ARTICLES

How Inorganic Anions Affect the Inclusion of Hexanoic and Decanoic Acid in *β*-Cyclodextrin

Sérgio Lima, Brian J. Goodfellow, and José J. C. Teixeira-Dias*

Department of Chemistry, University of Aveiro, CICECO, P3810-193 Aveiro, Portugal

Received: July 14, 2004

NMR chemical shift variations of β-cyclodextrin (βCD) H5 protons are used as probes in various experiments in which the initial concentrations of decanoic or hexanoic acids and/or of various sodium salts (NaCl, NaBr, $NaClO_4$, $NaNO_3$) are varied. The host−guest systems are considered to be in the NMR fast exchange chemical shift limit, and the slopes of the measured chemical shift variations as functions of the mole fractions of the free and complexed states, $\Delta\delta^\circ_i = (\partial\Delta\delta/\partial x_i)_{xj(j\neq i)}$, are determined in a systematic way using experiments delineated for that purpose. The evaluated $\Delta\delta^\circ$ coefficients provide quantitative interpretations of the shielding due to the inclusion of the amphiphilic guests in βCD and the deshielding resulting from the inclusion of hydrated anions in βCD. The influence of the amphiphilic guests on the inclusion of the hydrated anions in βCD and vice versa is also evaluated. By recording the chemical shift changes of the methyl protons of decanoic acid in a carefully delineated experiment, the $\Delta\delta^\circ$ coefficients for the aggregation and inclusion of decanoic acid were obtained. A shielding effect was observed for both of these processes although a relative deshielding effect was found for the inclusion in βCD relative to decanoic acid aggregation.

1. Introduction

The ability of β-cyclodextrin (cyclomalto-heptaose, βCD) to include guests of suitable size is derived mainly from the form of its cavity (a hollow truncated cone), which provides a large number of close contacts with the guest and thus increases the dispersion energy for the host−guest interaction (Figure 1).[1,2] The topology of the βCD macrocycle and the mode and extent of host−guest interactions can be effectively probed by ^{1}H NMR, in particular, by the chemical shift variations of the H3 and H5 protons situated inside the cavity.[3] When the guest displays amphiphilic behavior, its hydrophobic moiety has a tendency to enter the βCD cavity, leaving the hydrophilic head to interact mainly with the hydroxyl groups of the βCD rims and the solvent.[4] If, in addition, the guest exhibits surfactant behavior, then aggregates of varying dimensions may also be formed with sizes depending critically on the presence and concentration of electrolytes.[5] By varying the concentration of various alkali metal chlorides, the influence of different alkali metal cations on the βCD inclusion and aggregation processes of decanoic acid has been considered and interpreted.[6] In particular, the observed chemical shift variations of the βCD H5 and H3 protons were found to be consistent with the progressive self-association of decanoic acid and the simultaneous decrease in the amount of included decanoic acid, leading to the global deshielding of βCD H5 and H3 protons, as decanoic acid was replaced by water in the βCD cavity.[6]

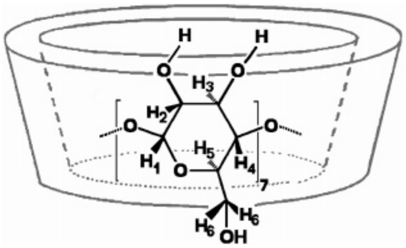

Figure 1. βCD macrocycle with a glucopyranose unit highlighted.

The present work considers the effects of increasing the concentration of various sodium salts (NaCl, NaBr, $NaClO_4$, $NaNO_3$) on the inclusion of hexanoic and decanoic acids in βCD and looks at the observed chemical shift changes of the βCD H5 protons. Although these chemical shift variations taken from the zero value of the electrolyte concentration were found to be positive and thus to correspond to deshielding, they should also include the shielding contribution of the amphiphilic guest inclusion in the βCD cavity. Thus, the main question raised in this paper concerns the identification and evaluation of the various shielding and deshielding contributions to the measured chemical shift changes when the concentration of various electrolytes is progressively increased. Although the identification and evaluation of the various terms contributing to the observed chemical shift changes is a prerequisite for quantitatively assessing the effects of anions, this is not a straightforward task because the βCD−guest system is in the NMR fast exchange chemical shift limit (i.e., the observed chemical shifts of the host and guest resonances are averages of the chemical shifts for the free and complexed states weighted by the mole fractions of each state[7]).

Salts are known to affect the solubilities of proteins in water by modulating hydrophobic interactions (Hofmeister effect).[8] However, the ranking of ions by the extent to which they

---

* Corresponding author. E-mail: tdias@dq.ua.pt. Fax: 351-234370084.

10.1021/jp046882w CCC: $27.50

modulate hydrophobicity (Hofmeister or lyotropic series) has been mainly dealt with on a qualitative basis. The main objective of the present paper is to address this problem on a quantitative basis for a typical βCD−guest system. Because it has been previously suggested that the water cluster in the βCD cavity parallels water clusters in protein crystal structures,[9] the βCD cavity may turn out to be a good model system for studying the effect of electrolytes in hydrophobic sites of proteins, thus contributing to a better understanding of the hydration in macromolecular systems with biological relevance.

