NMR Study of Amino Acids and Their Derivatives. V. Structures and Formation Constants of Zinc–L-Aspartic Acid Complexes in Aqueous Solution

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NMR spectra of L-aspartic acid coordinated with Zn ions were measured in aqueous solution at various pH values. Three species of zinc–L-aspartic acid complexes were identified *i.e.*, 1 : 1 zinc–L-aspartic acid(zwitter ionic form) complex (MAH), and 1 : 1 and 1 : 2 zinc–aspartic acid(anionic form) complexes (designated as MA and MA_2, respectively). It is shown that, in MAH, the amino acid coordinates with Zn as a monodentate ligand, whereas in MA and MA_2, approximately 65% of the molecules of amino acid coordinate as a tridentate ligand. From the chemical shift data, the equilibrium constants for these complexes are determined as follows: $pK_a(MAH) = 5.3 \pm 0.2$ and $\log K_2 = 4.5 \pm 0.2$ where $K_a(MAH) = [MA][H^+]/[MAH]$ and $K_2 = [MA_2]/[MA][A]$.

Nuclear magnetic resonance (NMR) has been successfully used in investigating the structure of amino acids in aqueous solution.[1] We have previously investigated the structure of L-cysteine,[2] L-phenylalanine[3] and L-serine[4]. The present paper reports an NMR study of L-aspartic acid–zinc ion system at various pH values. Structures of Zn–L-aspartic acid complexes which possibly exist in solution are presented together with their equilibrium constants.

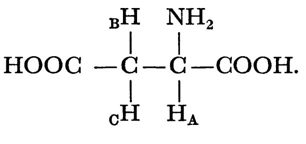
Experimental

Reagent grade L-aspartic acid, zinc nitrate, tetramethylammonium chloride and sodium hydroxide were used to prepare solutions of zinc-free L-aspartic acid, 1 : 1 and 1 : 2 zinc–L-aspartic acid in water. The concentration of aspartic acid in each solution is 0.10 M. Five mM of tetramethylammonium chloride is added to each solution as an internal reference.

The pH of the solution was controlled by adding concentrated sodium hydroxide solution and measured with a Toa Dempa pH meter, Model HM-5A. NMR spectra were recorded with a JNM 4H-100 spectrometer operating at 100 MHz.

Results and Discussion

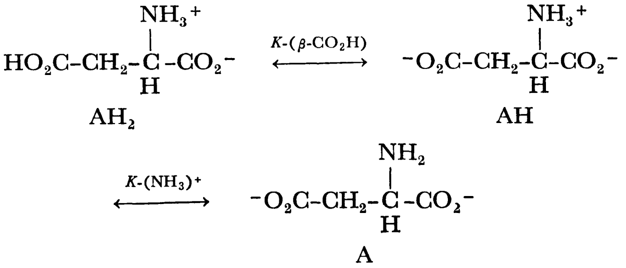
The α- and β-protons of L-aspartic acid are designated as follows:



$$HOOC-\underset{_{C}H}{\overset{_{B}H}{C}}-\underset{H_A}{\overset{NH_2}{C}}-COOH.$$

NMR spectra of this ABC three spin system were analyzed as described previously.[4]

Chemical Shift. *Zinc-free Aspartic Acid Solution:* Chemical shifts for zinc-free aspartic acid solutions are shown in Fig. 1, where stepwise shifts are observed in the pH range 4—5 and 9—11. These shifts are attributed to the dissociation of the β-COOH and NH_3^+ groups, respectively.[5] The three species and dissociation constants involved are designated as follows:



$$\underset{AH_2}{HO_2C\text{-}CH_2\text{-}\overset{NH_3^+}{\underset{H}{C}}\text{-}CO_2^-} \xleftrightarrow{K\text{-}(\beta\text{-}CO_2H)} \underset{AH}{^-O_2C\text{-}CH_2\text{-}\overset{NH_3^+}{\underset{H}{C}}\text{-}CO_2^-} \xleftrightarrow{K\text{-}(NH_3)^+} \underset{A}{^-O_2C\text{-}CH_2\text{-}\overset{NH_2}{\underset{H}{C}}\text{-}CO_2^-}$$

