Formation and Molecular Dynamics of Cycloamylose Inclusion Complexes with Phenylalanine

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The molecular dynamics of the inclusion complexes of cycloamyloses with phenylalanine in aqueous solution at different pD have been studied by means of carbon-13 NMR spectroscopy. As host cycloamyloses we have chosen cyclohexaamylose, cycloheptaamylose, and cyclooctaamylose. The influences of the cavity size of cycloamyloses and of medium pD values on the molecular dynamics of inclusion complexes were studied by using the $NT_1\eta$ values, where T_1 is the carbon-13 spin-lattice relaxation time, N is the number of directly bonded proton to a given carbon, and η is the viscosity of solution. Upon complexation, the $NT_1\eta$ values of phenyl carbons of guest phenylalanine show larger decreases than those of other carbons, indicating that the guest forms the inclusion complexes with the host by the insertion of its phenyl ring into the host cavity even in the case of cyclooctaamylose. It was found that the strength of the dynamic coupling between host and guest depends on the cavity size and pD values of medium. The strongest coupling was observed for the cycloheptaamylose–phenylalanine system at pD 11.3, where the phenyl ring is deeply and tightly included into the cavity. The inclusion of phenyl ring is shallow and loose in the case of cyclohexaamylose and deep and loose in the case of cyclooctaamylose.

The cycloamyloses, well known as cyclodextrins, are a series of cyclic oligosaccharides containing six and more α-1,4-linked D-glucopyranose units. Cycloamylose has the shape of a hollow truncated cone with primary and secondary hydroxyl groups crowning opposite ends of its torus. The CH groups of carbon 3 and 5 of each glucose unit compose the inside of the hollow torus. Thus the inside and the outside of the cycloamylose cavity should be relatively hydrophobic and hydrophilic, respectively. One of the most interesting properties of cycloamyloses are their ability to make a inclusion complex with a variety of guest molecules in their hydrophobic cavity in solution as well as in the solid state, and in some cases they catalyze the reaction of the guest molecule.[1] Because of this property, the cycloamyloses are attractive for many investigators as enzyme recognition and active site models. In particular, cycloamyloses are adequate models for hydrolytic enzyme such as esterase and protease in which the hydroxyl group of a catalytic serine residue attacks the acyl group of a bound substrate.[1,2] It is well known that a typical serine protease chymotrypsin selectively cleaves peptide bonds on the carboxyl side of residues with aromatic side chains and of bulky hydrophobic residues. In these enzymatic reaction, aromatic and bulky nonpolar side chains of substrates are considered 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 cycloamylose and aromatic amino acids as models for enzyme-substrate specific binding. Determination of the geometry of the cycloamylose–substrate complexes as well as the tightness of complexation is essential for a right understanding of the mechanism of enzymatic catalysis of the cycloamylose.

Because of the symmetry of the cycloamyloses and the simplicities of amino acid structures, their carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectra are very simple to analyze. The measurements of carbon-13 spin-lattice relaxation times (T_1) are particularly useful for the investigation of molecular dynamics of cycloamylose inclusion complexes in detail. In a preceding paper,[3] the molecular motions of both host and guest molecules in the inclusion complexes of cyclohexaamylose (α-cyclodextrin, α-CD) with L-phenylalanine (Phe), L-tyrosine, L-tryptophan, glycyl-L-phenylalanine, and L-phenylalanyl-L-lysine in acidic solution have been studied by means of carbon-13 spin-lattice relaxation. It was confirmed there that the complexation of the guest amino acids with the host α-CD are induced by an insertion of aromatic side chain into a cavity of α-CD. The overall correlation times of the substrates are about one third to one seventh shorter than those of the host molecule. It suggests the binding force between the host and the guest is relatively weak and the aromatic ring is shallowly trapped into the cavity. The shallowness of the insertion is also suggested by building a space-filling model in which the diameter of α-CD's cavity is too small for a deep insertion of phenyl ring. Thus the shape matching between the host cavity and the substrate is one of the important factors determining the strong dynamic coupling between host and substrate.

In this paper, we will study the influences of cavity size of the cycloamylose and of pH value of medium on the molecular dynamics of cycloamylose inclusion complexes with L-phenylalanine. As host cycloamyloses we will choose cyclohexaamylose (α-CD), cycloheptaamylose (β-CD), and cyclooctaamylose (γ-CD). Their internal diameters are *ca.* 4.5, 7.0, and 8.5×10^{-10} m, respectively.[1] Molecular model suggests that β-CD makes the most stable and the dynamically strongest inclusion complex with the guest phenylalanine among these cycloamyloses because of its best fitting geometry. The diameter of phenyl ring including the van der Waals radii of proton is about 6.8×10^{-10} m. The cavity sizes of α- and γ-CD may be slightly smaller and larger, respectively, for the fitted complexation with the guest phenylalanine. Since the cycloamylose has the ionizable secondary hydroxyl groups ($pK=12.1$[4,5]) on the entrance region for the guest insertion, it is interesting to study the pH dependence of the molecular dynamics of the inclusion complexes between the cycloamyloses and the substrates with ionic

groups such as amino acids. In this paper, carbon-13 spin-lattice relaxation times will be measured in acidic (pD=2), phosphate buffer (pD=11), and highly alkaline (1 mol dm^{-3} NaOD) solutions.

