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Raman and Solid State NMR Study on an Inclusion Compound of Aspartame with Cyclodextrin

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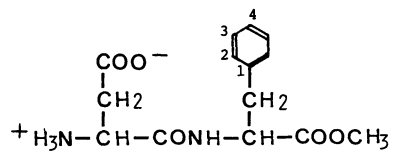
A crystal of inclusion compound of Aspartame (α-L-aspartyl-L-phenylalanine methyl ester, APM) with β-cyclodextrin (β-CD) was obtained from their aqueous solution. The Raman spectrum of this crystal indicates some differences from the spectral sum of APM and β-CD. An analysis of these spectral differences suggests that the phenyl moiety of APM is included in the cavity of β-CD in the inclusion compound. In addition, a Raman spectroscopic study of APM with ^{15}N-substitution and N-deuteration indicates that the peptide backbone of APM undergoes some conformational change on APM going into the inclusion compound. Solid state ^{13}C CP MAS- and gated-decoupling MAS-NMR spectra for the inclusion compound indicate that the phenyl ring rotates at a higher frequency than 270 MHz, that the Phe Cβ and phenyl ring C1 move at the frequency of about 270 MHz, and that the molecular motion of the other parts of APM occurs at lower frequency than 270 MHz.

APM (Scheme 1) is a dipeptide sweetener. We have recently succeeded in obtaining a crystal of an inclusion compound of APM with β-CD which has a higher dissolving rate into H_2O than APM itself. For finding out the mechanism of such a change in that property, a single crystal X-ray diffraction study of the inclusion compound would be powerful. Unfortunately, however, our attempt to obtain a sufficiently large crystal has not so far been successful. Other methods such as Raman spectroscopy[1,2] and solid-state ^{13}C NMR spectroscopy[3–5] may also be useful. We have used the Raman method to elucidate the inclusion mechanism of APM–β-CD and the solid-state ^{13}C NMR method to determine the rate of motion of APM molecule. Such studies would provide not only new pieces of information on the particular inclusion compound now in question but also data for constructing a general view of the CD inclusion compounds, in conjunction with comparison our results with others' for different guest molecules.



Scheme 1 APM

Experimental

Materials. Crystals of inclusion compound of APM with β-CD was prepared from their aqueous solutions, i.e., 6.7×10^{-2} M(=mol dm^{-3}) of APM and 5.5×10^{-2} M of β-CD in water were sealed in a flask and stirred gently at 60 °C for 15 min. The solution was cooled gradually and kept at 4 °C for two weeks. The crystals formed of the compound were precipitated, collected by filtration and dried in a desiccator at room temperature. The composition of this crystal was found by means of an amino acid analyzer, ^1H NMR and Karl Fischer's analysis to be 1:1:6 of APM:β-CD:water. Phenylalanine-^{15}N was obtained from SHOKO Co., Ltd. Phenylalanine-^{15}N methyl ester hydrochloride (**1**) was prepared by the thionyl chloride method. *N*-benzyloxycarbonyl-β-benzyl-L-aspartyl-L-phenylalanine-^{15}N methyl ester (**2**) was synthesized from β-benzyl-*N*-benzyloxycarbonyl-L-aspartate and **1** via dicyclohexylcarbodiimide in the presence of triethylamine in chloroform. α-L-Aspartyl-L-phenylalanine-^{15}N methyl ester (**3**) was prepared from **2** by hydrogenation at room temperature in the presence of 2% palladium charcoal in aqueous methanol. **3** was purified by YMC-PAK SH-343 column chromatography using methanol/0.1 M phosphate buffer pH 5.0 (30:70 by vol) as eluent. The fraction of **3** was collected, concentrated three times with a rotary evaporator, desalted by a SEP-PAK C18 cartridge, and lyophilyzed to dryness.

Apparatus. Raman spectra were recorded on a JASCO R-800 spectrometer. The 514.5 nm exciting line from an argon ion laser (NEC model GLS 3300) was used. Wavenumbers were calibrated against the spontaneous emission (521 cm^{-1}) of Ar^+.

Solid state ^{13}C NMR was recorded on a JEOL GX-270 spectrometer operating at 67.8 MHz and on a JEOL FX-60 spectrometer at 15.1 MHz.

