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Ultrasonic Relaxation Study of the Interaction of β-Cyclodextrin with Benzoic Acid in an Aqueous Solution

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Benzoic acid is an antifungal agent. In this study, we studied the interaction of β-cyclodextrin (β-CD) and non-ionized benzoic acid in an aqueous solution by using ultrasonic relaxation in the frequency range of 0.2 - 45 MHz. The interaction of β-CD with non-ionized benzoic acid showed the typical spectrum of a single relaxation process at frequencies below 1 MHz. We determined the backward rate constant (k_b), the equilibrium constant (K), and the standard volume change (ΔV) of the reaction as $k_b = 1.81 \times 10^6$ s^{-1}, $K = 137$ M^{-1}, and $\Delta V = 15.9 \times 10^{-6}$ m^3 mol, respectively. The backward rate constant and the thermodynamic constants were compared with those for the interaction of aspirin and 2-methoxy benzoic acid.

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I. INTRODUCTION

Cyclodextrins (CDs) play important roles in biology, medicine, and pharmaceutical applications [1, 2]. CDs consist of glucopyranose units linked by an α-(1→4) glucosidic bond forming a cyclic compound with hydrophilic outer surface and hydrophobic inner cavity [1]. Tyree types of CDs exist with 6, 7, and 8 glucopyranose units, referred to as α-, β-, and γ-CDs, respectively [1]. CDs are soluble in water and form inclusion complex with many guest molecules. Kinetic studies of β-CD and guest molecules provide valuable information understanding molecule-to-molecule interactions.

Benzoic acid is an antifungal agent in medicine, commonly used as a component of Whitfield's ointment [3]. Elucidating the interaction of CDs and drug molecules is important for the application of CDs in drug-delivery systems. Comparison of the kinetic and thermodynamic results obtained from β-CD and non-ionized aspirin [4] raised the need for clarification of the dynamics of the complex stabilization and how the rate constants of the formation and disruption of the complex are affected as a function of the guest molecules. The inclusion of aspirin, a benzoic acid derivative, into the β-CD cavity was previously reported using ultrasonic relaxation (0.8 - 7.5 MHz) [4], but the interactions occurring below 0.8 MHz is largely unknown.

Here, we studied the interaction of β-CD with non-ionized benzoic acid in an aqueous solution using ultrasonic relaxation. We used ultrasonic frequency range of 0.2 - 45 MHz and particularly focused on the low-frequency range below 1 MHz. The results were compared with the complex stabilization of β-CD with non-ionized aspirin [4] or ionized 2-methoxy benzoic acid [5].

II. EXPERIMENTAL METHODS

Benzoic acid and β-CD were acquired from Sigma (USA). Non-ionized benzoic acid solution (pH 1.7) was

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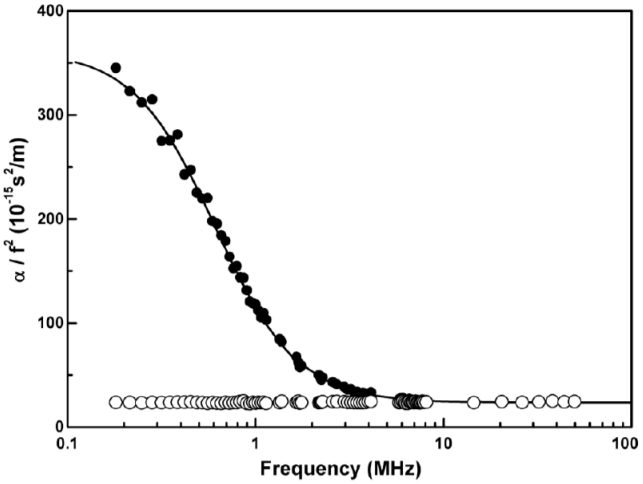

Fig. 1. Ultrasonic absorption α/f^2 versus frequency in aqueous solution of non-ionized benzoic acid at 25 °C. (○) 2 mM benzoic acid, (●) 10 mM benzoic acid + 8.7 mM β-CD. The solid line represents the single relaxation curve. The high-frequency limiting value represents $(\alpha/f^2)_\infty = 25 \times 10^{-15}$ s^2/m.

