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Aqueous Solution Inclusion of the Nonionic Surfactant $C_{12}E_4$ in β-Cyclodextrin: Implications of Micellization in Stoichiometry Determination and Model Calculations

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Abstract

The inclusion of α-*n*-dodecyl-ω-hydroxytetra(oxyethylene), $C_{12}E_4$, in β-cyclodextrin (βCD) has been studied in aqueous solution. Guest encapsulation is recognized by the upfield variations in the chemical shifts of the H3 and H5 inner protons of βCD, and the chemical shift differences of the H5 protons are used for determining the complex stoichiometry ($2\beta CD : 1C_{12}E_4$) by the continuous variation method. Self-association (micellization) of the surfactant molecules is considered, and the relative amount of surfactant involved in micellar systems at the stoichiometric point estimated. A two-layered integrated molecular orbital and molecular mechanics approach with the PM3 and UFF model chemistries for the guest and host, respectively, was used to perform full geometry optimizations and frequency calculations on the host-guest systems. Energies for the optimized structures were subsequently obtained by single point calculations at the Hartree–Fock level using the STO-3G basis set. These calculations showed that one $C_{12}E_4$ molecule encapsulated by a head-to-head βCD dimer is a stable model system in consonance with the experimentally determined stoichiometry, and that the 1 : 1 complex is not stable with respect to dissociation. In the stable 2 : 1 model system, the guest molecule is appreciably tilted with respect to the βCD dimer axis and presents a gradually bent alkylic chain in clear manifestation of conformational flexibility. Model calculations for βCD inclusion complexes of other oligo(oxyethylene) molecules further indicate that the number and strength of H$\cdots$H intermolecular close contacts reflect the position and conformational flexibility of the guest hydrophobic chain inside βCD.

Introduction

β-Cyclodextrin (cyclomalto-heptaose, βCD) is a cyclic oligosaccharide composed of seven α(1–4) linked glucopyranose residues in normal chair conformations. Its ability to form inclusion complexes by accommodating guest molecules of suitable size in its cavity derives mainly from its gross geometrical shape in the form of a hollow truncated cone [1]. Inclusion complexes involve noncovalent guest–host interactions. Since these are weak, molecular association is made possible by positive cooperativity of these interactions [2]. When the guest displays hydrophobic-hydrophilic behaviour, the βCD cavity has a tendency to host the hydrophobic fragment or part of it, leaving the hydrophilic moiety to interact mainly with hydroxyl groups of the βCD rims and with the solvent.

α-*n*-Dodecyl-ω-hydroxytetra(oxyethylene), hereafter referred to as $C_{12}E_4$, belongs 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 —$(OCH_2CH_2)_mOH$ moieties, respectively) [3]. Consideration of $C_{12}E_4$ as a guest molecule for inclusion in βCD in aqueous solution raises a few questions which will be addressed in this work.

Considering the lengths of the hydrophobic and hydrophilic moieties of $C_{12}E_4$, one might ask whether a true inclusion complex exists in solution and what will be its stoichiometry. Both of these questions will be addressed considering the induced upfield chemical shifts in the H3 and H5 protons of βCD caused by the anisotropic shielding by the encapsulated guest. Since these protons form two inner crowns inside the βCD cavity, the observation of these induced shifts is an indication of inclusion complex formation [4]. In addition, since the shifts vary monotonically with the host : guest molar ratio, they can be used to determine the complex stoichiometry [4]. We will resort to the continuous variation method for stoichiometry determination [5]. However, in an aqueous solution containing both βCD and $C_{12}E_4$, a competition is set up between complexation of the surfactant by βCD and self-association (micellization) of the surfactant monomers [6]. This competition inevitably leads to an increase in the amount of free (uncomplexed) βCD [6], thus affecting the interpretation of the continuous variation results and prompting a reanalysis of the method.

