Cyclodextrin Inclusion Complexes: Studies of the Variation in the Size of Alicyclic Guests

Maurice R. Eftink,*,† Maria L. Andy,† Katarina Bystrom,† Howard D. Perlmutter,‡ and David S. Kristol‡

Contribution from the Department of Chemistry, University of Mississippi, University, Mississippi 38677, and the Department of Chemistry and Chemical Engineering, New Jersey Institute of Technology, Newark, New Jersey 07102. *Received November 18, 1988*

Abstract: We have determined thermodynamic parameters for the interaction of a congener series of alicyclic carboxylic acids (e.g., adamantanecarboxylic acid, bicyclo[2.2.2.]octanecarboxylic acid, etc.) with α- and β-cyclodextrin hosts in aqueous solution. The side chains of these guests are roughly spherical and vary in carbon number from 11 to 5. With β-cyclodextrin, we find that 1:1 hosts–guest inclusion complexes are formed with all the guests at both low and high pH, with stronger binding occurring as low pH, where the carboxylic acid forms of the guests predominate. With α-cyclodextrin, 1:1 complexes are formed with the carboxylate forms of the guests, but 2:1 bis(α-cyclodextrin)–guest complexes are formed, in a cooperative manner, with the carboxylic acid forms of the guests. A strong guest-size dependence is found for both the free-energy change and enthalpy change for complex formation. The variation of these thermodynamic parameters with guest size is used to draw inferences about contributions to the overall binding free-energy change from the hydrophobic effect and van der Waals forces. Data were obtained by using flow microcalorimetry, pH potentiometric measurements, and spectral displacement methods.

The formation of guest–host inclusion complexes between small organic compounds and cyclodextrins (CDs) has proven to be an excellent model system for studying the nature of noncovalent bonding forces in aqueous solution.[1-5] As such, they have provided valuable insights concerning the hydrophobic effect and London dispersion forces[6-11] and are good models for understanding the specificity of enzyme–substrate interactions.[12] For example, CDs have been found to preferentially associate with the ionized form of para-substituted phenols[4,13,14] and the neutral form of several aromatic and aliphatic carboxylic acids.[7,15,16]

We have characterized the interaction of adamantanecarboxylate (AC) with CDs, with emphasis on the effects produced by variation of the size of the cavity within the series: α-CD (cyclohexaamylose, 5.5-Å cavity), β-CD (cycloheptaamylose, 7.0-Å cavity), and γ-CD (cyclooctaamylose, 9.5-Å cavity).[10,11] The adamantyl group of AC has a diameter of $\sim$7 Å, and we have found the strongest binding to occur with β-CD, consistent with the near-perfect match between the cavity and guest diameter. In this case, the association constant is approximately 3×10^4 M^{-1}, making the stability of this complex comparable to that found in protein–ligand systems.

The binding of AC to β-CD was found to occur at a 1:1 ratio and to be driven almost entirely by an exothermic enthalpy change, $\Delta H°$. Thus this is a prime example of what Jencks[17] has termed a "nonclassical hydrophobic interaction", since one would have expected the association of these molecules to be hydrophobically (and entropically) driven. From solvent dependency studies,[10] we have concluded that the hydrophobic force accounts for about 30% of the free energy for the binding of AC to β-CD but that this contribution is masked by a binding force having a negative $\Delta H°$ and $\Delta S°$ (i.e., a strong van der Waals interaction resulting from the snug fit of AC into the β-CD cavity). Interestingly, the binding of AC to γ-CD is found to be characterized by a small endothermic $\Delta H°$ and a large positive $\Delta S°$, the pattern for the classical hydrophobic effect. Since the diameter of the γ-CD cavity is 1–2 Å larger than the diameter of the adamantyl group, we concluded that van der Waals contacts are suboptimal in this case since the guest can touch only a small portion of the internal cavity at a time.[11]

From studies of the pH dependence of AC binding to the CDs, we have found the protonated (neutral) carboxylic acid form of the guest to bind preferentially to both β-CD and γ-CD. This is consistent with findings with other carboxylic acid guests.[7,15,16] Binding studies of AC with α-CD, on the other hand, indicate that a 2:1 α-CD–AC complex forms at low pH.[7,11] Furthermore, the formation of this 2:1 complex is a cooperative process.

The above information, along with the determined values of heat capacity changes, $\Delta C_p°$, for binding, illustrate that the interaction of AC with CDs is a very valuable model system for understanding the specificity and forces involved in forming protein–ligand complexes and for understanding how thermodynamic ($\Delta G°$, $\Delta H°$, $\Delta S°$, $\Delta C_p°$) information can be interpreted.

In this manuscript, we will continue our studies of such model systems by studying the binding of a congener series of alicyclic carboxylic acids to α- and β-CD. The alicyclic side chains of these guests are shown in Figure 1. The side chains are roughly spherical, rigid, and vary in size from 11 to 5 carbons.

Materials and Methods

Materials. The following compounds were obtained commercially: α- and β-cyclodextrin (Sigma Chemical Co.), norbornaneacetic acid (NBA), adamantane-1-carboxylic acid (AC), cyclohexaneacetic acid (CHA), cyclohexanecarboxylic acid (CHC), and cyclopentanecarboxylic acid (CPC) (Aldrich Chemical Company). The hydrated molecular weights

† University of Mississippi.
‡ New Jersey Institute of Technology.

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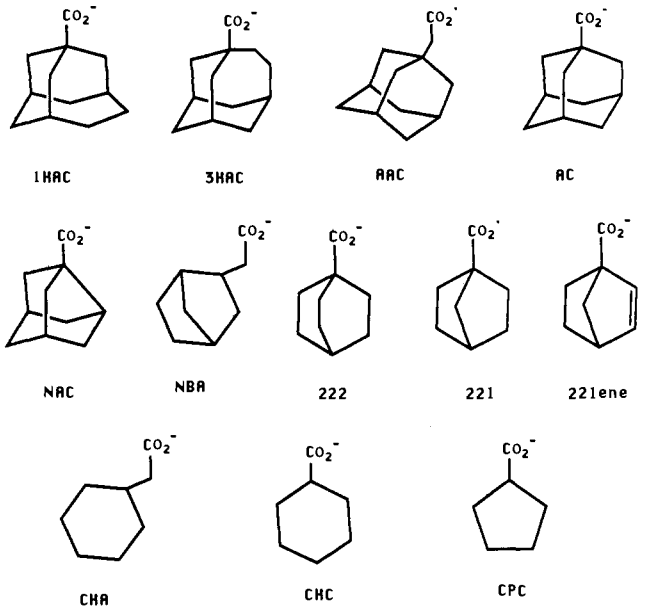


Figure 1. Structures of the alicyclic guests used in this study. Carboxylate forms are shown. Abbreviations are as follows: 1HAC, 1-homoadamantanecarboxylate; 3HAC, 3-homoadamantanecarboxylate; AAC, adamantaneacetate; AC, 1-adamantanecarboxylate; NAC, 3-noradamantanecarboxylate; NBA, norbornaneacetate; 222, 1-bicyclo[2.2.1]octanecarboxylate; 221, 1-bicyclo[2.2.1]heptanecarboxylate; 221ene, 1-bicyclo[2.2.1]heptenecarboxylate: CHA, cyclohexaneacetic acid; CHC, cyclohexanecarboxylic acid; and CPC, cyclopentanecarboxylic acid.

for both α-CD (1081.0) and β-CD (1297.1) were used when preparing solutions.[11] Homoadamantane-1-carboxylic acid (1HAC) and homoadamantane-3-carboxylic acid (3HAC) were prepared by the method of Schleyer et al. Bicyclo[2.2.2]octane-1-carboxylic acid (222) and bicyclo[2.2.1]heptane-1-carboxylic acid (221), and bicyclo[2.2.1]heptene-1-carboxylic acid (221ene) were donated samples.

