Exploring the Origins of Enthalpy–Entropy Compensation by Calorimetric Studies of Cyclodextrin Complexes

Schönbeck, Jens Christian Sidney; Holm, René

Published in:
Journal of Physical Chemistry Part B: Condensed Matter, Materials, Surfaces, Interfaces & Biophysical

DOI:
10.1021/acs.jpcb.9b03393

Publication date:
2019

Document Version
Peer reviewed version

Citation for published version (APA):
Schönbeck, J. C. S., & Holm, R. (2019). Exploring the Origins of Enthalpy–Entropy Compensation by Calorimetric Studies of Cyclodextrin Complexes. *Journal of Physical Chemistry Part B: Condensed Matter, Materials, Surfaces, Interfaces & Biophysical*, *123*(31), 6686-6693. https://doi.org/10.1021/acs.jpcb.9b03393

Exploring the Origins of Enthalpy-Entropy Compensation by Calorimetric Studies of Cyclodextrin Complexes

Christian Schönbeck[a,*] and René Holm[a,b]

[a] Department of Science and Environment, Roskilde University, Universitetsvej 1, DK-4000, Roskilde, Denmark

[b]Drug Product Development, Janssen Research and Development, Johnson & Johnson, Turnhoutseweg 30, 2340 Beerse, Belgium

*Corresponding author:

E-mail: jechsc@ruc.dk, Telephone: +45 46742345

Department of Science and Environment

Roskilde University

Universitetsvej 1

DK-4000 Roskilde

Denmark

Abstract

Cyclodextrin complexes were used as simple model systems to explore the enthalpy-entropy compensation phenomenon which is often observed in biomolecular processes, e.g. in protein-ligand binding. The complexation thermodynamics for the binding of a series of adamantane derivatives to several cyclodextrin hosts were determined by isothermal titration calorimetry in the temperature range 10-55 °C. As for other cyclodextrin complexes, the thermodynamic parameters depended systematically on the structural modifications of the cyclodextrins. Hydroxypropyl chains at the rims of the cyclodextrin hosts changed the thermodynamic fingerprint of binding to all guests by inducing significant increases in the complexation enthalpies and entropies. Similarly, the heat capacity changes upon complexation also showed a linear dependence on the number of hydroxypropyl chains. The altered complexation thermodynamics was ascribed to the increased dehydration of polar groups on the guest by the hydroxypropyl chains on the host. This unfavorable interaction destabilized the complexes as the enthalpic penalty was only partially compensated by the gain in entropy. The degree of enthalpy-entropy compensation depended on the guest molecule and seems to be related to the hydrophilicity/hydrophobicity of the desolvated molecular surface.

Introduction

Molecular recognition depends on numerous noncovalent interactions between the associating molecules. When the association takes place in a solvent the interactions with the solvent molecules also contribute. Given the complexity of such systems it is no surprise that binding free energies (ΔG°) are extremely difficult to predict, even for simple binary host-guest systems. It is often challenging to obtain even a qualitative understanding of the main driving forces for formation of host-guest complexes. Driving forces that may contribute to the formation of a complex include electrostatic/coulombic forces, van der Waals interactions, hydrogen bonding, etc. In aqueous solution, the hydrophobic effect also plays an important, and often dominant, role.

The binding free energy, ΔG°, can be divided into enthalpic and entropic contributions, which are often interpreted in terms of driving forces. Attractive electrostatic and van der Waals forces are assumed to result in an enthalpy-driven binding, while hydrophobic attraction supposedly is characterized by a large gain in entropy. This simplistic interpretation of binding thermodynamics has caused some confusion and seemingly contradictory observations. For example, there are many experimental indications that the hydrophobic effect is a crucial driving force for the formation of cyclodextrin (CD) inclusion complexes.[1] However, the thermodynamic fingerprint of these interactions does not conform to the conventional view that hydrophobic interactions are entropy-driven. On the contrary, the vast majority of CD complexes are enthalpy-driven.[2] This “nonclassical hydrophobic effect” is not only observed for CDs but may be a general characteristic of the solvent-driven association of molecules of moderate polarity,[3] keeping in mind that the CD cavity is considered semipolar.[1] Release of water molecules from the CD cavity constitutes another explanation of the enthalpy-driven complexation.[4] The latter explanation is hypothesized to constitute a general driving force in systems where water molecules are confined in small cavities.[5]

The example above illustrates how the experimentally observed thermodynamic fingerprint plays a pivotal role in the arguments for the driving forces and their molecular interpretation. Similarly, thermodynamic data play a central role in the recent arguments for the existence of a distinct chaotropic effect, orthogonal to the hydrophobic effect.[6] But is it really possible to conclude anything about the driving forces based on the enthalpic and entropic contributions? Possibly, but it requires a thorough understanding of the various contributing factors to these thermodynamic functions. Especially, the phenomenon called enthalpy-entropy compensation (EEC) needs to be understood as it can mask the thermodynamic fingerprint of the mechanisms that are responsible for the binding free energy. As the name indicates, EEC refers to processes where an enthalpic contribution is partially or wholly offset (compensated) by a similar but oppositely directed entropic contribution. Consequently, such processes do not contribute much to the free energy although they may dominate the total thermodynamic fingerprint. Reorganization of water molecules near solute surfaces have been suggested to be a source of EEC.[7,8]

EEC is a long known and much debated topic, but its underlying causes are not clear.[9–12] We have previously reported the complexation thermodynamics of numerous natural and modified CD host molecules with a number of bile salt guests.[13–15] For those complexes, a strong EEC effect was observed. Complexes with natural βCDs were driven by a large negative enthalpy ($\Delta H^\circ \approx$ -25 to -30 kJ/mol) and slightly destabilized by entropy ($T\Delta S^\circ \approx$ 0 to -7 kJ/mol). Chemical modification of the CD hosts resulted in a significant increase in ΔH° but also in a compensating increase in $T\Delta S^\circ$, so that the effect on the binding free energy was relatively small (see Eq. 3).

This effect scaled with the size and number of substituents at the rims of the CDs so that formation of complexes with some of the highly substituted CDs were mainly driven by entropy (($T\Delta S^\circ \approx$ 10 to 15 kJ/mol) and to a much lesser extent by enthalpy ($\Delta H^\circ \approx$ -5 kJ/mol). A similar picture was obtained for complexation with natural and modified γCDs.[16] Although the presence of substituents at the rims of the CDs completely altered the thermodynamic fingerprint of the complexation process, this was not interpreted as a change in the driving force. Rather, the observed EEC was attributed to the increased hydrophobic contacts between the guest and the extended host molecule. This interpretation was supported by molecular dynamics (MD) simulations, measured heat capacity changes, and osmotic stress measurements.[17–19] On the other hand, a detailed analysis of the MD simulations revealed that altered energetics of the water in the cavity of the free CD constituted at least part of the explanation for the observed EEC.[20] Upon formation of the complex, release of the cavity water into the bulk will thus exhibit an altered thermodynamic fingerprint.