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In addition, one would like to know to what extent is the conformational freedom of $C_{12}E_4$ in the micellar state [7] restricted by the inclusion in βCD, and how does this conformation compare with the solid state conformation in the crystalline state at liquid nitrogen temperature [8]. It has been previously shown by Raman Optical Activity, that for βCD a tighter guest binding leads to an additional reduction in conformational flexibility of the cyclodextrin macrocycle [9]. Finally, the conformational flexibility of the alkylic chain in $C_{12}E_4$ can be used to enhance the host–guest cavity interaction. In general, the strength of the βCD-alkylic fragment interactions increases with the length of the alkylic chain [10]. Considering the large diameter of the βCD cavity and the flexibility of the alkylic fragment, it has been previously suggested that the hydrocarbon chain should coil inside the cavity [11]. In this work, the above questions will be addressed by taking advantage of the strategic positions of the βCD inner crowns of CH bonds, namely, those involving the H5 and H3 hydrogen atoms. These protons can be used as ^{1}H-NMR probes to report on close contact H$\cdots$H interactions with the guest, since the corresponding inner crowns of protons stay at different levels inside the hollow truncated cone of the βCD cavity. We will look for a correlation between the variations in the NMR shifts of these protons and the close contact distances evaluated with our model system calculations.

Materials and methods

βCD, kindly donated by Wacker-Chemie (Germany), was recrystallised prior to use. α-*n*-Dodecyl-ω-hydroxytetra(oxyethylene), $C_{12}E_4$, and C_4E_1, C_4E_2, C_6E_2, and phE_1 (= phenoxyethanol), briefly referred to in this study for comparison purposes, were obtained from Aldrich and used as received. Preparation of solutions in deuterium oxide from Aldrich, 99.9% deuterium, for ^{1}H-NMR studies, resorted to sonification by ultrasonic waves for 1 hour.

^{1}H-NMR spectra of deuterated solutions were recorded at 300 MHz, on a Bruker Avance 300 spectrometer, at 20 °C. The water chemical shift (δ = 4.63 ppm) was used as internal reference.

Molecular modelling for the inclusion complexes was carried out using the Gaussian 98 system of programs [12]. Considering the large number of atoms in the largest molecular system in this study, $\beta CD{\cdot}C_{12}E_4$ (365 atoms, 183 of which are non-hydrogen atoms), an adequate choice of the model chemistry had to inevitably result from a compromise between the available computer power and the desired level of calculation. This compromise is particularly stringent for full geometry optimization calculations, since these tasks are computationally expensive if carried out at the Hartree–Fock level for such a large system. However, this level of calculation is relevant for the guest molecule, in particular, for expressing the conformational flexibility of its hydrocarbon chain which is expected to coil inside the βCD cavity, as previously suggested [10, 11]. On the other hand, the MO level of calculation is neither affordable nor critical for the geometry optimization of the βCD dimer with its 294 atoms. Along this reasoning, we have chosen to use an integrated MO-MM method for the full geometry optimization of the host-guest complexes in this study. Gaussian enables this to be done through the use of the ONIOM approach which allows the definition of different layers of calculation in the studied system and deals with them in an integrated way [12]. In contrast with previous approaches, the ONIOM method is especially designed to allow the introduction of molecular mechanics corrections in full geometry optimizations [13]. Since the host–guest interaction is of the non-covalent type, the distinct layers of calculation were naturally defined to correspond to the host and guest molecules. In particular, in the full geometry optimization calculations, the cyclodextrin moiety – in the largest system herein considered, a βCD dimer – was dealt with at the molecular mechanics level with the UFF force field, and the guest molecule was treated at the Hartree–Fock level using the PM3 semi-empirical method. So, we have performed full geometry optimizations and frequency calculations on the host-guest systems using the ONIOM(PM3:UFF) keyword of Gaussian which specifies the higher (for the guest) and the lower (for the host) model chemistries, respectively. Energies for all the optimized structures were subsequently obtained by single point calculations at the Hartree–Fock level with the STO-3G basis set. GaussView was used to visualize the optimized structures and identify the vibrational modes whenever necessary [14].

