOL MH-100 spectrometer and Varian EM-360 60 MHz NMR spectrometer and deuterium oxide was used as a solvent. The chemical shift was measured relative to the sodium salt of trimethylsilylpropane sulfonic acid taken as an internal standard. The pD-adjustment was carried out by the addition of concentrated NaOD-heavy water solution. A Hitachi-Horiba M-5 pH meter equipped with a No. 6026 combined electrode was used after standardization with a Nakarai standard buffer solution (pH 6.86 and 9.18 at 25 °C).

Results and Discussion

We have newly prepared four nickel(II) complexes of the Schiff bases obtained from salicylaldehyde and glycylglycylglycine, glycyl-DL-α-alanylglycine, glycylglycyl-DL-α-alanine, and DL-α-alanylglycylglycine. The complexes were prepared by reactions of each tripeptides with bis(salicylaldehydato)nickel(II) in the pH range 8—10. The visible and ultraviolet absorption spectrum for potassium *N*-salicylideneglycylglycylglycinatonickelate(II) in aqueous solution is shown in Fig. 1. Similar absorption spectra were found also for the nickel(II) complexes of *N*-salicylidene-glycyl-DL-α-alanylglycine, -glycylglycyl-DL-α-alanine, and -DL-α-alanylglycylglycine. Their absorption curves resemble those observed for the nickel(II) complexes which are considered to have the square planar structure.[4,5] On the basis of this finding as well as the results of the elemental analyses (Table 1), it is inferred that the

Table 1. Analytical data for $K_2[Ni(Sal{=}tripeptide)]\cdot nH_2O$

Complex	C, %		H, %		N, %	
	Found	Calcd	Found	Calcd	Found	Calcd
$K_2[Ni(Sal{=}Gly\cdot Gly\cdot Gly)]\cdot 2.5H_2O$	33.02	33.13	3.01	3.43	9.06	8.91
$K_2[Ni(Sal{=}Gly\cdot Gly\cdot \text{DL-}\alpha\text{-Ala})]\cdot H_2O$	36.58	36.69	3.57	3.31	9.21	9.17
$K_2[Ni(Sal{=}Gly\cdot \text{DL-}\alpha\text{-Ala}\cdot Gly)]\cdot 3H_2O$	34.06	34.02	3.68	3.88	8.50	8.51
$K_2[Ni(Sal{=}\text{DL-}\alpha\text{-Ala}\cdot Gly\cdot Gly)]\cdot H_2O$	36.79	36.69	3.41	3.31	9.18	9.17

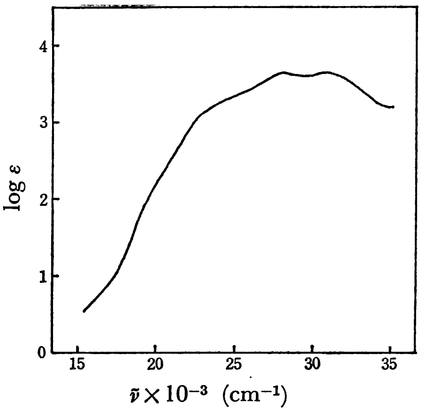
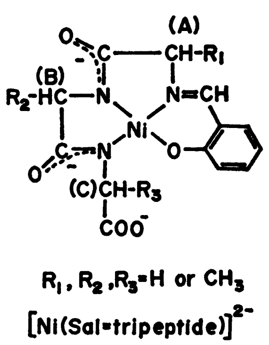


Fig. 1. Visible and ultraviolet absorption spectrum of K$_2$[Ni(Sal=Gly·Gly·Gly)] in aqueous solution.

structural formulas of nickel(II) complexes produced from salicylaldehyde and each tripeptide can be represented as **1**, in which the symbols (A), (B) and (C)



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indicate methylene or methine protons in the N-terminal, central and C-terminal amino acid residue, respectively.

