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Thermodynamics of the interaction of α-cyclodextrin with monocarboxylic acids in aqueous solutions: a calorimetric study at 25 °C

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Abstract

The interaction in water of cyclomaltohexaose (α-cyclodextrin) with monocarboxylic acids from C_3 to C_{12} has been studied calorimetrically at 25 °C in phosphate buffer, pH 11.3. When a complex forms, calorimetry enables the calculation of both the enthalpy and the association constant, from which the free energy and the entropy of the process can be obtained. For these acids, 1:1 inclusion complexes form, characterized by values of the association constants which do not follow a regular trend with increasing length of the alkyl chain. A model is proposed to explain this unusual behaviour. The association occurs through the insertion of the guest's alkyl chain into the host's cavity. However, for longer alkyl chains two forms of the acid are possible, each one associated with a different constant and enthalpy. The forces involved in the association process are discussed in the light of the analysis of the signs and values of the thermodynamic parameters. © 1996 Elsevier Science Ltd.

Keywords: α-Cyclodextrin; Calorimetry; Inclusion; Monocarboxylic acids

1. Introduction

The most important property of cyclomalto-oligosaccharides (cyclodextrins) is their ability to form complexes with a great variety of organic substances either in solution or in the solid state [1–3]. However, notwithstanding the great number of papers on these complexes, much remains to be clarified about the forces involved in these interactions [4,5].

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The smallest of these cyclodextrins, cyclomaltohexaose (αCD), is especially interesting. In the solid state it has two water molecules entrapped in the cavity, hydrogen-bonded to each other and to two glucopyranose rings [6]. These two water molecules diffuse to the medium when an inclusion complex forms. At the same time αCD undergoes a conformational transition from a "tense" to a "relaxed" conformation [7]. There are few hypotheses concerning the forces involved in these inclusion processes, and many problems regarding the mechanism and the changes experienced by the water in the hydration shells of the "guest" and "host" molecules are still unsolved.

In preceding papers we have reported on the binary aqueous solutions of αCD [8] and on its interaction with hydroxylated substances [8,9], alkylureas [10], amino acids [11–13], and other small molecules [14,15]. Our present contribution continues the programme aimed at understanding the forces involved in the interaction of the cyclodextrins with some monocarboxylic acids in aqueous solution. A calorimetric study, at 25 °C, is now reported on the interaction of αCD with the following acids: propanoic, butanoic, pentanoic, hexanoic, heptanoic, octanoic, nonanoic, decanoic, undecanoic, and dodecanoic. These systems have been studied in phosphate buffer, at pH 11.3. The aim of this study is to analyze the role of the functional group in the inclusion process, and to verify whether a stoichiometry different from 1:1 holds for longer chain acids. As well as the detection of the thermal effect, calorimetry shows whether an association process occurs and allows the evaluation of its equilibrium constant. From that, the free energy and entropy of the process can be derived.

2. Experimental

Materials.—α-Cyclodextrin and the acids employed were purchased from Sigma. The optical rotation of αCD was in agreement with those reported in the literature. Solutions were prepared by weight using doubly distilled water.

For solutions at pH 11.3, a 0.5 m NaH_2PO_4–NaOH buffer was employed. The choice of this buffer is determined by the need to avoid anions interfering with the inclusion process. It is reported [16] that phosphate and sulfate anions satisfy this requirement in the pH range 2–11. The concentration of carboxylic acids varies between 2.1×10^{-3} and 3.6×10^{-3} m for acids up to decanoic, while it is 7.8×10^{-4} m for undecanoic and 2.6×10^{-4} m for dodecanoic acid.

Calorimetry.—The values of the experimental heats of mixing, ΔH^{mix}, of two binary solutions containing any one of the solutes were determined at 25 °C by means of a Thermal Activity Monitor (TAM) from Thermometric. Experimental details are extensively reported in the earlier papers [8–11,17–21].

