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Correlation between Thermodynamic Behavior and Structure in the Complexation of Modified β -Cyclodextrins and Bile Salts

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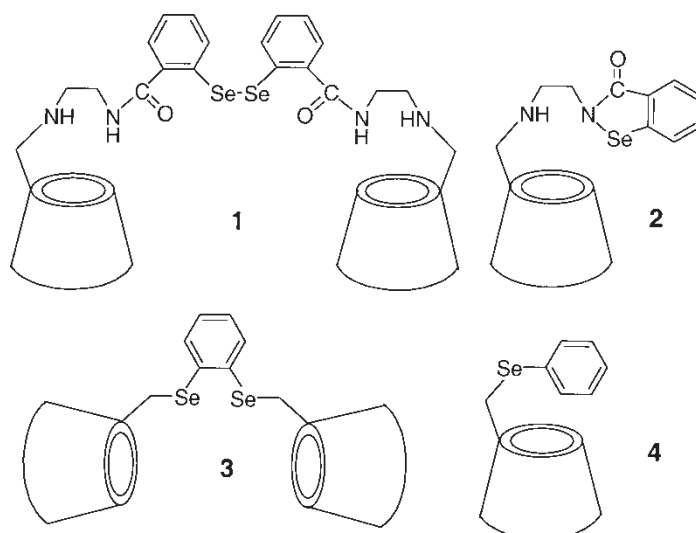
Complex stability constants (K_S), standard molar enthalpy changes (ΔH^0) and entropy changes (ΔS^0) for the inclusion complexation of two cyclodextrin dimers, 6,6'-[2,2'-diselenobis[2-(benzoylamino)ethyl-amino]]-bridged bis(β -cyclodextrin) (1) and *o*-phenylenediseleno bridged bis(β -cyclodextrin)s (3), and their monomer analogs, 6-deoxy-6-[2-(2,3-dihydro-3-oxo-1,2-benzisoselenazol-2-yl)ethyl]amino- β -cyclodextrin (2) and mono[6-(phenylseleno)-6-deoxy]- β -cyclodextrin (4), with two bile salt guests, sodium cholate (CA) and sodium deoxycholate (DCA), were determined at 25°C in Tris buffer solutions (pH 7.4) at 298.15 K by means of isothermal titration microcalorimetry (ITC). The interactions and binding modes between the host cyclodextrins and the guest bile salts were further studied by ROESY spectroscopy. The thermodynamic parameters obtained, together with the ROESY spectra, were used to examine the correlations between thermodynamic behavior and binding modes of the host–guest complexation. The results indicate that the length, structure and conformation of the tethers linked to the cyclodextrins determine the binding modes and the binding abilities between hosts and guests to a great extent, leading to a reversion in binding ability when comparing the corresponding dimer and its monomer analog.

INTRODUCTION

Cyclodextrin dimers are known to show strong binding ability toward certain substrates that can be cooperatively bound into two nearby located cyclodextrin rings, giving double, or even higher, free energies compared with the corresponding monomers [1–6]. It has been reported that sterols are good substrates for cyclodextrin dimers [4], and therefore, bile salts, as important biological amphiphiles possessing a steroid skeleton, have also

been used as suitable guest molecules for examining their inclusion complexation behavior with both mono-modified cyclodextrins [7] and cyclodextrin dimers [8–10]. However, to the best of our knowledge, a comparative investigation of the binding behavior of bile salts with cyclodextrin dimers and monomers has not yet been reported, although comparing the spacer effect of dimers and the substituent effect of monomers is important to understand the molecular recognition mechanism of cyclodextrin. Herein, the complexation behavior of two bile salts, sodium cholate (CA) and sodium deoxycholate (DCA) (Scheme 1), with two cyclodextrin dimers and their monomer analogs (Scheme 2) was investigated by isothermal titration microcalorimetry (ITC) and ROESY experiments. Our special interest is to determine the correlation between the structure of modified β -cyclodextrins and their thermodynamic behavior upon complexation with bile salts. Unlike in the usual case, the two dimers failed to achieve cooperative binding of one guest by the two nearby located cavities of one cyclodextrin molecule. It is found that, although the binding abilities of dimer 3 are stronger than those of its monomer analog 4, cyclodextrin dimer 1 binds with two guests independently by its two identical cavities, consequently displaying an apparent reversion in binding ability compared with its homologous monomer 2. As such binding modes exist generally in biological systems [11,12] and have rarely been reported for synthetic receptors, it is of great importance for understanding the actions of biological molecules bearing multiple noninteracting binding sites.

