^{1}H and ^{13}C NMR Studies of Formation and Molecular Dynamics of Methylated-Cyclodextrin Inclusion Complexes with Phenylalanine

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The formation and the molecular dynamics of the host-guest inclusion complexes in aqueous solution have been studied for hosts α- and permethylated α-cyclodextrin and permethylated β-cyclodextrin and guest phenylalanine by ^{1}H and ^{13}C NMR spectroscopy. The changes in ^{1}H chemical shift, ^{1}H spin-coupling constant, and ^{13}C spin-lattice relaxation time for the guest phenylalanine on addition of the host cyclodextrin show that they form the inclusion complexes in a similar manner, that is, by insertion of the guest's phenyl ring into the host's cavity. The strength of the host–guest dynamic coupling varies with the cavity size of cyclodextrins.

The cyclodextrins are a series of cyclic oligosaccharides composed of at least six(1→4)-linked α-D-glucosyl residues. Each cyclodextrin molecule has a toroidal, hollow, trancated cone with primary and secondary hydroxyl groups crowning the narrower and wider rims, respectively. The interior of the cavity of each cyclodextrin contains two bands of C–H groups and a band of glucosidic oxygens. Hence the interior of the cavity is relatively hydrophobic, whereas the exterior is relatively hydrophilic.

Cyclodextrins can admit various guest molecules into the cavity, without any covalent bonds being formed, and in some cases they catalyze reactions of included guest compounds.[1-4] Because of these properties, cyclodextrins serve as models for studying topochemical aspects and catalytic reactions of enzymes. In particular, cyclodextrins are good models for such hydrolytic enzymes as esterases and proteases in which the hydroxyl group of serine residue attacks the acyl group of a bound substrate.[5] It is well known that a typical serine protease, chymotrypsin, cleaves selectively at a significant rate the peptide bonds on the carboxyl side of residues with bulky side chain such as phenylalanine, tryptophan, and tyrosine. In these residues, the bulky side chains are assumed to be fitted neatly into a nonpolar pocket of chymotrypsin. Cyclodextrins also exhibit specificity for the guest inclusion.

The complexation and catalytic properties of chemically modified cyclodextrins have been extensively investigated.[2,3] Partially and fully methylated cyclodextrins can also form inclusion complexes in aqueous solution,[2,6] some of which are more stable than those of the parent cyclodextrins. Recently, these derivatives have become of great interest as their solubility in water as well as in organic solvents is very high and inclusion behaviors of them are not always the same as those of parent cyclodextrins.[7]

In order to characterize the inclusion behaviors, it is relevant to investigate the stoichiometry, the host–guest orientation, and the molecular dynamics of inclusion complex. In preceding papers,[8-11] the molecular dynamics of the inclusion complexes of α-, β-, and γ-cyclodextrin(α-, β-, and γ-CD, which are composed of **6**, **7**, and **8** glycosyl residues respectively) with some amino acids and dipeptides having aromatic side chain have been studied in aqueous solution by ^{13}C NMR. It was found that the strength of the host–guest dynamic coupling depends on the cavity size of the host cyclodextrin. We now report on the formation and molecular dynamics of inclusion complexes of methylated cyclodextrins with L-phenylalanine(Phe) in aqueous solution by the measurements of ^{1}H NMR parameters and ^{13}C spin-lattice relaxation times. The influence of the methylation of hydroxyl groups of cyclodextrins on the formation and the molecular dynamics of the complexes may be investigated by comparing the results with those obtained for unmodified cyclodextrin–Phe systems. As hosts we chose α-CD, hexakis(2, 3, 6-tri-*O*-methyl)-α-cyclodextrin(α-TMCD), and heptakis(2, 3, 6-tri-*O*-methyl)-β-cyclodextrin(β-TMCD). β-CD could not be studied because its solubility in neutral water is too poor to observe its natural abundance ^{13}C NMR spectrum with high quality.

Experimental

Materials α-CD, β-CD, and Phe were purchased from Nakarai Chemicals Ltd., Kyoto. α-TMCD and β-TMCD were synthesized by the procedure of Casu et al.[12] from α-CD and β-CD, respectively. They were recrystallized twice from hot water. Their structures and purities were confirmed by ^{1}H and ^{13}C NMR spectra as well as by thin-layer chromatography. Their ^{1}H NMR spectra were shown in Fig. 1, in which the previous assignments[13] were partly revised based on the ^{1}H–^{1}H and ^{1}H–^{13}C COSY spectra. The details of assignments will be published elsewhere.[14] $^{2}H_2O$ was purchased from Merck Sharp & Dohme, Canada, Ltd.

