Journal of Inclusion Phenomena and Macrocyclic Chemistry (2006) 56: 183-191
DOI 10.1007/s10847-006-9081-6

Selective Binding Thermodynamics of Bile Acids by Oligo (ethylenediamino)-β-Cyclodextrins and Their Copper (II) Complexes

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Key words: bile acids, calorimetric titration, cyclodextrin, molecular recognition, thermodynamics

Abstract

Complex stability constants (K_S), standard molar enthalpy changes ($\Delta H°$) and entropy changes ($T\Delta S°$) for the inclusion complexation of native β-cyclodextrin (β-CD) (**1**) and some modified β-CDs, i.e., mono(6-ethylenediamino-6-deoxy)-β-CD (**3**), mono[6-diethylenetriamino-6-deoxy]-β-CD (**4**) and their corresponding copper complexes **5** and **6**, with four representative bile acid guests, i.e., cholate (CA), deoxycholate (DCA), glycocholate (GCA) and taurocholate (TCA), were determined at 25 °C in aqueous phosphate buffer solution (pH 7.20) by means of isothermal titration microcalorimetry (ITC). The stoichiometry of resulting inclusion complexes between CDs and bile acids was demonstrated by UV and conductivity as well as ITC experiments, showing 1:1 binding model upon all inclusion complexation except for metal-mediated dimer **5**. The complex stability constants for modified β-CD **2-4** are dramatically magnified with the extended length of amino tether. As compared with **3** and **4,** copper(II) complexes **5** and **6** significantly enhance not only binding ability but also molecular selectivity toward bile guest molecule CA through multipoint recognition, but decreased complexes stability toward TCA could be attributed to the decreased hydrophobic microenvironment of CDs cavity due to the introduction of copper(II) coordination center. Thermodynamically, the resulting complexes between hosts and bile guests are driven absolutely by enthalpy, accompanied by entropy gain or loss. Using the present data and those previously reported for mono(6-amino-6-deoxy)-β-CD (**2**), thermodynamic behavior and enhanced molecular selectivity could be discussed from the viewpoint of hydrophobic interactions, electrostatic cooperation and van der Waals between the hosts and guests.

Introduction

Cyclodextrins (CDs), named cyclic oligosaccharides, have the ability to selectively bind various inorganic or organic guests, which leads to widespread applications of CDs in pharmaceutical chemistry, food technology, analytical chemistry, chemical synthesis, and catalysis [1]. The presence of potential coordinating groups attached to CD rim can promote the formation of stable metal complexes between modified CDs and metal ions [2]. Therefore, metal complexes of CDs functionalized with polyamines have been shown to act as metal enzyme models endowed with specific metal-substrate-binding interaction [3]. Meanwhile, the coordination of some metal ions with CDs amino tethers may enhance the binding abilities of CDs toward appropriate guests. Recently, Kano *et al.* [4] reported the extremely stable 1:2 inclusion complexes of water-soluble *meso*-tetraarylporphyrins possessing Fe(III) ion center with heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin (TMe-β-CD) in aqueous solutions, which showed strong binding ability. More recently, they studied the mechanism for the formation of the 1:2 inclusion complexes by means of NMR spectroscopy and isothermal titration calorimetry (ITC) [5]. We recently reported metallobridged bis(β-CD)s with additional binding size, showing multipoint binding model, enhanced binding ability and molecular selectivity [6]. However, these pioneering works are on the basis of the coordination of CDs dimers and metal ion, and mainly focus on the binding investigations of modified CDs with dyes by fluorescence spectrum. In the present work, we wish to report our investigation results on the thermodynamic behavior of the complexation between CDs bearing ethylenediamine substituents as transition-metal binding sites (Chart 1) and bile acids guests (Chart 2). Herein, a reason for choosing bile acids as guests is that bile acids belong to a group of physiologically important steroids and play a crucial role in lipid digestion, transportation, and absorption due to their amphiphilic nature. It is our

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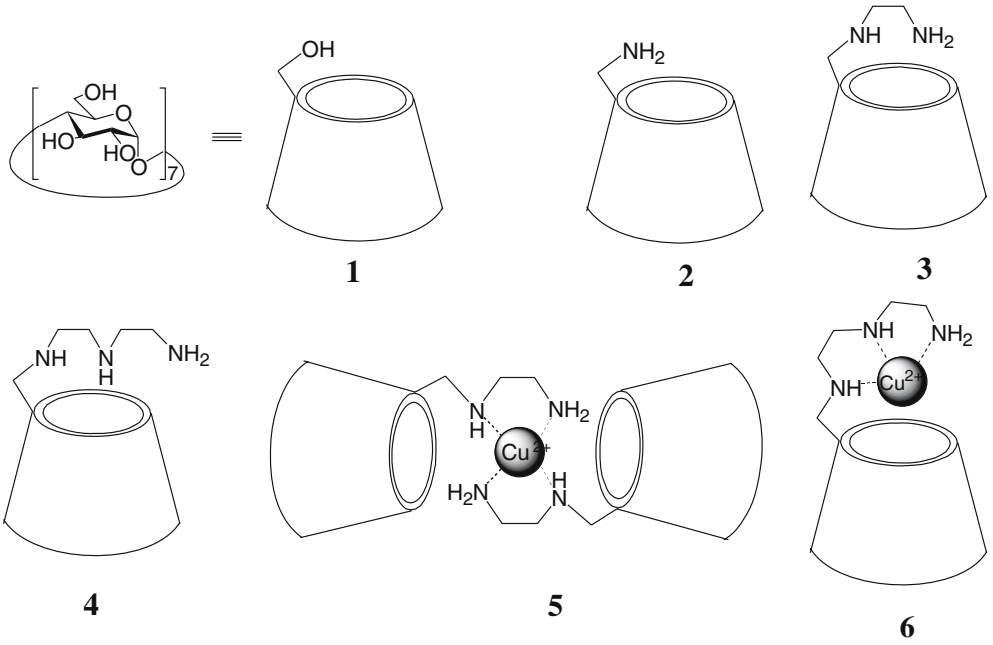

