Molecular Recognition Kinetics of Leucine and Glycyl-Leucine by β-Cyclodextrin in Aqueous Solution in Terms of Ultrasonic Relaxation

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Ultrasonic absorption coefficients in the frequency range of 0.8−95 MHz were measured in aqueous solution of leucine and glycyl-leucine in the presence and absence of β-cyclodextrin (β-CD) at 25 °C. A single relaxational absorption was found in both solutions although it was not observed in the absence of β-CD. The cause of the relaxation was attributed to a perturbation of a chemical equilibrium associated with an interaction between β-CD and the amino acid or the dipeptide. The rate and thermodynamic constants for the association and dissociation reaction of the complex in the system of glycyl-leucine and β-CD were determined from the concentration dependence of the relaxation frequency and the maximum absorption per wavelength. On the other hand, another analysis was applied for the system with leucine and β-CD to calculate the rate and thermodynamic constants due to the indistinguishable concentration dependence of the relaxation frequency. Therefore, first the equilibrium constant was estimated from the concentration dependence of a maximum absorption per wavelength and then the rate constants were calculated. The results obtained were compared with that for the isoleucine and β-CD system. In addition, the rate constant for the formation of the complex was discussed in relation to the diffusion controlled reaction.

Introduction

Cyclodextrins (host) are cyclic oligosaccharides consisting of glucopyranose units linked by a glucosidic bond. Naturally occurring α-, β-, and γ-cyclodextrin are composed of 6, 7, and 8 glucopyranose units, respectively. Their cavity diameter increases proportionally as the number of glucopyranose units increase (ca. 0.52, 0.66, and 0.84 nm) while the depth remains constant (ca. 0.80 nm).[1] They are doughnut-shaped molecules that have hydrophobic cavities that can form inclusion complexes with various kinds of molecules (guest). That is, cyclodextrins can recognize precisely the guest molecule. The extent of the recognition reflects upon the rate and equilibrium constants for complex formation. Accordingly, there are many reports that are concerned with the formation constants determined by various static methods.[2−5] However, kinetic study for molecular recognition is considerably limited, although it is very important to understand the precise mechanism of the complexation.

In our series of kinetic studies for the interaction between host and guest by means of ultrasonic relaxation, it has been clarified that the stability of the complex is strongly controlled by the structure of the guest molecules (e.g., hydrophobicity, functional group, charge effect, and so forth) and the size of the cavities of the cyclodextrins as well.[6−10] The recent kinetic study for amino acids reveals that the ultrasonic relaxation associated with the inclusion complex reaction is found in a system with isoleucine and β-CD, while it is not observed in a system with glycine and β-CD.[9] The molecular recognition of CDs for amino acids or peptides is very important for biological systems. Our goal was to extend our kinetic study to the recognition of peptides by supramolecules. In the present study, dipeptide (glycyl-leucine) was chosen as a model system. It is expected that an isomerization effect on the molecular recognition kinetics also plays an important role for understanding of molecular recognition mechanisms. For this purpose, leucine was taken as the guest for β-CD in order to compare with the results from isoleucine.

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Experimental Section

Chemicals. β-Cyclodextrin was purchased from Wako Pure Chemical Co. Ltd., and the purification procedure of β-CD was reported elsewhere.[7] Glycyl-leucine and leucine were also purchased from Wako Pure Chemical Co. Ltd. as the purest grade and were used without further purification. Sample solutions were prepared by weighing, and distilled and filtered water by a Milli-Q SP-TOC filter system from Japan Millipore Ltd. was used as a solvent.

Apparatus. Ultrasonic absorption measurements were performed by a resonance method in the frequency range from about 0.8 to 9 MHz and a pulse method in the range from 25 to 95 MHz. More details about the absorption apparatus and the procedure for determining the absorption coefficient, α, are described elsewhere.[11,12] Sound velocity values were obtained by the resonator at around 3 MHz, and the solution densities were measured by a vibrating density meter (Anton Paar DMA 60/602). All measurements were performed at 25 °C.