Results

Raman spectra of the inclusion compound of APM with β-CD and their mechanical mixture are shown in Fig. 1. The Raman bands assigned to the APM molecule in the inclusion compound indicate some frequency differences from those of free APM. Thus the bands at 819, 1005, and 1197 cm^{-1} of free APM are shifted to 825, 1002, and 1207 cm^{-1}, respectively, and the bands at 747, 765, 890, and 971 cm^{-1} are caused to disappear. From a comparison with Raman bands of toluene,[6] the shifted bands can be assigned to the normal vibrations at ν_1, ν_{12}, and ν_{13} of monosub-

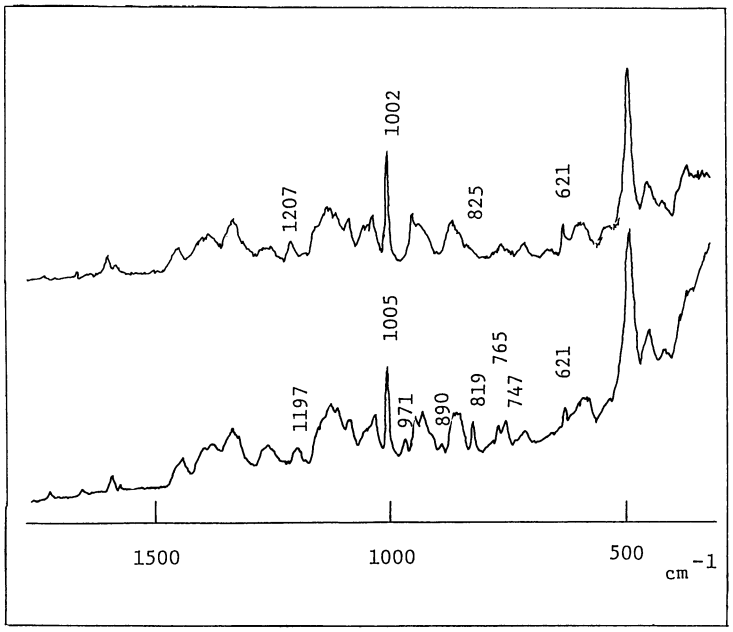


Fig. 1. Raman spectra of the inclusion compound of Aspartame with β-CD (top) and their mechanical mixture (bottom).

Table 1. Raman Bands/cm^{-1} of APM and the Inclusion Compound of APM with β-CD

APM	Inclusion compound	Assignment[a]
819	825	ν_1
1005	1002	ν_{12}
1197	1207	ν_{13}
747	Disappear	
765	Disappear	
890	Disappear	
971	Disappear	

a) Normal vibration of monosubstituted benzene.

Table 2. Raman Bands/cm^{-1} in 600—100 cm^{-1} of APM and Its Derivatives

APM	Asp-Phe-^{15}N-OMe	N-deuterated APM
621	621	621
747	747	745
819	816	812
890	890	886
971	967	960
1005	1005	1004

stituted benzene, respectively. Results of such observations are summarized in Table 1. The observed shifts suggest that the phenyl ring of APM gets located in the cavity of β-CD on the formation of the inclusion compound. The Raman bands observed in the 600—1000 cm^{-1} range with Asp-Phe-^{15}N-OMe and N-deuterated APM are shown in Table 2. The band at 971 cm^{-1} of normal APM is shifted to 967 cm^{-1} on ^{15}N-substitution and to 960 cm^{-1} on N-deuteration. The band width of the 960 cm^{-1} line of N-deuterated APM has been found to increase and this is probably because the band of amide III is overlapping here. The Raman band around 940 cm^{-1} of polypeptides has been assigned to the backbone stretching vibration,[7] and its frequency is reported to be sensitive to the backbone conformation.[8-10] The ^{15}N and N-deuteration shifts of the band (971 cm^{-1}) indicate that it is assignable to this backbone stretching vibration. The disappearance of the band in the inclusion compound suggests that when APM is included in the cavity of β-CD, its backbone undergoes a conformational change.