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SCHEME 1

EXPERIMENTAL SECTION

Materials

6,6'-[2,2'-Diselenobis[2-(benzoylamino)ethylamino]]-bridged bis(β -cyclodextrin) (**1**) [13] was synthesized from 2,2'-diselenobis(benzoic acid) and mono[6-(2-aminoethylamino)-6-deoxy]- β -cyclodextrin. 6-Deoxy-6-[[2-(2,3-dihydro-3-oxo-1,2-benziselenazol-2-yl)ethyl] amino]- β -cyclodextrin (**2**) [14] was synthesized from 2-(chloroseleno)benzoyl chloride and mono[6-(2-aminoethylamino)-6-deoxy]- β -cyclodextrin. The *o*-phenylenediseleno-bridged bis(β -cyclodextrin) (**3**) [15] was prepared from mono[6-*O*-(*p*-tolylsulfonyl)]- β -cyclodextrin (6-OTs- β -CD) and poly(*o*-phenylenediselenide). Mono[6-(phenylseleno)-6-deoxy]- β -cyclodextrin (**4**) [16] was synthesized from diphenyl diselenide and 6-OTs- β -CD. Commercially available sodium cholate and deoxycholate (Acros) were used without further purification. A 10 mM Tris buffer solution (140 mM NaCl, pH 7.4) was used as solvent throughout the measurements.

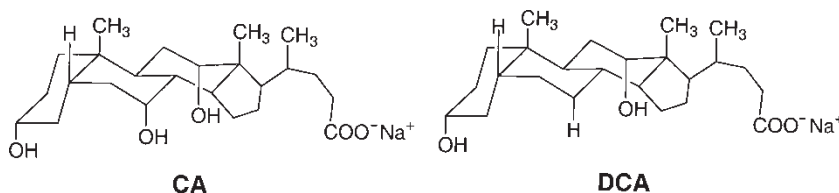
Microcalorimetric Titration

An isothermal calorimeter (VP-ITC), purchased from Microcal Co. (Northampton, MA, USA), was used for all microcalorimetric experiments. The instrument was calibrated chemically by performing

the complexation reaction of β -cyclodextrin with cyclohexanol, which gave thermodynamic parameters in good agreement with the literature data [17,18].

The ITC experiments were performed in 10 mM Tris buffer solution (pH 7.4, 140 mM NaCl) at 25°C by titrating (10 μ l/injection, 29 injections total) the 2.06–4.23 mM solution of cyclodextrins **1–4** into the 0.20 mM (below the critical micelle concentration of bile salts examined) solution of bile salts CA and DCA using a VP-ITC calorimeter (Microcal Co., Northampton, MA) [19], and a typical titration curve is shown in Fig. 1. The thermodynamic parameters for the complexation of **1–4** with bile salts CA and DCA were obtained directly by using the one set of binding sites model [20], and are listed in Table I, giving 1:1 stoichiometry for hosts **2–4** but 1:2 stoichiometry for host **1**. The 1:1 stoichiometry for the resulting complex of **2** and CA was further confirmed by a UV titration experiment (Fig. 2).

A control experiment was performed to determine the heat of dilution by injecting a cyclodextrin buffer solution into a pure buffer solution without bile salt. The dilution enthalpy was subtracted from the apparent enthalpy obtained in each titration run, and the net reaction enthalpy was analyzed by using the one set of binding sites model, as exemplified in Fig. 3 for the complexation of DCA–**2** and DCA–**3** pairs.



SCHEME 2

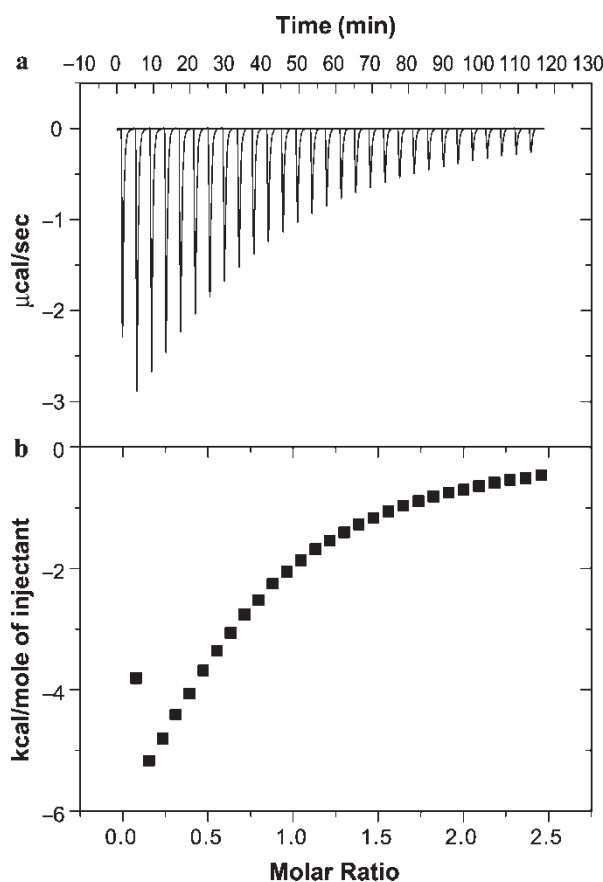


FIGURE 1 Calorimetric titration of dimer **1** with bile salt DCA in Tris buffer (pH 7.4) at 25°C. (a) Raw data for sequential 10 μ L injections of cyclodextrin (2.19 mM) into bile salt solution (0.2 mM). (b) Heats of reaction as obtained from the integration of the calorimetric traces.