Results and discussion

Stoichiometry of the inclusion complex

In principle, the stoichiometry of the inclusion complex in aqueous solution can be determined by a method due to Job [5] and generally known as the continuous variation method or Job's method. 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 ($r = [\beta CD]_0/\{[\beta CD]_0+[G]_0\}$), while maintaining constant the sum of the initial molar concentrations of host and guest ($[\beta CD]_0 + [G]_0$). In particular, 10 mM D_2O solutions of the guest and βCD were mixed

(i) to constant volume, i.e., the sum of the initial concentrations of βCD and G remained equal to 10 mM ($[\beta CD]_0 + [G]_0 = 10$ mM), and

(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 $r \cdot \Delta\delta$ against r, and finding the r value for the maximum of the distribution.

The H3 and H5 protons of βCD 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. Both H3 and H5 are shifted upfield due to anisotropic shielding caused by the encapsulated guest [4]. The experimental observation of these shifts enable us to infer that the host–guest

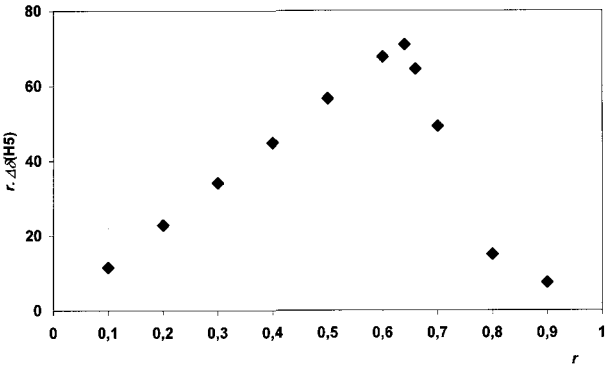

Figure 1. Continuous variation plot (Job's plot) for aqueous solutions in deuterated water of $C_{12}E_4$ (10 mM) and βCD (10 mM), where r is the mole fraction of βCD, and $\Delta\delta$(H5) is the variation in the chemical shift of the βCD H5 protons.

association is of the inclusion type, since the corresponding hydrogen atoms point towards the interior of the βCD cavity. In addition, no distinct resonances for the free host and the host–guest species could be observed, and the chemical shifts changed monotonically as the host : guest molar ratio was varied. Hence, the host-guest complexation system was considered to be in the NMR chemical shift fast exchange limit [15]. For a 300 MHz spectrometer, and a typical value of the largest observed chemical shift difference ($\Delta\delta_{\max} \approx 0.1$), 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 inclusion and release of the guest should occur at least 30 times/s. In this case, the observed chemical shifts of the host resonances are averages of the chemical shifts for the free and complexed states, weighted by the fractions of host molecules in each state [15].

Figure 1 presents the continuous variation results (Job's plot) for deuterated aqueous solutions of $C_{12}E_4$ and βCD, where r is the initial mole fraction of βCD, and $\Delta\delta$(H5) is the variation in the chemical shift of the βCD H5 protons. As can be seen, the maximum occurs at $r = 0.64$, a value which is intermediate to the r values for the 3 : 2 ($r = 0.60$) and 2 : 1 ($r = 0.66$) stoichiometries. One can then ask which of these corresponds to the true complex stoichiometry.

Application of the Job's method is straightforward for a single equilibrium, i.e., when there are no competing processes involving any of the complexation species. This is not the case in this study, since there is a competition for $C_{12}E_4$ due to its self- association (micellization) process [6]. In fact, there are two competing equilibria, one of the surfactant inclusion by βCD and the other for the surfactant micellar aggregation. The presence of βCD raises the surfactant cmc, and this effect is dependent on βCD concentration because the cmc is now the sum of the free and complexed surfactant [6].

