γ-Cyclodextrin Forms a Highly Compressible Complex with 1-Adamantanecarboxylic Acid

Nicolas Taulier and Tigran V. Chalikian*

Department of Pharmaceutical Sciences, Leslie Dan Faculty of Pharmacy, University of Toronto, 144 College Street, Toronto, Ontario M5S 3M2, Canada

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We report temperature-dependent ultrasonic velocimetric and densimetric data on changes in volume, expansibility, and adiabatic compressibility associated with the binding of 1-adamantanecarboxylic acid (AD) to γ-cyclodextrin (γ-CD). We compare these results with our previous data on the binding of AD to β-cyclodextrin (β-CD) [Taulier, N.; Chalikian, T. V. *J. Phys. Chem. B* 2006, *110*, 12222−12224]. The comparison reveals that, in contrast to the tight AD−β-CD complex with little void space left inside the cavity, AD forms a loose complex with γ-CD with ∼30 Å^3 of void space between the guest molecule and the inner walls of the cavity. The presence of the void renders the AD−γ-CD complex highly compressible; the intrinsic coefficient of compressibility of the AD−γ-CD complex is 37×10^{-6} bar^{-1} at 18 °C and decreases to 23×10^{-6} bar^{-1} at 55 °C. Such large compressibility is suggestive of only weak contacts between the interacting AD and γ-CD atomic groups in the cavity. Our results are consistent with the notion that the AD−γ-CD complex is predominantly stabilized by the hydrophobic effect with only modest contribution from intermolecular van der Waals interactions. This notion is in contrast to the AD−β-CD complex which is stabilized by strong host−guest van der Waals interactions in addition to the hydrophobic effect.

Introduction

Inclusion complexes between cyclodextrins and a variety of guest molecules have been extensively studied by physical chemists and molecular biophysicists as a relatively simple model that mimics protein binding events.[1] However, as noted by Connors, our current level of understanding of cyclodextrins "is not yet well enough to provide much confident guidance for the interpretation of macromolecular phenomena".[1] In this respect, we have only a rudimentary understanding of the contributions of different interactions (desolvation of interacting surfaces, a variety of host−guest intermolecular interactions, entropic factors) to the net thermodynamics of host−guest association. Inspection of the literature reveals that host−guest complexation events exhibit a wide range of associated changes in free energy, ΔG_b, enthalpy, ΔH_b, entropy, ΔS_b, and volume, ΔV_b.[1,2] This thermodynamic diversity involves not only the magnitude of the binding constant, K_b (or the binding free energy, ΔG_b) but also the magnitudes and the signs of the binding enthalpy, ΔH_b, entropy, ΔS_b, and volume, ΔV_b.[1,2] Clearly, the balance of forces regulating host−guest complexation depends on the specific pair of interacting species with no universal breakdown of the relative magnitudes of the thermodynamic contributions of different forces. Comparative studies of closely related inclusion complexes provide one possibility to categorize and characterize these forces. The binding of 1-adamantanecarboxylic acid (AD) to β-cyclodextrin (β-CD) and γ-cyclodextrin (γ-CD) represents an interesting example of such a possibility.

The thermodynamic profiles of the binding of AD to β-CD and γ-CD are markedly distinct.[3–5] The binding of AD to β-CD is stronger and accompanied by a negative change in enthalpy, ΔH_b, and an insignificant change in entropy, ΔS_b.[3,4] These results are inconsistent with the "classical" entropy-driven energetics of hydrophobic interactions-controlled events. In contrast, the binding of AD to γ-CD is associated with a small change in enthalpy, ΔH_b, and a large increase in entropy, ΔS_b, a thermodynamic profile characteristic of hydrophobic hydration.[4] These thermodynamic discrepancies have been rationalized in terms of the difference in the cavity size between β-CD (which consists of seven covalently linked glucose units) and γ-CD (which consists of eight glucose units) and the ensuing difference in the van der Waals interactions between the guest molecule and the interior wall of the cavity of the host molecule.[4] In particular, it has been suggested that the binding of AD to β-CD includes, in addition to the hydrophobic effect, strong van der Waals interactions.[4] This notion is in contrast to the binding of AD to γ-CD which has been suggested to be predominantly driven by the hydrophobic effect in the absence of significant van der Waals interactions.[4] Although these are attractive suggestions, additional data are required to support or refute them.