Methods. Potentiometric pH titration and proton uptake binding studies were performed as described by Cromwell et al.[11] Most of these studies were performed at pH 4.5, 0.1 M NaCl, but a few measurements were made at higher pH.

A spectral displacement method, using methyl orange as the chromophoric guest for β-CD and *p*-nitrophenol as the chromophoric guest for α-CD, was used to study the binding of certain alicyclic guests.[18]

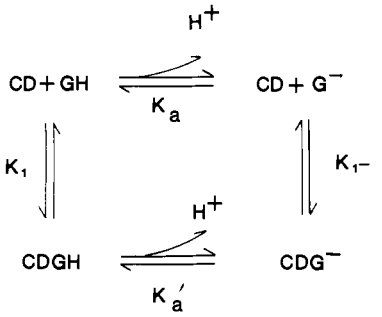
Flow microcalorimetric titrations were carried out with the various alicyclic guests at three different pHs and 25 °C. The experimental method of study is explained in our previous publications.[10,11] Briefly, binding profiles were obtained by measuring the heat of reaction as a constant guest concentration was mixed in a LKB flow microcalorimeter with an exponential gradient of CD concentration.[19] From the resulting profiles of heat signal versus CD concentration, an association constant and enthalpy change for binding can be obtained by fitting analogue of eq 4 or 7. For the smaller guests, 222, 221, and 221ene, it was important to use very high CD concentations in order to approach saturation. For these cases, we used 5×10^{-2} M α-CD and 7.5×10^{-3} M β-CD as the highest CD concentrations (in the calorimeter). The heat of dilution of the CDs was measured separately. No significant signal was observed for the dilution of a 0.015 M β-CD solution; for α-CD, an endothermic heat of dilution signal was observed, and these signals were subtracted from those for the mixing of α-CD with guest (see Figure 4A). The following buffers were used: 0.1 M sodium acetate at pH 4.05, 0.03 M sodium phosphate at pH 7.2, and 0.05 M sodium borate at pH 8.5. Since these buffers have negligible heats of protonation, apparent $\Delta H°$ values were not corrected for proton transfer to the buffers.

Equations for 1:1 or 2:1 binding were fitted to proton uptake and microcalorimetric binding data by using a modified Simplex nonlinear least-squares program or by using the program ENZFITTER.

Results

Models for Guest–CD Interactions. The interactions between the guests and cyclodextrins were studied by three different methods, potentiometric proton uptake measurements, competitive displacement of a chromophore, and microcalorimetric titrations. These results are presented below. First we will present general models for the interactions.

Scheme I



Thermodynamic Scheme I describes the formation of 1:1 complexes between a CD and either the protonated or unprotonated form of the guest. The free guest has an acid dissociation constant, K_a ($=(H^+)(G^-)/(GH)$). K_1 and K_{1-} are the association constants of GH and G^-, respectively, with the cyclodextrin, CD. K_a' is the acid dissociation constant of the bound guest. In binding studies in which the concentration of the guest species remains constant and the concentration of CD is varied, the saturation parameter, ν (=moles of bound guest divided by total moles of guest), is given as

$$\nu = \frac{(CD)K_1(1 + K_a'/[H^+])}{1 + K_a/[H^+] + (CD)K_1(1 + K_a'/[H^+])} \quad (1)$$

where (CD) is the free CD concentration and is related to the total CD concentration, $(CD)_0$, and total guest concentration, $(G)_0$, by

$$(CD) = (CD)_0 - \nu(G)_0 \quad (2)$$

In proton uptake binding studies, ν is experimentally determined as $\nu = \Delta n/\Delta N_1$, where Δn is the measured proton uptake per mole of total guest as CD is added and ΔN_1 is the maximum proton uptake at saturation of the guest. ΔN_1 is related to the acid dissociation constant of the free and bound guests as follows:

$$\Delta N_1 = \frac{(H^+)}{(H^+) + K_a'} - \frac{(H^+)}{(H^+) + K_a} \quad (3)$$

Thus, when Δn is measured as a function of total CD concentration, a hyperbolic binding profile should be obtained for this case, as given by the following equation:

$$\Delta n = \frac{K_{1,app}(CD)\Delta N_1}{1 + K_{1,app}(CD)} \quad (4)$$

where (CD) is given by eq 2 and K_{app} in this scheme is

$$K_{1,app} = \frac{K_1(1 + K_a'/(H^+))}{1 + K_a/(H^+)} \quad (5)$$

Substitution of eq 2 into eq 4 yields a quadratic expression, which we fitted to data via a nonlinear least-squares program. For flow microcalorimetric binding studies, a similar hyperbolic binding profile should result for systems described by Scheme I. The ν values are measured as $Q/Q_{1,max}$, where Q is the observed (corrected) heat of mixing (in microvolts per second per mole of guest) of CD with a fixed guest concentration and $Q_{1,max}$ is the maximum heat effect at saturating CD concentration. The value of $Q_{1,max}$ is related to the enthalpy change for the reaction by the relationship $Q_{1,max}\epsilon/f(G)_0 = \Delta H°_{1,app}$, where f is the flow rate through the microcalorimeter and ϵ is a calibration constant. The relationship for the binding isotherm is similar to eq 4, with Q and $Q_{1,max}$ substituted for Δn and ΔN_1.

For either proton uptake or microcalorimetric studies, the K_{1app} and the maximum signals (i.e., ΔN_1 or $\Delta H°_{1,app}$) may be pH

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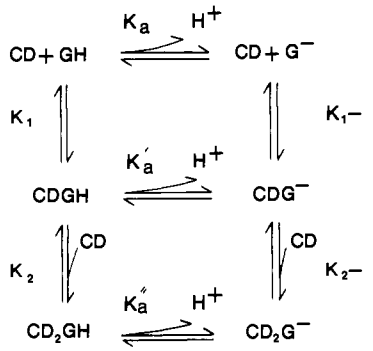
Scheme II



dependent, if there is a preferential binding of either the protonated or unprotonated form of the guest. The pH dependences of $K_{1,app}$ and ΔN_1 are given by eq 5 and 3. At pH values below pK_a and pK_a', K_{app} will approach K_1, the association constant for the protonated form of the guest to the CD; at pH values above pK_a and pK_a', $K_{1,app}$ will approach K_{1-}, the association constant for the unprotonated form of the guest. Likewise, $\Delta H°_{1,app}$ will approach $\Delta H°_1$ and $\Delta H°_{1-}$ at low and high pH, respectively. At intermediate pH values, $K_{1,app}$ and $\Delta H°_{1,app}$ will have contributions from the binding of both forms of the guest.

