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Thermodynamics of Binding of α-Cyclodextrin to Straight-Chain Alkyl Derivatives in Aqueous Solution

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Abstract

Apparent standard Gibbs energy, enthalpy, entropy, and heat capacity data of the interactions of α-cyclodextrin (αCD) to some *n*-carboxylates $H(CH_2)_nCOO^-$ ($n = 4$–6), are determined by isothermal titration microcalorimetry at different temperatures in phosphate buffer, pH 9.0, assuming a 1 : 1 model in dilute solution. Modelling of contributions of the thermodynamic properties of the solution indicates that αCD undergoes conformational change upon binding to homologous series of *n*-carboxylates, *n*-alcohols, α, ω-alkane dicarboxylates and α, ω-alkane diols.

Abbreviations: αCD = α-cyclodextrin, Å = Ångstrom (10^{-10} m)

Introduction

Cyclodextrins comprise a family of macrocyclic carbohydrates built up from D(+)-glucopyranose units linked together via α-(1–4) bonds. They are the products of degradation of starch and related compounds by the action of an amylase of *Bacillus macerans*. The cyclodextrins most studied are α-, β-, and γ-cyclodextrins, which consist of six, seven, and eight glucopyranose units, respectively. All of the hydroxyl groups are located on the exterior of the cyclodextrin molecule and the interior is considered as a largely apolar binding site. Cyclodextrin complexes, involving many different types of guest molecules, have found applications in a variety of fields, e.g., food technology, pharmaceuticals, cosmetics, catalysis, chiral separation, extraction of pollutants and polymer functionalization [1–3].

The formation of inclusion complexes of alkyl chains and α-cyclodextrin (αCD) has been strongly supported by NMR-measurements, recently reported by Watanabe *et al.* [4]. Cyclodextrins have also been looked upon as model compounds for studying processes in which non-polar compounds are transferred from aqueous solution to a non-polar environment, e.g., in protein-ligand binding studies. In our earlier investigations on binding of cyclodextrins to non-polar or partially polar compounds, i.e., *n*-alcohols [5], α, ω-alkanediols [6], α, ω-alkanedicarboxylates [7], benzene [8], and 1,4-bicyclo[2.2.2]octane diol [9], large and negative heat capacity changes were observed. This is typical for processes in which non-polar solutes are transferred from aqueous solution to organic solvents.

In this article, we report isothermal microcalorimetric titration data on the interactions of αCD to the *n*-carboxylates ranging from pentanoate to heptanoate. Changes in solvent accessible surface areas and entropy contributions were estimated by employment of an empirical model initially reported by Murphy and Freire [10]. In the calculations our thermodynamic data on 1 : 1 complex formation to αCD for the homologous series of *n*-alcohols [5], α, ω-alkanediols [6], *n*-carboxylates, and α, ω-alkanedicarboxylates [7] have been included.

Experimental

αCD of 98% purity grade was purchased from Sigma. The sample was further purified according to French *et al.* [11]. In the calorimetric binding experiments no significant difference was observed between the purified and the non-purified sample. In the measurements reported here, the purified αCD batch was used. The compound was equilibrated for several days over a saturated $Ca(NO_3)_2$ solution providing for 51% relative humidity [12]. From Karl–Fischer titration experiments (Metrohm Coulometer) the number of water molecules per αCD was determined to be 6.50 ± 0.18. The molar mass of αCD was therefore taken as 1090 g mol^{-1}. Heptanoic acid (99%) was purchased from Aldrich. The sodium salts of pentanoic acid (approx. 99%), hexanoic acid (99–100%), and octanoic acid (99–100%) were obtained from Sigma, the purities as stated by the manufacturer. These samples were used as received. The solutions for titration experiments were prepared in a 0.1 mol l^{-1} glycine

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buffer at pH 9.5, using boiled reagent grade water produced by a Milli-Q filtration system (Millipore). Sodium ion was in all cases employed as the counter ion. At this pH the deprotonated fraction of the carboxylate is essentially unity. Earlier calorimetric studies have shown that neither glycine [7] nor sodium chloride[13] bind to αCD in aqueous solution.

Isothermal titration experiments were carried out in a stainless-steel microcalorimetric titration vessel of 1 ml, kept in a four-channel microcalorimetric system [14] (Thermometric AB, Järfälla, Sweden). At each titration series, 12–15 consecutive aliquots of αCD solution were injected to a carboxylate solution at 4–5 min intervals. The power response was dynamically deconvoluted [15, 16] by use of the time constant of the instrument. Electrical calibrations were performed using an insertion heater immersed in the vessel. Further, dissolution of propan-1-ol [17] was also used for calibration and the two methods agreed within 0.5%. As a test process, the titration of 18-crown-6 with $BaCl_2$ was employed. The enthalpy change, ΔH°, and the equilibrium constant, K_c, agreed, within limits of error with results earlier reported by Briggner and Wadsö ($K_c = 5900 \pm 200$ mol l^{-1} and $\Delta H^\circ = -31.42 \pm 0.20$ kJ mol^{-1}) [17].

