Kinetic Study for Molecular Recognition of Amino Acid by Cyclodextrin in Aqueous Solution

Takaho Ugawa and Sadakatsu Nishikawa*

Department of Chemistry and Applied Chemistry, Faculty of Science and Engineering, Saga University, Saga 840-8502, Japan

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A system of β-cyclodextrin and L-isoleucine in aqueous solution was studied by the ultrasonic relaxational method to obtain kinetic parameters for the complexation reaction at 25 °C. Ultrasonic absorption coefficients were measured as a function of frequency in the range 0.8−95 MHz and solute concentration at three different pH's. The frequency dependence of the absorption observed as a function of frequency was analyzed by a Debye-type equation with a single relaxation time at neutral pH. The cause of the relaxation was attributed to a perturbation of a chemical equilibrium due to formation of inclusion complex consisting of β-cyclodextrin and L-isoleucine. The forward and backward rate constants, the equilibrium constant, and the standard volume change of the reaction were determined from the ultrasonic parameters. Comparing these obtained results with those at low pH, it was found that the complexation reaction at low pH is the same as that at neutral pH range. However, at the high pH, another reaction is generated, in addition to the complexation reaction presumably due to a proton transfer reaction. Further, the results for the complexation reaction were compared with those reported previously for several nonelectrolytes, and the structure of amino acid was considered to influence the complexation reaction process considerably, especially the process for the departure of guest from the host cavity.

Introduction

Recently, much attention has been paid for complexation between cyclodextrins (CD) (host) and a variety of other compounds (guest) in various fields, such as drug delivery systems, fabric softeners, antibacterial sheets, removal of cholesterol in food production processes, etc. Among other things, due to the importance of amino acids as basic components of protein, which is indispensable in the natural living body, molecular recognition of amino acids by CD has been one of the more attractive topics. Several interesting papers have been reported on both theoretical and experimental studies about molecular recognition between amino acids and CD.[1−5] However, relatively little kinetic study has been achieved, even though the time range for the complexation processes spreads very widely.[6−11] On the other hand, a lot of equilibrium studies have been carried out using various methods in order to obtain the stability of inclusion complexes.[12−15]

In our series of the kinetic studies for interactions between CD and nonelectrolytes by the ultrasonic relaxation method,[11,12] it has been clarified that the rate of the decomplexation of the guest is very dependent on both the cavity size of the host and the structure of the guest. Then, it is very interesting and very important to focus on how the amino acid, which consists of the ampholyte bearing amino, carboxyl and hydrophobic groups, affects the complexation and decomplexation reaction by CD in aqueous solution. In this study, β-cyclodextrin has been chosen as the host and L-isoleucine has been selected as the guest. The ultrasonic absorption, sound velocity, and density of the solutions have been measured as a function of the solute concentrations. This kinetic information will serve as an important part of the molecular recognition in the host−guest interaction between CD and amino acid.

* To whom correspondence should addressed. E-mail: nishikas@cc.saga-u.ac.jp.

Experimental Section

β-Cyclodextrin was purchased from Wako Pure Chemical Co. Ltd. It was recrystallized once from water, then dried in a vacuum oven at 45 °C until the weight of the sample powder reached a constant value, and stored in a desiccator for further use. L-Isoleucine (L-Ile) and Glycine (Gly) were also purchased from Wako Pure Chemical Co. Ltd. as the purest grade and were used without further purification. The concentrated aqueous solutions of sodium hydroxide and hydrochloric acid were used to adjust the solution pH to the desired values. Water for preparing sample solutions was distilled, deionized, and filtered through a Milli-Q SP-TOC filter System from Japan Millipore Ltd. The desired concentrations of the samples were determined by weighting.

