

Thermodynamic Studies on the Cyclodextrin Complexation of Aromatic and Aliphatic Guests in Water and Water–Urea Mixtures. Experimental Evidence for the Interaction of Urea with Arene Surfaces

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The thermodynamic functions that describe the complexation of a series of four carboxylic acid guests and two related quaternary ammonium derivatives with β -cyclodextrin were measured using flow calorimetry at 298 K in aqueous media in the presence and in the absence of large concentrations of urea. The relative differences of the thermodynamic parameters of complexation revealed the existence of specific interactions between urea and the aromatic surfaces of some of the nonpolar guests. As expected, the relative changes in the complex stability constants upon addition of urea have been found to depend on the balance of opposing enthalpic and entropic contributions. The interpretation as well as the magnitude of the interaction between urea and aromatic surfaces are in line with previous experimental and computational studies. The experimental evidence presented in this work provides insight into the mechanism by which large amounts of urea denature globular proteins in aqueous media.

Introduction

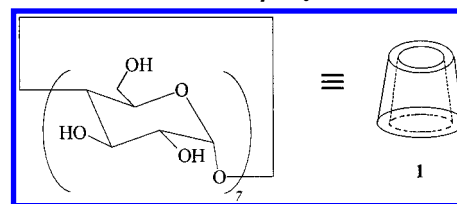
Denaturing agents, such as urea or guanidinium chloride, have been widely used in concentrated aqueous solutions to induce conformational changes in proteins.¹ Although the exact mechanism of interaction of these compounds with the different hydrophobic sites of the polypeptide chain of proteins (as well as with the water molecules that solvate them) remains unclear,² a large body of experimental evidence on the subject has been accumulated over the last 30 years.³

It has been suggested that urea increases the aqueous solubility of nonpolar compounds by destroying the highly structured network of solvent molecules that characterize the water medium.⁴ Such perturbation extends into the “iceberg-like” structure of hydrogen-bonded water molecules that surround a hydrophobic molecule in solution. Since the increase in the entropy of the released water molecules upon association of nonpolar solutes is the main driving force of the classical hydrophobic effect,⁵ the presence of urea has been postulated to decrease the intramolecular hydrophobic interactions of the protein residues and, therefore, provoke the unfolding of the biomolecule.

In 1990 Breslow and co-workers suggested that urea and other denaturing agents are not simple water structure breakers.⁶ On the basis of an observed slight increase of the surface tension of water upon addition of urea, they argued that the structure-breaking properties of urea cannot be the only factor responsible for its denaturing properties. According to the description of Sinanoglu on the classical hydrophobic effect,⁷ the dissolution of a nonpolar solute in aqueous media starts by the creation of a “cavity” in the solvent structure to accommodate the solute. The surface tension data of Breslow and co-workers showed that the presence of urea increases the energetic cost associated with cavity formation. Therefore, an *intrinsic interaction* between the denaturing agent and the nonpolar substrate must exist to overcome the increased thermodynamic difficulty to produce cavities in the aqueous medium.

Recently, the idea of specific interactions between urea molecules and some aromatic substrates in aqueous solution was

SCHEME 1: Structure of the β -Cyclodextrin Host



further supported by computational studies carried out by Jorgensen and co-workers.⁸ Using Monte Carlo simulations, an intrinsic interaction of the order of 2 kcal/mol between a benzene aromatic surface and a molecule of urea in aqueous media was clearly identified. Obviously, experimental studies on the postulated difference in the interaction forces of urea with aliphatic and aromatic compounds were needed.