2. Materials and Methods

β-Cyclodextrin (Fluka, ≥99%), hexanoic acid (Aldrich, 99%), decanoic acid (Fluka, 99%), NaCl (Panreac, ≥99.5%), NaBr (Aldrich, >99%), $NaClO_4$ (Sigma, 99.9%), $NaNO_3$ (RPE, 99.5%), and D_2O (Aldrich, 99.9%) were used as received without further purification. The ^{1}H NMR spectra were recorded on a Bruker DRX 300 spectrometer.[6] Solutions for NMR were prepared as previously described.[6] The NMR spectra were always recorded using freshly prepared unbuffered solutions. This precaution was taken to avoid any effect between the buffer anions and the βCD protons.[10] At the time of recording the spectra, a βCD(2.5 mM)/Dec(2.5 mM) mixture had a pH of 4.56. At this pH, the decanoate fraction amounts to 0.34 (the pK_a of decanoic acid is 4.84). The decanoate anions in the formed aggregates (cmc of decanoic acid = 0.141mM)[11] are likely to be stabilized by Na^+ counterions because these have the effect of reducing the repulsion between the carboxylate headgroups, thus lowering the cmc.[12] Hence, the extent of the aggregation increases, and the βCD inclusion complex subsequently dissociates to some extent, thus causing the replacement of decanoic molecules in the βCD cavity by water molecules and contributing to the deshielding of the H5 and H3 βCD protons.

3. Results and Discussion

In general, the addition of an amphiphile to an aqueous βCD solution leads to the shielding of H5 and H3, generally interpreted as an indication of the guest inclusion in βCD, because H5 and H3 are located inside the βCD cavity (Figure 1). When hexanoic acid and decanoic acid concentrations are increased in aqueous solutions of βCD (Figure 2), the inclusion of these guests is confirmed by the negative slopes of the H5 and H3 chemical shift changes, taken from the zero $\Delta\delta$ point corresponding to the absence of a guest. It can be seen from Figure 2 that an increase in the carboxylic acid concentration leads to the separation of the various βCD protons through their chemical shift variations. These distinct slopes follow the same relative order for both guests: from negative to positive $\Delta\delta$ values, the order is H5 > H3 > H6 > H4, with H4 exhibiting positive chemical shift changes. For the H5 and H3 protons and the same guest concentration, the absolute values of the chemical shift changes are larger for hexanoic acid than for decanoic acid.

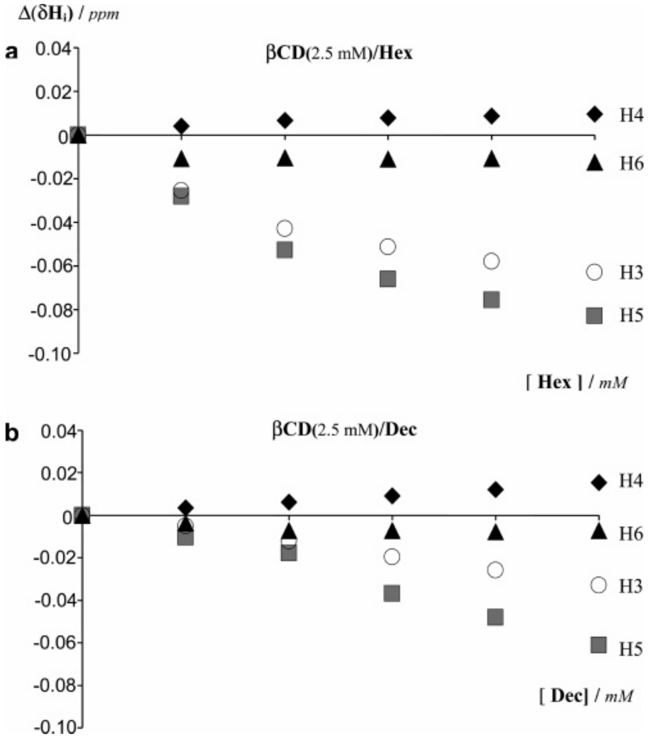

Figure 2. Chemical shift changes of βCD protons (H4, H6, H3, H5) in D_2O solutions, where $[\beta\text{CD}]_0 = 2.5$ mM and $[\text{G}]_0/\text{mM} = \{0, 1, 2, 3, 4, 5\}$: (a) G = Hex; (b) G = Dec.

To probe the effect of electrolytes on this system, a solution of βCD and an amphiphile (2.5 mM in both) was prepared. The electrolyte concentration was increased in steps of 50 mM up to 250 mM; it was found that both H4 and H6 yield approximately zero $\Delta\delta$ differences (these protons are on the outside of the βCD cavity; see Figure 1) and that H5 protons yield larger positive $\Delta\delta$ differences (not shown). Among the βCD CH protons, H5 was found to be most effective in discriminating among the various salts, a result possibly associated with the fact that these protons are located in a crown close to the narrower βCD rim where the host−guest contacts are necessarily shorter and the corresponding interactions are stronger. Because the $\Delta\delta$ differences were taken between the chemical shifts of the βCD/amphiphile solution (amphiphile = Hex, Dec) and those of the βCD solution, the positive slopes indicate deshieldings relative to the βCD aqueous solution (Figure 3). In the difference plot for hexanoic acid (Figure 3a), it can be seen that the H5 protons yield a positive slope for ClO_4^- and approximately zero slopes for Cl^-, NO_3^-, and Br^-. For decanoic acid (Figure 3b), positive slopes are clearly observed for ClO_4^- and Cl^-.