Experimental

Materials. Cyclohexa-, cyclohepta-, and cycloocta-amyloses, D-glucose, and L-phenylalanine were purchased from Nakarai Chemicals, Ltd., Kyoto. The values of specific rotation $[\alpha]_D^{25}$ of α-, β-, and γ-CD's were +149.9, +161.9, and +177.2, respectively, which agreed well with previously reported values (*i.e.*, +150.5±0.5, +162.5±0.5, and +177.4±0.5).[1,6] D_2O (isotopic purity 99.7 atom%), 38% DCl solution in D_2O, 40% NaOD solution in D_2O, and 85% D_3PO_4 solution in D_2O were purchased from Merck Sharp and Dohme Canada Ltd..

Methods. ^{13}C-NMR spectra were measured on a JEOL JNM PS-100 spectrometer (25 MHz) equipped with a PFT-100 Fourier transform system and proton noise decoupler. Data were accumulated in a JEOL JEC-6 computer and JEOL CM-219 IC core memory (8 K) using 4000 Hz sweep widths in 4096 points. ^{13}C spin-lattice relaxation times (^{13}C-T_1) were measured by the inversion-recovery method using a 180°-t-90° pulse sequence, where t is time in seconds between the 180° and 90° pulses. The 90° pulse recycle times were chosen to be at least five times longer than the longest ^{13}C-T_1 to be measured. The estimated error in T_1 was less than ±10%. Unless otherwise specified, the molar concentrations of cycloamyloses were 1.2 times larger than those of phenylalanine to measure the relaxation times of phenylalanine and *vice versa* to measure the relaxation times of cycloamyloses.

The buffer solutions were made up with 85% D_3PO_4, 40% NaOD, and solute phenylalanine in D_2O and the final pD value was 11.3±0.1 and the ionic strength was about 0.5. The acidic solutions were prepared with 38% DCl in D_2O and they were adjusted to be pD 2.0. The 1 mol dm^{-3} NaOD solutions were prepared with 40% NaOD. The pH values were read on a pH-meter Toko Model TP-101 with a micro combination electrode CE103, which enabled the measurement of pH value of solution contained in a NMR sample tube. The pD value was obtained by adding 0.4 to the pH-meter reading for the correction of isotope effect.[7]

The macroscopic viscosities of the solutions used in the ^{13}C relaxation experiments were measured using a Cannon-Finske viscometer. The values of viscosity η were calculated from the formula $\eta=B t\rho$, where B is the calibration constant for viscometer, t is the efflux time between the two predetermined marks, and ρ is the density of the solution. The constant B was determined by using extremely pure water and the η value of water reported in literature.[8]

Ultraviolet absorption (UV) spectra were measured on a Beckman-25 spectrometer.

The temperature was kept at 34±1 °C for all experiments.

Results

Carbon-13 Chemical Shifts. All peaks appeared in the ^{13}C-NMR spectra of both cycloamyloses and phenylalanine have been assigned previously.[3] Among three cycloamyloses, any conspicuous differences in spectral pattern were not observed. As found previously, the spectra of each cycloamylose–phenylalanine system consists of only one set of peaks, indicating that only one type of complexation occurs and/or the chemical exchange of Eq. 1 is rapid process compared with the ^{13}C-NMR time scale,

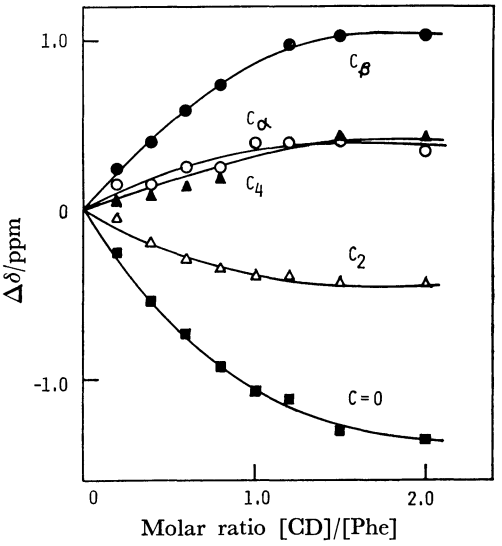


Fig. 1. ^{13}C chemical shifts displacements $\Delta\delta$ of phenylalanine carbons upon addition of β-CD in 1 mol dm^{-3} NaOD solutions as a function of molar ratio [β-CD]/[Phe].