Chart 1.

special interest to examine the heterotopic cooperativity of those metal-centered CDs, which will serve the understanding on the controlling factors for the binding behavior of oligo(ethylenediamine)-β-CD possessing metal coordination center.

Experimental

Material

All bile salt guests, i.e., sodium deoxycholate (DCA), sodium cholate (CA), sodium glycocholate (GCA), and sodium taurocholate (TCA), were purchased from Sigma and used as received. Mono(6-ethylenediamino-6-deoxy)-β-CD (**3**) and mono[6-diethylenetriamino-6-deoxy]-β-CD (**4**) were synthesized according to the reported procedures [3, 7]. Their copper complexes **5** and **6** were prepared by a literature method [8]. UV-vis titration and conductivity measurements were performed to examine the stoichiometry for the resulting complexes of modified β-CDs (**3** and **4**) with copper (II). The results obtained indicate that the coordination stoichiometry of copper with these CDs is 1:2 for complex **5** and 1:1 for complex **6**, which is also consistent with the elemental analysis as well as the previous literature report [8]. Disodium hydrogen phosphate and sodium dihydrogen phosphate were dissolved in distilled, deionized water to make a 0.1 M phosphate buffer solution of pH 7.20 for microcalorimetric titrations.

Measurements

^{1}H NMR experiments were recorded on a Varian Mercury VX300 instrument. All NMR experiments were

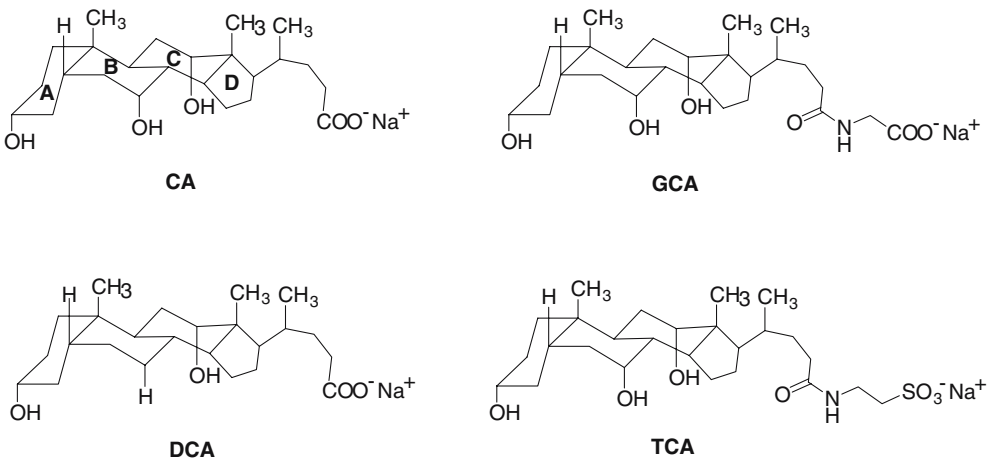

Chart 2.

carried out in D_2O. UV-vis spectra were recorded in a conventional quartz cell (light path 10 mm) on a Shimadzu UV-2401PC spectrophotometer equipped with a PTC-348WI temperature controller to keep the temperature at 25 ± 0.1 °C. The conductivity measurements were carried out on a DDS-307 instrument.

Microcalorimetric titration

All the microcalorimetric titrations experiments were performed on a Microcal VP-ITC titration microcalorimeter, which permits us to calculate the enthalpy and equilibrium constant from a single titration curve simultaneously. The instrument was calibrated chemically by performing the complexation reaction of β-CD with cyclohexanol, which gave thermodynamic parameters in good agreement with the literature data [9]. In each injection, 10 μl of modified β-CD buffer solution was released into the sample cell containing a buffer solution of bile salt with stirring at 300 rpm at 25 °C under atmospheric pressure. The sample cell volume was 1.4227 ml in all experiments. Each titration experiment was composed of 25 successive injections. All solutions were degassed and thermostated using a ThermoVac accessory before the titration experiments were performed. Bile salt solutions were applied at a concentration range of 0.167-0.320 mM, which is below their critical micelle concentration (CMC). (The CMC of the steroids employed here are >1 mm [10]). Each addition of CD into the sample cell gave rise to a heat of reaction, caused by the formation of inclusion complexes between bile salt molecules and CDs. The heats of reaction decrease after each injection of host CD because less and less bile salt molecules are available to form inclusion complexes. Typical titration curves are shown in Figure 1.

A control experiment was performed to determine the heat of dilution by injecting a host buffer solution into a pure buffer solution containing no bile salt molecules. 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 for all the β-CD derivatives.

