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Solubility of cyclomaltooligosaccharides (cyclodextrins) in H_2O and D_2O: a comparative study

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Abstract—Cyclomaltooligosaccharides (cyclodextrins, CDs) are cyclic oligomers having six, seven, or eight units of α-D-glucose, named as cyclomaltohexaose (α-CD), cyclomaltoheptaose (β-CD) and cyclomaltooctaose (γ-CD), respectively. The molecule of CD has a cavity in which the interior is hydrophobic relative to its outer surface. The solubility of cyclodextrins in water is unusual, as an irregular trend is observed in the series of the cyclic oligomers of glucose. β-CD is at least nine times less soluble than the others CDs. This intriguing behavior has been investigated, and some interesting explanations in terms of the effect caused by CD on the water lattice structure have been proposed. In this work a comparative study on the solubility of α, β, and γ-cyclodextrins was carried out in H_2O and D_2O and reveals a much lower solubility of the three CDs in D_2O. The solid-phase structure of the CDs in equilibrium with the solution is quite similar with both solvents. The results are discussed in terms of the CD molecular structure and the differences in the hydrogen bonds formed between H_2O and D_2O.

Keywords: Solubility of cyclodextrins in H_2O and D_2O; Hydrogen bonding; Hydrophobic effect

1. Introduction

Cyclomaltooligosaccharides (cyclodextrins, CDs) are cyclic oligosaccharides consisting of six (cyclomaltohexaose, α-CD), seven (cyclomaltoheptaose, β-CD), or eight (cyclomaltooctaose, γ-CD) glucose units linked by 1,4-α-glucosidic bonds. The structural consequence of this bonding mode is the formation of a shallow truncated 'cone shape' with *n* (number of glucose residues) primary hydroxy groups lined in one rim of the cone, and 2*n* secondary hydroxy groups lined at the other rim. A cavity arises from this structure containing rows of CH groups (C-3 and C-5) and a row of glucosidic oxygens. Therefore, the cavities are nonpolar relative to their outer surface.[1,2] The cavities can act as hosts for a great variety of monomer guests,[3] and also to polymeric chains. In the latter case supramolecular adducts are produced by the threading of the polymer chain into the cavity of the CD, forming interesting structures such as *necklaces*.[4–8] In spite of the great scientific interest in such supramolecular chemistry, the understanding of the driving forces that promote the guest–host complex formation in water is unclear. The role of the interaction between the CDs and water molecules, and even their anomalous solubility is still inconclusive.

The solubility of cyclodextrins in water is unusual, as an irregular trend is observed in the series of the cyclic oligomers of glucose. β-CD is nine and 11 times less soluble in water in comparison to α and γ-CD, respectively.[3,9] This intriguing behavior has been discussed in terms of the effect caused by CDs on the water lattice structure. The local water density around the molecules of the three CDs was determined using molecular dynamics, and the result reveals that β-CD induces stronger ordering on the surrounding water in comparison with the others.[10] The enthalpy of solutions of the three CDs is very similar, but the entropy of solution of β-CD is lower relative to the others, as shown in Table 1.[9] Therefore, the lower solubility of β-CD

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Table 1. Thermodynamic parameters for dissolution of cyclodextrins in aqueous saturated solution (adapted from Ref. 9)

Cyclodextrin	$\Delta G°$/kcal mol^{-1}	$\Delta H°$/kcal mol^{-1}	$\Delta S°$/cal mol^{-1} K^{-1}
α-CD	3.58	7.67	13.8
β-CD	4.81	8.31	11.7
γ-CD	3.34	7.73	14.7

in water is driven by the less favorable entropy.[11] The effect can be even reinforced if D_2O is considered. D_2O forms stronger hydrogen bonds than H_2O, as a consequence of the smaller quantum mechanical zero-point energy. The effects that arise from the difference in the hydrogen bonding between D_2O and H_2O influence a wide range of molecular phenomena in aqueous solution, ranging from gas solubility, formation of membranes and folding of proteins.[12]