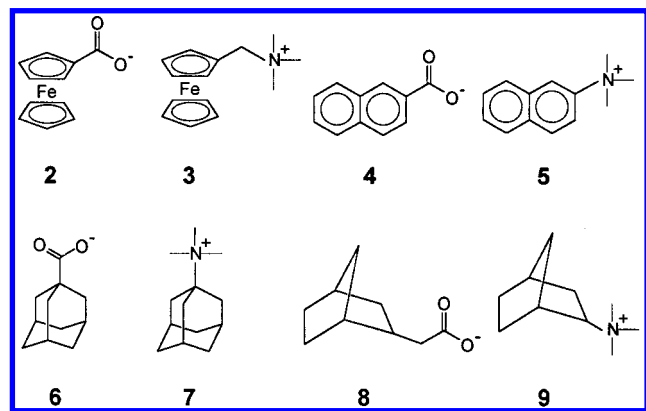
The cyclodextrins (CDs) constitute a very popular family of molecular hosts, capable of binding a wide variety of nonpolar compounds.⁹ Due to the well-defined hydrophobic cavity that characterizes these molecular receptors and their relatively high solubility in water, CDs are ideal host compounds to study hydrophobic interactions in aqueous medium through inclusion complexation (Scheme 1).¹⁰ Composed of seven glucopyranose units, β -CD (**1**) is characterized by its ability to complex aromatic as well as aliphatic guests in aqueous media with binding constants (K) in the order of 10^2 – 10^4 M⁻¹.¹¹

Thermodynamic studies on the binding affinities of CDs with different aromatic substrates such as naphthalene and ferrocene derivatives have been carried out by our group and others in water and water–urea mixtures. The resulting equilibrium data have showed that, in agreement with previous solubilization studies, the binding constant decreases in the presence of large amounts of urea.¹² Whether the observed stabilization of the free forms of the host and guest molecules in solution is caused by the water structure breaking effect of the urea or by a specific interaction of the denaturing agent with the hydrophobic surface of the guest has not been, to the best of our knowledge, addressed so far.

In this paper, we present the results of detailed thermodynamic studies on the complexation of a series of aromatic (**2**–

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SCHEME 2: Structures of the Aromatic and Aliphatic Guests



5) and aliphatic (6–9) guests (Scheme 2) with β -CD in aqueous media, both in the absence and in the presence of large concentrations of urea. All the surveyed guests are characterized by a hydrophobic residue which is known to fit tightly inside the cavity of the β -CD receptor. The experimental results support the presence of specific interactions between urea molecules and aromatic surfaces.

Experimental Section

Materials. The host β -CD was a gift from American Maize-Products Co. and was used without further purification. The molecular weight of the hydrated cyclodextrin (1297.1 g/mol) was used throughout this work.^{9b} Urea (99%) as well as compounds 2, 4, 6, and 8 were purchased from Aldrich and were of the best grade commercially available. The ferrocene derivative 3 was prepared as the iodide salt from ((dimethyl-amino)methyl)ferrocene (Aldrich) according to a reported procedure.¹³ The analog quaternary ammonium salts 5, 7, and 9 were also prepared as the iodides (from the commercially available amine derivatives) by the exhaustive methylation procedure reported by Sommer and co-workers.¹⁴ Further passage of concentrated aqueous solutions of compounds 3, 5, 7, and 9 through an Amberlite ion-exchange column (previously treated with HCl to render it in the Cl^- form) yielded the chloride salts of the quaternary ammonium guests. The purity of the resulting compounds was verified by NMR and elemental analysis.

The inorganic reagents used to prepare the buffer solutions were of the best grade commercially available. All the solutions used in this work were freshly prepared using distilled water further purified by passage through a four-cartridge Barnstead Nanopure System ($\rho = 18 \text{ M}\Omega \text{ cm}$).

Calorimetry. The thermochemical measurements were carried out in an LKB-2107 flow microreaction calorimeter immersed in a water bath at 25°C . The temperature of the bath was controlled to $\pm 0.001 \text{ K}$ by means of a coupled cooling system and a Tronac (model PTC-40) temperature controller.

In order to ensure quantitative deprotonation in solution of all the carboxylic acid guests compounds,¹⁵ the solvent used in the preparation of all the solutions consisted of a $\text{pH} = 8.6$ phosphate buffer ($I = 0.05$) also containing 0.1 M NaCl to maintain a constant ionic strength.

