NMR Study on Inclusion Complexes of L-Phenylalanine and Aspartame with Cyclodextrins in Aqueous Solution

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The interactions of Phenylalanine and Aspartame (*N*-L-α-Aspartyl-L-phenylalaninel-methyl ester, APM) with α- and β-cyclodextrins (CD) in aqueous solutions were investigated by ^{1}H and ^{15}N NMR techniques. The changes in chemical shift of CD protons on going from free CD to the mixture with phenylalanine and APM indicate that the phenyl ring is included in the cavity of CD in the complex states. The changes in the chemical shift of the β-protons of phenylalanine and APM following the binding to α- and β-CD indicate that the simple association constants (K_a) are not applicable to these systems except for the case of APM/β-CD (K_a=90), and that the large changes of the rotamer fractional population of APM side-chain occur for APM/β-CD system. The analysis with Asp-Phe(^{15}N)-OMe suggests that the torsional angles of the main chain of APM are unchanged on the formation of the inclusion complex. The low-field shifts of amide proton and its nitrogen resonances of Asp-Phe(^{15}N)-OMe in the complex formation suggest that the amide part of APM is hydrogen-bonded to the C2 or C3 hydroxyl group of β-CD.

APM (*N*-L-α-Aspartyl-L-phenylalaninel-methyl ester) is a well-known dipeptide used as a sweetner. We have obtained a crystal of the equimolar inclusion compound of APM with β-CD, and examined the crystal by Raman and solid-state ^{13}C NMR spectroscopy.[1] In the crystal state the phenyl ring of APM was found to be included in the cavity of β-CD. Also in aqueous solution the relatively hydrophobic environment of CD cavity seems to be favorable for the inclusion of the phenyl ring of phenylalanine and APM. We tried to elucidate the intermolecular interaction in aqueous solution by ^{1}H and ^{15}N NMR. In the past a number of papers investigated about the guest/CD interaction in solution by NMR examination.[2-10] Most of them pay attention to the conformational changes of CD molecule with which guests are included. In the present paper we report mainly on the conformational change of APM molecule which is included with β-CD as studied by the use of NMR techniques.

Experimental

Materials. Phenylalanine and APM were prepared in our laboratories. CD were purchased from Sanraku-Ocean CO., LTD. Asp-Phe(^{15}N)-OMe synthesized in the previous study[1] was used.

Apparatus. ^{1}H and ^{15}N NMR spectra were recorded on a JEOL GX-400 spectrometer operating at 399.55 MHz for ^{1}H and 40.40 MHz for ^{15}N. The proton chemical shifts were referenced to internal sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) and the nitrogen chemical shifts were to external 5 M† ammonium nitrate.

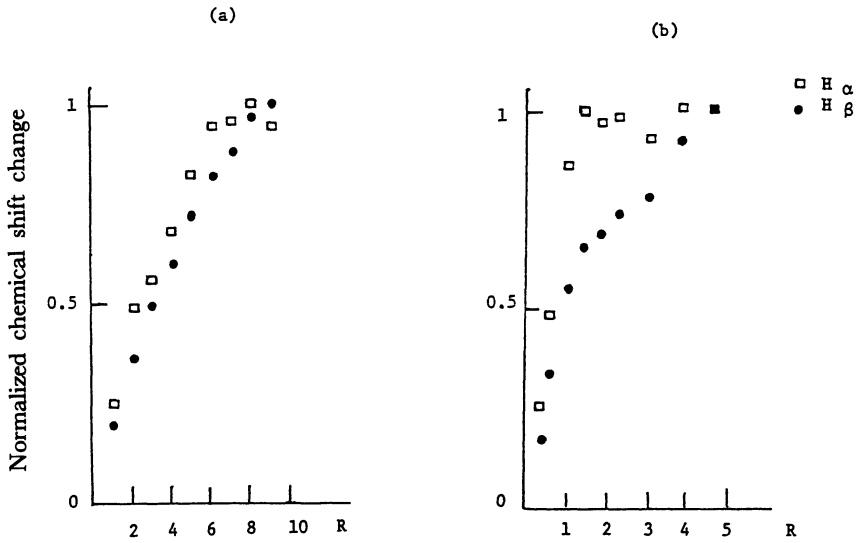


Fig. 1. CD-induced shifts of the chemical shifts of β-protons of Phe (a: α-CD, b: β-CD) in an aqueous solution at pH 6.0. R is [CD]:[Phe] molar ratio. The chemical shift changes are normalized with that at the highest molar ratio [CD]/[Phe].

