Low-Frequency Ultrasonic Relaxation of β-Cyclodextrin and Adenosine 5'-Monophosphate in Aqueous Solution

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Nucleotides are the building blocks of nucleic acids and essential for many cellular functions. In this study, ultrasonic absorption spectra of β-cyclodextrin (β-CD) and adenosine 5'-monophosphate (AMP) in aqueous solution were measured over the broad frequency range 0.1-40 MHz with emphasis on the low-frequency range below 1 MHz. Here we show that the interaction of β-CD and AMP complies with a typical spectrum of a single relaxation process. We determined reliable rate (k_b) and equilibrium (K) constants and a standard volume change (ΔV) of the reaction. They are $k_b = 2.3\times10^{-6}$s^{-1}, K = 89 M^{-1}, and ΔV = 13.8 (10^{-6}m^{3}mol^{-1}), respectively.

Key Words: Cyclodextrin, Nucleotide, Host-guest interaction, Ultrasonic wave

Introduction

Cyclodextrins (CDs) are cyclic oligosaccharides composed of glucopyranose units linked by an α-(1→4) glucosidic bond.[1] Tyree types of CDs naturally occur with 6, 7, and 8 glucopyranose units, usually referred to as α-, β-, and γ-CD, respectively.[1] CDs, which are unique in characteristic due to their hydrophilic outer surface and hydrophobic inner cavity, are soluble in water and able to form inclusion complexes with a variety of guest molecules containing hydrophobic functional groups. CDs play important roles in biology, medicine, and pharmaceutical applications, such as extracting cholesterol from the cell surface or delivering water-insoluble drugs to target tissues.[1,2] Kinetic studies on β-CD as a host and a number of molecules as guests have provided valuable information on the use of β-CD as a probe to study molecule to molecule interactions.

Nucleotides are the structural units of DNA and RNA and play important roles in energy metabolism and cellular signaling.[3] Adenosine 5'-triphosphate (ATP) serves not only as an ubiquitous supply of energy in cells, but also as an extracellular signaling molecule via binding to ATP receptors on the cell surface.[4] The ratio of AMP/ATP in the cell acts as a metabolic sensor in the regulation of whole-body metabolism by switching on and off the anabolic and catabolic pathways.[5] Extracellular ATP, ADP or AMP, released from cells or created extracellularly from the breakdown of ATP in the case of ADP and AMP, works as a signaling molecule playing an important role in the determination of cell fate and metabolism.[6]

Several studies on the complex formation of β-CD and nucleotide have been reported. Formoso calculated the equilibrium constant, K, of β-CD and AMP using the circular dichroism method and showed that hydrophobic interaction does not play a significant role.[7] Seno *et al.* showed that more hydrophilic ATP and ADP were eluted faster than AMP using a chromatographic method.[8] Kondo and Nishikawa measured the relaxational absorption of β-CD and AMP using the ultrasonic relaxation method in the frequency range 0.8 - 95 MHz.[9] Although the cause of the relaxation was attributed to a perturbation of a chemical equilibrium associated with a complex formation,[9] their determination of forward (k_f) and backward (k_b) rate constants and the equilibrium constant, K, was limited in the low frequency range by relying upon extrapolation of experimental data.

Low-frequency ultrasound absorption in aqueous solution provides valuable information on the kinetic properties of biological molecules such as hemoglobin, myoglobin, and albumin. Barnes *et al.* showed that the dissociation equilibrium of the quaternary $\alpha_2\beta_2$ structure of hemoglobin to αβ dimers occurs at a relaxation frequency of 600 kHz.[10] Choi *et al.* showed that bovine serum albumin undergoes conformational changes in the relaxation frequencies of 200-300 kHz and the cause of the relaxation is attributed to helix-coil transitions in the tertiary structure of bovine serum albumin.[11]

In this study we sought to elucidate reliable values for the rate and equilibrium constants and a standard volume change for the interactions of β-CD and AMP using plano-concave resonator, plano-plano resonator, pulse-echo overlap, and the beam reflection methods in the frequency range of 0.1-40 MHz with emphasis on the frequency range below 1 MHz.

