Molecular Dynamics of the Inclusion Complexes of Cyclohexaamylose with Some Aromatic Amino Acids and Dipeptides

Yoshio INOUE,* Yukio KATÔNO, and Riichirô CHÛJÔ

Department of Polymer Chemistry, Tokyo Institute of Technology, O-okayama 2-12-1, Meguro-ku, Tokyo 152

(Received November 29, 1978)

The molecular dynamics of the inclusion complexes of cyclohexaamylose as a model of enzyme have been studied by means of carbon-13 NMR spectroscopy. As substrates we have chosen L-phenylalanine, L-tyrosine, L-tryptophan, glycyl-L-phenylalanine, and L-phenylalanyl-L-lysine. The molecular motion of both the cyclohexaamylose and the substrates in D_2O–DCl solutions have been investigated by dividing the spin-lattice relaxation time into two contributions, the overall molecular reorientation and the anisotropic internal rotation. Upon complex formation, the correlation times for the internal motion of the phenyl ring of phenylalanine residue increase by a factor of up to 8, while those for the overall reorientation increase by a factor of only 2. These results indicate that the complex formation of the substrate with the cyclohexaamylose are induced by the insertion of aromatic side chain into a cavity of cyclohexaamylose even in the aqueous solution. The overall correlation times of the substrates are about three to seven times shorter than those of the host molecule. Thus the forces which bind the host cyclohexaamylose and the substrate are relatively weak. It was observed that the tightness of the inclusion varies with the types of aromatic amino acids and dipeptides.

Cycloamylose (cyclodextrin, CD) is known to form inclusion complexes with various types of small molecules of appropriate size in solution as well as in the crystalline state.[1-3] Cyclohexaamylose (α-CD, cyclohexaglucopyranose) has the shape of a hollow truncated cone with 6 primary and 12 secondary hydroxyl groups crowning opposite ends of its torus. All the glucose units in CD are present in the Cl chair conformation, and the CH groups of carbons 3 and 5 of each unit compose the inside of the hollow torus, even in the aqueous solution.[1,2,4-6] Thus, the hollow space should be relatively hydrophobic.

Cycloamyloses have been used as models for enzymes[7] because their structures are well defined and because they can specifically bind substrates into their hydrophobic cavity. Cycloamyloses are especially good models at present for hydrolytic enzyme, chymotrypsin.[2-7] It is well known that chymotrypsin does not cleave all peptide bonds at a significant rate, and rather it is selective for peptide bonds on the carboxyl side of residues with aromatic side chains, tryptophan, tyrosine, and phenylalanine, and of large bulky hydrophobic residues such as methionine.[8] In these residues aromatic or bulky nonpolar side chains are assumed to be fitted neatly into a nonpolar pocket on chymotrypsin chain.

It is of great interest to investigate the molecular dynamics of inclusion complexes between CD and aromatic amino acids and peptide as models for enzyme-substrate specific binding. Interactions between phenylalanine and α-CD and β-CD (cycloheptaamylose) in aqueous solution have been studied using ^{1}H NMR.[5,6] It has been also studied the thermodynamics of binding of the aromatic guest molecules, including L-phenylalanine (Phe), L-tyrosine (Tyr), and L-tryptophan(Try), to α- and β-CD.[9] These studies have been providing the evidence for the inclusion nature of CD's complex formation with the aromatic side chain of amino acids. Since measurements of carbon-13 spin-lattice relaxation times are particularly useful for the investigation of molecular dynamics of CD inclusion complexes in detail,[10,11] we will study the molecular motion of both guest and host molecules in the inclusion complexes of α-CD with amino acids and dipeptides having aromatic side chain in aqueous solution by means of carbon-13 spin-lattice relaxation. As substrates we will chose Phe, Tyr, Trp, glycyl-L-phenylalanine(Gly–Phe), and N^{α}-(*N*-acetyl-L-phenylalanyl)-L-lysine methyl ester(Phe–Lys) for studying the effect of the types of aromatic ring and of the peptide sequence on the dynamics of the inclusion complex.

Experimental

Materials. α-CD, Phe, Tyr, Try, and Gly–Phe were purchased from Tokyo Kasei Kogyo Co., Ltd. Phe–Lys was supplied by the courtesy of Mr. M. Sakurai of our laboratory. D_2O (isotopic purity: 99.7 atom %D) and 38% (w/w) DCl solution in D_2O were used as solvents and the deuterium field-frequency lock signal in ^{13}C NMR measurement. These deuterated compounds were purchased from Merck Sharp and Dohme Canada Ltd.