The PMR spectra at 1—4 ppm of those nickel(II) chelates in deuterium oxide under various conditions are illustrated in Figs. 2—5. The peak identification of the methylene or methine protons for the four chelates, [Ni(Sal=Gly·Gly·Gly)]$^{2-}$, [Ni(Sal=DL-α-Ala·Gly·Gly)]$^{2-}$, [Ni(Sal=Gly·Gly·DL-α-Ala)]$^{2-}$ and [Ni(Sal=Gly·DL-α-Ala·Gly)]$^{2-}$, were carried out on the basis of a comparative inspection of Figs. 2-, 3-, 4- and 5-(a), which were recorded within an hour after preparing each solution. The results of peak assignment necessary for the discussion are inserted in each figure. It is interesting to note that the assignment for the (A)- and (C)-methylene peaks of the coordinated tripeptide-Schiff bases **1** is the inverse of that for the same peaks of the coordinated simple tripeptides **2**.[3)]

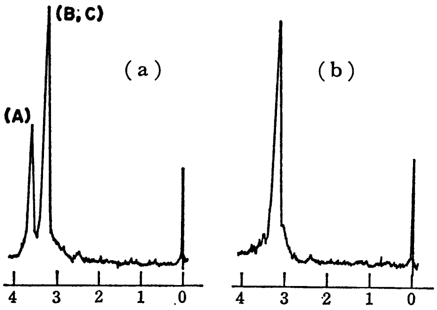
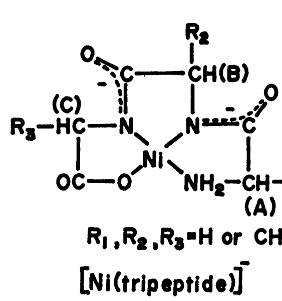


Fig. 2. PMR spectra of [Ni(Sal=Gly·Gly·Gly)]$^{2-}$: (a) pD 11.0, room temp., after 1 h; (b) pD 13.5, 40 °C, after 7 days.



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It is clearly shown from a comparison of Figs. 2(a) and (b) that the peak at 3.50 ppm arising from the N-terminal methylene protons of triglycine moiety for nickel(II) chelate **1**(R$_1$=R$_2$=R$_3$=H) almost disappears on allowing the solution to stand at 40 °C, pD 13.5 for 7 days. On the other hand, the peak (3.12 ppm) for the central and C-terminal methylene protons were unchanged under the same condition, though the decrease of the peak(3.12 ppm) was observed in a more severe condition (40 °C, pD 13.5, after 22 days).

In case of [Ni(Sal=DL-α-Ala·Gly·Gly)]$^{2-}$, the doublet methyl signals (1.22 and 1.30 ppm) collapsed on allowing the solution to stand at 40 °C, pD 13.7 for a day, producing a singlet on account of the deuteration of the methine proton of (A), whereas the signals due to the methylene protons of (B) and (C) were almost unchanged (Fig. 3(b)).

Fig. 4(b) illustrates the PMR spectrum of [Ni(Sal=Gly·Gly·DL-α-Ala)]$^{2-}$ recorded after standing the solution at 40 °C and at pD 13.3 for a day, which indi-

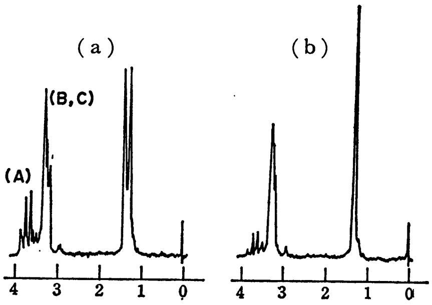


Fig. 3. PMR spectra of [Ni(Sal=DL-α-Ala·Gly·Gly)]$^{2-}$: (a) pD 11.2, room temp., after 1 hr; (b) pD 13.7, 40 °C, after 1 day.

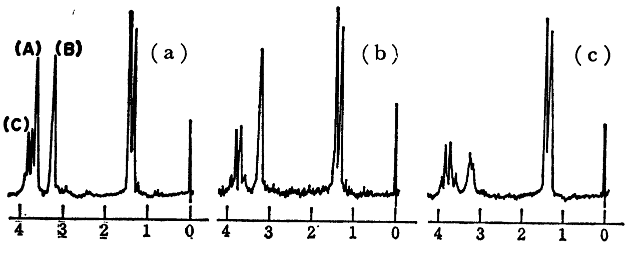


Fig. 4. PMR spectra of [Ni(Sal=Gly·Gly·DL-α-Ala)]$^{2-}$: (a) pD 11.5, room temp., after 1 h; (b) pD 13.3, 40 °C, after 1 day; (c) pD 13.1, 40 °C, after 12 days.