The titrations were carried out at three different concentration ranges: (i) aliquots of 31 μl 52 mmol l^{-1} αCD were injected to 0.7 ml 5 mmol l^{-1} heptanoate or hexanoate. (ii) portions of 30 μl of 0.1 mol l^{-1} αCD were injected to 0.4 ml 40 mmol l^{-1} pentanoate. (iii) injection steps of 31 μl 0.1 mol l^{-1} α-CD were added to 0.5 ml 30 mmol l^{-1} heptanoate. To correct for heat of dilution of the compound injected, separate dilution experiments were performed.

If we assume that we have a host-molecule, A, in the calorimetric vessel and add the ligand, B, the distribution of free and bound host-molecule, AB, in the calorimetric vessel changes as the titration proceeds. The total concentration, C_A and C_B, of the reacting species, A and B, can be described by mass law action:

$$C_A = [A] + [AB] \tag{1}$$

$$C_B = [B] + [AB]. \tag{2}$$

The equilibrium constant is given by

$$K_c = \frac{[AB]}{[A][B]}. \tag{3}$$

The concentration of the complex, AB, is calculated from the degree of reaction, α, which is the molar ratio of the complex containing A can be defined as

$$\alpha = [AB]/C_A. \tag{4}$$

Substituting (1)–(2) and (4) into (3) we obtain

$$K_c = \frac{\alpha}{(1-\alpha)(C_B - C_A\alpha)}. \tag{5}$$

We have now expressed the equilibrium constant in terms of total concentration of the reactants. For simplifying the expression we define the molar ratio between A and B, $r = C_B/C_A$, and the inverse of the product between the equilibrium constant and the stoichiometric concentration of A, $v = 1/K_cC_A$. We can solve α from

$$\alpha = \frac{r + v + 1 - \sqrt{(r+v+1)^2 - 4r}}{2}. \tag{6}$$

The procedure to calculate equilibrium constants and enthalpy of binding is based upon fitting differential measured heats, q_i, to

$$q_i = \Delta H^\circ([AB]_i V_i - [AB]_{i-1} V_{i-1}), \tag{7}$$

where V is the total volume in the calorimetric vessel and i is the number of injection.

The calorimetric data were tested for three different stoichiometric models, 1 : 1, and step-wise formation of 1 : 2, and 2 : 1, referring to the ratio αCD : carboxylate. The activity coefficient of αCD was assumed to be unity in all cases, whilst the Debye–Hückel limiting law approximated the activities of the carboxylates and the 1 : 2 complexes. Apparent equilibrium constants and apparent enthalpies of complex formation were calculated by non-linear regression analysis according to Marquardt [18], including an algorithm to eliminate linear parameters [19]. For each carboxylate and at each temperature, three to five titration series were carried out and simultaneously used in the regression analysis. From the variance-covariance matrix of the regressions, uncertainties in the fitting parameters were obtained. The uncertainties in ΔS° were estimated by propagation of error calculations, treating ΔG° and ΔH° as independent properties, being aware of that they are statistically correlated. The uncertainties in ΔC_p° were estimated graphically [20].

Results

Fitting experimental data to a 1 : 1 stoichiometric model resulted in random noise residuals within the range expected for the instrumentation used in this study. Other stoichiometric models resulted in non-random residuals and residuals much higher than what would be expected with the used instrumentation. The results reported herein, summarised in Table 1, are thus results from calculations of experimental data fitted to a 1 : 1 stoichiometric model. An example of fitting curve with data points is shown in Figure 1. The enthalpy and entropy of the 1 : 1 binding reactions of the n-carboxylates to αCD show compensating temperature dependencies leading to weak temperature dependences of the standard Gibbs energy changes, Figure 2. This behaviour is typical for processes involving apolar compounds in aqueous solution. The standard Gibbs energy change is linearly dependent of the alkyl chain length at the three temperatures examined. The Gibbs energy increment per methylene group is -2.83 ± 0.07 kJ mol^{-1}. This result is in agreement with our earlier results involving the interactions between αCD and n-alcohols [5], α,ω-diols [6] and α,ω-dicarboxylates [7]. Taking all these data into account gives an increment