Methods. ^{13}C spin-lattice relaxation times (^{13}C-T_1) were measured by the inversion-recoverly method using a 180°-*t*-90° pulse sequence as described by Freeman and Hill,[12] where *t* is time in seconds between the 180° and 90° pulses, on a JEOL JNM PS-100 spectrometer (25 MHz) equipped with a PFT-100 Fourier-transform system and a proton noise decoupler. Data were accumulated in a JEOL JEC-6 computer using 4000 Hz sweep widths in 4096 points (resolution: 2.0 Hz). The 90° pulse recycle times were chosen to be at least five times the longest ^{13}C-T_1 to be measured. The molar concentrations in D_2O–DCl solutions of both α-CD and the substrates for the ^{13}C NMR measurements were kept *ca.* 0.1 M, namely the molar ratio of α-CD to the substrate was 1: 1 in the mixture. Samples in D_2O–DCl solutions were thoroughly deoxygenated with nitrogen in order to prevent from the paramagnetic effect of oxygen molecules on T_1 values, because ^{13}C-T_1 of protonated carbon nucleus obtained on such samples may be identical with those from samples degassed by repeated freeze-pump-thaw cycles.[13,14] In all experiments the decrease in amplitude of each resonance with increasing *t* followed an exponential curve characterized by a single T_1, and there was no evidence of heterogeneous relaxation times. The estimated error in T_1 was less than ±10%.

Ultraviolet absorption (UV) spectra were taken on a Beckman-25 spectrometer. For the UV measurements, the

molar concentrations of the aromatic substrates were kept *ca.* 5×10^{-5} M and those of α-CD were varied from zero to 10^{-2} M.

The pH values, read on a TOA pH meter HM-7A, of all samples were adjusted to *ca.* 2 unless otherwise stated. The temperature was kept at 32±2 °C for all experiments.

Results and Discussion

Association Constants. The association constants (K_a) for the complexation of Phe, Tyr, and Trp with α-CD have been estimated by the UV spectral changes induced by the addition of α-CD.[15] The K_a values obtained by assuming the 1:1 complexation[5,6,9] were *ca.* 2×10^2—6×10^2 M for these three α-CD-substrate systems. These values are comparable with those of closely related systems,[2,3,6,9] where the complexes are of the 1:1 inclusion types. Thus, in this paper, the values of ^{13}C-T_1 were analysed by assuming that the complexation of the substrates with α-CD are induced by the insertion of an aromatic side chain of the substrate into a cavity of α-CD.

Carbon-13 NMR Spectra. All peaks in the ^{13}C NMR spectra of both α-CD and the substrates have been assigned based on the assignments previously given by several authors.[16] The ^{13}C NMR spectrum of each α-CD-substrate system consists of only one set of peaks, indicating that only one type of complexation occur and/or the chemical exchange process expressed by Eq. 1 is rapid on the ^{13}C NMR time scale,

$$\alpha\text{-CD} + \text{S} \rightleftarrows [\alpha\text{-CD, S}] \tag{1}$$

where S and [α-CD, S] are the substrate and α-CD-substrate complex, respectively. The ^{13}C chemical shifts induced in the spectra of α-CD by complexation with the substrates were less than 0.40 ppm of upper-field shifts, which could be attributed to the hydrophobic nature of the interaction between α-CD and the substrate.[11,17]

^{13}C Relaxation Times. In the limit of rapid exchange process of Eq. 1, one measures an average relaxation rate[18]

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

where T_{1f} and T_{1c} are intramolecular spin-lattice relaxation times for a spin at the free and complex states, and p_f and p_c ($=1-p_f$) are the probabilities that α-CD or the substrate is found in the free and complex states, respectively. Eq. 2 is valid only if the relaxation times T_{1f} and T_{1c} are much larger than the life times in the free and complex states. Since the association-dissociation process between α-CD and the aromatic substrates in the aqueous solution is performed in the microsecond to millisecond range[3] and the observed values of ^{13}C-T_1 are in the order of 1—10^{-1} s (see below), the Eq. 2 is applicable to the present study.