The ORIGIN software (Microcal), used for the calculation of the binding constant (K_S) and standard molar reaction enthalpy (ΔH°) from each titration curve, gave the relevant standard derivation on the basis of the scatter of data points in a single titration experiment. The binding stoichiometry was also given as a parameter when fitting the binding isotherm (panel b in Figure 2). Knowledge of the binding constant (K_S) and molar reaction enthalpy (ΔH°) enabled the calculation of the standard Gibbs free energy of binding (ΔG°) and entropy change (ΔS°), according to equation

$$\Delta G^\circ = -RT \ln K_s = \Delta H^\circ - T\Delta S^\circ$$

where R is the gas constant and T is the absolute temperature.

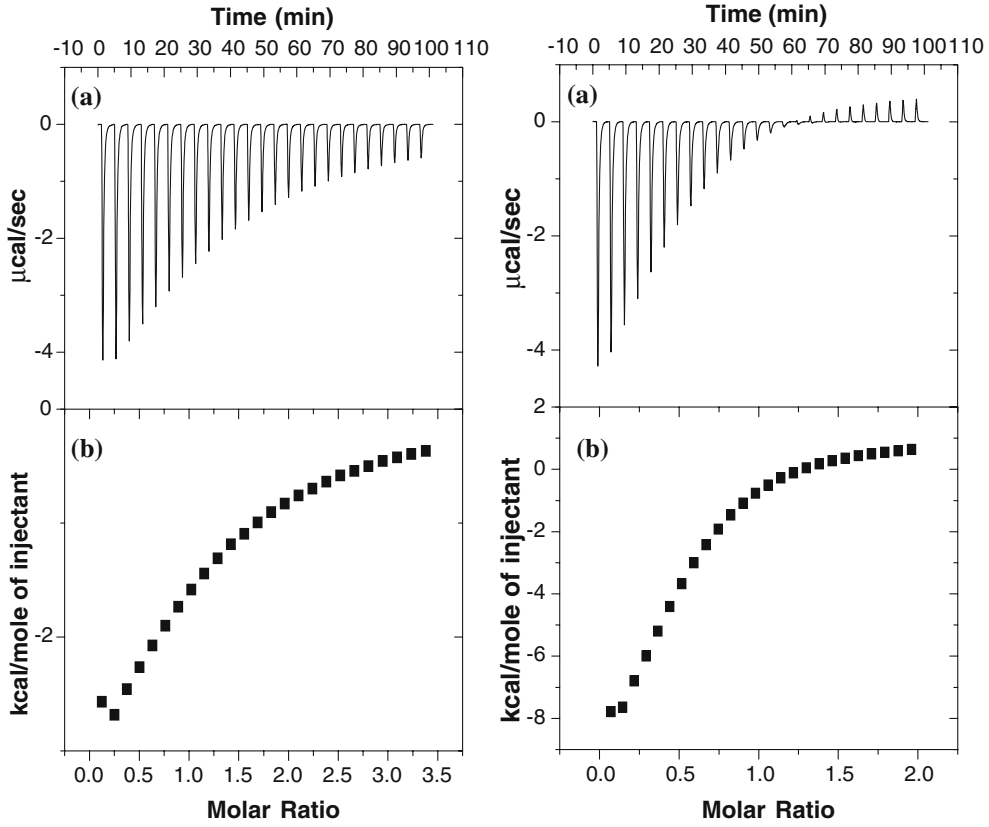

Figure 1. Calorimetric titrations of host **3** with GCA (left) and host **5** with DCA (right) in phosphate buffer (pH 7.20) at 25 °C. (a) Raw data for sequential 10 μl injections of CD solution (5.66 and 2.00 mM) into bile salt solution (0.312 and 0.2 mM). (b) Heats of reaction as obtained from the integration of the calorimetric traces.

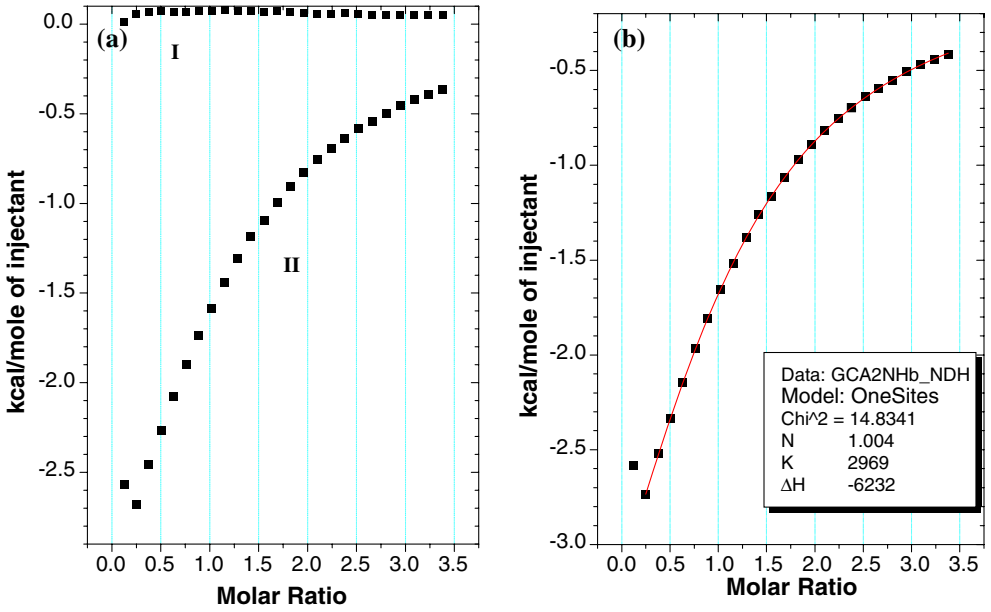

Figure 2. (a) Heat effects of dilution (I) and of complexation (II) of **3** with GCA for each injection during titration microcalorimetric experiment. (b) "Net" heat effect obtained by subtracting the heat of dilution from the heat of reaction, which was analyzed by computer simulation using the "one set of binding sites" model.

