*Journal of Inclusion Phenomena and Macrocyclic Chemistry* **42:** 209–212, 2002.



# Ternary $\beta$-Cyclodextrin Complexes as Models of Allosteric Effects

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(Received: 1 May 2001; in final form: 31 July 2001)

*Key words:* $\beta$-cyclodextrin, ternary complexes, formation constants, solubility changes, allosteric effect, allosteric enzyme systems

**Abstract**

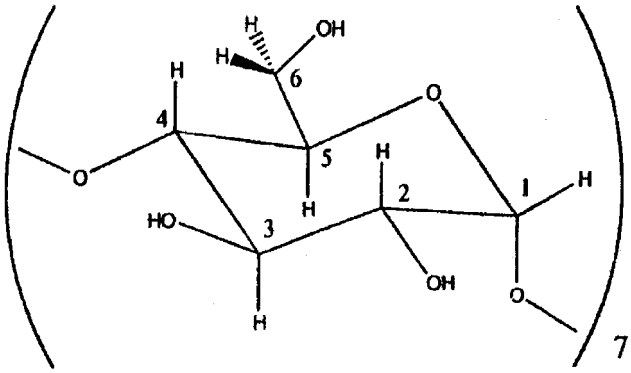
Two guests reacting with cyclodextrins (CDs) may form ternary complexes, in addition to the common competition of 1:1 complexes. One of the guests can really be included into the cavity of the CD, while the second guest molecule is either inserted close to the first one or attached to the outer surface of the supramolecule by H-bonding. There is a further possibility when the included guest bears a substituent outside the cavity and the second guest can interact with it. The properties of the ternary species formed are highly influenced by the solely (or primarily) included guest. The changes are attributed to the altered properties of the hydrophilic domain of the CD. The phenomena can be proved by NMR data obtained for some binary systems of $\beta$-CD inclusion complexes and acetic acid and by the stability constants of the ternary complexes formed. Allosteric effects as well as coenzyme/apoenzyme/substrate interactions could be well modelled by these types of CD complexes.

## Introduction

Cyclodextrins (CDs) are known to form inclusion complexes with a large variety of guests [1–6] and the physical and chemical properties of guests (solubility, volatility, light-sensitivity, etc.) are highly modified due to the complex formation. CDs are also frequently mentioned as enzyme mimicking models as they are really very good and relatively simple model molecules.

The most important native CD, the cyclomaltoheptaose ($\beta$-CD, Scheme 1) consists of seven glucopyranose units with $\alpha$(1,4) glycosidic bonds, and (in contrast to the guests) hardly anything is known about the effect of guest inclusion on its reactivity. Even the strong inhibitory effect of some guest molecules on the acid catalysed ring-opening of $\beta$-CD [7] has been explained by mere steric hindrance, i.e., by simple competition.

Much data are published on the increasing solubility of the guests in the presence of $\beta$-CD [2–6], but less attention is paid to the solubility of the supramolecule. The available values are significantly lower in all known cases than the solubility of the parent $\beta$-CD. Investigating the problem in detail, the solubility of the supramolecule can be correlated with that of the guest, as if the guest forced its solubility upon the $\beta$-CD ("guest enforced solubility") [8]. Since the solubilities of both $\beta$-CD and its inclusion complexes must be connected to and dependent on the same hydrophilic domain, i.e., the hydroxy groups on both rims of the truncated cone structure [3], the guest enforced solubility means that




*Scheme 1.* Atom-and-bond representation of one of the $\alpha$-D-glucopyranose units of $\beta$-cyclodextrin.

the H-bonding abilities of hydroxy groups must be highly influenced by the inclusion of the guest. It follows that the properties of the supramolecular species formed differ from those of the parent molecules. However, no direct experimental proof is known about the change, caused by guest inclusion, in the properties of the hydrophilic region of $\beta$-CD, although several data, showing the changes, can be found in NMR studies [2–6].

After all, the coenzyme – apoenzyme – substrate interaction [9] can also be regarded as ternary complex formation and the ternary CD complexes would be very good and relatively simple examples for modelling these extremely important processes.

As a first model system, the possibility of ternary complex formation of some well known binary inclusion com-

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plexes with a second guest, e.g., with acetic acid (HAc), has been investigated, in order to reveal the change of the hydrophilic domain in $\beta$-CD inclusion complexes.

## Experimental

$\beta$-CD of over 99% purity (received from Cyclolab Ltd., Hungary, as a gift), and other materials of analytical grade were used. Distilled water was redistilled from alkaline $KMnO_4$ solution. During the investigations the temperature was kept strictly constant (generally 25.0 ± 0.1 °C). In solubility experiments (published in detail earlier [10]), the solid material was equilibrated with CD solutions of different concentrations. The quantity of the guest needed for saturation had been determined in preliminary tests and 120% of this amount was weighed in each experiment, because we found that the real (and reproducible) phase equilibria can be reached only this way. The equilibration required normally 10–24 hours when the solid phase contained only one component, but over a whole week was necessary when the solid phase was a mixture also containing precipitated products.