For the reaction of Eq. 1, the probability p_c to find a α-CD or a substrate molecule in the complex state can be expressed by

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

where k is the molar ratio of α-CD to substrate S (or converse). In the present case, $K_a\simeq10^2\gg1$ and $k=1$, hence $p_c\simeq1$. Eq. 2 can be, therefore, simplified as,

$$\frac{1}{T_1} \simeq \frac{1}{T_{1c}} \tag{4}$$

that is, the ^{13}C-T_1 values observed in the present α-CD-substrate mixed system can be considered to be exclusively that of the complex state.

The values of ^{13}C relaxation time measured for the free substrates, free α-CD, and the complexes between them are listed in Table 1. From this table we shall analyse the molecular motions in the inclusion complexes.

For molecules of medium size whose motion is rapid on the ^{13}C NMR time scale, it is likely that the ^{13}C-T_1 value of the carbon atom directly linked at least one proton is governed by ^{13}C-^{1}H dipole-dipole relaxation brought about by a rotational motion.[19] In this case, if the overall molecular reorientation is isotropic, ^{13}C-T_1 is given by

$$\frac{1}{T_1} = \hbar^2\gamma_C^2\gamma_H^2 N r_{CH}^{-6}\tau_{eff} \tag{5}$$

where τ_{eff} is the effective correlation time for overall molecular reorientation, 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. This τ_{eff} can be related to the isotropic rotational diffusion constant D, and according to the Brownian diffusion model[20—22]

$$\tau_{eff} = \frac{1}{6D} = \frac{8\pi\eta f_r r_0^3}{6kT} = \frac{V_m\eta f_r}{kT} \tag{6}$$

where k is Boltzmann's constant, T is the absolute temperature, η is the viscosity of the solution in poise, f_r is a microviscosity factor, r_0 is the radius of a spherical solute molecule, and V_m is the molecular volume $V_m=4/3\pi r_0^3$. From Eqs. 5 and 6, a relationship between NT_1 value and the molecular volume is derived as follows

$$\frac{1}{NT_1} = \hbar^2\gamma_C^2\gamma_H^2 r_{CH}^{-6}\eta f_r V_m/kT. \tag{7}$$

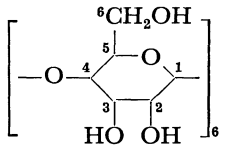
If α-CD is considered to be a spherical molecule of 7.31×10^{-10} m radius,[2,3] and the viscosity of the solution is assumed to be determined mainly by the solvent D_2O ($=1.04\times10^{-2}$ poise at 32 °C[24]), NT_1 values for the ring carbons of α-CD in the free states can be calculated from Eq. 7 using a value of f_r equal to unity[20] and the temperature equal to 305 K. We have 0.122 s for NT_1 value which agrees well with the observed mean value of 0.132 s for ring carbons C_{1-5} within experimental error. For the carbons of the free amino acids and dipeptides NT_1 values were calculated by a similar way. Here, the values of molecular volume of these molecules were calculated by the atomic increments method based on the van der Waals radii of the constituent atoms.[25] Since the molecules investigated here have the freedom of local internal rotation in the side chain and the backbone in addition to the overall motion, it is difficult to find the NT_1 value of the carbon which is determined mainly by the overall reorientation of the molecule. In general, the additional internal

Table 1. Values of ^{13}C NT_1[a,b] for cyclohexaamylose (α-CD), substrates, and molecular inclusion complexes between them measured in D_2O–DCl solutions at 32 °C and pH 2

