

Comparison of Association Constants of Cyclodextrins and Their *tert*-Butyl Derivatives With Halogenbenzoic Acids and Acridine Derivatives.

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Abstract: Association constants of complexes of a new class of cyclodextrin alkyl derivatives - randomly substituted *tert*-butyl derivatives (TB-CDs) with complete set of mono-halobenzoic acids and acridine derivatives were measured using capillary electrophoresis and compared with association constants of natural cyclodextrins. In most cases the association constants of natural cyclodextrin and its corresponding *tert*-butyl derivative were comparable. Strong dependence of association constants on actual cyclodextrin concentration was observed for complexes of *tert*-butyl- α -cyclodextrin with acridine derivatives. Significant increase of the association constant occurred below the critical micelle concentration of the cyclodextrin derivative, while an increase above this value was less significant.

Keywords: association constant, cyclodextrins, *tert*-butyl-cyclodextrins, capillary electrophoresis, benzoic acids, acridines, critical micelle concentration

Introduction

Natural cyclodextrins (CDs) comprise a family of macrocyclic oligosaccharides, formed by α -1,4-linked glucopyranose subunits, and shaped as truncated cones [1, 2]. CDs are typical host molecules and they form inclusion complexes with a great variety of compounds consisting of one or two benzene rings, or even with larger polycyclic ones carrying a side chain of suitable size to form inclusion complexes.

Besides natural cyclodextrins, the growing number of semi-synthetic derivatives and co-polymers has been prepared and is already commercially available. Many of them found use as structural and chiral selectors, with new properties given by the type and number of substituents [3-5].

The aim of this work was to study the complexation behavior of a new class of alkyl-cyclodextrin derivatives, randomly substituted *tert*-butyl-cyclodextrins [6]: *tert*-butyl- α -cyclodextrin (TB- α -CD), *tert*-butyl- β -cyclodextrin (TB- β -CD) and *tert*-butyl- γ -cyclodextrin (TB- γ -CD). Association constants of natural cyclodextrins (α -, β - and γ -CD) and these new derivatives with a complete set of mono-halogenbenzoic acids and newly synthesized derivatives of acridine were measured and compared.

Equilibrium constants for molecular association (binding constants, also called complexation constants, association constants, or formation constants) have been measured using a variety of experimental approaches, including microcalorimetry [7-10], spectroscopy [11], circular dichroism [12], fluorescence [13-15], nuclear magnetic resonance (NMR) [16], gas- [17] and liquid chromatography [18-20]. In principle it is easy to obtain value of association constant by employing capillary zone electrophoresis (CZE) [21].

During electrophoretic separation cyclodextrins, as host molecules, form intermediate complexes with chiral or achiral guest molecules. Selectivity of the separation is given by the differences in stabilities of these complexes. The association constants can be calculated from the relationship between concentration of ligand and the measured electrophoretic mobility of the solute. The equation that relates mentioned quantities was introduced by Alberty and King [22]. For a single binding scenario, this relationship is:

$$K[L] = \left(\frac{\mu_f - \mu_i}{\mu_i - \mu_c} \right) \quad (1)$$

where K is the binding constant, $[L]$ is the equilibrium concentration of uncomplexed ligand and μ_f , μ_c are the electrophoretic mobilities of free and complexed solute; μ_i is the solute mobility measured at ligand concentration $[L]$.

For many systems μ_c cannot be accurately measured due to experimental limitations, such as ligand solubility or capillary wall-ligand interactions. Capillary electrophoresis computation of association constants is done by solving Eq. 1 for the experimentally measured variable, μ_i , to give:

$$\mu_i = \frac{\mu_f + \mu_c K[L]}{1 + K[L]} \quad (2)$$

Eq. 2 can be transformed into the linear form (Eq. 3) by referencing all measured electrophoretic mobilities:

$$\frac{[L]}{(\mu_i - \mu_f)} = \frac{1}{(\mu_c - \mu_f)} [L] + \frac{1}{(\mu_c - \mu_f)K} \quad (3)$$

This linear plotting method does not require knowledge of the complex mobility, which can be difficult or impossible to measure.

