Molecular recognition study of a supramolecular system. Part 4.[1] Molecular recognition thermodynamics of amino acids by modified β-cyclodextrins

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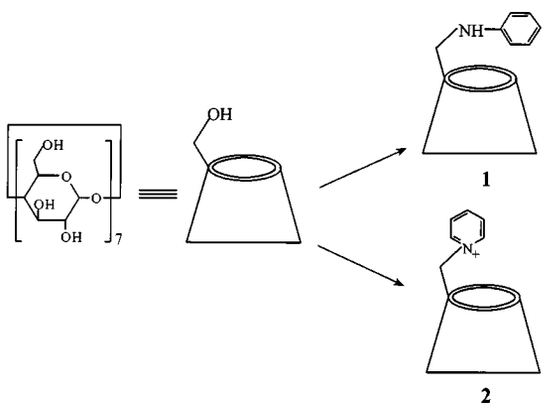
The novel β-cyclodextrin derivative mono-(6-anilino-6-deoxy)-β-cyclodextrin 1 bearing a single anilino moiety has been synthesized by a convenient method in 45% yield. Spectrophotometric titrations have been performed in buffer aqueous solution (pH 7.20) at 25.0–40.0 °C in order to obtain the complex stability constants (K_s) and the thermodynamic quantities (ΔH° and ΔS°) for the stoichiometric 1:1 inclusion complexation of various amino acids with the host compound 1 and mono-(6-1-pyridinio-6-deoxy)-β-cyclodextrin 2. The molecular recognition abilities and enantioselectivity for guest L- or D-amino acids of the host β-cyclodextrin derivatives 1 and 2 are discussed from the thermodynamic point of view. The thermodynamic parameters obtained indicate that the modified β-cyclodextrins 1 and 2 carrying one chromophoric anilino or pyridinio moiety as a probe for differential UV spectrometry can recognize not only differences between the molecular size and shape of amino acids, but also the chirality of the L- or D-amino acid isomer. The host compound 1 possesses higher molecular inclusion ability as well as enantioselectivity than the β-cyclodextrin derivative 2 as the size and shape of the L- or D-amino acids is varied, which is probably attributable to the increased enthalpic gain. The inclusion complexation is mainly enthalpy driven for 1, while for 2 it is mainly entropy driven. The larger enthalpic and entropic gains are attributed to the complexes' stabilities as a consequence of compensation effects.

Introduction

Cyclodextrins (CDs) having fairly rigid and well-defined hydrophobic cavities can act as molecular receptors (hosts) for a wide variety of organic and inorganic, as well as biological, guest molecules, forming host–guest complexes or supramolecular species.[1–8] A great deal of effort has been devoted to the investigation of the complexation thermodynamics of several types of guest molecules with cyclodextrins in order to understand the nature of the noncovalent interactions between them and to gain insight into the thermodynamic factors involved.[9–15] However, thermodynamic studies of molecular recognition by chemically modified cyclodextrins are so far rare, except for recent work using naphthalene-2-sulfonate and phenol derivatives as guests by Inoue[16] and Harata.[17]

Our recent study on the complexation thermodynamics of some naphthalene derivatives with natural α-, β- and γ-cyclodextrins has shown that the thermodynamic parameters obtained are sensitive functions of the position, number and type of anionic substituent(s) introduced into the guest molecules.[15] More recently, we have demonstrated that modifications to β-cyclodextrin for complexation with naphthalene-2-sulfonate led to substantial decreases in complex stability, which may be attributed to the highly negative entropy changes ($T\Delta S$) that exceed the increased enthalpic gains ($-\Delta H$) arising from the enhanced hydrophobic interaction with lipophilic side chain(s) in the modified cyclodextrin. The enthalpy–entropy compensation effect was always observed for host–guest complexation.[16] These results improve our understanding of several weak forces working on receptor and substrate, which include van der Waals forces, hydrogen bonding and hydrophobic interactions, prompting us to investigate the molecular recognition mechanism of amino acids by modified β-cyclodextrins.