In general terms, these experimental results suggest that the anions compete to different extents with the amphiphilic guest for inclusion in βCD. Two factors intervene in the outcome of this competition. One concerns the ratio of equilibrium constants for the inclusion of the amphiphilic guest and that of the hydrated anion species. The other is the concentration factor. Although it is expected that the apparent equilibrium constant for the inclusion of the hydrated anion species is a few orders of magnitude smaller than that of the amphiphilic guest, the concentration factor strongly favors the inclusion of the anion species. This competition leads to the partial dissociation of the βCD−amphiphile inclusion complex and the replacement of the amphiphilic molecules in the βCD cavity by the hydrated anions, thus resulting in deshielding with respect to βCD. In comparing the hexanoic and decanoic difference plots (Figure 3a and b), one finds that the relative deshieldings are stronger for ClO_4^- and Cl^- in the presence of decanoic acid, despite the fact that decanoic acid is included more extensively than hexanoic acid. (The apparent inclusion constant is larger for decanoic acid.[13]) This is an interesting result that will be quantitatively interpreted (vide intra).

Experiments and Expressions for $\Delta\delta°$ Coefficients. Because no distinct βCD proton resonances are observed for the free and complexed βCD states, the host−guest complex system is considered to be in the NMR fast exchange chemical shift

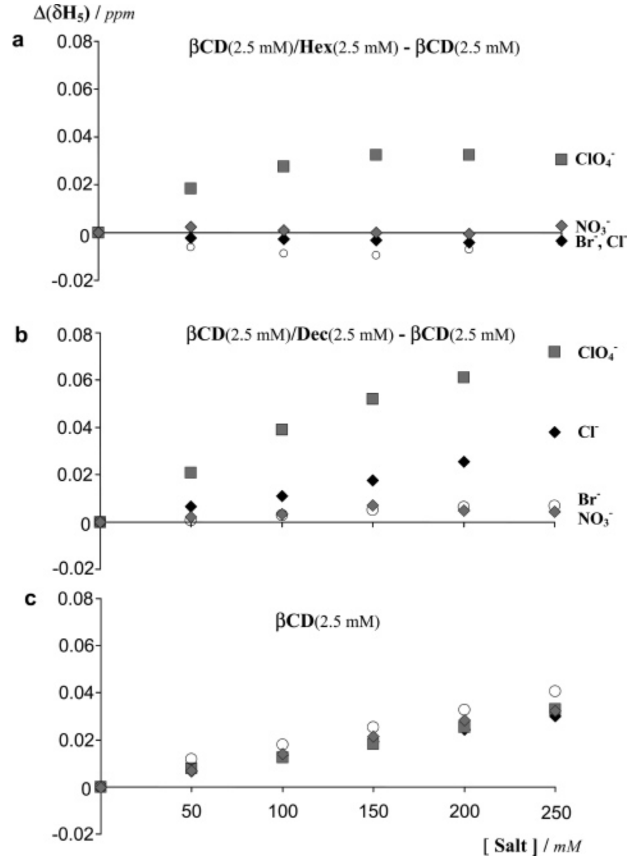

Figure 3. Chemical shift changes of βCD H5 protons in D_2O solutions for $[\beta CD]_0 = [G]_0 = 2.5$ mM vs the electrolyte (a sodium salt, NaX) concentration, $[X]_0$/mM = {0, 50, 100, 150, 200, 250}, where X = {ClO_4^-, NO_3^-, Cl^-, Br^-}: (a) difference plot βCD/Hex − βCD; (b) difference plot βCD/Dec − βCD; (c) βCD.

limit. If the probed protons belong to the host (in this work, βCD), then the observed chemical shifts of the host resonances for the host−guest system are averages of the chemical shifts for the free and complexed states, weighted by the mole fractions x_H and x_{HG} of each state,[7]

$$\delta = x_H\delta^\circ_H + x_{HG}\delta^\circ_{HG} \qquad (1)$$

where δ°_H and δ°_{HG} represent the chemical shifts for the free host, H, and the host−guest inclusion complex, HG. To eliminate the redundancy in the mole fractions ($x_H + x_{HG} = 1$), x_H is replaced by $1 - x_{HG}$ in expression 1 to yield

$$\Delta\delta = x_{HG}\Delta\delta^\circ_{HG} \qquad (2)$$

where $\Delta\delta = \delta - \delta^\circ_H$ and $\Delta\delta^\circ_{HG} = \delta^\circ_{HG} - \delta^\circ_H$. The chemical shift values, δ, were measured in an experiment in which the initial concentration of the host was kept constant and the initial concentration of the guest was varied (experiment 1a: H = βCD, G = Hex; $[H]_o = 2.5$ mM, $[G]_o$/mM = {0, 5, 7.5, 10, 12.5, 15}; see Table 1).

It has been shown that the water molecules in the βCD cavity form a cluster with normal hydrogen-bonding interactions with themselves and their oxygen atoms preferentially oriented toward the CH bonds in the interior of the βCD cavity.[9] The similarity between the orientation of water molecules in the βCD cavity and in the first layer of a hydrated anion strongly suggests that, unlike cations, anions may compete for inclusion in the βCD cavity.[14] The chemical shifts of host resonances for a βCD−anion system are given by

$$\delta = x_H\delta^\circ_H + x_{HX}\delta^\circ_{HX} \qquad (3)$$

If $[H]_o$ is kept constant and the initial concentration of the electrolyte is varied, then one obtains

$$\Delta\delta = x_{HX}\Delta\delta^\circ_{HX} \qquad (4)$$

where HX represents the inclusion complex of the anion, $\Delta\delta = \delta - \delta^\circ_H$, and $\Delta\delta^\circ_{HX} = \delta^\circ_{HX} - \delta^\circ_H$ (experiment 1b: H = βCD, X = {ClO_4^-, NO_3^-, Cl^-, Br^-}; $[H]_o = 2.5$ mM, $[NaX]_o$/mM = {0, 50, 100, 150, 200, 250}; see Table 1).

