THE JOURNAL OF PHYSICAL CHEMISTRY B LETTERS

2006, *110,* 12222−12224
Published on Web 06/08/2006

Hydrophobic Hydration in Cyclodextrin Complexation

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

Received: April 21, 2006; In Final Form: May 16, 2006

We report temperature-dependent acoustic and densimetric data on changes in volume, expansibility, and adiabatic compressibility accompanying the binding of 1-adamantanecarboxylic acid (AD) to β-cyclodextrin (β-CD). We interpret our volumetric results in terms of hydration. Based on our compressibility and expansibility data, we estimate that, at 25 °C, the binding of AD to β-CD is accompanied by displacement of 20 to 25 water molecules from the hydration shells of the two interacting species. Comparison of the temperature-dependent compressibility changes accompanying the binding of AD to β-CD with the compressibility contribution of aliphatic groups suggests that displaced water molecules predominantly come from the hydrophobic loci of AD and β-CD. Thus, we conclude that hydrophobic interactions play a major role in stabilizing the AD−β-CD complex. Our estimate of the number of water molecules released to the bulk is consistent with structural considerations. There is also good agreement between our volumetric data and osmotic stress results reported by Harries et al. (Harries, D.; Rau, D. C.; Parsegian, V. A. *J. Am. Chem. Soc.* **2005,** *127,* 2184). This observation is consistent with the picture in which the two techniques probe the same population of water molecules solvating AD and β-CD.

Cyclodextrins consist of six to eight covalently linked glucose units which are arranged into a hollow truncated cone.[1] Nonpolar molecules of an appropriate size can bind into the central cavity of cyclodextrins, thereby forming "guest−host" inclusion complexes. The thermodynamics of cyclodextrin−ligand interactions has been extensively studied.[1−5] Despite these efforts and the seeming simplicity of cyclodextrins and their complexes, we still have a rather rudimentary understanding of the nature and relative magnitude of the forces that govern the stability of cyclodextrin complexes. In particular, there is disagreement among the researchers concerning the role of hydrophobicity in stabilizing guest−host complexes.[1] This confusion stems in part from the observations that most but not all cyclodextrin−ligand association events are accompanied by negative changes in enthalpy, ΔH, and negative or close to zero changes in entropy, ΔS.[1,2,4−6] These observations are inconsistent with the "classical" entropy-driven energetics of hydrophobic interaction-controlled events.

Hydrophobicity is intimately related to hydration. If hydrophobic interactions play a major role in a binding process, the latter must be accompanied by substantial release of water molecules from the hydration shells of nonpolar groups. In line with this notion, we employed volumetric measurements to characterize changes in hydration accompanying the binding of 1-adamantanecarboxylic acid (AD) to β-cyclodextrin (β-CD) which contains seven glucose units. Specifically, we performed β-CD-into-AD densimetric and ultrasonic velocimetric titration experiments to determine the partial molar volume, V°, and relative molar sound velocity increment, [U], of AD in the absence and presence of β-CD at 18, 25, 35, 45, and 55 °C ($[U] = (U - U_0)/(U_0C)$ where U and U_0 are the sound velocities in solution and the solvent, respectively; and C is the molar concentration of a solute). Figure 1 plots the relative molar sound velocity increment, [U] (●), and partial molar volume, $V°$ (○), of AD against the β-CD-to-AD ratio, r, at a representative temperature of 25 °C. The partial molar adiabatic compressibility, $K°_S$, of solute can be calculated from $V°$ and [U] using $K°_S = \beta_{S0}(2V° - 2[U] - M/\rho_0)$, where ρ_0 and β_{S0} are, respectively, the density and coefficient of adiabatic compressibility of the solvent, and M is the molecular weight of a solute.[7] The plots shown in Figure 1 and similar plots obtained at other experimental temperatures (not shown) were used to calculate changes in volume, ΔV, relative molar sound velocity increment, $\Delta[U]$, and adiabatic compressibility, $\Delta K_S = 2\beta_{S0}(\Delta V - \Delta[U])$, accompanying the association of AD with β-CD. Figure 2 graphically illustrates the temperature dependences of changes in volume, ΔV (○), and adiabatic compressibility, ΔK_S (●), accompanying the binding of AD to β-CD. A change in expansibility, $\Delta E = (\partial \Delta V/\partial T)_P$, upon the binding can be calculated from the data shown in Figure 2 as the temperature slope of ΔV.