The present work aims to explore these hypotheses about the origins of the EEC observed for CD complexes. Is the EEC brought about by increased hydrophobic contacts between the guest and the CD substituents and the concomitant release of surface water? Or is there another explanation, such as altered energetics of the cavity water that is expelled upon complexation? The hypotheses and the experimental strategy are illustrated in Figure 1. Increased hydrophobic contacts occur when the guest molecule protrudes from the CD cavity and interacts with the substituents at the rims of the CD, but a sufficiently small guest molecule will not protrude from the CD cavity and will not form hydrophobic contacts with the substituents. Thus, if the hydrophobic contacts hypothesis is valid, no substituent-induced EEC is expected for the complexation with small guest molecules. Testing this hypothesis requires a suitable guest molecule. Adamantane is a spherical hydrocarbon that is able to fit almost perfectly into the cavity of βCD without protruding from the cavity.[21–23] As the adamantane moiety also has a strong affinity for βCD this binding motif is an excellent model for testing the hydrophobic contacts hypothesis. The present work explores the complexation thermodynamics of several water-soluble adamantane derivatives (AD) upon binding to natural and modified βCDs.

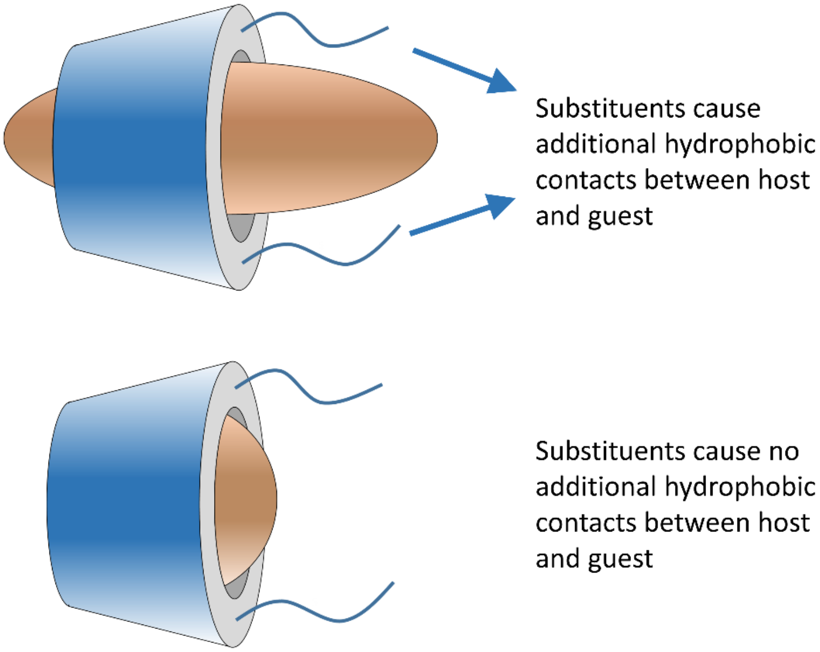


Figure 1 Small guest molecules are not expected to interact with the substituents at the CD rims. The small guest could be adamantane and the large guest could be a bile salt.

Experimental Section

Chemicals

Natural β-cyclodextrin, randomly substituted (2-hydroxypropyl)-β-cyclodextrin (HP093), and 1-Adamantylamine were purchased from Adamas-Beta, Shanghai, China. Randomly substituted (2-hydroxypropyl)-β-cyclodextrin (HP063) was from Alfa Aesar, Shanghai, China. Adamantanecarboxylic acid (AdCOO) and 1-adamantaneacetic acid ($AdCH_2COO$) were from J&K Chemical, Shanghai, China. 1-Adamantanol (AdOH) and 1-Adamantanemethanol ($AdCH_2OH$) were purchased from Sigma-Aldrich. The water content of the three CD samples were determined by Karl-Fischer titration to 11.2, 7.1, and 6.4 wt% for βCD, HP063 and HP093, respectively. The water content was taken into account when calculating the concentration of the cyclodextrins.

Nuclear Magnetic Resonance Spectroscopy

Spectra were recorded at 25°C on a Bruker Avance-600 spectrometer, equipped with a cryoprobe. For structural characterization of HPβCDs, 1D ^{1}H and ^{13}C and 2D HSQC, HMBC, H2BC spectra were recorded on samples containing around 10 mM CD in D_2O. For structural characterization of the complexes, 2D ROESY spectra (16 scans, 400 ms mixing time) were recorded on samples with around 10 mM of CD and AD dissolved in D_2O based phosphate buffer, pH 7.2.

Isothermal titration calorimetry

Titrations were made on a VP-ITC (Malvern Panalytical, Malvern, UK) with a cell volume of 1.4 ml. Aqueous solutions of CDs were titrated into solutions of ADs in aliquots of 10µL, in total 28 injections. The first injection of only 2 µL was not included in the data analysis. All solutions were buffered with a 50 mM phosphate buffer, pH 7.20. At this pH the ADs with carboxylic acid or amino groups carry one negative or positive charge, respectively. AD concentrations were in the range 0.3-1 mM and CD concentrations were approximately 10 or 20 times higher, resulting in a molar ratio of 2 or 4 at the end of the titration. The lower the binding constant, the higher the concentrations. Titrations were conducted at 10, 25, 40 and 55 °C, except for the adamantols which caused some problems at elevated temperatures, possibly due to problems with solubility or chemical stability.

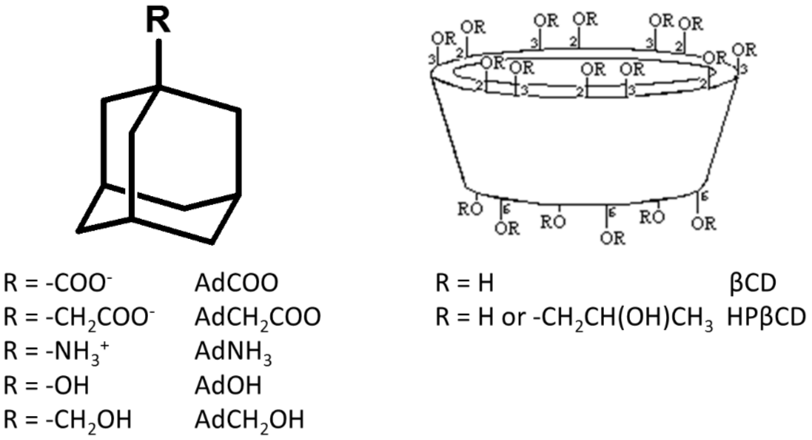
Molecular dynamics simulations

Complexes of natural βCD and a HPβCD with the two anionic guests were simulated, as well as the uncomplexed species. Each of the 4 complexes were simulated in two binding modes where the carboxylate group protruded from either the primary or the secondary CD rim. For the native CD structures, bonded and nonbonded parameters were from the CHARMM carbohydrate force field.[24,25] Parameters for the 2-hydroxypropyl substituents and the adamantane derivatives were generated by the CGenFF program version 1.0.0,[26,27] with the CHARMM General Force Field version 3.0.1.[28] See ref. 23 for details of the AD parameters. Molecules and complexes were solvated in cubic boxes of TIP3P water with a side length of 34-36 Å. If necessary, charge neutrality was obtained by adding a sodium ion.

Initial structures of the complexes were generated by placing the AD guest at the opening of the secondary CD rim with the charged group pointing away from the cavity. Simulations were run with 2 fs time steps in the NAMD software.[29] Complexes and free HPβCDs were simulated with a 10 ns equilibration run and a 20 ns production run. Free ADs and free natural βCD have fewer structural degrees of freedom and required less simulation time. ASA was determined in the VMD software[30] using a spherical probe of radius 1.4 Å at a sampling frequency of 10 ps and averaged.