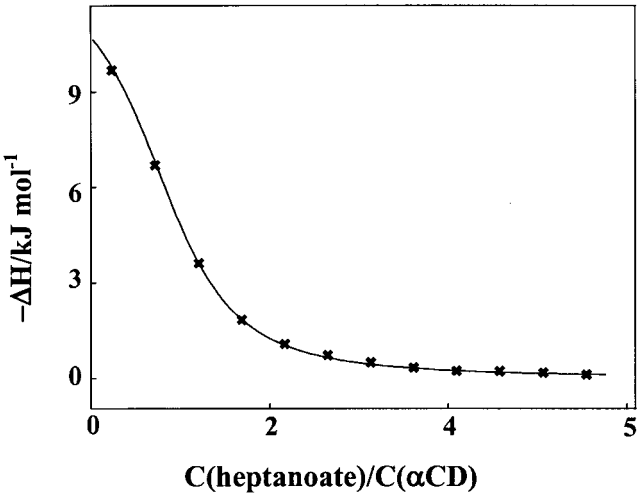

Figure 1. Microcalorimetric titration data for the titration of αCD with heptanoate at 288.15 K and pH 9.5. The plot shows the amount of heat measured per mole of heptanoate injected versus accumulated molar ratio of heptanoate added per αCD in the calorimetric vessel. Solid curve is the best fit for a 1 : 1 binding model. Best fit parameters were $K = 1069 \pm 14$*l* mol^{-1}, $\Delta H^\circ = -12.83 \pm 0.04$ kJ mol^{-1}.

per methylene group equal to -2.8 ± 0.1 kJ mol^{-1}; the uncertainty is twice the standard deviation of the mean. This value is rather close to the value for the transfer of a methylene group from water to a liquid organic phase, -3.4 to -3.7 kJ mol^{-1} [21–24].

The heat capacity values are the same, within experimental error, for pentanoate and hexanoate, while for heptanoate the heat capacity change is 130 J mol^{-1} K^{-1} more negative, cf. Table 1 and Figure 3. This is in contrast to other systems, like *n*-alcohols and diols, where the heat capacity changes are linearly dependent on the number of methylene groups in the straight alkyl chain. We have earlier reported the increments per methylene group to be -102 and -90 J K^{-1} mol^{-1}, for *n*-alcohols and diols, respectively [5, 6].

For octanoate as well as for elevated concentrations of pentanoate to heptanoate, as exemplified by the range (iii) of the αCD/heptanoate system, cf. Experimental, it was not possible to rationalise the experimental data to any simple stoichiometric model. When applying simple stoichiometric models consisting of a 1 : 1 complex and step-wise formation of a 1 : 2 or 2 : 1 complex, the residuals obtained from the regressions were, for all these cases, non-random and significantly larger than what would be expected from the properties of the instrument. Similar behaviour has been observed for the binding of αCD to some α, ω-dicarboxylates [7]. The concentration-dependent phenomena observed in the present systems do not allow an interpretation of the calorimetric data at higher concentrations, as normally preferred to minimise the statistical correlation between the fitting parameters [25].

Discussion

We suggest four tentative explanations for the discrepancy from a 1 : 1 model, observed at high concentrations: (i)