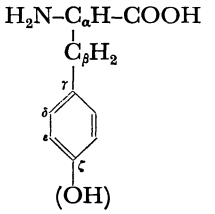
Compd[c]	^{13}C NT_1(s)±10%						
	1	**2**	**3**	**4**	**5**	**6**	$\langle T_1\rangle_{1-5}$ [α-CD][d]
[α-CD]	0.127	0.136	0.128	0.133	0.135	0.168	0.132
[α-CD, Phe]	0.115	0.119	0.131	0.120	0.119	0.140(0.83)	0.121(0.92)
[α-CD, Tyr]	0.124	0.113	0.110	0.105	0.118	0.164(0.98)	0.114(0.86)
[α-CD, Trp]	0.121	0.124	0.127	0.127	0.119	0.164(0.98)	0.124(0.94)
[α-CD, Gly–Phe]	0.097	0.114	0.115	0.114	0.099	0.124(0.74)	0.108(0.82)
[α-CD, Phy–Lys]	0.083	0.090	0.098	0.081	0.088	0.100(0.60)	0.088(0.67)
	α	β	δ	ε	ζ		
[Phe]	1.34	1.60	1.44	1.44	1.08		
[α-CD, Phe]	1.06(0.79)	1.18(0.74)	0.91(0.63)	0.91(0.63)	0.82(0.76)		
[Tyr]	0.99	1.26	1.16	1.16			
[α-CD, Tyr]	0.67(0.68)	0.86(0.68)	0.87(0.75)	0.87(0.75)			
	α	β	δ_1	ε_3	ζ_2	η	ζ_1
[Trp]	0.81	0.84	0.76	0.73	0.84	0.73	0.73
[α-CD, Trp]	0.61(0.75)	0.76(0.90)	0.40(0.53)	0.36(0.49)	0.41(0.49)	0.41(0.56)	0.37(0.51)
	Phe-α	β	δ	ε	ζ	Gly-α	
[Gly–Phe]	0.99	1.14	1.05	1.02	0.75	1.42	
[α-CD, Gly–Phe]	0.56(0.57)	0.82(0.72)	0.66(0.63)	0.62(0.61)	0.51(0.68)	0.64(0.45)	
[Phe–Lys][e]	Phe-α	β	δ	ε	ζ		
	0.34	0.42	0.53	0.54	0.37		
	Lys-α	β	γ	δ	ε		
	0.39	0.40	0.72	0.92	1.14		
[α-CD, Phe–Lys][e]	Phe-α	β	δ	ε	ζ		
	0.28(0.82)	0.34(0.81)	0.25(0.47)	0.24(0.44)	0.22(0.59)		
	Lys-α	β	γ	δ	ε		
	0.24(0.62)	0.34(0.81)	0.48(0.67)	0.54(0.59)	0.78(0.68)		

a) Here, T_1 is the spin-lattice relaxation time and N is the number of hydrogens attached to the carbon. The measured NT_1 values correspond to the underlined species. b) The values shown in parentheses are the NT_1 ratios of the complexed states to the free states. c) Assignments of carbon atoms are as follows. d) The mean values of T_1 for C_{1-5}. e) Measured at pH 7.0.

α-CD



Phe(Tyr)



Trp

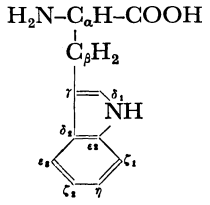


Table 2. Observed and calculated NT_1 values, in seconds, of substrate amino acids and dipeptides

Compd		Observed NT_1	Calculated NT_1	V_m[a]
Phe	C_ζ	1.08	1.01	148
Tyr	C_α	0.99	0.96	156
Trp	C_α	0.81	0.85	176
	$C_{\varepsilon,\eta,\zeta}$	0.73		
Gly–Phe	C_ζ	0.75	0.76	197
Phe–Lys	C_α(Phe)	0.34		
	C_ζ(Phe)	0.37	0.45	329
	C_α(Lys)	0.39		

a) The molecular volume used for the calculation of NT_1 value, estimated by the atomic increments method.[25]

motion faster than the overall one makes the NT_1 value lengthen.[19] Therefore, we regarded the smallest NT_1 values as the representative of the measure of the overall reorientation. Agreements between the calculated and the observed NT_1 values are well as shown in Table 2, notwithstanding the values of molecular volume used in the calculation of NT_1 are only approximate. Thus we can use Eq. 5 for the analyses of the overall molecular reorientation of both α-CD and the substrates in the free states. Eq. 5 might be applicable, at least qualitatively, to the analyses of molecular motions of both α-CD and substrate molecules in the complex states.