In a different type of experiment, $[G]_o$ was kept constant, and $[H]_o$ was varied with $[H]_o > [G]_o$, yielding

$$\Delta\delta = (x_{HG} - x^\circ_{HG})\Delta\delta^\circ_{HG} \qquad (5)$$

where $\Delta\delta^\circ_{HG} = \delta^\circ_{HG} - \delta^\circ_H$ and x°_{HG} represents the mole fraction of the host−guest complex when $[H]_o$ has its lowest significant value (note that the probed protons are in the host) (experiment 2: H = βCD, G = Dec; $[G]_o = 2.5$ mM, $[H]_o$/mM = {3, 4, 5, 6, 7, 8, 9, 10, 12}; see Table 1).

In the above experiments, either the amphiphilic guest or the sodium salt was present. In the next series of experiments, both the host and the amphiphilic guest were present, and their initial concentrations were kept constant and equal whereas the initial concentration of the sodium salt was varied. The zero value of the recorded chemical shift differences corresponds to $[X]_o = 0$. After eliminating the redundancy in the mole fractions ($x_H + x_{HG} + x_{HX} = 1$), one easily arrives at the measured $\Delta\delta$ as a function of x_{HG} and x_{HX},

$$\Delta\delta = x_{HG}\,\Delta\delta^\circ_{HG\_X} - x^\circ_{HG}\Delta\delta^\circ_{HG} + x_{HX}\Delta\delta^\circ_{HX\_G} \qquad (6)$$

where the subscripts HG_X and HX_G stand for the HG and HX inclusion complexes in the presence of X and G, respectively, $\Delta\delta^\circ_{HG} = \delta^\circ_{HG} - \delta^\circ_H$, $\Delta\delta^\circ_{HG\_X} = \delta^\circ_{HG\_X} - \delta^\circ_H$, and $\Delta\delta^\circ_{HX\_G} = \delta^\circ_{HX\_G} - \delta^\circ_H$ (experiment 3: H = βCD, G = {Hex, Dec}; X = {ClO_4^-, NO_3^-, Cl^-, Br^-}; $[H]_o = [G]_o = 2.5$ mM, $[NaX]_o$/mM = {0, 50, 100, 150, 200, 250}; see Table 1). The $\Delta\delta^\circ_{HG}$ coefficient in the second member of this equation was evaluated in experiment 1a.

The determination of the $\Delta\delta^\circ$ coefficients in expressions 2, 4, 5, and 6 by a least-squares linear fitting of the measured $\Delta\delta$ values requires prior evaluation of the mole fractions. These are obtained by solving the system of equations that includes the apparent inclusion constants and the conservation of the initial concentrations (experimental constraints).

TABLE 1: Experimental Constraints and $\Delta\delta$ Expressions with Evaluated $\Delta\delta^\circ$ Coefficients (H = βCD, G = {Hex, Dec}, X = {ClO_4^-, NO_3^-, Cl^-, Br^-})

experiment	probed protons	experimental constraints	$\Delta\delta$
1a	H5 (βCD)	$[H]_o$ = constant, $[G]_o$ variable	$x_{HG}\Delta\delta^\circ_{HG}$
1b	H5 (βCD)	$[H]_o$ = constant, $[X]_o$ variable	$x_{HX}\Delta\delta^\circ_{HX}$
2	H5 (βCD)	$[G]_o$ = constant, $[H]_o$ variable and $>[G]_o$	$(x_{HG} - x^\circ_{HG})\Delta\delta^\circ_{HG}$
3	H5 (βCD)	$[H]_o = [G]_o$ = constant, $[X]_o$ variable	$x_{HG}\Delta\delta^\circ_{HG\_X} - x^\circ_{HG}\Delta\delta^\circ_{HG} + x_{HX}\Delta\delta^\circ_{HX\_G}$
4	CH_3 (G)	$[G]_o$ = constant, $[H]_o$ variable and $>[G]_o$	$(x_{G,agg} - x^\circ_{G,agg})\Delta\delta^\circ_{G,agg} + (x_{HG} - x^\circ_{HG})\Delta\delta^\circ_{HG}$

TABLE 2: Apparent Inclusion Constants, *K*, and $\Delta\delta°$ Coefficients for Various Inclusion Complexes (G = {Hex, Dec}; X = {ClO_4^-, NO_3^-, Cl^-, Br^-})

inclusion complex	*K*	$\Delta\delta°$
βCD·Hex	3.9×10^2	−0.115
βCD·Dec	8.6×10^2	−0.048
βCD·ClO_4^-(aq)	2.04	0.088
βCD·NO_3^-(aq)	0.26	0.551
βCD·Cl^-(aq)	0.46	0.293
βCD·Br^-(aq)	0.81	0.240

Apparent Inclusion Constants. Values for the inclusion constants can be determined from the measured $\Delta\delta$ values.[15] Assuming that the activity coefficients and water activity are equal to 1, the apparent equilibrium constant for the HG inclusion complex is

$$K_{HG} = \frac{[HG]}{[H][G]} \tag{7}$$

where $[H] = [H]_o - [HG]$ and $[G] = [G]_o - [HG]$. Considering experiment 1a, the maximum $\Delta\delta$ value, $\Delta\delta_{max}$, is attained in the limit when $[HG] = [H]_o$ (i.e., when all βCD cavities are occupied by the guest). Hence, one can write

$$\frac{[HG]}{[H]_o} = \frac{\Delta\delta}{\Delta\delta_{max}} \tag{8}$$

By substituting expression 8 into expression 7, one obtains, after rearranging,

$$\frac{1}{\Delta\delta} = \frac{1}{\Delta\delta_{max}} + \{\Delta\delta_{max} K_{HG} [G]_o \phi\}^{-1} \tag{9}$$

where

$$\phi = 1 - \left(\frac{\Delta\delta}{\Delta\delta_{max}}\right)\left(\frac{[H]_o}{[G]_o}\right) \tag{10}$$