RESULTS AND DISCUSSION

To elucidate the difference in binding behavior between the cyclodextrin dimer and monomer, two cyclodextrin dimers and their monomer analogs were used for titration microcalorimetry. It can be seen clearly that the substituent of **2** (or **4**) possesses almost half the structure of the bridge linker of dimer **1** (or **3**), which could be thought of as a logical choice for this study as it increases the structural comparability between the dimer and the monomer

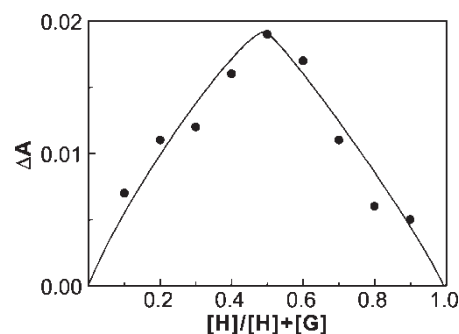


FIGURE 2 A Job plot for the complexation of **2** (H) with bile salt CA (G) in a Tris buffer solution (pH 7.4) at 25°C ($[H] + [G] = 5.0 \times 10^{-4}$ M).

to the greatest extent. It is interesting that the results of the thermodynamic measurements show a 1:1 binding stoichiometry for hosts **2–4** but 1:2 stoichiometry for host **1**. In addition, although the stability constants for the complexation between dimer **3** and the bile salts are much larger than those for monomer **4** ($K_{3-CA}/K_{4-CA} = 5.4$ and $K_{3-DCA}/K_{4-DCA} = 3.8$, respectively), the long-linked dimer **1** unusually displays a lower cavity binding ability than its corresponding monomer **2** upon complexation with both guests CA and DCA ($K_{2-CA}/K_{1-CA} = 2.7$ and $K_{2-DCA}/K_{1-DCA} = 2.0$, respectively).

Two-dimensional NMR Spectra

To investigate the correlation between thermodynamic behavior and structure, ROESY experiments for the complexes of cyclodextrins **1–4** and DCA were performed to illustrate the binding modes between the cyclodextrins and bile salts. The ROESY spectra for the complexation of DCA with dimers **1** and **3** are shown in Figs. 4 and 5, respectively.

It is known that the peaks of protons of DCA mainly fall into the high field ($\delta < 2.5$ ppm) [10]. In the ROESY spectra of the complexes of both dimers **1** and **3** with DCA, no NOE cross-peaks exist between the bridge linkers' protons ($\delta = 7-8$ ppm) of the dimers and the protons of DCA as expected, indicating that the bridge linker does not interact with DCA and the bile salt molecule is not

TABLE I Complex stability constants (K_S), standard enthalpy changes (ΔH^0 , kJ mol $^{-1}$), and entropy changes ($T\Delta S^0$, kJ mol $^{-1}$) for inclusion complexation of the bile salts CA and DCA with cyclodextrins **1–4** in Tris buffer solution (pH 7.40) at $T = 298.15$ K

Host ^a	Guest ^b	n^{\ddagger}	K_S/M^{-1}	ΔH^0	$T\Delta S^0$
1	CA	1.99	2700 ± 100	-27.1 ± 0.5	-7.5 ± 0.6
2	CA	0.90	7400 ± 500	-22.3 ± 0.1	-0.2 ± 0.0
3	CA	1.02	6860 ± 50	-30.5 ± 0.1	-8.6 ± 0.1
4	CA	1.0 [§]	1280 ± 10	-28.3 ± 0.1	-10.5 ± 0.1
1	DCA	2.08	3300 ± 100	-35.7 ± 0.5	-15.7 ± 0.5
2	DCA	0.94	6700 ± 300	-32.1 ± 0.4	-10.2 ± 0.6
3	DCA	1.04	9700 ± 200	-37.0 ± 0.1	-14.3 ± 0.2
4	DCA	1.0 [§]	2570 ± 10	-33.3 ± 0.1	-13.8 ± 0.1

^a[Host] = 2.06–4.23 mM. ^b[Guest] = 0.2 mM. [†]Stoichiometry given by fitting program. [§] n value was fixed.