Assuming that the surfactant micellar aggregation can be represented by a single process,

$$mS \rightleftarrows S_m, \tag{1}$$

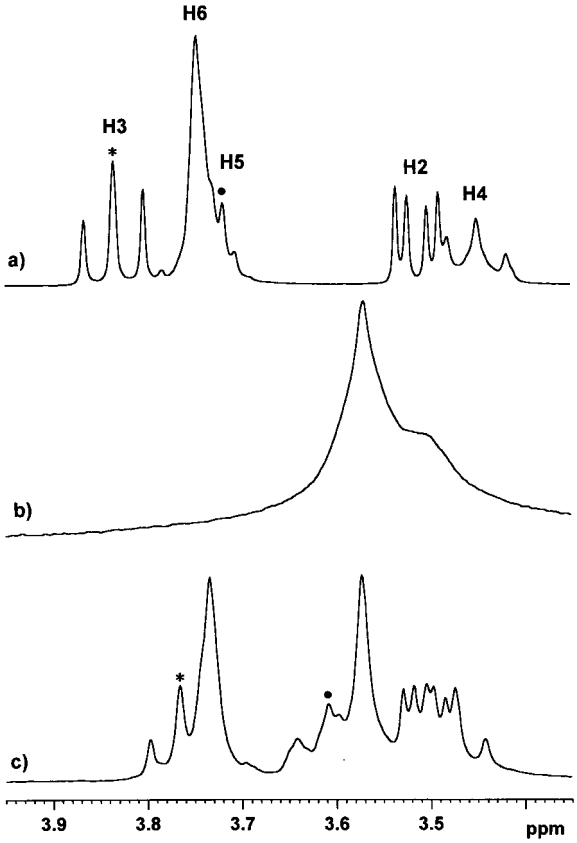

Figure 2. ^{1}H-NMR spectra for 10 mM aqueous solutions in deuterated water of βCD (a) $C_{12}E_4$ (b), and for the physical mixture of βCD and $C_{12}E_4$ which corresponds to $r_{\max} = 0.64$ (c).

where S stands for the surfactant monomer, and considering that the inclusion by βCD is described by

$$S + n\,\beta\text{CD} \rightleftarrows \beta\text{CD}_n \cdot S \tag{2}$$

the condition for a maximum in Job's plot, $d[\beta\text{CD}_n \cdot S]/dr = 0$, is accompanied by $d[S_m]/dr = 0$, since the above equilibria are coupled through the concentration of S, $[S]$, which appears in both equilibrium constants. It can be easily shown that these conditions lead to a maximum r value, $r_{\max}$, given by

$$r_{\max} = [n/(n+1)]\{1 - m[S_m]/C_0\}, \tag{3}$$

where C_0 is the sum of the initial concentrations of βCD and surfactant.

As can be seen from this expression, $r_{\max}$ differs from the usual maximum value at $n/(n+1)$ by a factor which deviates from 1 by the ratio of $m[S_m]$ and C_0. Introducing the experimentally determined maximum value, $r_{\max} = 0.64$, in the above expression, one arrives at an estimate for $m[S_m]$ which amounts to 3.5% of C_0. This shows that the true stoichiometry for the inclusion complex is 2 : 1, not the most unlikely 3 : 2 stoichiometry. In fact, self-association in the guest had the effect of deviating the Job's plot maximum from $r = 0.66$ to $r = 0.64$. As shown, this deviation in the maximum r value enabled us to estimate the relative amount of self-associated surfactant.