The 67.8 MHz solid state ^{13}C CP MAS-NMR spectra of the inclusion compound crystal of APM with β-CD and their mechanical mixture are shown in Fig. 2. The observed resonances are assigned by comparing with the spectrum in aqueous solution. The line widths of resonances of the atoms in APM are found larger than those of β-CD in the inclusion compound, especially, the resonances of phenyl ring Cl and Phe Cβ are too broad to be detected. Figure 3a shows a 67.8 MHz ^{13}C gated-decoupling MAS-NMR spectrum of the inclusion compound. In general, resonances of nuclei of higher frequency motion shoud be sharper than those of lower one in this method. A comparison of Figs. 2 (upper) and 3a indicates that the resonances of the phenyl ring carbons except Cl become obviously sharper on gated-decoupling. This fact indicates that the phenyl ring in the cavity of β-CD is rotating rapidly, whereas the other part of the molecule is moving slowly Figure 3b shows a 15.1 MHz solid state ^{13}C CP

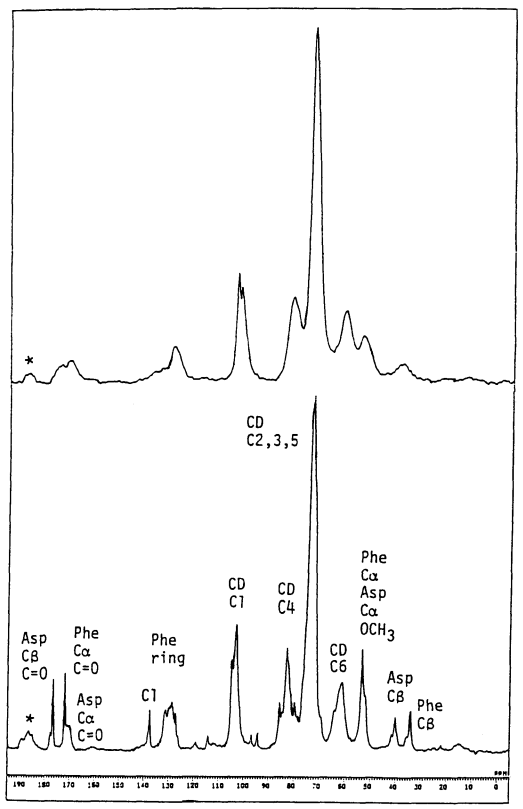


Fig. 2. 67.8 MHz solid state ^{13}C CP MAS-NMR spectra of the inclusion compound of APM with β-CD (top) and their mechanical mixture (bottom). (* denotes spinning side band)

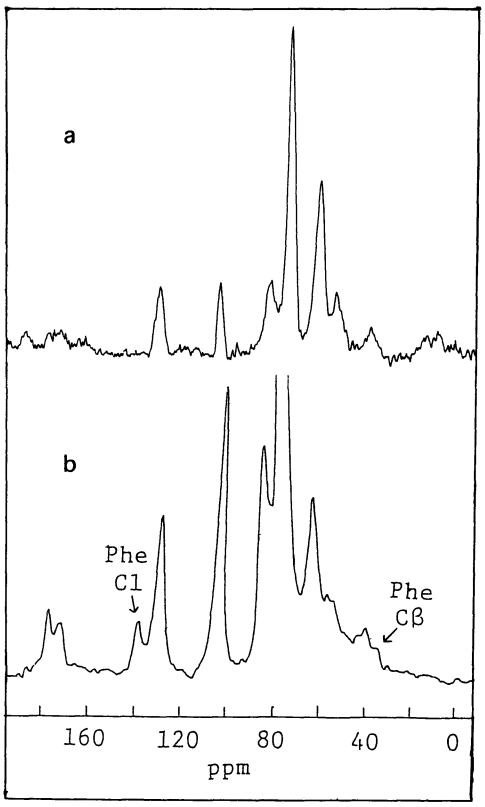


Fig. 3. (a) 67.8 MHz solid state ^{13}C gated-decoulping MAS-NMR spectrum of the inclusion compound of Aspartame with β-CD. (b) 15.1 MHz solid state ^{13}C CP MAS-NMR spectrum of the inclusion compound of Aspartame with β-CD.