Results and discussion

In this work, the binding constants of newly synthesized *tert*-butyl- α -CD, *tert*-butyl- β -CD and *tert*-butyl- γ -CD with a complete set of mono-halobenzoic acids (Fig. 1) were determined, as well as binding constants for native α -CD, β -CD and γ -CD complexes. The binding constants were also determined for the complexes of newly synthesized 9-alkylthioacridine derivatives (Table 1) to TB- α -CD and α -CD.

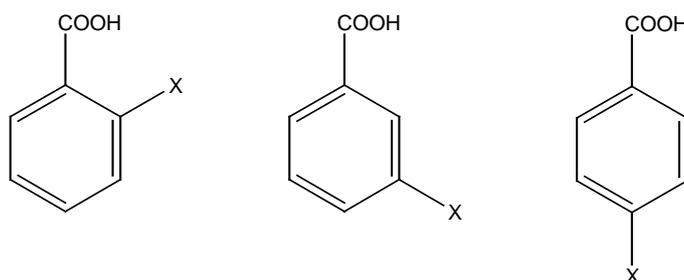
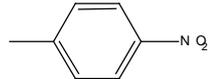
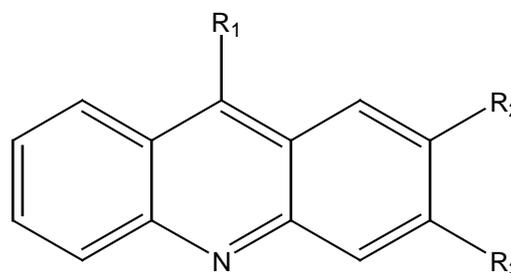


Fig. 1. The structures of the studied set of halobenzoic acids. X= F, Cl, Br, I.

Table 1.-The structures of studied derivatives of acridine

Code	R ₁	R ₂	R ₃
BG 138	-SCH ₃	-OCH ₃	-H
BG 981	-SCH ₂ CH ₃	-OCH ₃	-H
BG 980	-SCH ₂ CH ₂ CH ₃	-OCH ₃	-H
BG 463		-H	-NH ₂



Using the pendant drop method the following CMC values of TB-CDs were found in 20 mM phosphate buffer, pH 8,0 : $CMC^1_{TB-\alpha-CD} = 1.03 \times 10^{-2} \text{ mmol.l}^{-1}$, $CMC^1_{TB-\beta-CD} = 2.64 \times 10^{-2} \text{ mmol.l}^{-1}$, $CMC^1_{TB-\gamma-CD} = 1.48 \times 10^{-3} \text{ mmol.l}^{-1}$; in the buffer containing 20 mM aspartic acid and 20 mM β -alanine,

pH 3.6: $CMC^2_{TB-\alpha-CD} = 1.43 \times 10^{-2} \text{ mmol.l}^{-1}$, $CMC^2_{TB-\beta-CD} = 8.81 \times 10^{-3} \text{ mmol.l}^{-1}$, $CMC^2_{TB-\gamma-CD} = 1.48 \times 10^{-3} \text{ mmol.l}^{-1}$. Using capillary electrophoresis [31] the CMC of TB- α -CD was found to be in the range 10.0-12.5 mmol.l⁻¹ (buffer containing 20 mM aspartic acid and 20 mM β -alanine, pH 3.6 with the presence of $3 \times 10^{-4} \text{ mol.l}^{-1}$ 9-thioalkylacridine). The association constants were graphical estimated obtained by plotting method. It was found that there is non-linearity of the plot of TB- α -CD with 9-alkylthioacridine (see Fig. 2).

