

## Competition between the Hydrated Fluoride Anion and Hexanoic Acid for Inclusion in $\beta$ -Cyclodextrin: An $^1\text{H}$ -NMR Study in Aqueous Solution

SÉRGIO LIMA, BRIAN J. GOODFELLOW, and JOSÉ J. C. TEIXEIRA-DIAS\*

Department of Chemistry University of Aveiro, CICECO, 3810-193, Aveiro, Portugal

(Received: 24 September 2004; in final form 09 March 2005)

**Key words:**  $\beta$ -cyclodextrin, fast exchange regime, fluoride ion, hexanoic acid, inclusion complexes, NMR chemical shifts

### Abstract

$\beta$ -cyclodextrin ( $\beta$ CD) and two guests (hexanoic acid and the hydrated fluoride ion) are considered as a system in the NMR chemical shift fast exchange regime. The measured chemical shift variations for the  $\beta$ CD H5 protons are expressed as functions of the mole fractions for the free and complexed states, and their slopes,  $\Delta\delta_i^0 = (\partial\Delta\delta/\partial x_i)_{x_j(j\neq i)}$ , are evaluated. When the NaF concentration is varied ( $[\text{NaF}]_0/\text{mM} = \{50, 100, 150, 200, 250\}$ ) for a defined value of the hexanoic acid concentration ( $[\text{Hex}]_0/\text{mM} = \{5, 7.5, 10, 12.5, 15\}$ ), the chemical shift variations of the  $\beta$ CD H5 protons for 2.5 mM  $\beta$ CD solutions in  $\text{D}_2\text{O}$  present a second order polynomial variation with a minimum, suggesting two opposing trends. The first trend corresponding to the decrease of  $\Delta\delta$  values (relative shielding) is traced to increasingly negative values of  $\Delta\delta_{\beta\text{CD}\cdot\text{Hex}\cdot\text{F}^-}^0$ . Corresponding to the increase of  $\Delta\delta$  values (relative deshielding), the second trend is mainly associated with the increase of the mole fraction for the inclusion complex  $\beta\text{CD}\cdot\text{F}(\text{H}_2\text{O})_6^-$ , as the concentration of NaF increases. The kosmotropic, stabilizing features of the fluoride ion are consistent with the results of HF/6–31G\* single point energy calculations showing that  $\beta\text{CD}\cdot\text{F}(\text{H}_2\text{O})_6^-$  is energetically favourable, in contrast with  $\beta\text{CD}\cdot\text{Cl}(\text{H}_2\text{O})_6^-$  and  $\beta\text{CD}\cdot\text{Br}(\text{H}_2\text{O})_8^-$ . Due to its smaller size,  $\text{F}(\text{H}_2\text{O})_6^-$  is not appreciably distorted inside the  $\beta$ CD cavity as compared with  $\text{Cl}(\text{H}_2\text{O})_6^-$  and  $\text{Br}(\text{H}_2\text{O})_8^-$ .

### Introduction

Salts have been long known to affect the solubilities of proteins in water [1]. The ranking of ions by the extent to which they modulate hydrophobicity – the Hofmeister or lyotropic series – has been mainly dealt with on a qualitative basis. The large dimension and inherent complexity of macromolecular systems makes difficult or impossible to go beyond a simple qualitative picture. To overcome this difficulty and reach a quantitative interpretation for the effect of ions, the dimension of the studied system should be considerably reduced while the main structural features of the real system retained.

The structural features of  $\beta$ -cyclodextrin (cyclomaltoheptaose,  $\beta$ CD) make it a suitable model for much larger host-guest systems: it is soluble in water, provides a hydrophobic cavity that includes guests of suitable size and gives a number of close contacts for host-guest non-covalent interactions [2]. When the guest displays amphiphilic behaviour, its hydrophobic moiety has a tendency to enter into the  $\beta$ CD cavity, leaving the hydrophilic head to interact mainly with the hydroxyl groups of the  $\beta$ CD rims and with the solvent through hydrogen bond interactions [3]. In  $\beta$ CD hydrates, the

water cluster in the  $\beta$ CD cavity parallels water clusters in protein crystal structures [4]. Therefore,  $\beta$ CD may turn out to be a good host system model for studying the effect of electrolytes in hydrophobic sites of proteins, thus contributing to a better understanding of hydration in macromolecular systems with biological relevance.

The mode and extent of  $\beta$ CD-guest interactions can be effectively probed by  $^1\text{H}$ -NMR. In particular, chemical shifts variations of the H3 and H5 protons (Figure 1) inside the  $\beta$ CD cavity can be used to monitor the guest inclusion process [5]. By varying the concentration of various alkali-metal chlorides, the influence of different alkali-metal cations on  $\beta$ CD inclusion processes has been considered and interpreted [6]. In a separate work, the effects of the inorganic anions of various sodium salts ( $\text{NaCl}$ ,  $\text{NaBr}$ ,  $\text{NaClO}_4$ ,  $\text{NaNO}_3$ ) in the  $\beta$ CD inclusion of hexanoic and decanoic acids have been studied [7]. For the concentrations of the decanoic acid used in this paper, self-aggregation of this guest was also considered and estimated [7].