Chemical shifts of the three protons ν_A, ν_B and ν_C for AH and A forms are listed in Table 1. The $pK(NH_3^+)$ value estimated from the pH dependence of ν_A is in good agreement with values determined by the potentiometric titration method[6—8] (Table 2). The present result obtained above pH 7 agree well with those reported by Pachler[9] and Taddei and Pratt.[10]

Zn(II)–L-Aspartic Acid Solution: Figure 1 shows the chemical shift of the three protons in the 1 : 1 and 1 : 2 solutions, for the 1 : 1 solution the average values of ν_B and ν_C are obtained by analyzing the

Table 1. Chemical shifts of α- and β-protons of L-aspartic acid[a]

Form[b]	Zinc-free solution		1 : 1 Solution		1 : 2 Solution
	AH	A	MAH	MA	MA_2
pH	7.2	12.2	4.4	6.7	8.6
ν_A	71.8 Hz	37.4	72.1	50.2	50.9
ν_B	−36.9	−53.6	−37.7[c]	−39.9[c]	−41.4[c]
ν_C	−50.7	−90.1			

a) Chemical shift are in Hz unit from tetramethylammonium chloride. They are accurate to ±0.1 Hz. b) See text.
c) The average value of ν_B and ν_C. The difference between ν_B and ν_C is smaller than 4Hz.

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1) *Cf. e. g.*, J. J. M. Rowe, J. Hinton, and K. L. Rowe, *Chem. Rev.*, **70**, 1, (1970).

2) S. Fujiwara and Y. Arata, This Bulletin, **36**, 578, (1963).

3) S. Fujiwara and Y. Arata, *ibid.*, **37**, 344, (1964).

4) H. Ogura, Y. Arata, and S. Fujiwara, *J. Mol. Spectrosc.* **23**, 76, (1967).

5) The α-carboxyl group dissociates at a pH value which is outside the pH range covered in the present study.

6) H. Kroll, *J. Amer. Chem. Soc.*, **74**, 2034 (1952).

7) A. Albert, *Biochem. J.*, **50**, 690 (1952).

8) S. Chaberek, Jr. and A. E. Martell, *J. Amer. Chem. Soc.*, **74**, 6021 (1952).

9) K. G. R. Pachler, *Z. Anal. Chem.*, **224**, 211, (1967).

10) F. Taddei and L. Pratt, *J. Chem. Soc.*, **1964**, 1553,

Table 2. Equilibrium constants of zinc ion-L-aspartic acid complex

	This work[a]	Kroll[6]	Albert[7]	Martell[8]
pK(NH_3^+)	9.8±0.1	9.60	9.98	9.46
pK(MAH)[b]	5.3±0.2	—	—	—
log K_2[c]	4.5±0.2	—	—	4.31

a) The ionic strength is about 0.1.

b) $K(\mathrm{MAH}) = \dfrac{[\mathrm{MA}][\mathrm{H}^+]}{[\mathrm{MAH}]}$. c) $K_2 = \dfrac{[\mathrm{MA_2}]}{[\mathrm{MA}][\mathrm{A}]}$.

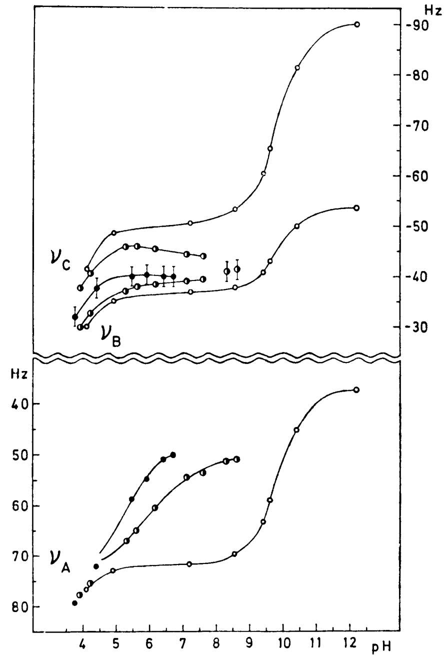

Fig. 1. The pH dependence of the chemical shifts for CH protons of L-aspartic acid.