Treatment of the data.—The enthalpy of formation of a complex, ΔH^*, is related to the heat of mixing of two binary solutions, ΔH^{mix}, and to the heats of dilution experienced by the two solutes, ΔH^{dil}, as follows [22]:

$$\Delta H^* = \Delta H^{mix}\{[(m_{ix})(m_{iy})] \rightarrow (m_x, m_y)\} - \Delta H^{dil}(m_{ix} \rightarrow m_x) - \Delta H^{dil}(m_{iy} \rightarrow m_y) \quad (1)$$

where m_{ix}, m_{iy}, m_x, and m_y are the initial and final molalities of the x and y solutes. The determination of the ΔH^* value then requires a knowledge of the heats of dilution of the binary solutions. These are known from the following relation:

$$\Delta H^{dil}(m_i \rightarrow m) = h_{xx} m(m - m_i) + h_{xxx} m(m^2 - m_i^2) + \ldots \quad (2)$$

where ΔH^{dil} (J kg^{-1}) is the heat of dilution of a solute from the initial (m_i) to the final molality (m). The h coefficients appearing in Eq (2) are determined by the experimental heats of dilution of binary solutions. To fit the data, a least-squares method was employed choosing the polynomial of highest degree, whose coefficients still exceed their own 95% confidence limits.

In order to interpret the calorimetric data, four association schemes will be considered. The first scheme refers to the formation of 1:1 αCD–acid complexes in the presence of only one form of the acid. The second refers to the formation of 2:1 αCD–acid complexes. The last two schemes refer to the formation of 1:1 complexes, with the acid molecule being present in two forms.

The observable enthalpy is given by

$$\Delta H = -R\,\delta \ln P/\delta(1/T) \quad (3)$$

and the degree of association is

$$\bar{x} = RT\delta \ln P/\delta\mu_x = \delta \ln P/\delta \ln[x] \quad (4)$$

where [x] is the concentration of free cyclodextrin, while P is the association polynomial, namely the partition function of the system referred to free acid [23]. This function is the sum of the concentrations of all species of the acid referred to the concentration of the free acid:

$$P = \sum_{k=0}^{n} [M_k]/[M_o] \quad (5)$$

P depends on the hypothesis about the association mechanism and is the key function for simulations of the enthalpy to verify the model from the experimental data. The most simple hypothesis is to consider that the association occurs through a 1:1 stoichiometry. Denoting the two associating substances as M (the acid) and X (α-cyclodextrin), the association reaction is:

$$M + X \rightarrow MX$$

In this case the association polynomial is:

$$P = ([M] + [MX])/[M] = 1 + K[x] \quad (6)$$

where K is the association constant: it depends on temperature, pressure, and chemical potential as follows [24]:

$$K = K_o e^{-(\Delta H_a/R)(1/T - 1/T_o)} e^{-(\Delta V/RT)(P - P_o)} e^{(\Delta n_x^o/RT)(\mu_x - \mu_{x_o})} \quad (7)$$

where K_o is the association constant at T_o, P_o, and μ_o, ΔH_a is the association enthalpy per mole of acid, ΔV is the volume variation, while Δn_x^o is the variation of the number of cyclodextrin molecules bound per acid molecule in the reference state and in the actual state. For a process having a 1:1 stoichiometry $\Delta n_x^o = 1$.

From the preceding equations we obtain:

$$\Delta H = K[x]\Delta H_a/(1 + K[x]) \quad (8)$$

$$\bar{x} = K[x]/(1 + K[x]) \quad (9)$$

$$\Delta H = \bar{x}\,\Delta H_a \quad (10)$$

Since

$$[x] = X_{tot} - \bar{x}M_{tot} \quad (11)$$

we obtain $\bar{x}$ as a function of X_{tot} and M_{tot}:

$$\bar{x}(M_{tot},X_{tot}) = \left\{(KX_{tot} + KM_{tot} + 1) - \sqrt{\left[(KX_{tot} + KM_{tot} + 1)^2 - 4K^2M_{tot}X_{tot}\right]}\right\}/2KM_{tot} \quad (12)$$

From that it is possible to obtain ΔH as a function of the X_{tot}/M_{tot} ratio. This function must be fitted to the experimental data employing as parameters K and ΔH_a. This analysis holds well for the acids up to heptanoic, and for undecanoic and dodecanoic acids, but it fails for octanoic, nonanoic, and decanoic acids.

