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ORIGINAL ARTICLE

Inclusion complexes of 2-phenoxyethanol and alkoxyethanols in cyclodextrins: an ^{1}H NMR study

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Abstract The association in aqueous solutions of small amphiphilic molecules [2-phenoxyethanol, PhE_1, and some α-*n*-alkyl-ω-hydroxyoligo(oxiethylenes), C_4E_1, C_4E_2 and C_6E_2] with β-cyclodextrin (βCD), heptakis(2,6-di-*O*-methyl)-β-cyclodextrin (DIMEB) and heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin (TRIMEB) was investigated by ^{1}H NMR spectroscopy. The upfield shifts observed for the H3 and H5 NMR signals due to anisotropic shielding confirm that the host–guest associations are of inclusion type. The stoichiometries and the apparent inclusion constants, K_{app}, were determined by ^{1}H NMR spectroscopy using the H5 and H3 signals. The relative differences in the K_{app} values for βCD inclusion complexes seem to reflect the hydrophobic/hydrophilic balance of the guests. The K_{app} values for the PhE_1 inclusion complexes can be related to the degree of methylation and hydrophobicity variation within the considered hosts. In addition, a comparative study between βCD and TRIMEB inclusion complexes using 2D ROESY (Rotating-frame Overhauser Enhancement SpectroscopY) NMR spectra provides structural features for these complexes which are inaccessible by other experimental methods.

Keywords β-Cyclodextrin · DIMEB · TRIMEB · Amphiphilic molecules · Inclusion complex · Stoichiometry · Apparent inclusion constant · ROESY

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Introduction

Natural cyclodextrins (cycloamyloses, CDs) are cyclic oligosaccharides consisting of six (αCD), seven (βCD) and eight (γCD) $\alpha(1 \rightarrow 4)$ linked D-glucopyranose units in normal chair conformations, produced from enzymatic degradation of the linear amylase component of starch [1]. These natural CDs are relatively rigid molecules and offer limited utility in terms of size, shape and chemical availability. They are moderately water soluble due to hydroxyl groups in the cavity rims. Chemical modifications of CDs, ranging from achieving solubility in a desired solvent to investigating the mechanisms of enzyme-catalyzed reactions, offer many opportunities and challenges to chemists [2].

The most important reason why CDs are well known and studied stems from their ability to form inclusion complexes in aqueous solution with a variety of guest molecules [3]. Usually, the formation of these complexes involves noncovalent host-guest interactions such as electrostatic, Van der Waals, hydrophobic and/or hydrogen bonding. However, the inclusion of guests of suitable size in a CD derives fundamentally from the CD cavity gross geometrical shape in the form of a hollow truncated cone (Fig. 1a) and from the chemical nature of guest molecules [1, 4, 5]. The lipophilic cavity of CDs provides a microenvironment into which appropriately size nonpolar molecules or molecular fragments can enter to form inclusion complexes. Actually, when the guest displays amphiphilic behaviour, the CD cavity has a tendency to host the hydrophobic fragment or part of it, leaving the hydrophilic fragment to interact mainly with the hydroxyl groups of the CD rims and with hydration water [6].

The guest compounds considered in this work are amphiphilic molecules, such as 2-phenoxyethanol (PhE_1) and some α-*n*-alkyl-ω-hydroxyoligo(oxiethylenes) (C_4E_1, C_4E_2 and C_6E_2; Fig. 1b) that belong to a family of nonionic surfactants of general formula C_nE_m (C_n and E_m stand for $CH_3(CH_2)_{n-1}-$ and $-(OCH_2CH_2)_m$ OH, respectively) [7, 8]. Considering the balance between the lengths of the hydrophobic and hydrophilic moieties of these small amphiphilic molecules, PhE_1 and the distinct C_nE_m, significant questions concerned with the formation of true inclusion complexes, their stoichiometry and their structural features will be addressed recording the induced upfield chemical shifts in the H3 and H5 protons of CD caused by the anisotropic shielding of the encapsulated guest [9]. Since these protons form two inner crowns inside the CD cavity, the observation of these induced shifts is an indication of inclusion complex formation [10]. In addition, as the shifts vary monotonically with the host:guest molar ratio, they can be used to determine the complex stoichiometry and the apparent inclusion constant. The continuous variation method (Job's method) is applied for the inclusion complexes stoichiometry determination [11], and 2D ROESY is used to clarify the structure of the inclusion complex.

