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Thermochimica Acta 470 (2008) 108–112
thermochimica acta
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Short communication

Thermodynamics of complexes between nucleobase-modified β-cyclodextrins and bile salts

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Received 3 January 2008; received in revised form 15 February 2008; accepted 19 February 2008
Available online 23 February 2008

Abstract

The binding of three nucleobase-modified β-CDs, (i.e., mono(6-ade-6-deoxy)-β-CD **2**, mono(6-thy-6-deoxy)-β-CD **3**, and mono(6-ura-6-deoxy)-β-CD **4**) with four bile salts (deoxycholate, DCA; cholate, CA; glycocholate, GCA; and taurocholate, TCA) were investigated by means of circular dichroism, 2D NMR spectroscopy and calorimetric titration. The results show the binding of host **2** with bile salts is weaker and different from hosts **3** and **4**. Enthalpy changes between hosts **2–4** and bile salts are much more favorable than those of native β-CD **1**, whereas the entropy changes are unfavorable.

Keywords: Microcalorimetric titration; Molecular recognition; Cyclodextrin; Bile salt

1. Introduction

Molecular recognition based on cyclodextrins (CDs) and their derivatives is of current interest in supramolecular chemistry [1]. Many efforts have been devoted to the design and synthesis of new CD derivatives to investigate binding behavior for various guests [2–5]. The thermodynamics of CD complexation with guests has been widely studied by our group and others [4c,6,7].

The present work investigates the thermodynamics of nucleobase-modified β-CDs **2–4** toward bile salt (Chart 1) binding by circular dichroism, 2D NMR spectroscopy and isothermal titration calorimetry (ITC). Nucleobase-functionalized β-CDs may be viewed as nucleoside analogues [8] that can provide additional binding interactions with guests. Bile salts are typical biological amphiphiles.

2. Experimental

2.1. Materials

β-CD of reagent grade was recrystallized twice from water and dried in Vacuo at 100 °C for 24 h prior to use. Cholate (CA), deoxycholate (DCA), glycocholate (GCA), and taurocholate (TCA) (Chart 1) were purchased from Sigma and used as received. Mono(6-ade-6-deoxy)-β-CD **2**, mono(6-thy-6-deoxy)-β-CD **3**, mono(6-ura-6-deoxy)-β-CD **4** (Chart 1) were prepared according to the procedures reported [8b,9]. Disodium hydrogen phosphate and sodium dihydrogen phosphate were dissolved in distilled, deionized water to make a 0.1 mol dm^{-3} phosphate buffer solution of pH 7.2 for calorimetric titrations.

2.2. Apparatus and procedures

2D ROESY (rotating frame Overhauser effect spectroscopy) spectra were recorded on a Varian Mercury VX300 instrument in D_2O solution. UV–vis and circular dichroism spectra were performed on a Shimadzu UV 2401 spectrophotometer and a JASCO J-715S spectropolarimeter in aqueous phosphate buffer solution (pH 7.2) at 298.15 K.

Calorimetric titrations were performed with a VP-ITC calorimeter from Microcal Inc., Northampton, MA. The calorimeter was calibrated chemically by the complexation reaction of β-CD with cyclohexanol. The thermodynamics obtained were in good agreement with literature data, measured $K = 707\ M^{-1}$, $\Delta H^\circ = -6.1\ kJ\ mol^{-1}$, literature $K = 704\ M^{-1}$, $\Delta H^\circ = -6.6\ kJ\ mol^{-1}$ [10]. Each solution was degassed in a ThermoVac accessory before titration. Twenty-five injections (10 μL) of host solution were injected with stirring at 300 rpm

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doi:10.1016/j.tca.2008.02.013