† 1 M=1 mol dm^{-3}.

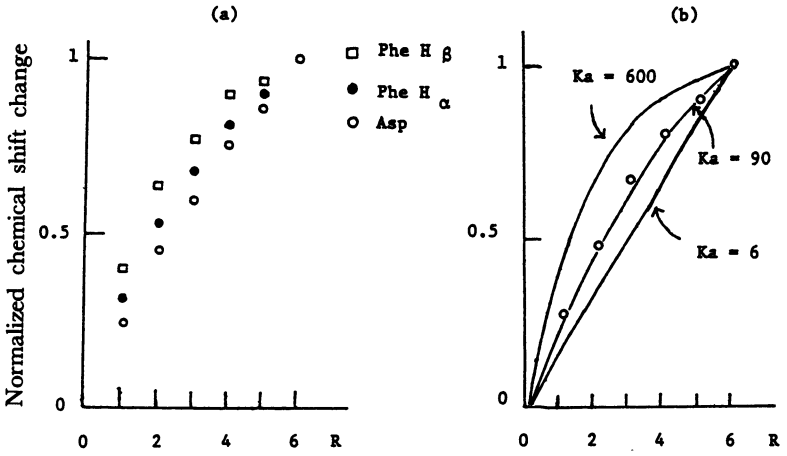


Fig. 2. CD-induced shifts of the chemical shifts of β-protons of Asp and Phe residues of APM (a: α-CD, b: β-CD) pH 6.0 in an aqueous solution. Solid lines in Fig. 2 b show variation of chemical shift changes with R for three association constants, calculated assuming a 1:1 adduct stoichiometry.

Results

(a) Binding of Phenylalanine and APM to CD. Figure 1 shows the chemical shift of β-protons of phenylalanine as a function of [α-CD]/[Phe] (Fig. 1(a)) and [β-CD]/[Phe] (Fig. 1(b)). The changes in the chemical shift are normalized to the values obtained at the highest molar ratio [CD]/[Phe]. The values for the two β-protons do not lie on the same line for α- and β-CD indicating that the simple association constant (K_a) for the complexation with CD is not applicable to these models. Figure 2 shows the chemical shift of methylene protons of Asp and Phe residues of APM as a function of [α-CD]/[APM] (Fig. 2(a)) and [β-CD]/[APM] (b). It is seen from Fig. 2(a) that the curves for four protons in [α-CD]/[APM] system are different and do not give the simple association constant while in Fig. 2(b) it is seen that in [β-CD]/[APM] the data points lie on the same curve so that the description on one association constant applies to [β-CD]/[APM] system. In Fig. 2(b) the curves corresponding to three K_a values (K_a=6, 90, 600 M^{-1}) are also shown. From those the value K_a=90 is obtained as a best fit.

(b) Depth of Inclusion of APM in the Cavity of β-CD. Figure 3 shows the changes of the ^1H NMR spectrum of β-CD on the complexation with APM in D_2O solution at pH 6.0. The upfield shifts of H3 and H5 located in the inner surface of β-CD were observed. We interprete that the shifts are due to the phenyl ring current and that the phenyl ring of APM is included in the cavity of β-CD. Then the most probable position of APM in the β-CD cavity in an aqueous solution can be determined from the Johnson-Bovey equation[11] when the intrinsic chemical shifts (δ_{int}) of H3 and H5 as defined as $\delta_b-\delta_o$ with δ_b and δ_o being the chemical shifts in fully

