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Inclusion complex formation constants of α-, β-, γ-, δ-, ε-, ζ-, η- and θ-cyclodextrins determined with capillary zone electrophoresis

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Abstract

In contrast to the well known small cyclodextrins (CD), cyclomaltohexaose (α-CD), cyclomaltoheptaose (β-CD) and cyclomaltooctaose (γ-CD), very limited information is available on larger CD and their inclusion complex forming properties. Using capillary electrophoresis, the binding constants of inclusion complexes formed with cyclomaltononaose (δ-CD), cyclomaltodecaose (ε-CD), cyclomaltoundecaose (ζ-CD), cyclomaltododecaose (η-CD) and cyclomaltotridecaose (θ-CD) and various anions were determined and compared to the corresponding binding constants of α-, β- and γ-CD. All of the large CD were capable to form inclusion complexes. Dependent on the type of guest molecules, δ- and ε-CD were the weakest complex formers. The complex forming ability of ε, ζ-, η-, and θ-CD increased with increasing size of the ring structure and θ-CD displayed inclusion complex formation constants comparable to γ-CD.

Keywords: Large cyclodextrins; Inclusion complex formation constants; Capillary electrophoresis

1. Introduction

Enzymatic degradation of starch by the extracellular microbial enzyme cyclodextrin glycosyltransferase (CGTase) (E.C. 2.4.1.19) yields a mixture of cyclic and linear oligosaccharides. The cyclic oligosaccharides, denoted cyclodextrins (CD), are composed of a number of (1→4)-linked α-D-Glc units of which CD with 6, 7 and 8 glucose units are well known as α-, β-, and γ-CD, respectively. CGTases predominantly produce α-, β-, and γ-CD and only a small fraction of large CD are formed. The existence of large CD with up to 12 (1→4)-linked α-D-Glc units was first described by French et al. [1,2]. Due to low yields and difficulties in their purification, they have not been studied in detail. Only recently, large CD ranging form 9 to 17 (1→4)-linked α-D-Glc units, denoted δ-, ε-, ζ-, η-, θ-, ι-, κ-, λ-, and μ-CD, respectively, have been

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purified and characterised [3–7]. The complex forming capacity of the larger CD has been assumed to be negligible due to an expected unsuitable cavity size and increased flexibility of the macrocycles [8]. However, the crystal structures of δ-, ε-, and ι-CD have shown a distorted ring structure and the cavity sizes are therefore not enlarged in proportion to the increased number of glucose units [3,5,9]. Furthermore, data recently obtained by NMR also suggest a distorted cyclic structure of the large CD [7]. Among the large CD, only the inclusion complex forming properties of δ-CD has been investigated previously and was found to have a significant effect on the solubility of digitoxin and spironolactone [10].

The large interest in cyclodextrins is based on their ability to form inclusion complexes with a wide range of molecules. α-, β-, and γ-CD are capable to complex molecules ranging from gases to proteins and other biopolymers. CD are able to modify the physicochemical properties of the guest molecules by increasing their solubility and stability, as well as by modifying their reactivity [8]. They have found numerous applications in the agricultural, food, chemical, and pharmaceutical industries [8,11–14]. Furthermore, CD have been shown to be valuable as selectivity reagents for the resolution of structural, positional and stereo isomers in analytical chemistry [15,16]. Several hypotheses have been proposed to explain the mechanism of inclusion complex formation. These include a relief from conformational strain, release of cavity-bound water, hydrophobic interaction, dipole–dipole and hydrogen-bonding interactions as well as induction and dispersion forces [8,12,17,18]. It is believed that hydrophobic interactions, dipole–dipole interactions and dispersion forces are the major factors in inclusion complex formation [18]. However, the size of the guest molecule and the size and flexibility of the CD cavity is also considered to be important in the formation of strong inclusion complexes, since too wide and flexible CD cavities would be unable to hold small guest molecules and to prevent a dissociation of the complex. On the other hand, a guest molecule which is too large will not be able to enter the CD cavity and thus a strong complex will not be formed [8,12,18].