The partial molar adiabatic compressibility, $K°_S$, and expansibility, $E°$, of a solute are the sums of the intrinsic and hydration contributions:[7−9]

$$K°_S = K_M + n_h(K_{Sh} - K_{S0}) \quad (1)$$

$$E° = E_M + n_h(E_h - E_0) \quad (2)$$

where K_M and E_M are, respectively, the intrinsic compressibility and expansibility of a solute molecule; K_{S0} and K_h are the partial

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

10.1021/jp062467n CCC: $33.50

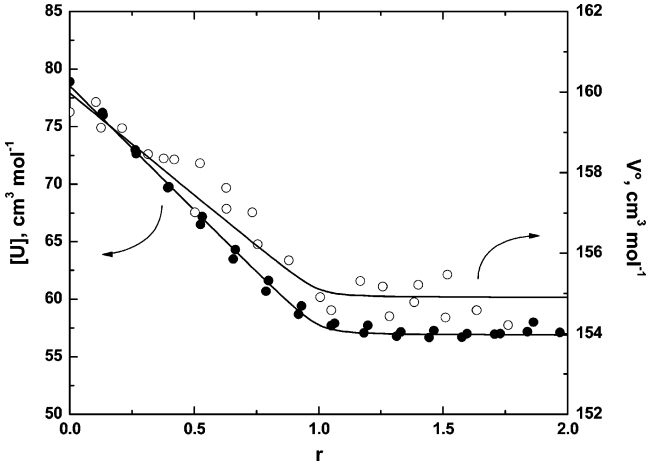

Figure 1. Relative molar sound velocity increment, [*U*] (●), and partial molar volume, *V*° (○), of AD as a function of the *β*-CD-to-AD ratio at 25 °C. The initial concentration of AD is 2.5 mM. Errors of [*U*] and *V*° are ±1.1 and ±0.9 cm^3 mol^{-1}, respectively. Acoustic and densimetric titration measurements were performed in a 20 mM phosphate buffer at pH 6.9 following previously described protocols.[15] Acoustic measurements were carried out with a previously described ultrasonic resonator.[16] Densimetric measurements were performed using an Anton Paar model DMA 5000 densimeter (Gratz, Austria). The plots were fit with a noncooperative one-to-one stoichiometric binding model.

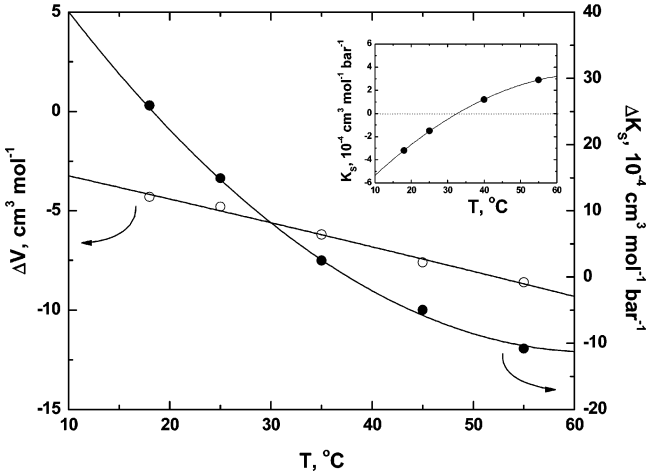

Figure 2. Temperature dependences of changes in volume, Δ*V* (○), and adiabatic compressibility, ΔK_S (●), accompanying the binding of AD to *β*-CD. Errors in Δ*V* and ΔK_S are ±0.7 cm^3 mol^{-1} and ±1.0 × 10^{-4} cm^3 mol^{-1} bar^{-1}, respectively. The inset shows the temperature dependence of the compressibility contribution of an independently hydrated −CH_2− group in long α,*ω*-aminocarboxylic acids.[10] All plots are approximated by second-order polynomials.

molar adiabatic compressibilities of water in the bulk state and the hydration shell of a solute, respectively; and E_0 and E_h are the partial molar expansibilities of water in the bulk state and the hydration shell of a solute, respectively. For low molecular weight compounds (such as AD and *β*-CD), the intrinsic compressibility, K_M, and expansibility, E_M, are small compared to the compressibility of liquid water and, consequently, can be ignored in analyses. Differentiating eqs 1 and 2, one obtains the following relationships for changes in compressibility, ΔK_S, and expansibility, Δ*E*, accompanying the association of AD with *β*-CD:

$$\Delta K_S = \Delta n_h(K_{Sh} - K_{S0}) \quad (3)$$

$$\Delta E = \Delta n_h(E_h - E_0) \quad (4)$$

Note that an alteration in hydration is the only type of molecular interaction to which our measured changes in compressibility, ΔK_S, and expansibility, Δ*E*, are sensitive. Thus, the compressibility contribution of an individual atomic group to $n_h(K_{Sh} - K_{S0})$ describes a change in the state of water upon its uptake from the bulk into the hydration shell of that group. The inset in Figure 2 shows the temperature dependence of the compressibility contribution of independently hydrated −CH_2− groups in long α,*ω*-aminocarboxylic acids.[10] It increases from negative to positive, passing zero at ∼35 °C. This temperature-dependent behavior of aliphatic groups is quantitatively and qualitatively distinct from that exhibited by charged or polar atomic groups and represents a volumetric signature of hydrophobic hydration.[9,11,12] Hence, if water molecules released to the bulk come predominantly from nonpolar domains of AD and *β*-CD, the value of ΔK_S is expected to change from positive to negative as temperature increases, passing zero at around 35 °C. In striking agreement with this expectation, inspection of Figure 2 reveals that a change in compressibility, ΔK_S, accompanying formation of the AD−*β*-CD inclusion complex is positive at low temperatures but decreases with temperature increasing and becomes negative above ∼37 °C. Using this volumetric behavior as diagnostic tool, we conclude that the binding of AD to *β*-CD is accompanied by displacement of water molecules from the hydration shells of nonpolar groups of the two reacting species. It should be noted, however, that while rearrangement in the waters interacting with the hydrophilic groups of the reacting species cannot be excluded, such rearrangements do not appear to result in any significant release or uptake of water molecules from the hydrophilic domains.

At 25 °C, a compressibility change, ΔK_S, accompanying the binding of AD to *β*-CD is (14.9 ± 1.3) × 10^{-4} cm^3 mol^{-1} bar^{-1}. This value can be used in conjunction with eq 3 to evaluate the number of water molecules, Δn_h, released to the bulk. For such an evaluation, one needs to know the differential partial molar adiabatic compressibility of water of hydrophobic hydration and bulk water, ($K_{Sh} - K_{S0}$). To this end, we use reported data on the compressibility contribution of a −CH_2− group that directly contacts approximately three water molecules (based on the solvent accessible surface area of a −CH_2− group of 25 Å^2 and the effective cross-section of a water molecule of 9 Å^2, n_h = 25/9 ≈ 3).[10,11] At 25 °C, the average compressibility contribution of a −CH_2− group is −(1.8 ± 0.2) × 10^{-4} cm^3 mol^{-1} bar^{-1}.[10,11] Hence, for practical calculations, ($K_{Sh} - K_{S0}$) can be taken to be −(0.60 ± 0.05) × 10^{-4} cm^3 mol^{-1} bar^{-1} (−1.8 × 10^{-4}/3). With this value and eq 3, we estimate the number of water molecules released to the bulk, Δn_h, to be −25 ± 6 (−14.9 × 10^{-4}/0.60 × 10^{-4}).

A similar estimate can be made from our measured expansibilty data, Δ*E*, using eq 4. From the linear temperature dependence of Δ*V* data presented in Figure 2, we calculate a temperature-independent value of $\Delta E = \Delta V/\Delta T$ equal to −0.12 ± 0.01 cm^3 mol^{-1} K^{-1}. The negative sign of Δ*E* is in agreement with pressure-perturbation calorimetric results of Cameron and Cooper.[5] Note that all atomic groups, independent of their chemical nature, positively contribute to the partial molar expansibilities, *E*°, of solutes.[13,14] Thus, the negative sign of Δ*E* is suggestive of the binding-induced dehydration of AD and *β*-CD. At 25 °C, the expansibility contribution of an independently hydrated −CH_2− group in long α,*ω*-aminocarboxylic acids is 0.018 ± 0.003 cm^3 mol^{-1} K^{-1}.[10] Hence, the value of ($E_h - E_0$) is −(6.0 ± 0.7) × 10^{-3} cm^3 mol^{-1} K^{-1} (0.018/3). With this value and eq 4, Δn_h equals −20 ± 5 (−0.12/6.0 × 10^{-3}).

One can perform analogous calculations using our ΔK_S and ΔE data obtained at other experimental temperatures studied in this work. These calculations reveal that Δn_h shows a tendency to decrease in absolute magnitude with an increase in temperature. For example, at 55 °C, when ΔK_S is $-(10.7 \pm 1.3) \times 10^{-4}$ cm^3 mol^{-1} bar^{-1} and the compressibility contribution of a $-CH_2-$ group is $(2.9 \pm 0.2) \times 10^{-4}$ cm^3 mol^{-1} bar^{-1},[10] the calculated value of Δn_h equals -11 ± 6. From expansibility data, one estimates an identical value of Δn_h of -12 ± 5 [at 55 °C, the expansibility contribution of an independently hydrated $-CH_2-$ group is 0.030 ± 0.003 cm^3 mol^{-1} K^{-1} [10]]. The observed decrease in Δn_h is not unexpected and consistent with partial dehydration of the reacting species at elevated temperatures.