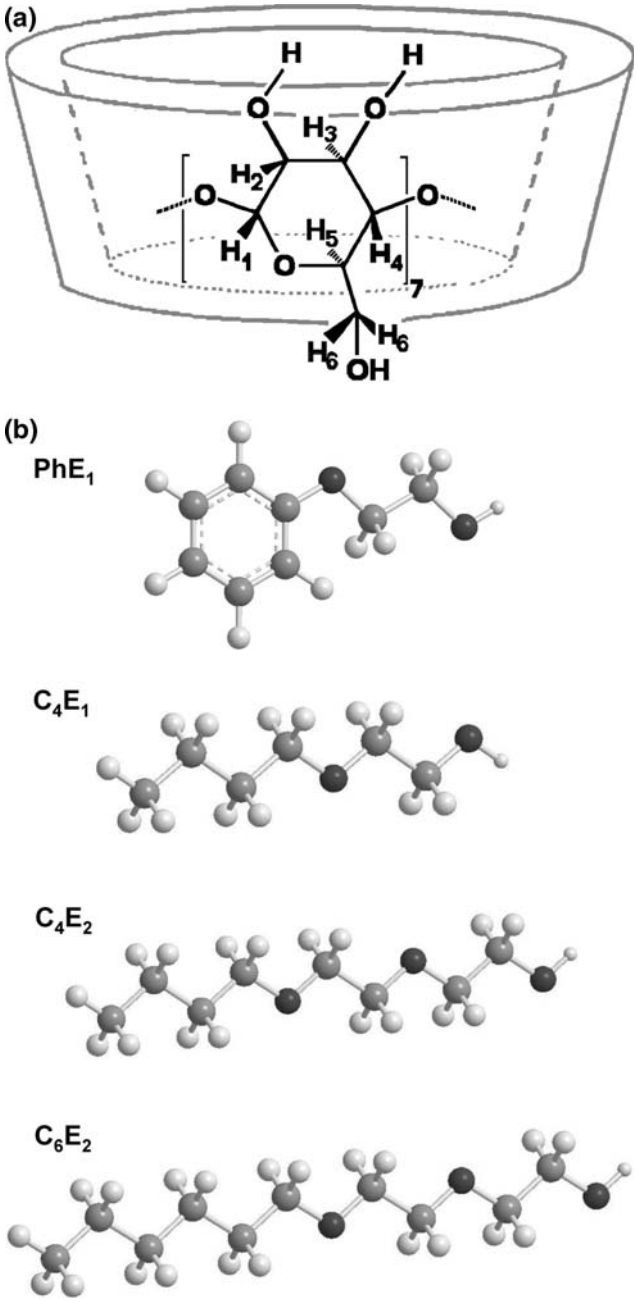

Fig. 1 (**a**) βCD macrocycle with one glucopyranose unit highlighted; (**b**) structures of amphiphilic guests (PhE_1, $C4E_1$, C_4E_2 and C_6E_2) studied

Experimental section

Materials

βCD (Roquete, 98%), kindly donated by Roquete, DIMEB (Fluka, 98%), TRIMEB (Fluka, 98%), C_4E_1 (Aldrich, 99%), C_4E_2 (Aldrich, 99%), C_6E_2 (Aldrich, 97%) and PhE_1 (Aldrich, 90%) were used as received. Preparation of solutions in deuterium oxide (Eurisotop, 99.9% D) for ^{1}H NMR studies, were resorted by ultra-sonification during 1 h.

Methods

^{1}H NMR spectra of deuterated solutions were recorded at 300 MHz, on a Bruker DRX 300 spectrometer, at 20 °C. The water chemical shift (δ = 4.83 ppm) was used as internal reference [12]. ROESY spectra were obtained on a Bruker DRX 500 spectrometer, using a 3 kHz mixing field applied for 300 ms. All the spectra were recorded at 20 °C.