In the present study, we synthesized mono-(6-anilino-6-deoxy)-β-cyclodextrin **1** and mono-(6-1-pyridinio-6-deoxy)-β-cyclodextrin **2**, and investigated the thermodynamics of molecular recognition by the two modified β-cyclodextrins carrying one pyridinio or one anilino moiety, which is used as a probe for differential UV spectra. Amino acid guest molecules



are employed in order to examine the possible participation of several weak interaction forces, working between modified β-cyclodextrins and amino acid molecules to form host–guest complexes, from the thermodynamic point of view, which will enhance our further understanding of this thermodynamically less investigated area of modified cyclodextrin chemistry.[16]

Experimental

General procedure

Elemental analyses were performed on a Perkin-Elmer 240 instrument. ^{1}H NMR spectra were recorded at 400 MHz in [$^2H_6$]dimethyl sulfoxide on an ARX400 spectrometer. IR and UV spectra were obtained on Nicolet FTIR 5DX and Shimadzu UV-240 spectrometers, respectively. Circular dichroism (CD) spectra were recorded on a JASCO J-720 spectropolarimeter.

Materials

Commercially available amino acids (Tianjin Chemical Reagent Plant) were used without further purification. β-Cyclodextrin of reagent grade was recrystallized twice from water and dried for 12 h *in vacuo* at 100 °C. *N*,*N*-Dimethylformamide (DMF)

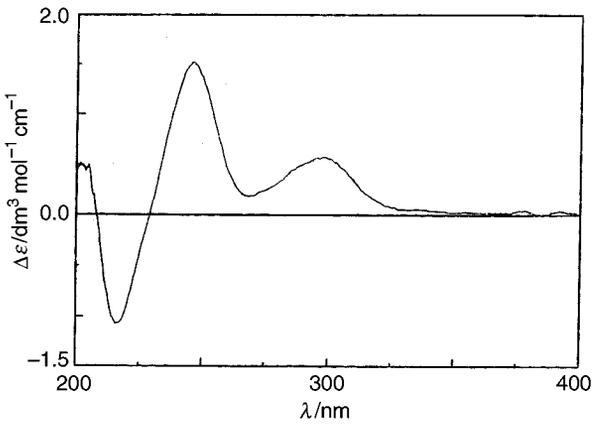


Fig. 1 Circular dichroism spectra of β-cyclodextrin derivative **1** (9.9×10^{-5} mol dm^{-3}) in aqueous solution at room temp.

was dried over calcium hydride for 2 d, and then distilled under reduced pressure prior to use. Aniline was distilled at reduced pressure before use. Analytical reagent grade pyridine was dried over calcium hydride for 2 d and distilled just before use. Mono-(6-1-pyridinio-6-deoxy)-β-cyclodextrin **2** was synthesized according the procedures reported by Matusi *et al.*[18] Disodium hydrogen phosphate and sodium dihydrogen phosphate were dissolved in distilled, deionized water to make a 0.10 mol dm^{-3} aqueous solution. Mixing 72 ml Na_2HPO_4 solution and 28 ml NaH_2PO_4 solution gave the phosphate buffer solution for spectral titration (pH 7.20). The pH values of the buffer solution at 25–40 °C were measured, with pH variation only on average 0.002 pH units per degree.

Synthesis of mono-(6-anilino-6-deoxy)-β-cyclodextrin 1

Mono-(6-*O*-tolylsulfonyl)-β-cyclodextrin (6-OTs-β-CD) was prepared by the reaction of β-cyclodextrin with toluene-*p*-sulfonyl chloride in dry pyridine.[19] Compound **1** was prepared by the reaction of 6-OTs-β-CD (2 g) with aniline (10 ml) in DMF (20 ml) at 85 °C with stirring for 12 h under N_2. The reaction mixture was evaporated *in vacuo* at 40 °C to dryness. The residue was dissolved in water and then acetone was added to the solution to give a grey precipitate. After drying, the grey precipitate was recrystallized twice from water, and dried *in vacuo* to give 0.85 g of a pale yellow solid (yield 45%); *ν*/cm^{-1} (KBr) 3386, 2927, 1639, 1606, 1507, 1417, 1368, 1335, 1302, 1253, 1237, 1155, 1081, 1032, 941, 859, 753, 704, 572. δ_H[$(CD_3)_2SO$, Me_4Si, ppm]: 0.83 (m, 1H N–H); 1.23 (d, 2H CH_2); 3.3–5.6 (m, C–H, O–H); 6.5–7.3 (m, 4H, Ar–H); Calc. for $C_{48}H_{75}O_{34}N_3 \cdot H_2O$: C, 45.61; H, 6.4; N, 1.11; Found: C, 45.9; H, 6.51; N, 1.14%.