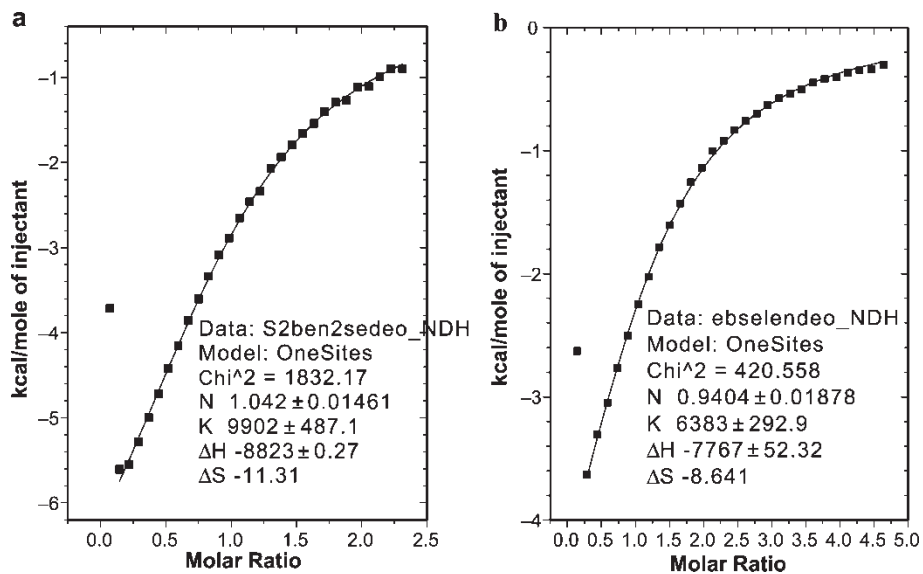


FIGURE 3 'Net' heat effect of complexation of (a) 2 with DCA and (b) 3 with DCA obtained by subtracting the heat of dilution from the heat of reaction, which was analyzed by computer simulation using the one set of binding sites model ($n = 1$).

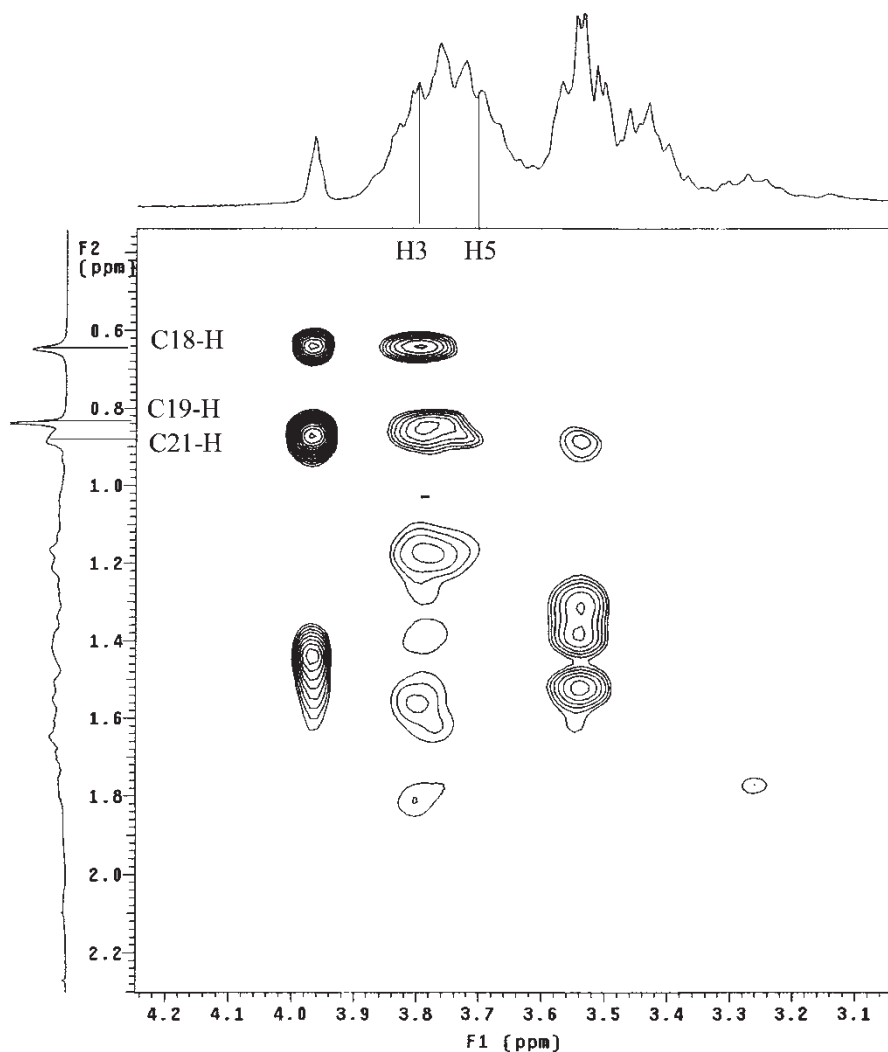


FIGURE 4 Partial ^1H ROESY spectrum (300 MHz) of a mixture of cyclodextrin 1 (1.5 mM) and DCA (1.5 mM) in D_2O at 298.1 K with a mixing time of 600 ms.