In a previous work, we have employed volumetric measurements to characterize the complexation of β-CD with AD.[6] Our measured temperature-dependent changes in volume, adiabatic compressibility, and expansibility were consistent with the picture in which ∼25 waters are released from nonpolar loci upon the binding of AD to β-CD.[6] In the present work, we extend these investigations to the binding of the same guest molecule to γ-CD. The differential analysis of the binding of AD to γ-CD versus β-CD allows us to compare and characterize the individual volumetric properties of the two host−guest complexes. Our combined volumetric results are consistent with the picture in which the AD−β-CD complex is stabilized by the hydrophobic effect and strong host−guest van der Waals contacts, whereas the binding of AD to γ-CD is predominantly driven by the hydrophobic effect with fewer and, probably, weaker intermolecular van der Waals interactions.

* Author to whom correspondence should be addressed. Tel.: (416) 946-3715. Fax: (416) 978-8511. E-mail: chalikan@phm.utoronto.ca.

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Materials and Methods

γ-CD and AD were purchased from Fluka (Buchs, Switzerland) and used without further purification. These compounds were dissolved in 20 mM sodium phosphate adjusted to pH 6.9 with 0.1 M HCl or 0.1 M NaOH. The concentrations of the samples were determined by weighing 10−20 mg of solute with a precision of ±0.03 mg and then dissolving the material in a known amount of buffer. Before weighing, AD and γ-CD were dried for 72 h under vacuum in the presence of phosphorus pentoxide. To prevent the formation of air bubbles at elevated temperatures (at 45 and 55 °C), the initial AD solution was preheated to 5 °C above the temperature at which the measurements were to be conducted prior to filling the ultrasonic or densimetric cells.

Solution sound velocities, U, were measured at 18, 25, 35, 45, and 55 °C at a frequency of 7.2 MHz using the resonator method[7–9] and a previously described differential technique.[9] The analysis of the frequency characteristics of the ultrasonic resonator cells required for sound velocity measurements was performed by a Hewlett-Packard model E5100A network/spectrum analyzer (Mississauga, ON, Canada). For the type of ultrasonic resonators used in this work, the accuracy of the sound velocity measurements is about $\pm 10^{-4}$ %.[9,10]

All densities were measured at 18, 25, 35, 45, and 55 °C with a precision of $\pm 1.5 \times 10^{-4}$ % using a vibrating tube densimeter (DMA-5000, Anton Paar, Gratz, Austria). The partial molar volumes, $V°$, of AD were calculated from the relationship

$$V° = M/\rho_0 - (\rho - \rho_0)/(\rho_0 C) \quad (1)$$

where M is the molecular weight of the solute, C is its molar concentration, and ρ and ρ_0 are the densities of the solution and the solvent (buffer solution), respectively.

The resulting values of U were used in conjunction with the $V°$ values derived from eq 1 to calculate the partial molar adiabatic compressibility, $K°_S$, using the relationship[11]

$$K°_S = \beta_{S0}(2V° - 2[U] - M/\rho_0) \quad (2)$$

where β_{S0} is the coefficient of adiabatic compressibility of the solvent and $[U]$ is the relative molar increment of the sound velocity of the solute, which is equal to $(U - U_0)/(U_0 C)$, where U and U_0 are the sound velocities in a solution and the solvent, respectively.

Ultrasonic and densimetric titration experiments (in which aliquots of γ-CD were incrementally added to the initial volume of the AD solution) were conducted following previously described protocols.[12] In these experiments, the initial concentration of AD was ∼2.5 mM. Each densimetric or ultrasonic velocimetric titration experiment was repeated three to five times, with the average values of $[U]$, $V°$, and $K°_S$ being reported.

Results and Discussion

Panels a and b of Figure 1 show, respectively, changes in the relative molar sound velocity increment, $[U]$, and partial molar volume, $V°$, accompanying the titration of the AD solution with the aliquots of γ-CD solution at 18, 25, 35, 45, and 55 °C. Sound velocity data in Figure 1a were approximated by a one-to-one stoichiometric binding model which yields the binding constants, K_b, and changes in the relative molar sound velocity increment, $\Delta[U]_b$, accompanying the formation of the AD−γ-CD complex. Our determined binding constants, K_b, are 315 ± 14, 324 ± 11, 426 ± 29, 542 ± 42, and 624 ± 47 M^{-1} at 18, 25, 35, 45, and 55 °C, respectively, which correspond to the binding free energies, $\Delta G_b = -RT \ln K_b$, of −3.3 ± 0.1, −3.4 ± 0.1, −3.7 ± 0.2, −4.0 ± 0.2, and −4.2 ± 0.2 kcal mol^{-1} at 18, 25, 35, 45, and 55 °C, respectively. We calculate a temperature-independent binding enthalpy, ΔH_b, of 3.8 ± 0.8 kcal mol^{-1} from the van't Hoff equation $\Delta H_b = -R[\partial \ln K_b(1/T)]_P$, and an average binding entropy, ΔS_b, of 24 ± 2 cal mol^{-1} K^{-1} from $\Delta S_b = -(\partial \Delta G_b/\partial T)_P$. Our binding constants, K_b, are lower compared to the calorimetric data of Cromwell et al.[4] but are in excellent agreement with the spectrophotometric results reported by Gelb et al.[13]