Ultrasonic absorption coefficients, α, were measured by a pulse method in the frequency range 25−95 MHz using a 5 MHz x-cut fundamental quartz crystal. To obtain α in the low-frequency range, 0.8−9 MHz, a resonance method was used. A new resonance cell with a 5 MHz x-cut fundamental crystal (2 cm diameter) mounted with silicone rubber rings was constructed in order to obtain the absorption coefficient in the range 3−8 MHz. More details about the absorption apparatus and the procedure for determining the absorption coefficient are described elsewhere.[11,16,17] Sound velocity values were obtained by the resonator at around 3 MHz. The densities of the solutions were achieved through an Anton Paar vibrating density meter (DMA 60/602). The solution pH was determined by using a glass electrode (HM-60s Toa Denpa). Water bath

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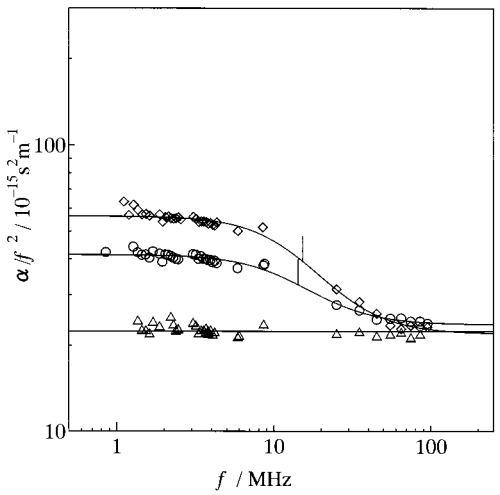

Figure 1. Ultrasonic absorption spectra in aqueous solutions of L-Ile with β-CD and in aqueous solution of L-Ile at neutral pH at 25 °C: (○) 0.075 mol dm^{-3} L-Ile + 0.0087 mol dm^{-3} β-CD; (◇) 0.15 mol dm^{-3} L-Ile + 0.0087 mol dm^{-3} β-CD; (△) 0.10 mol dm^{-3} L-Ile.

controlled within ±0.1 °C for the pulse apparatus cell (Eyela Uni Ace Bath NCB-2200) and circulating water maintained within ±0.01 °C for the resonance cells (Lauda, RM20) were utilized to keep the temperature at 25 °C.

Results

Figure 1 shows ultrasonic absorption spectra in aqueous solutions of L-Ile with β-CD at neutral pH. In the solution of L-Ile, the absorption coefficient divided by square of the frequency, α/f^2, is independent of the measuring frequency (no excess absorption). It has been reported that the relaxational absorption is found for aqueous β-CD solution at concentrations more than 0.013 mol dm^{-3}.[18] However, there is no excess absorption in the more dilute solutions of β-CD[11] in which the present experiments have been carried out. When β-CD is added into L-Ile aqueous solution, the excess absorption is observed. The frequency dependence of the absorption has been tested by a Debye-type relaxation equation,

$$\alpha/f^2 = A/\{1 + (f/f_r)^2\} + B \qquad (1)$$

where A is the relaxation amplitude, f_r is the relaxation frequency and B is the contribution from other sources. The dependence of α/f^2 on frequency is fitted to the slightly modified equation in order to obtain f_r, A, and B using a nonlinear least-mean squares method.[11] The solid curves in Figure 1 are generated from the obtained ultrasonic parameters, which are listed in Table 1 along with the sound velocity and density values. It is seen that the calculated curves have given good agreement with the experimental data. The fact that the relaxation is only found when the two solutes coexist in the solution at the neutral pH range means that the cause of the observed relaxational absorption is associated with the interaction between β-CD and L-Ile. The mechanism of the relaxation is considered to relate to a perturbation of the following equilibrium by ultrasonic wave,

$$\mathrm{CD} + \mathrm{Ile}^{\pm} \underset{k_b}{\overset{k_f}{\rightleftharpoons}} \mathrm{CDIle}^{\pm} \qquad (2)$$

where CD is β-CD, Ile$^{\pm}$ is L-Ile, CDIle$^{\pm}$ is the complex, and k_f and k_b are the forward and backward rate constants, respectively. At the neutral pH, most of amino and carboxylic groups are ionized. The rate equation for the above reaction is given using activities for the individual reactants as

$$\frac{da_{\mathrm{CDIle}\pm}}{dt} = k_f a_{\mathrm{CD}} a_{\mathrm{Ile}\pm} - k_b a_{\mathrm{CD}} a_{\mathrm{Ile}\pm} \qquad (3)$$