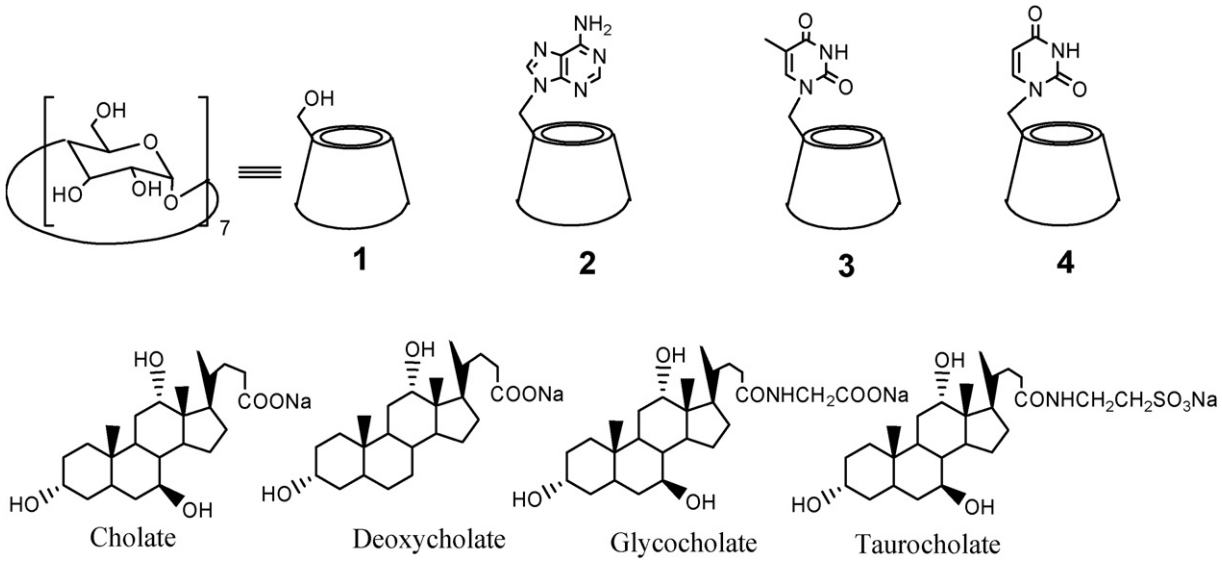

Chart 1. The structures of cyclodextrin hosts **1–4** and bile guests.

into the reaction cell (1.4227 mL) charged with guest solution in the same buffer solution. The concentration of bile salt was 0.15–0.51 mM, well below the critical micelle concentration (CMC) [4e,4f].

Since the dilution heat of CDs was not negligible, the dilution heat was determined by injecting the host solution into a buffer solution without guest under the same condition. The dilution enthalpy was subtracted from the enthalpy measured in the titration experiment to obtain the net reaction heat.

ORIGIN software (Microcal Inc.) was used to simultaneously compute the equilibrium constant (K) and enthalpy of reaction (ΔH°) from each titration curve by fitting with the 'one set of binding sites' model. The first point was excluded from the fitting procedure, but the titrant added in the first injection was included in calculating the total amount of titrant in the solution [11]. A typical curve fitting result for the complexation of **2** with DCA is shown in Fig. 1. Two independent titration experiments were carried out and their average values used to calculate the complex stability constant and thermodynamic parameters. The obtained complex stability constant (K), standard free energy (ΔG°), enthalpy change (ΔH°) and entropy change ($T\,\Delta S^\circ$) for the host–guest inclusion complexation are listed in Table 1.