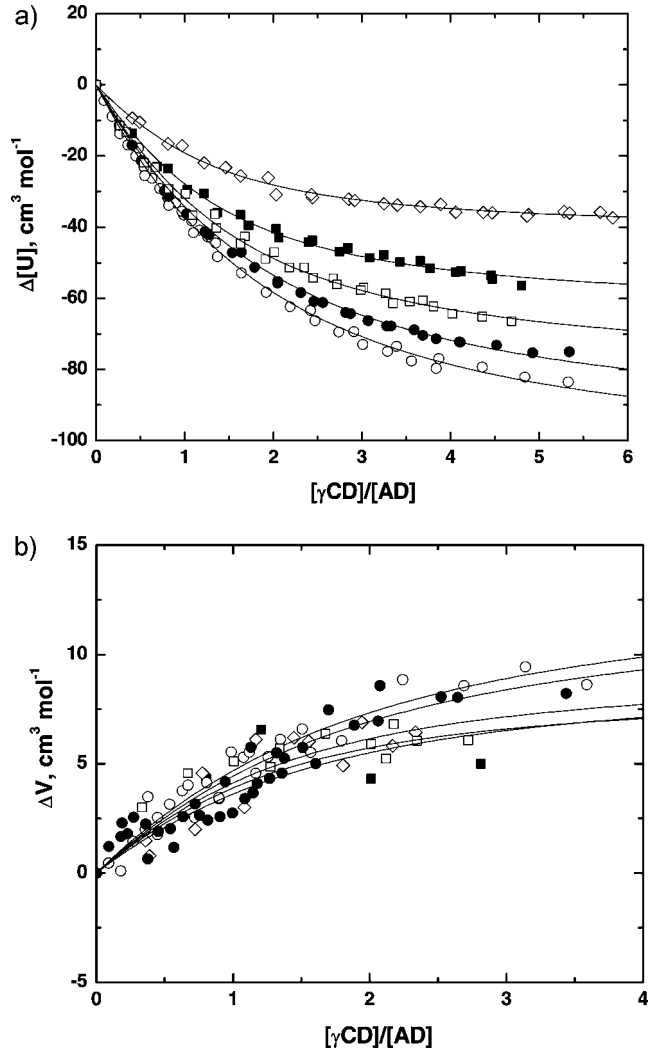

Figure 1. (a) Changes in relative molar sound velocity increment, $\Delta[U]$, of AD as a function of the γ-CD-to-AD ratio at 18 (○), 25 (●), 35 (□), 45 (■), and 55 °C (◇); (b) changes in partial molar volume, ΔV, of AD as a function of the γ-CD-to-AD ratio at 18 (○), 25 (●), 35 (□), 45 (■), and 55 °C (◇). The initial concentration of AD is ∼2.5 mM. Errors of $\Delta[U]$ and ΔV are ±1.1 and ±1.3 cm^3 mol^{-1}, respectively. The plots were fit with a noncooperative one-to-one stoichiometric binding model.

The binding constants, K_b, determined from the acoustic titration measurements were subsequently used in conjunction with the one-to-one binding model to fit the more noisy volume data presented in Figure 1b and evaluate changes in volume, ΔV_b, accompanying the association of AD with γ-CD. The values of $\Delta[U]_b$ and ΔV_b were combined to calculate changes in adiabatic compressibility, $\Delta K_{Sb} = 2\beta_{S0}(\Delta V_b - \Delta[U]_b)$, accompanying the binding. Table 1 presents our determined values of $\Delta[U]_b$, ΔV_b, and ΔK_{Sb} at 18, 25, 35, 45, and 55 °C.