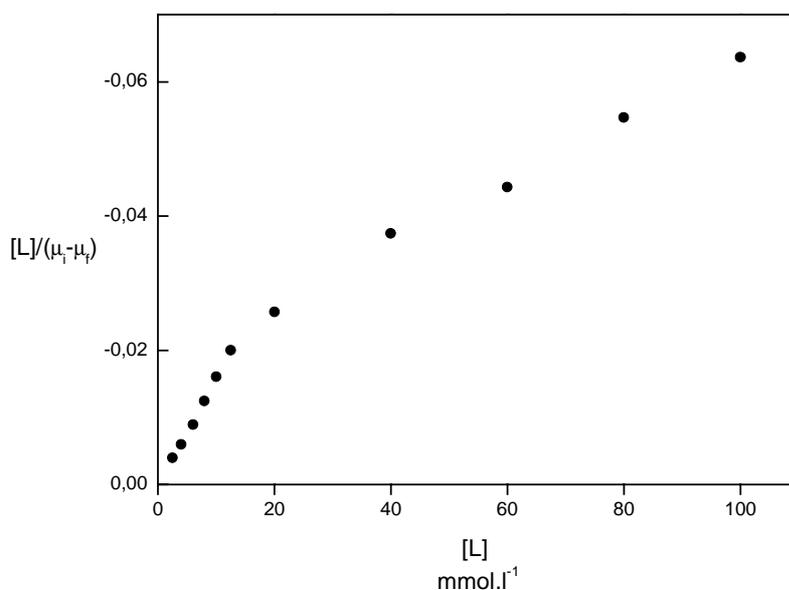


Fig. 2. The plot for determination of the association constant of TB- α -CD with BG 463.

This fact results in conclusion that the value of association constant of TB- α -CD with 9-alkylthioacridine derivatives depends strongly on the concentration of the TB- α -CD. If concentration of TB- α -CD is lower than its critical micelle concentration (CMC), the TB- α -CD shows much stronger complexation capability than native α -CD. On the other hand, the association constant of TB- α -CD becomes even slightly lower than of α -CD above CMC (Table 2).

Table 2. Association constants for 1:1 inclusion complexation of 9-alkylthioacridine derivatives with TB- α -CD and α -CD.

Substance	$K(TB-\alpha-CD;$ $c_{TB-\alpha-CD} \sim 0-10 \text{ mmol})$ $[M^{-1}]$	$K_t(TB-\alpha-CD;$ $c_{TB-\alpha-CD} \sim 20-100 \text{ mmol})$ $[M^{-1}]$	$K(\alpha-CD;$ $c_{\alpha-CD} \sim 0-100 \text{ mmol})$ $[M^{-1}]$
BG138	341.6±3.2	3.1±0.6	6.1±1.6
BG981	571.0±5.6	5.8±0.8	12.0±1.9
BG463	556.2±19.4	31.0±3.1	79.6±5.6
BG980	276.9±4.5	7.7±1.2	21.1±4.6

Probably, the values differ significantly due to decreased accessibility of TB- α -CD cavity associated in micelles, Fig. 3. The single binding scenario failed when micelles were formed. Therefore, association constants calculated according to Eq 3 are presented as formal association constants (K_f).

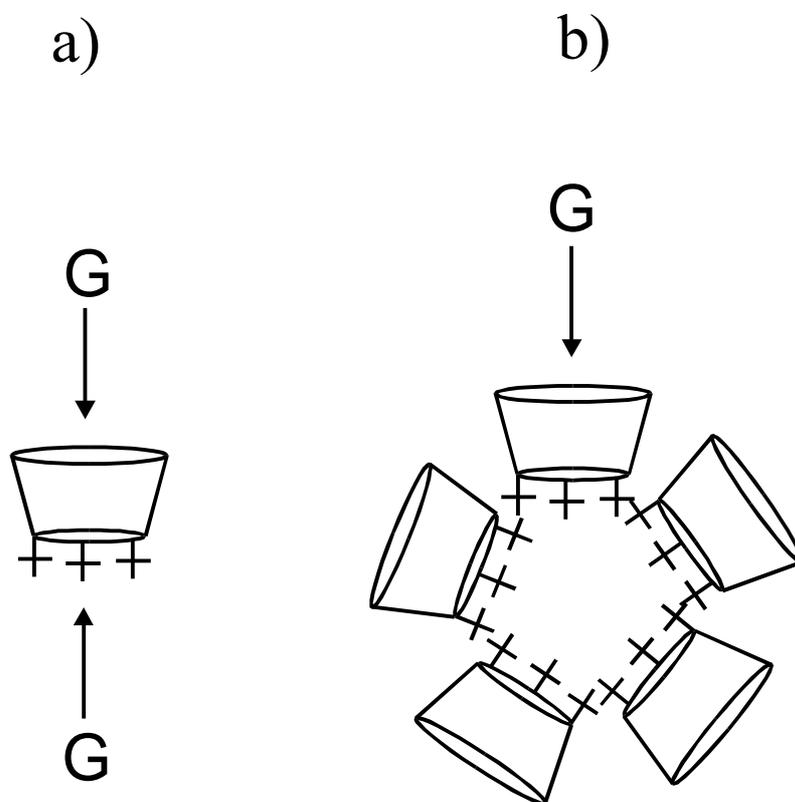


Fig. 3. Scheme of accessibility of TB- α -CD's cavity under [a)] and over [b)] CMC; G is guest molecule.