Capillary electrophoresis has been used for the estimation of the binding constants between CD and a range of molecules. In general, two methods have been applied. The direct absorbance detection method (DAD) estimates the inclusion complex formation constant between CD and a charged guest molecule by measuring the change of mobility of the guest molecule in buffers containing various concentrations of CD [19–24]. In the indirect absorbance detection method (IAD), the CD is run as an analyte and the mobility of the CD is measured as a function of the concentration of the charged guest molecule [20]. In this paper, we have measured the binding constants between CD ranging from α-CD to θ-CD and several anions using the IAD method and compared the complex forming properties of the larger CD with the well known α-, β-, and γ-CD.

2. Results and discussion

Inclusion complex formation constants between CD and various anions were measured using the IAD method according to Lee and Lin [20] and calculated using a y-reciprocal plotting method with least squares linear regression [25]. An example of such a plot is shown in Fig. 1. A linear relationship between the concentration of the anion and the concentration of the anion divided by the effective modility was obtained for all combinations of CD and anions in the y-reciprocal plots which supports the assumption that 1:1 complexes were predominating. The CD–aromatic anion complexes were easy to identify by UV absorbance detection, while the CD complexes with adamantane carboxylate gave poor detector responses. Inclusion complex formation constants could be determined for most combinations of CD and the

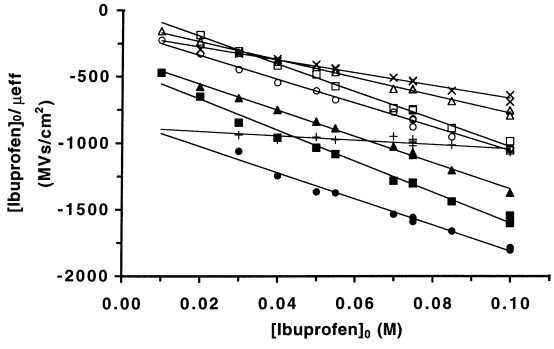

Fig. 1. Y-reciprocal plot of two data series obtained by using different concentrations of the ibuprofen anion for the calculation of the inclusion complex formation constants with CD. Open circle: α-CD; open square: β-CD; open triangle: γ-CD; tilted cross: δ-CD; cross: ε-CD; filled circles: ζ-CD; filled squares: η-CD; filled triangles: θ-CD.

Table 1
Inclusion complex formation constants of the 1:1 complexes between cyclodextrins and various anions measured by capillary electrophoresis at 25 °C

Compound	Inclusion complex formation constant (M^{-1})							
	α-CD	β-CD	γ-CD	δ-CD	ε-CD	ζ-CD	η-CD	θ-CD
Benzoate	16	23	3	3	3	5	4	5
2-Methyl benzoate	13	13	7	6	6	5	6	7
3-Methyl benzoate	26	40	6	3	5	6	7	8
4-Methyl benzoate	36	66	8	2	4	6	6	7
2,4-Dimethyl benzoate	45	42	8	3	4	5	7	6
2,5-Dimethyl benzoate	41	27	6	4	5	5	6	6
3,5-Dimethyl benzoate	39	9	7	2	5	7	8	8
3,5-Dimethoxy benzoate	47	63	10	8	9	10	9	12
Salicylate	11	65	13	9	8	8	9	10
3-Phenyl propionate	35	79	7	2	3	5	4	6
4-*tert*-Butyl benzoate	51	382	74	47	3	9	15	25
Ibuprofen anion	56	>2500[a]	67	27	2	12	29	39
1-Adamantane carboxylate	114	501	42	8	—[b]	4	4	8

[a] Too high to be accurately determined.
[b] Could not be determined.

aromatic anions (Table 1). Most of the inclusion complex formation constants were quite low. However, aromatic anions such as benzoate derivatives, are known to display inclusion complex formation constants 10–100 times lower than neutral molecules [18,26,27]. When comparing the binding constants of the different CD, β-CD was found to be the best complex forming compound, followed by α- and γ-CD. Only in the case of 2,5 dimethyl benzoate and 3,5 dimethyl benzoate, the binding constants were higher for α-CD compared to β-CD. The superiority of β-CD in forming strong complexes has also been confirmed by statistical analysis of CD-guest inclusion complex populations [28]. The larger CD showed overall lower binding capacities. δ-CD displayed the lowest complex formation capacity with substituted benzoates, whereas ε-CD was the poorest complexant with 4-*tert*-butyl benzoic acid, adamantane carboxylate and the ibuprofen anion. Surprisingly, ε-, ζ-, η-, and θ-CD, exhibited an increasing binding ability with an increasing number of glucose units in the rings and θ-CD obtained inclusion complex formation constants comparable to γ-CD and in some cases even to α-CD.