As it is well known, the fluoride ion has a relevant and important impact on health [8]. The present work considers the effects of increasing the concentration of sodium fluoride on the inclusion of hexanoic acid in  $\beta$ CD and looks at the observed chemical shift changes of the  $\beta$ CD H5 protons. Unlike  $\text{Cl}^-$ ,  $\text{Br}^-$ ,  $\text{NO}_3^-$  and  $\text{ClO}_4^-$ ,

\* Author for correspondence.: E-mail: tdias@dq.ua.pt

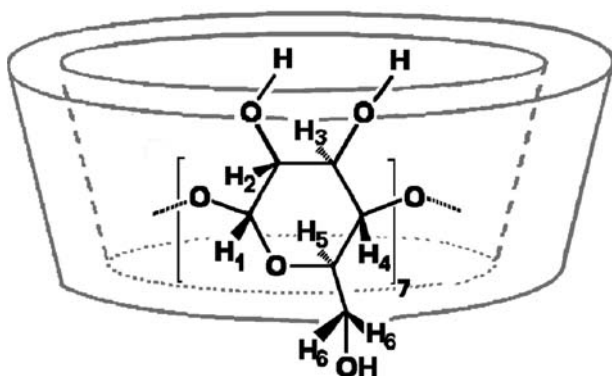


Figure 1. The  $\beta$ CD macrocycle with a glucopyranose unit highlighted.

the fluoride ion does not induce significant chemical shift variations in the  $\beta$ CD protons of a simple  $\beta$ CD aqueous solution. However, the observed chemical shift variations for solutions containing both  $\beta$ CD and hexanoic acid suggest a competition between the hydrated fluoride anion and hexanoic acid for inclusion in  $\beta$ CD, with an inclusion constant in  $\beta$ CD estimated to be two orders of magnitude larger than those for the inclusions of  $\text{Cl}^-$ ,  $\text{Br}^-$ ,  $\text{NO}_3^-$  and  $\text{ClO}_4^-$ . In addition,  $\text{F}^-$  clearly distinguishes itself from the latter inorganic anions since it leads to non-linear chemical shift variations when hexanoic acid is present. Understanding these peculiar features of the fluoride ion is the main objective of the present work.

## Materials and methods

$\beta$ -Cyclodextrin (Fluka,  $\geq 99\%$ ), hexanoic acid (Aldrich, 99%), NaF (Sigma, 99.9%) and  $\text{D}_2\text{O}$  (Aldrich, 99.9%) were used as received without further purification. The  $^1\text{H}$ -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 in order to avoid any effect between the buffer anions and the  $\beta$ CD protons [9]. Molecular modelling was carried out using the Gaussian 94 system of programs [10]. Full geometry optimization was carried out for the isolated hydrated anions ( $\text{F}(\text{H}_2\text{O})_6^-$ ,  $\text{Cl}(\text{H}_2\text{O})_6^-$ ,  $\text{Br}(\text{H}_2\text{O})_8^-$ ) at the MP2 level with the 6-31G\* basis set. During geometry optimization for the inclusion of these hydrated anions in  $\beta$ CD, the  $\beta$ CD coordinates were kept constant and all the remaining coordinates were optimized at the PM3 semi-empirical level ( $\beta$ CD had been previously optimized at this level of calculation). Single point energy calculations were carried out at the Hartree-Fock level with the 6-31G\* basis set, for all the considered systems.

## Results and discussion

In general, the addition of an amphiphile to an aqueous  $\beta$ CD solution leads to the shielding of H5 and H3, usually interpreted as an indication of guest inclusion in  $\beta$ CD, since H5 and H3 are located inside the  $\beta$ CD cavity (Figure 1). When the concentration of hexanoic acid (Hex) is increased in aqueous solutions of  $\beta$ CD, the various  $\beta$ CD protons can be separated from each other through their chemical shift variations. The slopes follow the order  $\text{H5} > \text{H3} > \text{H6} > \text{H4}$ , from negative values for H5, H3 and H6 protons, to positive  $\Delta\delta$  values for H4 protons (Figure 2) [7]. The inclusion of hexanoic acid is confirmed by the negative slopes of the H5 and H3 chemical shift changes whose zero value corresponds to the absence of guest, i.e., to a  $\beta$ CD solution [7].