To check the accuracy of the observed thermodynamic quantities and afford self-consistent parameters, at least two independent titration experiments were carried out. The average values obtained for the complex stability constant (K_S), standard free energy ($\Delta G°$), enthalpy ($\Delta H°$), and entropy changes ($T\Delta S°$) for inclusion complexation of various guest molecules with native β-CD **1** and modified β-CDs **3-6** are depicted in detail in Table 1. For comparison purpose, the thermodynamic quantities reported for mono(6-amino-6-deoxy)-β-CD (**2**) are also included in Table 1.

Results and discussion

Binding modes

The microcalorimetric experiments of β-CD **1** and modified β-CDs **2**, **3**, **4**, **6** with bile acids, i.e., CA, DCA, GCA, and TCA, showed typical titration curves of 1:1 complex formation. The stoichiometric ratio n that is observed from curve-fitting results of the binding isotherms fall within the range of 0.9-1.1:1. This clearly indicates that the majority of the inclusion complexes have a 1:1 binding stoichiometry between bile acids guests and CDs hosts. Moreover, our prediction that the titration curves of **5** displays a 1:2 host-guest binding stoichiometry is also validated from the 1.9-2.2 of n values from curve-fitting results of its binding isotherms for the four guests.

According to the previous report, bile acids, which possess A, B, C and D rings, are able to penetrate and interact with the hydrophobic CD cavity from either the primary or the secondary side [11], and bile acids can also enter the CD cavity by either the A-ring of the steroid body or the carboxylate and sulfonate group (tail) [12, 13]. Therefore, such uncertain penetrating model and binding sites of guests may greatly affect the conformation and binding pattern of the resulting complex of bile acids with CDs, giving different binding constants [13-15]. In the present study, the inclusion complexes conformation of amino-modified β-CDs with steroids is also determined on the forming complex stability to some extent. To investigate binding modes of these complexes, we performed 2D NMR experiments. A typical ROESY spectrum of host **3** and CA in D_2O is shown in Figure 3.

The symbols used are Hn for CD protons and Pn for steroid protons, where n is the carbon number in CD and steroid [14, 16]. As shown in Figure 3, the ROESY spectrum of CA-**3** complex exhibits clear NOE cross-peaks (peaks A) between the side-chain protons (P21) of CA and H3 protons of CD. Meanwhile, cross-peaks B corresponds to the interaction of the protons (P18) at D-ring of CA with both H3 and H5 protons of CD cavity, showing that the D-ring of CA is accommodated in the cavity. However, there are no NOE signals between the protons (P19) at A-ring of CA and CD's interior protons (H3/H5). Moreover, the cross-peaks C represent the NOE signals of steroid's self-correlation. According to these evidences, we could conclude that steroid body enters the CD cavity from the second side with its tail and D-ring parts, which is consistent with our previous report [16].

Complexation Thermodynamics

It is well known that aminated β-CDs, which possess positive charge at pH 7.2 [17], could improve the original binding ability of native CD toward negatively charged guest molecules due to the additional

Table 1. Complex stability constant (K_S) and standard enthalpy ($\Delta H°$) and entropy changes ($T\Delta S°$) for 1:1 or 1:2 inclusion complexation of bile salt guests with native β-CD **1** and modified β-CDs **2-6** in phosphate buffer solutions of pH 7.20 at T = 298.15 K.