With the exception of adamantane carboxylate, the investigated molecules can be divided into two groups according to their binding properties. The first group consists of substituted benzoates which were weakly bound. The second group which was strongly bound consists of aromatic anions substituted with bulky side groups (*tert*-butyl or isobutyl) in the para position. Normally, charged groups are repelled from the CD cavity and reduce the complex stability [18]. This may explain why the small benzoate derivatives have a low complex stability, since due to their anionic group they fail to be adequately included in the CD macrocycle. 4-*tert*-butyl-benzoate and the ibuprofen anion, on the other hand, have large bulky para substituents with high electron densities. This results in higher complex stabilities [18] and reduces the repellant forces of the anionic group by moving it further away from the CD cavity. The complex stability of 3-phenyl propionate with α-, β-, and γ-CD is 2 to 4 times higher than that of the corresponding benzoate. This may also be attributed to the anionic group which is further away from the binding site (the phenyl group) in 3-phenyl propionate compared to benzoate. The highest binding constant was obtained with (β-CD and the ibuprofen anion (Fig. 1). However, a precise estimation of this constant was not possible since the y-reciprocal plotting method is not precise for the determination of high inclusion complex formation constants. In these cases, the intercept of the regression line will be close to zero. Since the inclusion complex formation constant is calculated from the slope of the regression line divided by the intercept, the values of high inclusion complex formation constants will be more affected by errors in the mobility determination than those of small inclusion complex formation constants. A precise estimation of the high inclusion complex formation constants was also not possible using double-reciprocal and x-reciprocal plotting methods [25].

Table 2
Comparison of inclusion complex formation constants of 1:1 complexes measured at 25 °C or room temperature using different methods

Compound	Detection method	Inclusion complex formation constants (M^{-1})			
		α-CD	β-CD	γ-CD	References
Benzoate		16	23	3	This work
	Spectrophotometry	13 ± 1			[30]
	Microcalorimetry	10 ± 1	15.9 ± 1.2		[31]
	Potentiometry		60 ± 10		[32]
	Potentiometry	11.2 ± 0.35			[33]
	Microcalorimetry		20		[34]
2-Methyl benzoate		13	13	7	This work
	Capillary electrophoresis (DAD)		13.3 ± 2.0		[22]
3-Methyl benzoate		26	40	6	This work
	Capillary electrophoresis (DAD)		48.2 ± 0.6		[22]
4-Methyl benzoate		36	66	8	This work
	Capillary electrophoresis (DAD)		100.3 ± 1.0		[22]
	Potentiometry		110 ± 1		[32]
Salicylate		11	65	13	This work
	Capillary electrophoresis (IAD)	8 ± 0.3	82 ± 3		[20]
	Capillary electrophoresis (DAD)	15 ± 6	50 ± 7		[20]
4-*tert*-butyl benzoate		51	382	74	This work
	Potentiometry	64 ± 19			[35]
	Microcalorimetry		18400		[34]
Ibuprofen anion		56	> 2500	67	This work
	Spectrophotometry		2900 ± 500		[36]
	Capillary electrophoresis (DAD)		1280 ± 5[a]		[19]
1-Adamantane carboxylate		114	501	42	This work
	Spectrophotometry	140 ± 14	> 1000000		[37]
	Microcalorimetry	140	20000	3000	[38]
	Spectrophotometry		42000 ± 1000		[39]
	Spectrophotometry		16000 ± 1000		[39]
	Microcalorimetry		36000 ± 20000		[39]
	Spectrophotometry		36000 ± 600	268 ± 9	[40]

[a] Measured at 37 °C.