Thermodynamic Scheme II describes the formation of 2:1 complexes between CD and guest. K_2 and K_{2-} are stepwise association constants for binding a second CD to a CD–GH or CD–G$^-$ binary complex; K_a'' is the acid dissociation constant for the ternary complex. The saturation parameter is given by

$$\nu = [(CD)K_1(1 + K_a'/(H^+)) + (CD)^2K_1K_2(1 + K_a''/(H^+))]/[1 + K_a/(H^+) + (CD)K_1(1 + K_a'/(H^+)) + (CD)^2K_1K_2(1 + K_a''/(H^+))] \quad (6)$$

where the first term in the numerator relates the probability of forming the 1:1 complexes and the second term relates the probability of forming the 2:1 complexes. The free CD concentration in eq 6 is related to the total CD concentration by straightforward (but lengthy) mass balance expressions given as eq 5 and 6 in ref 11.

As with the above 1:1 scheme, experimentally measured proton uptake data are described by the following expression:

$$\Delta n = \frac{K_{1,app}(CD)\Delta N_1 + K_{1,app}K_{2,app}(CD)^2\Delta N_2}{1 + K_{1,app}(CD) + K_{1,app}K_{2,app}(CD)^2} \quad (7)$$

where ΔN_1 and $K_{1,app}$ are given by eq 3 and 5 and ΔN_2 and $K_{2,app}$ are given by

$$\Delta N_2 = \frac{(H^+)}{(H^+) + K_a''} - \frac{(H^+)}{(H^+) + K_a} \quad (8)$$

$$K_{2,app} = \frac{K_2(1 + K_a''/(H^+))}{1 + K_a/(H^+)} \quad (9)$$

Binding eq 7 was fitted to proton uptake data by nonlinear least squares. For microcalorimetric binding studies, an equation analogues to eq 7 was used, with Q, $Q_{1,max}$, and $Q_{2,max}$ substituted for Δn, ΔN_1, and ΔN_2. Note that ΔN_2 and $Q_{2,max}$ are defined to be the total proton uptake and total heat effect for binding two CD molecules to one guest molecule (i.e., for the process 2CD + G $\rightleftharpoons$ CD_2–G). The stepwise values, for binding the second guest to the CD–G complex, are given as $\Delta N_2 - \Delta N_1$ and $Q_{2,max} - Q_{1,max}$.

pH Titrations and Proton Uptake Studies. The pK_a values of some of the carboxylic acids and their complexes with near-saturating concentrations of α- and β-CD are given in Table I. The apparent pK_a values in the presence of the CDs should slightly underestimate the pK_a' and pK_a'' values in Schemes I and II, but these apparent pK_a values do indicate that some very large increases occur upon CD binding. The increases in the pK_as make it possible to determine association constants for inclusion complexes by adding aliquots of a CD solution to an unbuffered guest solution. In Figure 2 are shown typical plots of the proton uptake, Δn, upon adding α- and β-CD to a guest solution.

Table I. Proton Uptake Results for the Formation of CD–Guest Complexes[a]

α-CD

guest	pK_a	K_1, M^{-1}	ΔN_1	K_2, M^{-1}	ΔN_2
AC[b]	4.90–6.6	218 (150 ± 70)	⟨0⟩	770 (820 ± 50)	0.265
NAC		135 (122 ± 15)	⟨0⟩	2220 (2230 ± 10)	0.281
NBA	4.87–7.40	340 (330 ± 40)	⟨0⟩	7400 (5700 ± 1500)	0.425
222	4.89–5.82	30	⟨0⟩	200	0.310
221	4.68–5.89	94	⟨0⟩	265	0.385
221ene	4.36–5.61	150	⟨0⟩	200	0.690

β-CD

guest	pK_a	K_1, app	ΔN_1	K_1
AC[c]	4.90–6.2	(7 ± 3) × 10^4	0.270	9.5 × 10^5
NAC		(1.0 ± 0.3) × 10^5	0.279	1.4 × 10^5
NBA	4.87–5.55	(9.0 ± 1) × 10^3	0.279	1.2 × 10^4
222	4.89–5.98	(7.0 ± 3) × 10^4	0.300	9.7 × 10^4
221	4.68–5.88	(9.6 ± 0.5) × 10^3	0.371	1.5 × 10^4
221ene	4.36–5.60	(5.0 ± 1.0) × 10^3	0.529	1.1 × 10^4
CHA[b]	4.80	(6.8 ± 0.3) × 10^3	0.360	8.6 × 10^3
CHC[b]	4.90	(3.4 ± 0.1) × 10^3	0.297	2.4 × 10^3
CPC[b]	4.99	(1.3 ± 0.1) × 10^3	0.265	1.6 × 10^3

[a] pK_a values in column 2 were obtained at 25 °C, 0.1 M NaCl. The first pK_a value is for the free acid; the second value is in the presence of 0.1 M α-CD or 0.01 M β-CD. Association constants were obtained potentiometrically at 25 °C in 0.1 M NaCl, pH 4.5 solution, unless indicated otherwise. [b] pH 4.39. [c] pH 6.0. Values indicated as ⟨0⟩ were fixed in the fitting procedure. Values in parentheses are the average K_1 and K_2 values determined in three pH values. The K_1 and K_2 values for α-CD complexes are from fits with eq 5, 7, and 8 and Scheme II. For β-CD complexes, the $K_{1,app}$ is from a fit of eq 2 and 4 and Scheme I, where $K_{1,app}$ is related to K_1 by eq 5. Values of K_1 and CHA, CHC, and CPC were obtained by determining $K_{1,app}$ values at both pH 4.39 and 6.0 and simultaneous analysis of eq 5. Only $K_{1,app}$ values at pH 4.39 are shown for these. pK_a values for CHA, CHC, and CPC are from *Lange's Handbook of Chemistry*, 12th ed.; McGraw-Hill Co.: New York.

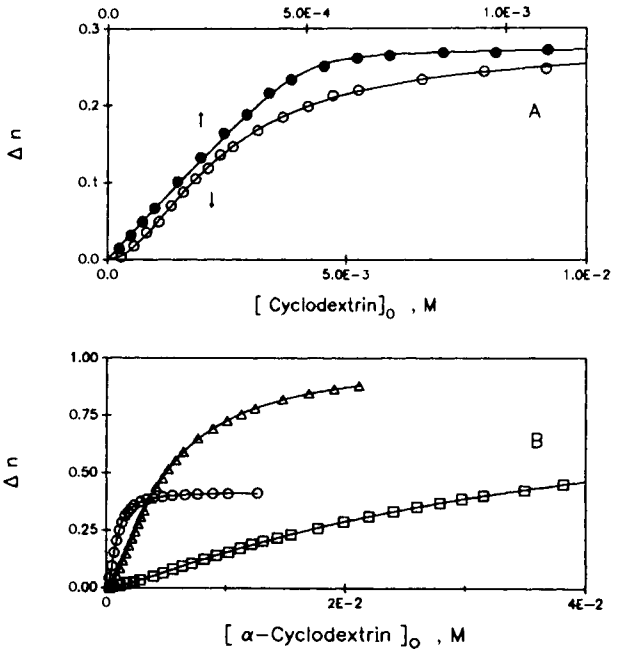


Figure 2. (A) Proton uptake binding isotherms for the interactions of α-CD (○, bottom abscissa) and β-CD (●, top abscissa) with NAC at pH 4.5, 25 °C. Solid lines are theoretical fits of Schemes I and II as described in the text. The fitting parameters are given in Table I. (B) Proton uptake data for the interaction of α-CD with NBA at pH 4.5 (○), 5.9 (△), and 7.27 (□).