MAS-NMR spectrum of the inclusion compound. The resonance of phenyl ring Cl carbon is now found sharper than that at 67.8 MHz, and the resonance of Cβ of Phe residue is detectable here, although its *S/N* ratio is very low. This phenomenon can be explained in terms of the formula proposed by Rothwell and Waugh.[11] In their paper the Hamiltonian of a two-spin system (I=^1H and S=^{13}C) is written as

$$H=-\omega_{0I}I_z-\omega_{0S}S_z-2\omega_1 I_x\cos\omega_{0I}t+d.d., \quad (1)$$

where ω_{0I} and ω_{0S} are the Larmor frequencies of I and S, respectively ω_1 is rf irradiation intensity, and *d.d.* is the term of the dipole-dipole interaction between I and S. If the motion of spin I takes place with a frequency ω_{0I} in the order of magnitude, the line width of the resonance of spin S should be large because I_x of the third term of Eq. 1 decays rapidly during the CP condition. Thus the large line widths of the resonances of Phe Cβ and phenyl ring Cl in 67.8 MHz ^{13}C NMR and the smaller one in 15.1 MHz indicate that the molecular motions of Phe Cβ and phenyl ring Cl occur at about 270 MHz (^1H Larmor frequency in 67.8 MHz ^{13}C NMR). In summary, it may be concluded that the phenyl ring is considered to rotate at higher frequencies than 270 MHz, that the Phe Cβ and phenyl ring Cl move at about 270 MHz, and that the molecular motions of the other parts of APM occurr at lower frequencies than 270 MHz in the inclusion compound.

Discussion

It is an interesting problem to find out what type and magnitude of distortions a guest molecule will undergo on being brought it into an inclusion compound with CD. Several pieces of information have been obtained on this point:[2-5] For example Sato et al.,[2] examined in 1979 some CD inclusion compounds of azo dyes in aqueous solutions. They detected frequency shifts for some Raman lines, and assigned them to Ph-N stretching and ring vibrations of the dye molecules having been brought into the inclusion compounds. There have been a number of reports on the broadening of signals of guest molecules in solid state ^{13}C NMR studies of their inclusion compounds with CD. In general, there may be two possible causes for such broadening: chemical shift dispersion and molecular mobility.[12]

Hall and Lim[13] demonstrated, by the deuterium quadrupolar echo method, that benzene-d_6 molecule is rapidly rotating around the six-fold symmetry axis in the cavity of CD where it is included.

By the use of the particular type of guest molecule, our present study has enlightened several new aspects of the problem. It should be pointed out, first of all, that our present study was made on a crystal in which 100% of the guest molecules are considered to be in the regular inclusion sites. At the same time, however, the guest molecules may be involved in an inter-molecular interaction, besides the interaction with CD, in such a crystal. In our present study some localized changes in the guest molecule have been suggested. Thus, the phenyl group is considered to be located in the CD cavity but seems to rotate rapidly in the cavity. The other part of the molecule seems to be moving slowly (<270 MHz), probably because this part is located outside the CD cavity and fixed by an inter-molecular interaction with the adjacent APM molecule. The inbetween portion, involving the Phe Cβ and Cl atoms, seems to be moving with a medium rate (close to 270 MHz). Probably because of its environmental change, on going from free to included APM, the Raman lines assignable to the phenyl group showed frequency shifts. Of them, the strong and sharp line at 1005 cm^{-1} is considered to be caused by the phenyl ring breathing vibration of the phenylalanine residue. This Raman line is always prominent in the Raman spectrum of a protein as long as it contains phenylalanine residues.[14] A review of such Raman spectra reveals that its frequency is generally insensitive to the conformation of the protein molecule and to the intra-molecular environment of the phenylalanine residue.[15] It is therefore noteworthy that this is shifted from 1005 to 1002 cm^{-1} on going from free to included APM. From this amount of frequency shift, as well as those given in Table 1, we shoud be able to derive a set of force constant changes of the phenyl group on going from free APM to the APM located in the CD cavity. This, however, is not yet to be done.

In conclusion, we have been able to ensure by Raman examination that the phenyl ring is included in the cavity of β-CD in the inclusion compound of APM with β-CD, and by solid state ^{13}C NMR that the phenyl ring rotates rapidly in the cavity.

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