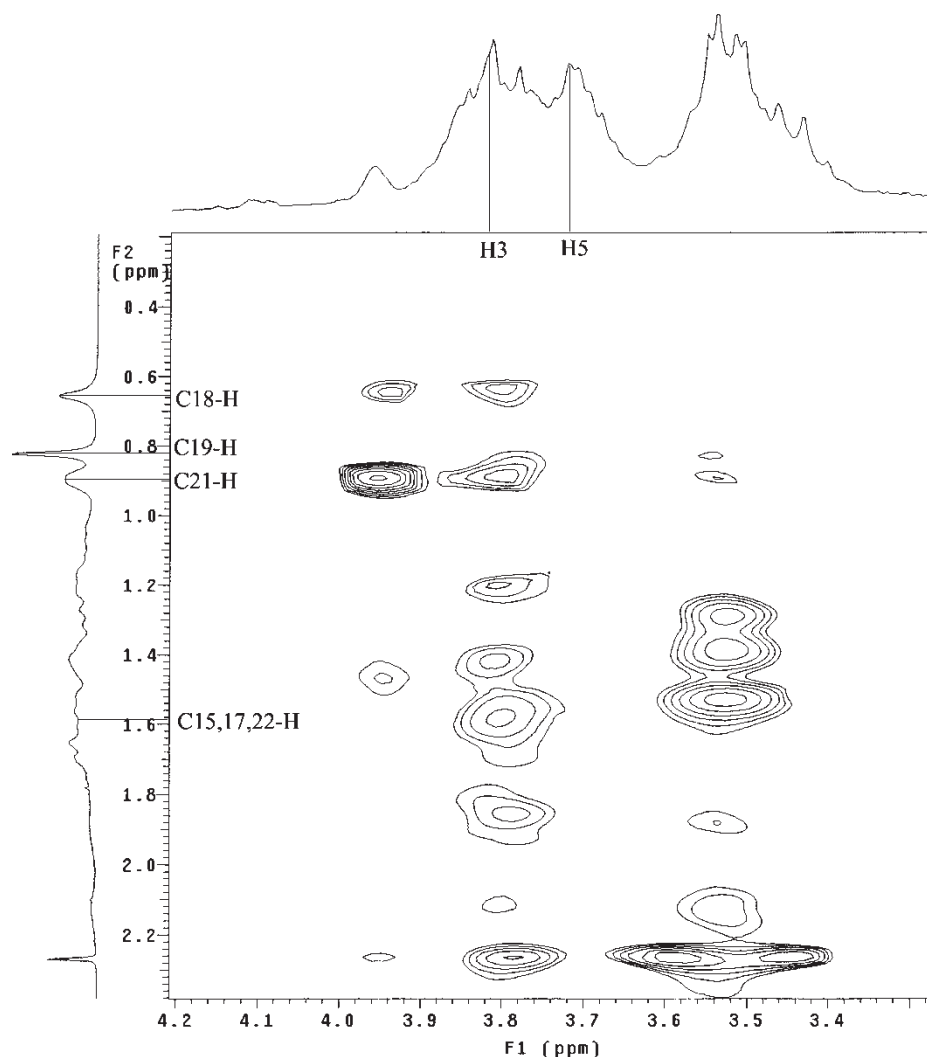


FIGURE 5 Partial ^1H ROESY spectrum (300 MHz) of a mixture of cyclodextrin 3 (1.5 mM) and DCA (1.5 mM) in D_2O at 298.1 K with a mixing time of 600 ms.

cooperatively bound by the two cavities of one dimer molecule. On the other hand, the NOE cross-peaks are observed only between the protons on C18, C21, C15, C17 and C22 of DCA and the C3 protons of cyclodextrin, but not the C5 protons of cyclodextrin. These observations further confirm that DCA is not included in the cavity of the dimer from the primary side (narrow open), but penetrates slightly into the cavity from the secondary side (wide open) using the side chain and D-ring moiety (Fig. 6), which indicates confirmation of the 1:2 stoichiometry of dimer 1 with the bile salts given by the ITC experiments. However, considering the 1:1 stoichiometry for the complexation between dimer 3 and DCA, it is possible that the A-ring moiety of DCA is simultaneously shallowly (no NOE correlations between the C19 proton of the bile salt and the C3(C5) protons of cyclodextrin) included in one of the cavities of another cyclodextrin to form a liner structure ($n:n$ stoichiometry) [10].

Circular dichroism showed that the substituent of 4 penetrates into the cyclodextrin cavity to form a self-inclusion conformation [16], which might influence the binding mode of the bile salt with cyclodextrin. This must be the case as there are obvious NOEs (peaks A) between the benzyl protons of 4 and C3 and C5 protons of the cyclodextrin cavity in the ROESY spectrum for the resulting 4-DCA complex (Fig. 7). Strong correlations of the C19 protons of DCA with both

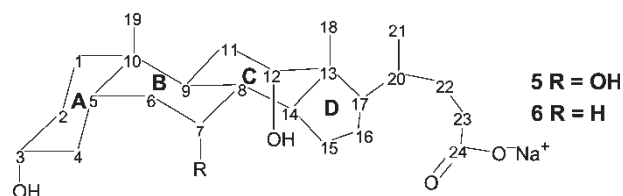


FIGURE 6 Framework of bile salts.