Results

A total of 15 AD:CD complexes were characterized with respect to structure and complexation thermodynamics. Two neutral ADs, AdOH and $ADCH_2OH$, two negatively charged ADs, AdCOO and $AdCH_2COO$, and a single positively charged AD, $AdNH_3$, were chosen as guest molecules. Natural βCD and two HPβCDs were used as host molecules. Structures are shown in Figure 2.



$R = -COO^-$ AdCOO
$R = -CH_2COO^-$ $AdCH_2COO$
$R = -NH_3^+$ $AdNH_3$
$R = -OH$ AdOH
$R = -CH_2OH$ $AdCH_2OH$

R = H βCD
R = H or $-CH_2CH(OH)CH_3$ HPβCD

Figure 2 Structures of the investigated adamantane derivatives and cyclodextrins.

Characterization of CDs and their complexes

The two HPβCD samples were characterized by NMR according to a previously described method,[13] using the areas of selected peaks in the ^1H and ^{13}C spectra (Figures S1 and S2 in the Supporting Information). The average degree of substitution, expressed as the number of HP chains per glucose unit, was 0.63 and 0.93 for HP063 and HP093. Around 58% of the HP chains were located on O2 of the secondary CD rim, while around 10% were located on O6 of the primary rim. The remaining 30% were assumed to be attached to O3 on the secondary rim.

Previous analyses of HPβCD samples by other analytical techniques yielded similar patterns of substitution.[31,32]

NMR ROESY spectra confirmed the inclusion of adamantane derivatives into the CD cavity, as evidenced by strong interactions between the interior CD protons, H3 and H5, and all of the protons on the adamantyl moiety (Figures S3 and S4). The complexity of the HPβCD samples resulted in significantly broadened and overlapping signals, which prevented an unambiguous determination of the orientation of the adamantane derivatives. For complexes with natural βCD, it was recently established that the charged group of $AdNH_3$ protrudes from the secondary rim, while AdCOO and $AdCH_2COO$ are oriented in both directions.[23] Those orientations were also observed in methylated βCDs,[23] and it seems likely that HPβCDs behave the same way. Very weak ROESY crosspeaks were observed between the protons on the AD guests and the methyl group on the HP chains of HP093, indicating limited interactions between these moieties (Figure S5)

Molecular Dynamics Simulations

Molecular dynamics simulations of four of the investigated complexes were conducted to explore their structure and dynamics. In particular, the influence of the HP substituents was explored to test the validity of the hypothesis expressed in Figure 1. To this end, inclusion complexes with a hydroxypropylated βCD were compared to the corresponding inclusion complexes with natural βCD. The HPβCD was named HP086 as its degree of substitution was 0.86 (6 HP chains distributed over 7 glucose units). To resemble the substitution pattern of the experimental CD samples as closely as possible, three HP chains were attached to O2's on the glucose units, two to O3's, and a single HP chain to an O6.

Each combination of host and guest was simulated with two different guest orientations in which the carboxylate groups on the guest protruded from either the wider secondary rim of the CD or from the primary rim. Which one is the correct guest orientation is difficult to determine experimentally, and both secondary and primary complexes may coexist, as observed for natural βCD and methylated βCDs.[23]

During the 30 ns of simulation, none of the complexes dissociated. Some mobility of the guests was observed, but in all complexes the adamantyl moiety stayed inside the CD cavity. The HP chains on HP086 were very mobile and interacted with the guest and each other. To quantify their interaction with the guest molecule the water accessible surface area (ASA) was used. This parameter is also expected to be correlated with experimentally determined thermodynamic functions such as ΔC_p.[33] Numerous interactions between the HP-chains and the guest would result in a large burial of surface area, that is, a large negative change in the water accessible surface area.

All ASA values for free and complexed species are presented in Table S1 in the Supporting Information. The change in ASA upon complexation was calculated as the difference in ASA between the complexes and the free species:

$$\Delta ASA = ASA_{complex} - (ASA_{CD} + ASA_{AD}) \qquad \text{Eq. 1}$$

The buried surface area (ΔASA) was grouped into polar (ΔASA_p) and nonpolar surface area (ΔASA_{np}). The resulting values appear in Table 1 for both secondary and primary complexes. It is clear that more nonpolar than polar surface area was buried upon complexation, which is not surprising given the nonpolar nature of the adamantyl moiety and the CD cavity. The presence of HP chains on the CD hardly resulted in increased burial of surface area, i.e. there were few direct contacts between the HP chains and the guest. In the most extreme case, the secondary HP086:AdCH$_2$COO complex, the presence of 6 HP chains only resulted in increased burial of 21 Å^2 of nonpolar surface when compared to the corresponding complex with natural βCD. For comparison, *each* HP chain in complexes with bile salt guests dehydrated an estimated 12-16 Å^2 of nonpolar surface due to numerous contacts between the HP chains and the protruding guest molecules.[17] Thus, the MD simulations essentially confirmed the picture in Figure 1, in which the HP chains interact with large protruding bile salt guests but not with small cavity-included adamantane guests.

Table 1 Buried polar (ΔASAp) and nonpolar (ΔASAnp) surface area of the simulated complexes. All values are in Å^2. It is not clear whether the ADs are oriented with the charged carboxylate group protruding from the secondary or primary rim of the CDs, therefore both orientations were simulated.

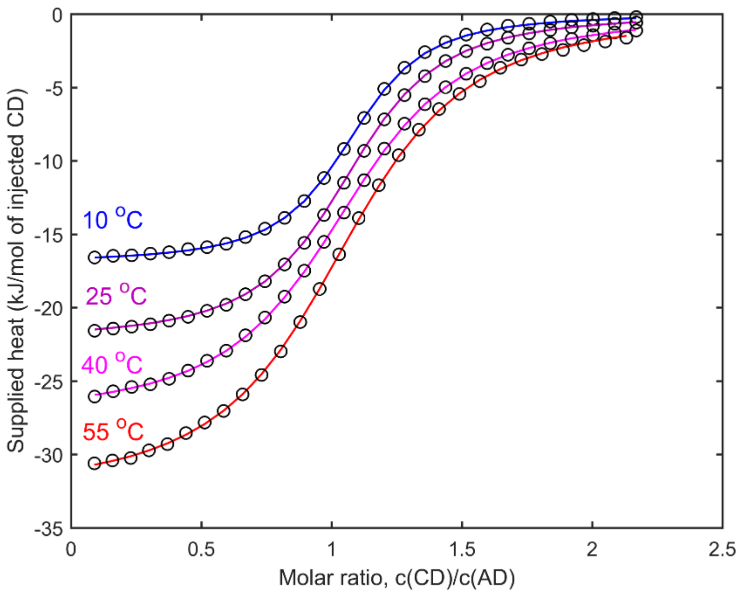
Complex	Secondary complex		Primary complex	
	ΔASA_p	ΔASA_{np}	ΔASA_p	ΔASA_{np}
βCD:AdCOO	-68	-306	-130	-290
HP086:AdCOO	-75	-316	-102	-307
βCD:AdCH$_2$COO	-76	-311	-130	-311
HP086:AdCH$_2$COO	-67	-332	-121	-322