Host[a]	Guest[b]	N[c]	K_S/M^{-1}	$-\Delta G°$/kJ mol^{-1}	$-\Delta H°$/kJ mol^{-1}	$T\Delta S°$/kJ mol^{-1}	Ref.
1	CA	4	4068 ± 84	20.60 ± 0.05	22.98 ± 0.45	−2.38	d
	DCA	4	4844 ± 16	21.03 ± 0.01	25.79 ± 0.00	−4.76	d
	GCA	4	2394 ± 69	19.29 ± 0.07	22.99 ± 0.08	−3.7	d
	TCA	4	2293 ± 13	19.18 ± 0.01	23.77 ± 0.08	−4.59	d
2	CA	2	11160 ± 75	23.10 ± 0.02	25.53 ± 0.25	−2.43	d
	DCA	2	7705 ± 3	22.18 ± 0.00	32.16 ± 0.08	−9.98	d
	GCA	4	2075 ± 19	18.93 ± 0.03	25.90 ± 0.03	−6.97	d
	TCA	2	2309 ± 82	19.20 ± 0.08	26.89 ± 0.07	−7.69	d
3	CA	4	11060 ± 520	23.08 ± 0.03	36.44 ± 0.8	−13.36	e
	DCA	4	11350 ± 110	23.14 ± 0.07	41.15 ± 0.0	−18.01	e
	GCA	4	3050 ± 81	19.89 ± 0.05	25.48 ± 0.2	−5.59	e
	TCA	4	3061 ± 32	19.90 ± 0.02	18.43 ± 0.3	1.47	e
4	CA	2	25315 ± 505	25.13 ± 0.03	34.26 ± 0.3	−9.13	e
	DCA	2	30300 ± 240	25.58 ± 0.15	38.13 ± 1.0	−12.55	e
	GCA	2	3098 ± 37	19.93 ± 0.02	25.82 ± 0.2	−5.89	e
	TCA	4	4659 ± 299	20.94 ± 0.18	14.86 ± 0.2	6.08	e
5	CA	2	13330 ± 50	23.54 ± 0.03	29.77 ± 0.0	−6.23	e
	DCA	2	12065 ± 115	23.30 ± 0.07	34.02 ± 0.1	−10.72	e
	GCA	2	2925 ± 54	19.78 ± 0.03	23.36 ± 1.1	−3.58	e
	TCA	2	2478 ± 21	19.37 ± 0.01	21.46 ± 0.1	−2.09	e
6	CA	2	25850 ± 70	25.18 ± 0.04	23.53 ± 0.3	1.65	e
	DCA	2	24785 ± 125	25.08 ± 0.08	27.59 ± 0.2	−2.51	e
	GCA	2	4722 ± 241	20.97 ± 0.15	21.22 ± 0.3	−0.25	e
	TCA	2	3022 ± 45	19.86 ± 0.03	24.29 ± 0.4	−4.43	e

[a][Host] 2.01-5.66 mM.
[b][Guest] 0.167-0.312 mM.
[c]Number of titration runs performed.
[d]Ref [1].
[e]This work.

electrostatic interactions among the oppositely charged host-guest complexation [18]. According to the data presented in Table 1, mono(6-amino-6-deoxy)-β-CD (**2**), mono(6-ethylenediamino-6-deoxy)-β-CD (**3**), mono(6-diethylenetriamino-6-deoxy)-β-CD (**4**) and their corresponding copper(II) complexes **5** and **6** give distinct binding abilities and molecular selectivities towards bile guests. As compared with native β-CD **1**, oligo(ethylenediamino)-β-CDs **2, 3** and **4**, which possess positively charged amino group in the tether, show enhanced molecular binding abilities and guest selectivities towards bile acids. However, the inclusion complexation of their copper(II) complexes **5** and **6** with bile acids give a relatively complicated but consentaneously enhanced binding abilities towards all bile acids as compared with native β-CD **1**. For example, **5** gives enhanced binding abilities towards CA and DCA but smaller binding constants towards GCA and TCA as compared with its precursor **3**, while **6** shows enhanced binding ability towards CA and GCA but decreased binding ability towards DCA and TCA. Thermodynamically, the inclusion complexation of bile acids with native β-CD **1** and their derivatives **2-6** is absolutely driven by favorable enthalpy changes accompanying with moderate unfavorable or slightly favorable entropy changes ($\Delta H° < 0$; $|T\Delta S°| > 0$). The favorable enthalpy change is attributed to the dominant contribution of the hydrophobic interactions deriving from the size/shape fit and geometrical complement between host and guest. Meanwhile, the unfavorable entropy given by most of

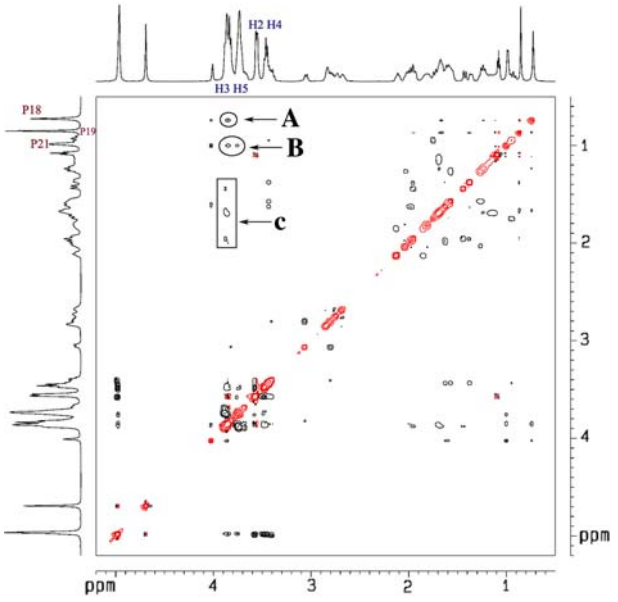

Figure 3. ROESY spectrum for CA and host **3** with a mixing time of 200 ms at 298 K.

the complexes is due to the decrease of rotational and structural freedom upon complex construction, and further leads to the decreased binding stability between hosts and guests. To compare the contributions of electrostatic and hydrophobic interactions as well as the additional binding sites upon inclusion complexation with bile acids, the binding behavior and thermodynamic parameters are respectively discussed according to different hosts series.