In the second scheme, we have considered that, for the acids from C_8 to C_{12}, two association sites, independent but not equivalent, are present on the guest molecule (acid). In this case, the partition function is given by:

$$P = 1 + K_1[x] + K_2[x] + K_3K_1[x]^2 \quad (13)$$

and

$$\Delta H = 1/P\left\{(\Delta H_1K + \Delta H_2K_2)[x] + (\Delta H_1 + \Delta H_3)K_1K_3[x]^2\right\} \quad (14)$$

The quantities K_1, K_2, K_3, ΔH_1, ΔH_2, and ΔH_3 have been used to fit the experimental data. However, notwithstanding the large number of parameters, the fit is rather poor.

We have, then, supposed that the acid molecule can exist in two conformations, A_E and A_B (E stands for extended and B for bent), each one having an association site for αCD. In this case, the association polynomial, normalized with respect to the unligated species $[1 + L_o]$, is given by:

$$P = 1 + [(K_E + L_oK_B)/(1 + L_o)][x] \quad (15)$$

where L_o is the equilibrium constant A_B/A_E. Eq (15) is analogous to Eq (6), thus falling in the first scheme.

Finally, we considered that the acid can exist in two conformations, A_E and A_B: however, these two forms are in a frozen equilibrium [23]. Namely, they remain in their original proportions in the absence of cyclodextrin. In this case:

$$P = (1 + K_E[x])^{a^o_E} \cdot (1 + K_B[x])^{a^o_B} \quad (16)$$

and $a^o_E + a^o_B = 1$. Here a^o_E and a^o_B are the fractions of guest molecule present in the E and B forms. In this case:

$$\bar{x} = a^o_E K_E[x]/(1 + K_E[x]) + a^o_B K_B[x]/(1 + K_B[x]) \quad (17)$$

and

$$\Delta H = a_E^o K_E[x]\Delta H_E/(1 + K_E[x]) + a_B^o K_B[x]\Delta H_B/(1 + K_B[x]) \quad (18)$$

Using as parameters a_E^o, K_E, ΔH_E, K_B, and ΔH_B, we obtain ΔH as a function of the ratio X_{tot}/M_{tot}, the function to fit to the experimental data. This model has led to a very good fit of the data relative to octanoic, nonanoic, and decanoic acids. The fit was accomplished using the non-linear Levendberg–Marquardt method [25], constraining the a_E^o parameter to vary in the 0–1 range. The errors on the single-fitting parameters were calculated with a 95.4% confidence limit by the Monte Carlo simulation method.

The nature of the cooperativity for these systems has been explored considering the homotropic derivative [23]:

$$d\bar{x}/d\ln[x] = a_E^o K_E[x]/(1 + K_E[x])^2 + (1 - a_E^o) K_B[x]/(1 + K_B[x])^2 \quad (19)$$

The left-hand term represents the slope of the $\bar{x}$ vs. ln[x] curve. It depends on the association parameters, and it is less than the slope relative to the association in the presence of only one species ($a_E^o = 1$ or $a_E^o = 0$). Then, cooperativity is negative, except at saturation, where it approaches neutral cooperativity.

3. Results

In Table 1 the association constant and enthalpy are shown for the inclusion process involving αCD and the monocarboxylic acids in phosphate buffer, pH 11.3. These values have been obtained using the hypothesis that association occurs with a simple 1:1 stoichiometry, only one form of the acids being present. While the enthalpies increase with increasing length of the alkyl chain, the association constants increase at first, then decrease, beginning with C_9, and increase again at C_{11}. It is to be noted that the lack of

Table 1
Thermodynamic parameters for the association between αCD and monocarboxylic acids at 25 °C, pH 11.3, derived according to a simple 1:1 model

Acid	$-\Delta H_a$ [a]	K' [b]
Propanoic	N.A. [c]	N.A.
Butanoic	0.7 ± 0.1	86 ± 14
Pentanoic	4.5 ± 0.1	145 ± 7
Hexanoic	6.1 ± 0.1	511 ± 54
Heptanoic	12.2 ± 0.1	1234 ± 68
Octanoic	18.6 ± 0.1	1353 ± 111
Nonanoic	38.4 ± 0.8	253 ± 20
Decanoic	50.7 ± 0.3	253 ± 6
Undecanoic	58.9 ± 0.2	845 ± 17
Dodecanoic	98 ± 3	1066 ± 186

[a] kJ mol^{-1}; errors are the standard deviations.
[b] kg mol^{-1}.
[c] N.A. means that measurements have been performed, but no association was detected.

