Hydroxypropyl-β-cyclodextrins: induced circular dichroism spectra of included phenolphthalein as a function of the degree of substitution

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The hydroxypropylation of β-cyclodextrin does not change the essential nature of its interaction with phenolphthalein but the pattern and degree of substitution do alter both the stability constant of the inclusion complex and its induced circular dichroism spectrum. The reason for this phenomenon is that substitution of the hydroxy groups (especially that of the primary ones) in cyclodextrin may cause increased steric hindrance and provide modified possibilities for hydrogen bonding.

Introduction

Among recently synthesized cyclodextrin derivatives 2-hydroxypropyl-β-cyclodextrins (HP-βCDx) are the most promising, since they can be manufactured on a large scale at a fair price; their solubility, solubilization power and toxicology/safety properties are excellent.[1]

βCDx (cyclomaltoheptaose) is hydroxypropylated using propylene oxide in alkaline solution.[1,2] The degree of substitution (DS) means either the average number of HP units per glucose units or (more often) that of the HP/CDx ratio. Consequently, the DS value gives no information either on the position (2, 3 or 6) and type (primary: 6, secondary: 2, 3) of the substituted hydroxy groups or on the kind of substituent {monomeric or oligomeric: $[-CH_2CH(CH_3)O]_n-H$}. The DS is generally measured by NMR spectroscopic or GC methods.[1] Plasma desorption mass spectrometry[2] provides the possibility of detecting the differently-substituted species, but an analysis covering every detail is rather complex.[3,1] Differential scanning calorimetry is a simple and rapid method which is able to detect over and above the average DS the individual DS values of the various fractions in one sample.[4]

These investigations prove the high variability of HP-βCDx samples in chemical composition. The samples are always complex mixtures of differently substituted isomers which is reflected in their amorphous nature and high solubility.[1] The capability of βCDx to form inclusion complexes is retained, but the stability constants are somewhat decreased with increasing DS. This effect has been measured using phenolphthalein (PP) as an example for large guest molecules.[5] In some cases we obtained significantly different association constant values for different samples of the same average DS, showing that an identical DS value can cover different patterns of substitution.

Circular dichroism spectroscopy is a very good tool for investigating inclusion complexes, since induced circular dichroism (ICD) at the absorption bands of the guest molecule depends not only on the strength (thermodynamics) of the interaction but also on the method of inclusion.[6] This method was used to study the PP-βCDx complex itself[7] and it seemed to have the potential to reveal the discrepancies found in the stability constants.

Experimental

The HP-βCDx samples were from Cyclolab Ltd. (Hungary), prepared in different ways (including different alkalinities which influence mostly the pattern of substitution). All other materials were of analytical grade and were used without further purification, except for phenolphthalein, which was recrystallized twice from an ethanol–water mixture.

The absorption spectra were recorded on a Perkin-Elmer Lambda 15 spectrophotometer and the stability constants of the complexes were measured as published earlier.[5] All PP-HP-βCDx inclusion complexes have 1 : 1 stoichiometries.

ICD spectra were measured using a Jobin–Yvon–Dichrograph Mark VI. The concentration of phenolphthalein was (1.5–3.0) × 10^{-5} mol dm^{-3}, and the appropriate HP-βCDx was in a relatively large excess so as to achieve almost full complexation of phenolphthalein wherever possible. The temperature was 25.0 ± 1.0 °C while the pH was kept constant at pH = 10.5.

Results and discussion

As has been mentioned, the stability constants of PP-HP-βCDx inclusion complexes are systematically decreased by increasing DS. The maximum values measured for samples of different DS are represented in Fig. 1. [For unsubstituted βCDx[7] K = (2.3 ± 0.2) × 10^4, and the standard error for other K values was ±12% or better.] All other K values (K = [PP•HP-βCDx]/[HP-βCDx][PP]) found are lower and fall within the hatched part of the Figure. It seems that the continuous line connects the formation constants of PP complexes with 'regular' HP-βCDx representatives of the given DS. 'Regular' means in this respect the best pattern of βCDx substitution for the complex formation with PP and up to now, these 'regular' HP-βCDx complexes have been found to be those characterized by higher substitution on the secondary rim than on the primary one. We have not measured any constant falling on the line beyond DS = 14.