In a typical run, a 2 mM solution of host 1 was continuously fed at a constant flow rate of 0.211 mL/min into the calorimeter reaction chamber from two independent channels using a Harvard Instruments syringe pump. While the solution was being mixed, a voltage signal produced by a thermopile attached to the reaction chamber was amplified 10^5 times (using a DC amplifier (Keithley instruments)) and the corresponding base

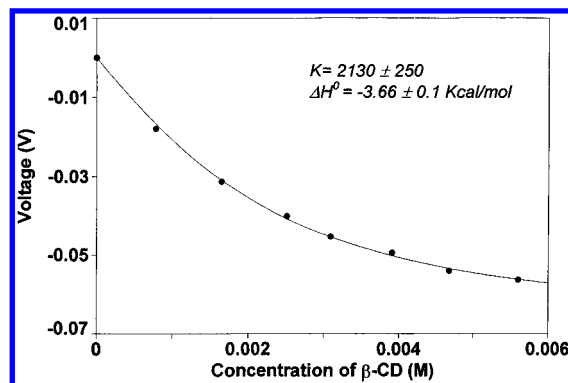


Figure 1. Voltage signal from the flow-calorimeter mixing chamber vs concentration of host 1. The concentration of guest 2 was held constant at 2 mM . The curve was calculated using the ΔH° and K values given in the figure.

line was recorded by a computerized data acquisition system. Using a chromatographic valve and a parallel sample loop (7 cm^3), a set of at least seven solutions of the guest under study (concentration between 0.6 – 6 mM in the same buffered medium) were successively injected into the calorimeter by one of the two channels feeding the reaction chamber. Since in the other channel there is a continuous flow of the host solution, the complexation reaction takes place upon mixing and the associated heat (Q) produces a voltage signal (ΔV) that, as expressed by eq 1, must be proportional to the ΔH° of the reaction and to the concentration of complex formed.

$$\Delta H = Q/\varphi\omega = \epsilon\Delta V/\varphi\omega \quad (1)$$

In eq 1, φ is the concentration of complex formed by the reaction, ϵ the instrument constant that relates the voltage signal to the heat produced by the association reaction, and ω the flow rate of solution at the exit of the calorimeter cell. If a 1:1 stoichiometry is assumed, the concentration of the complex φ is related to the equilibrium binding constant (K) by the following equation:

$$K = \frac{\varphi}{\left(\frac{c}{2} - \varphi\right)\left(\frac{c_0}{2} - \varphi\right)} \quad (2)$$

where c and c_0 are the concentrations before mixing of the guest and β -CD solutions, respectively.

From eqs 1 and 2, the heat of complexation (ΔH°) and the binding constant (K) of the system can be extracted from a series of measurements of the voltage signal (ΔV) at different concentrations (c) of the guest by standard regression analysis. Further use of basic thermodynamic relations leads to the values of the ΔG° and ΔS° for complexation of the host–guest system under study. In Figure 1, for example, the binding isotherm of the complexation of β -CD with guest 2 is presented along with the corresponding thermodynamic parameters of association at 298 K .

Correction for heat of dilution effects was carried out when needed by subtracting the voltage signals of independent dilution experiments from the voltage data obtained in the complexation runs at the corresponding concentrations. When the solubility of the guest was low enough so that the preparation of solutions up to the 6 mM range was not possible, the more soluble β -CD species was used as the variable concentration reagent. Further details on the accuracy of the calibration procedure of the instrument have been reported elsewhere.^{12b}

Results and Discussion

Initially, calorimetric experiments were carried out on the complexation of β -CD with the carboxylic acid guests 2, 4, 6,