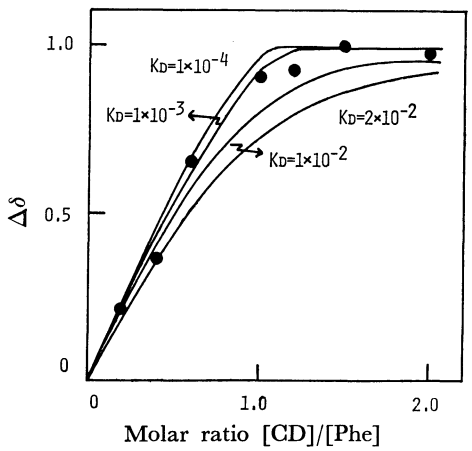


Fig. 2. Theoretically calculated binding curves for a simple CD+Phe⇄[CD,Phe] equilibrium with different dissociation constants K_d. Observed ^{13}C chemical shifts displacements shown in Fig. 1 are normalized and plotted (●).

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

where CD, S, and [CD, S] are host cycloamylose, guest (substrate) phenylalanine, and cycloamylose–phenylalanine complex, respectively.

Complexation induced small ^{13}C chemical shifts of cycloamyloses and phenylalanine because ^{13}C-NMR is not so much sensitive to perturbation through space as ^{1}H-NMR. By an addition of β-CD in alkaline solution, the resonances of phenylalanine showed exceptionally large shifts, although it is not easy to interpret the magnitudes and the directions of the shifts. Figure 1 shows the chemical shift displacements of phenylalanine carbons upon addition of β-CD in 1 mol dm^{-3} NaOD solutions. No substantial changes in

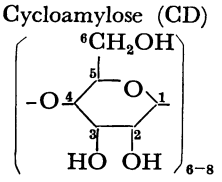
Table 1. Values of ^{13}C NT_1[a] for free cyclohexa-, cycloheptа-, and cycloocta-amyloses (α-, β-, and γ-CD), phenylalanine, and molecular inclusion complexes between them at 34 °C

Compd[b]	Solvent[c]	Concn(M)	^{13}C NT_1(s)±10% Cycloamyloses						
			1	2	3	4	5	6	$\langle T_1\rangle_{1-5}$[d]
[α-CD]	1 mol dm^{-3} NaOD	0.20	0.094	0.086	0.085	0.085	0.094	0.116	0.089
[α-CD, Phe]	1 mol dm^{-3} NaOD	0.20, 0.24	0.074	0.061	0.063	0.067	0.061	0.094	0.065
[β-CD]	1 mol dm^{-3} NaOD	0.20	0.066	0.068	0.071	0.070	0.068	0.096	0.069
[β-CD, Phe]	1 mol dm^{-3} NaOD	0.20, 0.24	0.064	0.054	0.061	0.057	0.054	0.072	0.058
[γ-CD]	1 mol dm^{-3} NaOD	0.20	0.058	0.059	0.059	0.059	0.059	0.080	0.059
[γ-CD, Phe]	1 mol dm^{-3} NaOD	0.20, 0.24	0.057	0.053	0.053	0.055	0.053	0.084	0.054
[α-CD]	buffer	0.10	0.110	0.107	0.112	0.104	0.098	0.132	0.106
[α-CD, Phe]	buffer	0.10, 0.12	0.099	0.093	0.101	0.113	0.090	0.126	0.099
[β-CD]	buffer	0.02, 0.024	0.102	0.083	0.088	0.097	0.083	0.102	0.091
[β-CD, Phe]	buffer	0.025, 0.030	0.110	0.111	0.097	0.103	0.111	0.094	0.106
[γ-CD]	buffer	0.08, 0.096	0.078	0.077	0.084	0.082	0.073	0.118	0.079
[γ-CD, Phe]	buffer	0.08, 0.096	0.082	0.080	0.082	0.094	0.082	0.104	0.084