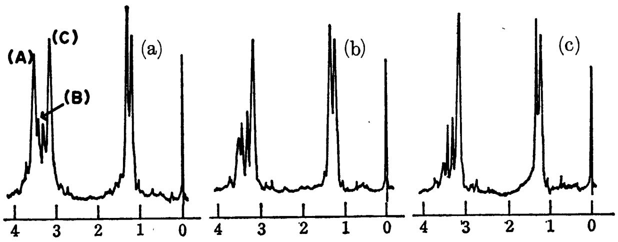


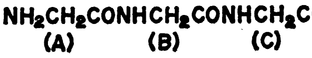
Fig. 5. PMR spectra of $[Ni(Sal{=}Gly\cdot DL\text{-}\alpha\text{-}Ala\cdot Gly)]^{2-}$: (a) pD 11.3, room temp., after 1 h; (b) pD 12.5, 40 °C, after 1 day; (c) pD 10.7, 40 °C, after 31 days.

cates that the deuteration is going on (A) methylene groups, whereas the (B) methylene protons do not show any tendency of activation under the condition. However, at 40 °C and at pD 13.1 after 12 days the signal (3.20 ppm) for the (B) methylene group almost disappeared. Even under this condition the methyl doublet at 1.37 ppm remains unchanged, as is seen from a comparison of Fig. 4(a)—(c). The fact suggests that the exchange reaction does not occur at (C) methine group in the *N*-salicylideneglycylglycyl-DL-α-alaninatonickelate(II).

Figure 5(a) shows the PMR spectrum of $[Ni(Sal{=}Gly\cdot DL\text{-}\alpha\text{-}Ala\cdot Gly)]^{2-}$, which was recorded within an hour after the preparation of the solution. In this spectrum the signals for the methylene or methine protons of (A), (B) and (C) are not well-separated. The resonance signals at 3.55 ppm and 3.17 ppm are supposed to arise from the methylene protons of (A) and (C), respectively. The signals at 3.33 ppm and 3.43 ppm are attributed to the (B) methine proton, and the remaining two peaks for the (B) methine proton are possibly considered as overlapping with peaks for the (A) and (C) methylene protons. After a day at 40 °C (pD 12.5), the signal at 3.55 ppm due to the (A) methylene proton decreased, but not that at 3.17 ppm (Fig. 5(b)). The methyl doublet at 1.23 ppm and 1.33 ppm remained unchanged under the same condition. Further deuteration of the (A) methylene proton could also be observed, on allowing the solution to stand at 40 °C and pD 10.7 for 31 days. Here the doublet methyl signals exhibited an indication of collapsing to give a singlet, indicating that deuteration began to occur at the (B) methine proton.

The foregoing description on the results of PMR study of nickel(II) chelates of Schiff bases derived from salicylaldehyde and tripeptides can be summarized as follows: (i) the N-terminal methylene or methine protons are more easily activated than the others; (ii) the C-terminal methylene or methine protons cannot be activated in the range of our experimental condition. The former, (i), can be explained by taking into consideration the electron-withdrawing effect of nickel(II) through the azomethine nitrogen atom. And the latter, (ii), may be interpreted as resulting from the absence of the electron-withdrawing effect of nickel(II) through the terminal carboxylate group.

Of interest is the fact that the preferential activation of the methylene or methine protons of tripeptides occurs in the different position (N-, C-terminal or central amino acid residue, respectively) depending upon the modes of tripeptides (chelate **1**, **2** or uncoordinated triglycinate **3**,⁶⁾ respectively).



$$\underset{(A)}{NH_2CH_2}CO\underset{(B)}{NHCH_2}CO\underset{(C)}{NHCH_2}COO^-$$

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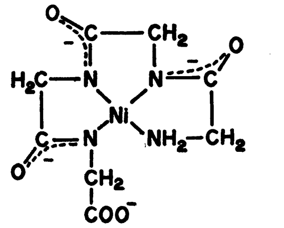
The results of our series of investigations on the selective activation of dipeptides[2,8] and tripeptides[9] in different structures can be summarized as follows: (i) in order to activate the C-methylene or -methine protons of oligopeptides, the coordination of the terminal carboxylate groups of peptides to metal ions is necessary; (ii) in order to activate the N-methylene or -methine groups, the terminal nitrogens of peptides have to be coordinated to metal ions in the form of Schiff bases.

The validity for the foregoing conclusion is also demonstrated by the fact that all the methylene protons in the tetraglycine-nickel(II) chelate **4** shows no tendency of activation after 4 days at 40 °C (pD 13.0).[10]



$[Ni(Gly\cdot Gly\cdot Gly\cdot Gly)]^{2-}$

4

This work was supported in part by a grant from the Ministry of Education.

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