TABLE 1: Thermodynamic Complexation Parameters of β -CD with Several Carboxylate Guests in pH = 8.6 Phosphate Buffer ($I = 0.05$), 0.1 M NaCl at 298 K

guest	K (M^{-1})	ΔG^0 (kcal/mol)	ΔH^0 (kcal/mol)	$T\Delta S^0$ (kcal/mol)
2	2130 \pm 250	-4.54 ± 0.06	-3.66 ± 0.10	0.88 ± 0.16
4	320 \pm 20	-3.41 ± 0.04	-4.60 ± 0.20	-1.19 ± 0.24
6	10 440 \pm 1350	-5.48 ± 0.07	-5.44 ± 0.06	0.04 ± 0.13
8	4510 \pm 470	-4.98 ± 0.06	-2.55 ± 0.04	2.43 ± 0.10

TABLE 2: Thermodynamic Complexation Parameters of β -CD with Several Carboxylate Guests in pH = 8.6 Phosphate Buffer ($I = 0.05$), 0.1 M NaCl, and 8 M urea at 298 K

guest	K (M^{-1})	ΔG^0 (kcal/mol)	ΔH^0 (kcal/mol)	$T\Delta S^0$ (kcal/mol)
2	700 \pm 100	-3.88 ± 0.08	-2.65 ± 0.15	1.23 ± 0.23
4	3990 \pm 1160	-4.91 ± 0.51	-0.40 ± 0.02	4.51 ± 0.53
6	2600 \pm 330	-4.66 ± 0.07	-6.64 ± 0.18	-1.98 ± 0.25
8	1880 \pm 330	-4.46 ± 0.10	-4.29 ± 0.20	0.17 ± 0.30

and 8. Due to the basic character of the buffer solutions, the anionic form of the acids is present at all times.¹⁵ The resulting complexation parameters at 298 K are presented in Table 1.

The naphthalene derivative (guest 4) is the only one which exhibits an unfavorable entropic term. The remaining guests show small (guests 2 and 6) or clearly favorable (guest 8) entropic terms. In any event, the results demonstrate that the inclusion complexation of the four guests surveyed is enthalpically driven. This is in good agreement with previous CD complexation studies that have suggested that the entropic contributions, which in the classical hydrophobic effect are the dominating factor, are overtaken by larger enthalpic terms.^{9c,11a} Several studies have shown that the favorable enthalpic contributions arise from intramolecular CD reorganization processes¹⁶ as well as from poorly hydrogen-bonded water molecules which are displaced from the CD cavity and incorporated into the bulk solvent upon guest inclusion.¹⁷

As can be seen in Table 2, addition of large concentrations of urea (8 M) to the reaction medium exerts, as expected, a clear effect on all the thermodynamic parameters that define the complexation of the four anionic guests with β -CD.

In agreement with previous complexation studies in water–urea mixtures, three of the four systems surveyed showed a decrease in the overall host–guest binding affinity in the presence of the denaturing agent.¹² The only exception to this trend is guest 4, the naphthalene derivative, for which a very favorable entropy change is responsible for the overall stabilization of the inclusion complex. However, the direction of the free energy changes upon addition of urea must be analyzed in terms of the effect that it has on the entropy and enthalpy complexation functions. Such effects must have their origin on specific perturbations that the urea molecules exert on the solvent structure as well as on the recently suggested guest–urea interactions.

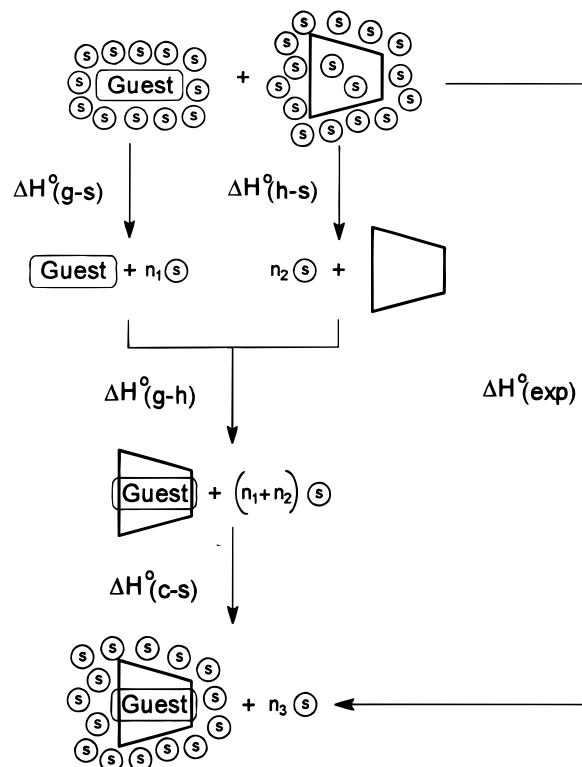
For each one of the carboxylate guests under study, the difference in the measured enthalpy of CD binding in 8 M urea and water can be defined as