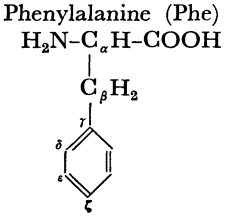
			Phenylalanine				
			α	β	δ	ε	ζ
[Phe]	1 mol dm^{-3} NaOD	0.20	1.41	1.48	1.91	1.87	1.38
[α-CD, Phe]	1 mol dm^{-3} NaOD	0.24, 0.20	0.56	0.70	0.61	0.60	0.39
[β-CD, Phe]	1 mol dm^{-3} NaOD	0.24, 0.20	0.20	0.24	0.29	0.26	0.17
[γ-CD, Phe]	1 mol dm^{-3} NaOD	0.24, 0.20	0.36	0.42	0.39	0.38	0.25
[Phe]	buffer	0.12, 0.10	1.42	1.82	1.82	1.89	1.20
[α-CD, Phe]	buffer	0.12, 0.10	0.45	0.68	0.62	0.61	0.40
[β-CD, Phe]	buffer	0.036, 0.03	0.32	0.52	0.47	0.41	0.28
[γ-CD, Phe]	buffer	0.12, 0.10	0.40	0.42	0.52	0.54	0.37
[Phe][e]	DCl (pD 2)	0.10	1.34	1.60	1.44	1.44	1.08
[α-CD, Phe][e]	DCl (pD 2)	0.10, 0.10	1.06	1.18	0.91	0.91	0.82

a) Here, T_1 is the spin-lattice relaxation time and N is the number of protons attached to the carbon. The measured NT_1 values correspond to the underlined species. b) Assignments of carbon atoms are as follows.

Cycloamylose (CD)



Phenylalanine (Phe)



c) Solvent "buffer" is made up with D_3PO_4 and NaOD and the final pD value is 11.3. d) The mean values of T_1 for C_{1-5}. e) The data were reported by Inoue, *et al.*[3]

chemical shifts occur above molar ratio R=[CD]/[Phe] larger than unity, indicating a formation of a 1:1 complex. From this figure, it is possible to determine the value of dissociation constant K_d (reciprocal of the association constant K_a) for cycloamylose–phenylalanine complex by assuming the 1:1 complexation and by using a modified Hildebrand-Benesi equations.[9,10]

Figure 2 shows theoretically calculated binding curves for a simple CD+S⇄[CD,S] equilibrium with different dissociation constants. They realized by the plot of normalized ^{13}C chemical shift changes $\Delta\delta$ against molar ratios [CD]/[Phe]. In Fig. 2 the experimental $\Delta\delta$ corresponding to cycloheptaamylose–phenylalanine system in 1 mol dm^{-3} NaOD solutions were also included. The dissociation constant K_d for this system was estimated to be approximately 10^{-3} M ($K_a=10^3$ M^{-1}:1 M=1 mol dm^{-3}), which was comparable with K_d values of about 3×10^{-3} M for phenylalanine–cyclohexaamylose[3] and –cyclooctaamylose complexes in acidic solutions at pD 2 measured using UV spectral changes.[3,11]

Carbon-13 Spin-Lattice Relaxation Times. In Table 1 are given the values of ^{13}C spin-lattice relaxation times measured for free cycloamyloses, free phenylalanine, and cycloamylose–phenylalanine complexes.

In the limit of rapid exchange process of Eq. 1, one measures an average relaxation rate $(1/T_1)$[12]

$$1/T_1 = P_f\frac{1}{T_{1f}} + P_c\frac{1}{T_{1c}}, \qquad (2)$$

where T_{1f} and T_{1c} are intramolecular spin-lattice

TABLE 2. VALUES OF ^{13}C NT_1 OF PHENYLALANINE(0.4 M) IN 1 mol dm^{-3} NaOD SOLUTIONS IN WHICH VARIOUS AMOUNTS OF D-GLUCOSE WAS CONTAINED AT 34 °C

Concentration of D-glucose(M)		^{13}C NT_1(s)±10%				
		α	β	δ	ε	ζ
0.0		1.301	1.284	1.542	1.518	0.977
0.6		1.080	0.934	1.142	1.151	0.721
1.5		0.742	0.670	0.713	0.675	0.456
2.5		—[a]	—[a]	0.467	0.451	—[a]
Concentration of D-glucose(M)	Viscosity(η) of solution(cp)	$NT_1\times\eta$				
0.0	1.51	1.96	1.94	2.33	2.29	1.48
0.6	2.06	2.22	1.92	2.35	2.37	1.48
1.5	3.24	2.40	2.18	2.31	2.19	1.48
2.5	4.91	—[a]	—[a]	2.29	2.21	—[a]

a) Not measured accurately.

relaxation times for a ^{13}C spin at the free and complexed states, and P_f and P_c ($P_f+P_c=1$) are the probabilities that cycloamyloses or the substrates are found in the free and complexed states, respectively. For the reaction (1), the values P_c and P_f can be determined from the association constant.[3] For example, if K_a is 2×10^2 M^{-1} and molar ratio [CD]/[S] is 1.2, the substrate of about 99% exists in the complexed state. In our experimental condition, the values of K_a were larger than 10^2 M^{-1} and the molar concentrations of the cycloamyloses were 1.2 times larger than those of phenylalanine to measure the relaxation times of phenylalanine and *vice versa* to measure the relaxation times of cycloamyloses. Thus we can conclude that the T_1 values observed in the cycloamylose–phenylalanine mixtures are exclusively those of the complexed states. From the T_1 values in Table 1 we shall analyze the molecular motions and discuss the dynamical properties of cycloamylose–phenylalanine complexes.