Equation 9 expresses a linear plot of $^1/_{\Delta\delta}$ as a function of $\{[G]_o\phi\}^{-1}$ and can be solved iteratively, converging smoothly providing ϕ is kept below 1 (i.e., when $[G]_o > [H]_o$).[15]

K_{HG} and the corresponding $\Delta\delta_{max}$ values have distinct yet interrelated physical meanings. On one hand, K_{HG} measures the extent of the inclusion reaction, thus being influenced by all host−guest close contacts, not just by those of the NMR probes, and on the other hand, $\Delta\delta_{max}$ mainly measures a particular type of close-contact interaction in the limit when all of the host cavities are occupied by guest molecules (i.e., when the corresponding mole fraction is 1). In this limit, $\Delta\delta$ becomes equal to $\Delta\delta_{max}$ in expression 9, and the second term of the second member can be neglected ($[G]_o$ becomes sufficiently large for ϕ to be $\simeq 1$).

When experiment 1a was applied to decanoic acid, the evaluation of $K_{\beta CD \cdot Dec}$ yielded a negative value. Because in this experiment the lowest initial concentration of decanoic acid was well above its critical micellar concentration (0.141 mM),[11] the impossible result was interpreted as an indication of wrongly evaluated mole fractions for decanoic acid due to its aggregation. Therefore, a new experiment was carried out (experiment 2, see Table 1), in which the initial concentration of decanoic acid was kept constant ($[Dec]_o = 2.5$ mM) and $[\beta CD]_o$ was increased starting from 3.0 mM. (The extent of decanoic acid aggregation is expected to decrease with the increasing $[\beta CD]_o$.)

Table 2 presents the values of the apparent inclusion constants, *K*, for the inclusion complexes of hexanoic and decanoic acid and the anions in βCD. It can be seen that the ratio of the $K_{\beta CD \cdot G}$ values for decanoic and hexanoic acids is roughly 2, the value for decanoic acid being larger possibly as a result of a longer and more conformationally flexible hydrocarbon chain leading to an increased number of close contacts with the βCD atoms.[6] In turn, the $K_{\beta CD \cdot X}$ values for the considered anions are of the same order of magnitude, 3 orders of magnitude below those of the amphiphilic guests, above 1 for ClO_4^-, and below 1 for Cl^-, Br^-, and NO_3^-. As expected, the $\Delta\delta_{max}$ values obtained in the evaluation of the inclusion constants coincide with the corresponding $\Delta\delta°_i$ values, within their standard deviations (not shown).

$\Delta\delta°$ Coefficients. In the fast exchange chemical shift regime, the measured $\Delta\delta$ values are linear combinations of $\Delta\delta°$ differences weighted by the corresponding mole fractions (Table 1),

$$\Delta\delta = \Sigma x_i \Delta\delta°_i \tag{11}$$

Hence,

$$\Delta\delta°_i = \left(\frac{\partial \Delta\delta}{\partial x_i}\right)_{xj(j \neq i)} \tag{12}$$

(i.e., each $\Delta\delta°_i$ is a slope, thus expressing the rate of change of $\Delta\delta$ with respect to variations in x_i). The definition of a $\Delta\delta°$ coefficient as a derivative of the observed $\Delta\delta$ with respect to a particular mole fraction, keeping the remaining nonredundant mole fractions constant (expression 12 is a partial derivative), stresses the possible parametric dependence between the $\Delta\delta°$ coefficient and the constant mole fractions. The $\Delta\delta°$ coefficients determined in experiments 1a, 1b, and 2 where only one nonredundant mole fraction variable and one $\Delta\delta°$ coefficient is to be determined per experiment (in these cases, the $\Delta\delta°$ coefficients are total derivatives; see Table 1) do not have this problem. However, this possibility occurs with the $\Delta\delta°$ coefficients evaluated in experiments 3 and 4. One should point out that only $\Delta\delta°$ coefficients obtained from the same experiment or from experiments with the same δ zero value may be directly compared with each other. Being a derivative, a positive (negative) $\Delta\delta°_i$ indicates an increasing (decreasing) function of x_i and a relative deshielding (shielding) effect. When there is only one $\Delta\delta°$ coefficient to be determined per experiment (in this work, experiments 1a, 1b, and 2) and in the limiting situation in which the considered mole fraction x_i is 1, the measured $\Delta\delta$ becomes equal both to $\Delta\delta_{max}$ and to $\Delta\delta°_i$ (i.e., $\Delta\delta_{max}$ and the corresponding $\Delta\delta°_i$ coincide). In these cases, the ranking orders for the $\Delta\delta°$ coefficients and for the corresponding inclusion constants are inverse because the product of a particular $\Delta\delta_{max}$ (coincides with the corresponding $\Delta\delta°$) with the corresponding *K* is constant. (See expression 9 for the slope of the linear plot of $^1/_{\Delta\delta}$ as a function of $\{[G]_o\phi\}^{-1}$.)