Table 2
Thermodynamic parameters for the association of αCD with monocarboxylic acids at 25 °C, pH 11.3, evaluated according to the "frozen equilibrium" model [a]

Acid	a°	$-\Delta H_a$ [b]	K' [c]	$-\Delta G^{o\prime}$ [b]	$T\Delta S^{o\prime}$ [b]
Butanoic	$a_E^o = 1$	0.7±0.1	86±14	11.0	10.3
Pentanoic	$a_E^o = 1$	4.5±0.1	144±7	12.3	7.8
Hexanoic	$a_E^o = 1$	6.1±0.1	513±54	15.5	9.4
Heptanoic	$a_E^o = 1$	12.2±0.1	1234±68	17.6	5.4
Octanoic	$a_E^o = 0.82 \pm 0.02$	20.0±0.5	2225±134	19.1	−0.5
	$a_B^o = 0.18 \pm 0.02$	44±3	27±2	8.2	−36.1
Nonanoic	$a_E^o = 0.39 \pm 0.02$	43±2	2390±150	19.3	−23.7
	$a_B^o = 0.61 \pm 0.02$	49±2	60±3	10.1	−39
Decanoic	$a_E^o = 0.21 \pm 0.02$	47±3	940±60	17.0	−30.3
	$a_B^o = 0.79 \pm 0.02$	52±2	200±7	13.1	−39.1
Undecanoic	$a_B^o = 1$	58.9±0.2	845±17	16.7	−42.2
Dodecanoic	$a_B^o = 1$	98±3	1066±186	17.3	−80.7

[a] For octanoic, nonanoic, and decanoic acids, the values of the thermodynamic parameters on the first line refer to the E form, those on the second line to the B form. Errors are the 68% confidence limits.
[b] kJ mol^{-1}.
[c] kg mol^{-1}.

information about activity coefficients leads to the evaluation of apparent association parameters.

In Table 2 the same thermodynamic parameters, together with the a_B^o, a_E^o, free energy, and entropy values, are reported for the same acids. They are evaluated according to the model postulating that the guest molecule is present in two forms in a

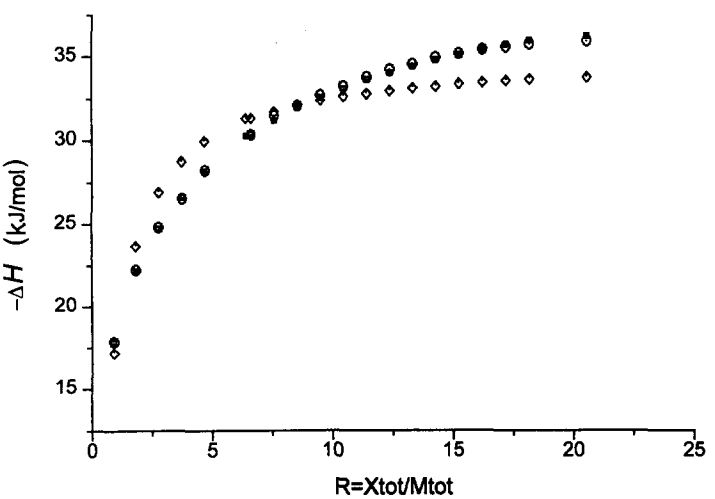

Fig. 1. Enthalpies of association, ΔH, as a function of the ratio X_{tot}/M_{tot}, for nonanoic acid at 25 °C: experimental points (⊙); the calculated points were obtained by employing a simple 1:1 association model (◇) and the frozen equilibrium model (□).