The results of CE measurements, together with surface tension measurements and observed excessive foaming of TB-CD water solutions, lead to the conclusion, that the TB-CDs are not only complexation but also surface active agents. In addition, *tert*-butyl groups increase stability of complexes with those molecules, that are not included in cyclodextrin cavity completely but contain lipophilic group fitting well (e.g alkyl group of alkylthioacridine derivatives to α -CD). *Tert*-butyl groups can also interact with lipophilic part of the complexed guest that is not included in the cavity. This can be explained by prolongation of the lipophilic CD cavity or extension of the lipophilic surface of α -CD by the *tert*-butyl groups of the CD molecule. The surface activity can be explained by a predominant substitution at the 6-O-positions of glucose units, i.e. the narrower side of the CD cone bears lipophilic *tert*-butyl groups, the wider side contains hydrophilic secondary hydroxyl groups of glucose units. This can be expected due to higher sterical accessibility and reactivity of primary hydroxyl groups in the described reaction.

The stabilities of the CD complexes with *meta*- and *para*- isomers of halobenzoic acids increase with increasing diameter of substituent (F<Cl<Br<I), and decrease indirectly with the diameter of the CD cavity (Table 3). There is a slight difference between interaction with native cyclodextrins and their *tert*-butyl derivatives; the native cyclodextrins have slightly stronger complexation ability to halogenbenzoic acid derivatives. The *ortho*- isomers constitutes a molecular shape which is not able to interact effectively with the CD cavity. Association constant of *tert*-butyl α -cyclodextrin with halogen derivatives of benzoic acid, that do not contain any aliphatic chain, do not show any dependence on the concentration, both below and above its CMC.

Table 3. Association constants for 1:1 inclusion complexation of benzoic acid derivatives with TB- α -CD, TB- β -CD, TB- γ -CD, α -CD, β -CD and γ -CD.

Substance	K (α -CD) [M ⁻¹]	K (β -CD) [M ⁻¹]	K (γ -CD) [M ⁻¹]	K (TB- α -CD) [M ⁻¹]	K (TB- β -CD) [M ⁻¹]	K (TB- γ -CD) [M ⁻¹]
m-F-b	9.6±7.2	7.3±6.1	9.8±6.3	8.3±6.0	5.3±3.6	6.5±5.1
m-Cl-b	75.5±4.2	10.4±4.2	16.1±3.1	53.8±4.2	9.8±2.3	17.5±2.4
m-Br-b	95.7±7.5	35.2±21.1	21.2±1.9	110.7±4.9	40.2±4.1	23.4±0.6
m-I-b	340.2±30.5	115.5±15.2	34.0±2.6	260.5±12.6	88.6±6.3	31.6±1.9
p-F-b	52.6±36.2	30±18.2	27.9±11.1	19.9±3.2	12.2±1.7	22.7±3.1
p-Cl-b	114.5±8.3	72.5±20.2	25.7±5.6	114.6±0.6	65.2±3.0	24.3±4.8
p-Br-b	282.0±12.1	130.2±26.8	28.6±4.5	228.8±0.6	126.2±3.1	30.5±3.2
p-I-b	580.8±20.5	322.6±32.7	41.2±2.5	570.8±2.7	323.6±6.2	44.1±1.7

Conclusions

Complexation properties of the new class of alkyl cyclodextrin derivatives - randomly substituted *tert*-butyl-cyclodextrins were studied. It was observed that these compounds can act not only as complexation but also as surface active agents. Their association constants with halogenbenzoic acids were comparable to those of natural CDs. The example of strong association constant of TB- α -CD with alkylthioacridine derivatives below CMC concentration of TB- α -CD shows that these compounds can be used as selective complexation agents.