Results

Figures 1 and 2 show representative ultrasonic absorption spectra in aqueous solutions of leucine and glycyl-leucine. The pH of both solutions were about 5.8, which is an isoelectric point. When β-CD does not exist in the solution (only the guest molecule is dissolved in water), the absorption coefficients divided by the square of the measurement frequency, α/f^2, are independent of the frequency: no excess absorption is observed. Also, the excess absorption is not found in β-CD solution when

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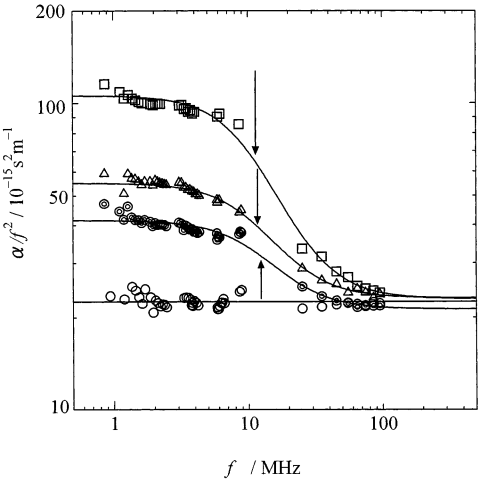

Figure 1. Representative ultrasonic absorption spectra in aqueous solution of leucine in the presence and in the absence of β-CD at 25 °C; (◎): 0.020 mol dm^{-3} leucine + 0.0087 mol dm^{-3} β-CD, (△): 0.042 mol dm^{-3} leucine + 0.0087 mol dm^{-3} β-CD, (□): 0.16 mol dm^{-3} leucine + 0.0087 mol dm^{-3} β-CD, (○): 0.100 mol dm^{-3} leucine. The arrows show the locations of relaxation frequency.

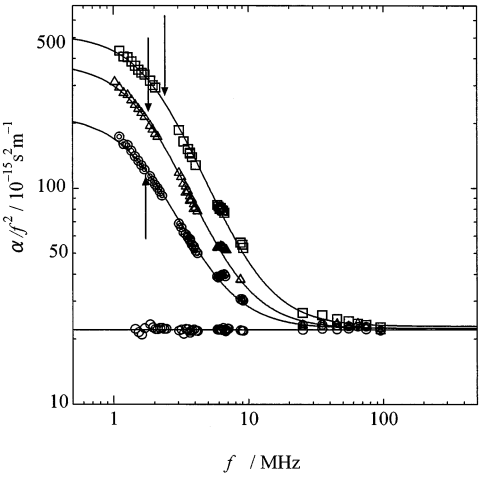

Figure 2. Representative ultrasonic absorption spectra in aqueous solution of glycyl-leucine in the presence and in the absence of β-CD at 25 °C; (◎): 0.0050 mol dm^{-3} glycyl-leucine + 0.0087 mol dm^{-3} β-CD, (△): 0.010 mol dm^{-3} glycyl-leucine + 0.0087 mol dm^{-3} β-CD, (□): 0.040 mol dm^{-3} glycyl-leucine + 0.0087 mol dm^{-3} β-CD, (○): 0.040 mol dm^{-3} glycyl-leucine.

the concentration is below 0.01 mol dm^{-3}.[13] However, the excess absorption was clearly observed only when leucine or glycyl-leucine was dissolved in a solution in the presence of β-CD.

An analytical equation used conveniently to make clear the frequency dependence of the absorption is a Debye-type relaxational one

$$\alpha/f^2 = A/[1 + (f/f_r)^2] + B \quad (1)$$

where f_r is the relaxation frequency, A is the amplitude of excess absorption, and B is the background absorption. Actually, a slightly modified version of eq 1 was used to determine the parameters f_r, A, and B, using a nonlinear least-mean square method.[6] The solid curves shown in Figures 1 and 2 indicate the calculated ones, and it is clearly seen that the Debye's equation can reasonably be applied to the experimental data. The obtained values are listed in Table 1 along with solution density, ρ, and the sound velocity, c.