A comparison of the calculated inclusion complex formation constants with previously published data showed that comparable constants could be found using the IAD method with CE (Table 2). Inclusion complex formation constants measured with different methods can vary by a factor of 2 [18]. However, when comparing good complex forming compounds (e.g., 4-*tert*-butyl benzoate and 1-adamantane carboxylate), CE gave values which were lower than those obtained using other methods. The described method for the estimation of inclusion complex formation constants gave good results in estimating small inclusion complex formation constants, whereas high stability constants were underestimated.

The results strongly suggest that the cavities of the large CD are much smaller than what would be expected if they had a planar structure like α-, β-, and γ-CD [8]. Indeed, the crystal structures which have been obtained for δ- [3], ε- [5], and ι-CD [9] have indicated that the larger CD are elliptical macrocycles with a saddel-like structure. NMR measurements have also suggested that the large CD have a distorted cyclic structure [7]. However, no information is presently available on the structure and flexibility of the larger CD in solution.

3. Conclusion

Although the described method is susceptible to the same errors as other methods for the estimation of inclusion complex formation constants [8], it excells in requiring only minute amounts of sample (less than 1 mg). Using this method we have found that large CD have the ability to form complexes similar to the small CD (α-, β-, and γ-CD). θ-CD gave the highest inclusion complex formation constants among the large CD. δ-CD was the weakest complex forming compound with benzoate derivatives with small substituents. Benzoate derivatives with large para substituents and adamantane

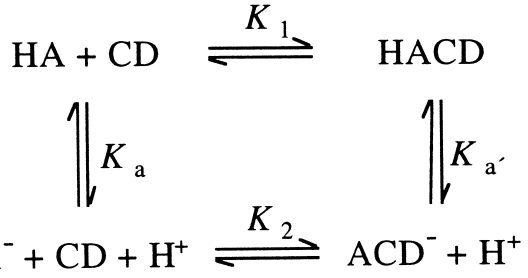

Fig. 2. Equilibria for the 1:1 inclusion complex formation between CD and acidic BGE. K_a and $K_{a'}$ are the acid dissociation constants of the BGE and the CD–BGE complex, respectively. K_1 and K_2 are the complex formation constants of the unionized and the ionized BGE and CD, respectively.

carboxylate formed the weakest complexes with ε-CD. The complex forming ability of ε-, ζ-, η-, and θ-CD increased with an increasing number of glucose molecules in their ring structure, suggesting potential applications of these molecules comparable to α-, β-, and γ-CD.

4. Experimental

Theory.—The separation of CD by capillary electrophoresis relies on the formation of inclusion complexes between the CD and a charged background electrolyte (BGE) [20,29]. Differences in the formation constants between the various CD and the BGE leads to the separation of the CD. Given that 1:1 inclusion complexes are formed between CD and the BGE, the following equlibria can be considered (Fig. 2). While the pH of the CE solution is kept above the pK_a of the BGE, the anionic form of the BGE, $[A^-]$, predominates. Therefore, only the equlibrium expression for the formation of inclusion complexes between CD and the anionic BGE needs to be considered [eq (1)].

$$K_2 = \frac{[\mathrm{ACD}^-]}{[\mathrm{A}^-][\mathrm{CD}]} \quad (1)$$

The mole fraction of the complexed form of BGE (α_{ACD^-}) can be expressed by eq (2).

$$\alpha_{\mathrm{ACD}^-} = \frac{[\mathrm{ACD}^-]}{[\mathrm{CD}] + [\mathrm{ACD}^-]} \quad (2)$$

Combining eqs (1) and (2) yields eq (3)

$$\alpha_{\mathrm{ACD}^-} = \frac{K_2[\mathrm{A}^-]}{1 + K_2[\mathrm{A}^-]} \quad (3)$$

The effective electrophoretic mobility of the ACD^- complex can be expressed by the molar fraction of the complexed form of BGE multiplied by the mobility of the ACD^- complex (4).

$$\mu_{\mathrm{eff}} = \alpha_{\mathrm{ACD}^-}\mu_{\mathrm{ACD}^-} \quad (4)$$

Inserting eq (3) into (4) gives eq (5).

$$\mu_{\mathrm{eff}} = \frac{K_2[\mathrm{A}^-]\mu_{\mathrm{ACD}^-}}{1 + K_2[\mathrm{A}^-]} \quad (5)$$