When the activity coefficient for Ile$^{\pm}$ is similar to that for CDIle$^{\pm}$, and the activity of CD is assumed to be unity, then eq 3 is simply approximated by

$$\frac{d[\mathrm{CDIle}^{\pm}]}{dt} = k_f[\mathrm{CD}][\mathrm{Ile}^{\pm}] - k_b[\mathrm{CDIle}^{\pm}] \qquad (4)$$

The equilibrium constant, K, defined as $K = k_f/k_b = [\mathrm{CDIle}^{\pm}]/[\mathrm{CD}][\mathrm{Ile}^{\pm}]$ is also approximated. Then, the relationship for the relaxation time, τ, or the relaxation frequency with the reactant concentrations is derived as

$$\tau^{-1} = 2\pi f_r = k_f([\mathrm{CD}] + [\mathrm{Ile}^{\pm}]) + k_b$$
$$= k_b\{(KC_{\mathrm{CD}} + KC_{\mathrm{Ile}} + 1)^2 - 4K^2C_{\mathrm{CD}}C_{\mathrm{Ile}}\}^{1/2} \qquad (5)$$

where C_{CD} and C_{Ile} are the analytical concentrations for β-CD and L-Ile, respectively. When the concentration of β-CD is fixed, the f_r is only a function of the L-Ile concentration. Consequently, the parameters K and k_b can be estimated using a nonlinear least-mean squares method. Thus, determined values are tabulated in Table 2 along with those for solutions of other nonelectrolytes as the guests that have been reported previously.[11] Figure 2 shows the plots of $2\pi f_r$ vs $[\{KC_{\mathrm{CD}} + KC_{\mathrm{Ile}} + 1\}^2 - 4K^2C_{\mathrm{CD}}C_{\mathrm{Ile}}]^{1/2}$ for an aqueous solution of L-Ile with β-CD. It can be seen that a straight line, which goes through a zero intercept, agrees with the experimental data, which helps confirm that the cause of relaxation is due to the perturbation of equilibrium expressed by eq 2.

A standard volume change of the reaction, ΔV, is related to the maximum absorption per wavelength, $\mu_{\max}$, which is given by the next relation under the assumption that a thermal

TABLE 1: Ultrasonic and Thermodynamic Parameters for Aqueous Solutions of L-Isoleucine with β-CD

C_{CD}, mol dm^{-3}	C_{Ile}, mol dm^{-3}	f_r, MHz	$10^{15}A$, s^2 m^{-1}	$10^{15}B$, s^2 m^{-1}	ρ, Kg dm^{-3}	c, ms^{-1}	pH
0.0087	0.034	12 ± 1	14 ± 1	21.3 ± 0.1	1.00174 ± 0.00001	1504.6 ± 0.7	6.72
0.0087	0.050	9.7 ± 1.0	15.1 ± 0.8	21.9 ± 0.1	1.00214 ± 0.00001	1506.1 ± 0.9	5.64
0.0087	0.075	14.3 ± 0.5	17.8 ± 0.4	23.6 ± 0.1	1.00280 ± 0.00001	1509.1 ± 0.6	6.03
0.0087	0.10	13.7 ± 0.6	24.0 ± 0.7	22.8 ± 0.1	1.00342 ± 0.00001	1511.2 ± 0.8	6.47
0.0087	0.15	15.3 ± 0.5	34.6 ± 0.8	21.9 ± 0.1	1.00472 ± 0.00001	1516.9 ± 0.7	6.44
0.0087	0.17	17.9 ± 0.4	35.3 ± 0.5	24.2 ± 0.1	1.00533 ± 0.00001	1518.8 ± 0.8	6.00
0.0087	0.20	15.6 ± 0.6	41 ± 1	23.2 ± 0.1	1.00593 ± 0.00001	1521.9 ± 1.0	6.34
0.0087	0.10	17.1 ± 0.5	40.8 ± 0.8	28.3 ± 0.1	1.00457 ± 0.00001	1509.0 ± 1.0	1.81
0.0087	0.10	3.7 ± 0.9	331 ± 9	25.3 ± 0.2	1.00511 ± 0.00005	1514.0 ± 1.0	9.82
0	0.10	3.16 ± 0.07	209 ± 5	21.51 ± 0.05	1.00126 ± 0.00001	1511.6 ± 0.9	9.81