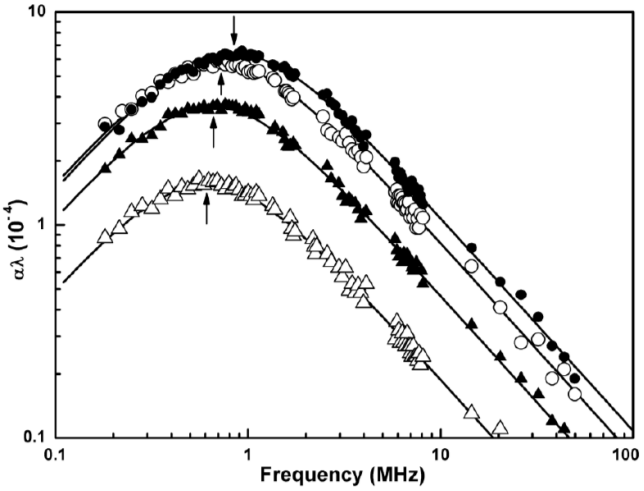

Fig. 2. Excess absorption per wavelength μ versus frequency in aqueous solutions of varying concentrations of non-ionized benzoic acid and β-CD (8.7 mM) at 25 °C. (△) 2 mM benzoic acid; (▲) 5 mM benzoic acid; (○) 10 mM benzoic acid; (●) 15 mM benzoic acid. The solid lines represent single relaxation curves and the arrows indicate relaxation frequencies.

freshly prepared in each experiment. Solution densities were measured using a vibrating density meter (Anton Paar DMA 5000M). We used a high-Q ultrasonic resonance apparatus equipped for a lower frequency range at 25 °C. Three ultrasonic absorption methods were use in the frequency range of 0.2 - 45 MHz: a plano-concave resonance method (0.2 - 1.7 MHz), a plano-plano resonance method (2.4 - 8.1 MHz) and optical beam deflection method (15 - 45 MHz). A pulse-echo method was used to measured velocity at 3 MHz. Briefly, standing waves were generated in a cylindrical cavity (56 mm diameter and 50 cm^3 volume) that consists of a 2-MHz fundamental X-cut quartz transducer and a concave reflector. A resonance spectrum was obtained with an optical heterodyne detection system using the Raman-Nath light diffraction method. The absorption coefficient of the sample liquid was obtained from the half-bandwidth of each resonance curve. Reliable absorption measurements below 1 MHz were attained from the high-quality factor attained from the resonator cell. Frequency above 300 kHz was insignificant and the loss below 300 kHz was calibrated using deionized H_2O. To measure the frequency range of 2.4 - 8.1 MHz, a plano-plano resonator cell (2-MHz fundamental frequency X-cut quartz crystals with 2-cm diameter) was used.

III. RESULTS AND DICUSSION

The following formula describes the inclusion complex formation of non-ionized benzoic acid (guest) with β-CD:

$$\text{Guest} + \beta\text{–}CD \underset{k_b}{\overset{k_b}{\rightleftharpoons}} \text{Guest} \cdot \beta - CD$$

where k_f and k_b are the forward rate constant and backward rate constant, respectively. When the ultrasonic relaxations of β-CD and non-ionized benzoic acid was measured at low frequencies below 1 MHz, β-CD showed ultrasonic relaxation at concentrations above 13 mM, whereas non-ionized benzoic acid showed no relaxation (Fig. 1). The ultrasonic absorption coefficient α at frequency f is shown as a function of frequency (Fig. 1). The solid line in Fig. 1 is the single relaxation curve of a Debye-type single relaxation equation [6], $\alpha/f^2 = A/[1+(f/f_r)^2] + B$ (α: the ultrasonic absorption coefficient, A: the relaxation amplitude, and B: constant resulting from the classical absorption and other sources). As frequency increased at the low frequency range, the value of absorption (α/f^2) decreased and reached the high-frequency limiting value $(\alpha/f^2)_\infty = 25 \times 10^{-15}$ s^2/m, which is similar to the absorption value in water, 23×10^{-15} s^2/m [7]. The relaxation process did not occur at frequencies greater than 10 MHz. The excess

Table 1. Ultrasonic and thermodynamic parameters for non-ionized benzoic acid and β-CD (8.7 mM) at 25 °C.