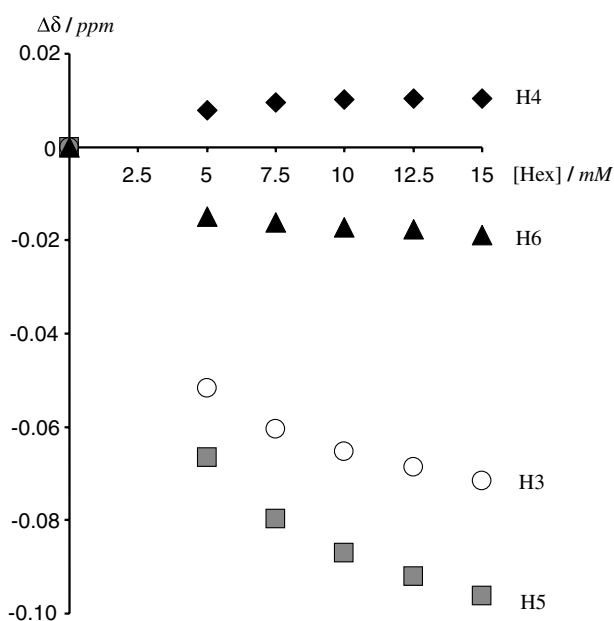


Figure 2. Chemical shift changes of  $\beta$ CD protons, in  $\text{D}_2\text{O}$  solutions, for  $[\beta\text{CD}]_0 = 2.5$  mM and  $[\text{Hex}]_0/\text{mM} = \{5, 7.5, 10, 12.5, 15\}$ .

In order to consider the effect of sodium fluoride on the inclusion of hexanoic acid in  $\beta$ CD, a series of 5 experiments was carried out with 5 points each, thus comprising 25 points on a ( $[\text{Hex}]_0$ ,  $[\text{F}^-]_0$ ,  $\Delta\delta$ ) surface. In each of these experiments, the initial concentration of  $\beta$ CD was kept constant at 2.5 mM, the initial concentration of NaF took a defined value among the following  $[\text{NaF}]_0/\text{mM} = \{50, 100, 150, 200, 250\}$ , and the initial concentration of hexanoic acid was varied ( $[\text{Hex}]_0/\text{mM} = \{5, 7.5, 10, 12.5, 15\}$ ). The chemical shift differences for the  $\beta$ CD H5 protons,  $\Delta\delta$ , were obtained by subtracting the chemical shift for a  $\beta$ CD 2.5 mM solution.  $\Delta\delta$  values were found to have negative values, i.e., to express shielding, and follow approximate linear trends (approximately constant slopes). However, using the same experimental data but considering instead the variation of  $[\text{NaF}]_0$  for defined values of  $[\text{Hex}]_0$ , non linear  $\Delta\delta$  trends were obtained, with minima about  $[\text{NaF}]_0 = 150$  mM (Figure 3). Comparing these results with those of the other sodium salts (NaCl, NaBr, NaClO<sub>4</sub>, NaNO<sub>3</sub>)<sup>7</sup> clearly points to the fluoride ion, not to the hexanoic acid, as the origin of the observed non linear trends.

A system composed of  $\beta$ CD and two guests (an amphiphilic guest, hexanoic acid, and the hydrated fluoride ion) comprises three states for the  $\beta$ CD H5 protons: free  $\beta$ CD,  $\beta$ CD-amphiphile and  $\beta$ CD-hydrated fluoride ion. In order to quantitatively interpret the above mentioned experimental results and understand the peculiar features of the fluoride ion in aqueous solutions where both hexanoic acid and  $\beta$ CD are present, the measured  $\delta$  values should be expressed, in the fast exchange chemical shift regime, as linear combinations of  $\delta^0$  coefficients weighted by the corresponding mole fractions [11],

$$\delta = x_{\beta\text{CD}}\delta_{\beta\text{CD}}^0 + x_{\beta\text{CD}\cdot\text{Hex}}\delta_{\beta\text{CD}\cdot\text{Hex}\cdot\text{F}^-}^0 + x_{\beta\text{CD}\cdot\text{F}^-}\delta_{\beta\text{CD}\cdot\text{F}^-}^0 \quad (1)$$

where  $\beta\text{CD}$ ,  $\beta\text{CD}\cdot\text{Hex}$  and  $\beta\text{CD}\cdot\text{F}^-$  represent the three states of the  $\beta$ CD H5 protons and the subscripts  $\beta\text{CD}\cdot\text{Hex}\cdot\text{F}^-$  and  $\beta\text{CD}\cdot\text{F}^-_{\text{Hex}}$  stand for the  $\beta\text{CD}\cdot\text{Hex}$  and  $\beta\text{CD}\cdot\text{F}^-$  inclusion complexes in the presence of  $\text{F}^-$  and Hex, respectively. Since  $[\beta\text{CD}]_0 = [\beta\text{CD}] + [\beta\text{CD}\cdot\text{Hex}] + [\beta\text{CD}\cdot\text{F}^-]$ , the mole fractions satisfy  $x_{\beta\text{CD}} + x_{\beta\text{CD}\cdot\text{Hex}} + x_{\beta\text{CD}\cdot\text{F}^-} = 1$ , that is, one of these mole fractions is mathematically redundant. To eliminate this redundancy and simultaneously ascribe the  $\delta$  zero value to the  $\beta\text{CD}$  2.5 mM solution in the absence of Hex and NaF ( $\delta_{\beta\text{CD}}^0$ ),  $x_{\beta\text{CD}}$  was chosen as the redundant mole fraction and substituted by  $1 - x_{\beta\text{CD}\cdot\text{Hex}} - x_{\beta\text{CD}\cdot\text{F}^-}$  in (1), yielding