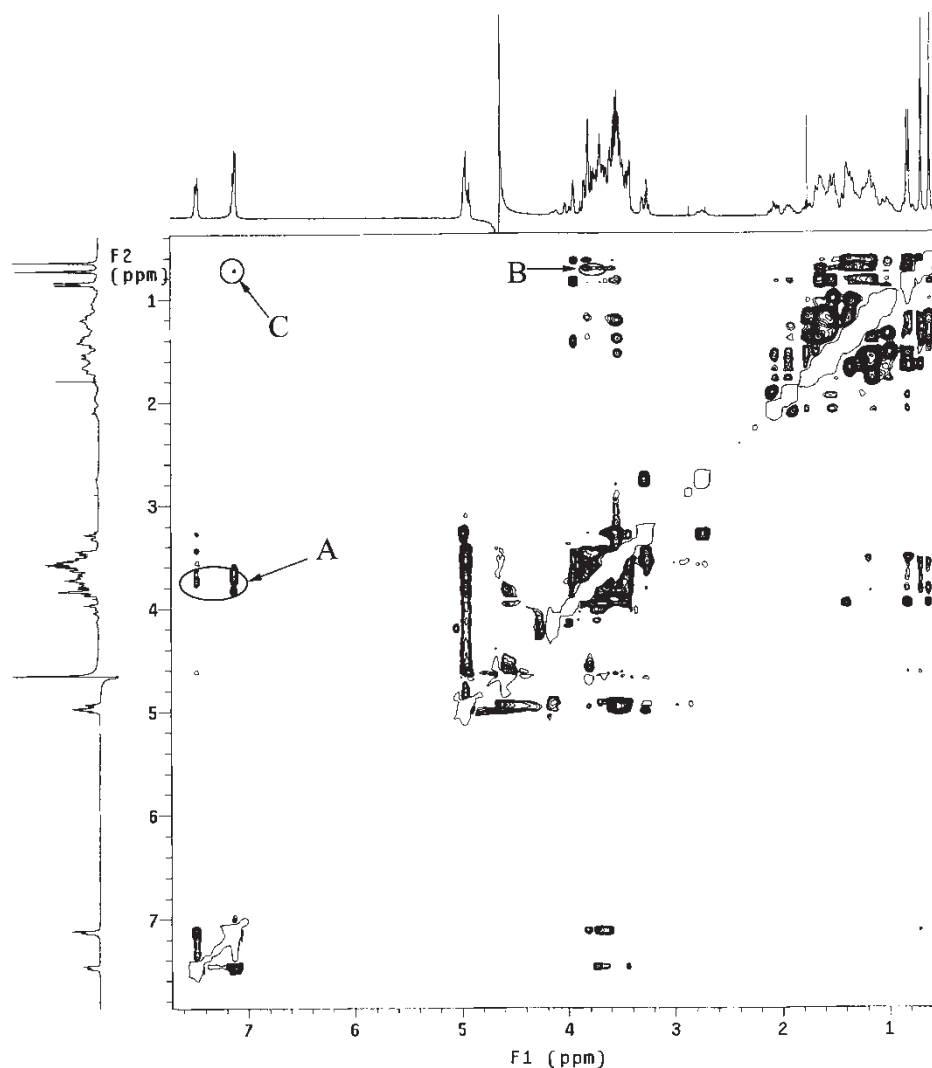


FIGURE 7 ^1H ROESY spectrum (300 MHz) of a mixture of cyclodextrin **4** (1.5 mM) and bile salt DCA (1.5 mM) in D_2O at 298.1 K with a mixing time of 600 ms.

C3, C5 protons of the cyclodextrin cavity (peaks B) and the benzyl protons of **4** (peaks C) can still be seen, which indicates that DCA is included in the cavity of **4** from the secondary side by its A-ring moiety, differing from other cyclodextrins (by D-ring moiety).

However, it has also been confirmed by circular dichroism that, different from that of monomer **4**, the substituent of **2** is located outside the cyclodextrin cavity [14], allowing the bile salts to be included deep in the cavity of **2**. This is validated by the ROESY spectrum for the complexation of host **2** with DCA. In Fig. 8, strong correlations are shown between the C18 protons of DCA and the C3 protons of **2** (peaks D), and between the C22 protons of DCA and the C3, C5 protons of **2** (peaks E), indicating that the D-ring moiety of DCA penetrates deep into the cavity of **2** from the secondary side. Based on these results,

the binding modes for each host with DCA were simulated, and are shown in Fig. 9.