Complexation Thermodynamics

Complexation thermodynamics was determined by isothermal titration calorimetry for all combinations of the three host molecules (βCD, HP063 and HP093) and the 5 guest molecules (AdCOO, AdCH$_2$COO, AdNH$_3$, AdOH and AdCH$_2$OH), in total 15 complexes. Binding thermodynamics are usually obtained by fitting the 'one set of sites' model to each enthalpogram, yielding the binding constant, K, binding enthalpy, $\Delta H°$, and stoichiometry, N, as best fit parameters. Conducting titrations at different temperatures allows for the change in heat capacity, ΔC_p, to be determined as the temperature derivative of $\Delta H°$. By exploiting that the temperature dependence of the binding constant is governed by $\Delta H°$, as expressed by the van't Hoff equation, more accurate thermodynamic data can be obtained by doing a global fit to multiple titrations at different temperatures.[34] In the global fit ΔC_p is assumed to be temperature independent. This assumption is based on personal experience with CD complexes,[15,18,34–36] and there is no indication in the literature that ΔC_p for CD complexes should exhibit any significant temperature dependence within the currently investigated temperature range.[37–39] Assuming a temperature independent ΔC_p the binding constant can be expressed as:

$$\ln(K) = \frac{\Delta H_0 - T_0 \Delta C_p}{R}\left(\frac{1}{T_0} - \frac{1}{T}\right) + \frac{\Delta C_p}{R}\ln\left(\frac{T}{T_0}\right) + \ln(K_0) \qquad \text{(Eq. 2)}$$

where ΔH_0 and K_0 are the complexation enthalpy and binding constant at a reference temperature, T_0, here chosen to be 25 °C. The global fitting procedure then yielded ΔH_0, K_0 and a temperature independent ΔC_p as global best fit parameters. Additionally, a set of local parameters, N, one for each titration, were varied to fit the data. An example of a global fit is shown in Figure 3 together with the obtained fitting parameters and their statistics. More examples are shown in the Supporting Information, and all obtained thermodynamic parameters are presented in Table S2. In most cases, excellent fits of the enthalpograms were obtained, and the errors on the obtained thermodynamic parameters were very small.



Parameter	Best fit value	Error at 95% CL	Correlation Matrix		
K at 25 °C (M^{-1})	109789	1173	1	0.38	0.28
ΔH at 25 °C (kJ/mol)	-23.52	0.04		1	-0.31
ΔC_p (J/mol/K)	-374	2			1

Figure 3 Global fit of 4 calorimetric titrations of AdCH$_2$COO with βCD, conducted at 10, 25, 40 and 55 °C. The fitting parameter, N, was local and assumed the values 0.942, 0.943, 0.938, and 0.936.

Once the binding constant and binding enthalpy was determined, the binding free energy and binding entropy was calculated:

$$\Delta G° = -RT\ln(K) = \Delta H° - T\Delta S° \qquad \text{(Eq. 3)}$$

where R is the gas constant.

The obtained binding thermodynamics for the complexes of natural βCD with the three charged ADs are in good agreement with values previously reported by Cameron *et al.*, with the exception of $\Delta H°$ for the βCD:AdCH$_2$COO complex where the previous value is too negative by around 5 kJ/mol.[40] The complexes of βCD with the two neutral ADs are less characterized in the literature, and only the βCD:AdCH$_2$OH complex has been studied by calorimetry at a single temperature (25 °C), at which slightly deviating values of K and $\Delta H°$ were reported.[41] None of the complexes with HPβCDs have previously been described in the literature

The thermodynamic parameters, $\Delta G°$, $\Delta H°$, and $T\Delta S°$, for all 15 complexes are plotted in Figure 4, along with previously published data for complexes of HPβCDs with three bile salt guests.[13] Although the most apparent feature is the large linear increase in $\Delta H°$ and $T\Delta S°$ with the number of HP chains on the host CD, it is also noteworthy that the HP chains severely destabilized the complexes. Going from βCD to HP091, the binding free energy increased 5-6 kJ/mol, meaning that the binding constants were reduced by a factor of 10 in the highly modified CD complexes (corresponding to an increase in $\Delta G°$ of 5.7 kJ/mol). Complexes with charged ADs were destabilized slightly more than complexes with neutral ADs.

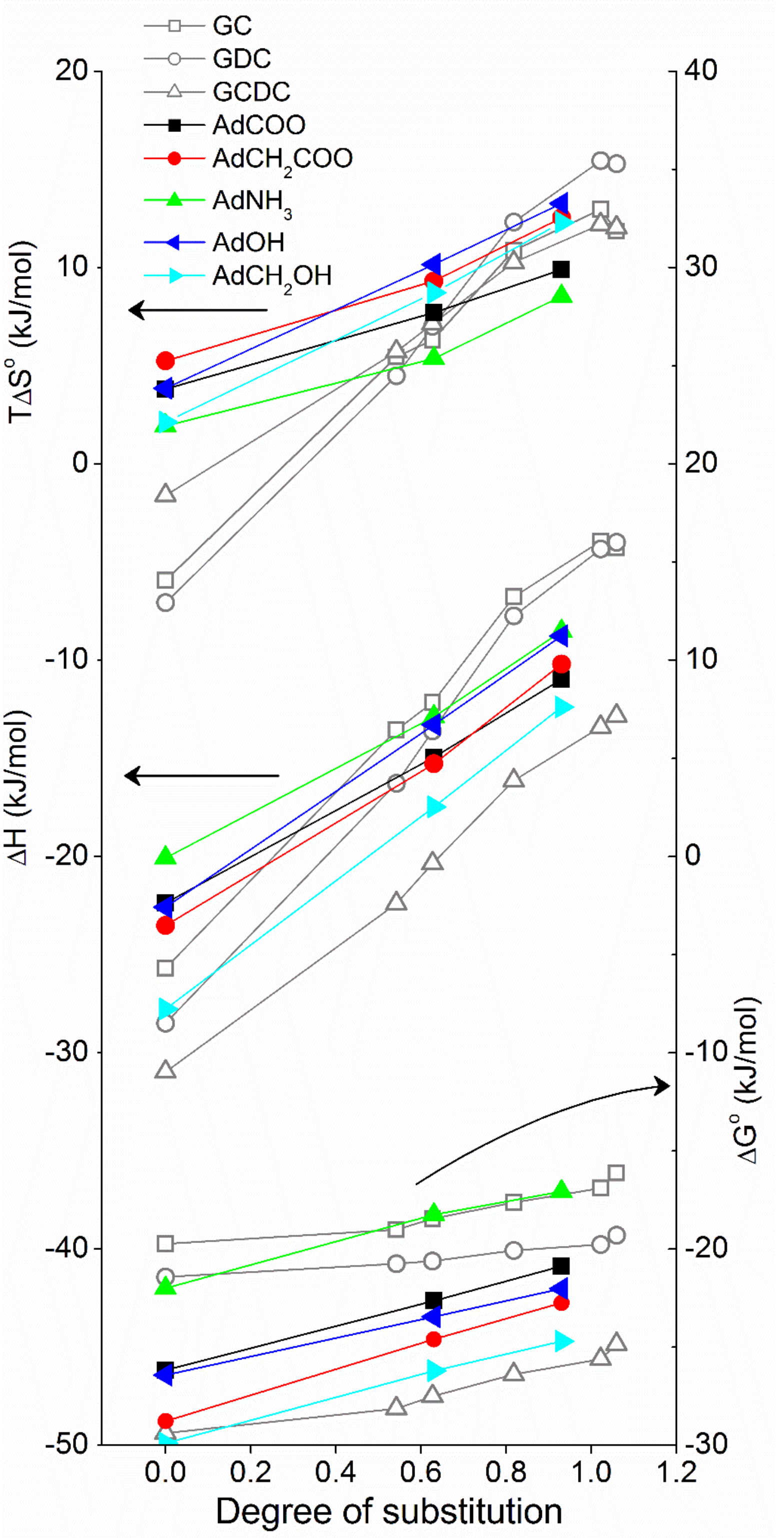


Figure 4 ΔH°, TΔS° (left axis) and ΔG° (right axis) for the formation of complexes with βCD and HPβCDs, plotted as a function of the number of HP chains per glucose unit of the CD hosts. Data for AD guests are shown with filled symbols while previously reported data[13] for three bile salt guests, glycocholate (GC), glycodeoxycholate (GDC) and glycochenodeoxycholate (GCDC), are shown with open symbols. All data are from Table S2.