$$\Delta(\Delta H^0) = \Delta H^0_{(\text{exp})u} - \Delta H^0_{(\text{exp})} \quad (3)$$

where $\Delta H^0_{(\text{exp})}$ and $\Delta H^0_{(\text{exp})u}$ are the calorimetrically obtained ΔH^0 values of the complexation reaction in the absence and in the presence of 8 M urea, respectively. By extending the definition of $\Delta(\Delta H^0)$ to $\Delta(\Delta S^0)$ and $\Delta(\Delta G^0)$, the differential thermodynamic functions of β -CD complexation with each one of the four carboxylate guests under study are presented in Table 3.

TABLE 3: Differential Thermodynamic Complexation Parameters of β -CD with Different Carboxylate Guests in the Presence (8 M) and in the Absence of Urea

guest	$\Delta(\Delta G^0)$ (kcal/mol)	$\Delta(\Delta H^0)$ (kcal/mol)	$\Delta(T\Delta S^0)$ (kcal/mol)
2	0.66 ± 0.14	1.01 ± 0.25	0.35 ± 0.39
4	-1.50 ± 0.55	4.20 ± 0.22	5.70 ± 0.77
6	0.82 ± 0.14	-1.20 ± 0.24	-2.02 ± 0.38
8	0.52 ± 0.16	-1.74 ± 0.24	-2.26 ± 0.40

SCHEME 3: Thermodynamic Cycle for the Complexation of a Nonpolar Guest by β -CD

Clearly, the $\Delta(\Delta H^0)$ and $\Delta(T\Delta S^0)$ functions defined by eq 3 show a different trend for compounds 2 and 4 than for guests 6 and 8. Positive differences for both the enthalpy and entropy functions were measured for the complexation of the two aromatic guests (2 and 4), whereas negative $\Delta\Delta$ values characterize the aliphatic compounds (6 and 8).

As can be inferred from the cycle presented in Scheme 3, the measured enthalpy of complexation $\Delta H^0_{(\text{exp})}$ for each one of the host–guest systems obeys the following equation

$$\Delta H^0_{(\text{exp})} = \Delta H^0_{(\text{g-s})} + \Delta H^0_{(\text{h-s})} + \Delta H^0_{(\text{g-h})} + \Delta H^0_{(\text{c-s})} \quad (4)$$

where $\Delta H^0_{(\text{h-s})}$ and $\Delta H^0_{(\text{g-s})}$ are the desolvation enthalpies of the host and guest molecules, respectively, $\Delta H^0_{(\text{c-s})}$ is the solvation enthalpy of the complex, and $\Delta H^0_{(\text{g-h})}$ is the enthalpy change associated with the host–guest interaction in the gas phase. Obviously, the extrapolation of eq 4 to the other two thermodynamic state functions ΔG^0 and ΔS^0 is straightforward.

The $\Delta(\Delta H^0)$ function defined in eq 3, upon combination with eq 4, becomes

$$\Delta(\Delta H^0) = (\Delta H^0_{(\text{g-s})u} - \Delta H^0_{(\text{g-s})}) + (\Delta H^0_{(\text{h-s})u} - \Delta H^0_{(\text{h-s})}) + (\Delta H^0_{(\text{c-s})u} - \Delta H^0_{(\text{c-s})}) \quad (5)$$

where the subscript “u” refers to the thermodynamic function for complexation in the presence of 8 M urea and the difference $\Delta H^0_{(\text{g-h})u} - \Delta H^0_{(\text{g-h})}$ has been omitted since the host–guest

interaction term is independent of the presence of urea in the aqueous solvent.