● Zn(II)—Asp 1 : 1 solution

◑ Zn(II)—Asp 1 : 2 solution

○ Zn(II) free solution

The average values of ν_B and ν_C are plotted for the 1 : 1 solution, and pH≥8.3 for the 1 : 2 solution.

system as an AB_2 spin system and plotted, since $\Delta\nu_{BC}$, the difference between ν_B and ν_C, is too small (≲4 Hz) for the spectra to be analyzed as an ABC spin system.

In the titration curve for the A proton, high field shift due to dissociation of the NH_3^+ group occurs at a pH much lower than in the zinc-free aspartic acid solution, suggesting that the following complex formation takes place:

$$\text{-}NH_3^+ \rightarrow \text{-}NH_2\text{-}Zn^{2+}.$$

Figure 1 shows that the coordination of the amino group is complete at pH 6.7 and at pH 8.6 for the 1 : 1 and the 1 : 2 solutions, respectively.[11] It should also be noted that the A-proton chemical shifts for these systems (columns 4 and 5, Table 1) are lower than that of zinc-free aspartic acid in A form. This low field shift may be due to participation of the amino group in the complex formation with zinc ion.

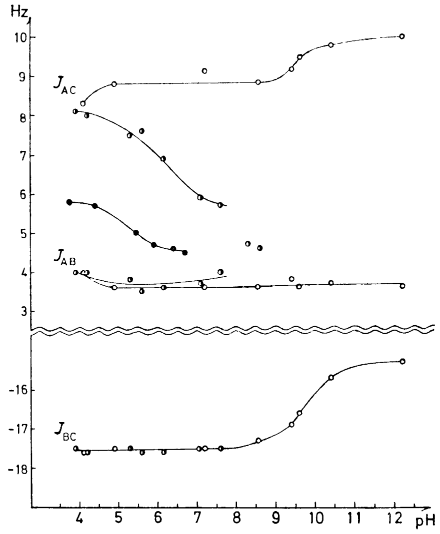

Fig. 2. The pH dependence of the spin coupling constant between CH protons of L-aspartic acid.

● Zn(II)—Asp 1 : 1 solution

◑ Zn(II)—Asp 1 : 2 solution

○ Zn(II) free solution

The average values of J_{AB} and J_{AC} are plotted for the 1 : 1 solution, and at pH≥8.3 for the 1 : 2 solution.

The difference $\Delta\nu_{BC}$ at pH 3.7—4.9 decreases in the following order: zinc-free, 1 : 2, 1 : 1 solutions. This suggests that at least one zinc–L-aspartic acid complex with a smaller $\Delta\nu_{BC}$ value exists in the pH range.[12] It is assumed that the observed chemical shifts are the average of contributions from zinc-free and coordinated (1 : 1 and 1 : 2 complexes) aspartic acid as follows:

$$\Delta\nu_{BC(obs)} = f\Delta\nu_{BC(free)} + (c_1+c_2)\Delta\nu_{BC(coord)} \quad (1)$$

where

$$f + c_1 + c_2 = 1 \quad (2)$$

Here, f, c_1 and c_2 are the fractional population of zinc-free and coordinated (1 : 1 and 1 : 2 complexes) aspartic acid, respectively. The values of $\Delta\nu_{BC(free)}$ and $\Delta\nu_{BC(coord)}$ were obtained by the data for the zinc-free and 1 : 1 solutions respectively, as follows: $\Delta\nu_{BC(free)}$=11.4(pH 4.1)~13.4 Hz(pH 4.9) and $\Delta\nu_{BC(coord)} \lesssim 4$ Hz. Comparison of these values with $\Delta\nu_{BC(obs)}$ for the 1 : 2 and 1 : 1 solutions indicates that c_2 is negligibly small, $f \fallingdotseq c_1 \fallingdotseq 0.5$, $c_2 \fallingdotseq 0$ for the 1 : 2 solution and $f \fallingdotseq c_2 \fallingdotseq 0$, $c_1 \fallingdotseq 1$ for 1 : 1 solution (*cf.* Fig. 1). Thus, it may be concluded that a 1 : 1 complex (designated as MAH) is dominant in the pH range examined.