Table II. Spectral Displacement Results for the Formation of CD–Guest Complexes[a]

guest	$K_{app} = K_{1-}$, M^{-1} α-CD	β-CD
1HAC		5.5×10^4
3HAC		4.4×10^4
AC		4.2×10^4
NAC		8.2×10^3
NBA	70	4.3×10^3
222	100	8.7×10^3
221	38	5.9×10^2
221ene	33	8.5×10^2

[a] Values obtained at 25 °C in 0.1 M sodium borate buffer, pH 9.0. At this pH, it is assumed that the apparent association constant is K_{1-}, that for the 1:1 binding of the carboxylate form of the guest.

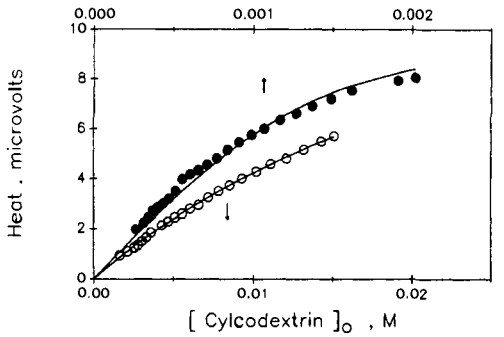


Figure 3. Microcalorimetric binding isotherms for the interaction of α-CD (○) and β-CD (●) with NAC at pH 7.2. Note the different x axes for the two data sets. Solid lines are a fit to Scheme I with parameters given in Table III.

Hyperbolic binding curves were always found for the interaction of β-CD with these guests (most studies at pH 4.5), indicating that only 1:1 binding (Scheme I) occurs. Using eq 2 and 4, we obtained the apparent association constants, $K_{1,app}$, and maximum proton uptake, ΔN_1, values given at the bottom of Table I for the binding of β-CD to the guests. According to Scheme I, $K_{1,app}$ is related to K_1, pK_a, and pK_a' via eq 5. Assuming that the pK_a values in the presence of 0.01 M β-CD are approximately equal to pK_a', values of K_1 were calculated and are listed in Table I. We note that, for the guests AC, NAC, NBA, and 222, the K_1 for β-CD binding must be considered with caution, since we have approached the limits of the proton uptake method in reporting association constants of such a large magnitude.

For α-CD, a sigmoidal binding curve has been observed for every guest studied at pH 4.5. As shown in Figure 2B for α-CD binding to NBA, sigmoidal patterns can persist at higher pH, as the profiles shift toward higher α-CD concentration. Such sigmoidal behavior was previously found for α-CD binding to AC at low pH.[7,11] A 2:1 binding scheme (Scheme II) is required to describe these data. Using eq 7, we fitted the α-CD binding data for the various guests to obtain the thermodynamic parameters given in Table I. To simplify the analysis, we assumed that $\Delta N_1 = 0$, that is, that there is no pK_a shift upon binding the first α-CD to the guests but that the binding of the second α-CD results in the entire pK_a shift and causes the ΔN_2. With this assumption, eq 7 was fitted for the three parameters K_1, K_{1-}, and ΔN_2, as listed in the top of Table I.

Spectral Displacement Studies. This technique[18] was used as an auxiliary method to determine CD–guest association constants at pH 9.0. The results are given in Table II. At this pH, the $K_{1,app}$ are essentially equal to K_{1-} in Scheme I and only 1:1 binding appears to be significant. (These $K_{1,app}$ values were derived from data at only a single guest concentration, and therefore, these studies alone cannot distinguish the 1:1 and 2:1 models. This distinction is made by the other data.)

Flow Microcalorimetric Studies. Binding profiles were also obtained for α- and β-CD binding to the various alicyclic guests using this method at pH 8.5, 7.2, and 4.05. The higher pH was selected to enable the 1:1 binding K_{1-} equilibrium step to be characterized. The lower pH was selected as a compromise between the solubility limit of some of the larger carboxylic acids (1HAC, 3HAC, and AAC were too insoluble to study at pH 4.05) and the desire to measure primarily the K_1 binding step (and to study the cooperativity of α-CD binding). Measurements were made at pH 7.2 in order to compare with published values.

With β-CD, hyperbolic binding profiles of the heat effect, Q, versus β-CD concentration were obtained for all guests at each pH. In Figures 3 and 4 are representative binding curves (see also Figure 1 of ref 10 and Figure 2 of ref 11). Using an analogue of eq 4, we obtained $K_{1,app}$ and $\Delta H°_{1,app}$ for binding. These are

Table III. Microcalorimetric Titration Results for the Formation of CD–Guest Complexes[a]

guest	$K_{1,app}$, M^{-1}	$\Delta H°_{1,app}$, kcal/mol	$\Delta S°_{1,app}$, cal/(mol·deg)
		α-CD	
		At pH 8.5	
AC	140	−3.2 ± 0.1	−1.0
NBA	100	−3.4 ± 0.3	−2.3
222	30	−3.4 ± 0.4	−4.8
221	22	−3.1 ± 0.3	−4.2
221ene	24	−2.4 ± 0.4	−0.5
		At pH 7.2	
1HAC	700	−5.3 ± 0.3	−4.8
3HAC	350	−4.6 ± 0.2	−3.8
AAC	200	−3.0 ± 0.1	0.4
AC	220	−3.10 ± 0.05	0.3
NAC	120	−3.25 ± 0.5	−1.3
NBA	164	−6.5 ± 0.7	−11.7
222	45	−2.8 ± 0.2	−1.9
221	25	−3.1 ± 0.5	−4.0
221ene	30	−2.0 ± 0.6	0.0
		At pH 4.05	
AC	(K_1) 130	(−3.2)	(−1.1)
	(K_2) 420	−8.8 ± 0.3	−17.6
NAC	(K_1) 195	(−3.1)	(0.0)
	(K_2) 345	−11.9 ± 0.8	28.4
NBA	(K_1) 625	(−2.8)	(3.4)
	(K_2) 2000	−5.8 ± 0.2	−7.7
221	(K_1) 31	(−3.1)	(−3.6)
	(K_2) 157	−7.0 ± 1.0	−13.5
221ene	(K_1) 180	(−2.4)	(2.2)
	(K_2) 150	−5.0 ± 0.3	−6.9
		β-CD	
		At pH 8.5	
AC	1.8×10^4	−4.85 ± 0.22	3.1
NAC	5.0×10^3	−3.75 ± 0.25	4.3
NBA	6.2×10^3	−1.95 ± 0.10	10.7
222	7.0×10^3	−3.8 ± 0.2	4.8
221	8.3×10^2	−1.9 ± 0.2	6.9
221ene	5.6×10^2	−1.8 ± 0.2	6.5
		At pH 7.2	
1HAC	1.6×10^4	−7.8 ± 0.1	−7.0
3HAC	1.4×10^4	−7.5 ± 0.1	−6.3
AAC	2.0×10^4	−5.3 ± 0.3	1.8
AC	4.0×10^4	−5.4 ± 0.1	2.9
NAC	5.4×10^3	−3.6 ± 0.2	4.9
NBA	3.7×10^3	−2.5 ± 0.2	7.9
222	6.3×10^3	−3.1 ± 0.1	6.9
221	1.1×10^3	−1.9 ± 0.3	7.5
221ene	4.3×10^2	−1.8 ± 0.3	8.5
		At pH 4.05	
AC	$3.0 \pm 3 \times 10^5$	−7.53 ± 0.002	−0.3
NAC	$7.0 \pm 3 \times 10^4$	−5.8 ± 0.1	2.6
222	$5.0 \pm 3 \times 10^4$	−6.2 ± 0.3	0.6
221	$7.0 \pm 1 \times 10^3$	−5.5 ± 0.2	−0.9
221ene	$3.2 \pm 0.2 \times 10^3$	−5.0 ± 0.1	−0.8