Benzoic acid (mM)	f_r (MHz)	μ_m (10^{-4})	ν (m/s)	ρ (kg/l)
2	0.61	1.54	1495.2	1001.56
5	0.65	3.58	1495.7	1001.63
8	0.68	5.14	1496.1	1001.71
10	0.72	5.72	1496.6	1001.76
13	0.76	6.28	1497.2	1001.82
15	0.85	6.35	1497.5	1001.85

absorption was obtained by subtracting the experimental values $(\alpha/f^2)_\infty$ from the experimental data, and drawn in the form of absorption per wavelength μ (Fig. 2).

$$\mu = 2\mu_m \frac{f}{f_r}\left\{1+\left(\frac{f}{f_r}\right)^2\right\}^{-1} \quad (1)$$

where μ_m represents maximum absorption per wavelength at the relaxation frequency, f_r. The ultrasonic parameters (f_r, A, and B) were determined from the experimental data using the Eq. (1) with a good fit (see Table 1). The results indicate single relaxation absorption between β-CD and guest in aqueous solution as was previously suggested by Fukanori *et al.* that the observed relaxation is associated with a perturbation of the chemical equilibrium [8].

Based on the analysis of ultrasonic relaxation, the backward rate constant (k_b) can be obtained from f_r:

$$\begin{aligned} 2\pi f_r &= k_f\{[\beta - CD] + [\text{Guest}]\} + k_b \\ &= k_b\{(KC_{\beta-CD} + KC_{\text{Guest}} + 1)^2 \\ &\quad -4K^2C_{\beta-CD}\cdot C_{\text{Guest}}\}^{\frac{1}{2}} \end{aligned} \quad (2)$$

where K is the equilibrium constant defined as $K = k_f/k_b$. Thus, f_r is a function of C_{Guest} and the initial concentration of C_{Guest} is the only variable for the relaxation frequency when the concentration of β-CD is kept constant (8.7 mM). We used a non-linear optimization method to estimate the kinetic parameters, k_b and K, using Eq. (2) for the data points of C_{Guest} and f_r (Table 1). The plots and calculated line of $2\pi f_r$ *vs.* $\{(KC_{\beta-CD}+KC_{\text{Guest}}+1)^2-4K^2C_{(\beta-CD)}\cdot C_{\text{Guest}}\}^{1/2}$ were shown in Fig. 3 with good correlation using values of k_b and K obtained from Eq. (2). The forward rate constant (k_f) was obtained by $K = k_f/k_b$. The

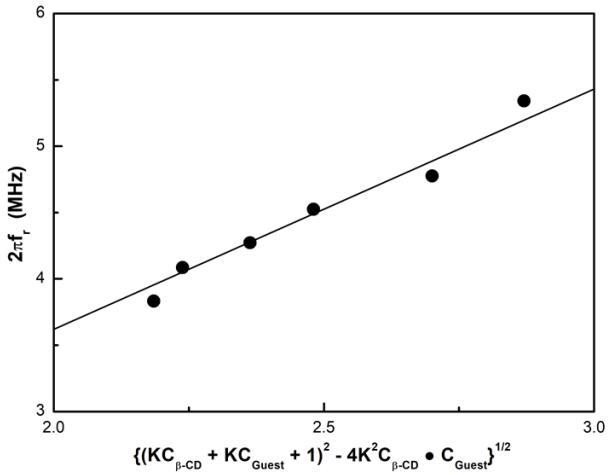

Fig. 3. Plots of $2\pi f_r$ *vs.* $\{(KC_{\beta-CD} + KC_{\text{Guest}} + 1)^2 - 4K^2C_{\beta-CD}\cdot C_{\text{Guest}}\}^{1/2}$ for non-ionized benzoic acid and β-CD (8.7 mM) in aqueous solution at 25 °C.

complex formation not only include the entry of the guest molecule into β-CD cavity but also include the accompanying non-covalent interactions between the guest molecule and β-CD inside the cavity [9]. Van der Waals forces and hydrophobic interactions mainly account for the inclusion complex formation [9] and the inclusion of guest molecule into β-CD cavity occurs by diffusion with k_f values of approximately 10^8 $M^{-1}s^{-1}$ [10–12]. The k_f values of benzoic acid and non-ionized aspirin were 2.47 $\times$ 10^8 $M^{-1}s^{-1}$ and 7.21 $\times$ 10^8 $M^{-1}s^{-1}$, respectively (Table 2), similar to the values of diffusion-controlled reactions. On the contrary, as the guest molecule becomes more hydrophobic, the lower is the k_b value as the result of host-guest complex stabilization. The k_b value of non-ionized benzoic acid (1.81 $\times$ 10^{-6} s^{-1}) is larger than that of non-ionized aspirin (1.31 $\times$ 10^{-6} s^{-1}), indicating high affinity of non-ionized aspirin than non-ionized benzoic acid in the β-CD cavity. As a result, non-ionized aspirin was released slower than non-ionized benzoic acid. We also studied the interaction of ionized 2-methoyx benzoic acid with β-CD. The k_b value of ionized 2-methoxy benzoic acid (7.48 $\times$ 10^{-6} s^{-1}) was 4-fold higher than that of non-ionized benzoic acid (Table 2), indicating that 2-methoxy benzoic acid is readily separated from β-CD cavity because of the interaction with water molecules.