Discussion

Disrupted solvent interactions with polar groups determine binding affinity

The measured binding constants can be easily rationalized. For the βCD complexes, the binding affinity correlates with the distance between the nonpolar adamantyl moiety and the attached polar group. The adamantyl moiety is located in the hydrophobic cavity of the CD with the polar group protruding from the cavity. The complexes are presumably destabilized when the polar group is too close to the CD host, resulting in reduced interactions between the polar group and the solvent. The charged $–NH_3$ group is directly attached to the adamantyl moiety and is therefore strongly dehydrated upon complexation, thus $AdNH_3$ had the lowest binding affinity. The charged carboxylate oxygens are separated from the adamantyl moiety by two and three bonds in AdCOO and $AdCH_2COO$, respectively, thus fewer interactions with the solvent are broken upon complexation, and the binding affinity increases in the order $AdNH_3$ < AdCOO < $AdCH_2COO$. Same pattern is seen for the two neutral guests. Although the hydroxyl groups in AdOH and $AdCH_2OH$ are separated from the adamantyl moiety by only one and two bonds, respectively, the destabilizing effect is not as severe as for the charged guests due to the weaker interactions of the hydroxyl group with the solvent.

Similar reasoning can be used to rationalize the destabilizing effect of the HP chains. The chains presumably disrupt the attractive interactions between the polar guest groups and the solvent. This may explain why the HP chains caused a larger destabilization of complexes with charged ADs than neutral ADs. The partial dehydration of the neutral hydroxyl groups in AdOH and $AdCH_2OH$ presumably cost less energy than dehydration of the charged carboxylate and amino groups. This interpretation is supported by the relatively small destabilizing effect of HP chains on complexes with bile salts,[13] where solvent interactions with the polar hydroxyl groups on the guest probably are less disrupted by the HP chains.

Cyclodextrin substituents induced large increase in *ΔH*° and *TΔS*°

As seen on Figure 4, the HP chains significantly altered the thermodynamic fingerprint of the complexation process. Upon going from βCD to HP091, *ΔH*° increased with 11-15 kJ/mol, depending on the guest. The increase in *TΔS*° was a little smaller: 6-7 kJ/mol for complexes with charged ADs and around 10 kJ/mol for neutral ADs. Whereas the complexes with natural βCD were almost exclusively driven by enthalpy, the binding free energy of complexes with HP091 was defined by almost equal contributions from enthalpy and entropy. The observed increase in *ΔH*°, and especially the increase in *TΔS*°, were less pronounced than for previously studied complexes with bile salt guests, also shown in Figure 4.

Interestingly, it seems to be a general trend that substituents at the rims of the CD host increase the enthalpy and entropy of complexation. This has been observed for ibuprofen binding to HPβCD,[39] adamantane-based drugs binding to sulfobutylether-βCD,[42] rimantadine and amantadine ($AdNH_3$) binding to sulfobutylether-βCD (compare data for sulfobutylether-βCD[43] to data for βCD[41]), numerous drugs binding to sulfobutylether-βCD,[44] aminobenzoic acids binding to HPαCD and HPβCD[45], and the already mentioned binding of bile salts to methylated, hydroxypropylated and sulfobutylated βCDs and γCDs. Despite the large experimental evidence of such effects on the complexation thermodynamics the underlying reasons are still somewhat speculative. As outlined in the introduction, the working hypothesis ascribes the substituent-induced increments in *ΔH*° and *ΔS*° to increased hydrophobic contacts between substituents and guest. The current MD simulations indicated limited contacts between the HP chains and AD guests, so the observed increase in $\Delta H°$ and $\Delta S°$ seems to disprove this hypothesis. However, the measured heat capacities creates a more ambiguous picture, as discussed below.

Decrease in ΔC_p indicates hydrophobic contacts between HP-chains and guest

The change in isobaric heat capacity, ΔC_p, is an often used measure of the molecular surface area that becomes exposed to water during a molecular process. Exposure of nonpolar surface is characterized by a large positive ΔC_p while exposure of polar surface gives a negative ΔC_p.[33,46] The measured negative values of ΔC_p, plotted in Figure 5 are thus consistent with the large burial of hydrophobic surface upon insertion of the adamantyl moiety into βCD.

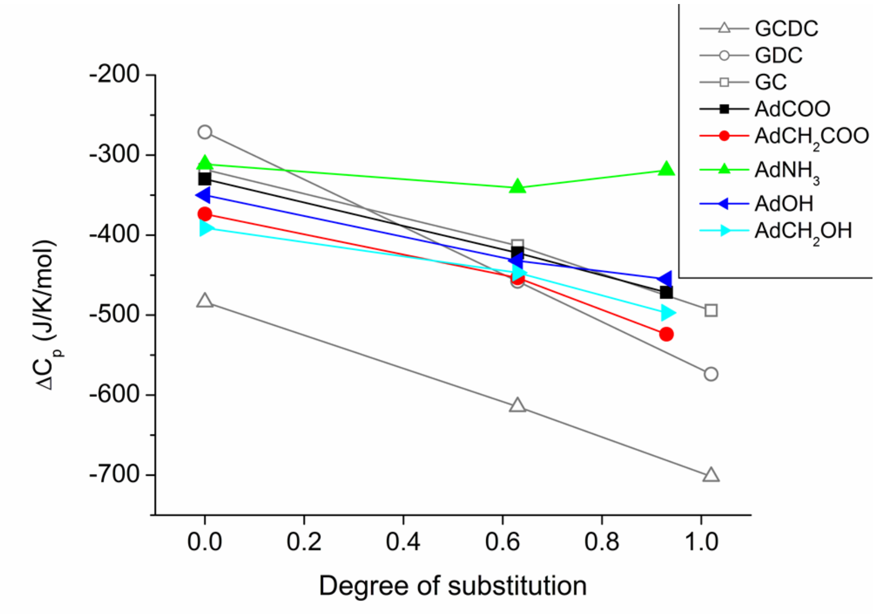


Figure 5 ΔC_p for the formation of complexes with βCD and HPβCDs. Data for AD guests are shown with filled symbols while previously reported data[17] for three bile salt guests, glycocholate (GC), glycodeoxycholate (GDC) and glycochenodeoxycholate (GCDC), are shown with open symbols. All data are from Table S2.