The experimental fact that the relaxation was observed only when both solutes coexist in an aqueous medium indicates that the relaxation process was associated with the interaction between the two solutes. As was shown in our previous report, the relaxation is not found in an aqueous solution when the guest is glycine.[9] It can be derived from this result that a hydrophobicity of a guest molecule is greatly responsible to a host−guest complex. In the solution with glycyl-leucine, the relaxation frequency is increased with the guest concentration. This tendency has been also observed in other systems with various guests.[6−9] However, the concentration dependence of the relaxation frequency is not clear for the system with leucine as is seen in Table 1. As it is certain that the cause of the relaxation is due to the interaction between β-CD and leucine or glycyl-leucine, the perturbation of the following chemical equilibrium is considered as the cause of the relaxation,

$$\mathrm{CD} + \mathrm{PEP} \underset{k_b}{\overset{k_f}{\rightleftharpoons}} \mathrm{CDPEP} \quad (2)$$

where CD, PEP, and CDPEP are the host, guest, and the complex molecule, and k_f and k_b are the forward and backward rate constants, respectively. Moreover, from an analytical procedure of a chemical relaxation, we obtain the relation between the relaxation frequency and the reactant concentration as follows[9]

$$2\pi f_r = k_f\{[\mathrm{CD}] + [\mathrm{PEP}]\} + k_b \quad (3)$$

$$= k_b\{(KC_{\mathrm{CD}} + KC_{\mathrm{PEP}} + 1)^2 - 4K^2C_{\mathrm{CD}}C_{\mathrm{PEP}}\}^{1/2} \quad (3')$$

where C_{CD} and C_{PEP} are the analytical concentrations of the host and the guest, respectively. Here, K is defined as $K = k_f/k_b$. As is already indicated above, in the solutions including β-CD and glycyl-leucine, the relaxation frequency increases with the guest concentration. When a fixed concentration is used for β-CD, the independent variable in eq 3′ for the relaxation frequency is only the concentration of the guest. Consequently, a nonlinear least-mean square computer program was applied to determine the constants, k_b and K for the glycyl-leucine system. Figure 3 shows the plots of $2\pi f_r$ vs the concentration term in eq 3′, and the great linear relationship going through original point was obtained. This result confirms that the cause of the relaxation is due to the interaction between β-CD and glycyl-leucine. Thus, the calculated values are listed in Table 2 along with those for other systems to make a comparison. It is now possible to calculate the relaxation frequency at different concentrations of β-CD using the determined rate and equilibrium constants. The calculated and observed values are indicated in Table 1, and they are seen to be very close each other. In the leucine system, however, the observed relaxation frequency seems to be independent of the concentrations as can be seen in Table 1. Therefore, the same analytical procedure is not applicable to the leucine system.

The amplitude of the excess absorption, A, is also related to the reaction parameter. In the ultrasonic relaxation study, a maximum absorption per wavelength, μ_{max}, is widely used and it is expressed for the reaction under consideration as[9]

$$\mu_{\max} = 0.5Af_rc$$

$$= \pi\rho c^2 (1/[\mathrm{CD}] + 1/[\mathrm{PEP}] + 1/[\mathrm{CDPEP}])^{-1} (\Delta V)^2/2RT \quad (4)$$

where ΔV is the standard volume change of the reaction. Since the rate constants have been determined from the concentration

TABLE 1: Ultrasonic Relaxation and Thermodynamic Parameters for Aqueous Solutions of Leucine and Glycyl-Leucine with β-CD at 25 °C