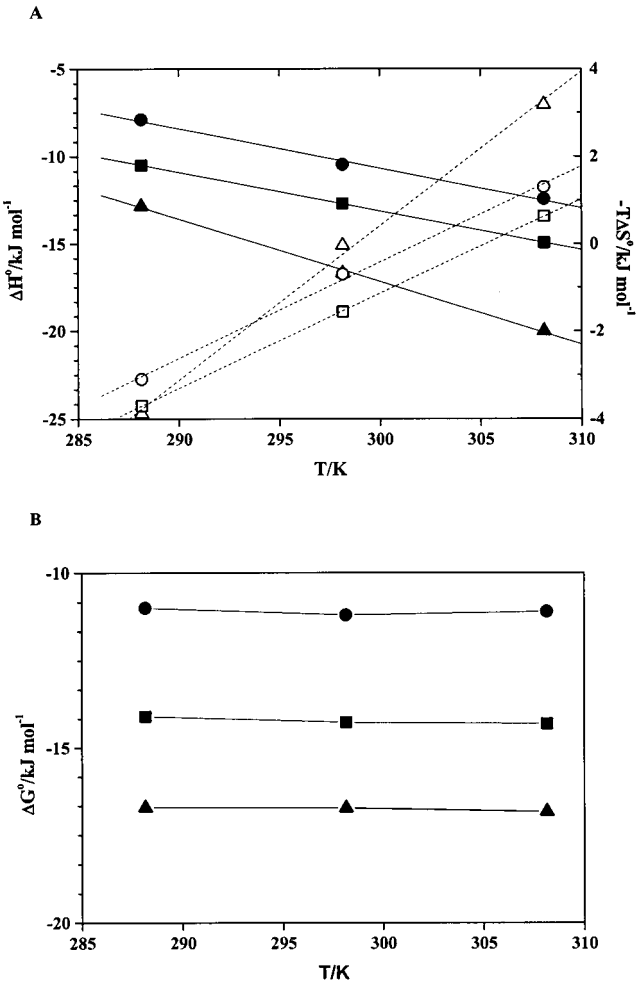

Figure 2. The graphs show a summary of the thermodynamic properties for the formation of 1 : 1 complexes between αCD and some *n*-carboxylates, $H(CH_2)_nCOO^-$, plotted against temperature, T. In the plots are $n = 4$ circle, $n = 5$ square and $n = 6$ triangle. (A) The enthalpy, ΔH°, filled symbols, and the entropy contribution to the Gibbs free energy, $-T\Delta S^\circ$, open symbols, are plotted against temperature, T. (B) Gibbs free energy, ΔG°, is plotted against temperature, T.

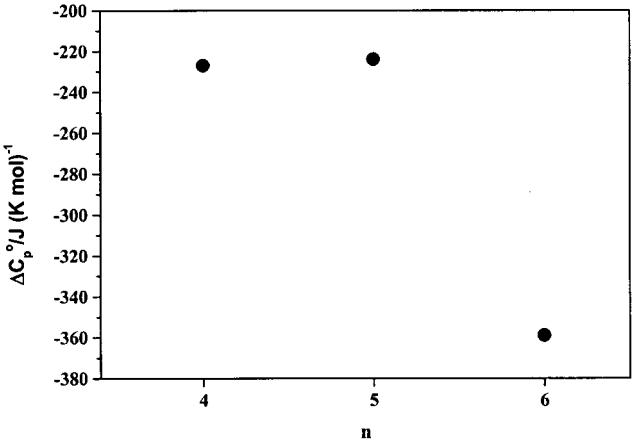

Figure 3. A plot showing ΔC_p° for the 1 : 1 binding between αCD and some *n*-carboxylates, $H(CH_2)_nCOO^-$, against the number of methylene groups, *n*.

Table 1. Apparent thermodynamic properties for 1 : binding of the carboxylates $H(CH_2)_nCOO^-$ to α-cyclodextrin in dilute aqueous solution.

$n(CH_2)$	T K	K_c l mol^{-1}	ΔH° kJ mol^{-1}	ΔG°a kJ mol^{-1}	$T\Delta S^\circ$ kJ mol^{-1}	ΔS° J(mol K)$^{-1}$	ΔC_p^0 J(mol K)$^{-1}$
4	288.15	100 ± 12	-7.9 ± 0.2	-11.0 ± 0.2	3.1 ± 0.4	10.4 ± 1	
	298.15	91 ± 5	-10.47 ± 0.03	-11.2 ± 0.1	0.7 ± 0.1	2.4 ± 0.4	-227 ± 30
	308.15	76 ± 5	-12.43 ± 0.03	-11.1 ± 0.1	-1.3 ± 0.2	-4.3 ± 0.6	
5	288.15	370 ± 14	-10.5 ± 0.1	-14.1 ± 0.1	3.7 ± 0.1	12.8 ± 0.5	
	298.15	316 ± 3	-12.71 ± 0.04	-14.27 ± 0.02	1.56 ± 0.04	5.2 ± 0.2	-224 ± 20
	308.15	267 ± 4	-14.94 ± 0.06	-14.31 ± 0.04	-0.63 ± 0.07	-2.0 ± 0.2	
6	288.15	$1069 \pm$	-12.83 ± 0.04	-16.71 ± 0.03	3.88 ± 0.05	13.5 ± 0.2	
	298.15	854 ± 5	-16.69 ± 0.03	-16.73 ± 0.02	0.04 ± 0.04	0.1 ± 0.1	-359 ± 6
	308.15	714 ± 10	-20.01 ± 0.08	-16.83 ± 0.04	-3.18 ± 0.08	-10.3 ± 0.2	

[a] $\Delta G^\circ = -RT \ln(K_c/\text{l mol}^{-1})$.

we have in the data treatment assumed that the activity coefficients of the "free" carboxylate and the carboxylate-αCD complex cancel out. Because activity coefficients are concentration-dependent and diverge from unity for increasing concentration, the assumption (i) may hold true more accurately at lower concentration regimes. (ii) Higher-order interactions involving carboxylate and αCD may exist, or 1 : 1, 1 : 2 and 2 : 1 complexes may all participate in the solution. (iii) Non-specific interactions between the complex or complexes and the free species may contribute to the heat measured, that may best be described by virial coefficients of non-ideality. (iv) The complex formed is in each consecutive titration step diluted and may have a large heat of dilution. The differences between these explanations may be a matter of definition. However, neither of these possibilities can in any meaningful sense be included in any of the models used. Taking more than two interactions into account in the fitting procedure would lead to parameters suffering from large statistical correlation. The heat of dilution of the complex (iv) cannot be measured by titrating the complex saturated with the ligand. The concentrations needed for such experiments exceed by far the solubility of αCD.