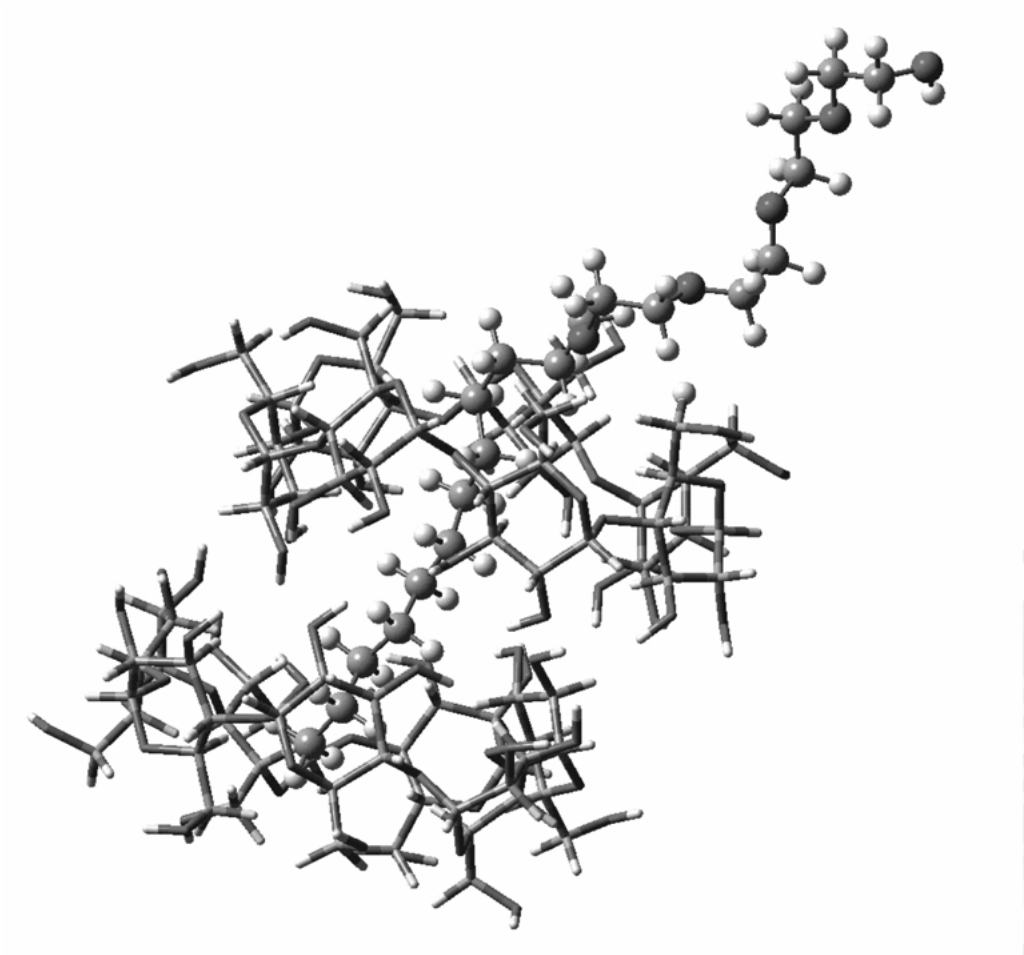

Figure 3. Calculated inclusion mode for the $\beta CD_2 \cdot C_{12}E_4$ complex. See text for details.

Figure 2 presents the ^{1}H-NMR spectra of 10 mM solutions of βCD, $C_{12}E_4$ and a physical mixture of βCD and $C_{12}E_4$ for $r_{max} = 0.64$, in deuterated water. The upfield variations in the chemical shifts of the H3 and H5 protons are 0.069 and 0.111 ppm, respectively. Since the H5 NMR shift is larger than for the H3 protons, one can infer stronger host-guest interactions with H5, which are closer to the narrower βCD rim.

The ^{1}H-NMR spectrum for the above mentioned aqueous solution of $C_{12}E_4$ presents very broad signals without any fine structure. In particular, the most intense signal of $C_{12}E_4$ in the chemical shift region of the βCD protons (Figure 2) shows an appreciably broad signal with a shape resembling that of an anisotropic system. In fact, the initial concentration of $C_{12}E_4$ (10×10^{-3} M) is more than two orders of magnitude larger than the $C_{12}E_4$ cmc ($\approx 6 \times 10^{-5}$ M) [16]. By contrast, in the spectrum of the physical mixture where βCD is also present, the same signal (slightly upfield shifted) becomes much narrower, exhibiting a typical isotropic liquid NMR shape. This further suggests that the presence of βCD had the effect of drastically increasing the cmc of $C_{12}E_4$, in consonance with previous studies with other surfactants [6].