The $\Delta\delta°$ coefficients for the βCD inclusion of hexanoic acid (experiment 1a), decanoic acid (experiment 2), and the anions (experiment 1b) are shown in Table 2. In most cases, the squares of the correlation coefficients, R^2, were ca. 0.99, and in all cases, they were above 0.94. The standard deviations of the $\Delta\delta°$ coefficients did not excede 0.012. It can be seen that the $\Delta\delta°_{\beta CD \cdot G}$ values are negative (i.e., express relative shielding) and that the $\Delta\delta°_{\beta CD \cdot X}$ values are positive (i.e., indicate relative deshielding). In addition, a comparison of the $\Delta\delta°_{\beta CD \cdot X}$ coefficients enables us to rank the anions by their relative deshielding effects: $NO_3^- > Cl^- > Br^- > ClO_4^-$. This is the inverse order of the corresponding inclusion constants.

TABLE 3: $\Delta\delta°$ Coefficients Obtained from Experiment 2 ($[\beta CD]_o = [G]_o = 2.5$ mM, $[X]_o$/mM = {0, 50, 100, 150, 200, 250})

evaluated quantity	X	ClO_4^-	NO_3^-	Cl^-	Br^-
$\Delta\delta°_{\beta CD\cdot Hex\_X}$		−0.074	−0.110	−0.119	−0.132
$\Delta\delta°_{\beta CD\cdot Dec\_X}$		−0.039	−0.042	−0.055	−0.044
$\Delta\delta°_{\beta CD\cdot X\_Hex}$		0.191	0.814	0.385	0.471
$\Delta\delta°_{\beta CD\cdot X\_Dec}$		0.515	1.166	1.296	0.504

As mentioned above, $\Delta\delta°_{\beta CD\cdot G\_X}$ indicates the effect of anion X on the $\Delta\delta°_{\beta CD\cdot G}$ coefficient, whereas $\Delta\delta°_{\beta CD\cdot X\_G}$ expresses the effect of amphiphile G on the $\Delta\delta°_{\beta CD\cdot X}$ coefficient. Their values, obtained from experiment 3 and expression 6, are shown in Table 3. As expected, the $\Delta\delta°_{\beta CD\cdot X\_G}$ values are positive (i.e., they express relative deshielding), whereas the $\Delta\delta°_{\beta CD\cdot G\_X}$ values are negative (i.e., they express relative shielding). In addition, the $\Delta\delta°_{\beta CD\cdot X\_G}$ values dominate the corresponding $\Delta\delta°_{\beta CD\cdot G\_X}$ values, thus explaining why the recorded $\Delta\delta$ values in expression 6 are positive (deshielding). From the determined values of the $\Delta\delta°_{\beta CD\cdot G\_X}$ coefficients (G = Hex or Dec), one concludes that the considered anions have little effect on the inclusion of hexanoic and decanoic acid. On the contrary, the presence of either decanoic or hexanoic acid appreaciably affects the inclusion of the anions: $\Delta\delta°_{\beta CD\cdot X\_Hex}$ ranks the anions by the order of their relative deshielding effects in the presence of hexanoic acid ($NO_3^- > Br^- > Cl^- > ClO_4^-$). Decanoic acid leads to a different order of anions in $\Delta\delta°_{\beta CD\cdot X\_Dec}$ ($Cl^- > NO_3^- > ClO_4^- \approx Br^-$).

The apparent inclusion constants in expression 7 assume activity coefficients equal to 1 and ignore the removal of water molecules from the βCD cavity. Because these appear in the second member of the amphiphilic guest inclusion equilibrium, one obtains

$$K_{\beta CD\cdot G} = K_{\beta CD\cdot G,app}\Gamma \tag{13}$$

where $\Gamma = a_w^m$, a_w represents the water activity, and m represents the amount of water that left the cavity because of guest inclusion.[16] Assuming that $m = 6$,[17] and using a_w values reported in the literature for NaCl and $NaClO_4$,[18] we evaluated the $K_{\beta CD\cdot G,app}$ values for various concentrations of these electrolytes. The mole fractions were then calculated for the various points in experiment 3, and the $\Delta\delta°_{\beta CD\cdot X\_G,a_w}$ and $\Delta\delta°_{\beta CD\cdot G\_X,a_w}$ coefficients were evaluated. To determine the effect of water activity on the relative shielding or deshielding effects, the $\Delta\delta°_{\beta CD\cdot X\_G,a_w} - \Delta\delta°_{\beta CD\cdot X\_G}$ and $\Delta\delta°_{\beta CD\cdot G\_X,a_w} - \Delta\delta°_{\beta CD\cdot G\_X}$ differences were evaluated. It can be seen that in the presence of hexanoic acid the changing water activity caused by ClO_4^- exerts a relative deshielding effect ($\Delta\delta°_{\beta CD\cdot ClO_4^-\_Hex,a_w} - \Delta\delta°_{\beta CD\cdot ClO_4^-\_Hex} = 0.053$), suggesting an increase in strength for the close-contact interactions between ClO_4^- and the βCD H5 protons. In addition, $\Delta\delta°_{\beta CD\cdot Hex\_ClO_4^-,a_w} - \Delta\delta°_{\beta CD\cdot Hex\_ClO_4^-} = -0.024$, suggests an increase in strength for the close-contact interactions between Hex and the H5 protons. In the presence of decanoic acid, small relative deshielding and shielding effects are produced by ClO_4^- and Cl^- ($\Delta\delta°_{\beta CD\cdot ClO_4^-\_Dec,a_w} - \Delta\delta°_{\beta CD\cdot ClO_4^-\_Dec} = 0.029$, $\Delta\delta°_{\beta CD\cdot Cl\_Dec,a_w} - \Delta\delta°_{\beta CD\cdot Cl^-\_Dec} = -0.022$).