Methods

β-CD and AMP were purchased from Sigma (St. Louis, MO). Sample solutions were freshly prepared at pH values around 6.9. Experiments were carried out at 25℃ with temperature control within ± 0.1°C. Solution densities were measured by a vibrating density meter (Anton Paar NMA 35N).

We used three experimental techniques to measure ultrasonic absorption covering the wide frequency range of 0.1-40 MHz: a plano-concave resonance method (0.1-2 MHz), plano-plano resonance method (3-8 MHz), and beam reflection method (10-40 MHz). The velocity was measured using a pulse-echo overlap method at 3 MHz. The key apparatus of the present work used the high-Q ultrasonic resonance method equipped for the lower frequency range, where data acquisi-

tion was unreliable previously. Experiments were carried out as follows: Briefly, standing waves were established in a cylindrical cavity, which was composed of a 2-MHz fundamental X-cut quartz transducer and a concave reflector. The diameter of the cavity was 56 mm and the sample volume size was 50 cm^3. Using the Raman-Nath light diffraction method, a resonance spectrum was obtained with an optical heterodyne detection system. This technique allows the bandwidth of one resonance curve to give the absorption coefficient of the sample liquid. The high-quality factor attained with this resonator allowed reliable absorption measurements below 1 MHz, where the conventional resonance method has poor accuracy. The instrumental loss above 300 kHz was negligible and the loss below 300 kHz was calibrated using water. A conventional resonator cell consisting of 5-MHz fundamental X-cut quartz crystals, 2 cm in diameter, was used for the frequency range between 3 and 8 MHz.

Results and Discussion

The complex formation of β-CD and AMP can be described as the following chemical equilibrium:

$$\beta\text{-CD} + \text{AMP} \underset{k_b}{\overset{k_f}{\rightleftharpoons}} \beta\text{-CD}\cdot\text{AMP} \qquad (1)$$

where β-CD is the host molecule, AMP is the guest, and β-CD · AMP is the host-guest inclusion complex. k_f and k_b are the forward and backward rate constant, respectively. To elucidate the ultrasonic relaxations of β-CD and AMP at frequencies of 1 MHz or below, we first measured relaxations of β-CD or AMP in aqueous solution. No relaxation was observed for AMP as shown in Fig. 1, whereas β-CD showed ultrasonic relaxation at concentrations above 13 mM. In the present study, we fixed the concentration of β-CD at 8.7 mM. The ultrasonic absorption coefficient α at frequency f for 10 mM AMP and 8.7 mM β-CD is shown in Fig. 1 as a function of frequency. The solid line represents the single relaxation curve of a Debye- type single relaxation equation,[12] $\alpha/f^2 = A/[1+(f/f_r)^2]+B$, where α is the ultrasonic absorption coefficient, A is the relaxation amplitude, and the constant B represents the contribution from the classical absorption and other sources. The value of absorption, α/f^2, decreases linearly as frequency increases in the lower frequency range, and approaches the high-frequency limiting value $(\alpha/f^2)_\infty = 25\times10^{-15}$ s^2/m. This value is similar to the absorption value in water,[13] 23.0×10^{-15} s^2/m, suggesting that the relaxation process does not play a role at frequencies above 30 MHz.

To examine only the relaxation process, we calculated the excess absorption by subtracting the experimental values $(\alpha/f^2)_\infty$ from the experimental data, and then plotted the results in the form of absorption per wavelength μ, as shown in Fig. 2.