Model inclusion complexes

Table 1 presents the STO-3G energies for the model systems considered in this study, with fully optimized geometries obtained at the ONIOM(PM3:UFF) level of calculation. From these values one can obtain the energy values for the gas phase reactions of formation of the 1 : 1 and 2 : 1 inclusion complexes, as well as for the gas phase dimerization of cyclodextrin (Table 2). The dimer system herein considered is the head-to-head dimer, in consonance with previous MD simulations which have shown that this is the most stable dimer, due to a larger number of intermolecular hydrogen bonds [17]. Our calculations have also confirmed this previous conclusion.

As can be seen from Table 2, the 1 : 1 model complex is not stable with respect to dissociation into one βCD and one guest molecules ($\Delta E = 21$ kJ mol^{-1}), whereas the 2 : 1 model complex is both appreciably stable with respect to dissociation into two βCD molecules and one guest molecule ($\Delta E = -252$ kJ mol^{-1}), as well as with respect to dissociation into the head-to-head βCD dimer and the guest molecule ($\Delta E = -236$ kJ mol^{-1}). These results are in agreement with the experimental results. In particular, the large negative ΔE value for the formation of the 2 : 1 inclusion complex in contrast with the small positive value for the 1:1 stoichiometric ratio are in consonance with the easy

Table 1. STO-3G//ONIOM(PM3:UFF) gas phase energies for the studied systems.

System	E/E_h[a]
$C_{12}E_4$	−1141.87885
βCD	−4196.40142
βCD·$C_{12}E_4$	−5338.27215
βCD_2	−8392.80872
βCD_2·$C_{12}E_4$	−9534.77760

[a] $1E_h = 2625.500$ kJ mol^{-1}.

Table 2. STO-3G//ONIOM(PM3:UFF) gas phase reaction energies for the studied systems.

Reaction	ΔE/kJ mol^{-1}
$\beta CD + C_{12}E_4 \rightarrow \beta$CD·$C_{12}E_4$	21
$2\,\beta CD \rightarrow \beta CD_2$	−16
$2\,\beta CD + C_{12}E_4 \rightarrow \beta CD_2$·$C_{12}E_{24}$	−252

preparation of the inclusion complex and with the marked maximum near the point $r = 2/3$ in the Job's plot.

It is generally accepted that the solid state molecular conformation should, in principle, be strongly correlated to the molecular forms of lowest energy in solution. Therefore, we used the helical conformation of $C_{12}E_4$ in the crystalline solid at liquid nitrogen temperature [8] as input geometry for building the complex model system. Nevertheless, the optimized geometry in the inclusion model system (Figure 3) deviates from it, resembling rather the predominant liquid form (intermediate or meander conformation) [7]. In addition, the process of inclusion in βCD had the effect of reducing by ca. 10° the tilt angle between the alkylic chain and the oxyethylene axes [18], a result that should reflect the geometrical constraints imposed on $C_{12}E_4$ by the less flexible βCD dimer.

Since our model system does not take account of the solvent which is likely to mostly affect the polar oxyethylene moiety, we restrict ourselves to considerations on the encapsulated guest alkylic chain. As can be seen from Figure 3, the guest molecule is appreciably tilted with respect to the βCD dimer axis and presents a gradually bent alkylic chain in clear manifestation of its conformational flexibility. By contrast, the *all-trans* form is the most stable form for the alkylic chain of the isolated molecule.

Finally, the above results on the model system for the inclusion of $C_{12}E_4$ in βCD and the results of additional model system calculations for other included guests with 1 : 1 stoichiometries, namely, for C_4E_1, C_4E_2, C_6E_2 and phE_1, suggest that the number and strength of H···H intermolecular close contacts reflect both the relative position and conformational flexibility of the guest hydrophobic chains inside the βCD cavity, in apparent confirmation of what has been previously concluded [10, 11].

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