The rate of threading of the poly(ethylene glycol) chain into the α-CD cavities, forming the polypseudorotaxanes, is higher in D_2O, in comparison to the rate of threading in H_2O. This result has been attributed to the stronger hydrogen bonding formed with D_2O compared to H_2O.[13,14] Harada pointed out that the formation of the complex is entropically unfavorable as the polymer chain must be fit into several host units.[5] The entropic gain related to the release of free water molecules to the bulk phase is not enough to drive the process. On the other hand, the difference in the kinetics of the complexation in light and heavy water suggests an important role of the hydrophobic effect. In order, to investigate the behavior of the CDs in both solvents, we developed a systematic study of the solubility and the kinetics of crystallization of CDs in H_2O and D_2O. In this work we present results in which remarkable differences in these properties are observed. The results are discussed in terms of the numbers of the glucose units that form the cyclic oligomers, and in the difference in the hydrogen bonds formed between H_2O and D_2O molecules.

2. Experimental

2.1. Purification of CDs

α-CD (lot 60T005), β-CD (lot 70P229), γ-CD (lot 80P200) were supplied by Wacker Chemical Co. α-CD and γ-CD were purified using five steps of recrystallization from concentrated aqueous solutions by adding small amounts of EtOH (E. Merck). Supersaturated solutions (25% w/w) of α-CD and γ-CD were prepared in water at 70 °C, and kept at this temperature for 24 h. The solutions were centrifuged for 10 min at 3,000 rpm at room temperature, and then the supernatants were separated from the solid phase. EtOH (E. Merck) was then added to the solutions, in order to precipitate the CDs. The solid phases were filtered, washed with cold water and dried at 80 °C for 24 h. Due to the low solubility of β-CD in water and the low recovery by addition of EtOH, it was purified by recrystallization from a saturated solution (7% w/w). Part of the purified samples were used to prepare supersaturated solutions in D_2O (Goss), and recrystallized in this solvent in order to prepare D_2O hydrates of the samples.

2.2. Equilibrium solubility studies

An adaptation of the method proposed by Jozwiakowski and Connors[9] was used in the determination of the solubility of the three CDs. The proportions of 15%, 4%, and 28% (w/w), respectively, of purified α, β, and γ-CD were placed in vials with H_2O and with D_2O. The samples were agitated by rotation in a constant-temperature bath at 25.00 ± 0.01 °C for 10 days. The supernatants were then separated from the solid phase at 25 °C and filtered with Milli-Q membrane filters (0.45 μm pore size) upon injection from 3-mL disposable plastic syringes. Supernatant (1 g) of each sample was placed in at least eight different Eppendorf vials. The samples were dried at 70 °C for 5 days and weighed to within ±0.0001 g.

2.3. Kinetics of crystallization

Supersaturated solutions of α-CD in H_2O and in D_2O were prepared by heating the solutions at 70 °C and then centrifuging for 1 h at 10,000 rpm at 25 °C. The supernatants of both solutions were then distributed among several Eppendorf vials and kept in a constant-temperature bath at 25 °C without agitation. The supernatant present in each Eppendorf vial was then sampled at different times. These samples were dried in an oven at 80 °C for 5 days, and the mass of solid was then measured. The concentration of α-CD (weight of α-CD/weight of solution) present in the supernatant was determined from the average of several duplicate runs.

2.4. Sorption experiments

Dried CD samples were kept in contact with the vapors of H_2O and D_2O for 10 days in a constant-temperature bath at 25 °C without agitation. Thermal gravimetric analysis (TGA) of the dehydrated and hydrated CD were obtained by using a TA instruments model 2950 for temperatures ranging from 25 to 300 °C using a heating rate of 10 °C/min in order to determine the number of water molecules (H_2O and D_2O) of the stable hydrates. X-ray diffraction measurements of the hydrates were performed with a powder diffractometer (Shimadzu XRD 7000, Cu $K_\alpha = 1.5406$ Å, 40.0 kV, 30.0 mA).