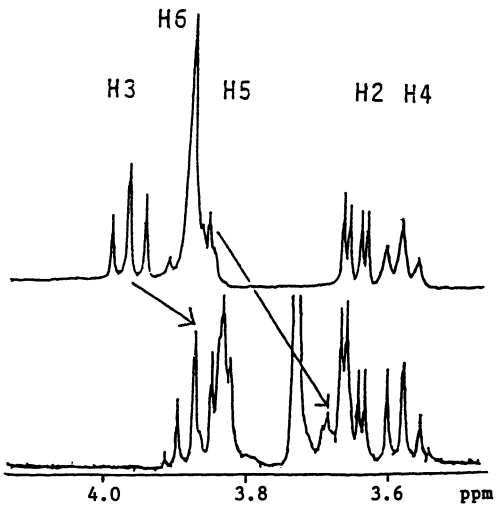


Fig. 3. 400 MHz ^1H NMR spectra of β-CD (top) and β-CD plus APM (bottom:molar ratio 1:20=CD:APM, [β-CD] 1.7 mM) in D_2O.

complexed and free state, respectively, are known. The values of δ_b and thus δ_{int} were estimated from the chemical shifts at known concentration of β-CD and APM with the aid of K_a value determined in section (a). Thus the estimated distance between the center of the phenyl ring and the plane containing seven H3 of β-CD is +1.7 Å. (the positive sign corresponds to the direction H3→H5 plane.) (Fig. 4)

(c) The Change of the Side-Chain and Backbone of APM in Complex Formation with β-CD. Table 1 summarized the vicinal coupling constants of the side-chain and the fractional populations of the rotamer in the free and complexed states in an aqueous solution. The fractional populations of three rotamers, PI, PII, and PIII were calculated using the following equations:

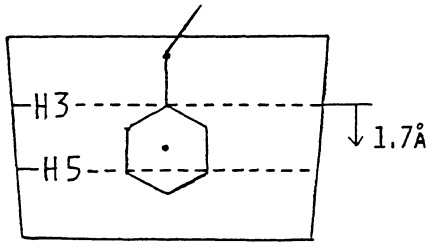
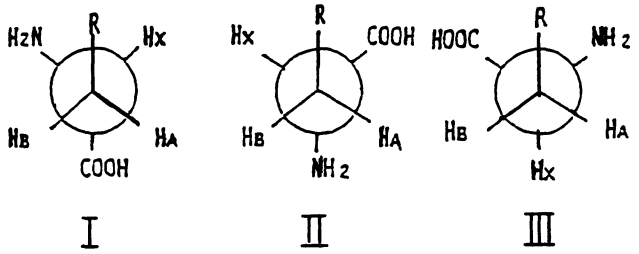


Fig. 4. Time-averaged conformation of the inclusion complex of APM with β-CD in an aqueous solution.

Table 1. Vicinal Coupling Constants (Hz) and Rotamer Fractional Populations for APM at pH 5.2

		Free	Complex
	J_{AX}	4.6	6.6
	J_{BX}	8.7	7.2
Asp	PI	0.56	0.43
	PII	0.19	0.36
	PIII	0.25	0.21
	J_{AX}	5.6	8.0
	J_{BX}	9.0	7.8
Phe	PI	0.59	0.47
	PII	0.27	0.49
	PIII	0.14	0.04



I II III

$$PI = (J_{BX}-J_g)/(J_t-J_g),$$

$$PII = (J_{AX}-J_g)/(J_t-J_g),$$

$$PIII = 1 - PI - PII,$$

where J_t=13.6 Hz, J_g=2.56 Hz.[12] The coupling constants and therefore the fractional population of the rotamer change on forming the inclusion with β-CD. In Table 2 are compared the three coupling constants $^1J15_{NH}$, $^3J15_{NCCH}$, and $^3J_H15_{NCH}$ of Asp-Phe(^{15}N)-OMe in the free and complexed state to indicate the effect of the backbone dihedral angles. The three coupling constants do not change on the complex formation. It suggests that the backbone dihedral angles of APM do not vary in the complex. Table 2 also shows the chemical shifts of amide proton and amide nitrogen of Asp-Phe(^{15}N)-OMe in the free and complexed states in H_2O. The low-field shifts in the complex formation were observed suggesting that the amide part of APM is hydrogen-bonded to the C2 or C3 hydroxyl group of β-CD in the complex.

Table 2. ^{1}H Coupling Constants (Hz) and Amide Proton and Nitrogen Chemical Shifts (ppm) for Asp-Phe(^{15}N)-OMe at pH 4.3

	Free	Complex with β-CD
$J15_{NH}$	93.1	93.1
J_H15_{NCH}	7.6	7.6
$J15_{NCCH}$	<0.2	<0.2
δ_{NH}	8.76	9.12
$\delta15_N$	119.6	120.0

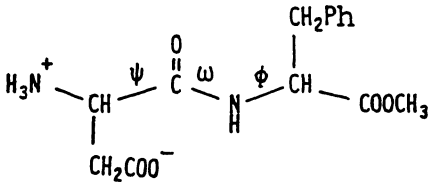


Fig. 5. The torsion angles of APM molucule.