For the group undergoing an additional internal motion, the NT_1 value of a protonated carbon with N directly bonded hydrogens is given by[19,26]

$$\frac{1}{NT_1} = \frac{\hbar^2\gamma_H^2\gamma_C^2}{r_{CH}^6}\tau_{eff}\left[A + B\frac{6\tau_G}{6\tau_G+\tau_{eff}} + C\frac{3\tau_G}{3\tau_G+2\tau_{eff}}\right] \quad (8)$$

where τ_G is the correlation time for internal motion and

$$A = 1/4(3\cos^2\theta - 1)^2 \quad (9)$$

$$B = 3\sin^2\theta\cos^2\theta \quad (10)$$

$$C = 3/4\sin^4\theta. \quad (11)$$

Here θ is the angle between the C–H vector and the axis of internal rotation. In the case of rotation of a tetrahedral C–H bond about the C–C bond, $\theta = 109°$, this yields $A = 1/9$, $B = 8/27$, and $C = 16/27$. Similarly, in the case of a phenyl ring rotation about C_β–C_γ bond of the Phe residue, C_δ–H and C_ε–H vectors intersect with the rotational axis at $\theta = 60°$ and 120°, respectively, and then $A = 1/64$, $B = 9/16$, and $C = 27/64$.

From Eqs. 5 and 8, it is clear that the greater the NT_1 value, the more mobile is the ^{13}C moiety, and that the additional internal rotation faster than the overall one makes the NT_1 value lengthen as mentioned in the preceding paragraph.

As shown in Table 1, for α-CD in the free and the complex states, the NT_1 values for the ring carbons (C_{1-5}) are equal within experimental error in each system, indicating the absence of specific fast internal motion in the pyranose ring.[10] Thus the mean value $\langle T_1\rangle_{1-5}$ could be used to calculate the effective correlation times for overall molecular reorientation of α-CD. The NT_1 values show the existence of rapid internal rotation of the primary alcohol group of α-CD, the phenyl group of Phe and Tyr residues, and all the C_β-methylene groups even in the complex states. Definite increase is observed in NT_1 values in Lys residues when going from the α-carbon to the terminal ε-carbon. These results also show the existence of the internal motion.[27] In the case of Phe residue, the axis of rotation of the phenyl ring about C_β–C_γ bond coincides with the C_ζ–H bond. Therefore, the rotation about C_β–C_γ bond cannot affects the T_1 value of C_ζ, since $\theta = 0$ and thus Eq. 8 is reduced to Eq. 5. Then we can estimate the τ_G value for the internal rotation of phenyl ring of Phe residue by using the τ_{eff} value for C_ζ. Since the phenyl ring of Tyr has no C–H bond on the prefered axis of internal rotation, we cannot estimate accurately the τ_G value. The results of calculation of the correlation times are shown in Table 3. Here the correlation time τ_{eff} for the substrate was calculated by adopting the smallest NT_1 value in each substrate as the representative of the overall molecular reorientation having no or a little contribution from the internal motion. Throughout these calculation we have assumed that all C–H bond lengths (r_{CH}) are 1.10×10^{-10} m.

Effect of Complex Formation on the Molecular Motion of α-CD and the Substrates. As can be seen in Tables 1 and 3, all the values of NT_1 (τ_{eff}, τ_G) for both α-CD and the substrates decrease (increase), namely, the motions of these molecules slow down by complexation between them. The reductions of NT_1 are qualitatively explainable as a consequence of the increases in apparent molecular volume by complexation as expected from Eq. 7. The change in molecular dimension induced by complexation is relatively small for α-CD and relatively large for substrates, namely the reduction in NT_1 value of the former is smaller than the latter. For the substrates, it is noticeable that the NT_1 values of aromatic groups show larger changes by complex formation than those of other groups. The effect of the complex formation on the molecular motion could be discussed quantitatively in terms of correlation time. Despite the lack of accurate C–H lengths, the results shown in Table 3 are giving some interesting facts.

Table 3. Rotational correlation time τ_{eff} and τ_G of α-CD, substrates, and molecular inclusion complexes between them[a]

Compd[b]	Correlation time (10^{-11} s)	
	α-CD overall (τ_{eff})[c]	$-CH_2OH$ internal (τ_G)
[α-CD]	37	55
[α-CD, Phe]	41(1.1)	100(1.8)
[α-CD, Tyr]	43(1.2)	36(0.7)
[α-CD, Trp]	40(1.1)	49(0.9)
[α-CD, Gly–Phe]	46(1.2)	130(2.4)
[α-CD, Phe–Lys]	56(1.5)	170(3.1)
	substrate overall (τ_{eff})[d]	phenyl internal (τ_G)[e]
[Phe]	4.6	4.6
[α-CD, Phe]	6.0(1.3)	19 (4.1)
[Tyr]	5.0	4.2[f]
[α-CD, Tyr]	7.2(1.4)	5.6(1.3)[f]
[Trp]	6.7	
[α-CD, Trp]	13 (1.9)	
[Gly–Phe]	6.6	5.6
[α-CD, Gly–Phe]	9.7(1.5)	13 (2.3)
[Phe–Lys]	13	9.0
[α-CD, Phe–Lys]	22 (1.7)	69 (7.7)