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

Here, μ_m denotes maximum absorption per wavelength at the relaxation frequency, f_r. The solid lines in Fig. 2 represent

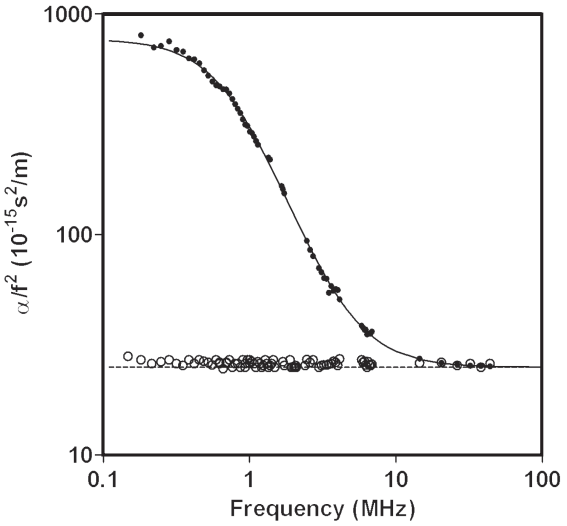

Figure 1. Ultrasonic absorption α/f^2 vs. frequency in aqueous solution of AMP at 25℃ in the presence and absence of 8.7 mM β-CD: (○) 10mM AMP only, (●) 10 mM AMP+8.7 mM β-CD. The solid line represents the single relaxation curve. The dotted line represents the high-frequency limiting value $(\alpha/f^2)_\infty = 25\times10^{-15}$ s^2/m.

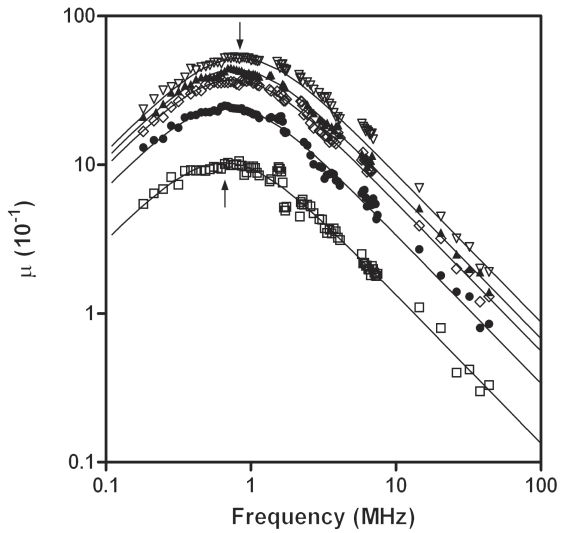

Figure 2. Excess absorption per wavelength μ vs. frequency in aqueous solutions of AMP in the presence of 8.7mM β-CD at 25℃. (□) 2mM AMP+8.7mM β-CD; (●) 5mM AMP+8.7mM β-CD; (◇) 8mM AMP+8.7mM β-CD; (▲) 10mM AMP+8.7mM β-CD; (▽) 13mM AMP+8.7mM β-CD. The solid lines represent single relaxation curves. The arrows indicate relaxation frequencies.

single relaxation curves of Eq. (2), and arrows indicate relaxation frequency. The experimental data are distributed close to the lines. Our results indicate that single relaxation absorption exists in aqueous solutions of β-CD and AMP.

In the analysis of ultrasonic relaxation, the relaxation time, τ, and the amplitude of the relaxation should be interpreted adequately. The relaxation time, τ, based on chemical relaxation analysis, is given by

$$\tau^{-1} = 2\pi f_r = k_b[(KC_{\beta\text{-}CD} + KC_{AMP} + 1)^2 - 4K^2 C_{\beta\text{-}CD}C_{AMP}]^{1/2} \qquad (3)$$

where K is the equilibrium constant defined as $K = k_f/k_b$ and $C_{\beta\text{-}CD}$ and C_{AMP} are the initial concentrations of host and guest,

Table 1. Ultrasonic and Thermodynamic Parameters for Aqueous Solutions of β-CD (8.7 mM) and AMP at 25 °C