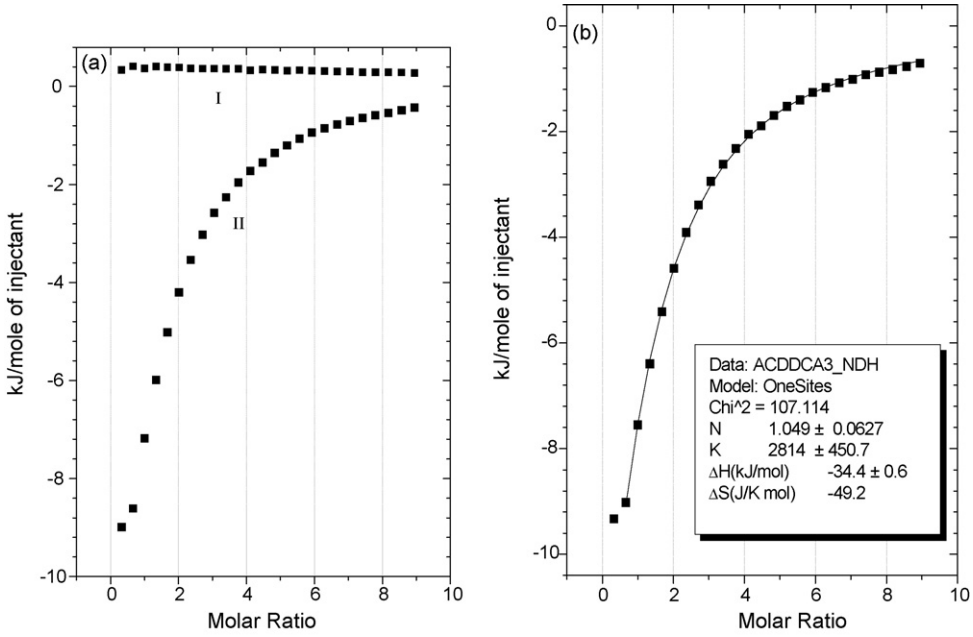

Fig. 1. (a) Heat effects of the dilution (I) and the complexation (II) of host **2** with DCA for each injection during titration microcalorimetric experiment. (b) 'Net' heats effect of the complexation of host **2** with DCA for each injection, obtained by subtracting the dilution heat from the reaction heat, which was analyzed by computer simulation using the 'one set of binding sites' model.

Table 1
Complex stability constant (K_s) and thermodynamic parameters for the inclusion complextion of bile salt guests with host **1–4** in phosphate buffer solution (pH 7.2) at $T = 298.15$ K

Guest[a]	Host[b]	K (M^{-1})	$-\Delta G^\circ$ (kJ mol^{-1})	$-\Delta H^\circ$ (kJ mol^{-1})	$T\Delta S^\circ$ (kJ mol^{-1})	Ref.
CA	**1**	4068 ± 84	20.6 ± 0.1	23.0 ± 0.45	−2.4 ± 0.2	[c]
	2	1276 ± 118	17.7 ± 0.2	31.0 ± 0.3	−13.3 ± 0.2	[d]
	3	2567 ± 20	19.4 ± 0.1	29.3 ± 0.1	−9.9 ± 0.2	[d]
	4	2605 ± 36	19.5 ± 0.1	28.6 ± 0.1	−9.1 ± 0.1	[d]
DCA	**1**	4844 ± 16	21.0 ± 0.1	25.8 ± 0.1	−4.8 ± 0.1	[c]
	2	2839 ± 25	19.7 ± 0.1	34.8 ± 0.2	−14.9 ± 0.2	[d]
	3	3137 ± 20	20.0 ± 0.1	34.0 ± 0.1	−14.0 ± 0.2	[d]
	4	3813 ± 21	20.4 ± 0.1	33.7 ± 0.2	−13.3 ± 0.1	[d]
GCA	**1**	2394 ± 69	19.3 ± 0.2	23.0 ± 0.1	−3.7 ± 0.2	[c]
	2	1032 ± 21	17.2 ± 0.2	25.7 ± 0.2	−8.5 ± 0.1	[d]
	3	2898 ± 43	19.8 ± 0.1	31.2 ± 0.1	−11.4 ± 0.2	[d]
	4	3140 ± 28	19.9 ± 0.1	29.6 ± 0.1	−9.7 ± 0.1	[d]
TCA	**1**	2293 ± 13	19.2 ± 0.1	23.8 ± 0.1	−4.6 ± 0.1	[c]
	2	1003 ± 45	17.1 ± 0.1	26.6 ± 0.2	−9.5 ± 0.2	[d]
	3	2284 ± 96	19.2 ± 0.1	30.0 ± 0.1	−10.8 ± 0.1	[d]
	4	2402 ± 21	19.3 ± 0.1	28.8 ± 0.1	−9.5 ± 0.1	[d]

[c]Reference [5b]. [d]This work.
[a] [guest] = 0.15–0.51 mM.
[b] [host] = 3.00–10.30 mM.

3. Results and discussion

3.1. Manner of binding

In host **2**, the adenine group is deeply inserted into the β-CD cavity with an orientation parallel to the C_7 axis of β-CD while the thymine and uracil groups are shallowly inserted in the β-CD cavity with an orientation perpendicular to the C_7 axis of β-CD [9]. Upon complexation with DCA guest, the induced circular dichroism (ICD) signal changed as shown in Fig. 2. The DCA guest gives UV absorption around 200–225nm so we only study the signal changes of ICD beyond 225 nm. The intensity of negative Cotton effect peak of host **2** undergoes a pronounced decrease (from −6.86 to −2.45 M^{-1} cm^{-1}), while the intensities of positive Cotton effect of hosts **3** and **4** just have a slight increase (from 6.27 to 6.82 M^{-1} cm^{-1} for **3**, from 8.97 to 10.98 M^{-1} cm^{-1} for **4**). These ICD results indicate that the conformation of host **2** changes markedly upon complexation with DCA guest, whereas hosts **3** and **4** almost maintain their original conformations. One rational explanation for these phenomena is that the deeply included adenine group in host **2** should be expelled from the cavity upon complexation with DCA guest, however, the shallowly included thymine and uracil groups in hosts **3** and **4** are hardly influenced by the inclusion of DCA guest.