a) The values shown in parentheses are the ratios of the correlation times of the complexed to the free states. b) The calculated values correspond to the underlined species. c) Calculated by using the mean values of T_1 for C_{1-5}. d) Calculated by using the smallest NT_1 values shown in Table 1. e) The average T_1 values for $C_{\delta,\varepsilon}$ and for τ_{eff} for C_ζ were used for the calculation of τ_G. f) The values of τ_{eff} were shown, so these values could not compare directly with other τ_G values.

The correlation times for the internal motion of the phenyl ring are clearly showing the mechanism of complex formation. The values of τ_G for the free substrates are comparable or slightly smaller than those of τ_{eff}, indicating the existence of rapid internal rotation of the phenyl ring. Upon complex formation with α-CD, the internal motion of the phenyl ring slows down by a factor of up to *ca.* 8, while the reduction factor of the overall motion is only less than 2. These results support the assumption that the substrates investigated here form the complexes with α-CD by the insertion of an aromatic side chain into a cavity of α-CD even in the aqueous solution. The τ_G values of the phenyl ring in the inclusion complexes show still the existence of apprecia-

bly rapid internal rotation. The results that the NT_1 values of C_δ and C_ϵ in phenyl ring agree with each other and they are always larger than those of C_ζ indicate that α-CD favors the axial inclusion in which the internal rotational axis C_γ–C_ζ of the phenyl ring of the guest is parallel to the axis of the α-CD cavity. According to the space-filling models, the diameter of the cavity of α-CD is about 6.0×10^{-10} m.[3] The molecular diameter, including the van der Waals radii of the proton, of the benzene nucleus is about 6.8×10^{-10} m, thus the axial inclusion is the most natural mode of inclusion.[5]

For the substrates themselves the overall correlation time τ_{eff} increases in the order Phe<Tyr<Gly–Phe≃Trp<Phe–Lys, and this order is also valid after the complex formation. The τ_{eff} value of the three substrates Phe, Tyr, and Trp in the free states are about same and are in the range expected for a molecule of similar size. The Trp, however, shows the prominent increase in τ_{eff} by the formation of complex. The peculiarity of Trp should be a partly due to the difference of the inclusion mode and a partly due to the bulkiness of the indolyl group. In the most probable inclusion mode of the indolyl group, the long axis of the benzene ring $C_{\epsilon3}$–$C_{\zeta1}$ may crosses at about right angles with the axis of the α-CD cavity. This inclusion mode induces larger steric interactions between the indolyl group of Trp and the internal and peripheral group of the α-CD's cavity due to the bulkiness of indolyl group as compared with the axial inclusion of the monocyclic aromatic ring of Phe and Tyr. Thus the bulkiness and type of the aromatic side chain are important factors determining the tight packing of the substrate into the cavity.

Several mechanisms have been proposed for the formation of CD inclusion complex,[2,3,28] but the force driving complex formation and the mechanism of inclusion are still unclear and a matter of speculation.[28] A formation of hydrogen bond between α-CD and substrate may promote complex formation and stabilize resulting complex. It is generally accepted that many benzene derivatives and α-CD form 1 : 1 complexes with benzene ring inserted into the cavity from the secondary hydroxyl side.[3,6,15] According to this model several groups of the substrates investigated here have the hydrogen-bonding capabilities to the α-CD's hydroxyl groups located on the outside of the torus. The existence of hydrogen bond may be confirmable from an analyses of NT_1 values.[29,30] The hydroxyl group of Tyr has no noticeable influence, as compared to the other substrates, on the molecular motions of both α-CD and Tyr in the complexed state, indicating the absence of strong hydrogen bonding interaction between Tyr hydroxyl group and α-CD primary hydroxyl group. The NT_1 value of Gly–C_α in Gly–Phe, in spite of the adjoining carbon to the end group, reveals a significant decrease of motion by complex formation, while that in the free state indicates an evidence of appreciable internal motion. The reduction of Gly–C_α motion may be due to the anchoring effect of the hydrogen bond at the chain end on the molecular motion.[29]