When comparing thermodynamic functions in different host–guest systems, the term $\Delta H^0_{(h-s)u} - \Delta H^0_{(h-s)}$, which represents the difference in host desolvation enthalpy in the presence and in the absence of urea, must have a constant value since for all the systems under study the same host (β -CD) was used. Furthermore, all of the encapsulated guests expose approximately the same structure to the solvent. Therefore, the complex solvation term ($\Delta H^0_{(c-s)u} - \Delta H^0_{(c-s)}$), while not constant, is not expected to change significantly when going from guest to guest. Moreover, based on solubility and β -CD fluorescence binding measurements in water and water–urea mixtures, Breslow and co-workers have recently suggested that the host's cavity (as well as the rest of the molecule, either complexed or uncomplexed) interacts with urea in a negligible fashion as compared to the effect of the denaturant on the guest molecule.^{12a} Because of this, the experimentally measured $\Delta(\Delta H^0)$, $\Delta(T\Delta S^0)$ and $\Delta(\Delta G^0)$ values represent, on a relative scale, the differences between the corresponding guest desolvation thermodynamic functions in the presence and in the absence of urea. In other words, the opposing trends observed in the thermodynamic functions of Table 3 reflect the differences in desolvation enthalpies and entropies between aromatic and aliphatic guests.

Following Breslow and co-workers, the difference in the desolvation enthalpy of a nonpolar guest in a water–urea mixture and water can be expressed as the sum of two independent contributions:^{6,18}

$$\Delta H^0_{(g-s)u} - \Delta H^0_{(g-s)} = \Delta(\Delta H^0)_{\text{cavity}} + \Delta(\Delta H^0)_{\text{interaction}} \quad (6)$$

Here $\Delta(\Delta H^0)_{\text{cavity}}$ describes the effect that urea has on the energy required to remove a cavity in the solvent upon desolvation, and $\Delta(\Delta H^0)_{\text{interaction}}$ is related to the difference in interaction enthalpies between the solvent molecules and the nonpolar guest in the presence and in the absence of urea.

Since the volume of the four carboxylate guests is very similar (79, 60, 72, and 64 Å³ for compounds **2**, **4**, **6**, and **8**, respectively),¹⁹ the $\Delta(\Delta H^0)_{\text{cavity}}$ term, while not zero, should in principle be very close for the four guests, and according to eqs 5 and 6, the relative differences in $\Delta(\Delta H^0)$ values for the carboxylate guests should reflect the differences in interaction energies between the solvent and the nonpolar guests.

As can be seen by inspection of Table 3, the differential complexation enthalpies are clearly larger for the aromatic guests **2** and **4** than for the aliphatic ones. Whereas the ferrocene carboxylate guest exhibits a more positive $\Delta(\Delta H^0)$ value of about 2.5 kcal/mol with respect to the average $\Delta(\Delta H^0) = -1.5$ kcal/mol for the two aliphatic guests, the naphthalene carboxylate derivative shows a significantly larger increase of 5.7 kcal/mol. These thermodynamic data reveal specific attractive interactions between aromatic surfaces and urea–water solvent molecules. The desolvation process that the aromatic guest molecules undergo prior to complexation is thus characterized by a larger ΔH^0 value than that observed with the aliphatic guests. According to the theoretical predictions of Jorgensen and co-workers,⁸ the interaction energy between one molecule of urea and a benzene ring in water should be of the order of 1.8 kcal/mol and that corresponding to an urea molecule and a larger naphthalene molecular surface would be approximately 3.5 kcal/mol. Considering that the ferrocene guest exposes to the solvent approximately two benzene surfaces and the naphthalene derivative exposes two even larger surfaces, the average interaction energies of 2.5 and 5.7 kcal/mol, respectively, correlate relatively well with the theoretical predictions if one molecule of urea interacts on average with each one of the aromatic surfaces of guests **2** and **4**.