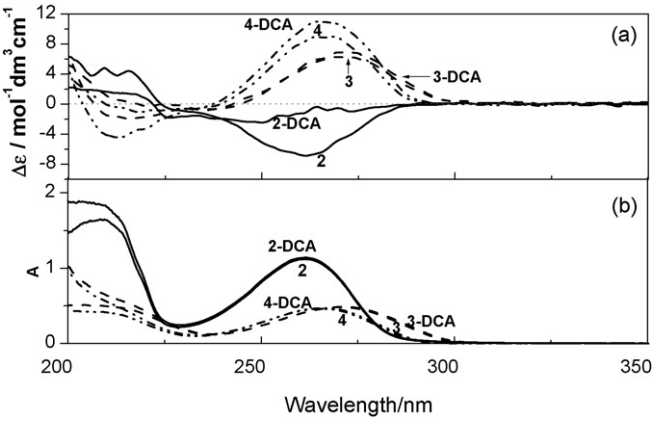

Fig. 2. Circular dichroism (a) and absorption (b) spectra of hosts **2–4** (0.1 mM) in the absence and presence of DCA (1 mM) in aqueous phosphate buffer solution (pH 7.2) at 298.15 K.

Furthermore, the complex structures are also identified by the ROESY experiments of **2**-DCA and **4**-DCA (Fig. 3). As can be seen from Fig. 3a, the NOE correlation between the adenine substitute and the interior protons of β-CD cavity disappeared [9] upon complexation with DCA, and clear NOE correlations between the DCA protons and the interior protons of β-CD cavity were observed (peaks A). It is reasonable to deduce that the DCA guest was included into the CD cavity and the adenine substitute was expelled out by DCA guests. Fig. 3b shows the clear NOE correlations between the DCA protons and the interior protons of β-CD cavity (peaks A) which means the DCA guest were included into the CD cavity. The NOE correlation between the uracil substituent and interior protons of β-CD cavity was not been observed and the possible reason is that the uracil substituent possesses less protons and enters the CD cavity shallowly. Therefore, combining the ICD and 2D NMR results together, it can be inferred that the complexation of host **2** with DCA follows the way of competitive inclusion between adenine substituent and DCA guest, and hosts **3** and **4** present the binding manner of co-inclusion that both the thymine and uracil substituents and the DCA guest molecule are located in the cavity.

