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 ($\Delta V$) 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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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 used 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 cm³ 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 H₂O. 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 DISCUSSION

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

Guest + β-CD \rightleftharpoons \frac{k_f}{k_b} \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 \(\alpha\) 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], \(\alpha/f^2 = A/[1 + (f/f_r)^2] + B\) (\(\alpha\): 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 \(\alpha/f^2\) decreased and reached the high-frequency limiting value \(\lim_{f \to \infty} (\alpha/f^2) = 25 \times 10^{-15} \text{s}^2/\text{m}\), which is similar to the absorption value in water, \(23 \times 10^{-15} \text{s}^2/\text{m}\) [7]. The relaxation process did not occur at frequencies greater than 10 MHz. The excess
where $K$ backward rate constant ($k_b$) 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 $\beta$-CD cavity. As a result, non-ionized aspirin was released slower than non-ionized benzoic acid. We also studied the interaction of ionized 2-methoxy benzoic acid with $\beta$-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 $\beta$-CD cavity because of the interaction with water molecules.

We determined the standard volume change ($\Delta V$) from the amplitude of relaxation ($A$) using the equation

$$A = 2\mu_m f_r \left(1 + \left(\frac{f_r}{f_b}\right)^2\right)^{-1}$$

Fig. 3. Plots of $2\pi f_r$ vs. $\{(KC_{\beta-CD} + KC_{Guest} + 1)^2 - 4K^2C_{\beta-CD} \cdot C_{Guest}\}^{1/2}$ for non-ionized benzoic acid and $\beta$-CD (8.7 mM) in aqueous solution at 25 °C.
on the maximum absorption per wavelength ($\mu_m$) [13]:

$$
\mu_m = 0.5 A f \nu
$$

$$
= \pi \rho \nu^2 (1/|\beta - CD| + 1/|Guest| + 1/|\beta - CD \cdot Guest|)^{-1}(\Delta V)^2/2RT 
$$

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

**Table 2. Rate and thermodynamic constants of benzoic acid derivatives with $\beta$-CD at 25 °C.**

<table>
<thead>
<tr>
<th>Guest</th>
<th>$k_f$ (10$^8$ M$^{-1}$s$^{-1}$)</th>
<th>$k_b$ (10$^6$s$^{-1}$)</th>
<th>$K$ (M$^{-1}$)</th>
<th>$\Delta V$ (10$^{-6}$m$^3$mol$^{-1}$)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzoic acid (pH≈1.7)</td>
<td>2.47</td>
<td>1.81</td>
<td>137</td>
<td>13.8</td>
<td>This study</td>
</tr>
<tr>
<td>Aspirin (pH≈1.7)</td>
<td>7.21</td>
<td>1.31</td>
<td>549</td>
<td>15.5</td>
<td>Fukahori et al. [4]</td>
</tr>
<tr>
<td>Aspirin (pH≈1.7)</td>
<td>2.08</td>
<td>2.88</td>
<td>73</td>
<td>11.5</td>
<td>Bae [15]</td>
</tr>
<tr>
<td>2-methoxy benzoic acid (pH≈7.0)</td>
<td>5.13</td>
<td>7.48</td>
<td>68.6</td>
<td>10.6</td>
<td>S. Park and J.-R. Bae [5]</td>
</tr>
</tbody>
</table>

**IV. CONCLUSION**

We investigated the interactions of non-ionized benzoic acid with $\beta$-CD in the low-frequency range below 1 MHz. A single relaxation resulting from the dynamic interaction between non-ionized benzoic acid and $\beta$-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 $\beta$-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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**REFERENCES**


