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Aggregation of Cyclodextrins: An Explanation of the Abnormal Solubility of β-Cyclodextrin

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Abstract. α-, β- and γ-Cyclodextrins have been shown to exist as aggregates in solution bound together by a network of hydrogen bonds. Removal of this network by ionisation of the hydroxyl groups leads to a greatly increased solubility and removal of aggregation. The presence of aggregates in solution of structure breaking solutes in which the solubility of β-cyclodextrin is greatly enhanced, leads to a proposal that the abnormally low solubility of β-CD may be explained by the presence of aggregates and the unfavourable interaction of these aggregates with the hydrogen bonded structure of water.

Key words. Cyclodextrins, solubility, aggregates, light scattering.

1. Introduction

The cyclodextrins (CDs), cyclic oligosaccharide molecular hosts, have been employed for the formation of a wide variety of inclusion compounds and are now beginning to be exploited commercially [1]. They are of interest as enzyme mimics [2], and in consequence considerable research has been undertaken on their chemical modification [3]. However, exploitation of the most readily available compound, β-cyclodextrin, has been limited by its low solubility in aqueous solution (18.5 g L^{-1}). It has been previously suggested that α- and γ-cyclodextrin may be associated in solution, at least as dimers, or possibly larger aggregates, and that these compounds may be of the structure making type. The aggregation process is supported by evidence from the concentration dependence of viscosity measurements [4]. However, surprisingly, no similar information has been published concerning the association of β-CD. The current work shows the presence of large aggregates for α-, β- and γ-CD. At low pH ionisation of the hydroxyl groups leads to dispersion of these aggregates, leading in turn to increased solubility. The solubility of β-CD is increased in the presence of structure breaking solutes but with retention of aggregates. The evidence leads us to propose that the solubility of the CDs is related to the interaction of these aggregates with the structure of water.

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2. Experimental

We have undertaken a series of light scattering experiments on aqueous solutions of the cyclodextrins and methyl derivatives of β-CD. The experiments at neutral pH were carried out on solutions of 5, 10 and 15 g L^{-1} for β-CD; 10, 20, 50 and > 100 g L^{-1} for α-CD, 10 g L^{-1} for γ-CD; 10 g L^{-1} for β-CD in varying concentrations of urea solution (2–8 M); and 20, 50 and 100 g L^{-1} for methyl-β-CD derivatives. For the variable pH studies the β-CD concentration was 10 g L^{-1}. All concentrations used, except for α-CD at >100 g L^{-1}, were well below saturation values.

3. Results and Discussion

The results obtained for a light scattering angle of 90° are given in Table I, together with the solubilities, for comparison.

Aggregate sizes of 2100 Å were observed for β-CD and were constant for samples from a number of different sources and for β-CD recrystallised, partially deuterated, treated at pH 14 and subsequently neutralised, and for light scattering apparatus from a variety of suppliers. The results of both dynamic and static experiments demonstrate the presence of large aggregates showing considerable polydispersity (>0.1), for the unsubstituted CDs in both aqueous and urea solutions. The angular variation of the scattering angle for β-CD shows a slight increase, as the angle decreases, to yield at an extrapolated zero angle an apparent diameter of 3000 Å.

The values obtained for aggregate size were observed to increase slightly with concentration for α-CD rising to 300 nm at 50 g L^{-1}; however, at $C > 100$ g L^{-1} extremely large aggregates (2700 nm) could be observed. As this value is approaching saturation values, we may expect the aggregates to be forming larger systems prior to the crystallisation processes. For α-CD at 10 and 20 g L^{-1} the aggregate sizes are 1940 and 2090 Å, with error limits of ±100 Å, and for β-CD the values at 10 and 15 g L^{-1} are 2000 and 2100 with error limits of ±100 Å, thus a slight increase in size may exist with increasing concentration at these low values.

With regard to the methylated CDs an apparent diameter of 40 Å is observed for the 2,6-dimethyl-β-CD. The presence of the OH(3) hydroxyl groups may allow dimerisation of the molecule, which would be in accord with the observed size.

Table I. Aggregate size and cyclodextrin solubility.

	Solubility g L^{-1}	Aggregate Size Å (25 g L^{-1}, ±100 Å)
α-CD	145	2000
β-CD	18.5	2100
γ-CD	232	2000
β-CD^{n-}	750	20
dimethyl β-CD	570	40
trimethyl β-CD	200	25