The non-linear ΔC_p° dependence on the number of methylene groups of the n-carboxylates, suggests that there may exist different binding modes for the different n-carboxylates, cf. Figure 2. Gelb and Schwartz [26] have reported results from potentiometric measurements on αCD and n-carboxylates. For butanoate, pentanoate, hexanoate and the corresponding acids, they used a 1 : 1 model, including acid-base equilibria of the carboxylic acids. For octanoate and decanoate, they added a second complex to the model, with two cyclodextrin molecules and one carboxylate or carboxylic acid. However, this model was reported as inadequate for decanoate. They suggested that higher order complexation may exist in this system, in agreement with one of our possible explanations to deviation from a 1 : 1 binding stoichiometry, for lower carboxylates at high concentrations. Their enthalpy data of complex formation were derived from the van't Hoff equation, without taking the heat capacity changes into account. Enthalpies and, in particular, heat capacities, derived from the van't Hoff equation in general suffer from large errors [27–30].

Other groups have earlier reported microcalorimetric binding studies [31–34] on the interactions between n-carboxylates and αCD. These studies were performed at a range of different pH conditions and different temperatures, leading to difficulties in comparing the results. Castronuovo *et al.* [31] have reported the interactions of αCD and n-carboxylates at 298.15 K. They applied a 1 : 1 model for butanoate to heptanoate, and for octanoate to decanoate they used a model, which assumes two forms of the free carboxylate. However, we have difficulties in visualising the physical relevance of the latter model. The measurements were performed at pH 11.3, which is close to the reported $pK_a \approx 12$ for the secondary hydroxyl groups on the αCD exterior [3]. At this pH a fraction of 0.2 of the secondary hydroxyl groups is deprotonated. Their experimental conditions are significantly different compared to what our group and other groups have used. Liu and Sturtevant [32] have reported calorimetric binding data at different temperatures for heptanoate in non-buffered solution at pH 8. Rekharsky *et al.* [33] and Ross and Rekharsky [34] have reported calorimetric binding data at different temperatures for hexanoate and heptanoate in sodium phosphate buffer at pH 6.9. The results for heptanoate, reported by these groups, are in close agreement to our results. The results for hexanoate reported by Rekharsky *et al.* [33] and by Ross and Rekharsky [34] differ significantly from our results. The differences are largest in enthalpies and heat capacity values; we report herein $\Delta H^\circ = -12.71$ kJ mol^{-1} and $\Delta C_p^\circ = -224$ J K^{-1} mol^{-1} at 298.15 K while Rekharsky *et al.* have reported $\Delta H^\circ = -14.50$ kJ mol^{-1} and $\Delta C_p^\circ = -310$ J K^{-1} mol^{-1} at 298.15 K. Whether or not these differences are due to different solution conditions, e.g., pH, is not clear.

We have earlier reported heat capacity increments per methylene group for the binding reactions to n-alcohols, -102 K^{-1} mol^{-1} [5] and diols, -90 J K^{-1} mol^{-1} [6]. The cited increments may be compared to the transfer of a methylene group from aqueous solution to an organic solvent, -50 to -60 J K^{-1} mol^{-1} [35–38]. We have earlier

suggested that conformational change of αCD may be involved in the excess heat capacity decrease of binding [5–7]. Change in the conformation of the glucose units of αCD, leading to changes in the hydration state, could contribute to the total heat capacity of binding observed. Based on circular dichroism spectroscopic data, Rees has earlier suggested that the conformation of αCD changes when alkanoic acids are included [39]. Crystal structures of complexes of αCD with methanol, n-propanol, iodine, polyiodide, krypton and *p*-iodoaniline have been reported by Saenger *et al*. [40], and a crystal structure of αCD including acetate has been determined by Hybl *et al.* [41]. The αCD structure was in all these solid-state complexes close to purely cyclic. An αCD structure including two water molecules in the cavity, hydrogen bonded to each other and to two hydroxyl groups of αCD was also determined [40]. In this case, one glucose unit was reported to be rotated out of the cone-shaped body described by the other five. Further, Puliti *et al.* have crystallised a more cyclic αCD from mother solutions containing salt or organic guest molecules but not from pure water [42]. Four ordered water molecules were placed outside of the αCD and disordered solvent, mainly in the oligosaccharide cavities, was also reported. A cyclic αCD has been proposed as a transition state of the binding process [42–44].