$$\Delta\delta = x_{\beta\text{CD}\cdot\text{Hex}}\Delta\delta_{\beta\text{CD}\cdot\text{Hex}\cdot\text{F}^-}^0 + x_{\beta\text{CD}\cdot\text{F}^-}\Delta\delta_{\beta\text{CD}\cdot\text{F}^-}^0 \quad (2)$$

where  $\Delta\delta$  is the measured chemical shift difference,  $\Delta\delta = \delta(\beta\text{CD}/\text{Hex}/\text{F}^-) - \delta_{\beta\text{CD}}^0$ , and

$$\Delta\delta_{\beta\text{CD}\cdot\text{Hex}\cdot\text{F}^-}^0 = \partial\Delta\delta/\partial x_{\beta\text{CD}\cdot\text{Hex}}$$

$$\Delta\delta_{\beta\text{CD}\cdot\text{F}^-}^0 = \partial\Delta\delta/\partial x_{\beta\text{CD}\cdot\text{F}^-}$$

It can be seen from (2) that  $\partial\Delta\delta/\partial x_{\beta\text{CD}\cdot\text{Hex}}$  stands for the partial derivative of  $\Delta\delta$  with respect to  $x_{\beta\text{CD}\cdot\text{Hex}}$ , keeping  $x_{\beta\text{CD}\cdot\text{F}^-}$  constant (the latter condition is omitted in the partial derivative, for simplicity of notation), whereas  $\partial\Delta\delta/\partial x_{\beta\text{CD}\cdot\text{F}^-}$  represents the partial derivative of  $\Delta\delta$  with respect to  $x_{\beta\text{CD}\cdot\text{F}^-}$ , keeping  $x_{\beta\text{CD}\cdot\text{Hex}}$  constant. In order to evaluate the mole fractions in (2), the apparent constants for the inclusions of the amphiphile and of the hydrated fluoride ion need to be known. The constant for  $\beta\text{CD}\cdot\text{Hex}$  was taken from our previous work [7], where it was determined from  $\Delta\delta$  data [12]. However, the fluoride anion yielded negligible chemical shift variations for the H5 protons in a  $\beta$ CD solution, the equilibrium constant for the inclusion of a hydrated

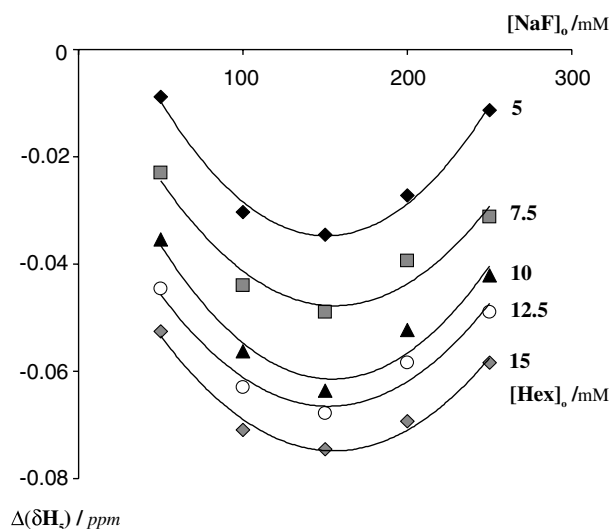


Figure 3. Chemical shift changes of  $\beta$ CD H5 protons, in D<sub>2</sub>O solutions, for  $[\beta\text{CD}]_0 = 2.5$  mM and defined values of  $[\text{Hex}]_0/\text{mM} = \{5, 7.5, 10, 12.5, 15\}$ , as functions of  $[\text{NaF}]_0$  ( $[\text{NaF}]_0/\text{mM} = \{50, 100, 150, 200, 250\}$ ).

fluoride anion in the  $\beta$ CD cavity,  $K_{\beta\text{CD}\cdot\text{F}^-}$ , could not be reliably determined by the same method. Having considered as trial values for  $K_{\beta\text{CD}\cdot\text{F}^-}$  1 and  $1 \times 10^2$ , the latter value was adopted as it yields sensible results, as it is shown below.

Once the equilibrium constants for the inclusion of hexanoic acid and the fluoride ion in  $\beta$ CD and the initial concentrations of the solutes are known, the mole fractions in (2) could be evaluated, and the  $\Delta\delta^0$  coefficients,  $\Delta\delta_{\beta\text{CD}\cdot\text{Hex}\cdot\text{F}^-}^0$  and  $\Delta\delta_{\beta\text{CD}\cdot\text{F}^- \cdot\text{Hex}}^0$ , determined by bilinear least squares fitting of the measured  $\Delta\delta$  values considered as functions of  $[\text{Hex}]_0$ , for defined values of  $[\text{NaF}]_0$ . In this case, the chemical shift differences follow approximate linear trends, that is, have constant slopes that parametrically depend on  $[\text{NaF}]_0$ , as mentioned above. Thus, five distinct pairs of  $\Delta\delta^0$  coefficients were obtained, one for each defined value of  $[\text{NaF}]_0$  (Table 1). The least squares fitting of the  $\Delta\delta^0$  coefficients as functions of  $[\text{NaF}]_0$  yields