Spin Coupling Constant. The spin coupling constants for the 1 : 1, 1 : 2 and zinc-free aspartic acid solutions are shown in Fig. 2, where the average of J_{AB} and J_{AC} was plotted for the 1 : 1 solution throughout the pH range examined, and for the 1 : 2 solution at pH≧8.3.

The vicinal coupling constants, J_{AB} and J_{AC} obtained can be used to discuss possible conformations of L-

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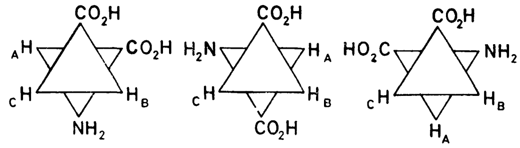
[11] NMR spectra could not be measured at pH>6.7 and at pH>8.6 for the 1 : 1 and the 1 : 2 solutions, respectively, because of limited solubility of the samples.

[12] $\Delta\nu_{BC(obs)}$ does not change by adding sodium nitrate to the zinc-free solution, indicating that $\Delta\nu_{BC}$ is independent of the ionic strength.



I II III

Fig. 3. The three rotational isomers of L-aspartic acid.

aspartic acid in the present systems. It is assumed that J_{AB} and J_{AC} are the statistical average of contributions from three rotational isomers I, II and III of staggered form, as shown in Fig. 3.

Thus

$$J_{AB} = p_I J_t + p_{II} J_g + p_{III} J_g \tag{3}$$

$$J_{AC} = p_I J_g + p_{II} J_t + p_{III} J_g, \tag{4}$$

where

$$p_I + p_{II} + p_{III} = 1. \tag{5}$$

Here, p_I, p_{II} and p_{III} are the fractional populations of the three rotational isomers, and J_t and J_g are the spin coupling constants between *trans* and *gauche* proton pairs, respectively. It is further assumed that J_t and J_g do not change when aspartic acid coordinates to zinc ion. This assumption may be confirmed by the following experiment. In L-alanine the spin coupling constant between alpha and beta protons was constant, *i. e.*, J=7.2 Hz, both at pH 4.2 and 6.7 in 1 : 2 zinc–L-alanine solution and at pH 2.5 and 6.0 in the absence of zinc ion. This result suggests that, since $p_I = p_{II} = p_{III}$ in L-alanine, J_t and J_g are not influenced by the coordination of the amino acid to zinc ion.

In the present study, J_t=13.6 Hz and J_g=2.6 Hz were used to obtain the fractional populations p_I, p_{II} and p_{III}.[4] Figure 4 shows the results for the 1 : 1, 1 : 2 and zinc-free aspartic acid solutions.[13]

In the pH region where MAH dominates, isomer I with the two carboxyl anions *gauche* to each other makes only a minor contribution in the 1 : 2 solution. This strongly suggests that in this pH region aspartic acid coordinates as a monodentate ligand using either one of the two carboxyl anions; aspartic acid as a bidentate ligand, coordinating with the α- and β-carboxyl groups, would result in a large populations the rotational isomer I. As Fig. 4 shows, p_{II} increases with pH and becomes constant and equal to about 65% at pH 6.7 and at pH 8.6 in the 1 : 1 and 1 : 2 solution, respectively; isomer III becomes dominant in the pH region where aspartic acid can coordinate with zinc ion in the A form (See Fig. 1). This result suggests that in this pH range L-aspartic acid behaves as a tridentate ligand, coordinating with the amino, α- and β-carboxyl groups to zinc ion. Although isomer III is dominant in these systems, p_I and p_{II} are also not negligibly small, suggesting that bidentate species possibly exist as minor components; one of the possible species may be the isomer II coordinating with the amino and β-carboxyl groups, and another isomer I or II which coordinates with the amino and α-carboxyl groups.