Complexation of DCA and CA by 3 and its copper(II) complex 5

As compared with native β-CD **1**, mono(6-ethylenediamino-6-deoxy)-β-CD **3** shows increased binding abilities toward negatively charged bile acids guest molecules, which should be mainly due to the additional electrostatic interactions between the amino tether moiety of hosts and anionic carboxylate or sulfonate tail of guests. As can be seen from Table 1, the binding abilities for the inclusion complexation of **3** with DCA and CA are much higher than those values for native β-CD **1**. Our previous study [14,19] demonstrated that the effective electrostatic interaction between host and guest usually led to a more exothermic reaction enthalpy. However, the higher heat enthalpy does not always lead to the higher complex stability for the inclusion complexation of **5** with CA and DCA, because the entropy loss caused by the rigidity of host-guest structures partly counteracts or over-compensated the enthalpy gain. As can be seen in Table 1, **5** shows larger binding constant upon inclusion complexation with CA and DCA than **3**, although the magnitude of increase is not very remarkable (approximate 10%) when we make a comparison between these two hosts according to single CD unit. This may be attributed to that the coordination of copper ion onto the amino tether of CD affords a more positive charged environment as compared with its precursor **3**. Unexpectedly, the thermodynamic parameters showed the smaller exothermic reaction enthalpy for **5** than for **3** ($\Delta\Delta H^\circ$ = 6.67-7.13 kJ mol^{-1}), while the entropy increased ($T\Delta\Delta S^\circ$ = 7.13-7.29 kJ mol^{-1}). This relatively larger differential reaction entropy compared with differential reaction enthalpy leads to the enhanced binding ability of **5** *versus* **3** upon inclusion complexation with CA and DCA. These results indicate that the introduction of copper ion not only provides additional binding sites, but also makes the copper(II)-coordinated side arm become more hydrophilic. Therefore, the decreased enthalpy and entropy gain reasonably response to the weaker van der Waals interaction and the extensive desolvation effect towards the inclusion complexation with **5**.

Complexation of DCA and CA by 4 and its copper(II) complex 6

Compared with **3**, host **4** also shows stronger binding abilities toward guest molecules, i.e., **4** gives 2.3-2.7 and 6.2-6.3 times higher binding constants than **3** and native β-CD **1**, respectively. By analyzing the thermodynamic parameters in Table 1, we can find that enthalpy changes of modified CDs upon inclusion complexation with CA and DCA display smaller negative value with the extension of the host tether, i.e., $\Delta H^\circ_{\mathbf{3}} < \Delta H^\circ_{\mathbf{4}}$ and $\Delta H^\circ_{\mathbf{5}} < \Delta H^\circ_{\mathbf{6}}$. However, with the extension of the host tether, the entropy changes generally increase but the enthalpy changes decrease. And then the enhanced binding ability ranging from 14255 M^{-1} ($K_s^{(\mathbf{4}\text{-CA})}$ - $K_s^{(\mathbf{3}\text{-CA})}$) to 18,950 M^{-1} ($K_s^{(\mathbf{4}\text{-DCA})}$ - $K_s^{(\mathbf{3}\text{-DCA})}$), and 12,520 M^{-1} ($K_s^{(\mathbf{6}\text{-CA})}$ - $K_s^{(\mathbf{5}\text{-CA})}$) to 12,720 M^{-1} ($K_s^{(\mathbf{6}\text{-DCA})}$ - $K_s^{(\mathbf{5}\text{-DCA})}$), is mainly controlled by entropy change.

It is notable that, attributed to the enthalpic losses, the introduction of copper actually decreases the original binding ability of **4** towards DCA and give comparable stability constant upon complexation with CA, although such modification of hosts supplies an additional binding site, which could result in the enhanced attractive electrostatic interactions. Thermodynamically, the enthalpic losses to some extent derive from the destroyed van der Waals interactions of host-guest complex and the decreased hydrophobic environment [19] of CD cavity caused by the introduction of metal ion. Even though, instead of the electrostatic interaction, the hydrophobic interaction is still the primary factor to control the process of the complexation in this case. The less negative or even positive entropy changes of **6** upon inclusion complexation with DCA and CA are larger (ca. 10.04-10.78 kJ mol^{-1} ($T\Delta\Delta S^\circ$)) than that of host **4**, which perfectly cancel out the dramatically changed $\Delta\Delta H^\circ$ (10.54-10.73 kJ mol^{-1}). This result makes us believe that the extended tether seems to be so flexible upon binding with guests as to structurally adapt to the tether and gain raising freedom detected from the entropy.

As to the inclusion complexation with CA, hosts **4** and **6** give similar binding affinities (K_s = 25315 M^{-1} by **4** and 25850 M^{-1} by **6**, respectively) and merely negligible host selectivity of 1.02 upon complexation with CA. However, upon inclusion complexation with DCA, they give the stability constants up to 30300 M^{-1} by **4** and 24785 M^{-1} by **6**, respectively, and show a relatively higher host selectivity of 1.2. On the other hand, host **6** gives a guest selectivity of 1.04 towards CA/DCA, while host **4** shows reversed molecular selectivity of 0.82.