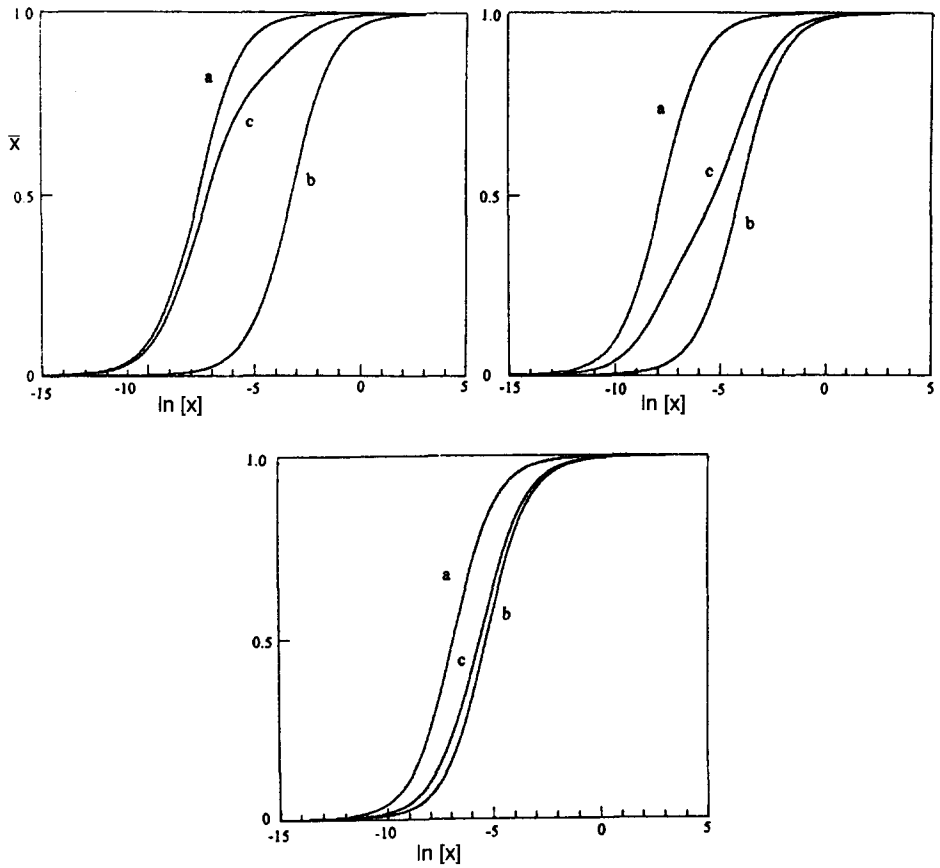

Fig. 2. Association curves, $\bar{x}$ vs. ln[x], of frozen mixtures of E and B molecules with fixed fractions a_E° and a_B° for octanoic, nonanoic, and decanoic (from left to right) acids interacting with αCD, pH 11.3, at 25 °C. The extreme curves (a and b) show association to the E and B forms, while the intermediate association curve (c) is obtained on the basis of the fractional amounts a_E° and a_B°. The overall curve (c) has a slope less than that of the curves relative to the association in the presence of only one species.

frozen equilibrium. Each form has only one site available for the association with αCD. As can be observed, up to heptanoic acid only one form is present. Beginning with C_8, there is the presence of another form, which becomes the only one for undecanoic and dodecanoic acids. This model agrees with the experimental data for octanoic, nonanoic, and decanoic acids better than any other model.

In Fig. 1, ΔH is reported as a function of the ratio X_{tot}/M_{tot} for nonanoic acid. The calculated points have been obtained employing a simple 1:1 association model at first, and then considering that the acid exists in two forms in a frozen equilibrium. The good agreement obtained, by using the latter model, between experimental and calculated values is apparent.

In Fig. 2 the association curves, $\bar{x}$ vs. ln[x], are shown for octanoic, nonanoic, and decanoic acids. In the same figure the curves relating to the pure E and B forms are also shown, imposing $a_E^o = 1$ or $a_E^o = 0$. In all cases, the slope of the overall curve obtained through the fitting parameters is consistently less than that relative to the E and B curves. That indicates a negative cooperativity, namely a competition of one form with respect to the other toward the association with αCD. Moreover, a shift from the E form for octanoic acid to the B form for decanoic acid is apparent.