Upon complex formation of Phe–Lys with α-CD, the NT_1 values of alkyl chain carbons of Lys residue show slightly larger reductions as compared with those of C_α and C_β of Phe and Lys residues of Phe–Lys, suggesting an existence of further weak interaction between alkyl chain of Lys residue and α-CD. In this case, two causes can be pointed out as the origin of interaction, *i.e.*, steric hindrance due to access of huge α-CD to Phe residue and $N_\epsilon H$ hydrogen bonding to α-CD. The changes of NT_1 values, however, are too small to clear the causes.

Phe dipeptide, Phe–Lys, in both free and complexed states shows the largest τ_{eff} and τ_G values among the corresponding values of all substrates. The magnitude of Phe–Lys's τ_{eff} is not unreasonable as compared with those of other substrates. However, Phe–Lys shows an unexpected increase in τ_G value of phenyl group by complex formation. Since, in the complex state, the phenyl τ_G value of Gly–Phe is shorter than that of Phe, a largeness of molecular volume or molecular weight of Phe–Lys is not a only reason for this unusual increase in τ_G value. From these results, we can say that the type of the amino acid residue adjoining to aromatic residue in peptide chain is also one of the factors for the tight packing of the substrate into the cavity.

It is noteworthy that, in the complexes, the overall correlation times of the five substrates are about three to seven times shorter than those of the host α-CD molecule. This result indicates the weakness of a dynamic coupling between α-CD and the substrates, namely the extensive independency of a overall motion between them. This result further suggests the shallowness of penetration of the aromatic ring of the substrates into the cavity of α-CD. If the penetration is deep, all motions of aromatic ring other than anisotropic internal rotation about C_β–C_γ bond are greatly restricted within narrow limits due to the steric hindrance. In this case, however, the NT_1 values of C_ζ of Phe residue are too large to explain because the internal rotation about C_β–C_γ bond cannot affects the T_1 values of C_ζ. Thus the penetration of the aromatic ring into the cavity may be an extent to allow the substrate a relatively rapid motion. The shallowness of the insertion is suggested by the absence of hydrogen bond between the hydroxyl group of Tyr and the primary hydroxyl group of α-CD. The results of ^1H NMR study also support the shallow insertion of phenyl ring of Phe into the α-CD cavity.[6] The inclusion of the phenyl ring of D- and L-Phe into the cavity is evident from the upperfield shift of the α-CD's H_3 resonances, but the H_5 resonances are not affected by complexation and furthermore the magnitude of the H_3 upperfield shift induced by complexation is only 0.10 ppm.[6] The three times larger shifts (0.31 ppm) of the H_3 resonances have been observed in the complex between α-CD and *p*-iodoaniline.[6] The strongest coupling between α-CD and the substrate overall motions is seen in [α-CD, Phe–Lys] system, where several intermolecular interaction may participate in the dynamic coupling.

In conclusion, the inclusion complexes between α-CD and the aromatic amino acids and dipeptides investigated here show some characteristics like general peculiarities of enzyme-substrate complex.[8] The host

α-CD has the cavity leading to substrate specifity and the shape matching between the cavity and the substrate is one of the factors determining the strong coupling between them. A nonpolar character of the host cavity and a intermolecular hydrogen bond enhance the binding of substrate and α-CD, but the forces which bind them are relatively weak. The strength of the dynamic coupling also depends on the types of dipeptides.