$$\Delta\delta_{\beta\text{CD}\cdot\text{Hex}\cdot\text{F}^-}^0 = -0.0012([\text{F}^-]_0/\text{mM}) - 0.0865 (R^2 = 0.9977)$$

$$\Delta\delta_{\beta\text{CD}\cdot\text{F}^- \cdot\text{Hex}}^0 = 4 \times 10^{-6}([\text{F}^-]_0/\text{mM})^2 - 0.0012([\text{F}^-]_0/\text{mM}) + 0.0853 (R^2 = 0.9572)$$

The first of these coefficients takes negative values and is a decreasing function of  $[\text{F}^-]_0$ , i.e., it expresses relative shielding (Figure 4). Unlike  $\Delta\delta_{\beta\text{CD}\cdot\text{Hex}\cdot\text{Cl}^-}^0$  and  $\Delta\delta_{\beta\text{CD}\cdot\text{Hex}\cdot\text{Br}^-}^0$  which have approximately constant values ( $\Delta\delta_{\beta\text{CD}\cdot\text{Hex}\cdot\text{Cl}^-}^0 = -0.119$  and  $\Delta\delta_{\beta\text{CD}\cdot\text{Hex}\cdot\text{Br}^-}^0 = -0.132$ ) [7],  $\Delta\delta_{\beta\text{CD}\cdot\text{Hex}\cdot\text{F}^-}^0$  depends linearly on the initial concentration of the anion. For  $[\text{F}^-]_0 = 50$  mM,  $\Delta\delta_{\beta\text{CD}\cdot\text{Hex}\cdot\text{F}^-}^0$  has the value  $-0.145$  (Table 1) which, incidentally, is close to the values of  $\Delta\delta_{\beta\text{CD}\cdot\text{Hex}\cdot\text{Cl}^-}^0$  and  $\Delta\delta_{\beta\text{CD}\cdot\text{Hex}\cdot\text{Br}^-}^0$ .

The second of the above coefficients,  $\Delta\delta_{\beta\text{CD}\cdot\text{F}^- \cdot\text{Hex}}^0$ , takes values about zero, both positive and negative, and

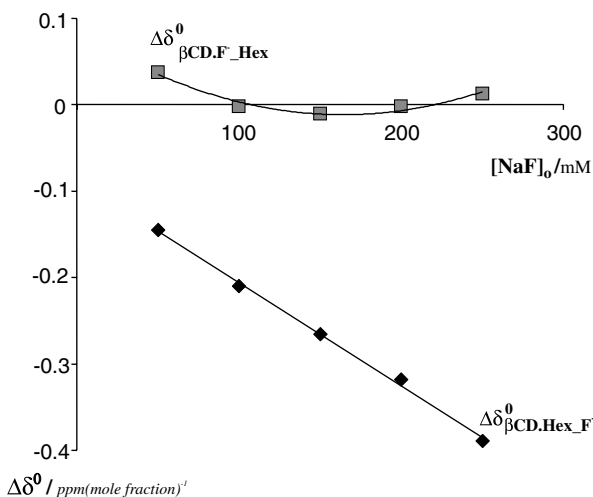


Figure 4.  $\Delta\delta_{\beta\text{CD}\cdot\text{Hex}\cdot\text{F}^-}^0$  and  $\Delta\delta_{\beta\text{CD}\cdot\text{F}^- \cdot\text{Hex}}^0$  coefficients as functions of  $[\text{NaF}]_0$ .

is a quadratic function of  $[\text{F}^-]_0$ , thus exhibiting a curvature with a minimum at  $[\text{F}^-]_0 = 150$  mM (Table 1, Figure 4). It is worth mentioning that the small values found for  $\Delta\delta_{\beta\text{CD}\cdot\text{F}^- \cdot\text{Hex}}^0$  are consistent with the negligible chemical shift variations found for a simple  $\beta$ CD solution with NaF, a fact that gives support at least to the order of magnitude of the value adopted for  $K_{\beta\text{CD}\cdot\text{F}^-}$  ( $1 \times 10^2$ ). In addition, the values of the corresponding  $\Delta\delta_{\beta\text{CD}\cdot\text{Hex}}^0$  coefficients for  $\text{X} = \text{Cl}^-$  and  $\text{X} = \text{Br}^-$  ( $\Delta\delta_{\beta\text{CD}\cdot\text{Cl}^-}^0 = 0.293$ ,  $\Delta\delta_{\beta\text{CD}\cdot\text{Cl}^- \cdot\text{Hex}}^0 = 0.385$ ,  $\Delta\delta_{\beta\text{CD}\cdot\text{Br}^-}^0 = 0.240$ ,  $\Delta\delta_{\beta\text{CD}\cdot\text{Br}^- \cdot\text{Hex}}^0 = 0.471$ ) [7] are appreciably distinct from  $\Delta\delta_{\beta\text{CD}\cdot\text{F}^- \cdot\text{Hex}}^0$ , confirming the peculiar behaviour of the fluoride ion. These findings are consistent with the classification of the fluoride ion as kosmotropic, with stabilizing (salting-out) features, in contrast with the destabilizing (salting-in) characteristics of chloride and bromide anions [13].