In an attempt to further rationalize our assumptions, we have applied the same sort of model treatment of the thermodynamic data for binding of alkane derivatives to αCD as has earlier been done for protein–protein interactions and protein unfolding. The origin of this model is based upon experimental data for the transfer of small organic compounds from their pure liquid state to aqueous solution and has later been further refined by use of the thermodynamic data of protein unfolding and binding processes. Protein molecules are large and contain many types of functional groups. For a protein–protein interaction there are many types of molecular interactions involved, both intramolecular and intermolecular, compared with systems like a small molecule–cyclodextrin interaction. Where there are subtle differences in interaction modes for protein–protein interactions, they are by necessity averaged as polar and apolar interactions. The present systems contain a smaller distribution of functional groups and smaller contact areas, and thus, few specific interactions compared with protein–protein interactions on which the model is based. For these reasons, the calculation is rather an attempt to apply a model, developed for protein thermodynamics, to small molecular systems, and to rationalise experimental data. In the following discussion we have in the modelling used data reported herein for the complex formation between αCD to *n*-carboxylates, as well as earlier reported data for the binding of αCD to *n*-alcohols [5], α, ω-alkane diols [6] and α, ω-alkane dicarboxylates [7].

There are two dominating models for structural parameterisation of solution thermodynamic properties of protein-protein interactions reported, one is given by Spolar *et al.* [45] and the other by Freire *et al*. [10, 46–48]. Only subtle formalism differences distinguish these models from each other. In these models, heat capacity changes and enthalpy changes are linear combinations of the changes in non-polar and polar solvent-accessible surface areas of the molecules binding, $\Delta\mathrm{ASA}_{n-\mathrm{pol}}$ and $\Delta\mathrm{ASA}_{\mathrm{pol}}$, respectively. We have in the present calculation chosen the model reported by Freire *et al*. Contributions from other interactions, among them specific ionic interactions and protonation reactions, are accounted by a separate term, $\Delta C_{p,\mathrm{other}}$. The heat capacity change is then parameterised as:

$$\Delta C_p = \Delta C_{p,n-\mathrm{pol}} + \Delta C_{p,\mathrm{pol}} + \Delta C_{p,\mathrm{other}} \tag{8}$$

$$\Delta C_p = a\Delta\mathrm{ASA}_{n-\mathrm{pol}} + b\Delta\mathrm{ASA}_{\mathrm{pol}} + \Delta C_{p,\mathrm{other}}, \tag{9}$$

where $a = 1.89 \pm 0.08$ J K^{-1} Å^{-2} mol^{-1} and $b = -1.09 \pm 0.13$ J K^{-1} Å^{-2} mol^{-1} [46–48].

The enthalpy change is written as:

$$\Delta H = \Delta H^* + (\Delta C_{p,n-\mathrm{pol}} + \Delta C_{p,\mathrm{pol}})(T - T_H^*) + \Delta H_{\mathrm{other}}, \tag{10}$$

where T_H^* is a system-dependent reference temperature and $\Delta H_{\mathrm{other}}$ is an enthalpy contribution analogous to $\Delta C_{p,\mathrm{other}}$. ΔH^* is proportional to the decrease in polar solvent-accessible surface area [46–48]:

$$\Delta H^* = c\Delta\mathrm{ASA}_{\mathrm{pol}}; \quad c = 147 \pm 12 \text{ J Å}^{-2} \text{ mol}^{-1}. \tag{11}$$

In the modelling on the data for the binding reactions of αCD to *n*-carboxylates, *n*-alcohols [5], α, ω-alkane diols [6] and α, ω-alkane dicarboxylates [7] we have attempted to estimate the changes in $\Delta\mathrm{ASA}_{n-\mathrm{pol}}$ and $\Delta\mathrm{ASA}_{\mathrm{pol}}$. The terms $\Delta C_{p,\mathrm{other}}$ and $\Delta H_{\mathrm{other}}$ were in the present calculations assumed to be zero. Further, the convergence temperature, T_H^*, for the binding of αCD to *n*-alcohols, α, ω-alkane diols and *n*- carboxylates was found to be 257 K, Figure 4. The data of the three compounds of dicarboxylates did not fit to any convergence temperature. However, because for the sake of tendencies we applied the value T_H^* = 257 K also for the dicarboxylate α-CD interactions.

The results of the calculations, using Equations (8)–(11) are shown in Table 2. The monofunctional *n*-alcohols and *n*-carboxylates have values for $\Delta\mathrm{ASA}_{n-\mathrm{pol}}$ which are parallel shifted toward lower alkyl chains by 0.5 to 1.2 methylene groups, compared to the difunctional diols and dicarboxylates. The end methyl group in the alkyl chain of the monofunctional compounds could explain the larger non-polar surface area obtained. The $\Delta\mathrm{ASA}_{\mathrm{pol}}$ values are small and essentially constant within each guest substance class. For the diols and the *n*-alcohols, the values of $\Delta\mathrm{ASA}_{n-\mathrm{pol}}$ and of ΔC_p° show linear chain length dependencies up to six or seven methylene groups. The slopes are of the same order, -45 Å^2 $(\mathrm{CH_2})^{-1}$ and -52 Å^2 $(\mathrm{CH_2})^{-1}$, respectively. These numbers are significantly larger than the methylene group surface area, -33 Å^2 $(\mathrm{CH_2})^{-1}$, as calculated by Hermann [49]. This would suggest that these $\Delta\mathrm{ASA}_{n-\mathrm{pol}}$ data involve the contribution of change in hydration of the cyclodextrin molecule.