AMP (mM)	f_r (MHz)	μ_m (10^{-4})	ν(m/s)	ρ(kg/l)
2	0.65	1.03	1499.1	1000.3
5	0.69	2.45	1499.7	1001.1
8	0.75	3.72	1499.1	1001.6
10	0.78	4.35	1500.2	1001.9
13	0.84	5.20	1500.5	1002.4

respectively. The initial concentration of guest, C_{AMP}, is the only variable for the relaxation frequency when the concentration of β-CD is kept constant (i.e., $C_{\beta\text{-}CD}$ = 8.7 mM in this study). Thus, the kinetic parameters, k_b and K, can be estimated from Eq. (3). Figure 3 shows the plots and calculated line of $2\pi f_r$ *vs.* $[(KC_{\beta\text{-}CD} + KC_{AMP} + 1)^2 - 4K^2C_{\beta\text{-}CD}C_{AMP}]^{1/2}$ using values of k_b and K obtained from Eq. (3) for β-CD and AMP in aqueous solution. The data correlates with the calculated line between β-CD and AMP. The forward rate constant, k_f, 2.1×10^8 $M^{-1}s^{-1}$, elucidated in this study is consistent with the observation by Debye that for a diffusion-controlled reaction in ionic solution the forward rate constant, k_f, is approximately 1.6×10^8 M^{-1} s^{-1}.[14] The backward rate constant, k_b, dependent on the structure of the guest molecules, is 2.3×10^6 s^{-1}, and is larger than k_b, 0.71×10^6 s^{-1} reported by Kondo and Nishikawa.[9]

The equilibrium constant, K, of β-CD and AMP reported varies depending upon experimental methods and conditions as shown in Table 2. Seno *et al.* determined the value to be 3130 M^{-1} by chromatography. The value determined using the ultrasonic relaxation method in this study is 89 M^{-1} and is 10 times smaller than the value determined by Kondo and Nishikawa, $(1.1 \pm 0.4) \times 10^3 M^{-1}$. The discrepancy of data is unclear. However, since experimental methods by Kondo and Nishikawa relied upon extrapolation of data to determine k_b, their value is not accurate below 1 MHz. The equilibrium constant, K, from circular dichroism spectra has been determined to be 41 M^{-1} by Formoso[7] and 44 M^{-1} by Suzuki *et al.*,[15] respectively, supporting our observation.

Table 2. Rate and Thermodynamic Constants for Interaction between β-CD and AMP 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^3mol^{-1})	
AMP	2.1 ± 0.2	2.3 ± 0.1	89 ± 5	13.8 ± 0.4	this study
AMP[a]	8.0 ± 2.6	0.71 ± 0.18	(11 ± 0.4)×10^3	19 ± 3	Kondo *et al.*[9]
AMP[b]			41		Formoso[7]
AMP[c]			44		Suzuki *et al.*[15]
AMP[d]			3130		Seno *et al.*[8]

The maximum absorption per wavelength, μ_m, is used for the analysis of the amplitude of the relaxation. This relationship is connected to the equilibrium concentrations of the reactants by the following equation.

$$\mu_m = \pi\rho\upsilon^2(1/[\beta\text{-CD}] + 1/[AMP] + 1/[\beta\text{-CD}\cdot AMP])^{-1} \times (\Delta V)^2/2RT \quad (4)$$

where, ΔV, is the standard volume change of reaction, R is the gas constant, and T is the absolute temperature. Once the equilibrium constant, K, was determined, it was possible to calculate the individual equilibrium concentration of the reactants. Next, the graph for $2RT\mu_m/\pi\rho v^2$ *vs.* $[(KC_{\beta\text{-}CD} + KC_{AMP}+1)^2 - 4K^2C_{\beta\text{-}CD}C_{AMP}]^{1/2}$ was drawn as shown in Fig. 4. The ΔV, the standard volume change of the reaction determined from the slope, is 13.8×10^{-6} m^3mol^{-1} and is consistent with the value of 19×10^{-6} m^3mol^{-1}, reported by Kondo and Nishikawa.[9]