ITC Experiment

The cooperative binding of the two cavities of cyclodextrin dimer is found to produce much greater van der Waals contacts compared with the monomer upon complexation with certain guest [2,6]. In the linear structure formed by dimer **3** and the bile salts, dimer **3** takes an unusual mode, achieving similar cooperative binding toward the guests and producing more negative enthalpy changes than its monomer analog **4** ($-\Delta\Delta H^0 = 2.2\text{--}3.7\text{ kJ mol}^{-1}$), as well as all other hosts. However, the enhancement of the binding ability of dimer **3** compared to monomer **4** could be ascribed not only to the cooperative binding but also partly to the peculiar self-inclusion conformation of **4**, as this conformation greatly

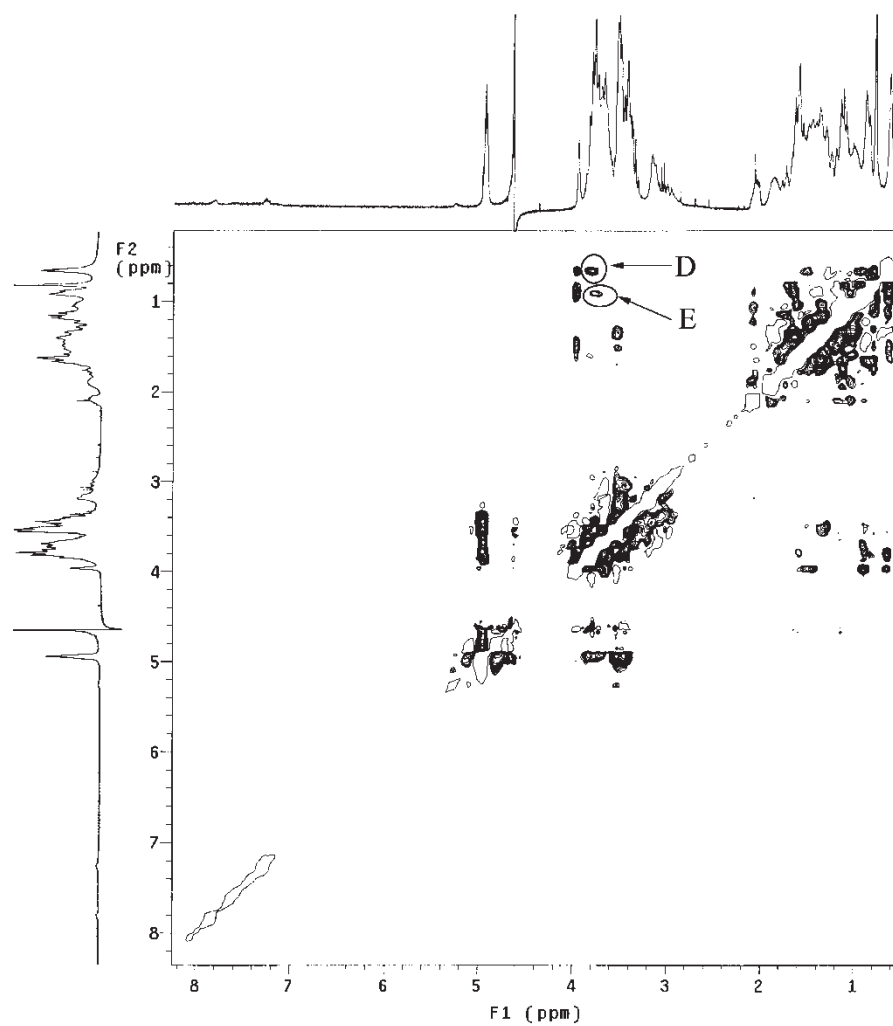


FIGURE 8 ^1H ROESY spectrum (300 MHz) of a mixture of cyclodextrin 2 (1.5 mM) and bile salt DCA (1.5 mM) in D_2O at 298.1 K with a mixing time of 600 ms.