We have measured the lowest formation constants with samples prepared in highly alkaline solution which promotes the formation of species substituted on primary hydroxy groups or by dimeric (or oligomeric) HP substituents.[1,8] For example, we investigated three samples of DS = 8.0 and measured a stability constant for the 'regular' one which was more than six times greater than that for the most 'irregular' one. Their absorption spectra are identical as shown in Fig. 2 —the difference at λ = 550 nm is caused by the different extent of complexation by identical concentrations of HP-βCDx. In fact, no band can be observed in their ICD spectra at λ = 550 nm but the characteristic bands[7] in the UV are

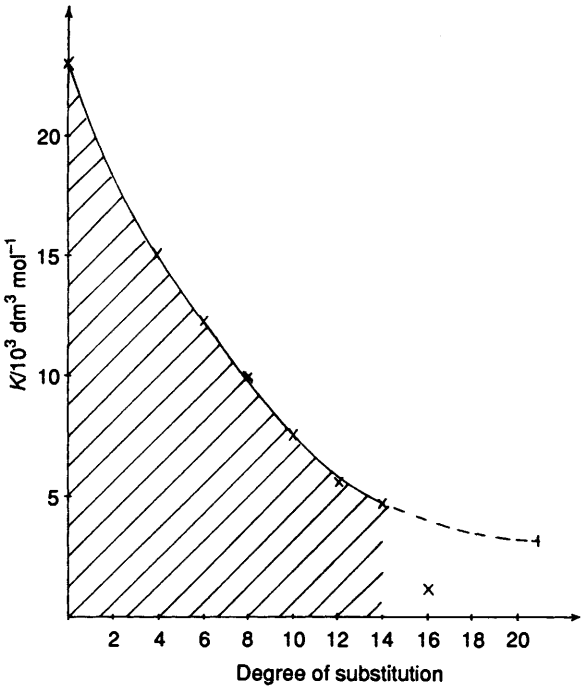

Fig. 1 Stability constants of PP-HP-βCDx inclusion complexes (K/dm^3 mol^{-1}) as a function of DS (pH = 10.5, T = 25 °C; x and — highest values found with 'regular' substitution; /// area for values of 'irregular' substitution)

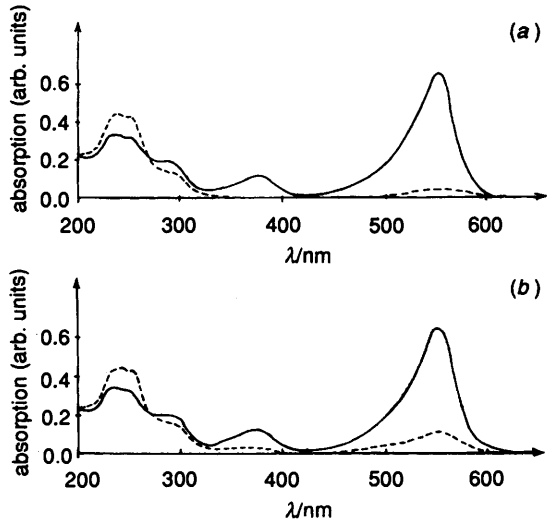

Fig. 2 UV-visible spectra of phenolphthalein at pH = 10.5 and T = 25 °C (— in the absence and - - in the presence of HP-βCDx of DS = 8.0 in similar excess; (*a*) with a 'regular' [K = (9.0 ± 0.3) × 10^3] and (*b*) with an 'irregular' [K = (1.29 ± 0.11) × 10^3] sample)

totally different for the two samples since they have opposite signs (Fig. 3).