Considering now the mole fractions in (2),  $x_{\beta\text{CD}\cdot\text{F}^-}$  and  $x_{\beta\text{CD}\cdot\text{Hex}}$ , as functions of  $[\text{NaF}]_0$ , it can be seen that they exhibit slight curvatures of opposite signs that approach defined plateaux (Figure 5). With comparable  $\beta$ CD inclusion constants,  $\text{F}^-$  is favoured over Hex by its much larger concentration, as this factor is multiplied by the inclusion constant in the equilibrium equation. Hence,  $x_{\beta\text{CD}\cdot\text{F}^-}$  becomes larger than  $x_{\beta\text{CD}\cdot\text{Hex}}$  (see Figure 5).

The minima exhibited by the negative  $\Delta\delta$  values in Figure 3 point to two opposing trends: one leading to the decrease of the chemical shift of the  $\beta$ CD H5 protons (a decreasing  $\Delta\delta$  function expressing a relative shielding trend), the other leading to the increase of the same chemical shift (an increasing  $\Delta\delta$  function expressing a relative deshielding trend). It can now be concluded that the relative shielding trend is associated with the first term of (2) and results mainly from the increasingly negative  $\Delta\delta_{\beta\text{CD}\cdot\text{Hex}\cdot\text{F}^-}^0$  values (Figure 4) that outweigh the decrease of  $x_{\beta\text{CD}\cdot\text{Hex}}$  (dissociation of the  $\beta\text{CD}\cdot\text{Hex}$  inclusion complex) as  $x_{\beta\text{CD}\cdot\text{Hex}}$  multiplies  $\Delta\delta_{\beta\text{CD}\cdot\text{Hex}\cdot\text{F}^-}^0$  in (2). The more negative is  $\Delta\delta_{\beta\text{CD}\cdot\text{Hex}\cdot\text{F}^-}^0$ , the more effective becomes the shielding term in (2), thus suggesting closer contacts between the included hexanoic acid molecules and the  $\beta$ CD H5 protons, i.e., stronger  $\beta\text{CD}\text{--Hex}$  interactions. On the contrary, an increase in  $x_{\beta\text{CD}\cdot\text{Hex}}$  would mean a more extensive Hex inclusion in  $\beta$ CD. However, it can be seen from Figure 5 that this situation is not observed.

The relative deshielding trend, i.e., the increase of the negative  $\Delta\delta$  values, is associated with the second term of

Table 1.  $\Delta\delta^0$  coefficients and  $\Delta\delta_{\text{calc}}$  rms errors, for the  $\beta\text{CD}\text{--Hex}\text{--F}^-(\text{aq})$  system, for defined values of  $[\text{NaF}]_0$

| $[\text{NaF}]_0/\text{mM}$ | $\Delta\delta_{\beta\text{CD}\cdot\text{Hex}\cdot\text{F}^-}^0$ | $\Delta\delta_{\beta\text{CD}\cdot\text{F}^- \cdot\text{Hex}}^0$ | rms error for $\Delta\delta_{\text{calc}}$ |
|----------------------------|---|--|--|
| 50                         | -0.145  | 0.038  | 0.000                                      |
| 100                        | -0.210  | -0.002   | 0.001                                      |
| 150                        | -0.266  | -0.011   | 0.003                                      |
| 200                        | -0.318  | -0.002   | 0.001                                      |
| 250                        | -0.389  | 0.013  | 0.003                                      |

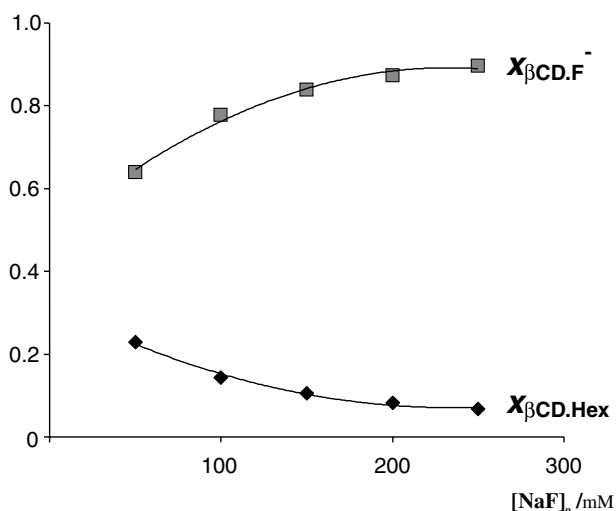


Figure 5. Mole fractions  $x_{\beta\text{CD}\cdot\text{Hex}}$  and  $x_{\beta\text{CD}\cdot\text{F}^-}$  as functions of  $[\text{NaF}]_0$ .