To check the results of the solubility measurements and those of further experiments, the most precise and appropiate methods of analytical chemistry were used [10]. The stability (equilibrium, formation or binding) constants of the complexes were evaluated using the measured concentrations and a computer program. The chemical and mathematical background of the computations was also summarized in an earlier publication [8].

The NMR spectra were recorded in $D_2O$ (internal reference: DSS) at 500.13 MHz by a Bruker DRX 500 instrument.

## Results

The system containing benzoic acid (HBz) and $\beta$-CD was considered to be worthy of further investigations, because the solubilities of HBz, $\beta$-CD and their inclusion complex are very close to each other (at 25.0 °C: $2.75 \times 10^{-2}$ M, $1.52 \times 10^{-2}$ M and $1.47 \times 10^{-2}$ M, respectively) [8]. The $\beta$-CD : HBz ratio in the precipitated complex, equilibrated at 25.0 °C with the mother liquor, in which the concentrations of $\beta$-CD and HBz are lower than their solubilities is reproducibly 1.00 : 1.07, showing that not only 1 : 1 but also 1 : 2 complexes exist. Of course, the guest : host ratio in the saturated mother liquor does not need to be identical with that measured in the solid phase, since the equilibrium concentrations are controlled by the formation constants while the composition of the precipitate depends on the solubility of different species. In our case, the HBz concentration is relatively higher than the concentration of $\beta$-CD, proving the existence of a 1 : 2 complex from this side, too.

One of the HBz molecules is known to be included in the cavity of $\beta$-CD [11], but the second HBz molecule must be bound differently, since 6.5% of the total HBz content, which is strictly equivalent to the amount over the 1.00 : 1.00 stoichiometry, can be sublimed and determined quantitatively [12]. It follows that the system is rather complex and at least two equilibria (over some less important ones) exist in the aqueous phase:

*Table 1.* Equilibrium constants for the different interactions

| Interaction[a] | Stability constant[b] | Ref. |
|---|---|---|
| $Bz^- + H^+$ | pK = 4.205 | 13 |
| $Ac^- + H^+$ | pK = 4.757 | 13 |
| HBz + HAc | $(7.4 \pm 0.9) \times 10^{-1}$ | this work |
| HBz + HBz | $(1.67 \pm 0.09) \times 10$ | 14 |
| HBz + $Bz^-$ | $(8.6 \pm 0.2) \times 10^{-1}$ | 14 |
| HBz + CD | $(7.94 \pm 0.27) \times 10^{2}$ | 12 |
| $Bz^-$ + CD | $(5.0 \pm 0.3) \times 10^{1}$ | this work |
| Bn + CD | $(1.3 \pm 0.2) \times 10^{2}$ | this work |
| HAc + CD | $(2.0 \pm 0.2) \times 10^{-1}$ | this work |
| $Ac^-$ + CD | $<10^{-2}$ | this work |
| CD·HBz + HAc | $(1.45 \pm 0.09) \times 10^{0}$ | this work |
| CD·Bn + HAc | $\sim 10^{-2}$ [c] | this work |
| CD·HBz + HBz | $(4.0 \pm 0.6) \times 10^{0}$ | 12 |

[a] HAc = acetic acid, HBz = benzoic acid, Bn = benzene, CD = $\beta$-cyclodextrin.
[b] According to the given interaction (e.g., see Equations 1–4), T = 25.0 °C.
[c] Poor reproducibility.

$$CD + HBz \rightleftharpoons CD(HBz) \quad (1)$$

$$CD \cdot HBz + HBz \rightleftharpoons CD \cdot (HBz)_2 \quad (2)$$

(where CD means $\beta$-CD for simplicity).

The interactions can be characterized by stability constants defined as

$$K_{11} = \frac{[CD \cdot HBz]}{[CD][HBz]} \quad (3)$$

$$K_{12} = \frac{[CD \cdot (HBz)_2]}{[CD \cdot HBz][HBz]} \quad (4)$$

(The values of these constants can be found in Table 1.)

In order to demonstrate the enhanced reactivity (H-bonding ability) of the hydrophilic domain of $\beta$-CD in the $\beta$-CD·HBz complex, the solid $\beta$-CD·HBz supramolecule of 1 : 1 stoichiometry was prepared, then its solubility was measured in HAc solution (as the concentration of given components, using appropiate analytical methods [8]) as a function of the HAc concentration (Figure 1). Similarly, the solubility of the specially prepared inclusion complex of benzene (Bn), $\beta$-CD·Bn was also measured as a function of the HAc concentration (and shown in Figure 1), as well as that of $\beta$-CD itself, for the sake of comparison.