What is really interesting is the effect of the hydroxypropyl substituents on ΔC_p. With the exception of $AdNH_3$, there was a substantial decrease in ΔC_p with increasing degree of substitution, indicating formation of hydrophobic contacts between the HP chains and the AD guests. The literature does not present any universal quantitative relationship between ΔC_p and ΔASA_{np} but previous studies suggest a proportionality constant of around 2 J/mol/K per Å^2 for extracavity interactions in cyclodextrins.[17,47] ΔC_p for complexes with HP093 were more negative than for the corresponding complexes with natural βCD by around 100-150 J/mol/K. This decrease in ΔC_p translates into an additional burial of 50-75 Å^2 in complexes with HP093 but analysis of the MD simulations, presented in Table 1, yields a much smaller number. Is something wrong with the MD simulations, or is the interpretation of ΔC_p in terms of accessible surface area too simplistic? While it is worth noting that there are other contributions to ΔC_p,[11] it is also questionable whether ΔASA is a sufficient measure of the hydration changes. Without going into a deeper discussion of these issues, it is noted that both the MD simulations and the ΔC_p values showed that the AD guests interacted less with the HP chains than did the previously studied BS guests. The HP chains also have a smaller impact on the enthalpies and entropies of binding to the AD guests, suggesting that the observed substituent-induced variations in complexation thermodynamics indeed are related to the interactions between the HP chains and the guest.

Disrupted polar interactions reduce the degree of enthalpy-entropy compensation

The studied AD:CD complexes were investigated to see whether the presence of HP chains at the CD rims would produce compensating changes to $\Delta H°$ and $T\Delta S°$. The HP chains brought about significant increases in $\Delta H°$ and $T\Delta S°$ but they also destabilized the complexes, i.e. the observed changes in $\Delta H°$ and $T\Delta S°$ were not fully compensating. The low degree of compensation is also apparent from the enthalpy-entropy compensation plot in Figure 6 where the slopes are much lower than unity. Only if the slope is unity there will be exact EEC and an unaltered $\Delta G°$.

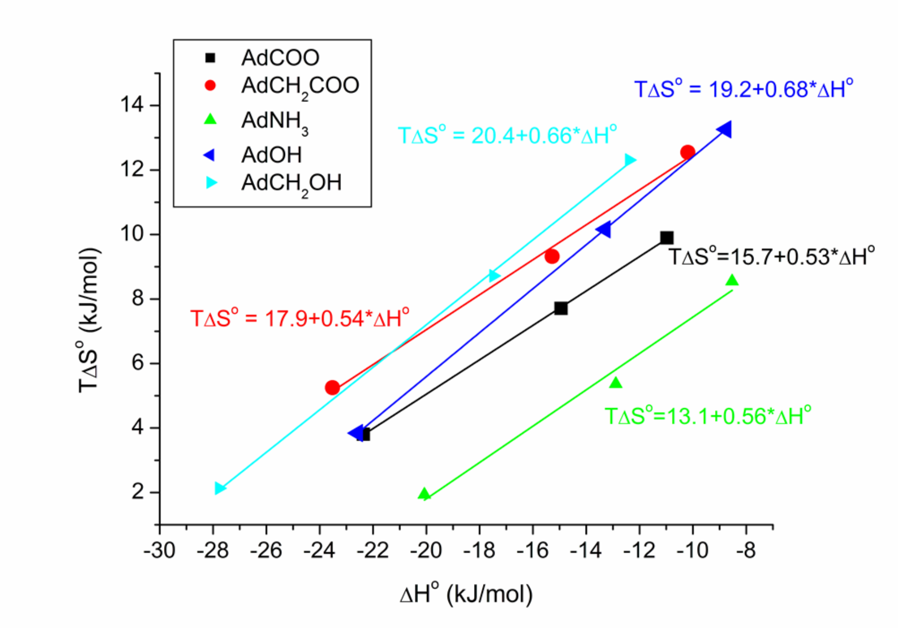


Figure 6 Enthalpy-entropy compensation plot for all AD:CD complexes. For each AD guest, the complexes with the three CD hosts lie very close to the linear regression lines.

It is noteworthy that the slopes in the EEC plot can be grouped in two. For the charged AD guests the slopes are in the range 0.53-0.56, meaning that only half of the destabilizing effect resulting from an increase in $\Delta H°$ is compensated by increased $T\Delta S°$. For the neutral hydroxyl containing ADs, the slopes are significantly higher, around 0.67. For a large number of complexes of bile salts with various modified CDs, the slopes in the EEC plot were closer to unity, between 0.75 and 0.90,[48] and even closer, 0.93-0.94, when only methylated CDs were considered.[18] It is tempting to interpret the slopes in terms of the hydrophilicity of the molecular surfaces that are dehydrated upon complexation. Partial dehydration of highly hydrophilic molecular moieties, like the charged carboxylate and amino groups, results in a low degree of EEC. Dehydration of nonpolar surface, as in the case of the bile salt complexes, yields an almost complete compensation with slopes close to unity. Dehydration of hydroxyl groups with intermediate hydrophilicity results in an intermediate degree of compensation. This interpretation is corroborated by a large study of the complexation thermodynamics of charged CDs with ionic guests.[49] No compensation was observed for complexes with significant electrostatic (coulombic) interactions between host and guest. Only the complexes with a neutral guest exhibited EEC, but with a slope of 0.5. According the interpretation above, this low degree of compensation is due to dehydration of the hydrophilic zwitterionic headgroup of the employed neutral guest.

The results presented in the present study add to the growing body of evidence that liberation of water molecules from the hydration layers of molecular surfaces is a major source of EEC.[50–52] Much of the evidence comes from protein-ligand interactions, but cyclodextrin complexes are in many ways better model systems due to their smaller and less complex structure which allows for a more detailed and precise structural and thermodynamical characterization.

Conclusion

The small AD guest molecules were anticipated to be completely included in the cavity of βCD and should therefore not interact with the sidechains at the rims of modified βCDs. However, the results suggest that the sidechains disturb the hydration of the polar moieties of the guest molecules that protrude from the CD cavity. Thus, the studied systems were not as ideal as anticipated and could not provide an unambiguous confirmation of the hypothesis that dehydration of guest and sidechains is the cause of previously observed examples of EEC. Nevertheless, the study resulted in several important findings. As for other CD complexes, the presence of substituents at the CD rims resulted in increased enthalpies and entropies of complexation as well as more negative values of ΔC_p, presumably due to the release of hydration waters from the guest and the substituents. Unlike other complexes, where the increase in enthalpy was almost completely compensated by increased entropy, a lower degree of enthalpy-entropy compensation was observed. Importantly, the degree of compensation seems to be related to the polarity of the dehydrated surface, with a low degree of compensation for hydrophilic surfaces and almost complete compensation for hydrophobic surfaces.

Acknowledgements

CS is grateful for a grant (DFF – 5054-00173) from the Danish Council for Independent Research.

Supporting Information. 1D and 2D NMR spectra. Water accessible surface areas. All thermodynamic data. Fitted enthalpograms.

References

(1) Connors, K. A. The Stability of Cyclodextrin Complexes in Solution. *Chem. Rev.* **1997**, *97*, 1325–1358.

(2) Rekharsky, M. V.; Inoue, Y. Complexation Thermodynamics of Cyclodextrins. *Chem. Rev.* **1998**, *98*, 1875–1918.

(3) Jencks, W. P. *Catalysis in Chemistry and Enzymology*; McGraw-Hill Book Company: New York, 1969, pp 425-427.

(4) VanEtten, R. L.; Sebastian, J. F.; Clowes, G. A.; Bender, M. L. Acceleration of Phenyl Ester Cleavage by Cycloamyloses. A Model for Enzymic Specificity. *J. Am. Chem. Soc.* **1967**, *89*, 3242–3253.