TABLE 2: Rate and Thermodynamic Constants for Complexation of β-CD with Some Guests at 25 °C

	$10^{-8}k_f$, mol^{-1} dm^3 s^{-1}	$10^{-7}k_b$, s^{-1}	K, mol^{-1} dm^3	K, mol^{-1} dm^3	$10^6\Delta V$, m^3 mol^{-1}
isoleucine	2.9 ± 0.3	5.9 ± 0.2	4.9 ± 0.4		10 ± 1
methyl propionate[a]	1.3 ± 0.1	8.66 ± 0.10	1.5 ± 0.1		24 ± 3
methyl butyrate[a]	3.7 ± 0.3	1.28 ± 0.03	29 ± 1		16 ± 2
1-butyramide[a]	2.7 ± 0.3	9.8 ± 0.7	2.7 ± 0.3		12 ± 2
1-propanol[b]	5.1 ± 0.7	12.1 ± 0.7	4.2 ± 0.6	3.72[c]	12.5 ± 0.3
1-butanol[b]	2.8 ± 0.8	3.8 ± 0.6	7.2 ± 2.0	16.6[c]	11.1 ± 1

[a] Reference 11. [b] Reference 10. [c] Reference 12.

relaxational term is negligible in aqueous solution.

$$\mu_{max} = 0.5Af_r c = \pi\rho c^2(1/[\mathrm{CD}] + 1/[\mathrm{Ile}^{\pm}] + 1/[\mathrm{CDIle}^{\pm}])^{-1}(\Delta V)^2/2RT \quad (6)$$

where c and ρ are the sound velocity and the solution density, respectively. The individual reactant concentrations are calculated from the analytical concentrations of the host and the guest, using the equilibrium constant, K. Also, c and ρ are determined independently. Therefore, ΔV is calculated from eq 6 and it is indicated in Table 2 along with those for other nonelectrolytes.

To see the effect of molecular charge on the reaction, the absorption measurements have been carried out at different pHs.

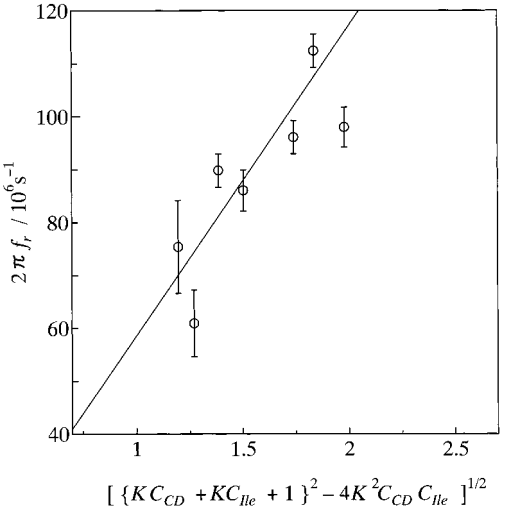

Figure 2. Plots of $2\pi f_r$ vs $[\{KC_{CD} + KC_{Ile} + 1\}^2 - 4K^2C_{CD}C_{Ile}]^{1/2}$ for aqueous solutions of L-Ile in the presence of β-CD at neutral pH.