The stoichiometry of the inclusion complexes in aqueous solution was determined by a method due to Job and generally known as the continuous variation method or Job's method [11]. This method involves running a series of experiments in which the ratio of host to guest initial concentrations is varied at well defined *r* values ($r = [H]_o/\{[H]_o + [G]_o\}$), while maintaining constant the sum of the initial molar concentrations of host and guest ($[H]_o + [G]_o$) [9]. In particular, 5 mM D_2O solutions of the guest and CD were mixed: (i) to constant volume, i.e., the sum of the initial concentrations of H and G remained equal to 5 mM ($[H]_o + [G]_o = 5$ mM); (ii) to defined values of

r, where r took values from 1/10 to 9/10, in steps of 1/10.

The stoichiometry was determined by plotting $r{\cdot}\Delta\delta$ versus r, and finding the r value for the maximum of the distribution [13].

The apparent association constants, K_{HG}, as measure of the extent of complex formation, were determinated by the Benesi-Hildebrand regression method [14], using ^{1}H NMR spectroscopy chemical shifts [15]. The host (CDs) concentration was kept constant and that for the guest was varied, thus keeping one of the species (host) in the presence of a large excess of the other component (guest), for the correct application of this method. In particular, the experiments were performed as the initial concentration of the guest, $[G]_o$, was varied from 0 to 20 mM, with the initial concentration of CD being kept constant ($[H]_o$ = 2 mM).

Results and discussion

Stoichiometry of the inclusion complex

The H3 and H5 protons of the cyclodextrin form two inner crowns of hydrogen atoms, near the wider and narrower rims, respectively. These crowns of protons have strategic positions for reporting host–guest interactions in the cavity. The upfield shifts observed for the H3 and H5 NMR signals due to anisotropic shielding confirm that the host–guest associations are of inclusion type since the corresponding hydrogen atoms point towards the cavity inside [10].

It can be seen in Fig. 2 that no distinct resonances are observed for the free host and the host-guest species. In addition, the chemical shifts of H3 and H5 change monotonically as the H:G molar ratio is varied. Hence, the studied systems are considered to be in the NMR fast exchange chemical shift limit [12]. For a 300 MHz spectrometer, and a typical value of the largest observed chemical shift difference ($\Delta\delta_{max} \approx 0.2$), the fast exchange condition (this condition states that the exchange rate is larger than the reciprocal of the largest observed shift in Hz) implies that the inclusion and release of the guest should occur at least 60 times/s. If the probed protons belong to the host (in this work, βCD, DIMEB or TRIMEB), then the observed chemical shifts of the 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}

$$\delta = x_H\delta^0_H + x_{HG}\delta^0_{HG} \quad (1)$$

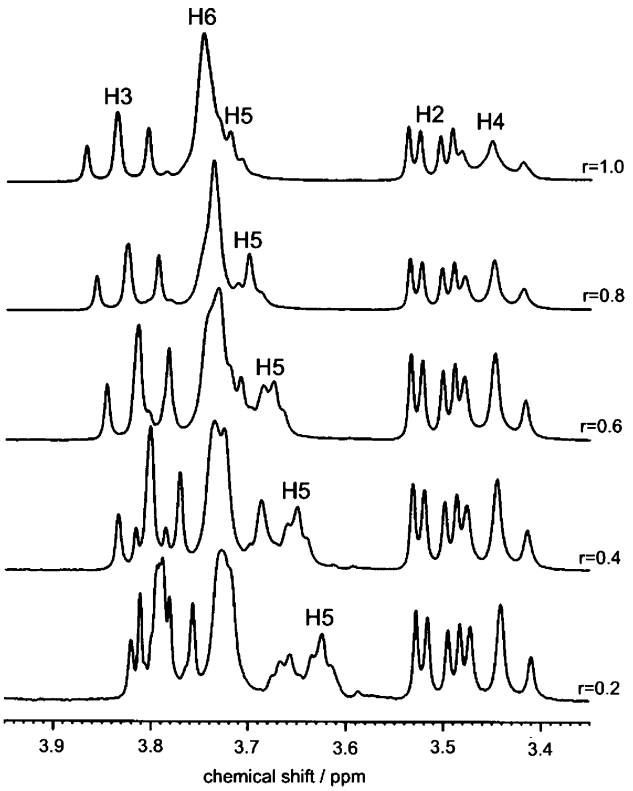

Fig. 2 NMR spectra of D_2O solutions of βCD and PhE_1, at 20 °C

where δ^o_H and δ^o_{HG} represent the chemical shifts for the free host, H, and the host-guest inclusion complex, HG [13, 15, 16].