Methods. ^{1}H NMR spectra were recorded with a JEOL GX-500 spectrometer operated at 500 MHz. ^{13}C NMR spectra were recorded with a JEOL JNM PS-100 spectrometer operated at 25.1 MHz. The ^{13}C spin-lattice relaxation

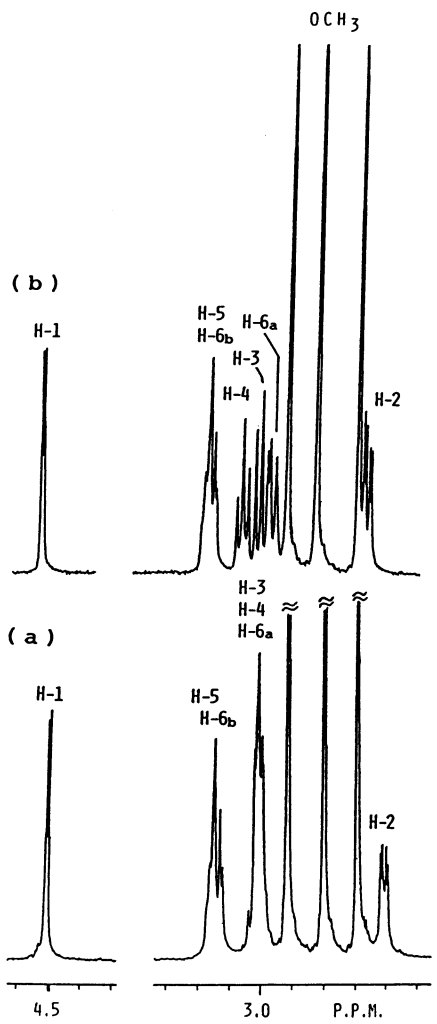


Fig. 1. 500 MHz ^1H NMR spectra of α-TMCD(a) and β-TMCD(b) in 0.01 M solution in ^2H_2O. The previous assignments[13] are partly revised[14] in this figure.

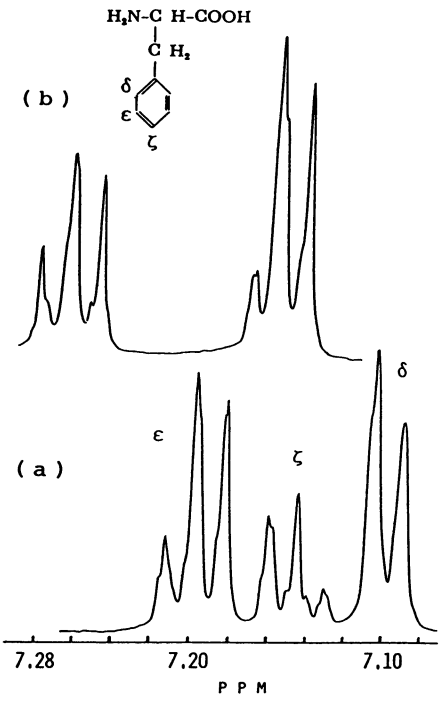


Fig. 2. 500 MHz ^1H NMR resonances of phenyl ring protons of phenylalanine(0.01 M) in the absence(a) and in the presence of α-TMCD(0.07 M)(b) in ^2H_2O.

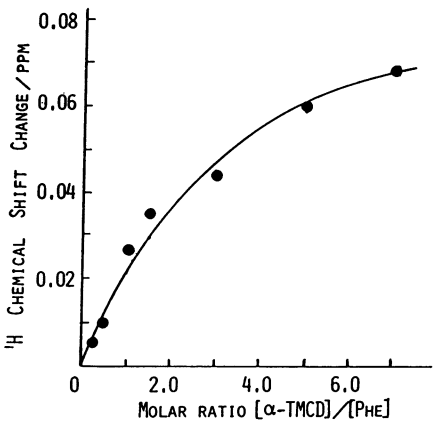


Fig. 3. ^1H chemical shift change of phenylalanine ε-proton on addition of α-TMCD in deuterium oxide, as a function of the molar ratio[α-TMCD]/[Phe]. The solid line indicates theoretically calculated chemical shift changes.