Since the experimentally obtained 2.5 and 5.7 kcal/mol differences for the ferrocene and naphthalene derivative guests are measured relative to an average aliphatic enthalpy change, a small van der Waals interaction term that reflects the interaction between the solvent and the aliphatic nonpolar guest should be added to these values in order to properly compare them with the computationally obtained ones. Although an accurate value of such interaction is not available, a rough estimate of about 1 kcal/mol for the van der Waals interaction between a nonpolar aliphatic compound dissolved in water would make the enthalpic urea interaction values for the two aromatic guests very close to the theoretically predicted ones.

Entropic differences arising from the comparison of the $\Delta(T\Delta S^0)$ values depicted in Table 3 further support the urea–aromatic surface interaction originally suggested in the work of Breslow⁶ and Jorgensen.⁸ Whereas the two aliphatic guests feature very similar entropic differences, the two aromatic carboxylates show positive $\Delta(T\Delta S^0)$ values relative to the values of the aliphatic changes. Such entropic differences for β -CD complexation in the presence and in the absence of urea are consistent with a picture in which a larger increase in entropy occurs when interacting urea molecules are released from the aromatic surface upon inclusion of the guest into the CD cavity. Moreover, the fact that there is a relatively large difference between the two $\Delta(T\Delta S^0)$ values for the ferrocene and the naphthalene derivatives is also consistent with the postulated interaction of urea molecules with aromatic surfaces since the naphthalene carboxylate guest features a larger aromatic area compared to the one that characterizes the ferrocene derivative.

Therefore, free energy changes of CD binding of aromatic guests in the presence of urea may be larger or smaller than the corresponding values in pure water. This may be surprising since recent reports on binding studies of CD derivatives in water and water–urea mixtures have indicated that the presence of the denaturing agent always decreases the stability of the inclusion complex.¹² In view of our results with guest **4**, however, the opposing thermodynamic contributions defined by the interaction between urea and nonpolar surfaces may sometimes lead to complex stabilization.

On one hand, ΔH^0 values of binding must be more positive in urea–water mixtures due to the energetic penalty that the system has to pay to desolvate the aromatic guest so that it can be included in the CD cavity. This destabilizing enthalpy effect, usually dominates over the opposing entropic contribution that tends to stabilize the complex by increasing the entropy of the system upon urea–water desolvation of the aromatic guest. In the case of guest **4**, however, the balance of enthalpic and entropic changes favors the complex stabilization effect due to the large entropic contribution and, thus, the CD binding of this derivative is favored in concentrated urea solutions.

We can speculate that the large aromatic surface area that characterizes guest **4** may be responsible for the stabilization effect that urea exerts on its β -CD inclusion complex. This is, in principle, possible because the opposing enthalpic and entropic stabilizing effects upon addition of urea may have different dependencies on the size of the aromatic surface exposed to the solvent at 298 K. Obviously further studies are needed in order to clarify this point.

It is important to note that the study of a larger number of carboxylate compounds using similar calorimetric complexation measurements with β -CD was unfortunately not possible. In this type of study, the basic requirements for guest molecules are that they must be relatively soluble in water, fit tightly into the β -CD cavity, and as a consequence, have binding constants with β -CD of the same order of magnitude as those surveyed in this work. These requirements seriously limit the possibility

TABLE 4: Thermodynamic Complexation Parameters of β -CD with Several Quaternary Ammonium Guests in pH = 8.6 Phosphate Buffer ($I = 0.05$), 0.1 M NaCl at 298 K

guest	K (M^{-1})	ΔG^0 (kcal/mol)	ΔH^0 (kcal/mol)	$T\Delta S^0$ (kcal/mol)
3	2900 \pm 330	-4.70 \pm 0.10	-6.80 \pm 0.30	-2.10 \pm 0.40
5				
7	3860 \pm 195	-4.89 \pm 0.03	-5.85 \pm 0.05	-0.96 \pm 0.08
9				