The ICD spectrum of 'regular' HP-βCDx with phenolphthalein resembles that of a PP-βCDx complex.[7] The structure of the very stable PP-βCDx complex was explained by a three-site interaction, where (i) one of the phenolic rings of phenolphthalein is included in the cavity of CDx, (ii) its phenolate (or phenolic, or quinoidal) oxygen forms a hydrogen bond with the primary hydroxy groups of CDx, while (iii) the secondary hydroxy groups of the other rim interact with the carboxylate substituent perturbing the planar symmetry of the central (methane or carbenium type) carbon atom (see Fig. 4, ref. 7 and references therein).

The opposite signs in the ICD spectrum of phenolphthalein complexed by 'irregular' HP-βCDx (Fig. 3) must mean that the inclusion of phenolphthalein is disturbed by the hydroxypropylation. (Nevertheless, the complex is indeed formed, as proved by the existence of an ICD spectrum and by the lack of a band at λ = 550 nm.) We can conclude that substitution of CDx hydroxy groups may cause increased steric hindrance and provide modified possibilities for hydrogen bonding. The 'irregular' hydroxypropylation can influence not only the direction but also the depth of inclusion which can result in a change of the ICD band sign.[9,10] Because of this hindrance, phenolphthalein can be fixed in an inclined position bound with hydrogen bonds to HP substituents as well.

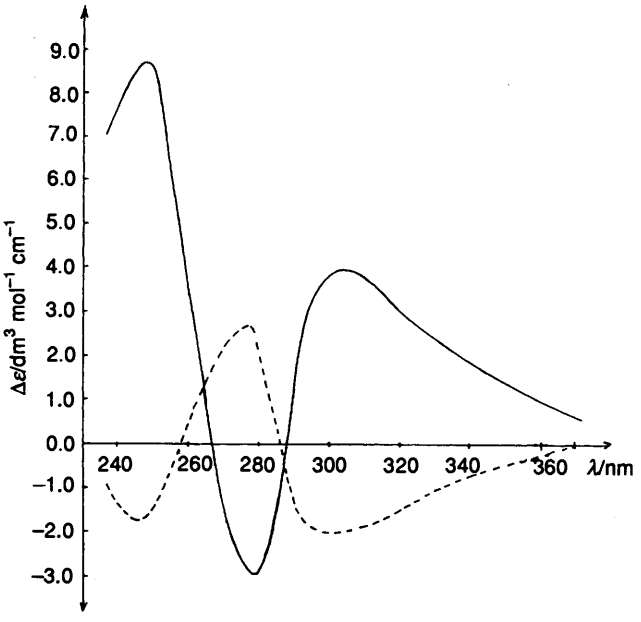

Fig. 3 ICD spectra of the inclusion complexes shown in Fig. 2 (- - 'regular' and — 'irregular' sample)

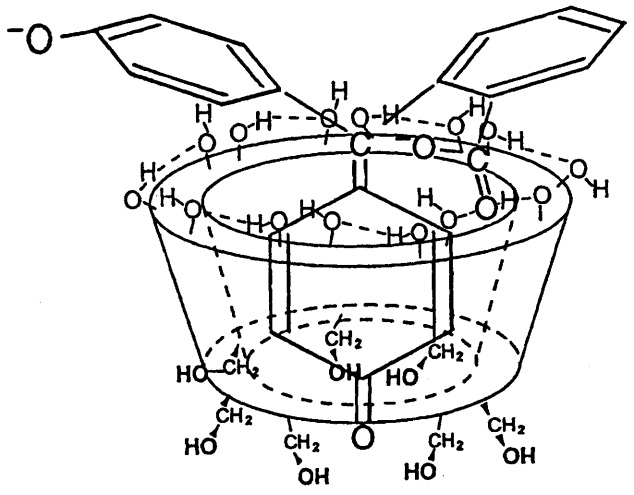

Fig. 4 Rough sketch of the PP–CDx inclusion complex

The stability constants and ICD spectra of phenolphthalein inclusion complexes have been investigated with an ample collection of HP-βCDx samples of different DS values prepared at different alkalinities, therefore the ratio of substituted primary to secondary hydroxy groups ranged from 2:1 to 1:9.[5] The two characteristic Δε values as well as their differences are represented in Fig. 5 as a function of DS. Similar to the K constants *versus* DS relations (illustrated in Fig. 1), a clear connection is found only with those samples originating from identical (or at least from similarly prepared) batches.