Spectral measurements

The stability constants of modified cyclodextrins **1** and **2** inclusion complexes with some selected amino acid biological molecules were determined using differential UV spectrometry. Differential absorption spectra were obtained directly using the instrument according to its normal procedures. The quartz cells (1 cm) were kept at constant temperature (25.0 ± 0.1 °C) with circulating water from a constant-temperature water bath. In order to determine thermodynamic parameters for the complexation equilibria, the spectral titrations were repeated at 25.0, 30.0, 35.0 and 40.0 °C.

Results and discussion

CD spectra

The electronic spectra of modified β-cyclodextrin **1** (9.9×10^{-5} mol dm^{-3}) in aqueous solution showed a strong negative Cotton effect peak, corresponding to the ^1L_a band at 216.2 nm ($\Delta\varepsilon = -1.07$) and a strong positive Cotton effect for the ^1L_b band at 246.2 nm ($\Delta\varepsilon = 1.52$), shown in Fig. 1. As can be seen in Fig. 2, according to the sector rule proposed by Kajtar,[20] the Cotton effects observed for the ^1L_a and ^1L_b bands indicate that the anilino moiety penetrates only a little into the hydrophobic cavity of cyclodextrin.[21]

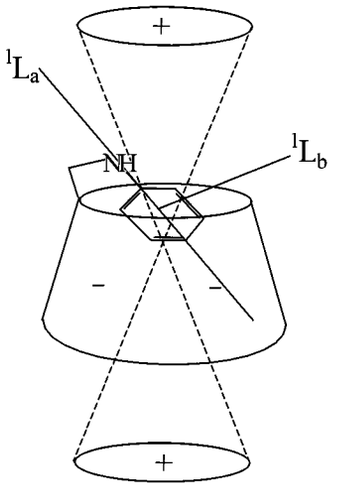


Fig. 2 Kajtar's sector rule applied to transition moments of ^1L_a and ^1L_b bands of the anilino moiety in **1**

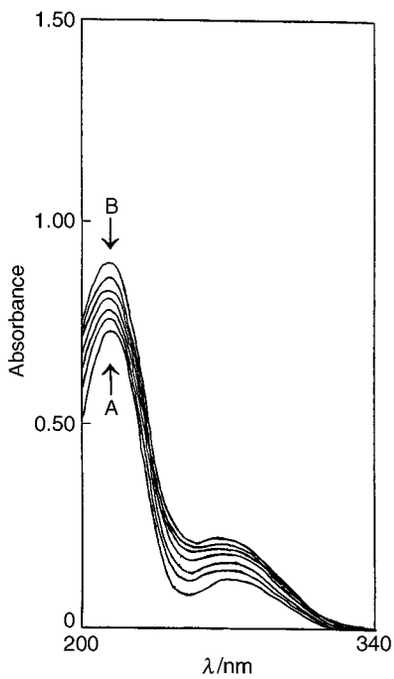


Fig. 3 Spectrum of **1** with varying amino acid (L-Ile) concentration: solvent, phosphate buffer, pH 7.20; 25.0 °C. The cyclodextrin derivative **1** concentration is 8×10^{-5} mol dm^{-3}. The concentrations of amino acid (mol dm^{-3}) are 0, 2×10^{-4}, 6×10^{-4}, 1×10^{-3}, 2×10^{-3}, 3×10^{-3} and 4×10^{-3} reading from A to B.