Discussion

A number of papers on the investigation of the host/CD interaction by NMR examination have been published.[2-10] Komiyama and Hirai estimated time-averaged position of phenol derivatives in the cavity of β-CD in aqueous solution with the Johnson-Bovey equation.[8] The estimated distance between the center of the aromatic ring of phenol and the plane containing the seven H-3 atoms of β-CD is 1.8 Å. The value is very close to the present result of 1.7 Å. A number of papers about the association constants of guest molecules with β-CD with ^{1}H and ^{13}C NMR[2-7] are published. The reported values range between 100 and 1000 and our result is within this range. The molecular size of the guest molecules examined in the past on complexation with β-CD is comparatively small. On the other hand the guest molecule now in question is a large-sized dipeptide and so we have the problem of the conformational change of the guest molecule on complexation with β-CD. ^{15}N labeling of the amide nitrogen is useful technique for the examination of the conformation of dipeptides. The study of the preferred conformation of APM in an aqueous solution by using the same method as that described in this paper was reported.[13] The torsional angles of ψ and ϕ, (Fig. 5) in the free state are −150° and −90°, respectively. The results differed from those by F. Lelj et al, which were derived from the potential energy calculations.[14] The present results (section c) suggest that the torsional angles ϕ, ψ, and ω of APM do not change on the complexation with β-CD. On the other hand the rotamer fractional populations of both Phe and Asp residues do change in the complex formation. Figure 6 shows the conformation of APM in the various states. The upper figure shows the preferred conformation in the free state and the middle one is the preferred conformation in the complex and the lower one is the least preferred conformation in the complex. The

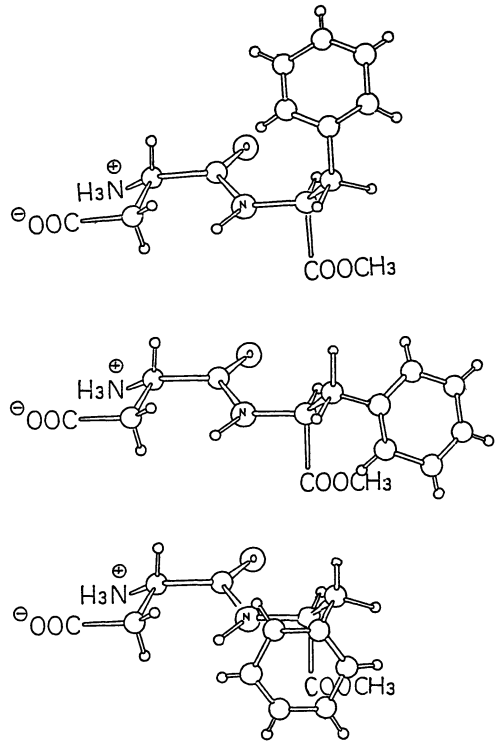


Fig. 6. The conformations of APM molucule in an aqueous solution, (upper) the preferred one in the free state (middle) the preferred one in the complexed state with β-CD (bottom) the least preferred one in the complexed state with β-CD.

preferred conformation in the complex is seen to be rod like and is suitable for the inclusion in the cavity of β-CD. The stereo model of this conformer based on Fig. 6 shows that the amide part of APM is so close to the C2 or C3 hydroxyl group that the hydrogen bonding is possible between them. The least preferred conformer in the complex formation is also reasonable because the phenyl ring is so close to the backbone of APM in this conformer that APM can not be included within β-CD.

In conclusion

(1) The phenyl ring of APM is included with β-CD with the association constant K_a=90 in an aqueous solution.

(2) The estimated distance between the center of the phenyl ring of APM and the plane containing seven H-3 of β-CD is 1.7 Å.

(3) The backbone conformation of APM is not varied in the complexed formation with β-CD.

(4) The side chain of APM is changed to the extented conformation in the complexed formation with β-CD.

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