time (T_1) was measured by the inversion-recovery method using a 180°-t-90° pulse sequence, where t is the time interval between the 180° and 90° pulses. The estimated error in the ^{13}C-T_1 values was approximately ±10%. The ^1H- and ^{13}C-chemical shifts were referenced to external tetramethylsilane. The digital resolution of ^1H shift was 0.0006 ppm. The macroscopic viscosity of solution was measured with a Cannon-Finske viscometer.[9] The temperature was kept at 30±2 °C for all measurements.

Results

Cyclodextrin-Induced ^1H-Chemical Shifts in Phe and Dissociation Constants. As found previously,[8-11] ^1H as well as ^{13}C NMR spectra of each cyclodextrin–Phe system consist of only one set of resonances, indicating that only one type of complexation occurs and/or the chemical exchange of the type

$$CD + S \rightleftarrows [CD, S] \quad (1)$$

is rapid process compared with the NMR time scale, where CD, S, and [CD, S] are host cyclodextrin, guest (substrate) Phe, and complex between them, respectively. The phenyl proton resonances of Phe in the 500 MHz ^1H NMR spectra were well resolved and were assigned easily as shown in Fig. 2. On addition of cyclodextrin, the phenyl proton resonances showed remarkably large changes, the example of which is also shown in Fig. 2. By plotting these cyclodextrin-induced ^1H chemical shift changes against the molar

ratio [CD]/[S], it is possible to determine the value of dissociation constant K_d (reciprocal of the association constant K_a) for CD–Phe complexation as shown in Fig. 3. In these experiments, the concentration of Phe was kept constant at 0.01 M and that of cyclodextrin was varied between 0 and ca. 0.07—0.10 M (1 M=1 mol dm^{-3}), depending on the solubility of cyclodextrins. The K_d value was estimated by fitting of the modified Hildebrand-Benesi equations[9,15,16] to the chemical shift change vs. [CD]/[S] curve. Here, it was assumed that a 1 : 1 complex between each CD and Phe was formed and that complexation was reversible as represented by Eq. 1. In Fig. 3 theoretically calculated shifts are also shown by the solid line, reproducing adequately the observed shift changes. Examination of the residuals obtained by subtraction observed shift changes from calculated ones revealed random but not systematic trends and the standard deviation was 0.003 ppm. The reversible dimerization has been suggested for α-TMCD in aqueous solution from its ^{13}C chemical shifts[17], but influence of dimerization was not found in the plot of ^{1}H shift change of guest Phe against [CD]/[S] molar ratio. That influence has also not been found for *p*-nitrophenol–α-TMCD system.[6]

The values of K_d and the cyclodextrin-induced ^{1}H chemical shift change $\Delta\delta$ were summerized in Table 1. Here, $\Delta\delta$ is defined as chemical shift difference between the shifts found in the fully complexed and in the uncomplexed states and was estimated from the chemical shifts at known concentrations of cyclodextrin and Phe, in conjunction with the predetermined K_d value.

The large chemical shift changes of phenyl proton resonances suggest that the Phe molecule forms the complex with each cyclodextrin by insertion of its phenyl ring into the cyclodextrin cavity.

The K_d values for three cyclodextrin–Phe systems are practically the same, although that for the α-CD–Phe system is the smallest and that for the β-TMCD one the largest.

Conformational Change of Phe by Complexation with Cyclodextrins. In order to investigate the steric host–guest interaction, a conformational change of the guest Phe on the occasion of complexation with cyclodextrin was analyzed by observing ^{1}H spin coupling constants.[18] The 500 MHz ^{1}H NMR resonances of the Phe α- and β-protons could be analyzed as an ABX three spin system. The ^{1}H coupling constants were observed at the host and guest concentrations of 0.07 and 0.01 M (in ^2H_2O solution), respectively. The coupling constants for Phe in the fully complexed states were estimated from the observed ones with the aid of K_d values. The results were given in Table 2, in which the rotamer populations for the side chain estimated with the use of the Feeney's approximation[19] were also included.