Table 2. Results from model calculations on calorimetric data for the binding of αCD to alkane derivatives: changes in non-polar and polar solvent accessible surface areas, ΔASA_{n-pol} and ΔASA_{pol}, respectively, and changes in entropy contributions from hydration/dehydration, ΔS_{hydr} and conformational change, ΔS_{conf}.

Guest	n	ΔASA_{n-pol}/Å^2	ΔASA_{pol}/Å^2	ΔS_{hydr}/J K^{-1} mol^{-1}	ΔS_{conf}/J K^{-1} mol^{-1}
$H(CH_2)_nCO_2^-$[a]	4	−125	−8	58	−16
	5	−133	−25	57	−13
	6	−198	−13	92	−52
$O_2C(CH_2)_nCO_2^-$[b]	6	−163	−26	71	−42
	7	−190	−38	81	−53
	8	−204	−52	84	−35
$H(CH_2)_nOH$[c]	3	−116	20	61	−19
	4	−171	23	89	−49
	5	−218	18	111	−76
	6	−280	29	144	−111
	7	−312	13	155	−133
$HO(CH_2)_nOH$[d]	4	−103	−31	41	−24
	5	−157	−47	63	−55
	6	−193	−26	86	−67
	7	−204	−54	82	−70
	8	−218	−51	91	−67
	9	−228	−51	96	−64
	10	−252	−51	108	−78

The calorimetric data were obtained from [a]the present work, [b]Ref. [7], [c]Ref. [5] and [d]Ref. [6].

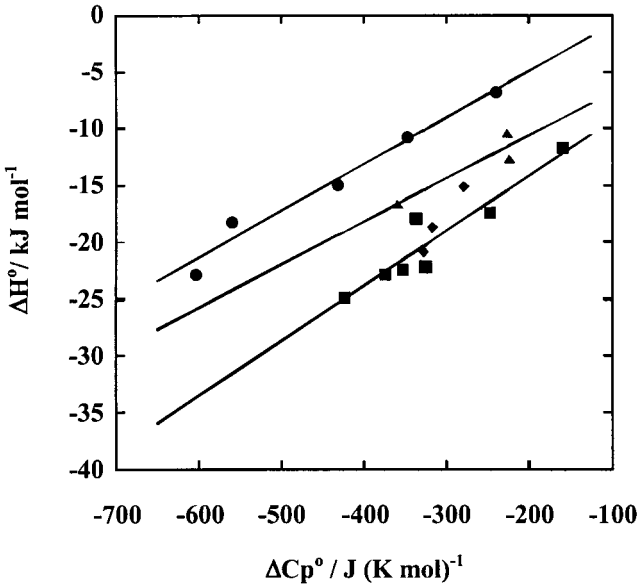

Figure 4. ΔH° *vs.* ΔC_p° for the binding of αCD to (●) n-alcohols, (▲) n-carboxylates, (■) α,ω-alkane diols and (♦) α,ω-alkane dicarboxylates at 298.15 K. The mean slope of the three least-squares fits is taken as the temperature difference $\Delta T = T_H^* - 298.15$ K. The mean value for the convergence temperature, T_H^*, among the three system classes involving n-alcohols, n-carboxylates and diols, is 257 K.

At above six methylene groups, ΔASA_{n-pol} for the diols levels off. This trend could reflect on the cavity depth of αCD, as earlier suggested on the basis of the ΔC_p° data [6]. Unfortunately, the limited range of the data would not allow an evaluation of a similar trend, if any, in the other systems of Table 2.