The solubility of $\beta$-CD·HBz increased most with increasing [HAc], the change was lower in the case of $\beta$-CD itself, while the HAc has practically no influence on the solubility of $\beta$-CD·Bn. Considering all possible equilibria among the components (Equations 1–4 and similar ones), as well as the mass balances, the equilibrium constants

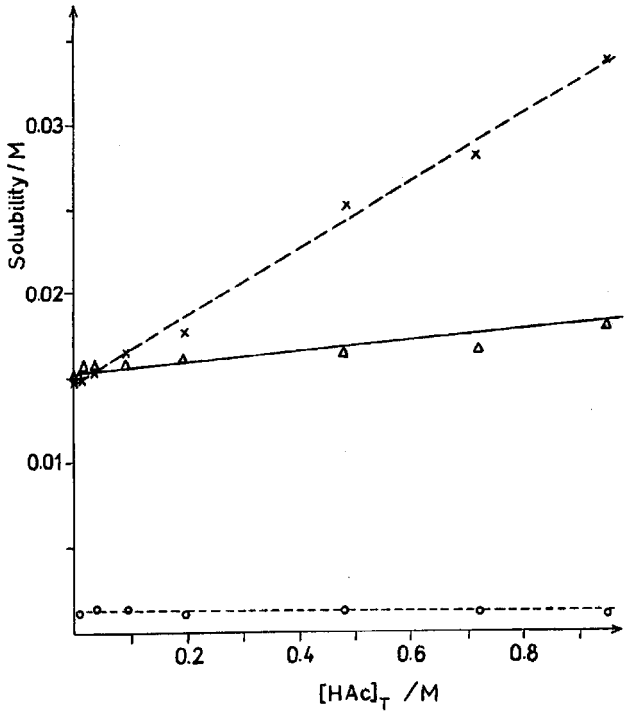



*Figure 1.* The solubility of different species as a function of $[HAc]_T$, × = [β-CD·HBz]; Δ = [β-CD·$(H_2O)_n$]; **o** = [β-CD·Bn].

(stepwise stability constants) were obtained by means of an iterative computer program. The calculated values are listed in Table 1, together with other constants used in the evaluations.

Comparing the data of $^1$H-NMR measurements on the different complex systems (Table 2), characteristic differences can be observed. The chemical shifts of the 'exo'-oriented H-1, H-2 and H-4 as well as the H-6,6′ protons (see Scheme 1) are nearly identical in the spectra of β-CD and its HBz complex, while a considerable upfield shift of the 'endo'-oriented H-3 and H-5 protons (0.17 and 0.18 ppm, respectively) can be recorded for the complex. This must obviously be due to the anisotropic shielding effect of the aromatic ring of the included HBz. Very interestingly, addition of HAc does not change the $^1$H-NMR spectrum of β-CD (or that of its Bn complex), but causes a small but significant downfield shift (0.04–0.04 ppm) again exclusively of the H-3 and H-5 protons of the complex formed with HBz. Parallel with these, extremely small (0.01–0.01) downfield shifts can also be observed on ortho and para protons of HBz.

*Table 2.* Data of NMR measurements

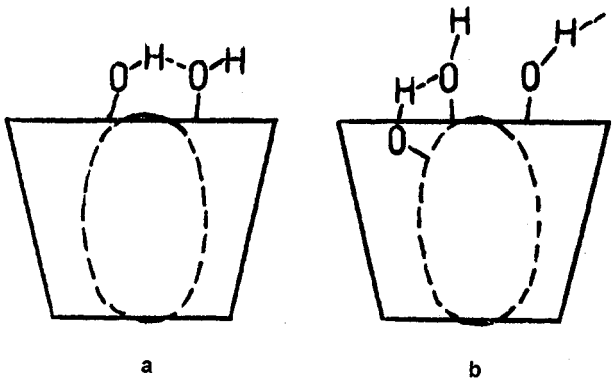
| System | Shift observed |
|---|---|
| β-CD | 1-H 4.99 *d* (3.7 Hz, 1H);<br>2-H 3.56 *dd* (9.8 Hz, 3.6 Hz, 1H);<br>3-H 3.89 *t* (9.4 Hz, 1H);<br>4-H 3.50 *t* (9.2 Hz, 1H);<br>5-H 3.78 br *d* (9.6 Hz) partly overlapped by H-6,6′ signals at 3.80 (br Σ: 3H). |
| β-CD + HBz | 1-H 4.98 *d* (3.7 Hz, 7H);<br>2-H 3.54 *dd* (9.8 Hz, 3.6 Hz, 7H);<br>3-H 3.72 *t* (9.4 Hz, 7H) partly overlapped by H-6,6′ signals at 3.80 (br Σ: 21H);<br>4-H 3.49 *t* (9.2 Hz, 7H);<br>5-H 3.60 br *d* (9.6 Hz, 7H); |
| *Phenyl group* | *ortho* 7.92 *d* (7.8 Hz, 2H);<br>*meta* 7.45 *t* (7.7 Hz, 2H);<br>*para* 7.60 *t* (7.8 Hz, 1H). |
| β-CD + HBz + HAc | 1-H 4.98 *d* (3.7 Hz, 7H);<br>2-H 3.54 *dd* (9.8 Hz, 3.6 Hz, 7H);<br>3-H 3.76 *t* (9.4 Hz, 7H) partly overlapped by H-6,6′ signals at 3.80 (br Σ: 21H);<br>4-H 3.49 *t* (9.2 Hz, 7H);<br>5-H 3.64 br *d* (9.6 Hz, 7H); |
| *Phenyl group* | *ortho* 7.93 *d* (7.8 Hz, 2H);<br>*meta* 7.45 *t* (7.7 Hz, 2H);<br>*para* 7.61 *t* (7.8 Hz, 1H); |
| HAc | 1.99 *s* (35 H). |