C_{CD} mol dm^{-3}	C_{PEP} mol dm^{-3}	f_r MHz	A 10^{-15} s^2 m^{-1}	B 10^{-15} s^2 m^{-1}	ρ kg m^{-3}	c m s^{-1}	pH
			Leucine System				
0.0087	0.010	12.1 ± 0.7	12.7 ± 0.4	20.8 ± 0.1	1001.15 ± 0.01	1502.2 ± 0.5	5.71
0.0087	0.016	13.5 ± 0.4	16.9 ± 0.3	20.4 ± 0.1	1001.27 ± 0.01	1503.0 ± 0.6	5.80
0.0087	0.020	12.7 ± 0.5	20.3 ± 0.4	21.3 ± 0.1	1001.39 ± 0.01	1503.4 ± 0.5	5.84
0.0087	0.026	13.8 ± 0.7	24.8 ± 0.8	20.0 ± 0.1	1001.55 ± 0.01	1503.9 ± 0.6	5.85
0.0087	0.042	12.0 ± 0.4	32.0 ± 0.6	23.2 ± 0.1	1001.91 ± 0.01	1506.1 ± 0.6	5.92
0.0087	0.071	13.2 ± 0.3	50.7 ± 0.7	22.5 ± 0.1	1002.62 ± 0.01	1509.0 ± 0.5	5.78
0.0087	0.079	11.9 ± 0.3	55.2 ± 0.9	22.2 ± 0.1	1002.77 ± 0.01	1509.7 ± 0.8	5.83
0.0087	0.100	12.1 ± 0.3	63.5 ± 1.0	23.0 ± 0.1	1003.30 ± 0.01	1512.0 ± 0.4	5.92
0.0087	0.120	12.9 ± 0.3	72.1 ± 1.0	21.5 ± 0.1	1003.76 ± 0.01	1514.2 ± 0.9	5.97
0.0087	0.130	12.8 ± 0.4	73.2 ± 1.0	22.5 ± 0.2	1004.08 ± 0.01	1515.0 ± 0.8	5.91
0.0087	0.150	12.6 ± 0.4	80.2 ± 2.0	23.3 ± 0.2	1004.45 ± 0.01	1517.0 ± 0.6	5.80
0.0087	0.160	11.3 ± 0.3	83.1 ± 2.0	23.0 ± 0.1	1004.47 ± 0.01	1518.2 ± 0.8	5.96
			Glycyl-Leucine System				
0.0087	0.0050	1.75 ± 0.05	197 ± 8	22.4 ± 0.1	1001.16 ± 0.01	1500.7 ± 0.8	5.93
0.0087	0.010	1.83 ± 0.04	359 ± 9	22.4 ± 0.1	1001.50 ± 0.01	1502.0 ± 0.5	5.95
0.0087	0.020	1.98 ± 0.03	470 ± 8	22.8 ± 0.1	1001.85 ± 0.01	1502.6 ± 0.8	5.83
0.0087	0.030	2.20 ± 0.03	496 ± 8	23.0 ± 0.1	1002.29 ± 0.01	1504.0 ± 0.9	5.85
0.0087	0.040	2.41 ± 0.03	490 ± 7	22.9 ± 0.1	1002.78 ± 0.01	1505.1 ± 0.6	5.81
0.0050	0.030	2.08 ± 0.03 (2.16)[a]	322 ± 5	22.6 ± 0.5	1000.72 ± 0.01	1503.6 ± 0.7	5.79
0.0070	0.020	1.93 ± 0.03 (1.99)[a]	389 ± 7	22.4 ± 0.1	1001.08 ± 0.01	1502.0 ± 0.7	5.94

[a] Those values are calculated from eq 3′ using determined parameters.

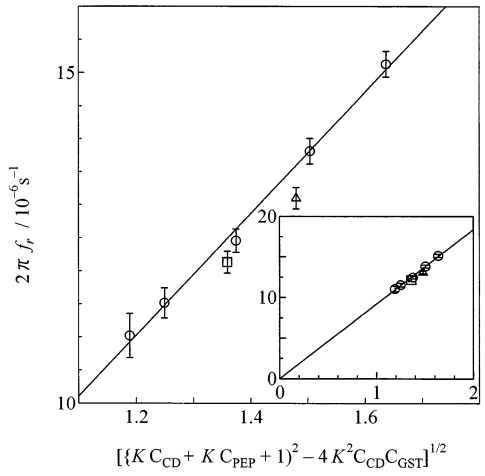

Figure 3. Plots of $2\pi f_r$ vs $\{(KC_{CD} + KC_{PEP} + 1)^2 - 4K^2C_{CD}C_{PEP}\}^{1/2}$ for aqueous solutions of glycyl-leucine in the presence of β-CD at 25 °C; (○): 0.0087 mol dm^{-3} β-CD, (△): 0.0050 mol dm^{-3} β-CD, (□): 0.0070 mol dm^{-3} β-CD.