The entropy change of binding can be expressed as a sum of different contributions

$$\Delta S = \Delta S_{hyd} + \Delta S_{r/t} + \Delta S_{np} + \Delta S_{conf} + \Delta S_{ion}. \quad (12)$$

The contributions to the entropy change of the binding process are: (i) change in hydration; ΔS_{hyd}, (ii) change in rotational and translational degrees of freedom; $\Delta S_{r/t}$, (iii) change in number of particles in the system, due to complexation; ΔS_{np}, (iv) change in conformational degrees of freedom; ΔS_{conf}, and (v) ionisation, ΔS_{ion}.

$$\Delta S_{hyd} = \Delta C_p \ln(T/T_R), \quad (13)$$

where $T_R = 385.15$ K is the convergence temperature of transferring apolar surfaces into water from a non-aqueous environment. This value for the convergence temperature is common to processes involving transfer of apolar groups from solid, liquid and gas phases as well as from the protein interior into water using mole fraction or molar concentration units for the model compound data [10, 50, 51]. The $\Delta S_{r/t}$ for a 1 : 1 complex has been estimated to be -33.5 J K^{-1} mol^{-1} [46, 52]. The third contribution listed, ΔS_{np}, is equal to $R\ln(1/2) = -5.8$ J K^{-1} mol^{-1} for a 1 : 1 complex, where R is the gaseous constant and in brackets is the ratio between the number of particles after and prior to complex formation. The values for ΔS_{ion} are here set to zero, since no ion-ion interactions are expected. The contribution to the total entropy change due to changes in the degrees of conformational freedom can then be calculated from

$$\Delta S_{cont} = \Delta S^\circ - (\Delta S_{hyd} + \Delta S_{r/t} + \Delta S_{np}). \quad (14)$$

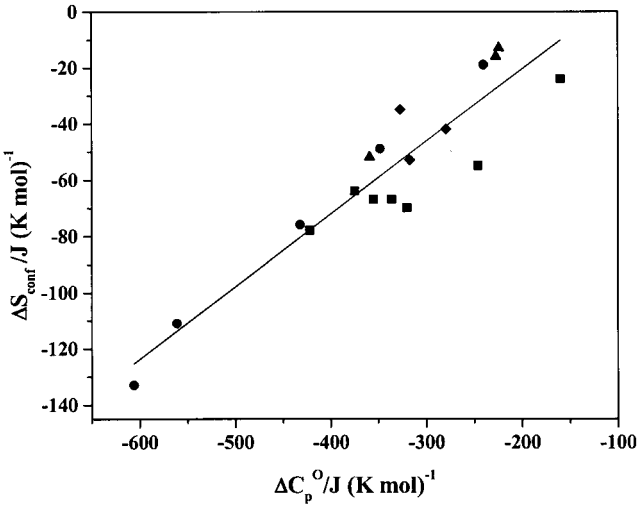

Figure 5. Entropy contribution from conformational change, ΔS_{conf}, *vs.* ΔC_p° for the binding of αCD to (●) *n*-alcohols, (▲) *n*-carboxylates, (♦) α, ω-alkane diols and (♦) α, ω-alkane dicarboxylates.

The entropy contributions obtained from this model are given in Table 2. A plot of ΔS_{conf} versus ΔC_p° is shown in Figure 5. Taking all data from the different alkane series into account and assuming a linear correlation between ΔS_{conf} and ΔC_p°, ΔS_{conf} is zero for ΔC_p° equal to -120 J K^{-1} mol^{-1}. This value could be compared to the heat capacity group additivity values of transfer from aqueous solution to non-polar solvent for a methylene group, -50 to -60 J K^{-1} mol^{-1}, and a methyl group, -114 J K^{-1} mol^{-1} [35]. One interpretation of the value of ΔC_p° at zero contribution of ΔS_{conf} would be that conformational change is induced by binding to a molecule of the size of a methyl group, or larger.

As mentioned above, the value for the methylene group heat capacity increment of binding an alkane analogue to αCD is more negative than compared to the transfer of a methylene group from aqueous solution into an organic solution. If we assume a mean methylene group increment value of binding of an alkane analogue to αCD, $\Delta C_p^\circ = -100$ J/(K mol CH$_2$)$^{-1}$, this heat capacity increment corresponds to a difference in ΔS_{conf} of 25 J/(K mol)$^{-1}$. A free hydrocarbon chain can exist in three different conformations per C—C bond. A C—C bond without any restrictions has a conformational entropy of $R \ln 3 = 9.1$ J K^{-1} mol^{-1}, where R is the gas constant ($R = 8.314$ J mol^{-1} K^{-1}). If we assume a restriction to solely one conformer of the alkyl chain when bound to αCD, it would result in a maximum contribution to $\Delta S_{\text{conf}} = -9.1$ J K^{-1} (mol C—C bond)$^{-1}$. This means that, according to the assumptions given by the model, there are entropy contributions stemming from decrease in degrees of freedom that can be accounted to the αCD molecule.

The model calculations indicate restrictions in the number of conformers of host-molecule and ligand molecule upon binding. As we have stressed previously, the model calculations performed have been an attempt to rationalise the experimental thermodynamic data. Whether the results from the calculations and the following interpretations reflect the binding phenomena of alkane analogues to αCD or not can only be proven by other experimental data. Dynamic data and more structural data would be beneficial for a better understanding of the molecular events occurring upon binding.

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