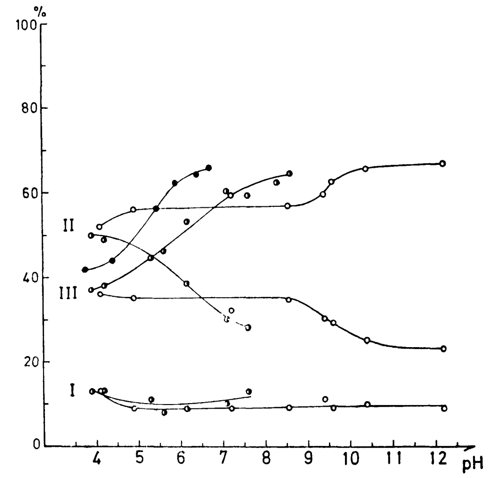


Fig. 4. pH dependence of the fractional populations of the fractional populations of the three rotational isomers.
● Zn(II)—Asp 1 : 1 solution
◑ Zn(II)—Asp 1 : 2 solution
○ Zn(II) free solution
Only the population of isomer III is plotted for the 1 : 1 solution and pH≥8.3 for the 1 : 2 solution.

In contrast to the above results, an x-ray diffraction study shows that only rotational isomer III is present in solid zinc aspartate trihydrate.[14] Martin and Mathur[15] suggested on the basis of their NMR study that in di(L-histidino)zinc complex a rotational isomer corresponding to the above mentioned isomer III is dominant in aqueous solutions. However, the pH of the solutions are not stated definitely, and the population of the rotational isomer is not given.

Equilibrium Constants among Zinc–L-Aspartic Acid Complexes. On the basis of the experimental results mentioned above, it is assumed that the following equilibria are dominant in the 1 : 1 and 1 : 2 zinc–aspartic acid solutions at pH≳4.5:

$$\mathrm{MAH} \xleftrightarrow{K(\mathrm{MAH})} \mathrm{MA} + \mathrm{H}^+,\ K(\mathrm{MAH}) = \frac{[\mathrm{MA}][\mathrm{H}^+]}{[\mathrm{MAH}]}, \tag{6}$$

$$\mathrm{MA} + \mathrm{AH} \xleftrightarrow{K(\text{MA-AH})} \mathrm{MA}_2 + \mathrm{H}^+,$$

$$K(\text{MA-AH}) = \frac{[\mathrm{MA}_2][\mathrm{H}^+]}{[\mathrm{MA}][\mathrm{AH}]}. \tag{7}$$

Here, MAH denotes the 1 : 1 zinc–aspartic acid(AH form) complex, and MA and MA_2 the 1 : 1 and 1 : 2 zinc–aspartic acid(A form) complexes, respectively. The equilibrium constants were determined from the pH dependence of ν_A by curve fitting. It is assumed that ν_A of MAH is equal to that of AH. The pH dependence of ν_A for the 1 : 1 and 1 : 2 solutions cal-

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[13] The assignment of the curves to isomers I and II in Fig. 4 may be reversed. Considering steric hindrance, however, one would expect isomer II with two carboxyl anions *trans* to each other to be more stable than isomer I.

[14] T. Doyne, R. Pepinsky, and T. Watanabe, *Acta Crystallogr.*, **10**, 438 (1957).

[15] R. B. Martin and R. Mathur, *J. Amer. Chem. Soc.*, **87**, 1065, (1965).

culated using pK(MAH)=5.3 and pK(MA-AH)= 5.3 is plotted in Fig. 1. These curves are in good agreement with the experimental data. The following stability constant for the 1 : 2 complex can be determined by using the values of pK(MA-AH) and pK-(NH_3^+):

$$K_2 = \frac{[MA][A]}{[MA_2]}$$

The values of pK(MAH) and log K_2 are given in the second and third row, respectively, of Table 2, together with the reported log K_2 value determined by the potentiometric titration method.[8)]

Conclusion

Three zinc–L-aspartic acid complexes, MAH, MA and MA_2, were identified in the zinc ion–aspartic acid systems. The dominant(~65%) species for MA and MA_2 is a tridentate complex with the amino acid coordinating with the amino, α- and β-carboxyl groups. As minor components, bidentate species are suggested to exist, where the ligand coordinates with the amino and α- or β-carboxyl groups. The dominant species for MAH is a monodentate complex coordinating with one of the two carboxyl groups. The equilibrium constants of these complexes, pK(MAH) and log K_2 were determined (Table 2).