Figure 2a graphically presents the temperature dependence of ΔV_b for the binding of AD to γ-CD along with the

TABLE 1: Changes in Relative Molar Sound Velocity Increment, $\Delta[U]_b$, Volume, ΔV_b, and Adiabatic Compressibility, ΔK_{Sb}, Accompanying the Binding of AD to γ-CD

T, °C	18 °C	25 °C	35 °C	45 °C	55 °C
$\Delta[U]_b$, cm^3 mol^{-1}	-109.0 ± 1.2	-98.8 ± 1.2	-81.5 ± 1.2	-64.1 ± 1.2	-41.8 ± 1.2
ΔV_b, cm^3 mol^{-1}	13.7 ± 1.0	12.8 ± 1.0	9.5 ± 1.0	8.5 ± 1.0	9.2 ± 1.0
ΔK_{Sb}, 10^{-4} cm^3 mol^{-1} bar^{-1}	112.9 ± 1.4	99.9 ± 1.4	80.3 ± 1.4	62.1 ± 1.4	43.2 ± 1.4

temperature dependence of ΔV_b for the binding of AD to β-CD from our previous work.[6] Inspection of Figure 2a reveals that, although the absolute values of ΔV_b are markedly distinct for the two binding events, the temperature slopes $\Delta\Delta V_b/\Delta T$ are practically identical. Recall that $\Delta\Delta V_b/\Delta T$ represents the change in expansibility accompanying the binding, ΔE_b. The values of ΔE_b are equal to -0.14 ± 0.04 and -0.12 ± 0.01 cm^3 mol^{-1} K^{-1} for the binding of AD to γ-CD and β-CD, respectively. Numerous experimental results suggest that partial molar expansibility, $E°$, predominantly reflects solute hydration; hence, a change in expansibility accompanying a chemical reaction should originate mainly from the related changes in hydration.[14–17] With this notion, one may plausibly conclude that the observed similarity in ΔE_b for the association of AD with β-CD and γ-CD is consistent with the picture in which the two binding events are accompanied by similar changes in hydration. This conclusion is further supported by structural considerations as explained below.

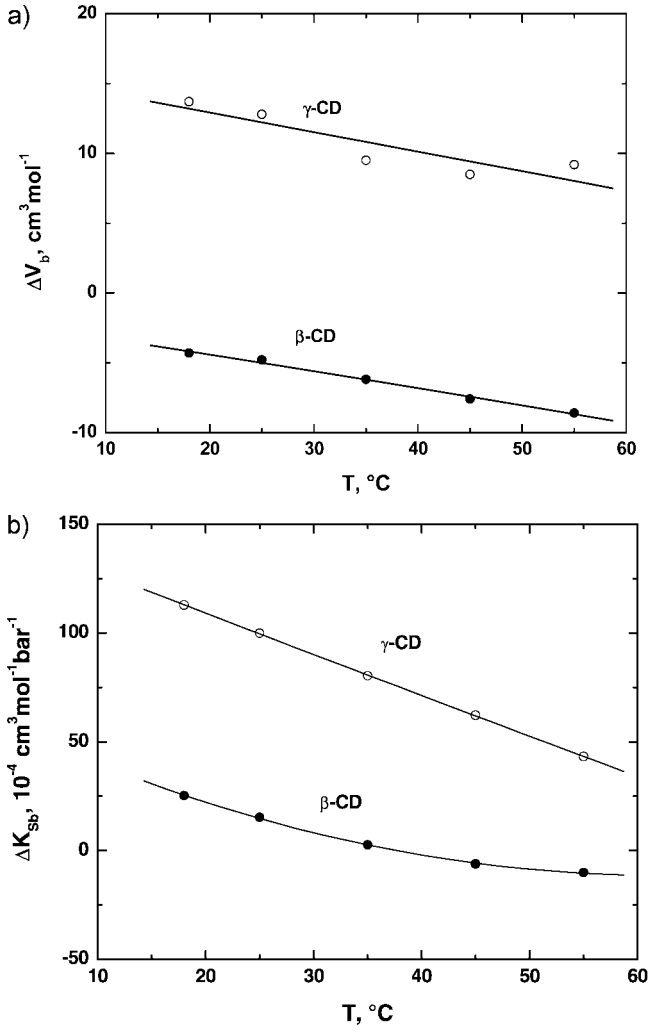

Figure 2. (a) Temperature dependences of changes in volume, ΔV_b, accompanying the binding of AD to β-CD (●) (ref 6) and γ-CD (○); (b) temperature dependences of changes in adiabatic compressibility, ΔK_{Sb}, accompanying the binding of AD to β-CD (●) (ref 6) and γ-CD (○). Errors in ΔV_b and ΔK_{Sb} are ± 1.0 cm^3 mol^{-1} and $\pm 1.4 \times 10^{-4}$ cm^3 mol^{-1} bar^{-1}, respectively. All plots are approximated by second-order polynomials.