Complexation of GCA and TCA by modified β-CDs 2-6

All the hosts, including native β-CD **1** and modified β-CDs **2-6**, show the weaker binding abilities upon inclusion complexation with GCA and TCA than that of CA and DCA. The highest binding constant towards GCA is 4722 M^{-1} given by host **6**, and that of towards TCA is 4659 M^{-1} given by host **4**. With respect to GCA, all hosts give moderate negative entropy with the scale of −0.25 to −6.97 kJ mol^{-1}. As for guest TCA, it can be divided into two groups according to the entropy. Hosts **2**, **5** and **6** give negative entropy of −7.69, −2.09 and

−4.43 kJ mol^{-1}, respectively, like native β-CD (−4.59 kJ mol^{-1}). Nevertheless, the other two hosts **3** and **4** show positive entropy of 1.47 and 6.08 kJ mol^{-1}. Possessing positively charged tether as compared with native β-CD and more hydrophobic cavities than copper(II) modified β-CD hosts **5** and **6**, aminated β-CDs **3** and **4** happen solvent release process upon inclusion complexation to result in positive entropy. Meanwhile, hosts **1-6** show different guest selectivity upon binding with these four bile acids. For example, for native β-CD, the best guest selectivity is 2.1 for DCA/TCA, but the situation is quite different for the modified β-CDs due to the remarkably enhanced binding ability toward CA and DCA. Consequently, the best guest selectivity for the modified β-CDs could reach 5.4 for CA/GCA with **2**, 3.7 for DCA/GCA with **3**, 9.8 for DCA/GCA with **4**, 5.4 for CA/TCA with **5** and 8.6 for CA/TCA with **6**, respectively.

Comparison of the enlarged binding abilities of 5 and 6

It is noted that the self-assembly of ethylenediamine modified β-CD by copper ion exhibits a dimeric structure **5**, which significantly enhances the original binding ability of parent β-CD and its derivative through the binding of two hydrophobic β-CD cavities with two guest molecules. Different from the 1:2 binding mode of host **5** with guest molecules, the 1:1 binding model for **6** is more favorable to wrap guest. Towards the same bile acid guests, host **6** gave almost two-folded binding constant than host **5** by comparing a single CD cavity. This result indicates that the complex stability, by comparing a single CD cavity, is greatly influenced by the binding model. Thermodynamically, as compared with host **6,** the conformation rigidity of host **5** may decrease the entropy gains for complexation with CA, DCA and GCA, leading to the relative lower binding stability.

Length effective of aminated β-CD

To investigate the influence of the amino tether length of aminated β-CD upon complexation with guests, we compare the binding ability of hosts **2-4** upon inclusion complexation with bile acids. It is found that complexes stabilities enhance with the extended length of spacer for the same guest except for **2**-CA to **3**-CA giving decreased complex stability ($K_s^{(2\text{-CA})}$ - $K_s^{(3\text{-CA})}$ = 100 M^{-1}). The largest enhancement of binding constant belongs to the **4**-DCA giving 18,950 M^{-1} difference to reach 30,300 M^{-1} when compared with **3**-DCA. Since the amide atom in aminated β-CD can participate in forming hydrogen bonding [18], which will contribute to the overall thermodynamic parameters, it is reasonable to believe the increased stability resulting from the extended length of amide atom-included space derives to the enlarged hydrogen binding system.

Molecular selectivity and binding ability toward bile acids

It is interesting to compare the "host selectivity" sequence obtained for each bile salt. The binding constants for the complexation of each bile salt by native β-CD **1**, aminated β-CDs **2-4** and their corresponding copper(II) complexes **5-6** increase in the order:

CA: **1** < **3** < **2** < **5** < **4** < **6**
DCA: **1** < **2** < **3** < **5** < **6** < **4**
GCA: **2** < **1** < **5** < **3** < **4** < **6**
TCA: **1** < **2** < **5** < **6** < **3** < **4**

As can be seen from Table 1 and Figure 4, the complexes stability constants of hosts **2-6** with guests CA and DCA molecules are larger than those of native β-CD, that is, the corresponding K_S values for the modified CDs are enhanced by factors of 2.7 and 1.6 for **2**, 2.7 and 2.3 for **3**, 6.2 and 6.3 for **4**, 3.3 and 2.5 for **5**, and 6.4 and 5.1 for **6,** respectively. After coordinating with copper(II), host **5** enhances the guest selectivity of DCA/GCA up to 4.1 ($K_S^{(5\text{-DCA})}/K_S^{(5\text{-GCA})}$) from 3.7 given by host **3**.

While the best DCA/GCA selectivity is given by host **4** as high as 9.8, because the inclusion complexes pair **4**·DCA and **4**·GCA gave the largest difference of the Gibbs free energy changes up to −5.65 kJ mol^{-1} ($\Delta\Delta G^\circ = \Delta G^\circ_{\mathbf{4}\cdot\mathrm{DCA}} - \Delta G^\circ_{\mathbf{4}\cdot\mathrm{GCA}}$). Opposite to the increasing trend of guest selectivity from **3** to **5**, the host **6** showed decreased DCA/GCA selectivity from 9.8 to 5.2 after coordinated with copper(II) as compared with **4**, since the relatively smaller $\Delta\Delta G^\circ$($\Delta G^\circ_{\mathbf{6}\cdot\mathrm{DCA}}$ - $\Delta G^\circ_{\mathbf{6}\cdot\mathrm{GCA}}$) value of −4.11 kJ mol^{-1} for host **6** is responsible for the decreased guest selectivity from **4** to **6**. From the view of thermodynamics, the complexation of GCA with **6** shows similar ΔH° and $T\Delta S^\circ$ values to