4. Discussion

From the earlier studies carried out in this laboratory on mono- [8] and poly-hydroxylated [9] substances and α-amino acids [11–13] reacting with αCD, it was inferred that it is the alkyl chain of the guest molecule that penetrates the cavity of the cyclodextrin. The functional group forms hydrogen bonds with the external hydroxyl groups of the macrocycle. This is supported by data on substances bearing a hydroxyl group in more central positions, which prevents the remainder of the alkyl chain from penetrating the cavity [9]. Moreover, from these studies it was also inferred that increasingly stronger complexes are obtained when the alkyl chain becomes longer.

The present data, concerning the interaction of αCD with monocarboxylic acids at pH 11.3, show a very unusual trend when treated as a simple 1:1 model and postulating that only one form of the acids is present. The association constants increase up to octanoic acid, then suddenly decrease, and increase again with undecanoic and dodecanoic acids. From this it can be inferred that the hypothesis of a simple 1:1 association holds only for the acids having an alkyl chain length up to about 6–7 CH_2 groups. For longer chains it is necessary to propose a more complex model in order to rationalize the experimental data.

The first such hypothesis proposes a two host:one guest association. The alkyl chain and the carboxyl group of the longer guest molecules could be inserted into the cavities of two different αCD molecules. However, that could hold only for processes at low pH, where the acids are in undissociated form. In fact, it has been observed [26] that the solvation requirements of the charged carboxylate group by water prevents the aliphatic chain from fully penetrating the cavity, although deeper penetration may be possible with the neutral carboxylic acid group. The alkyl chain can penetrate deeper into the cavity when the carboxylic acid group is neutral, while this functional group may also form hydrogen bonds with the hydroxyl groups on the rim of the CD cavity. We conclude that insertion of the charged carboxylate group of the ligand into the cavity is probably energetically prohibitive. In any case, the agreement with the present experimental data using the parameters calculated according to this model was not satisfactory.

In earlier studies concerning the formation of complexes between alkan-1-ols and αCD [8,9], it was observed that the curve ΔG° vs. the total number of CH_2 groups shows discontinuity around 6, thus indicating that the αCD cavity is saturated by that number of methylene groups. From other studies [27] of the αCD–alkanols interaction it was shown, on the basis of CPK models, that for unbranched chains longer than about

six carbons some changes in conformation of both the alkanol and αCD must occur to provide a fit. As reported in the Experimental section, the model which better describes the experimental data when the alkyl chain is longer than seven carbons is the one postulating that the acids are a mixture of two forms in a frozen equilibrium [23], independent of the concentration of αCD. Starting with octanoic acid, probably because of the flexibility of the alkyl chain, a "bent" form of the acid could exist together with the extended one: both would form 1:1 complexes with the cyclodextrin, penetrating the cavity through the extended and bent alkyl chain. The fraction of the bent molecules increases on passing from octanoic to decanoic acid. For a further lengthening of the alkyl chain (undecanoic and dodecanoic acids) only the bent form is present; for these last two substances, a simple 1:1 model again gives the best fit of the experimental data. The assumption that the longer chain acids exist in a frozen equilibrium between two forms may appear rather artificial, given the nature of the guest molecules. However, the trend of the data seems to indicate a transition from one form (extended) to another (bent); other conformers in solution cannot be excluded. The proposed model, then, must be considered as a first attempt to explain results that are unexplainable through other known models. Nevertheless, the very good consistency of the experimental data with this interpretation may be the basis of studies concerning the mechanistic implications of complexation and could suggest definitive methods for the investigation of the precise nature of these complexes. The question is whether the αCD cavity has dimensions that could accommodate a bent alkyl chain. It is reported that αCD forms complexes with a variety of cyclic compounds, benzoic acid [28] and L-phenylalanine [12,29] among them. A straightforward example is that of the αCD–adamantanecarboxylate complex [26]. It has been found that the bulky adamantyl group should fit into cyclomaltooctaose with room to spare, that it should fit very snugly into the cyclomaltoheptaose, and that it should be unable to fully penetrate into cyclomaltohexaose. Thus, with the last cyclodextrin it forms a weaker complex. To further verify this point, we studied the interaction of αCD with cyclopentanol, cyclohexanol, and cycloheptanol, that is, with substances that can simulate bent alkyl chains. Complexes were formed, but were characterized by small association constants [30]. However, an alkyl chain, free to bend, is much more flexible than the cycloalkanols examined, in that the ring formation leads to a more rigid structure. The greater flexibility of a bent alkyl chain would enable it to adapt better to the cavity. Moreover, the possibility that the cyclodextrin also undergoes a conformational transition favouring the formation of such complexes cannot be excluded.