As mentioned previously, the stoichiometry of the inclusion complex in aqueous solution can be determined by Job's method [11]. Figure 3 shows the continuous variation results (Job's plot) for deuterated aqueous solutions of PhE_1 and βCD, where r is the initial mole fraction of βCD, and $\Delta\delta$(H3) is the variation in the chemical shift of the βCD H3 protons. As can see, the $r.\Delta\delta$ maximum value was obtained at r = 0.5, corresponding to an inclusion complex with 1:1 stoichiometry (βCD · PhE_1). All the remaining systems investigated in this work show a similar result, i.e., $r.\Delta\delta$ maximum value at r = 0.5, a value which corresponds to a 1:1 stoichiometries (βCD.C_4E_1, βCD.C_4E_2, βCD.C_6E_2, DIMEB.PhE_1 and TRIMEB.PhE_1) [13].

Apparent inclusion constants

The equilibrium for the inclusion process in aqueous solution involves hydrated forms of cyclodextrins (H) and guests (G), and physically represents a substitution of water molecules in the CD cavity by the incoming

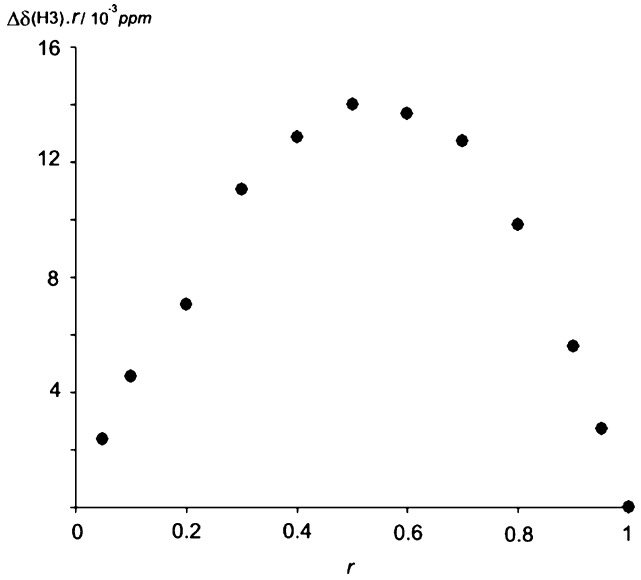

Fig. 3 Job's plot of D_2O solutions of βCD and PhE_1, at 20 °C

guest molecules. Hence, the corresponding inclusion equilibrium constant is given by

$$K = K_{HG}\, a_w^m \quad (2)$$

where a_w represents the water activity, the exponent m represents the amount of water released of the cavity during the inclusion process and

$$K_{HG} = [HG]/([H][G]) \quad (3)$$

is the apparent inclusion constant (hereafter also designated K_{app}). Considering the experimental conditions (low solute concentrations in the absence of electrolytes), one can assume that the activity coefficients and water activity are approximately constant. Hence, the apparent equilibrium constant can be adequately used to express the extent of the inclusion process [17, 18]. Since the maximum $\Delta\delta$ value, $\Delta\delta_{max}$, is obtained in the limit situation when $[HG] = [H]_o$ (i.e., when all the CD cavities are occupied by the guest), one can conclude that

$$\Delta\delta/\Delta\delta_{max} = [HG]/[H]_0 \quad (4)$$

Substitution of (4), $[H] = [H]_0 - [HG]$ and $[G] = [G]_0 - [HG]$ in (3) leads to the following equation

$$\Delta\delta^{-1} = \Delta\delta_{max}^{-1} + \{\Delta\delta_{max} K_{HG} [G]_0 \phi\}^{-1} \quad (5)$$

where

$$\phi = 1 - (\Delta\delta/\Delta\delta_{max})\,([H]_0/[G]_0) \quad (6)$$

The Eq. 5, exact under the assumption of a single 1:1 inclusion complex, represents a linear dependence of $\Delta\delta^{-1}$ as a function of $\{[G]_o\ \phi\}^{-1}$and can be solved iteratively, converging smoothly providing the second term in Eq. 6 is kept well below 1, i.e., when $[G]_o > [H]_o$ (see Experimental section) [15, 18].