References

1) F. Cramer, "Einschlussverbindungen," Springer-Verlag, Heiderberg (1954).
2) F. Cramer and H. Hettler, *Naturwissenschaften*, **54**, 625 (1967).
3) F. Cramer, W. Saenger, and H.-Ch. Spatz, *J. Am. Chem. Soc.*, **89**, 14 (1967).
4) C. A. Class, *Can. J. Chem.*, **43**, 2652 (1965).
5) P. V. Demarco and A. L. Thakkar, *Chem. Commun.*, **1970**, 2.
6) D. J. Wood, F. E. Hruska, and W. Saenger, *J. Am. Chem. Soc.*, **99**, 1735 (1977).
7) M. Komiyama and M. L. Bender, *J. Am. Chem. Soc.*, **99**, 8021 (1977); B. Siegel, A. Pinter, and R. Breslow, *ibid.*, **99**, 2309 (1977), and references cited therein.
8) L. Stryer, "Biochemistry," W. H. Freeman and Co., San Francisco (1975).
9) E. A. Lewis and L. D. Hansen, *J. Chem. Soc. Perkin Trans. 2*, **1973**, 2081.
10) J. P. Behr and J. M. Lehn, *J. Am. Chem. Soc.*, **98**, 1743 (1976).
11) K. Uekama, F. Hirayama, and H. Koinuma, *Chem. Lett.*, **1977**, 1393.
12) R. Freeman and H. D. W. Hill, *J. Chem. Phys.*, **53**, 4103 (1970).
13) Y. K. Levine, N. J. M. Birdsall, A. G. Lee, and J. C. Metcalfe, *Biochemistry*, **11**, 1416 (1972).
14) Y. Inoue, A. Nishioka, and R. Chujo, *J. Polym. Sci, Polym. Phys. Ed.*, **11**, 2237 (1973).
15) R. L. VanEtten, J. F. Sebastian, G. A. Clowes, and M. L. Bender, *J. Am. Chem. Soc.*, **89**, 3242 (1967).
16) (a) For α-CD: T. Usui, N. Yamaoka, K. Matsuda, T. Tuzimura, H. Sugiyama, and S. Seto, *J. Chem. Soc. Perkin Trans. 1*, **1973**, 2425; P. Colson, H. J. Jennings, and I. C. P. Smith, *J. Am. Chem. Soc.*, **96**, 8081 (1974); H. Friebolin, N. Frank, G. Keilich, E. Siefert, *Makromol. Chem.*, **177**, 845 (1976). (b) For amino acids and small peptides: W. J. Horsely and H. Sternlicht, *J. Am. Chem. Soc.*, **90**, 3738 (1968); M. Christl and J. D. Roberts, *J. Am. Chem. Soc.*, **94**, 4565 (1972); S. Fermandjian, S. Tran-Dinh, S. Saŭrda, E. Sala, R. Mermet-Bouvien, E. Bricas, and P. Fromageot, *Biochim., Biophys. Acta*, **399**, 313 (1975); O. W. Howarth and D. M. J. Lilley, *Prog. NMR Spectrosc.*, **12**, 1 (1978).
17) W. W. Conover and J. Fried, *Proc. Nat. Acad. Sci. USA*, **71**, 2157 (1974).
18) J. R. Zimmerman and W. E. Brittin, *J. Chem. Phys.*, **61**, 1328 (1957).
19) A. Allerhand, D. Doddrell, and R. Komoroski, *J. Chem. Phys.*, **55**, 189 (1971).
20) J. H. Noggle and R. E. Schirmer, "The Nuclear Overhauser Effect," Academic Press, New York, N. Y. (1971).
21) R. D. Deslauriers, A. C. M. Paiva, K. Schaumburg, and I. C. P. Smith, *Biochemistry*, **14**, 878 (1975).
22) R. D. Deslauriers, Z. Grzonka, K. Schaumburg, T. Shiba, and R. Walter, *J. Am. Chem. Soc.*, **97**, 5093 (1975).
23) A. Hybl, R. E. Rundle, and D. E. Williams, *J. Am. Chem. Soc.*, **87**, 2779 (1965).
24) C. F. Prutton and S. H. Maron, "Fundamental Principles of Physical Chemistry," Macmillan, New York, N. Y. (1951), p 679.
25) J. T. Edward, *J. Chem. Educ.*, **47**, 261 (1970).
26) D. Doddrell, V. Glushko, and A. Allerhand, *J. Chem. Phys.*, **56**, 3683 (1972).
27) H. Saito and I. C. P. Smith, *Arch. Biochem. Biophys*, **158**, 154 (1973).
28) W. Saenger, M. Noltemeyer, P. C. Manor, B. Hingerty, and B. Klar, *Bioorg. Chem.*, **5**, 187 (1976).
29) D. Doddrell, A. Allerhand, *J. Am. Chem. Soc.*, **93**, 1558 (1971).
30) U. Edlund, C. Holloway, and G. C. Levy, *J. Am. Chem. Soc.*, **98**, 5069 (1976).