(2) and results mainly from the progressive substitution of Hex by the hydrated fluoride anion in the  $\beta\text{CD}$  cavity, as  $x_{\beta\text{CD}\cdot\text{F}^-}$  necessarily increases with  $[\text{NaF}]_0$  (Figure 5). In fact,  $x_{\beta\text{CD}\cdot\text{F}^-}$  is multiplied by  $\Delta\delta_{\beta\text{CD}\cdot\text{F}^-}^0$  in the second term of (2), but the increase of  $x_{\beta\text{CD}\cdot\text{F}^-}$  outweighs the small variation of  $\Delta\delta_{\beta\text{CD}\cdot\text{F}^-}^0$  (Figure 4).

The above interpretation enable us to explain the main features exhibited by the experimental  $\Delta\delta$  values, namely, (i) the negative  $\Delta\delta$  values, (ii) the observed curvatures in the  $\Delta\delta$  values, and (iii) the negligible  $\Delta\delta$  values found for a 2.5 mM  $\beta\text{CD}$  solution containing NaF. Being relatively high in comparison with the corresponding inclusion constants of the chloride and bromide anions [7], the adopted value for the inclusion constant of  $\beta\text{CD}\cdot\text{F}(\text{H}_2\text{O})_6^-$  is consistent with the singular features of the fluoride ion [13], and with the

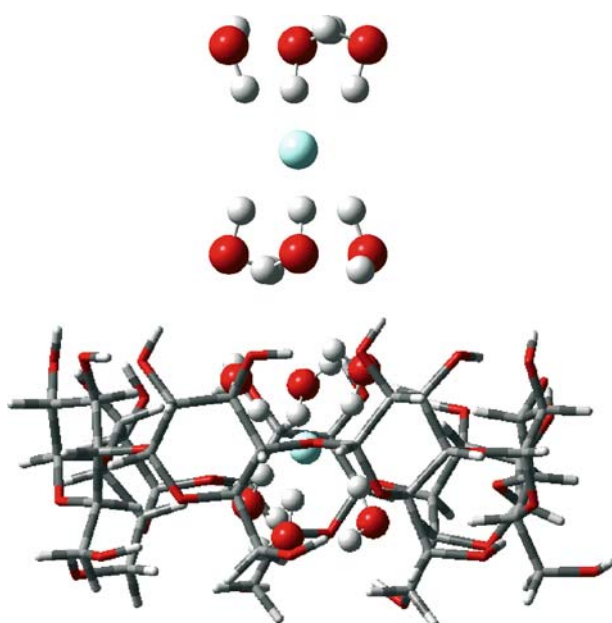


Figure 6. Structures for the inclusion complex  $\beta\text{CD}\cdot\text{F}(\text{H}_2\text{O})_6^-$ , optimized at the PM3 level, and the hydrated fluoride ion,  $\text{F}(\text{H}_2\text{O})_6^-$ , optimized at the MP2 level with a 6-31G\* basis set.

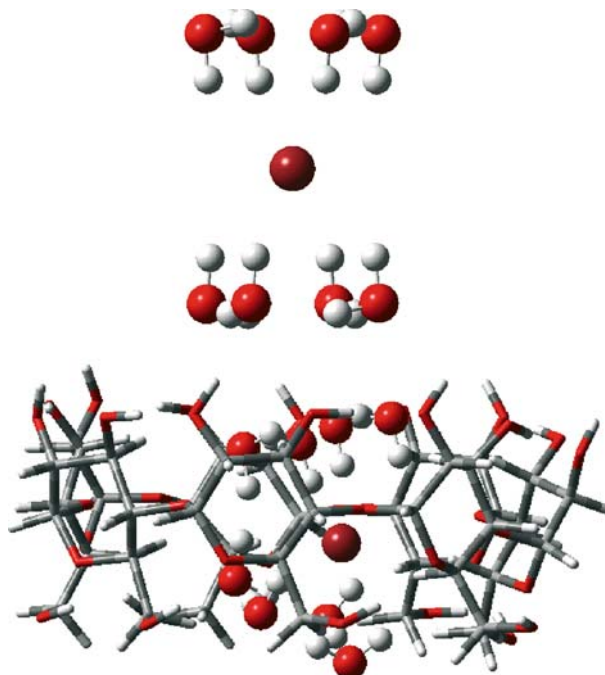


Figure 7. Structures for the inclusion complex  $\beta\text{CD}\cdot\text{Br}(\text{H}_2\text{O})_8^-$ , optimized at the PM3 level, and the hydrated bromide ion,  $\text{Br}(\text{H}_2\text{O})_8^-$ , optimized at the MP2 level with a 6-31G\* basis set.