## Discussion

The different bonding of two HBz molecules in the β-CD·$(HBz)_2$ complex (i.e., its ternary character) is directly proved by the quantitative sublimation of the excess benzoic acid, as well as by the characteristic and very different formation constants measured in aqueous solution. The first HBz molecule is bound two hundred times stronger than the second one, and the value of $K_{12}$ is too low for a real inclusion complex. Moreover, the β-CD cavity is too small [3] to accommodate two HBz molecules. On the other hand, the $K_{12}$ value of 4.00 seems to be high for a common hydrogen bonded carboxyl–carboxyl interaction (which could bind the second HBz molecule to the included one) since the dimerization constant of HBz in aqueous solution is only 1.67 [14]. We can conclude that one of the two HBz molecules is included in the β-CD cavity and the second one is attached to the hydrophilic domain of the surface, interacting with both the included HBz and the hydroxy groups of the β-CD. There are also IR evidences for the existence of two differently bound HBz molecules in the β-CD complex [15]. However, the other possibility when the second guest is bound to the substituent of the included one may result in similar effects.

The HAc molecule itself seems to be too small to be firmly included into the β-CD cavity, it competes only with the water molecules, as evidenced by the formation constant of the β-CD·HAc complex.

The inclusion of HBz and Bn is unambiguously proved by the $^1$H-NMR investigations. The observed shifts in the spectra of the β-CD–HBz system and their changes in the presence of HAc prove the formation of the ternary complex. On the other hand, the negligible effect of HAc on the spectra of the β-CD–Bn system and β-CD itself makes highly improbable the existence of similar associates in these cases. The results of equilibrium studies (Table 1 and Figure 1) also demonstrate clearly, that the reactivity of β-CD changes when any guest is included into its cavity, and the consequences are different with different guests.




*Scheme 2.* Representation of the effect of a hydrophobic (a) and hydrophilic (b) guest on the hydrogen bond system of $\beta$-cyclodextrin inclusion complexes.

## Conclusions

The behaviour of the supramolecules containing $\beta$-CD as host is highly influenced by the kind of included guest, in spite of the fact that the outer surface (influencing principally the properties) is thought to be – more or less – identical.

The relatively high stability constant of the $\beta$-CD·HBz inclusion complex is known to be the result of the cooperativity of van der Waals and H-bonded interactions [15], while no H-bonds exist between $\beta$-CD and Bn. The H-bonded interaction with the included guest seems to promote cooperatively the formation of further H-bondings of the supramolecule (Scheme 2), while the hydrophobic host–guest interaction does not. In general, the possibility of further interactions are highly influenced by the kind of different forces contributing to the inclusion itself.

The order of stability constants of ternary associates: $\{CD{\cdot}HBz + HAc\} > \{CD{\cdot}(H_2O)_n + HAc\}$ [or $\{CD{\cdot}HAc + HAc\}$] > $\{CD{\cdot}Bn + HAc\}$ is fully explained by this hypothesis, as well as the aqueous solubility of $\beta$-CD inclusion complexes. The reason for the latter is that the hydrophilicity of the outer sphere of the $\beta$-CD or the complex (H-bonded interactions with the solvent) are modified by the included guest.

The allosteric effect, the concept that enzymes contain separate binding sites for not only the substrate but the effector substances, which can inhibit or activate the catalysed reaction [9], is widely known and accepted. The hypothesis outlined and proved above corresponds to this concept, but the investigation of ternary $\beta$-CD containing supramolecules seems to be simpler, therefore they could be used for modelling more complex enzyme systems.

## Acknowledgement

We thank the Hungarian Research Foundation (OTKA T 032470) for financial support of this work.

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