The Models of Molecular Motion to Describe the Observed T_1 Values. For rapidly rotating molecules with medium size, the ^{13}C relaxation mechanism of the protonated carbon nucleus seems to be governed by ^{13}C-^1H dipole-dipole interaction.[12] In this case, ^{13}C-T_1 for the isotropically reorienting molecule is given by

$$1/NT_1 = \hbar^2\gamma_C^2\gamma_H^2 r_{CH}^{-6}\tau_{eff}, \quad (3)$$

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 ^1H and ^{13}C nuclei, and N is the number of directly bonded proton.

According to the Brownian diffusion model,[13] τ_{eff} can be related to the isotropic rotational diffusion constant D by the manner of

$$\tau_{eff} = 1/6D = \frac{8\pi\eta f_r r_o^3}{6kT} = \frac{V_m\eta f_r}{kT}, \quad (4)$$

where k is Boltzmann's constant, T is the absolute temperature, η is the viscosity of the solution in poise (1P=0.1 Pas), r_o is the radius of spherical solute molecule, V_m is the molecular volume $V_m=4/3\pi r_o^3$, and f_r is a microviscosity factor.

For anisotropic molecular motion two or more correlation times must be introduced to interpret the observed T_1 value and τ_{eff} represents a weight average of various correlation times. In general, lengthy procedure is required to construct a model of anisotropic molecular motion. In the case of a rigid ellipsoidal molecule, molecular motions are characterized by two rotational diffusion constants,[13,14] *i.e.*, the diffusion constant for rotation about the symmetry axis, D_a, and that for rotation about any axis perpendicular to the symmetry axis, D_b. For a rigid ellipsoidal molecule in which the C–H internuclear vector makes an angle θ with the symmetry axis, τ_{eff} is defined as a function of angle θ, viscosity η, diffusion constants D_a and D_b, and the lengths of the ellipse semiaxes.[14]

For the group undergoing an additional internal motion as well as isotropic overall molecular reorientation such as phenyl group of phenylalanine,[3] the NT_1 value of a protonated carbon with N directly bonded hydrogens is given by[12,15]

$$\frac{1}{NT_1} = \frac{\hbar^2\gamma_C^2\gamma_H^2}{r_{CH}^6}\tau_{eff}\left[A+B\frac{6\tau_G}{6\tau_G+\tau_{eff}}+C\frac{3\tau_G}{3\tau_G+2\tau_{eff}}\right], \quad (5)$$

where τ_G is the correlation time for internal motion and $A=1/4\ (3\cos^2\theta-1)^2$, $B=3\sin^2\theta\cos^2\theta$, $C=3/4\sin^4\theta$, and θ is the angle between the C–H vector and the axis of internal rotation.

From these discussions, it is clear that the NT_1 value depends on the viscosity of solution, molecular shape and size of rotor, and that the greater the NT_1 value, the more mobile is the ^{13}C moiety and the additional internal rotation faster than the overall one makes the NT_1 value lengthen.

Here we examine the viscosity dependences of ^{13}C-T_1 values. According to model predictions, $NT_1\eta$ values for a specified ^{13}C nuclei must be constant. As seen from Table 2, the expected constancy of $NT_1\eta$ values were certainly observed for phenylalanine in 1 M NaOD aqueous solutions, where the solution viscosities were controlled by adding various amounts of D-glucose. Thus the macroscopic viscosities (macroviscosities[16]) of these solutions, in which there are no specific interaction among solute phenylalanine,

Table 3. Values of $NT_1\eta$[a] for free cyclohexa-, cycloheptа-, and cycloocta-amyloses(α-, β-, and γ-CD), free phenylalanine, and molecular inclusion complexes between them at 34 °C