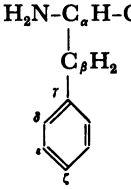
In the free as well as in the complexed states, the most stable rotamer is the gt one with the aromatic ring trans to the carboxyl group. These results are in agreement with the general observations that the aromatic side chain has a tendency to orient itself toward the amino group of the same residue.[20] Thus, the most stable rotamer, gt, in the free state is still the one in the complexed state.

The complexation with α-TMCD induces a small but a notable increase of gt rotamer population with a decrease of gg one. The influence of β-TMCD on the conformation is negligibly small. In the case of α-CD,

Table 1. Dissociation Constants K_d and Complexation-Induced ^{1}H Chemical-Shift Changes $\Delta\delta$ for Cyclodextrin–Phenylalanine System in Deuterium Oxide[a]

Compound	K_d/M[b]	$\Delta\delta$/ppm[c]		
		H_δ	H_ϵ	H_ζ
[Phe, α-CD]	3.0×10^{-2}	0.112	0.106	0.052
[Phe, α-TMCD]	3.3×10^{-2}	0.080	0.103	0.023
[Phe, β-TMCD]	3.5×10^{-2}	0.045	0.039	0.032

a) Assignments of carbon atoms of phenylalanine are as follows.

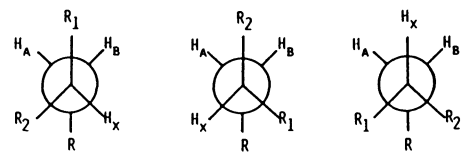


b) Estimated error is less than ±15%. c) $\Delta\delta$ is chemical shift difference between free and fully complexed states, and positive value indicates lowfield shift.

Table 2. ^{1}H Coupling Constants and Populations of Rotamers about C_α–C_β Bond of Phenylalanine in Free State and Complexed State with Cyclodextrin in Deuterium Oxide

Compound	Coupling constant/Hz			Rotamer population[a]		
	J_{AB}	J_{AX}	J_{BX}	gt	tg	gg
[Phe]	14.5	7.9	5.2	0.53	0.26	0.21
[Phe, α-CD]	14.5	7.5	5.5	0.49	0.28	0.23
[Phe, α-TMCD]	14.5	8.5	5.0	0.60	0.26	0.14
[Phe, β-TMCD]	14.5	8.0	5.1	0.54	0.25	0.21

a) Caluculated by Freeney's relation. Rotamer notations are as follows.



g t t g g g

where R=C_6H_5, R_1=COOH, and R_2=NH_2.

the complexation induces a small decrease of gt population. These results indicate that the steric perturbation of the host cyclodextrins on the conformation of the guest Phe is the largest in the α-TMCD system.

^{13}C Spin-Lattice Relaxation Times of Phe. The measurements of ^{13}C-T_1 values are particularly useful for the investigation of molecular dynamics of cyclodextrin inclusion complexes.[8-11] In Table 3 are given the values of ^{13}C-T_1 for Phe in the free state and in the cyclodextrin-Phe mixed systems in aqueous solution. In the limit of rapid exchange process of Eq. 1, one measures an average relaxation rate

$$\frac{1}{T_1} = p_f\frac{1}{T_{1f}} + p_c\frac{1}{T_{1c}} \tag{2}$$

where T_{1f} and T_{1c} are intramolecular spin-lattice relaxation times for a spin in the free and complexed states, and p_f and p_c ($=1-p_f$) are the probabilities that Phe is found in the free and complexed states, respectively.

For the complexation reaction of Eq. 1, the p_c value can be expressed by[8,9]

$$p_c = \frac{1}{2}(1+r)\left\{1-\left[1-\frac{4K_a}{K_a+1}\cdot\frac{r}{(1+r)^2}\right]^{1/2}\right\} \tag{3}$$

where r is the molar ratio of Phe to cyclodextrin and K_a is the association constant ($=1/K_d$).