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Experimental

Procedure

The association constants (K) were calculated using the plotting method of Eq. 3 listed in Table 4. This equation can be expressed in terms of migration times using the relationship [23].

$$(\mu_i - \mu_f) = \frac{L_D \times L_T}{U} \times \left(\frac{1}{t_i} - \frac{1}{t_f} \right) \quad (4)$$

where L_T and L_D are the total capillary length and distance to the detector, U is run voltage and t_i and t_f are the measured and free migration times of the analyte. The influence of viscosity was counted using Eq. 5 in Ref [24].

Table 4. Plotting method of Eq. 3

Plotting form	K	$\mu_c - \mu_f$
$\frac{[L]}{(\mu_i - \mu_f)} \text{ vs } [L]$	$\frac{\text{slope}}{\text{intercept}}$	$\frac{1}{\text{slope}}$

Materials

The α -, β - and γ -CD used were of the highest grade commercially available and were obtained from Sigma (St. Louis, MO, USA). The 9-alkylthioacridine derivatives (Table 1) were synthesized at the Universite de Marseille, France [25]; Sodium phosphate, sodium hydrogen phosphate and sodium dihydrogenphosphate were of analytical purity (Lachema Brno, Czech Republic), β -alanine and aspartic acid were obtained from Sigma (St. Louis, MO, USA); Deionized water was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA). Reagents used for synthesis of TB-CDs were purchased from Sigma-Aldrich and used without further purification.

tert-Butyl-cyclodextrins (TB-CDs)

The TB- α -CD, TB- β -CD and TB- γ -CD were synthesized using a patented procedure [26] that consists in reaction of CD (4g) dissolved in trifluoroacetic acid (10 mL) with isobutylene (2-10g) at 0°C for 15 min. The reaction mixture was evaporated at 25°C at 12 torr, treated with concentrated ammonia solution (24%, 20 mL) for 12 h at 25°C. The mixture was then evaporated to dryness, the residue dissolved in water (50 mL) and poured onto Dowex 1x8 (50/100 mesh) anion exchange column (volume 80 mL, OH⁻ cycle). The product was washed out with water (4 x 50 mL) and obtained in solid form by freeze drying. Yield ~ 3g. Average degree of substitution (DS) depends on the amount of isobutylene added (max. DS = 4.5) and was determined by ¹H-NMR by comparing integrals of *tert*-butyl protons and protons of sugar units. DS of TB- α -CD is 3.8, TB- β -CD 4.5 and

TB- γ -CD 4.4. CMC [27] were determined from surface tension measurements using pendant drop method [28] with evaluation procedure by Roe [29].

Instrumentation

Capillary electrophoresis was performed on a Crystal CE system (ATI Unicam, Great Britain) equipped with a Unicam 4225UV detector. $^1\text{H-NMR}$ measurements were carried out on the Varian UNITY-INOVA 400 instrument (400 MHz proton frequency) in DMSO at 25°C.

CE measurement of association constant of benzoic acid derivatives

Electrophoretic experiments were performed in a fused silica capillary tube 73.0 cm \times 75 μm i.d. (Composite metal services Ltd., UK). The distance to the detector was 60.0 cm. UV spectrophotometric detection was carried out at a wavelength of 200 nm. The running buffer solution was 20 mM phosphate (pH 8) [30]. Sample solutions were injected at a pressure 1000 Pa for 6 s. The separation voltage was 15 kV, which typically generated a capillary current of about 64.1 μA . All measurements were done at the temperature 25 °C.

CE measurement of association constant of 9-alkylthioacridine derivatives

Electrophoretic experiments were performed in a fused silica capillary tube 60.3 cm \times 75 μm i.d. (Composite metal services Ltd., UK). The distance to the detector was 47.3 cm. UV spectrophotometric detection was carried out at a wavelength of 256 nm. The running buffer solution was 20 mM aspartic acid and 20 mM β -alanine (pH 3.6). Sample solutions were injected at a pressure 1000 Pa for 6 s. The separation voltage was 25 kV, which typically generated a capillary current of about 19.5 μA . All measurements were done at the temperature 25 °C.

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Sample Availability: Samples are available from the authors