Compd	Solvent	Viscosity(cp)	$NT_1\eta$ Cycloamylose						
			1	2	3	4	5	6	$\langle T_1\eta\rangle_{1-5}$[b]
[α-CD]	1 mol dm^{-3} NaOD	2.18	0.205	0.187	0.185	0.185	0.205	0.252	0.19
[α-CD, Phe]	1 mol dm^{-3} NaOD	2.24	0.166	0.137	0.141	0.150	0.137	0.210	0.15
[β-CD]	1 mol dm^{-3} NaOD	2.77	0.183	0.188	0.197	0.194	0.188	0.266	0.19
[β-CD, Phe]	1 mol dm^{-3} NaOD	2.67	0.177	0.144	0.163	0.152	0.144	0.192	0.16
[γ-CD]	1 mol dm^{-3} NaOD	2.98	0.173	0.176	0.176	0.176	0.176	0.238	0.18
[γ-CD, Phe]	1 mol dm^{-3} NaOD	3.28	0.187	0.174	0.174	0.180	0.174	0.276	0.18
[α-CD]	buffer	1.57	0.172	0.168	0.176	0.163	0.154	0.208	0.17
[α-CD, Phe]	buffer	1.30	0.129	0.121	0.131	0.147	0.117	0.164	0.13
[β-CD]	buffer	1.28	0.131	0.106	0.113	0.124	0.106	0.130	0.12
[β-CD, Phe]	buffer	1.06	0.117	0.118	0.103	0.109	0.118	0.100	0.11
[γ-CD]	buffer	1.51	0.118	0.116	0.127	0.124	0.110	0.178	0.12
[γ-CD, Phe]	buffer	1.40	0.115	0.112	0.115	0.132	0.115	0.146	0.12
			Phenylalanine						
			α	β	δ	ε	ζ		
[Phe]	1 mol dm^{-3} NaOD	1.35	1.90	2.00	2.58	2.52	1.86		
[α-CD, Phe]	1 mol dm^{-3} NaOD	2.48	1.39	1.74	1.51	1.49	0.97		
[β-CD, Phe]	1 mol dm^{-3} NaOD	3.13	0.63	0.76	0.91	0.81	0.53		
[γ-CD, Phe]	1 mol dm^{-3} NaOD	3.78	1.36	1.58	1.47	1.44	0.95		
[Phe]	buffer	0.97	1.38	1.76	1.77	1.83	1.16		
[α-CD, Phe]	buffer	1.29	0.58	0.88	0.80	0.79	0.52		
[β-CD, Phe]	buffer	1.04	0.33	0.54	0.49	0.43	0.29		
[γ-CD, Phe]	buffer	1.63	0.65	0.68	0.85	0.88	0.60		
[Phe]	DCl (pD 2)	0.94	1.26	1.50	1.35	1.35	1.02		
[α-CD, Phe]	DCl (pD 2)	1.20	1.27	1.42	1.09	1.09	0.98		

a) Here, T_1 is the spin-lattice relaxation time, N is the number of protons attached to the carbon, and η is the solution viscosity. The $NT_1\eta$ values correspond to the underlined species. The corresponding NT_1 values are given in Table 1. See the footnotes of Table 1, also. b) The mean values of $T_1\eta$ for C_{1-5}.

glucose, and solvent water, could be related to the observed T_1 behavior. We have shown in the previous paper[3] that the isotropic diffusion model expressed by Eqs. 3 and 4 was applicable to analyses of the overall molecular reorientation of both free α-CD and aromatic amino acids in acidic aqueous solutions.

In this paper we shall adopt the value of $NT_1\eta$ to investigate the complexation effect on the molecular motions. The $NT_1\eta$ values were tabulated in Table 3 with the viscosities. As shown in Table 1, for cycloamyloses in the free and the complexed states, the T_1 values for the pyranose ring carbons C_{1-5} are equal within experimental error in each system, indicating the absence of specific fast internal motion. Thus the mean value $<NT_1\eta>_{1-5}$ could be used to discuss the overall motion of cycloamyloses. It is noticeable that α-, β-, and γ-CD in the free states have nearly the same $<NT_1\eta>_{1-5}$ values. These results contradict to the model prediction of Eqs. 3 and 4, which stand for the inverse dependence of ^{13}C-T_1 value on molecular volume V_m. The lack of inverse correlation between ^{13}C-T_1 and V_m have been found for several molecules with higher molecular weight in which the various local motions such as segmental motions (localized motion along a backbone of a chain molecule) play a predominant role in ^{13}C relaxation.[17,18] The $<NT_1\eta>_{1-5}$ values of cycloamyloses may be looked upon as standing for the measures of segmental motions.

In the case of phenylalanine, the axis of rotation of the phenyl ring about C_β–C_γ bond is identical with the C_ζ–H bond. Therefore, the rotation about C_β–C_γ bond cannot affect the T_1 values of C_ζ, since $\theta=0°$ and Eq. 5 is reduced to Eq. 3. Thus we can estimate the contribution of phenyl ring rotation to the T_1 values of ring carbons by comparing the T_1 values of $C_{\delta,\varepsilon}$ and C_ζ.[19,20] The $NT_1\eta$ values show the existence of rapid internal rotation of the primary alcohol group of cycloamyloses and the phenyl group of phenylalanine even in the complexed states. The $NT_1\eta$ values of free phenylalanine are not constant but increase greatly with pD values of solutions. According to model prediction, these pD dependences reflect the changes in apparent molecular volume of phenylalanine probably due to the pD depending changes in solvation states of charged groups. If the dynamic coupling between solute and solvent is strong, it is necessary to increase to molecular volume of the solute with that of strongly bound solvent molecules in order for it to fit the theoretical prediction.