TABLE 5: Thermodynamic Complexation Parameters of β -CD with Several Quaternary Ammonium Guests in pH = 8.6 Phosphate Buffer ($I = 0.05$), 0.1 M NaCl, and 8 M urea at 298 K

guest	K (M^{-1})	ΔG^0 (kcal/mol)	ΔH^0 (kcal/mol)	$T\Delta S^0$ (kcal/mol)
3	715 \pm 110	-3.90 \pm 0.10	-4.90 \pm 0.70	-1.00 \pm 0.80
5				
7	1160 \pm 190	-4.18 \pm 0.09	-4.46 \pm 0.23	-0.28 \pm 0.32
9				

of a more comprehensive study on the effect that urea has on the CD complexation of nonpolar guests because the number of available guests is rather small.

In the second part of the work, the series of quaternary ammonium guests **3**, **5**, **7**, and **9** were studied in the same way as the analog carboxylate derivatives in order to address the effect that the positive charge has on the difference of the thermodynamic parameters that describe CD complexation in the presence of urea. In this case however, the densely charged ammonium group proved to introduce serious differences in the calorimetric measurements.

The thermodynamic parameters of complexation of the cationic ammonium guests with β -CD are presented in Tables 4 (in the absence of urea) and 5 (in the presence of 8 M urea). The binding parameters of β -CD with guests **5** and **9** could not even be determined. In both cases the heat of mixing, as reflected in the voltage signal from the calorimeter reaction chamber, was so small that a meaningful analysis of the data could not be performed. Whether this is due to a low binding constant or to an extremely small heat of complexation was not determined.

Comparison of the differences in the thermodynamic functions defined in eqs 3 and 5 for the remaining two guests ($\Delta(\Delta H^0) = 1.9 \pm 1$, $\Delta(T\Delta S^0) = 1.1 \pm 1.2$ kcal/mol for the complexation of guest **3** and $\Delta(\Delta H^0) = 1.4 \pm 0.3$, $\Delta(T\Delta S^0) = 0.68 \pm 0.4$ kcal/mol for guest **7**), showed that, in agreement with the urea–aromatic surface interaction postulate, the ferrocene derivative **3** has a more positive desolvation heat $\Delta(\Delta H^0)$ and a more negative entropic contribution $-\Delta(T\Delta S^0)$ to the free energy of binding with host **1**.

A detailed comparison of the values obtained in these measurements with those for the carboxylate guests, however, is hampered by larger deviations on the experimentally determined values as well as by the presence of a densely charged region in the ammonium guest molecules located so close to the nonpolar part of the ion. The overlap of the cospheres of the polar and nonpolar parts of the ion must affect the desolvation process in a more complicated fashion.

Conclusions

In this work, the thermodynamic parameters that describe the association of β -CD with a series of aromatic and aliphatic guest molecules in aqueous media have been determined in the absence and in the presence of large concentrations of urea. The comparison of the complexation data afforded, to the best of our knowledge, the first experimental evidence for specific

interactions between urea molecules and aromatic surfaces, in very good agreement with previous computational studies.⁸

Although the magnitude of this interaction accounts for less than a hydrogen bond (in addition to the interaction between the solvent molecules and saturated surfaces), it can be very important for large biomolecules that feature many aromatic residues. In fact, the effect of urea on the thermodynamic parameters of complexation of the nonpolar guests with β -CD presented here sheds some light on the mechanism for the urea-induced denaturing of folded proteins in aqueous media.

As expected, the net effect that large concentrations of urea may have on the stability of CD inclusion complexes results from a balance between opposing enthalpic and entropic contributions. Their relative magnitude, however, and therefore the net stabilizing or destabilizing effect of urea, may depend on the size of the aromatic surface that the guest exposes to the solvent. Additional studies on this matter are certainly needed.

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