(5) Biedermann, F.; Nau, W. M.; Schneider, H. J. The Hydrophobic Effect Revisited - Studies with Supramolecular Complexes Imply High-Energy Water as a Noncovalent Driving Force. *Angew. Chemie - Int. Ed.* **2014**, *53*, 11158–11171.

(6) Assaf, K. I.; Nau, W. M. The Chaotropic Effect as an Assembly Motif in Chemistry. *Angew. Chemie Int. Ed.* **2018**, *57*, 13968–13981.

(7) Grunwald, E.; Steel, C. Solvent Reorganization and Thermodynamic Enthalpy-Entropy Compensation. *J. Am. Chem. Soc.* **1995**, *117*, 5687–5692.

(8) Breiten, B.; Lockett, M. R.; Sherman, W.; Fujita, S.; Al-Sayah, M.; Lange, H.; Bowers, C. M.; Heroux, A.; Krilov, G.; Whitesides, G. M. Water Networks Contribute to Enthalpy/Entropy Compensation in Protein-Ligand Binding. *J. Am. Chem. Soc.* **2013**, *135*, 15579–15584.

(9) Lumry, R.; Rajender, S. Enthalpy-Entropy Compensation Phenomena in Water Solutions of Proteins and Small Molecules : A Ubiquitous Property of Water. *Biopolymers* **1970**, *9*, 1125–1227.

(10) Chodera, J. D.; Mobley, D. L. Entropy-Enthalpy Compensation: Role and Ramifications in Biomolecular Ligand Recognition and Design. *Annu. Rev. Biophys.* **2013**, *42*, 121–142.

(11) Cooper, A.; Johnson, C. M.; Lakey, J. H.; Nöllmann, M. Heat Does Not Come in Different Colours: Entropy-Enthalpy Compensation, Free Energy Windows, Quantum Confinement, Pressure Perturbation Calorimetry, Solvation and the Multiple Causes of Heat Capacity Effects in Biomolecular Interactions. *Biophys. Chem.* **2001**, *93*, 215–230.

(12) Inoue, Y.; Hakushi, T.; Liu, Y.; Tong, L.; Shen, B.; Jin, D. Thermodynamics of Molecular Recognition by Cyclodextrins. 1. Calorimetric Titration of Inclusion Complexation of Naphthalenesulfonates with α-, β-, and γ-Cyclodextrins: Enthalpy-Entropy Compensation. *J. Am. Chem. Soc.* **1993**, *115*, 475–481.

(13) Schönbeck, C.; Westh, P.; Madsen, J. C.; Larsen, K. L.; Städe, L. W.; Holm, R. Hydroxypropyl-Substituted β-Cyclodextrins: Influence of Degree of Substitution on the Thermodynamics of Complexation with Tauroconjugated and Glycoconjugated Bile Salts. *Langmuir* **2010**, *26*, 17949–17957.

(14) Schönbeck, C.; Westh, P.; Madsen, J. C.; Larsen, K. L.; Städe, L. W.; Holm, R. Methylated β-Cyclodextrins: Influence of Degree and Pattern of Substitution on the Thermodynamics of Complexation with Tauro- and Glyco-Conjugated Bile Salts. *Langmuir* **2011**, *27*, 5832–5841.

(15) Holm, R.; Østergaard, J.; Schönbeck, C.; Jensen, H.; Shi, W.; Peters, G. H.; Westh, P. Determination of Stability Constants of Tauro- and Glyco-Conjugated Bile Salts with the Negatively Charged Sulfobutylether-β-Cyclodextrin: Comparison of Affinity Capillary Electrophoresis and Isothermal Titration Calorimetry and Thermodynamic Analysis Of . *J. Incl. Phenom. Macrocycl. Chem.* **2014**, *78*, 185–194.

(16) Køhler, J.; Schönbeck, C.; Westh, P.; Holm, R. Hydration Differences Explain the Large Variations in the Complexation Thermodynamics of Modified γ-Cyclodextrins with Bile Salts. *J. Phys. Chem. B* **2016**, *120*, 396–405.

(17) Schönbeck, C.; Holm, R.; Westh, P.; Peters, G. H. Extending the Hydrophobic Cavity of β-Cyclodextrin Results in More Negative Heat Capacity Changes but Reduced Binding Affinities. *J. Incl. Phenom. Macrocycl. Chem.* **2014**, *78*, 351–361.

(18) Schönbeck, C.; Westh, P.; Holm, R. Complexation Thermodynamics of Modified Cyclodextrins: Extended Cavities and Distorted Structures. *J. Phys. Chem. B* **2014**, *118*, 10120–10129.

(19) Holm, R.; Schönbeck, C.; Somprasirt, P.; Westh, P.; Mu, H. A Study of Salt Effects on the Complexation between β-Cyclodextrins and Bile Salts Based on the Hofmeister Series. *J. Incl. Phenom. Macrocycl. Chem.* **2014**, *80*, 243–251.

(20) Tidemand, K. D.; Schönbeck, C.; Holm, R.; Westh, P.; Peters, G. H. Computational Investigation of Enthalpy–Entropy Compensation in Complexation of Glycoconjugated Bile Salts with β-Cyclodextrin and Analogs. *J. Phys. Chem. B* **2014**, *118*, 10889–10897.

(21) Enright, G. D.; Udachin, K. a.; Ripmeester, J. a. Complex Packing Motifs Templated by a Pseudo-Spherical Guest: The Structure of β-Cyclodextrin–Adamantane Inclusion Compounds. *CrystEngComm* **2010**, *12*, 1450.

(22) Rüdiger, V.; Eliseev, A.; Simova, S.; Schneider, H.-J.; Blandamer, M. J.; Cullis, P. M.; Meyer, A. J. Conformational, Calorimetric and NMR Spectroscopic Studies on Inclusion Complexes of Cyclodextrins with Substituted Phenyl and Adamantane Derivatives. *J. Chem. Soc., Perkin Trans. 2* **1996**, *1*, 2119–2123.

(23) Schönbeck, C. Charge Determines Guest Orientation: A Combined NMR and Molecular Dynamics Study of β-Cyclodextrins and Adamantane Derivatives. *J. Phys. Chem. B* **2018**, *122*, 4821–4827.

(24) Guvench, O.; Greene, S. N.; Kamath, G.; Brady, J. W.; Venable, R. M.; Pastor, R. W.; Mackerell, A. D. Additive Empirical Force Field for Hexopyranose Monosaccharides. *J. Comput. Chem.* **2008**, *29*, 2543–2564.

(25) Guvench, O.; Hatcher, E.; Venable, R. M.; Pastor, R. W.; MacKerell, A. D. CHARMM Additive All-Atom Force Field for Glycosidic Linkages between Hexopyranoses. *J. Chem. Theory Comput.* **2009**, *5*, 2353–2370.

(26) Vanommeslaeghe, K.; MacKerell, A. D. Automation of the CHARMM General Force Field (CGenFF) I: Bond Perception and Atom Typing. *J. Chem. Inf. Model.* **2012**, *52*, 3144–3154.

(27) Vanommeslaeghe, K.; Raman, E. P.; MacKerell, A. D. Automation of the CHARMM General Force Field (CGenFF) II: Assignment of Bonded Parameters and Partial Atomic Charges. *J. Chem. Inf. Model.* **2012**, *52*, 3155–3168.