We determined the standard volume change (ΔV) from the amplitude of relaxation (A) using the equation

Table 2. Rate and thermodynamic constants of benzoic acid derivatives with β-CD at 25 °C.

Guest	k_f (10^8 $M^{-1}s^1$)	k_b ($10^6 s^{-1}$)	K (M^{-1})	ΔV ($10^{-6} m^3 mol^{-1}$)	
Benzoic acid (pH≈1.7)	2.47	1.81	137	13.8	This study
Aspirin (pH≈1.7)	7.21	1.31	549	15.5	T. Fukahori *et al.* [4]
Aspirin (pH≈1.7)	2.08	2.88	73	11.5	Bae [15]
2-methoxy benzoic acid (pH≈7.0)	5.13	7.48	68.6	10.6	S. Park and J.-R. Bae [5]

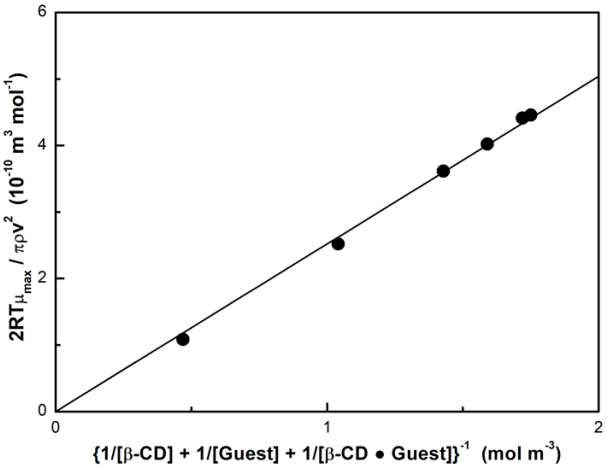

Fig. 4. Plots of $2RT\mu_m/\pi\rho^2$ *vs.* $(1/[\beta{-}CD]{+}1/[\text{Guest}]{+}1/[\beta - CD \cdot \text{Guest}])^{-1}$ for non-ionized benzoic acid and β-CD (8.7 mM) at 25 °C.

on the maximum absorption per wavelength (μ_m) [13]:

$$\begin{aligned}\mu_m &= 0.5Af_r\nu \\ &= \pi\rho\nu^2(1/[\beta - CD] + 1/[\text{Guest}] \\ &\quad +1/[\beta - CD \cdot \text{Guest}])^{-1}(\Delta V)^2/2RT \qquad (3)\end{aligned}$$

where R is the gas constant and T is the absolute temperature. The ΔV was determined from the slope of the graph for $\frac{2RT\mu_m}{\pi\rho\nu^2}$ *vs.* $(1/[\beta - CD] + 1/[\text{Guest}] + 1/[\beta - CD \cdot \text{Guest}])^{-1}$ (Fig. 4). Generally, five to seven water molecules exist in the β-CD cavity and some of them are forced to go out when the guest molecule enters the cavity [14]. The ΔV of non-ionized benzoic acid was 15.9×10^{-6} m^3mol^{-1} while that of non-ionized aspirin was 15.5×10^{-6} m^3mol^{-1}, suggesting that benzene ring is completely included in the β-CD cavity. In the case of ionized 2-methoxy benzoic acid, ΔV was 10.6×10^{-6} m^3mol^{-1} (Table 2), smaller than that of non-ionized benzoic acid. The charged group of 2-methoxy benzoic acid is considered to hinder the inclusion of benzene ring into β-CD cavity.

IV. CONCLUSION

We investigated the interactions of non-ionized benzoic acid with β-CD in the low-frequency range below 1 MHz. A single relaxation resulting from the dynamic interaction between non-ionized benzoic acid and β-CD in aqueous solution was observed. While the forward process of the complex (k_f) was a diffusion-controlled reaction, the backward rate constant (k_b) suggested that the complex was not stabilized by hydrogen bonds but by hydrophobic interactions between the two solutes. Furthermore, the inclusion complex of β-CD with non-ionized benzoic acid was more stable than that with ionized 2-methoxy benzoic acid. The results of this study suggest that the backward rate constant is affected by the charge group of guest molecules during inclusion complex formation.

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