The viscosity of the cyclodextrin solution varies widely, depending on the type and concentration of cyclodextrin.[9-11] In order to compare the ^{13}C-T_1 values of Phe in different solutions, a correction for the viscosity difference between the solutions is needed. For a molecule of medium size whose motion is rapid enough on the ^{13}C NMR time scale, it is likely that the ^{13}C-T_1 value of the carbon atom having at least one directly-bonded proton is governed by ^{13}C-^{1}H dipole-dipole relaxation brought about by a rotational motion.[21,22] In this case, if the overall molecular motion is isotropic, ^{13}C-T_1 is given by

$$\frac{1}{T_1} = \hbar^2\gamma_C^2\gamma_H^2 N r_{CH}^{-6}\tau_{eff} \tag{4}$$

where τ_{eff} is the effective correlation time for the overall molecular motion, r_{CH} is the carbon-hydrogen bond length, γ_H and γ_C are the gyromagnetic ratios of ^{1}H and ^{13}C nuclei, and N is the number of directly bonded proton. The τ_{eff} can be related to the viscosity of solution, η, by assuming the Brownian diffusion model.[21-23] As the result, ^{13}C-T_1 is given as follows

$$\frac{1}{NT_1} = \hbar^2\gamma_C^2\gamma_H^2 r_{CH}^{-6}\eta f_r V_m/kT. \tag{5}$$

where k is the Boltzman's constant, T is the absolute temperature, f_r is a microviscosity factor, and V_m is the molecular volume. We have shown in the previous paper[8] that the isotropic diffusion model expressed by Eq. 5 was applicable to the analysis of overall molecular motion of Phe in aqueous solution. According to Eq. 5, $NT_1\eta$ value for a given carbon nucleus must be constant, if all situations except the viscosity of solution remain unchanged. The expected

Table 3. Values of ^{13}C NT_1[a] for Free Phenylalanine and Its Inclusion Complexes with Cyclodextrins in Deuterium Oxide

Compound	Concn/10^{-1} M	^{13}C NT_1/s±10%				
		C_α	C_β	C_δ	C_ε	C_ζ
[Phe]	1.0	1.60	1.82	1.85	1.86	1.33
[Phe, α-CD]	1.0, 1.2	0.45	0.58	0.56	0.58	0.38
[Phe, α-TMCD]	0.83, 1.0	0.74	0.81	0.72	0.71	0.40
[Phe, β-TMCD]	0.83, 1.0	0.76	0.82	0.80	0.81	0.56

a) Here, N is the number of proton(s) attached to the carbon and T_1 is the spin-lattice relaxation time.

Table 4. Values of ^{13}C NT_1 and ^{13}C $NT_1\eta$ (in parenthesis) for Phenylalanine in Free State and in Fully Comlexed State with Cyclodextrins in Deuterium Oxide

Compound	Viscosity	^{13}C NT_1 (^{13}C $NT_1\eta$)/s(s cp)				
	η/cp	C_α	C_β	C_δ	C_ε	C_ζ
[Phe]	0.94	(1.50)	(1.71)	(1.74)	(1.75)	(1.25)
[Phe, α-CD]	1.27	0.42	0.54	0.52	0.54	0.35
		(0.53)	(0.69)	(0.66)	(0.69)	(0.44)
[Phe, α-TMCD]	1.43	0.71	0.77	0.68	0.67	0.37
		(1.02)	(1.10)	(0.97)	(0.96)	(0.53)
[Phe, β-TMCD]	1.70	0.75	0.80	0.78	0.79	0.54
		(1.28)	(1.36)	(1.33)	(1.34)	(0.92)

constancy of $NT_1\eta$ values has been certainly observed for aqueous solutions of Phe,[9] where the solution viscosity was controlled by adding various amounts of D-glucose. Thus, the ^{13}C-T_1 values of free Phe molecule in the cyclodextrin-Phe mixture can be estimated by multipling the ^{13}C-T_1 values observed in the solution containing only Phe by the value of viscosity ratio η(solution of cyclodextrin–Phe mixture)/η(solution of Phe). By using these T_1 values in Eq. 2 as T_{1f} values of Phe in the cyclodextrin–Phe solution, the T_{1c} values are calculated for each system. The results are shown in Table 4 with the values of solution viscosity.