A plot of ΔG° for both the E and B forms as a function of the total number of carbon atoms (nCtot) is shown in Fig. 3. The curve relative to the E form reaches a plateau and then decreases at nCtot = 10. This means that, beyond an alkyl chain of nine carbon atoms, the free energy tends toward less negative values. The formation of a complex where the alkyl chain is included into the cavity in its extended form becomes energetically unfavoured. The curve relative to the B form follows the same trend, and it seems to reach a plateau at nCtot = 12. It is likely that in this case also a decrease of the ΔG° values could occur for longer chains. The steric hindrance of the alkyl chain, bent in the interior of the dextrin, would give an unfavourable contribution to the formation of the complex.

The values of the enthalpies characterizing these complexes are spread over a wide

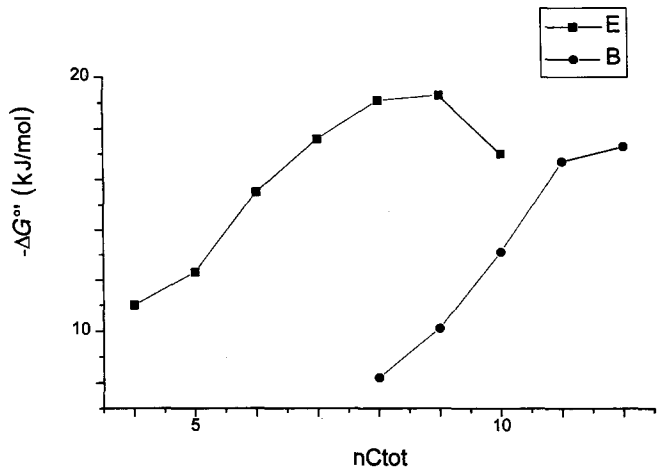

Fig. 3. Free energies as a function of the total number of carbon atoms (nCtot) of alkanoic acids interacting with αCD, pH 11.3, at 25 °C. Free energies were evaluated according to the model of frozen equilibrium between the E (extended) and B (bent) forms.

range: they vary regularly with the alkyl chain length. A discontinuity is noted passing from octanoic to nonanoic acid, with deviation from an almost linear trend. After that, the values increase steeply for every CH_2 group added, probably due to the presence of the bent form. ΔH_a values are negative, as for most of the complexes reported in the literature. They are the sum of several and contrasting effects determined primarily by the changes of water upon the inclusion process. For αCD, water molecules included in the cavity are displaced to the medium, whereas there are rearrangements in the external hydration shell. At the same time, the hydration shell of the hydrophobic part of the guest molecule loses some water molecules on entering the cavity. Both effects are endothermic. However, the reconstitution of the hydration shell of the complex, dipole-induced dipole and "host–guest" interactions, hydrogen bonds, and the decrease in energy when a hydrophobic residue fills the cavity make the value of the enthalpy negative. Thus, there are several effects involved in the formation of these complexes. Hydrophobic interactions do not always play the major role, as indicated by the positive or negative values of the entropies. In the present case, the values for entropies relative to the E form pass from positive to negative, and that could be due to the better fitting of the alkyl chain into the cavity with increasing length. The entropies relative to the B form are much more negative, probably because the adaptation of the bent alkyl chain to the cavity occurs with a large decrease of degrees of freedom of the alkyl chain. There is an enthalpy–entropy compensation, a phenomenon frequently observed in water, and ascribed to the modifications experienced by the solvent water in the hydration shells of the interacting substances [31–33]. This compensation leads to the same free energy for different compounds, even though the enthalpies and entropies are different. In the present case, the slope of the graph ΔH vs. ΔS, called the "compensation temperature",

gives 298 ± 11 K, a value consistent with the 250–320 K range characteristic of processes dominated by solvation phenomena. It can be concluded that changes in solvation of both the guest and host molecules play an important role in determining the stability of the inclusion complex.

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