Aggregation of Decanoic Acid. Experiments 2 and 4 were both used for the βCD/Dec system and have the same experimental constraints ($[Dec]_o = 2.5$ mM, $[\beta CD]_o$/mM = {3, 4, 5, 6, 7, 8, 9, 10, 12}), differing from each other in the NMR probes used: the βCD H5 protons are used in experiment 2, whereas the decanoic acid methyl hydrogen atoms are probed in experiment 4 (Table 3). Experiment 2 was used to determine $K_{\beta CD\cdot Dec}$, and experiment 4 enabled us to account for the decanoic acid aggregation. On the basis of the assumption that the chemical shift changes of the methyl protons of decanoic acid account for its self-aggregation and its inclusion in βCD, whereas the chemical shift changes of the βCD H5 protons account only for the inclusion, one can write

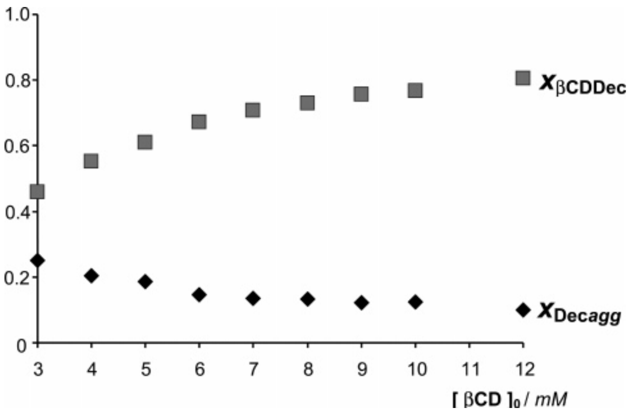

Figure 4. Mole fractions $x_{\beta CD\cdot Dec}$ and $x_{Dec,agg}$ vs $[\beta CD]_0$ for experiment 4 ($[Dec]_0 = 2.5$ mM and $[\beta CD]_0$/mM = {3, 4, 5, 6, 7, 8, 9, 10, 12}).

$$x_{Dec,agg} = \frac{\Delta\delta_{CH_3}}{\Delta\delta_{max,CH_3}} - \frac{\Delta\delta_{H5}}{\Delta\delta_{max,H5}} \tag{14}$$

where $x_{Dec,agg}$ corresponds to the mole fraction of decanoic acid involved in the formation of all types of aggregates: $\Delta\delta_{CH_3}$ and $\Delta\delta_{max,CH_3}$ stand for the chemical shift changes of the methyl protons in decanoic acid, and $\Delta\delta_{H5}$ and $\Delta\delta_{max,H5}$ indicate the chemical shift changes of H5 protons in βCD. The divisions by $\Delta\delta_{max,CH_3}$ and $\Delta\delta_{max,H5}$ normalize the $\Delta\delta_{CH_3}$ and $\Delta\delta_{H5}$ values because both terms in expression 14 range from 0 to 1. The plot of $x_{Dec,agg}$ and $x_{\beta CD\cdot Dec}$ as a function of the initial concentration of βCD (Figure 4) shows that the increase of $[\beta CD]_o$ leads to the increase of $x_{\beta CD\cdot Dec}$ and to the decrease of both $x_{Dec,agg}$ and x_{Dec} (not shown; points for x_{Dec} almost coincide with those of $x_{Dec,agg}$). In fact, the introduction of a term for $x_{Dec,agg}$ in the equation for the conservation of $[Dec]_o$ ($1 = x_{Dec} + x_{Dec,agg} + x_{\beta CD\cdot Dec}$) effectively buffers variations of both x_{Dec} and $x_{\beta CD\cdot Dec}$. A consideration of experiment 4 for decanoic acid leads to

$$\Delta\delta = (x_{Dec,agg} - x°_{Dec,agg})\Delta\delta°_{Dec,agg} + (x_{\beta CD\cdot Dec} - x°_{\beta CD\cdot Dec})\Delta\delta°_{\beta CD\cdot Dec} \tag{15}$$

(Table 1) where the zero value for $\Delta\delta$ corresponds to δ_{CH_3} for $[Dec]_o = 2.5$ mM and $[\beta CD]_o = 3$ mM. Both $\Delta\delta°$ coefficients have $\delta°_{Dec}$ subtracted, and the $x_{Dec,agg}$ values were evaluated by expression 14. The least-squares solution of expression 15 yields $\Delta\delta°_{Dec,agg} = -1.121$ ($R^2 = 0.8987$) and $\Delta\delta°_{\beta CD\cdot Dec} = -0.328$ ($R^2 = 0.9549$). Being negative, these values express shieldings due to either aggregation (the first value) or inclusion in βCD. Note that the latter value should not be directly compared with $\Delta\delta°_{\beta CD\cdot Dec}$ from Table 2 because the zero values of the corresponding measured $\Delta\delta$ values and the probed protons are different. By subtracting the first of the above values from the second, one obtains $\delta°_{\beta CD\cdot Dec} - \delta°_{Dec,agg} = 0.793$, thus showing that, overall, inclusion in βCD leads to relative deshielding if taken with respect to the aggregated decanoic acid, a point previously noticed.[6]

References and Notes

(1) Saenger, W. R. *Angew. Chem., Int. Ed. Engl.* **1980**, *19*, 344−362.
(2) Saenger, W. R.; Jacob, J.; Gessler, K.; Steiner, T.; Hoffmann, D.; Sanbe, H.; Koizumi, K.; Smith, S. M.; Takaha, T. *Chem. Rev.* **1998**, *98*, 1787−1802.