[a] Values determined microcalorimetrically at 25 °C. The $K_{1,app}$ are related to K_1, pK_a, and pK_a' by eq 5. At pH 8.5 and 7.2, the $K_{1,app}$ is approximately equal to K_{1-} in Scheme I. At pH 4.05, the $K_{1,app}$ are approximately equal to K_1. The symbols ( ) indicate that a value was fixed in the data analysis.

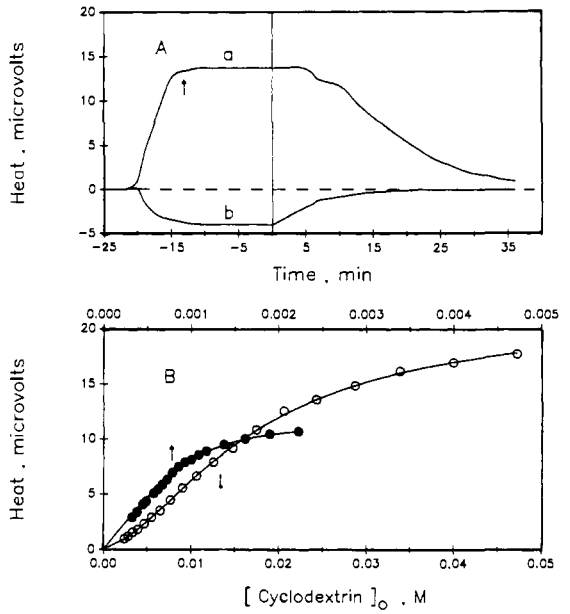


Figure 4. (A) Depiction of raw microcalorimetric data for the interaction of α-CD with 221 at pH 4.08, 25 °C. Curve a is the thermogram for the mixing of 1.03×10^{-3} M 221 (concentration within microcalorimeter) with a 10.3×10^{-3} M solution of α-CD. At a time indicated as about −20 min on the abscissa, the α-CD solution entered the cell and mixed with the 221 solution. A plateau signal was then reached (exothermic signal). At a time indicated by the arrow, an exponential concentration gradient of the α-CD solution was introduced. Approximately 13 min later, this gradient reaches the microcalorimetric cell and the signal begins to decrease. Curve b is the signal for mixing the same α-CD solution with buffer (i.e., heat of dilution of α-CD). This endothermic signal was subtracted from the mixing signal to get the true heat effect. The dashed line is the base line. (B) Microcalorimetric binding isotherms for the interaction of α-CD (○) and β-CD (●) with 221 at pH 4.08, 25 °C. The data for α-CD are from panel A. Solid lines are fits of Schemes I and II; fitting parameters are given in Table III.

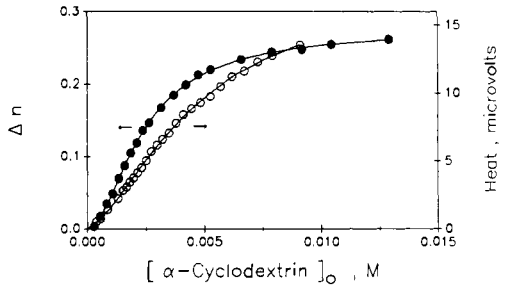


Figure 5. Comparison of proton uptake (●) and microcalorimetric (○) binding isotherms for the interaction of α-CD with NAC. The proton uptake data were obtained at pH 4.5: the microcalorimetric data were obtained at pH 4.08. Fitting parameters are given in Tables I and III.

listed at the bottom of Table III. The $K_{1,app}$ values are equal to $K_1(1 + K_a'/(H^+))/(1 + K_a/(H^+))$, but at pH 8.5 and 7.2, the $K_{1,app}$ values are approximately equal to K_{1-} (approximation is better at pH 8.5), and at pH 4.05 the $K_{1,app}$ values are approximately equal to K_1. At pH 4.05, the binding of the guests is very strong (except for 221ene), and again, the limit of the methodology has been approached. Reported values of $K_{1,app}$ above 5×10^4 must be viewed with caution; if anything, the true values are larger. The apparent entropy change, $\Delta S°_{app}$, for binding is also given in Table III.

For α-CD, hyperbolic binding curves were seen at pH 7.2 and 8.5, indicating that 1:1 complexes predominate (see also Figure 1 of ref 10). At pH 4.05, however, sigmoidal binding curves were again seen (see Figures 4 and 5). This is consistent with the proton uptake studies and indicates that the 2:1 model in Scheme II is required to fit the data. Using an analogue to eq 7, we obtained the thermodynamic parameters in Table III. Again, to make the fitting procedure tractable, we assumed that the $Q_{1,max}$ value, for the pH 4.05 data, is the same as the $Q_{1,max}$ value obtained for binding at pH 8.5 (i.e., that the 1:1 binding step has the same maximum heat effect at both pH ranges). The analogue of eq 7 was then fitted to the data to obtain the K_1, K_2, and $Q_{2,max}$ values listed in Table III.

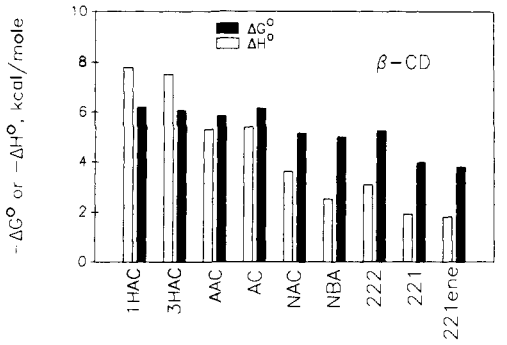


Figure 6. Variables in the $\Delta G°_{app}$ and $\Delta H°_{app}$ for the interaction of β-CD with various alicyclic carboxylates. Data primarily correspond to pH 7.2.

Figure 5 compares microcalorimetric and proton uptake binding data for the α-CD–NAC interaction at low pH. Clearly, both profiles are sigmoidal. However, the curves differ slightly. This may be due partially to the small contribution to the proton uptake by the binding of the first α-CD (i.e., $\Delta N_1 \approx 0$), whereas a large contribution is made to the heat effect by the binding of the first α-CD (i.e., $Q_{1,max} = 6$ μV). Also, there are systematic errors that may affect the two experiments in different ways. Nevertheless, the general features are similar for the two types of measurements.