UV spectral titrations

In the titration experiments using UV spectrometry, as can be seen from Fig. 3, the absorption maximum of the aromatic group, originally perched on the edge of the β-cyclodextrin cavity, gradually increased upon the addition of various concentrations of amino acid (0.2–4.0 × 10^{-3} mol dm^{-3}), and there were two characteristic absorptions at 241 and 286 nm, indicating that modified β-cyclodextrins must suffer substantial conformational change upon guest inclusion, and form inclusion complexes with amino acids. This substantial conformational change is used to determine complex stability constants. Although there is a slight shift in the absorbance wavelength at *ca.* 286 nm, due to the inclusion complexation of the host with

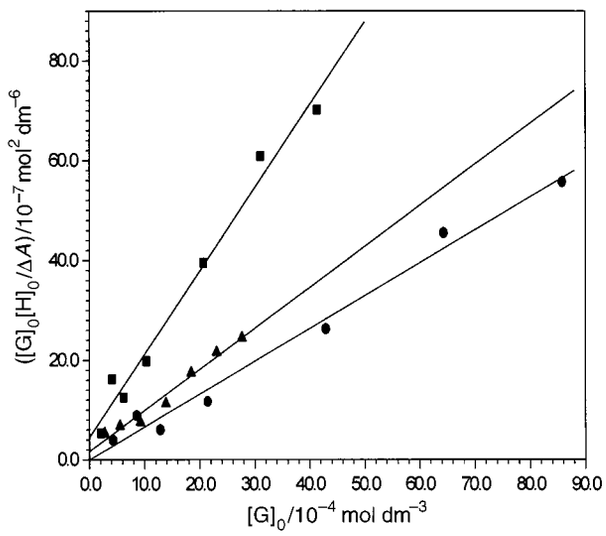


Fig. 4 Typical plots of $[G]_0[H]_0/\Delta A$ *versus* $[G]_0$ for the host–guest complexation of amino acids (▲ L-Val, ■ L-Ile, ● L-Glu) and derivative **1** in phosphate buffer solution (pH 7.20) at 25 °C

the guest, the K_s is calculated on the basis of the first characteristic peak at 241 nm. With the assumption of a 1:1 stoichiometry, the inclusion complexation of amino acids (G) with β-cyclodextrin derivatives (H) is expressed by eqn. (1).

$$H + G \overset{K_s}{\rightleftharpoons} G\cdot H \quad (1)$$

Under the conditions employed, the concentration of β-cyclodextrin derivatives (8.0×10^{-5} mol dm^{-3}) is much smaller than that of amino acids, *i.e.* $[H]_0 \ll [G]_0$. Therefore, the stability constant of the supramolecular system formed can be calculated according to the modified Hildebrand and Benesi equation,[22,23] eqn. (2), where $[G]_0$ denotes the total concentration of amino acid, $[H]_0$ refers to the total concentration of cyclodextrin derivatives, $\Delta\varepsilon$ is the difference between the molar extinction coefficient for the free and complexed β-cyclodextrin derivatives, ΔA denotes the changes in the absorption of the modified β-cyclodextrin on adding amino acid. For all guest molecules examined, plots of calculated $[H]_0[G]_0/\Delta A$ values as a function of $[G]_0$ give good straight lines. Typical plots are shown for inclusion complexation of compound **1** with some selected amino acids in Fig. 4. The experiments were performed at 25.0, 30.0, 35.0 and 40.0 °C and the stability constants ($\log K_s$) calculated from the slope and the intercept are listed in Table 1.

$$[G]_0[H]_0/\Delta A = 1/K_s\Delta\varepsilon + [G]_0/\Delta\varepsilon \quad (2)$$

Thermodynamic parameters

The free-energy change ($\Delta G°$) for inclusion complexes formed by modified β-cyclodextrins and guest amino acids is calculated from the equilibrium constant K_s by eqn. (3) and is related to

$$\Delta G° = -RT \ln K_s \quad (3)$$

the enthalpic and entropic changes ($\Delta H°$ and $\Delta S°$) through the Gibbs–Helmholtz eqn. (4). Combining eqns. (3) and (4), we obtain eqn. (5) which describes the temperature dependence of

$$\Delta G° = \Delta H° - T\Delta S° \quad (4)$$

$$\log K_s = (1/2.303R)(\Delta S° - \Delta H°/T) \quad (5)$$

K_s. Thus, plots of the $\log K_s$ values, shown in Table 1, as a function of the inverse of temperature gave good linear relationships. Typical plots for host compound **1** are shown in Fig. 5. The thermodynamic parameters obtained for each modified β-cyclodextrin inclusion complexation with amino acids are listed in Table 2.