β-CD and γ-CD both represent truncated cones with interior cavities 7.9 Å deep.[18] The adamantyl group of AD, which is nearly spherical with a diameter of ~7 Å, may penetrate the same depth into the cavities of β-CD and γ-CD. The volume of the adamantyl group of AD is ~180 Å^3. Consequently, AD forms a tight complex with β-CD (with the cavity volume of 270 Å^3) with little space left within the host−guest interface. On the other hand, due to the larger size of γ-CD (with the cavity volume of 400 Å^3), its complex with AD is loose with the average separation between the adamantyl group of AD and the internal walls of the cavity (on the order of ~0.5 Å) not being sufficient to allow for any water molecule. One may, therefore, speculate that the number of water molecules released to the bulk upon the binding of AD to γ-CD should be very similar to that observed for the binding to β-CD. Within the context of such a conjecture, the differential changes in volumetric properties associated with the binding of AD to β-CD and γ-CD should be reflective of the void space inside the γ-CD−AD inclusion complex. With this notion, the differential change in volume, $\Delta\Delta V_b$, accompanying the binding of AD to γ-CD and β-CD (see Figure 2a) is roughly equal to the volume of the void space inside the loosely packed AD−γ-CD complex; $\Delta\Delta V \approx 17$ cm^3 mol^{-1} (~30 Å^3). This result is in qualitative agreement with the volume of the structural void between AD and the internal walls of the γ-CD cavity that can be estimated from geometric considerations. Implicit in this treatment is the assumption that water molecules released to the bulk from the interior cavities of β-CD and γ-CD are characterized by similar changes in volume as well as in compressibility (see below). This is a plausible assumption given the similarity in the chemical nature of the functional groups lining the inner walls of the cavities of the two cyclodextrin types.

Figure 2b depicts the temperature dependences of compressibility changes, ΔK_{Sb}, accompanying the binding of AD to γ-CD and β-CD (the latter is taken from our previous work[6]). Inspection of Figure 2b reveals that the differential change in compressibility accompanying the binding, $\Delta\Delta K_{Sb}$, decreases from 87.5×10^{-4} cm^3 mol^{-1} bar^{-1} at 18 °C to 54.2×10^{-4} cm^3 mol^{-1} bar^{-1} at 55 °C. Assuming similarity of hydration changes associated with AD binding to β-CD and γ-CD, the value of $\Delta\Delta K_{Sb}$ reflects the compressibility of the void volume inside the AD−γ-CD complex. Normalizing $\Delta\Delta K_{Sb}$ by the volume of the γ-CD cavity (400 Å^3 or 240 cm^3 mol^{-1}), one obtains the intrinsic coefficient of adiabatic compressibility, β_M, of the AD−γ-CD inclusion complex. The value of β_M equals 37×10^{-6}, 36×10^{-6}, 33×10^{-6}, 29×10^{-6}, and 23×10^{-6} bar^{-1} at 18, 25, 35, 45, and 55 °C, respectively. To the best of our knowledge, these results represent the first estimate of the compressibility of host−guest inclusion complexes. The determined values of β_M, which are quite large and comparable to the compressibility of protein interior (~25×10^{-6} bar^{-1}), suggest loose packing inside the AD−γ-CD complex with weak van der Waals interactions.[17,19–21] This observation is in contrast

to the tightly packed core of the AD−β-CD complex with its strong van der Waals interactions that should not exhibit any significant intrinsic compressibility.

In the aggregate, our comparative analysis of the binding of AD to γ-CD versus β-CD suggests that the AD−γ-CD inclusion complex is loosely packed with ~30 Å^3 of void space between the guest molecule and the inner walls of the cavity. The presence of the void renders the AD−γ-CD complex highly compressible. Our estimated intrinsic coefficient of compressibility of the AD−γ-CD complex ranges from 37×10^{-6} bar^{-1} at 18 °C to 23×10^{-6} bar^{-1} at 55 °C. Such large compressibility is suggestive of only weak van der Waals contacts between the interacting AD and γ-CD atomic groups in the cavity. Our combined volumetric results support the notion that, whereas the binding of AD to β-CD is stabilized by the hydrophobic effect and strong host−guest van der Waals interactions, the binding of AD to γ-CD is predominantly driven by the hydrophobic effect with fewer intermolecular van der Waals contacts.

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