Discussion

Using three different methods (proton uptake, competitive spectral displacement, and microcalorimetry), we have obtained consistent determinations of the association constants for inclusion complex formation between α- and β-CD and a congener series of alicyclic carboxylic acids. The only guest, for which there is a literature value for the association constants, is AC. The $K_{1,app}$ we observe for the α-CD–AC complex at high pH and the sigmoidal binding profile at low pH are in agreement with that previously determined by Gelb et al.[7,8] and by our group.[10,11] The $K_{1,app}$ we report for the β-CD–AC complex at neutral pH has recently been reproduced by Dr. I. Wadso's group at the University of Lund.[20] The $K_{1,app}$ reported very early by Komiyama and Bender[6] for AC binding to both α- and β-CD are clearly in error by a factor of 10 in each case. The K_1 value for β-CD binding to protonated AC is very large ($\sim 5 \times 10^5$ M^{-1}) and difficult to determine by the methods employed here. The binding of β-CD to the other guests can be determined with greater confidence. A span of association constants from 30 to $\sim 5 \times 10^5$ M^{-1} are reported for the various CD–guest pairs. The larger association constants, i.e., those for β-CD with protonated guests, are in the range found for protein–ligand interactions. Thus, these systems are models for understanding the forces and specificity of such biomacromolecular interactions.

The thermodynamics of inclusion complex formation for the congener series of alicyclic carboxylates show a gradual decrease in $-\Delta G°_{app}$ and $-\Delta H°_{app}$ as the size of the guest decreases. This trend holds for both α-CD and β-CD and is shown by the bar graphs in Figures 6 and 7. In these graphs, the larger guests (i.e., 11-carbon alicyclic group for 1HAC and 3HAC) are given on the left and the smaller guests (i.e., 7 carbon group of 221 and 221ene) are given on the right. Figures 6 and 7 show results at pH 7.2, where our data are most extensive. A similar trend occurs for the data at pH 8.5 and 4.0 (or for the values of K_{1-} and K_1). While there is a gradual drop in $-\Delta G°_{app}$ and $-\Delta H°_{app}$ with decreasing guest size for both α- and β-CD, an exception to this trend is the large $-\Delta H°_{app}$ for the interaction of α-CD with NBA. For this

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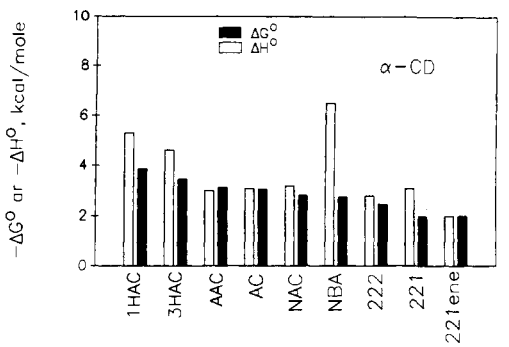

Figure 7. Variation in the ΔG°_{app} and ΔH°_{app} for the interaction of α-CD with various alicyclic carboxylates.

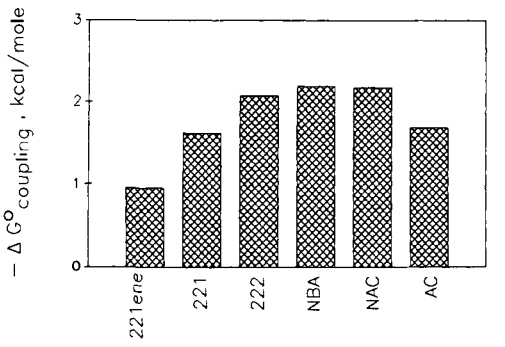

Figure 8. Free energy of coupling for the cooperative binding of α-CD to various guests.

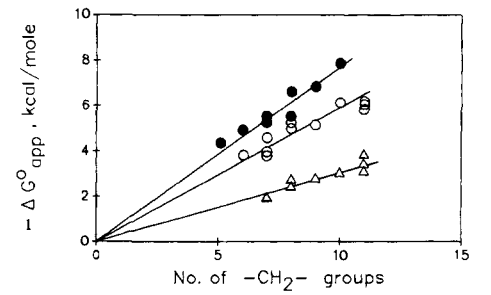

Figure 9. Dependence of ΔG°_{app} for binding on the number of methylene groups in the guests. Values for α-CD at pH 7.2 (△), β-CD at pH 7.2 (○), and β-CD at pH 4 (●). The lines are extrapolated to the origin.

pair, the $-\Delta H^\circ_{app}$ is much more exothermic than expected at pH 7.2. However, this can be explained by the very strong tendency to form a 2:1 complex with NBA, even at this pH. (Note the large pK_a for NBA in the presence of α-CD in Table I.) Apparently the $-\Delta H^\circ_{app}$ for the α-CD–NBA interaction at pH 7.2 includes a significant contribution from the K_2 equilibrium. Below we will discuss in more detail our interpretation of the size dependence of the thermodynamics of the CD–guest interactions.

For all of the guests, we find preferential binding of the protonated, carboxylic acid forms. For example, with β-CD complexes, the ΔG° value for binding the carboxylic acid forms is about 1.7 ± 0.5 kcal/mol more negative than the ΔG° value for the carboxylate forms. There is no obvious dependence of this free-energy increment on guest size. Also the improved binding of the carboxylic acid forms of the guests to β-CD is due to an improvement in the ΔH° of binding. The ΔH° for binding the carboxylic acid forms is 2.7 ± 0.5 kcal/mol more exothermic than is the ΔH° for the carboxylate forms. As discussed below, this improved ΔG° and ΔH° for binding the neutral guest forms may reflect a deeper penetration of the guest into the β-CD cavity and enhanced van der Waals interactions.