TABLE 2: Rate and Thermodynamic Constants for Host−Guest Complexation at 25 °C

guest	k_f 10^8 mol^{-1} dm^3 s^{-1}	k_b 10^7 s^{-1}	K mol^{-1} dm^3	ΔV 10^{-6} m^3 mol^{-1}
isoleucine	2.9 ± 0.3	5.9 ± 0.2	4.9 ± 0.4	10 ± 1
leucine	2.6	7.9 ± 0.3	3.3	16.3 ± 2.7
glycyl-leucine	1.38 ± 0.01	0.92 ± 0.03	15.0 ± 0.2	19.2 ± 0.6

dependence of the relaxation frequency, the individual equilibrium reactant concentrations are calculated from the analytical concentrations of the host and the guest, using the equilibrium constant, $K = k_f/k_b$ = [CDPEP]/[CD][PEP]. However, this was just for the case of the glycyl-leucine system. As a result, ΔV was calculated from eq 4 with the aids of c and ρ, which were measured independently. The obtained values are shown in Table 2. This analytical procedure for the standard volume change requires the equilibrium constant. Once the constant, K, is obtained, the rate constants for the leucine system are also calculated. Concerning the leucine system, for the first approximation we considered that the relaxation frequency reflects the backward rate constant, k_b, in eq 3, that is, the term concerning k_b is taken to be sufficiently dominant to the k_f-{[CD] + [PEP]} term in eq 3 since the equilibrium concentrations of host and guest were small.[14] Various values of the equilibrium constant, K, for the leucine system were attempted for the plots of the $2RT\mu_{max}/\pi\rho c^2$ vs the $(1/[CD] + 1/[PEP] + 1/[CDPEP])^{-1}$ term in order to obtain the best straight line going through a zero intercept. When K was used as 3.3 mol^{-1} dm^3, the best fit was received as is shown in Figure 4. Then, the standard volume change was calculated from the slope of the line, and the forward rate constant was also obtained from the definition of the equilibrium constant, $K = k_f/k_b$. They are listed in Table 2, although the probable errors were not estimated.

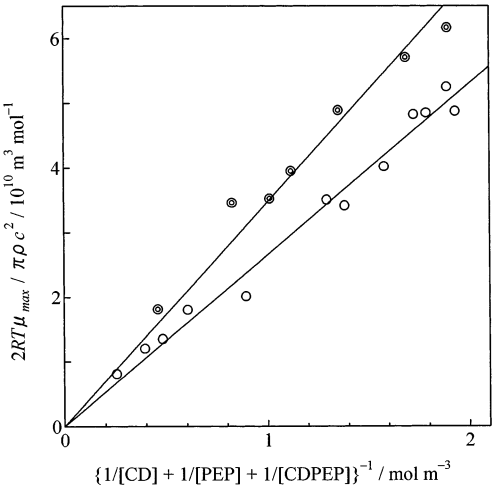

Figure 4. Plots of $2RT\mu_{max}/\pi\rho c^2$ vs $\{1/[CD] + 1/[PEP] + 1/[CDPEP]\}^{-1}$; (○): leucine system, (◎): glycyl-leucine system.

The above procedure was tested to obtain the standard volume change for the glycyl-leucine system, and it is also shown in

Figure 4. The estimated value is corresponding to that value from eq 4 shown in Table 2.