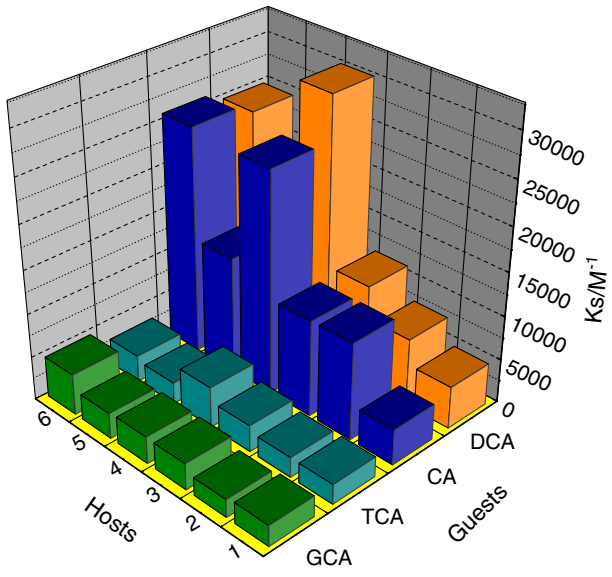

Figure 4. Complex stability constants (K_S) of bile acids upon inclusion complexation with β-CD **1** and β-CD derivatives **2-6** in aqueous phosphate buffer solutions at 298.15 K.

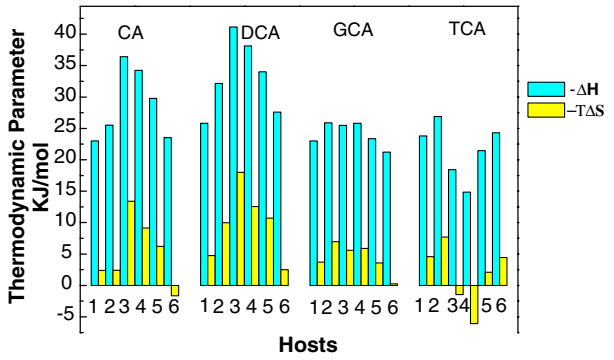

Figure 5. Standard enthalpy ($\Delta H°$) and entropy changes ($T\Delta S°$) for the 1:1or 1:2 inclusion complexation of hosts **1-6** in phosphate aqueous buffer solutions at 298 K.

those for native β-CD **1** (Figure 5), the enthalpic loss ($\Delta H°_{\mathbf{6}}$ - $\Delta H°_{\mathbf{1}}$ = 1.77 kJ mol^{-1}) is almost counterbalanced by the entropy changes ($T\Delta S°_{\mathbf{6}}$ - $T\Delta S°_{\mathbf{1}}$ = 3.45 kJ mol^{-1}). However, the complexation of DCA with **6** gives a moderate enthalpic gain than that for β-CD ($\Delta H°_{\mathbf{6}}$ - $\Delta H°_{\mathbf{1}}$ = −1.8 kJ mol^{-1}), which is increased by adding the comparable entropic gain ($T\Delta S°_{\mathbf{6}}$ - $T\Delta S°_{\mathbf{1}}$ = 2.25 kJ mol^{-1}). As a consequence of such accumulated effect of $\Delta H°$ and $T\Delta S°$, the negligible molecular selectivity of β-CD ($\Delta\Delta G° = \Delta G°_{\mathbf{1}\cdot\mathrm{DCA}} - \Delta G°_{\mathbf{1}\cdot\mathrm{GCA}}$ = −1.74 kJ mol^{-1}) is substantially enhanced to give a $\Delta\Delta G°$ value of −4.11 kJ mol^{-1}.

The copper(II) complexes reverse the binding ability towards CA and DCA, i.e., they form more stable complexes with CA, rather than with DCA when compared the binding ability of four hosts. Undoubtedly, such opposite binding ability is related to the conformation of host molecules and bile acids guests. Compared with CA, the structure of DCA is more hydrophobic accounting for the absence of C-7 hydroxyl group, and such structure characteristic distinguishes DCA from CA in terms of more favorable hydrophobic and van der Waals interactions in inclusion complexation process. As for the host conformation, the introduction of copper(II) decreased the hydrophobicity of hosts but increased electronic interaction with guests molecules to obtain enhanced stability constants.

Possessing the similar backbone but more hydrophilic tail, guests GCA and TCA present different binding behavior from CA and DCA. As can been seen from Table 1, only the moderate increases of binding abilities were given (1.2-2.0 times for GCA and 1.1-2.0 times for TCA) by **3-6** in comparison with native β-CD. Attributing to the more hydrophilic tail, which is attached to the end of the D ring, GCA and TCA are unfavorable to insert into the cavity from the second side of β-CD cavity with their D ring. Obviously, the hydrophobic interaction is the primary driving force for the inclusion complexation of this series of modified β-CDs and chosen bile salt guests.

Conclusion

Thermodynamic parameters of the inclusion complexation of amino modified β-CDs and their copper(II) complexes with bile acids have been determined and compared, indicating that the attachment of oligo(ethylenediamine) to β-CDs and/or extended binding size not only enhance the molecular binding ability but also molecular selectivity. Thermodynamically, the enhanced molecular recognition abilities and complexes stability along with the extension of amino tether are controlled by entropy change, but all the complex formations are driven by enthalpy without exception. Although the self-assembly of ethylenediamine modified β-CD by copper ion shows smaller binding constants compared with its counterpart diethylenetriamino modified β-CD copper(II) complex according to single CD unit, it still gives higher stability than that of native β-CD. Apparently, the coordination of metal or increased binding size can substantially enhance binding ability and molecular selectivity.

Acknowledgements

We are grateful to the National Natural Science Foundation (No. 90306009, 20572052, 20402008 and 20421202) and the Tianjin Natural Science Foundation (043604411) for financial support.

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