For α-CD complexes, there is also a preferential binding of the carboxylic acid forms of the guests, as is indicated by the pK_a shifts. What is more interesting, however, is the sigmoidal binding profiles observed at low pH (Figures 2, 4, and 5), which indicate that 2:1 α-CD–guest ternary complexes form (see Scheme II). Such a ternary complex between α-CD and AC has already been thoroughly studied by Gelb et al.[7] and our group.[10,11] The degree of positive cooperativity found for the formation of the 2:1 α-CD–guest complexes at low pH is remarkable. This behavior makes this system an excellent model for the cooperative phenomena that are so characteristic of protein–ligand systems. If there were no cooperative effect in the binding of the second α-CD in Scheme II, then the stepwise K_2 value would be expected to be one-fourth the value of K_1 (since K_1 and K_2 are stoichiometric constants; see chapter 2 of ref 30). Instead we find K_2 to be 1.3–20 times larger than K_1 for the various guests. This corresponds to a free energy of coupling, $\Delta G^\circ_{coupling}$, of −1.0 to −2.2 kcal/mol for this system (where $\Delta G^\circ_{coupling} = -RT \ln (4K_2/K_1)$). Connors and Pendergast[21] have previously studied the formation of 2:1 α-CD–guest ternary complexes for a number of aromatic guests. They found both positive and negative interactions in the binding of the second α-CD. The free energies of coupling that we find for the alicyclic guests studied here are among the most negative that have been found (i.e., compare to the values of $-RT \ln a_{xx}$ for Connors and Pendergast's data on 1,4-disubstituted benzenes as guests). The interpretation of positive free energies of couplings (negative interactions) is rather apparent; the second α-CD cannot interact with the guest in the α-CD–guest binary complex, as it can with the free guest. This would be due to some steric effects or, when the guest is asymmetric, due to the fact that the "high affinity" side of the guest binds into the first α-CD cavity, leaving the "low affinity" side for the second α-CD. For systems with negative free energies of couplings (positive interactions), such as the α-CD–alicyclic carboxylic acids studied here, the interpretation is less clear. We have suggested that, in forming the $\alpha\text{-CD}_2$–guest ternary complexes, the secondary (or primary) hydroxyl groups lining the rims of one α-CD will hydrogen bond with those of the second α-CD.[11] Figure 7 of ref 11 shows this predicted structure. The rather large and negative enthalpy changes (ΔH°_2 in Table III under pH 4.05 parameters) that are found for the stepwise binding of the second α-CD molecules are consistent with this hypothesis, since one would normally expect a negative enthalpy change contribution from the formation of hydrogen bonds. Evidence that α-CD undergoes self-aggregation further supports this explanation.[22]

We had anticipated that the α-CD ternary complexes would form only for the larger guests, such as AC, and that the smaller guests, such as 221, would penetrate deeply into the α-CD cavity and form only 1:1 complexes. Surprisingly, 2:1 complexes form between α-CD and the protonated state of each of the alicyclic guests that we have studied. The free energy of coupling appears to depend on the guest size, to a certain extent. In Figure 8 we plot $\Delta G^\circ_{coupling}$ (averaged from proton uptake and microcalorimetric studies) versus the number of methylene groups in the guest. The pattern is crude, but a maximum $\Delta G^\circ_{coupling}$ is seen for the guests whose side chains have 8 and 9 carbons. (Unfortunately, we were unable to study the binding of α-CD to the larger guests, 1HAC, 3HAC, and AAC, at low pH, due to the poor solubility of the protonated forms of these molecules.) NBA and NAC appear to be optimal sized alicyclic guests for the cooperative binding of two α-CDs. (This optimum binding of α-CD to NBA is also indicated in Table I by the very large shift in the pK_a of this guest in the presence of α-CD.)

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The dependence of the thermodynamics parameters for inclusion complex formation on the size of the alicyclic side chains should reflect the nature of the binding forces at play. The side chains of this congener series are all approximately spherical and apolar. One immediately considers the hydrophobic effect as being a significant binding force from consideration of the apolar nature of the guest side chains and the CD cavity. Also we have found negative heat capacity changes, ΔC°_p, for the binding of AC to α- and β-CD, which indicates the action of the hydrophobic effect.[11,23,24] A way to assess this contribution and to try to rationalize the size dependence is to consider the free-energy increment per methylene group for binding. In Figure 9 we have plotted the apparent free-energy changes for the binding (1:1 complexes in all cases; averages values from all the data) of the guests to α- and β-CD, versus the number of methylene groups in the guests side chain. In each case, a straight line can be drawn through the origin and the points for the guests. For β-CD, the slope of this plot is 0.60 and 0.78 kcal/CH_2 group for the carboxylate and carboxylic forms of the guests, respectively. These values fall within the range (0.4–1.0 kcal/CH_2 group) of such free-energy increments that have been found for other model systems for the hydrophobic effect.[25-27] These other model systems include the phase-transfer of organic molecules between water and octanol[26,27] and the interaction of organic molecules with detergent micelles.[25] In certain ways, our inclusion complex system is more similar to protein–ligand complex formation (i.e., defined stoichiometry, possible loss of rotational entropy) than is the other model systems.

For α-CD, the slope in Figure 9 is 0.31 kcal/CH_2 group for the carboxylate form of the guests. Thus, the free-energy increment is significantly larger in magnitude for β-CD complexes than for α-CD complexes. This suggests that the hydrophobic contribution is larger for β-CD complexes. This is consistent with structural and NMR evidence that the adamantyl group of AC can be completely inserted into the β-CD cavity[28] but can only partially enter the smaller α-CD cavity.[29] Possibly this greater degree of penetration into β-CD occurs for the other alicyclic groups as well.

The free-energy increment is also significantly larger for the protonated, carboxylic acid forms of the guests (−0.78 kcal/CH_2-group) than it is for the carboxylate forms (−0.6 kcal/CH_2 group) for binding to β-CD. This suggests a greater hydrophobic contribution for the carboxylic acid forms, which may be due to deeper penetration of the neutral guests into the cavity. This may be due to the fact that the carboxylate group requires interaction with water and thus does not allow full insertion of the alicyclic group into the cavity. Alternatively, this may indicate a favorable interaction of the carboxylic acid group with the cavity (i.e., hydrogen bonding with CD hydroxyl groups), which results in deeper penetration or even functional-group-first insertion into the secondary rim of β-CD. It is risky to draw structural insights from thermodynamic information, but the pattern in Figure 9 does implicate a strong hydrophobic contribution to inclusion complex formation.

We believe that the hydrophobic effect does not make the sole contribution to binding, however. In fact, the pattern in Figure 9 really indicates that there is a strong size dependence of the binding force; the hydrophobic force need not be the only binding force that would increase with the size of the guest. van der Waals interactions between the spherical alicyclic groups and the cylindrical CD cavity should also be important. Since the α- and β-CD cavities are more or less rigid and of internal diameters of ~4.5 and ~7 Å and since van der Waals forces are so critically dependent on separation distances, one would expect to find this binding force to depend on guest size. Indeed, an optimum guest size might be expected for this force. So the patterns in Figure 9 might be expected for either, or a combination of, hydrophobic and van der Waals forces.

In previous studies of the solvent dependence of the β-CD–AC interaction, we argued that about 30% of the binding free energy for this complex is attributable to a hydrophobic contribution; the remaining free-energy change we suggested to be due to van der Waals interactions.[10] In fact, the ΔH°_{app} values that are found for forming many of these inclusion complexes are exothermic (and the ΔS°_{app} are negative). This is not what is expected for a hydrophobically driven process. We have argued that a combination of hydrophobic ($\Delta H^\circ \approx 0$; ΔS° positive) and van der Waals (ΔH° negative; ΔS° negative) forces could account for the apparent thermodynamic parameters for AC binding to β-CD.[10,11]

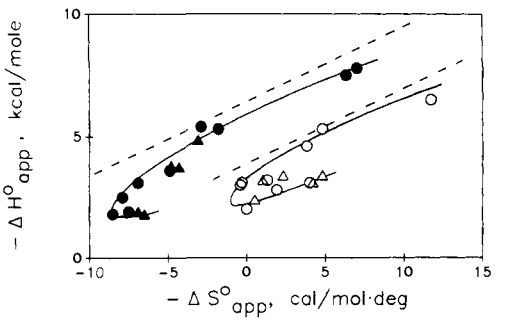


Figure 10. "Compensation" plot of ΔH°_{app} versus ΔS°_{app} for the interaction of α-CD (○,△) and β-CD (●,▲) with the congener series of guests in Figure 1. Circles are values at pH 7.2; triangles are values at pH 8.5. The curves are arbitrarily drawn and are based on arguments presented elsewhere.[24]