Our estimate of 20 to 25 waters displaced upon AD−β-CD binding at 25 °C corresponds to the estimate performed by Harries et al.[6] based on their osmotic stress measurements (15 to 25 waters). Our results are also consistent with structure-based estimates. The cylindrical internal cavity of β-CD has a volume of ∼270 Å^3 and may contain 11 water molecules.[1] Based on the solvent-accessible surface area of AD of 323 Å^2 and effective cross-section of a water molecule of 9 Å^2, the number of water molecules within the first coordination layer of AD is 35 (323/9). However, the number of water molecules released from the hydration shell of AD should be less than 35, since, in the complex, the negatively charged carboxyl group of AD is not enclosed within the cavity and remains accessible to water. Based on the solvent-accessible surface area of the carboxyl group, the number of water molecules within its first coordination layer is ∼10. Thus, the upper limit of the number of water molecules released to the bulk upon the binding of AD to β-CD is 36 (11 + 35 − 10). This value is qualitatively consistent with our estimate of 20 to 25.

In conclusion, our results suggest that significant dehydration accompanies the binding of AD to β-CD with the displacement of 20 to 25 water molecules. This estimate is consistent with structural considerations. Displaced water molecules come predominantly from hydrophobic loci of AD and β-CD. Thus, despite the nonclassical energetics profile of AD−β-CD association, hydrophobic interactions play a major role in stabilizing the AD−β-CD complex. The energetics of hydrophobic interactions apparently is offset by the energetics of other interactions, which is manifested in nonclassical profiles of ΔH and ΔS of binding. There is good agreement between our volumetric data and osmotic stress results reported by Harries et al.[6] This observation is consistent with the picture in which the two techniques probe the same population of water molecules solvating AD and β-CD.

Acknowledgment. We are grateful to Dr. Robert B. Macgregor, Jr. for stimulating discussions and useful comments. This work was supported by grants from the Natural Sciences and Engineering Research Council of Canada and Petroleum Research Fund of the American Chemical Society to T.V.C.

References and Notes

(1) Connors, K. A. *Chem. Rev.* **1997**, *97*, 1325.
(2) Cromwell, W. C.; Bystrom, K.; Eftink, M. R. *J. Phys. Chem.* **1985**, *89*, 326.
(3) Eftink, M. R.; Andy, M. L.; Bystrom, K.; Perlmutter, H. D.; Kristol, D. S. *J. Am. Chem. Soc.* **1989**, *111*, 6765.
(4) Harrison, J. C.; Eftink, M. R. *Biopolymers* **1982**, *21*, 1153.
(5) Cameron, D.; Cooper, A. *J. Incl. Phenom. Macrocycl. Chem.* **2002**, *44*, 279.
(6) Harries, D.; Rau, D. C.; Parsegian, V. A. *J. Am. Chem. Soc.* **2005**, *127*, 2184.
(7) Sarvazyan, A. P. *Annu. Rev. Biophys. Biophys. Chem.* **1991**, *20*, 321.
(8) Chalikian, T. V. *Annu. Rev. Biophys. Biomol. Struct.* **2003**, *32*, 207.
(9) Chalikian, T. V.; Sarvazyan, A. P.; Breslauer, K. J. *Biophys. Chem.* **1994**, *51*, 89.
(10) Chalikian, T. V.; Sarvazyan, A. P.; Breslauer, K. J. *J. Phys. Chem.* **1993**, *97*, 13017.
(11) Kharakoz, D. P. *J. Phys. Chem.* **1991**, *95*, 5634.
(12) Taulier, N.; Chalikian, T. V. *Biochim. Biophys. Acta* **2002**, *1595*, 48.
(13) Lin, L. N.; Brandts, J. F.; Brandts, J. M.; Plotnikov, V. *Anal. Biochem.* **2002**, *302*, 144.
(14) Kharakoz, D. P. *Biophys. Chem.* **1989**, *34*, 115−125.
(15) Chalikian, T. V.; Gindikin, V. S.; Breslauer, K. J. *J. Mol. Biol.* **1995**, *250*, 291.
(16) Sarvazyan, A. P. *Ultrasonics* **1982**, *20*, 151.