As $\mathrm{pH} \gg \mathrm{p}K_a$, $[\mathrm{A}^-] \approx [\mathrm{HA}]_0$, eq (5) can be rewritten in eq (6).

$$\mu_{\mathrm{eff}} = \frac{K_2[\mathrm{HA}]_0\mu_{\mathrm{ACD}^-}}{1 + K_2[\mathrm{HA}]_0} \quad (6)$$

The reciprocal form of eq (6) multiplied with $[\mathrm{HA}]_0$ is given in eq (7).

$$\frac{[\mathrm{HA}]_0}{\mu_{\mathrm{eff}}} = \frac{[\mathrm{HA}]_0}{\mu_{\mathrm{ACD}^-}} + \frac{1}{K_2\mu_{\mathrm{ACD}^-}} \quad (7)$$

A plot of $[\mathrm{HA}]_0/\mu_{\mathrm{eff}}$ against $[\mathrm{HA}]_0$ will give a straight line where the intercept and the slope is equal to $1/K_2\mu_{\mathrm{ACD}^-}$ and $1/\mu_{\mathrm{ACD}^-}$, respectively. K_2 is obtained by dividing the slope by the intercept.

Electrophoresis.—Instrumentation. Capillary electrophoresis was performed on a Beckman P/ACE 5010 system equipped with 200, 214, 254, 280 and 340 nm filters and a P/ACE UV absorbance detector (Beckmann Instruments Inc., Fullerton, CA). Electrophoresis and electropherogram analysis was performed using System Gold version 8.10 software (Beckmann Instruments Inc., Fullerton, CA). 50 μm i.d. fused silica capillaries from Composite Metal, CA were used.

Materials. 2-methyl benzoic acid, 3-methyl benzoic acid, 4-methyl benzoic acid, 2,4-dimethyl benzoic acid, 2,5-dimethyl benzoic acid, 3,5-dimethyl benzoic acid, 3,5-dimethoxy benzoic acid, 4-*tert*-butyl benzoic acid, 1-adamantane carboxylic acid and salicylic acid were obtained from Aldrich, Steinheim, Germany. Benzoic acid and ibuprofen (4-isobutyl-α-methyl phenyl acetic acid, sodium salt) were obtained from Sigma Chemical CO., St. Louis, MO. 3-phenyl propionic acid and Na_2HPO_4 were obtained from E. Merck, Darmstadt, Germany. All chemicals were of analytical grade. Pharmaceutical grade α-, β-, and γ-CD were a gift from Wacker Chemie. The large CD were purified as described previously [4].

Electrophoresis. The capillaries were daily conditioned with a high pressure purge (20 psi) of 1 M HCl followed by 1 M NaOH, both for 5 min. Prior to analysis, the capillaries where rinsed with 1 M NaOH for 0.5 min using high pressure purge (20 psi), followed by a high pressure purge (20 psi) of water for 0.5 min. The capillary was filled with background electrolyte (BGE) using a high pressure purge (20 psi) for 2 min. Before the sample was loaded onto the capillary, the outer surface of the anionic side of the capillary was washed with 2 mM phosphate buffer, pH 7.0 to prevent a carry over of BGE to the sample resevoir. Sample loading was performed by applying pressure (0.5 psi) to the anionic side of the capillary for 5 s. Prior to separation, a small amount of BGE was loaded onto the capillaries by pressure (0.5 psi) for 10 s in order to prevent an escape of sample at the begining of the separation. The separation was carried out at 30 kV constant voltage. The capillary temperature was maintained at 25 °C. The capillary length was 77 cm (70 to the detector). The absorbance was measured at 254 nm.

Stock solutions of 200–250 mM aromatic anions in 2 mM disodiumphosphate pH 7.0 or 8.0 were used to prepare the different BGE solutions. 2 mM phosphate buffer pH 7.0 or 8.0 was added to a final volume of 10 ml.

The inclusion complex formation constant was calculated from the effective mobilities of the individual CD separated in two series of at least seven different concentrations ranging from 10 to 200 mM aromatic anion [20] and calculated using a y-reciprocal plotting method with least squares linear regression [25].

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