We will discuss the complexation effect on the molecular motion of phenylalanine at different pD by comparing $NT_1\eta$ ratios between free and complexed states at same pD.

Discussion

Effect of Complexation on the Molecular Motion of Cycloamyloses and Phenylalanine. As can be seen in Table 3, all the $NT_1\eta$ values for phenylalanine greatly decrease by complexation with cycloamyloses, while those for cycloamyloses decrease slightly or remain unchanged. The reductions of the $NT_1\eta$ values are explainable with a term of the increase in apparent molecular volume by complexation as expected from Eqs. 3 and 4. Since the molecular volumes of cycloamyloses are six to nine times larger than that of phenylalanine, the change in molecular dimension induced by complexation is relatively small for cycloamyloses and relatively large for phenylalanine, namely the reductions in $NT_1\eta$ values of the former are smaller than the latter.

For the substrate phenylalanine, the greater the dynamic coupling with the host cycloamyloses, the greater is the increase in apparent molecular volume of phenylalanine. Thus the degree of $NT_1\eta$ reduction may be used as the measure of the strength of dynamic coupling of phenylalanine with cycloamyloses. In Table 4, it is shown the ratios of $NT_1\eta$ values of the complexed to the free states of phenylalanine. It is noticeable that the $NT_1\eta$ values of phenyl carbons show larger changes by complexation than those of other carbons indicating the larger slowing down of the internal rotation of the phenyl ring than that of the overall reorientation. These results are clearly showing the formation of the inclusion complexes of the guest phenylalanine with the host cycloamyloses by an insertion of the phenyl ring into the cavity of cycloamylose even in the case of γ-CD. The results that the NT_1 values of C_δ and C_ε in phenyl ring agree well with each other and they are always larger than those of C_ζ indicate that cycloamyloses favor the axial inclusion in which the internal rotation axis C_γ–C_ζ of the phenyl ring is parrallel to the axis of the cycloamylose cavity.

Table 4. Values of $NT_1\eta$[a] ratios of the complexed states to free states of phenylalanine

Compound	Solvent	$(NT_1\eta)_{complex}/(NT_1\eta)_{free}$			
		α	β	$\langle\delta, \varepsilon\rangle$[b]	ζ
[α-CD, Phe]	1 mol dm^{-3} NaOD	0.73	0.87	0.59	0.52
[β-CD, Phe]	1 mol dm^{-3} NaOD	0.33	0.38	0.34	0.29
[γ-CD, Phe]	1 mol dm^{-3} NaOD	0.72	0.79	0.57	0.51
[α-CD, Phe]	buffer (pD 11.3)	0.42	0.50	0.44	0.45
[β-CD, Phe]	buffer (pD 11.3)	0.24	0.31	0.26	0.25
[γ-CD, Phe]	buffer (pD 11.3)	0.47	0.39	0.48	0.52
[α-CD, Phe]	DCl (pD 2)	1.00	0.95	0.80	0.96

a) Here, T_1 is the spin-lattice relaxation time, N is the number of protons attached to the carbon, and η is the solution viscosity. The corresponding $NT_1\eta$ values are given in Table 3. See the footnotes of Table 1, also. b) The mean values of $NT_1\eta$ for C_δ and C_ε were used.

Effects of Cavity Size of Cycloamyloses and Medium pD on Complexation. As can be seen in Table 4, the phenyl $NT_1\eta$ values of phenylalanine suffer the largest reduction by complexation with β-CD. In these systems, the C_α- and C_β-$T_1\eta$ values also suffer the largest reductions. On the other hand, the phenyl $NT_1\eta$ values show the comparable reductions by complexation with α-CD and with γ-CD. These findings are observed in spite of the medium pD values, and can be explained with a term of the cavity size effect on complexation. According to the space-filling models, β-CD has the best fitting cavity size for the complete and tight inclusion of phenyl ring. The molecular model also suggests that the complete inclusion of the phenyl ring of phenylalanine into the α-CD cavity is sterically unfavorable and the extent of inclusion is shallow. On the contrary, γ-CD has enough cavity size for deep and loose inclusion of phenyl ring. As the results, the substrate phenylalanine is more strongly fixed in β-CD cavity and rather loosely in α- and γ-CD cavities. The shallow and deep inclusions of the phenyl ring into the cavities of α-CD and γ-CD, respectively, are also suggested by the reductions of $NT_1\eta$ values of C_β (Table 4), *i.e.*, those in [γ-CD, Phe] systems are larger than those in [α-CD, Phe] systems. The deep insertion of the phenyl ring obstructs a rotational motion of C_β around the C_α–C_β and C_β–C_γ bonds due to an increase in steric hindrance.