Discussion

Our previous reports showed that the larger the hydrophobic part of the guest molecule is, the more the stable complex is formed, leading to a smaller backward rate constant, k_b.[6−9] Furthermore, the guest molecule bearing a branched carbon chain can be incorporated in more stable form than that with normal carbon chain.[7] On the other hand, the forward rate constants, k_f, fall in the similar values, about 3×10^8 mol^{-1} dm^3 s^{-1} for most of the guest molecules used in our previous study.[6−10] This may be because the association process is considered to be a diffusion controlled reaction[15,16] for the following reason. The rate constant for a diffusion controlled reaction is given by the Smoluchowski's equation,[17]

$$k_D = 4\pi N_A (D_{CD} + D_{PEP}) (r_{CD} + r_{PEP}) \quad (5)$$

where N_A is the Avogadro number, D_{CD} and D_{PEP} are the diffusion coefficient for the reactants, r_{CD} and r_{PEP} are the radii of the molecules. To estimate the forward rate constant of leucine system as an example, the reported values are used for r_{CD} as 0.79 nm (radius of outer torus of β-CD),[1] and 3.2×10^{-6} cm^2 s^{-1} as D_{CD}.[18] For the diffusion coefficient of leucine, D_{PEP}, the value of alanine in about 0.1 mol dm^{-3} is taken alternatively as 9.0×10^{-6} cm^2 s^{-1} because we could not find that value for leucine.[19] The molecular size of leucine was estimated from the distances between the nuclei of bonded atoms to be 0.86 nm,[20] and the half value was used as r_{PEP}. Therefore, we obtained $k_D \approx 1.1 \times 10^{10}$ mol^{-1} dm^3 s^{-1}, which was more than 30 times greater than the value obtained for the forward rate constant, $k_f \approx 3 \times 10^8$ mol^{-1} dm^3 s^{-1}, in our series of kinetic study. However, it is needed for the guest molecules to enter into the cavity of the cyclodextrin from just both entrances. It seems reasonable that the probability of guest molecules forming a complex is proportional to the relative values of surface areas of inner torus to the total surface area of the cyclodextrin. Therefore, the needed surface areas are calculated[1] to estimate a more appropriate value for k_D. The calculated surface area of the inner torus of β-CD is 0.68 nm^2, and the calculated value of the total surface area is 8.45 nm^2. As a result, we have to multiply 1.1×10^{10} mol^{-1} dm^3 s^{-1} by 0.08 to yield $k_D \approx 8.8 \times 10^8$ mol^{-1} dm^3 s^{-1}, which seems to be in good agreement with the experimental value. Moreover, since the diffusion coefficients of the molecules for alcohols and amides are also on the order of 10×10^{-6} cm^2 s^{-1},[21] the suggestion that the association process for the complexation is in diffusion controlled reaction is strongly supported.

As a result, it can be deduced that the backward rate constant is the most important factor to see the stability of the complex. The backward rate constant, k_b, for the leucine system is slightly larger than for the isoleucine system. Despite the analogous structural formulas for the two amino acids, leucine and isoleucine, it was revealed that leucine forms more unstable complex than that between β-CD and isoleucine. The difference should also be noted that the relaxation frequencies look to be independent of the concentration of leucine while they are dependent on the concentration of isoleucine. In this regard, it might be suggested that β-CD can recognize the subtle structural difference.

Next, the result in the dipeptide system with glycyl-leucine is considered. It is found that the excess absorption, A, is considerably great, and the relaxation frequencies shift to a much lower frequency range when compared with other systems. These tendencies may reflect the existence of the peptide bond in the structure. In detail, because the charge effect of the ammonium cation on the hydrophobic side chain group is decreased through the peptide bond between glycine and leucine (i.e., the hydrophobic part and charged cation group are more distant compared with amino acids that do not have a peptide bond in their structure), the extent of incorporation of the dipeptide into the cavity of β-CD increases. Hence, the glycyl-leucine molecule can be accommodated firmly in the cavity of β-CD, resulting in the reduced backward rate constant, k_b, and consequently leading to the greater equilibrium constant, K. This speculation is supported by the observed greater values of the standard volume change of the reaction as is seen in Table 2.

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