The NT_{1c} values for phenyl ring C_ζ are always shorter than those of other ring protonated carbons. These results indicate the existence of rapid internal rotation of the Phe ring in addition to the overall molecular motion even in the complexed states. The rotational motion of Phe ring about the C_β–C_γ bond makes lengthen the NT_1 values of C_δ and C_ε. This motion, however, cannot affect that of C_ζ, since the C_ζ–H bond is on the extension line of rotational axis.[9]

To investigate strictly the influence of complexation on the molecular motion of the guest Phe, we used the $NT_1\eta$ values, which are shown also in Table 4 (in parenthesis). According to the theoretical prediction expressed by Eq. 5, a decrease in $NT_1\eta$ value corresponds to an increase in molecular volume Vm. For cyclodextrin–Phe inclusion systems, the greater the dynamic coupling between them, the greater is the increase in the apparent molecular volume of the Phe and hence the greater is the decrease in the $NT_1\eta$ value, as the molecular volume of cyclodextrins is about 6—9 times larger than that of Phe.[8] Thus, the extent of the decrease in the $NT_1\eta$ value may be used as a measure of the strength of dynamic coupling of Phe with cyclodextrins. As can be seen from the data in Table 4, all of the $NT_1\eta$ values show a decrease, to greater or lesser extents, on the addition of cyclodextrins. These results clearly indicate that the molecular motion of Phe is restricted by the cyclodextrins.

The degree of restriction can be clarified by estimating the ratios of $NT_1\eta$ values for the complexed and free states of Phe, which are shown in Table 5. It is noticeable that almost all phenyl-ring carbons in the three cyclodextrin–Phe systems show larger decreases in the $NT_1\eta$ values than those for other carbons in the same systems, indicating a larger slow-down of the internal rotation of the phenyl ring than that of the other part or of the overall molecular motion. The results clearly show that the Phe molecule forms truly the inclusion complexes with three cyclodextrins by insertion of its phenyl ring into the cyclodextrin cavities. This conclusion is supported by the large chemical shift changes of phenyl protons as shown in the preceding section.

Table 5. Values of ^{13}C $NT_1\eta$ Ratios for the Complexed and Free States of Phenylalanine[a]

Compound	$(NT_1\eta)_{complex}/(NT_1\eta)_{free}$				
	C_α	C_β	C_δ	C_ε	C_ζ
[Phe, α-CD]	0.35	0.40	0.38	0.39	0.36
[Phe, α-TMCD]	0.68	0.64	0.56	0.55	0.42
[Phe, β-TMCD]	0.85	0.80	0.76	0.77	0.74

a) The corresponding $NT_1\eta$ values are given in Table 4.

The $NT_1\eta$ values of the phenyl carbons suffer the largest reduction on complexing with α-CD. In this case, the values of C_α and C_β also suffer the largest reductions. On the other hand, the $NT_1\eta$ values of Phe show the intermediate and the smallest reductions by comlexation with α- and β-TMCD, respectively.

Discussion

The changes of chemical shifts and ^{13}C-T_1 of the guest Phe on addition of the hosts α-CD, α-TMCD, and β-TMCD show clearly that they form the inclusion complexes in a similar manner, namely, by insertion of the guest's phenyl ring into the host's cavity. The results that the NT_1 values of C_δ and C_ε in Phe phenyl ring agree well with each other and they are always larger than those of C_ζ indicate that all cyclodextrins investigated here favor the axial inclusion in which the internal rotation axis C_γ–C_ζ of the phenyl ring is parallel or nearly parallel to the axis of the cyclodextrin cavity. Thus, it is concluded that the permethylation of hydroxyl groups of cyclodextrin does not modify essentially the mode of Phe inclusion. It is generally accepted that many benzene derivatives form 1:1 complexes with α-CD by inserting their benzene rings into the α-CD's cavity from its secondary hydroxyl side, namely from the wider side of cavity. The same type inclusion has been also found for α-TMCD–*p*-nitrophenol system in aqueous solution.[6] The phenyl ring of Phe may be also inserted into the cavities of α- and β-TMCD as well as into that of α-CD[8–11] from the wider side with C_α as the head.

Several mechanisms have been proposed to interpret the formation of cyclodextrin inclusion complexes; such as van der Waals interactions between the guest and cyclodextrin, hydrogen bonding between the guest and the hydroxyl groups of cyclodextrins, release of strain energy in the macrocycle of cyclodextrin and so on.[1,2] But the driving force(s) for formation of cyclodextrin inclusion complexes is(are) still unclear and a matter of speculation.