(3) (a) Guo, Q. X.; Li, Z. Z.; Ren, T.; Zhu, X. Q.; Liu, Y. C. *J. Inclusion Phenom. Mol. Recognit. Chem.* **1994**, *17*, 149−156. (b) Schneider, H.-J.; Hacket, F.; Rüdiger, V. *Chem. Rev.* **1998**, *98*, 1755−1785. (c) Wilson, L. D.; Verrall, R. E. *Can. J. Chem.* **1998**, *76*, 25−34.

(4) (a) Lo Nostro, P.; Lopes, J. R.; Ninham, B. W.; Baglioni, P. *J. Phys. Chem. B* **2002**, *106*, 2166−2174. (b) Funasaki, N.; Ishikawa, S.; Neya, S. *J. Phys. Chem. B* **2003**, *107*, 10094−10099.

(5) (a) Funasaki, N. *J. Colloid Interface Sci.* **1978**, *67*, 384−386. (b) Funasaki, N. *J. Phys. Chem.* **1979**, *83*, 1979−2003. (c) Schott, H. J. *J. Colloid Interface Sci.* **1995**, *173*, 265−277. (d) Garcia-Rio, L.; Leis, J. R.; Mejuto, J. C.; Pérez-Juste, J. *J. Phys. Chem. B* **1997**, *101*, 7383−7389. (e) Garcia-Rio, L.; Leis, J. R.; Mejuto, J. C.; Pérez-Juste J. *J. Phys. Chem. B* **1998**, *102*, 4581−4587. (f) Dorrego, A. B.; Garcia-Rio, L.; Herves, P.; Leis, J. R.; Mejuto, J. C.; Pérez-Juste, J. *Angew. Chem., Int. Ed.* **2000**, *39*, 2945−2948. (g) Molina-Bolivar, J. A.; Aguiar, J.; Ruiz, C. C. *J. Phys. Chem. B* **2002**, *106*, 870−877.

(6) Lima, S.; Goodfellow, B. J.; Teixeira-Dias, J. J. C. *J. Phys. Chem. B* **2003**, *107*, 14590−14597.

(7) Gunther, H. *NMR Spectroscopy*, 2nd ed.; John Wiley & Sons: Chichester, England, 1995; pp 339−341.

(8) (a) Hofmeister, F. *Arch. Exp. Pathol. Pharmakol.* **1888**, *24*, 247−260. (b) Hippel, P. H.; Schleich, T. *Acc. Chem. Res.* **1969**, *2*, 257−265. (c) Baldwin, R. L. *Biophys. J.* **1996**, *71*, 2056−2063.

(9) (a) Betzel, C.; Saenger, W.; Hingerty, B. E.; Brown, G. M. *J. Am. Chem. Soc.* **1984**, *106*, 7545−7557. (b) Steiner, T.; Saenger, W.; Lechner, R. E. *Mol. Phys.* **1991**, *72*, 1211−1232. (c) Steiner, T.; Koellner, G. *J. Am. Chem. Soc.* **1994**, *116*, 5122−5128.

(10) Matsui, Y.; Ono, M.; Tokunaga, S. *Bull. Chem. Soc. Jpn.* **1997**, *70*, 535−541.

(11) Horozov, T.; Joos, P. *J. Colloid Interface Sci.* **1995**, *173*, 334−342.

(12) Hunter, R. J. *Foundations of Colloid Science*; Oxford University Press: Oxford, England, 1986; Vol. I.

(13) (a) Gelb, R. I.; Schwartz, L. M. *J. Inclusion Phenom. Mol. Recognit. Chem.* **1989**, *7*, 465−476. (b) Rekharsky, M. V.; Inoue, Y. *Chem. Rev.* **1998**, *98*, 1875−1918.

(14) (a) Gelb, R. I.; Schwartz, L. M.; Radeos, M.; Laufer, D. A. *J. Phys. Chem.* **1983**, *87*, 3349−3354. (b) Mu, P.; Okada, T.; Iwami, N.; Matsui, Y. *Bull. Chem. Soc. Jpn.* **1993**, *66*, 1924−1928. (c) Matsui, Y.; Ono, M.; Tokunaga, S. *Bull. Chem. Soc. Jpn.* **1997**, *70*, 535−541.

(15) (a) Moreira da Silva, A. M.; Empis, J.; Teixeira-Dias, J. J. C. *J. Inclusion Phenom. Macrocyclic Chem.* **1999**, *33*, 81−97. (b) Fielding, L. *Tetrahedron* **2000**, *56*, 6151−6170.

(16) Hamad, E. Z.; Mansoori, G. A. *J. Phys. Chem.* **1990**, *94*, 3148−3152.

(17) Starzak, M.; Peacock, S. D.; Mathlouthi, M. *Crit. Rev. Food Sci.* **2000**, *40*, 327−367.

(18) (a) Lynden-Bell, R. M.; Rasaiah, J. C. *J. Chem. Phys.* **1997**, *107*, 1981−1991. (b) Koneshan, S.; Rasaiah, J. C.; Lynden-Bell, R. M.; Lee, S. H. *J. Phys. Chem. B* **1998**, *102*, 4193−4204.