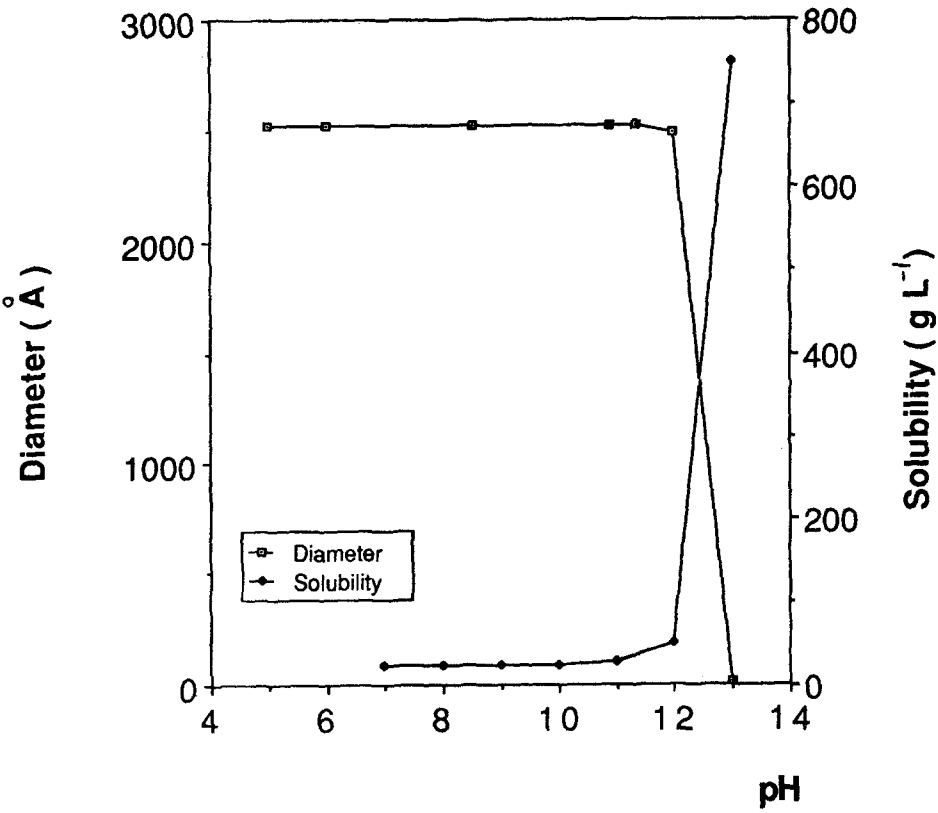

Fig. 1. Effect of pH variation on aggregate size and solubility of β-cyclodextrin.

For the trimethyl derivative no aggregation via hydrogen bonding is possible and the observed apparent diameter of 25 Å is in agreement with a monomeric structure.

The hypothesis that cyclodextrin aggregates are held together by intermolecular hydrogen bonding and bridging water molecules is supported by the variation of aggregate size as measured by dynamic light scattering and solubility as a function of pH, as shown in Figure 1. Both size and solubility remain constant up to pH 12.5, where the solubility rises rapidly to 750 g L^{-1} and the apparent diameter decreases sharply to 20 Å. The sharp diminution in size and increased solubility may be explained by ionisation of the hydroxyl groups and aggregate dissociation as a result of electrostatic repulsion. The pK_a value for β-CD has been reported by Gelb [5] as 12.2 at 25°C. Thus, at the pH values necessary for aggregate dissociation, we may expect that multiple ionisation of the hydroxyl groups has occurred. It is suggested that the first step in the ionisation process involves complexation of [OH^-] by the cyclodextrin [5b]. We have shown that in the KOH inclusion complex the OH^- group is found within the cavity: more importantly, this structure is isostructural with β-CD · 12 H_2O [6].

The ^1H NMR spectra of $NaOD/D_2O$ solutions of β-CD at pH 13 show upfield displacements of the H(2) and H(3) protons consistent with the presence of negatively charged O(2) and O(3) atoms. Neutralisation shows that no epimerization occurs [7]. Less dramatic changes in the solubility of α- and γ-CD are also observed at high pH values.

From the above evidence it appears that removal of aggregation from CDs or their derivatives leads to a considerable increase in the solubility. However, as both α- and γ-CD are also present in solution as aggregates the abnormally low solubility of β-CD may not be explained simply in terms of aggregation.

It has previously been reported that the solubility of β-CD increases in aqueous solutions of certain salts [8], and also in urea solutions [9]. We have observed that the solubility of β-CD increases in $CaCl_2$ and $Ca(NO_3)_2$ solution. Measurement of the aggregate size by light scattering as a function of urea concentration shows a nearly constant value for β-CD. The structure of β-CD shows double columns of CD molecules in a herringbone pattern held together by hydrogen bonds and separated by zones of water [6], and it may be proposed that the CD aggregates are present as relatively ordered, rod-like structures in solution, resembling an ordered helical structure of amylose [10]. This is further supported by the crystalline KOH inclusion complex which, as has previously been stated [6], is isostructural with β-CD. As stated by Gelb, events leading to the ionisation of CDs and hence aggregate dispersion, involve complexation of $[OH^-]$, thus the presence of identical herringbone structures for these compounds favours the presence of similar structures for the observed aggregates.

This is supported by an approximate aggregate mass of 7×10^5 mass units calculated from light scattering, which is in reasonable agreement with two chains of hydrated β-CD molecules forming a rod-like aggregate of the appropriate length.

The high solubility of monosaccharides in solution has been explained by the favourable geometry of the hydroxyl groups, which allows them to interact with water molecules in the hexagonal structure of water [11]. Treating first the non-aggregated CDs, it can be seen that the glucoside units may interact favourably with the water structure along the axis of the molecule. However, for the aggregates, the occurrence of such interactions will be restricted only to the ends, and it may be proposed that the axial symmetry may now become a determinant factor in the interaction between the CDs and water. For α- and γ-CD with 6- and 8-fold symmetry, respectively, an incompletely rigid rod may interact with little or no perturbation of the water structure. However, for β-CD, which has a 7-fold symmetry, any interaction will lead to a perturbation of the water structure. Hence, as previously noted, α- and γ-CD should be structure-making solutes [4], while it is expected that β-CD will be a structure-breaking solute. This is further confirmed by the solubility effects in urea, itself a structure breaker. As the structure of water is reduced, β-CD becomes much more soluble, whereas α-CD initially becomes less soluble.

The above results suggest that the abnormally low solubility of β-CD may be explained by the presence of aggregates in solution and the interaction of these aggregates with the surrounding water structure.

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