In the general cases of the enzyme–substrate complex formation the binding force is supposed to be provided, at least partly, by hydrophobic interaction. The methylation of the hydroxyl groups of cyclodextrin is expected to increase the extent of the low

polar or apolar sphere of its cavity. In fact, heptakis (2,6-di-*O*-methyl)-β-cyclodextrin is found to be a hydrophobic solute in contrast with α-CD which is hydrophilic.[24,25] Thus, the permethylation may result an increasing the binding tendency of the Phe's apolar phenyl ring to the cyclodextrin cavity, if the hydrophobic interaction is the major mechanism for the formation of the inclusion complexes. The values of K_d and ^{13}C relaxation time do not support this expectation. Instead, the K_d values for both permethylated cyclodextrin–Phe systems are more or less larger than that for the α-CD–Phe system. The strength of dynamic coupling between the host and the guest estimated from the ^{13}C-T_1 is the largest also in the α-CD complex. Thus; only the hydrophobic interaction, although it may play some role, may not be the major driving force for the formation of inclusion complexes between Phe and permethylated cyclodextrins investigated here.

The X-ray crystallographic studies of the inclusion complexes of α-CD[26] and α-TMCD[27] with *p*-nitrophenol have shown that the size of the secondary hydroxyl side of the cyclodextrin cavity is widened by the methylation of all hydroxyl groups. In these cases, the intermolecular hydrogen–hydrogen distances indicate that the benzene ring of the guest *p*-nitrophenol has weaker van der Waals contacts with the inside of the α-TMCD cavity than with that of the α-CD cavity. Similar situations must hold for the cyclodextrin–Phe systems investigated here. The orders of stability of complex manifested in K_d values and the strength of host–guest dynamic coupling estimated from the ^{13}C relaxation times are related to that of cavity sizes of cyclodextrins. Namely, according to the CPK space-filling molecular models, the cavity size of α-CD is the smallest and that of β-TMCD is the largest. α-TMCD has the cavity of intermediate size. The steric contacts between the host and the guest molecules are expected to become the greatest and the smallest in the α-CD and β-TMCD systems, respectively. Therefore, the van der Waals interactions seem to play a significant role for the formation of cyclodextrin inclusion complexes with Phe in aqueous solution. This is also supported by the facts that the greatest and the smallest $\Delta\delta$ values were observed for the Phe aromatic proton resonances in the α-CD and β-TMCD systems, respectively, although it is not easy to interpret the signs and the magnitudes of the complexation-induced shifts.

The formation of hydrogen bond between the peripheral secondary hydroxyl groups of α-CD and the carboxyl group of Phe may also contribute, more or less, to the stabilization of complex, as the population of gt rotamer decreased and those of tg and gg rotamers increased slightly, on complexation with α-CD. In the α-CD inclusion complex, where the phenyl ring or Phe is inserted into the cyclodextrin cavity from its secondary hydroxyl side, the carbonyl group of Phe can form the hydrogen bond with the cyclodextrin's hydroxyl groups when the Phe side chain takes the tg and gg rotamers. The existence of this hydrogen bond is also suggested from the ^{13}C $NT_1\eta$ values for Phe C_α and C_β, which reveal significant decrease on complexation with α-CD (Table 5), indicating the reduction of mobility of these carbons in the complexed state. This reduction may be due to the anchoring effect of the hydrogen bond on the molecular motion.[28]

In the case of α-TMCD complex, the tg and gg rotamers may become unfavorable because of the repulsive interactions between the Phe's carbonyl and α-TMCD's methoxyl groups. Thus, the gt rotamer, in which the carbonyl group is far away from the methoxyl groups, becomes more probable. On complex formation with β-TMCD, which has the largest cavity size, the rotamer populations of Phe side chain remain unchanged. The cavity size of β-TMCD may be large enough to avoid severe contact between the Phe carboxyl and β-TMCD's rim even in the tg and gg rotamers. This conclusion is supported again by the ^{13}C $NT_1\eta$ values of C_α and C_β in the complexed state, which are comparable to those in the free state.

In conclusion, permethylated cyclodextrins form inclusion complexes with phenylalanine in a manner similar to parent cyclodextrins, but stability and strength of host-guest dynamic coupling change significantly by methylation. The NMR is useful method for studies of formation and molecular dynamics of cyclodextrin inclusion complexes.

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