A radical change is observed when the Δε values mentioned are arranged as a function of the appropriate stability constants measured separately (Fig. 6). The good correlation proves the identical background of these basic characteristics as well as the

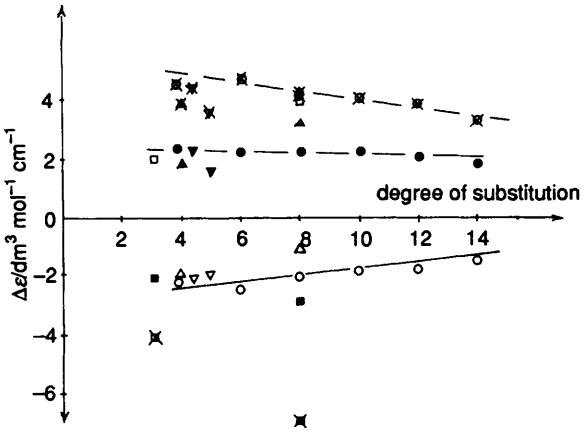

Fig. 5 $\Delta\varepsilon$ values of PP–HP-βCDx inclusion complexes as a function of DS (pH = 10.5; T = 25 °C; open symbols: values measured at λ = 300 nm; filled symbols: those at λ = 270 nm; crossed symbols: $\Delta\varepsilon_{270}$–$\Delta\varepsilon_{300}$; o, one batch prepared 'regular' samples; △ and ▽, two other, less 'regular' series; □, 'irregular' samples)

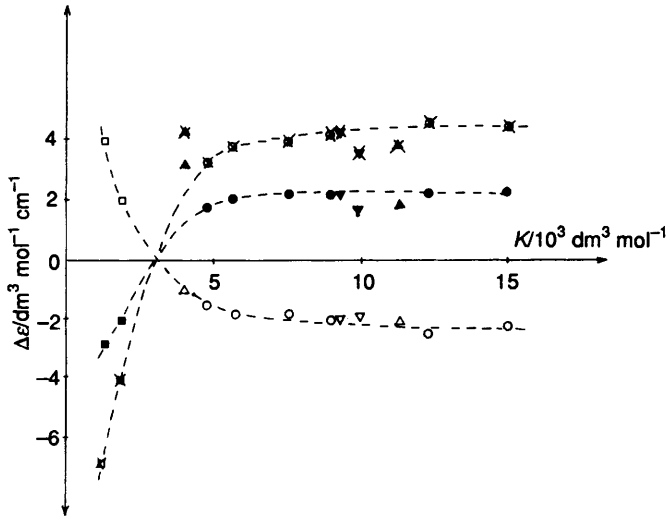

Fig. 6 $\Delta\varepsilon$ values of PP-HP-βCDx inclusion complexes as a function of stability constants measured (conditions and legends as in Fig. 5)

picture outlined for the perturbance of the three-site interaction between phenolphthalein and CDx (and the sign change in the ICD spectra).

To date we could find no PP-CDx inclusion complex with stability constant $K \approx 3000$, but the lack of an ICD spectrum can be predicted based on Fig. 6, and we are looking for this very interesting case.

Quantitative determination of most of the parent and chemically-modified CDx complexes in biological fluids is carried out by an HPLC method using a unique, post-column detection. This detection is based on the decoloration process of an alkaline solution of phenolphthalein observed in the presence of cyclodextrins,[11] and has been successfully used for years in pharmacokinetic studies of parenterally administered HP-βCDx drug complexes.[12]

The practical usefulness of our results will certainly be manifested in the pharmacokinetic studies of CDx complexed drugs, since the different DS and substitution pattern of different manufactured HP-βCDx products may result in alterations in detection sensitivity and reproducibility of the HPLC method.

On the other hand, since there is no simple possibility of determining directly the spatial distribution of substituents, the combined application of these two methods seems to be an appropriate tool for the qualification of HP-CDx products with respect to the similarity or difference in the disposition of substituent groups.

Acknowledgements

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