results of HF/6-31G\* single point energy calculations showing that  $\beta\text{CD}\cdot\text{F}(\text{H}_2\text{O})_6^-$  is an energetically favourable inclusion complex (the evaluated energy for the inclusion reaction is  $\Delta E = -26 \text{ kJ mol}^{-1}$ ), in contrast with  $\beta\text{CD}\cdot\text{Cl}(\text{H}_2\text{O})_6^-$  ( $\Delta E = 79 \text{ kJ mol}^{-1}$ ) and with  $\beta\text{CD}\cdot\text{Br}(\text{H}_2\text{O})_8^-$  ( $\Delta E = 92 \text{ kJ mol}^{-1}$ ). Due to its smaller size,  $\text{F}(\text{H}_2\text{O})_6^-$  is not appreciably distorted inside the  $\beta\text{CD}$  cavity (Figure 6), as compared with  $\text{Cl}(\text{H}_2\text{O})_6^-$  (not shown) and  $\text{Br}(\text{H}_2\text{O})_8^-$  (Figure 7).

## References

- (a) F. Hofmeister: *Arch. Exp. Pathol. Pharmacol.* **24**, 247 (1888); (b) P.H. Hippel and T. Schleich: *Acc. Chem. Res.* **2**, 257 (1969); (c) R.L. Baldwin: *Biophys. J.* **71**, 2056 (1996); (d) M.G. Cacace, E.M. Landau, and J.J. Ramsden: *Quart. Rev. Biophys.* **30**, 241 (1997).
- (a) W.R. Saenger: *Angew. Chem. Int. Ed. Engl.* **19**, 344 (1980); (b) W.R. Saenger, J. Jacob, K. Gessler, T. Steiner, D. Hoffmann, H. Sanbe, K. Koizumi, S.M. Smith, and T. Takaha: *Chem. Rev.* **98**, 1787 (1998).
- (a) P. Lo Nostro, J.R. Lopes, B.W. Ninham, and P. Baglioni: *J. Phys. Chem. B* **106**, 2166 (2002); (b) N. Funasaki, S. Ishikawa, and S. Neya: *J. Phys. Chem. B* **107**, 10094 (2003).
- (a) C. Betzel, W. Saenger, B.E. Hingerty, and G.M. J. Brown: *Am. Chem. Soc.* **106**, 7545 (1984); (b) T. Steiner, W. Saenger, and R.E. Lechner: *Molec. Phys.* **72**, 1211 (1991); (c) T. Steiner and G. Koellner: *J. Am. Chem. Soc.* **116**, 5122 (1994).
- (a) Q.X. Guo, Z.Z. Li, T. Ren, X.Q. Zhu, and Y.C.J. Liu: *Inclusion Phenom. Mol. Recog. Chem.* **17**, 149 (1994); (b) H.-J. Schneider, F. Hackett, and V. Rüdiger: *Chem. Rev.* **98**, 1755 (1998); (c) L.D. Wilson and R.E. Verrall: *Can. J. Chem.* **76**, 25 (1998).
- S. Lima, B.J. Goodfellow, and J.J.C. Teixeira-Dias: *J. Phys. Chem. B* **107**, 1459014597.
- S. Lima, B.J. Goodfellow, and J.J.C. Teixeira-Dias: *J. Phys. Chem. B* (2004) (in press).
- American Association: *J. Am. Diet. Assoc.* **100**, 1208 (2000).
- Y. Matsui, M. Ono, and S. Tokunaga: *Bull. Chem. Soc. Jpn.* **70**, 535541.

10. M.J. Frisch, G.W. Trucks, H.B. Schlegel, P.M.W. Gill, B.G. Johnson, M.A. Robb, J.R. Cheeseman, T. Keith, G.A. Petersson, J.A. Montgomery, K. Raghavachari, M.A. Al-Laham, V.G. Zakrzewski, J.V. Ortiz, J.B. Foresman, J. Cioslowski, B.B. Stefanov, A. Nanayakkara, M. Challacombe, C.Y. Peng, P.Y. Ayala, W. Chen, M.W. Wong, J.L. Andres, E.S. Replogle, R. Gomperts, R.L. Martin, D.J. Fox, J.S. Binkley, D.J. Defrees, J. Baker, J.P. Stewart, M. Head-Gordon, C. Gonzalez, and A. Pople: Gaussian 94, Revision E.2, Gaussian Inc., Pittsburgh, PA (1995).
11. H. Gunther: *NMR Spectroscopy*, 2nd ed., John Wiley & Sons, Chichester (1995), pp. 339–341.
12. (a) A.M. Moreira da Silva, J. Empis, and J.J.C. Teixeira-Dias: *J. Incl. Phenom. Macro.* **33**, 81 (1999); (b) L. Fielding: *Tetrahedron* **56**, 6151 (2000).
13. (a) P.W. Hochachka and G.N. Somero: *Biochemical Adaptation*. Princeton University Press, Princeton, NJ (1984); (b) D.L. Hall and P.L. Darke: *J. Biol. Chem.* **270**, 22697 (1995); (c) P. Terpstra, D. Combes, and A. Zwick: *J. Chem. Phys.* **92**, 65 (1990).