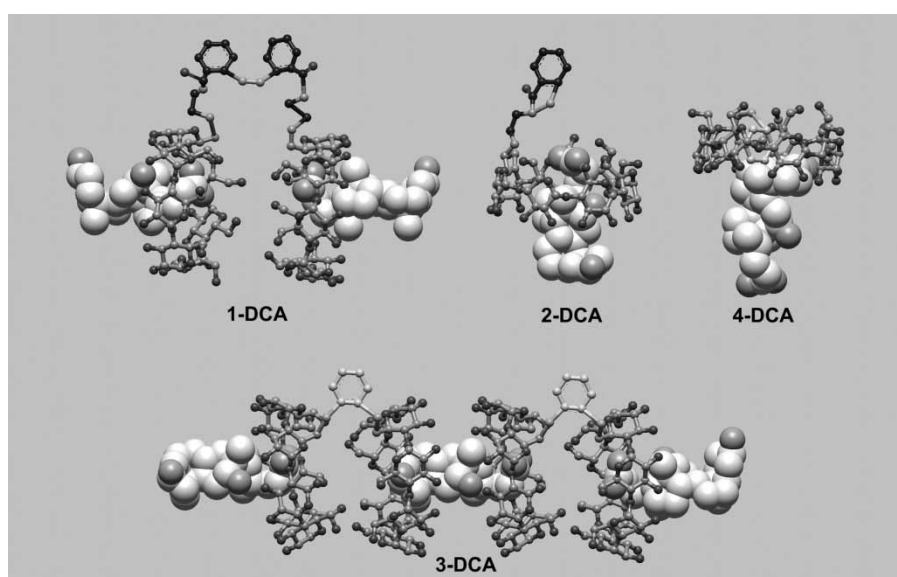


FIGURE 9 Schematic drawing of the possible binding modes for complexation of hosts 1–4 with DCA.

reduces the freedom for the complexation of **4** with the bile salts, leading to more unfavorable entropy changes, especially for the **4**-CA pair ($T\Delta S^0 = -10.5 \text{ kJ mol}^{-1}$). Therefore, the advantage of cooperative binding of **3**, together with the self-inclusion conformation of **4**, results in the binding ability sequence of hosts **3** and **4** ($K_3 > K_4$).

It is of interest to note that, by possessing the linker differing from that of dimer **3** in both length and structure, dimer **1** shows obviously different stoichiometry (1:2, dimer **1**-bile salts) for complexation with CA and DCA as the results of the thermodynamic measurements show, meaning that the two guest molecules are separately and independently included in the two cavities of **1**. This is reasonable because the longer linker, especially the ethylenediamino moiety of dimer **1**, makes it possess a relatively large conformational freedom. For dimer **1** to form a linear mode like dimer **3** during guest binding would require a considerable conformational change, which would certainly result in the extensive entropy loss. Then it could be that just the longer and more flexible linker of dimer **1** prevents the formation of a linear structure like dimer **3**, leading to the different binding mode. Therefore, the K_s value obtained for dimer **1** (listed in Table I) refers to the binding ability of one cavity toward one bile salt molecule [19].

Because DCA is included deep in the cavity of **2**, as has been validated by the ROESY spectrum, the resultant extensive desolvation effect compensates the entropy loss induced by conformation fixation to some extent, and then host **2** displays the smallest entropy loss for each guest ($T\Delta S^0 = -0.2 \text{ kJ mol}^{-1}$ for CA and $-10.2 \text{ kJ mol}^{-1}$ for DCA). Although dimer **1** gives the more favorable enthalpy changes ($-\Delta\Delta H^0 = 3.6$ and 4.8 kJ mol^{-1} for CA and DCA, respectively) compared with monomer **2**, it also gives the much more unfavorable entropy changes ($\Delta T\Delta S^0 = -7.3$ and -5.5 kJ mol^{-1} for CA and DCA, respectively) caused by the more frozen conformation influenced by the bridge linker. As the considerable entropy loss cancels the advantage of enthalpy gain, dimer **1** displays relatively weak binding abilities. Therefore, it is easy to understand the 'reversed' binding ability sequence for dimer **1** and its monomer analog **2**; that is, upon complexation with bile salts CA and DCA, monomer **2** uniformly gives significantly higher binding constants (7400 and 6700 M^{-1}) than those (2700 and 3300 M^{-1} , respectively) for dimer **1** (one cavity of dimer **1** in the exact manner).

However, if we compare the binding ability of cyclodextrin dimer toward DCA and CA from the guest aspect, then both hosts **1** and **2** show similar binding ability for DCA and CA ($K_{1\text{-DCA}}/K_{1\text{-CA}} = 1.2$, $K_{2\text{-CA}}/K_{2\text{-DCA}} = 1.1$). It is reasonable by considering

the binding mode of the bile salts with the two host molecules. Either binding with host **1** or host **2**, the two guest bile salts are included into the cavity of cyclodextrins by its D-ring and side-chain moiety, which reduces the influence of the substituent in C7 ($-\text{OH}$ for CA and $-\text{H}$ for DCA). However, while binding with hosts **3** and **4**, the A-ring moiety participates in the binding process, so the more hydrophobic C7 substituent of DCA makes it bind more strongly with the host cyclodextrins, giving the higher binding constants than with CA, especially for host **4** ($K_{4\text{-DCA}}/K_{4\text{-CA}} = 2.0$).

CONCLUSION

From the comparison of the thermodynamic parameters and binding modes for each dimer-monomer pair, it can be deduced that the length, structure and location of the tethers linked to cyclodextrins cooperatively lead to their observed binding abilities to present an apparent reversion ($K_1 < K_2$ and $K_3 > K_4$). The present investigation not only reveals the correlation between thermodynamic behavior and structure but also builds the binding mode of multiple noninteracting sites to serve to understand the interaction mechanism between the synthetic receptor and the substrate.

Acknowledgements

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