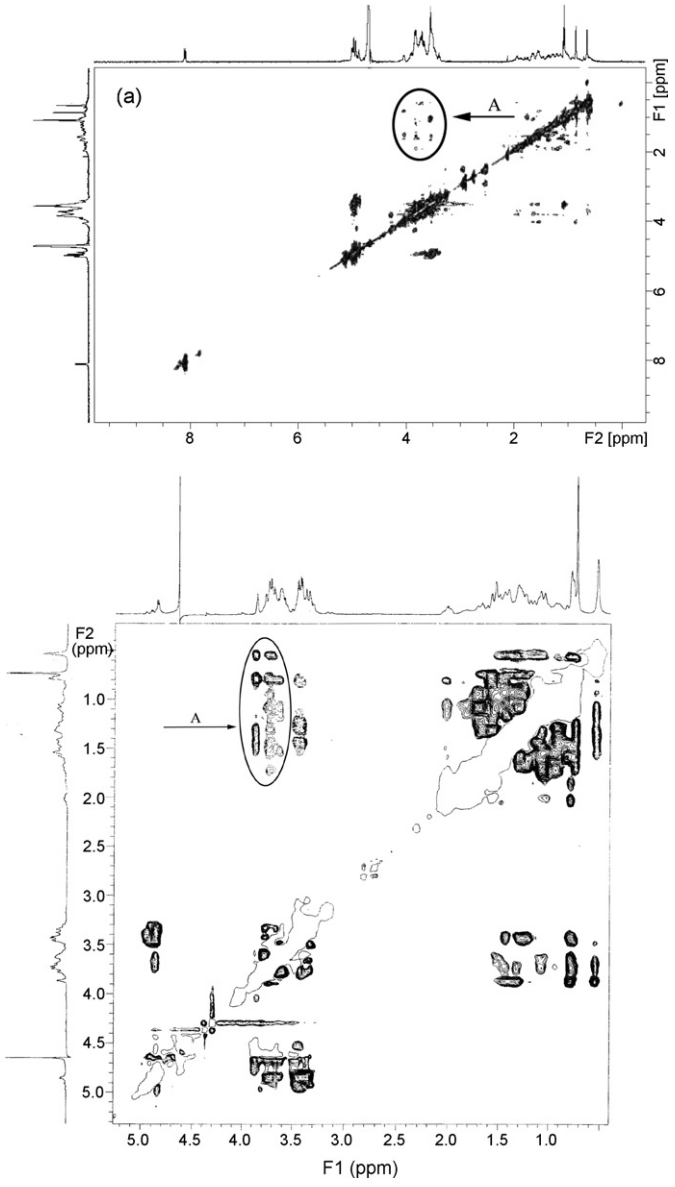

Fig. 3. ROESY spectra of the resulting complexes of **2**-DCA (a) and **4**-DCA (b) with a mixing time of 400 ms at 298.15 K.

3.2. Thermodynamics of binding

As can be seen from Table 1, the nucleobase-modified β-CDs **2–4** exhibit distinguishable binding abilities toward bile salts. Host **4** shows increased binding of TCA/GCA and host **3** exhibits increased binding of GCA while hosts **2–4** show less binding of the other bile salts used in present investigation. The inclusion complexation of hosts **2–4** with bile salts have favorable enthalpy changes, accompanied with unfavorable entropy changes. The association of hosts **2–4** with bile salts is driven by hydrogen-bonding and van der Waals interactions, simultaneously producing marked geometric configuration change [12]. Further investigation showed that the enthalpy changes for complexation of hosts **2–4** with bile salts are in the range of −25.7 to −34.8 kJ mol^{-1}, which are obviously more favorable than those of native β-CD **1**, it mainly ascribed to the host–guest hydrogen-bonding interactions [6a,7d,13].

Obvious differences among hosts **2–4** upon complexation with the same bile guest also exist. Host **2** displays weaker binding ability for every bile salt than hosts **3** and **4**. This is mainly owing to expelling adenine group from β-CD cavity to accommodate bile guests in hosts **2** as proved by circular dichroism experiments, which is unfavorable to the host–guest complexation. For the enthalpy term, the enthalpy changes upon complexation with CA and DCA guests are similar among the three hosts **2–4**. That is, host **2** gives the most negative enthalpy changes while host **4** gives the less negative enthalpy changes which originate from the stronger hydrogen-bonding and van der Waals interactions between host **2** and CA/DCA guest than hosts **3** and **4**. As far as hosts **2–4** binding with GCA/TCA is concerned, the enthalpy changes of hosts **3** and **4** with GCA and TCA guests are more favorable than those of host **2**. As a typical example, the absolute value of enthalpy change of **3** with GCA is 5.5 (kJ mol^{-1}) larger than that of **2** with GCA. Comparing the host–guest binding, the co-inclusion geometry of hosts **3** and **4**, the NH group in the side chain of GCA (or TCA) can also possibly form a hydrogen-bond with the C=O group in the thymine (or uracil) substituents of hosts **3** and **4**. Ross and Rekharsky investigated the contribution of hydrogen-bonding on thermodynamics of CDs binding guests and their results showed that hydrogen-bonding gave favorable enthalpy changes, though not always increasing the binding ability between hosts and guests [1b,13b].

4. Conclusion

The circular dichroism experiments indicate that complexation of host **2** with bile salts occurs with competitive inclusion, whereas hosts **3** and **4** show co-inclusion. Complexation of hosts **2–4** with bile salts is enthalpy-driven, accompanied with the unfavorable entropy changes. Besides the hydrophobic and van der Waals interactions between CDs and guests, hydrogen-bonding is the key factor controlling the stability of present inclusion complexes.

Acknowledgments

This work was supported by NNSFC (Nos. 20673061 and 20421202) and the Special Fund for Doctoral Program from the Ministry of Education of China (No. 20050055004), which are gratefully acknowledged.

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