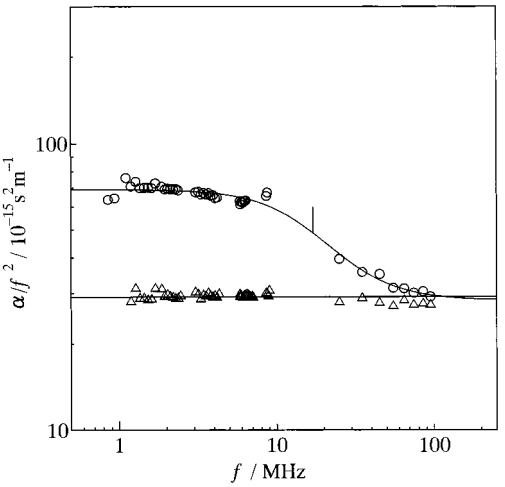

Figure 3. Ultrasonic absorption spectra in aqueous solutions of L-Ile with β-CD at pH 1.8 at 25 °C: (○) 0.10 mol dm^{-3} L-Ile + 0.0087 mol dm^{-3} β-CD; (△) 0.10 mol dm^{-3} L-Ile.

Figure 3 shows the absorption spectra at pH = 1.81. When β-CD does not exist in the solution, the relaxational absorption is not observed. However, when β-CD and L-Ile (the amino groups are mostly ionized while the carboxyl groups are partly ionized) coexist in the solution, the single relaxational absorption is found. The position of the relaxation frequency is close to that at the neutral pH, and the amplitude of this relaxation, A, is slightly greater, as is seen in Table 1. Furthermore, the absorptions have been measured at the alkaline solution adding sodium hydroxide. Figure 4 shows the absorption spectra at pH $\cong$ 9.8. Even if β-CD does not present, a clear relaxational absorption is observed. When β-CD is added, the amplitude of the relaxation increases. The spectra look to be analyzed by the single relaxational equation as eq 1.

The absorption measurements for an aqueous solution of Gly with β-CD have been also performed, examining how the hydrophobic group of the guest affects the relaxation. In this solution the excess absorption has not been observed, as is seen in Figure 4.

Discussion

It has been considered from the previous studies that the rate constant for the incorporation of small guests into the β-CD cavity, k_f, is not so dependent on guest structures, $\sim 3 \times 10^8$ mol^{-1} dm^3 s^{-1}. On the contrary, the change in the backward rate constant, k_b, has been found to be remarkably dependent on the guest structures. These mean that the stability of the complex formed by guests with a relatively small hydrophobic group is controlled by the rate of departure of the guest molecule from the host cavity.

In the neutral pH range, the k_f value for L-Ile is almost the same as those for other guests in our series of kinetic studies, as is seen in Table 2. Therefore, the L-Ile molecule may be incorporated into the β-CD cavity as other nonelectrolytes.

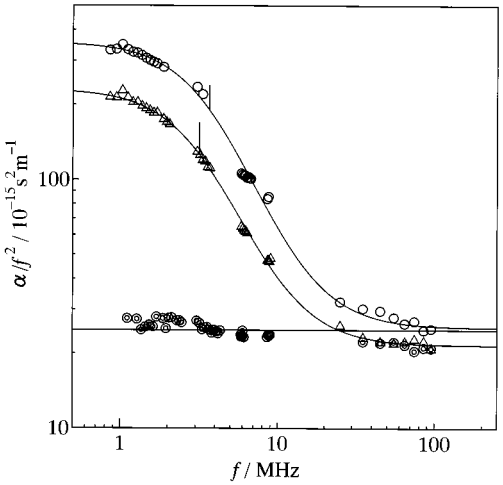

Figure 4. Ultrasonic absorption spectra in aqueous solutions of L-Ile with β-CD at pH 9.8 and in that of Gly with β-CD at pH 5.8 at 25 °C: (○) 0.10 mol dm^{-3} L-Ile + 0.0087 mol dm^{-3} β-CD; (△) 0.10 mol dm^{-3} L-Ile; (⊙) 0.10 mol dm^{-3} Gly + 0.0087 mol dm^{-3} β-CD.