Several mechanisms have been proposed for the formation of cycloamylose inclusion complexes,[21–24] but the forces leading the complexation are still unclear and a matter of speculation.[1,23] Among the proposed mechanisms, hydrophobic interaction seems to be the most probable as the driving force for cycloamylose–phenylalanine complexation investigated here. In this case, the presence of additional interactions will be alter the strength of dynamic coupling between cycloamylose and phenylalanine. The pD dependences of $NT_1\eta$ values of phenylalanine must be analysed by taking into account of at least three additional factors, *i.e.*, hydrogen bonding, electrostatic interactions between host cycloamylose and guest phenylalanine, and solvation of charged groups.[25]

Since the complexation geometry which disturbs a solvation of the protonated amino group (pK 1.8) or the carboxylate anion (pK 9.24) of phenylalanine is energetically unpreferable, the inclusions of these groups into the cavity of cycloamylose are excluded. It is noticeable that the $NT_1\eta$ values of phenylalanine show the largest complexation-induced reductions at pD 11.3. Especially the reductions of C_α-$NT_1\eta$ values in three phenylalanine–cycloamylose systems at pD 11.3 are pronounced as compared with those at other pD values. These results suggest the existence of the hydrogen bond between the peripheral secondary hydroxyl groups and the amino and carboxyl (carboxylate) groups of phenylalanine. The reduction of C_α motion of phenylalanine may be due to the anchoring effect of the hydrogen bond at the chain end.[25,26]

In 1 mol dm^{-3} NaOD solutions, secondary hydroxyl groups of cycloamyloses (pK 12.0) are dissociated and

they interact repulsively with carboxylate anion of phenylalanine. These interactions reduce the strength of complexation and are explaining the reason why the reductions of $NT_1\eta$ values in 1 mol dm^{-3} NaOD solution are smaller than those in buffer solution. It is not clear at present that the $NT_1\eta$ values of phenylalanine show the smallest reductions at pD 2. More informations, especially about the solvation states, are required for the definitive explanation.

Conclusion

The cycloamylose inclusion complexes with phenylalanine show some characteristics like general peculiarities of enzyme–substrate complex. The host cycloamyloses have the hydrophobic cavity leading to specific binding of guest. The hydrophobic interaction seems to be the most probable as the driving force for the complexation of cycloamylose with phenylalanine. For these systems, the shape matching between the host cavity and the guest inserting group is one of the factors determining the strong coupling between them. It was found that the strength of the dynamic coupling depends on the cavity size of cycloamylose and pD value of solution. The strongest coupling was observed for the β-CD–phenylalanine system in phosphate buffer solution at pD 11.3, where the phenyl ring of guest is deeply and tightly included into the cavity. The inclusion of phenyl ring into the cavity is shallow and loose in the case of α-CD and is deep and loose in the case of γ-CD. The dynamic coupling is strengthened by the hydrogen bonding between the secondary hydroxyl groups of cycloamylose and the amino and carboxyl groups of phenylalanine, and is weakened by the repulsive interaction between alkoxyl anion of cycloamylose and carboxylate anion of phenylalanine. It is noteworthy that, even in the tightly coupled [β-CD, Phe] system in phosphate buffer, the $NT_1\eta$ values of phenylalanine are about four times larger than the $<NT_1\eta>_{1-5}$ values of cycloamylose and phenyl ring rotate rapidly in the cavity. Thus the dynamic coupling of phenylalanine and cycloamylose is weak and they have extensive independency of molecular motions. The characterizations of cycloamylose complexation by molecular dynamics as well as thermodynamics should offer useful informations for an application of cycloamyloses in various fields.

Finally we should refer to the validity of assignment η appeared in Eq. 4 to the solution macroviscosity. It is known that in some cases the macroviscosities of solutions are not related to the observed ^{13}C-T_1 behavior.[16] For these cases the microviscosities as defined by the various local molecular motions and local molecular interactions should be used as the values of viscosity η. Thus the alternative explanations are possible for the causes of the changes in $NT_1\eta$ values of phenylalanine carbons induced by complexation; in the first one the macroviscosity is used as the η value and the changes in $NT_1\eta$ values are attributed to those in apparent molecular volume; in another the molecular volume is looked upon as constant and the changes are attributed to those in microviscosities. In this paper we discussed the changes in $NT_1\eta$ values based on the first one, because we have no mean to measure the microviscosity. Since the NT_1 values of phenylalanine in the free states at constant pD value were inversely proportional to the macroviscosity as expected from the diffusion model, we can safely discuss the complexation effects on the molecular motions based on the first explanation.

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