While the apparent inclusion constant (K_{HG} or K_{app}) and the corresponding $\Delta\delta_{max}$ are interrelated, they present distinct physical meanings. The K_{HG} value is influenced by all host-guest interactions and not just by those of the NMR probes. In turn, $\Delta\delta_{max}$ values mainly reflect a particular type of close-contact interaction in the limit when all the host cavities are occupied by guest molecules (i.e., when the corresponding mole fraction is 1).

The relative differences in the K_{app} values for βCD inclusion complexes seem to reflect the hydrophobic/hydrophilic balance of the guests. Thus, with the increase of the hydrophobic component, K_{app} values become higher, the highest value being achieved for βCD.PhE_1 where the guest includes an aromatic ring. In turn, the low value for βCD.C_4E_2 when compared with βCD.C_4E_1 should be due to the increase of the hydrophilic component of the guest. On the other hand, for the PhE_1 inclusion complexes, the K_{app} values can be related with the degree of methylation and hydrophobicity within the considered hosts. Also, it can be seen from Table 1 that the inclusion complex with the highest inclusion constant value is TRIMEB.PhE_1, suggesting that the inclusion constant increases with the degree of methylation of the βCD hydroxyl groups.

Structural features

The ROESY (Rotating-frame Overhauser Enhancement Spectroscopy) experiment provides structural information and allows the study of the geometry of the inclusion complex in aqueous solution. The intensities of the cross-peaks in a ROESY spectrum depend on the distance between interacting nuclei (the

Table 1 Apparent inclusion constants for the systems studied

Inclusion complex	$K_{app}/10^3$ M^{-1}
βCD.C_4E_1	0.7
βCD.C_4E_2	0.3
βCD.C_6E_2	1.3
βCD.PhE_1	4.9
DIMEB.PhE_1	12
TRIMEB.PhE_1	32

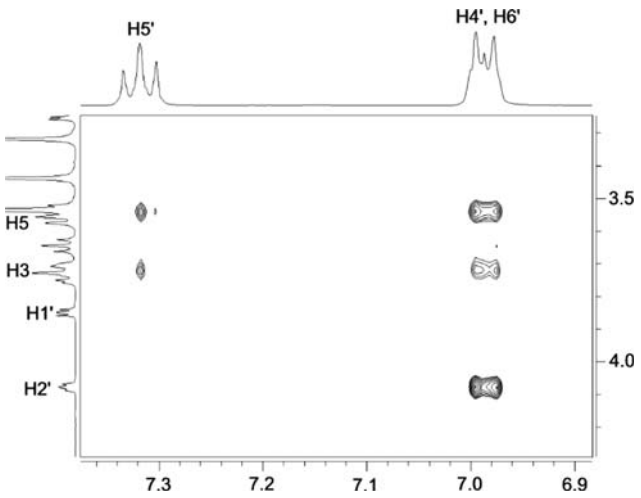

Fig. 4 ROESY spectrum for a stoichiometric (1:1) aqueous solution of PhE_1 and TRIMEB, measured at 20 °C

cross-peak intensity decreases with the internuclear distance r according to r^{-6}) [16].

To infer the geometry of the inclusion complex from the ROE intensity, 500 MHz ROESY spectra were recorded. For both systems, βCD.PhE_1 and TRIMEB.PhE_1, cross-peaks are displayed between the inner protons of cyclodextrin, H3 and H5 (see Fig. 1), and the aromatic protons of PhE_1, confirming the formation of a true inclusion complexes. Moreover, stronger cross-peaks between the cyclodextrin H5 protons and the aromatic guest protons point to an aromatic ring located near the larger crown. This conclusion is confirmed by: (i) the absence of cross-peaks between the guest alkyl protons, H1′ and H2′, and the cyclodextrin inner proton, H3 and H5; and (ii) the cross-seen peaks between guest alkyl protons and the methoxy groups of TRIMEB (Fig. 4).

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