On the other hand, the rate constant for decomplexaton of L-Ile is $k_b = (5.9 \pm 0.2) \times 10^7\ s^{-1}$, which falls between 1-propanol and 1-butanol values. Since L-Ile's pI = 6.04 (pI: the isoelectric point), L-Ile molecules in aqueous solution at the neutral pH exist as the amphoteric ions. It is well-known that the functional group of an amino acid, $H_3^+RCCOO^-$, has high hydrophilicity when compared with those of the guests of nonelectrolytes. Therefore, there should exist the effect of the hydrophilic interaction of the functional group with the hydroxyl groups locating at the rim of the β-CD molecule. As a result of the increasing interaction between the hydrophilic group of L-Ile and the hydroxyl groups of β-CD, it is considered that the rate of the departure of the L-Ile from the β-CD cavity increases. It is said that the inside of the CD cavity is relatively the hydrophobic environment. Then, it is considered that the larger the hydrophobic group the guest molecule has, the more stable is the host−guest complex formed. Glycine is one of the amino acids that has no hydrophobic group. As is seen in Figure 4, there is no excess absorption in the aqueous solution of Gly with β-CD. This means that the hydrophobic group of guest molecule plays an important role in the host−guest complex formation, which is also examined by static experiments.[1−5] Consequently, the hydrophobic group of L-Ile can enter into the cavity of β-CD and the complex is formed mainly by the hydrophobic interaction. As discussed above, however, although L-Ile will remain in the cavity by the hydrophobic interaction, the release of L-Ile from the CD cavity is compensated due to the effect of the hydrophilic interaction. This means that the stability of the complex depends not only on the hydrophilicity but also on the hydrophilic interaction between the host and guest. That is, the molecular recognition of CD is very sensitive to guest structures.

At the low pH range, the observation in the aqueous solution of L-Ile including β-CD indicates the same result as those in the neutral pH range represented in Figure 3. In the solution with only β-CD, no excess relaxation is observed. The structure of L-Ile at pH 1.8 is mostly the cation type consisting of an $-NH_3^+$ ionized amino group. This implies that the effect of the hydrophilic interaction is not more dominant than the hydrophobic one for the complex formation between β-CD and L-Ile. In contrast, the structure of L-Ile in the high pH is the anion type, $-COO^-$, rather than amphoteric ions. As shown in Figure 4, in both solutions in the presence and absence of β-CD at this pH, the absorption spectra obtained are considerably larger compared with those in the neutral and low pH. It is anticipated that there is a possibility that another reactions is proceeding, i.e., the proton-transfer reaction $R-NH_3^+ + OH^- \rightleftharpoons R-NH_2 + H_2O$, in addition to the complexation reaction.[19] The amplitude of the relaxation, A, in the solution of L-Ile without β-CD is smaller than that in the solution with the two solutes, as shown in Table 1. This difference probably arises from the absorption due to the complexation process between L-Ile and β-CD. However, the ultrasonic amplitude of the complexation reaction is too small to distinguish from the total absorption observed.

Next, we consider the result of the standard volume change of the reaction. It is considered that there are several water molecules in the β-CD cavity, which are released when the guest molecule is included in the cavity.[20,21] However, the observed volume changes are considerably small, even if some water molecules in β-CD are released from the cavity. Hence we have considered that only a part of the hydrophobic group of the guest molecule (L-Ile) enters into the β-CD cavity and that there are the interactions between the hydroxyl groups at the rims on β-CD and the functional group of the L-Ile. This is because the size of the cavity is big enough to include the L-Ile molecule since the depth and diameter of the cavity of β-CD are about 7×10^{-10} m, and the calculated molar volume of L-Ile is approximately $100 \times 10^{-6}\ m^3\ mol^{-1}$.

Further kinetic study for host−guest complex formation is necessary to obtain the qualitative relation between the structure and the rate parameter for amino acid and CD.

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