In Figure 10 is given a "compensation" plot of ΔH°_{app} versus ΔS°_{app} for the binding of the congener series we have studied to α-CD and β-CD (data at pH 7.2). Such plots are a popular way to present data of this kind. The slope of such plots is defined as a "compensation temperature", which is presumed to be characteristic of the system. We have favored interpreting compensation plots by drawing a line equal to the experimental temperature, tangential to the data point for the most negative free-energy change.[24] For all other data points, the farther, perpendicularly, that the point falls below the experimental temperature line, the lower will be the $-\Delta G^\circ_{app}$. Figure 10 shows that the plots of ΔH°_{app} versus ΔS°_{app} for these systems are not straight lines, but, instead, a hairpin pattern (most clearly apparent for the β-CD data) is found as one goes from larger to smaller guests. Similar hairpin curves have been found for protein–ligand interactions.

We are not inclined to report a compensation temperature for these data. Rather, we believe that the patterns seen in Figure 10 indicate nothing more than the fact that the ΔH° varies more than the ΔG° as one decreases the size of the alicyclic group in the series. We propose that the basis for this greater variation in the ΔH° is that there are two principal binding forces that operate, to different degrees, for the guests. One force is the hydrophobic effect (near zero ΔH°; positive ΔS°). Recall that this plot represents a congener series in which only the size of the roughly spherical alicyclic group varies. The carboxylate group is negatively charged (at pH 7.2) for each guest, and the interaction of this functional group with the solvent and CD should remain constant through the series. For small guests, the fit inside the β-CD cavities is not snug. The van der Waals contribution will be small, and binding, if it occurs, must be hydrophobically driven. The smaller, exothermic ΔH° and more positive ΔS° that are found for the smaller guests are consistent with this description. For the larger guests, on the other hand, the guests will fit very snugly into the CD cavity, and the van der Waals contribution may be equal to or larger than the hydrophobic contribution. The more exothermic ΔH° and more negative ΔS° found for the larger guests are consistent with the dominant role of van der Waals forces in the binding to these guests. Thus, contributions from these two forces—contributions that systematically vary with guest size—can explain the thermodynamic patterns that we observe.

Conclusions

Cyclodextrin inclusion complexes are one of the most valuable model systems available for understanding noncovalent bonding interactions in aqueous solution. They model protein–ligand

systems by showing defined stoichiometry, specificity, cooperative effects, heat capacity changes upon binding, and enthalpy–entropy compensation phenomena. Our present study with a congener series of carboxylic acid guests, having variable-size, roughly spherical, alicyclic groups, reveals the importance of both the hydrophobic effect and van der Waals forces in determining the affinity of the CD for the guest.

Acknowledgment. This work was support by research grant DMB 88-06113 from the National Science Foundation.

Structure and Reactivity of Lithium Diisopropylamide (LDA). The Consequences of Aggregation and Solvation during the Metalation of an *N,N*-Dimethylhydrazone

Angela S. Galiano-Roth and David B. Collum*

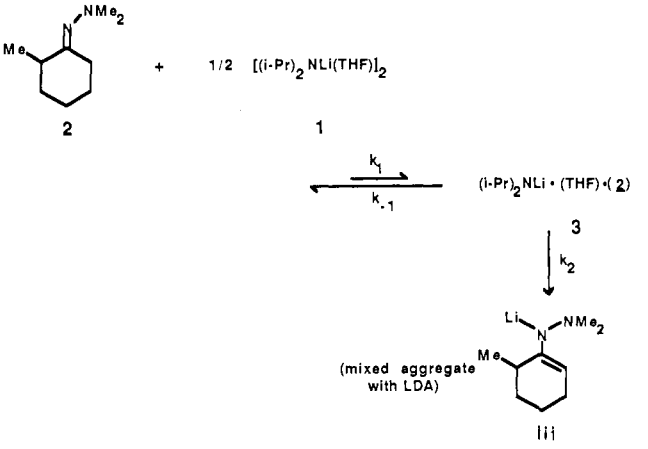
Contribution from the Department of Chemistry, Baker Laboratory, Cornell University, Ithaca, New York 14853-1301. *Received December 12, 1988*

Abstract: ^{6}Li and ^{15}N NMR spectroscopic studies of ^{6}Li-labeled and $^{6}Li,^{15}N$-doubly-labeled lithium diisopropylamide (LDA) are consistent with a disolvated dimer. Revision of a reported monomer–dimer equilibrium is suggested. Rate studies on the lithiation of 2-methylcyclohexanone *N,N*-dimethylhydrazone (**2**) provide a rate expression of the form $-d[2]/dt = k[2][LDA]^{1/2}[THF]^{0}$ and are interpreted in the context of a model involving the following: (1) spectroscopically invisible dissociation of disolvated LDA dimer to an LDA monomer bearing a single ethereal ligand and a complexed hydrazone ligand, followed by (2) rate-determining proton transfer. While the measured lithiation rate is insensitive to the free donor solvent concentration (i.e., [THF]), the nature of the ligands on the solvated LDA dimer in its ground state has a marked effect on the overall reaction rate.

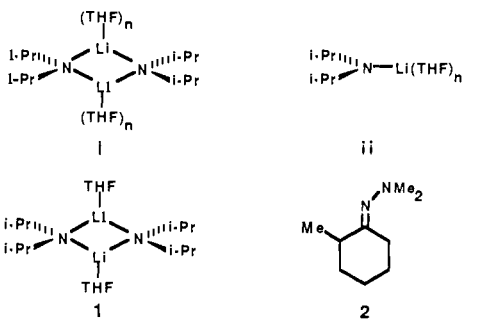
Lithium diisopropylamide (LDA) was first prepared by Hammell and Levine at the University of Pittsburgh in 1950.[1] It remained in relative obscurity[2] until 1967 when a report by Creger highlighted the advantages of LDA as a highly reactive base[3] and sent it on a meteoric rise to become one of the most prominent reagents in organic chemistry.[4] As information on the reactivity and selectivity of LDA accumulated, supportive mechanistic details fell into place far more slowly. Important rate studies of LDA-mediated metalations emanating from the laboratories of Newcomb,[5] Rathke,[6] Saunders,[7] Fraser,[8] Ahlbrecht,[9] and Rickborn[12] have unravelled some of the key parameters affecting LDA reactivity.[10,11] However, in no instance have the roles of solvation and aggregation been elucidated in any detail.[12]

The limitations of our understanding of the chemistry of LDA stem, at least in part, from a shortage of structural information.

Scheme I



It was only in 1984 that Seebach and Bauer provided the first potentially useable structural data.[13] Through colligative measurements, they concluded that 0.05–0.10 molar solutions of LDA in tetrahydrofuran (THF) at −108 °C contain appreciable concentrations of both dimeric and monomeric forms i and ii, respectively. In 1989, Williard and Salvino revealed by an X-ray crystallographic analysis that LDA crystallizes as disolvated dimer **1** from THF/hydrocarbon mixtures.[14]



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