The determination of reliable k_f and k_b values of β-CD and AMP is important for the understanding of the interactions of AMP with other molecules in cells. AMP and adenosine were shown to bind to the cell surface receptor and initiate G protein-mediated signals,[16] but the k_b value of AMP to its receptor has not been elucidated yet. Cyclic adenosine monophosphate (cAMP) is a secondary messenger in cells synthesized from ATP by adenylyl cyclase and leads to the activation of protein kinase A (PKA) by binding to the regulatory subunit of PKA.

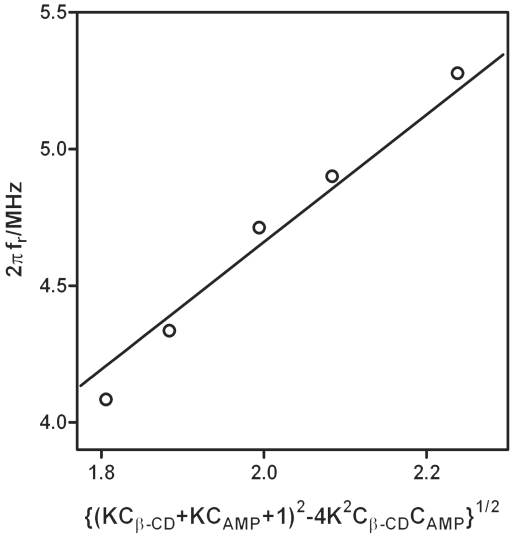

Figure 3. Plots of $2\pi f_r$ *vs.* $[(KC_{\beta\text{-}CD} + KC_{AMP} + 1)^2 - 4K^2C_{\beta\text{-}CD}C_{AMP}]^{1/2}$ for aqueous solution of AMP in the presence of 8.7 mM β-CD at 25°C.

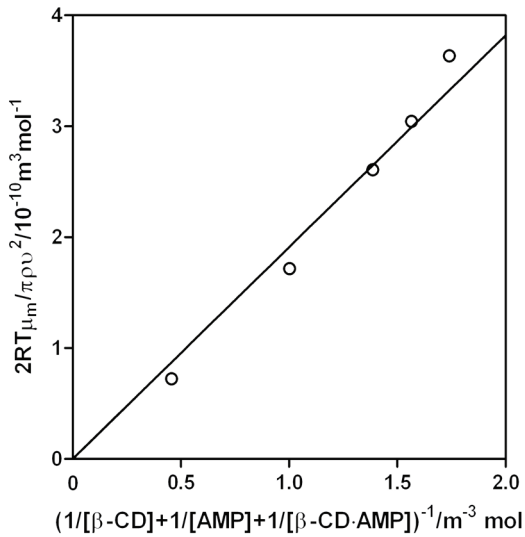

Figure 4. Plots of $2RT\mu_m/\pi\rho\upsilon^2$ *vs.* $(1/[\beta\text{-}CD] + 1/[AMP] + 1/[\beta\text{-}CD\cdot AMP])^{-1}$ for aqueous solution of AMP in the presence of 8.7 mM β-CD at 25°C.

Upon termination of cell signaling, cAMP is degraded into AMP by the enzyme phosphodiesterase. Ultrasonic relaxation method could be utilized to elucidate the kinetic properties of AMP and cAMP in cells including the interaction of cAMP with PKA. In this aspect, kinetic studies of β-CD with other nucleotides such as ADP, ATP, and cAMP using ultrasonic relaxation are highly sought to elucidate the hitherto unknown molecular interactions of adenosine nucleotides.

In conclusion, we studied the interactions of β-CD and AMP using ultrasonic relaxation in aqueous solution that resulted from host-guest interactions and determined the kinetic properties, especially in the frequency range below 1 MHz.

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