(28) Vanommeslaeghe, K.; Hatcher, E.; Acharya, C.; Kundu, S.; Zhong, S.; Shim, J.; Darian, E.; Guvench, O.; Lopes, P.; Vorobyov, I.; et al. CHARMM General Force Field: A Force Field for Drug-like Molecules Compatible with the CHARMM All-Atom Additive Biological Force Fields. *J. Comput. Chem.* **2010**, *31*, 671–690.

(29) Phillips, J. C.; Braun, R.; Wang, W.; Gumbart, J.; Tajkhorshid, E.; Villa, E.; Chipot, C.; Skeel, R. D.; Kalé, L.; Schulten, K. Scalable Molecular Dynamics with NAMD. *J. Comput. Chem.* **2005**, *26*, 1781–1802.

(30) Humphrey, W.; Dalke, A.; Schulten, K. VMD: Visual Molecular Dynamics. *J. Mol. Graph.* **1996**, *14*, 33–38.

(31) Pitha, J.; Trinadha Rao, C.; Lindberg, B.; Seffers, P. Distribution of Substituents in 2-Hydroxypropyl Ethers of Cyclomaltoheptaose. *Carbohydr. Res.* **1990**, *200*, 429–435.

(32) Trinadha Rao, C.; Pitha, J.; Lindberg, B.; Lindberg, J. Distribution of Substituents in O-(2-Hydroxypropyl) Derivatives of Cyclomalto-Oligosaccharides (Cyclodextrins): Influence of Increasing Substitution, of the Base Used in the Preparation, and of Macrocyclic Size. *Carbohydr. Res.* **1992**, *223*, 99–107.

(33) Myers, J. K.; Pace, C. N.; Scholtz, J. M. Denaturant m Values and Heat Capacity Changes: Relation to Changes in Accessible Surface Areas of Protein Unfolding. *Protein Sci.* **1995**, *4*, 2139-2148.

(34) Schönbeck, C.; Holm, R.; Westh, P. Higher Order Inclusion Complexes and Secondary Interactions Studied by Global Analysis of Calorimetric Titrations. *Anal. Chem.* **2012**, *84*, 2305–2312.

(35) Schönbeck, C.; Madsen, T. L.; Peters, G. H.; Holm, R.; Loftsson, T. Soluble 1:1 Complexes and Insoluble 3:2 Complexes – Understanding the Phase-Solubility Diagram of Hydrocortisone and γ-Cyclodextrin. *Int. J. Pharm.* **2017**, *531*, 504–511.

(36) Schönbeck, C.; Gaardahl, K.; Houston, B. Drug Solubilization by Mixtures of Cyclodextrins: Additive and Synergistic Effects. *Mol. Pharm.* **2019**, *16*, 648–654.

(37) Ross, P. D.; Rekharsky, M. V. Thermodynamics of Hydrogen Bond and Hydrophobic Interactions in Cyclodextrin Complexes. *Biophys. J.* **1996**, *71*, 2144–2154.

(38) Wenz, G. Influence of Intramolecular Hydrogen Bonds on the Binding Potential of Methylated β-Cyclodextrin Derivatives. *Beilstein J. Org. Chem.* **2012**, *8*, 1890–1895.

(39) Bertaut, E.; Landy, D. Improving ITC Studies of Cyclodextrin Inclusion Compounds by Global Analysis of Conventional and Non-Conventional Experiments. *Beilstein J. Org. Chem.* **2014**, *10*, 2630–2641.

(40) Cameron, D. L.; Jakus, J.; Pauleta, S. R.; Pettigrew, G. W.; Cooper, A. Pressure Perturbation Calorimetry and the Thermodynamics of Noncovalent Interactions in Water: Comparison of Protein-Protein, Protein-Ligand, and Cyclodextrin-Adamantane Complexes. *J. Phys. Chem. B* **2010**, *114*, 16228–16235.

(41) Carrazana, J.; Jover, A.; Meijide, F.; Soto, V. H.; Vázquez Tato, J.; Tato, J. V. Complexation of Adamantyl Compounds by β-Cyclodextrin and Monoaminoderivatives. *J. Phys. Chem. B* **2005**, *109*, 9719–9726.

(42) Perry, C. S.; Charman, S. A.; Prankerd, R. J.; Chiu, F. C. K.; Scanlon, M. J.; Chalmers, D.; Charman, W. N. The Binding Interaction of Synthetic Ozonide Antimalarials with Natural and Modified β-Cyclodextrins. *J. Pharm. Sci.* **2006**, *95*, 146–158.

(43) Leong, N. J.; Prankerd, R. J.; Shackleford, D. M.; Mcintosh, M. P. The Effect of Intravenous Sulfobutylether7-β-Cyclodextrin on the Pharmacokinetics of a Series of Adamantane-Containing Compounds. *J. Pharm. Sci.* **2015**, *104*, 1492–1498.

(44) Zia, V.; Stella, V. J. Thermodynamics of Binding of Neutral Molecules to Sulfobutyl Ether β-Cyclodextrins (SBE-β-CDs): The Effect of Total Degree of Substitution. *Pharm. Res.* **2000**, *17*, 936–941.

(45) Zielenkiewicz, W.; Terekhova, I. V.; Wszelaka-Rylik, M.; Kumeev, R. S. Thermodynamics of Inclusion Complex Formation of Hydroxypropylated α- And β-Cyclodextrins with Aminobenzoic Acids in Water. *J. Therm. Anal. Calorim.* **2010**, *101*, 15–23.

(46) Cabani, S.; Gianni, P.; Mollica, V.; Lepori, L. Group Contributions to the Thermodynamic Properties of Non-Ionic Organic Solutes in Dilute Aqueous Solution. *J. Solution Chem.* **1981**, *10*, 563–595.

(47) Vargas, C.; Schönbeck, C.; Heimann, I.; Keller, S. Extracavity Effect in Cyclodextrin/Surfactant Complexation. *Langmuir* **2018**, *34*, 5781–5787.

(48) Schönbeck, C. *Structure and Thermodynamics of Inclusion Complexes between Cyclodextrins and Bile Salts in Aqueous Solution*, Roskilde University: Roskilde, 2012, p 71.

(49) Wenz, G.; Strassnig, C.; Thiele, C.; Engelke, A.; Morgenstern, B.; Hegetschweiler, K. Recognition of Ionic Guests by Ionic β-Cyclodextrin Derivatives. *Chem. - A Eur. J.* **2008**, *14*, 7202–7211.

(50) Dragan, A. I.; Read, C. M.; Crane-Robinson, C. Enthalpy–Entropy Compensation: The Role of Solvation. *Eur. Biophys. J.* **2017**, *46*, 301–308.

(51) Fox, J. M.; Zhao, M.; Fink, M. J.; Kang, K.; Whitesides, G. M. The Molecular Origin of Enthalpy/Entropy Compensation in Biomolecular Recognition. *Annu. Rev. Biophys.* **2018**, *47*, 223–250.

(52) Portman, K. L.; Long, J.; Carr, S.; Briand, L.; Winzor, D. J.; Searle, M. S.; Scott, D. J. Enthalpy/Entropy Compensation Effects from Cavity Desolvation Underpin Broad Ligand Binding Selectivity for Rat Odorant Binding Protein 3. *Biochemistry* **2014**, *53*, 2371–2379.