Table 1 Stability constants (K_s) for 1:1 host–guest complexation of some amino acid guest molecules with modified β-cyclodextrin **1** and **2** at 25–40 °C (pH 7.20, 0.10 mol dm^{-3} phosphate buffer)[a]

		log K_s			
Host	Guest	25.0 °C	30.0 °C	35.0 °C	40.0 °C
1	L-Ala	3.62	3.74	3.84	3.90
	D-Ala	3.52	3.46	3.39	3.26
	L-Val	3.82	3.72	3.62	3.49
	L-Ile	3.79	3.71	3.56	3.39
	L-Pro	3.64	3.61	3.55	3.50
	L-Ser	3.49	3.53	3.62	3.71
	D-Ser	3.43	3.46	3.55	3.59
	L-Cys	2.65	2.53	2.38	2.08
	L-Glu	4.17	4.03	3.90	3.55
	L-Lys	2.57	2.64	2.71	2.83
	L-Asp	4.04	3.96	3.89	3.78
	L-Leu	3.70	3.83	3.88	3.96
	D-Leu	3.67	3.64	3.60	3.56
2	L-Ala	3.14	3.07	2.87	2.87
	D-Ala	3.06	3.01	2.95	2.89
	L-Val	3.65	3.64	3.63	3.62
	D-Val	3.34	3.48	3.59	3.70
	L-Ile	3.90	3.88	3.87	3.85
	L-Pro	3.08	3.07	3.10	3.11
	L-Ser	3.07	2.99	2.87	2.74
	D-Ser	2.82	2.74	2.60	2.37
	L-Lys	2.31	2.39	2.44	2.48
	L-Asp	3.61	3.64	3.66	3.69
	L-Leu	3.69	3.67	3.65	3.63
	D-Leu	3.46	3.52	3.57	3.64
	L-Cys	2.43	2.45	2.49	2.50
	L-Met	2.52	2.82	2.85	2.88
	L-Glu	2.88	2.90	2.95	2.99

[a] The log K_s values are the average of two or three independent runs: error <5% of the reported value.

Molecular recognition ability and enantioselectivity

Our recent study indicated that the size–fit relation between a host cavity and a guest molecule plays an important role in molecular recognition by cyclodextrins.[1,15,16,24] Therefore, the hydrogen bonding, van der Waals forces, and hydrophobic interactions should depend on how the size and/or shape of a guest molecule fit into the host cavity. As can be seen from Table 2, both molecular recognition ability and relative enantioselectivity are affected drastically by several structural factors of modified β-cyclodextrins and amino acid guests. In spectral titration, the characteristic absorption of host compounds **1** and **2** gradually increases upon the addition of amino acids, indicating that amino acid guest molecules can form inclusion complexes with modified β-cyclodextrins, even if their thermodynamic properties are quite different. The entropy changes ($T\Delta S°$) for the complexation of β-cyclodextrin derivatives with various amino acids are mostly positive. This fact indicates that formation of the supramolecular system is mostly entropy-driven, typically showing large positive entropy changes and somewhat smaller positive or negative enthalpy changes. Thus hydrophobic interactions play a crucial role in the complexation of β-cyclodextrin derivatives with amino acids. As a consequence of compensation between these positive or negative $T\Delta S°$ and $\Delta H°$ values, the molecular recognition ability and enantioselectivity may be more explicitly understood in terms of the size- or shape-fit and the hydrophobicity of the amino acid guest molecules, as discussed below.

Mono-(6-anilino-6-deoxy)-β-cyclodextrin 1

It is noted that the molecular recognition ability, the free-energy change ($-\Delta G°$) as well as enthalpy change ($-\Delta H°$) and entropy

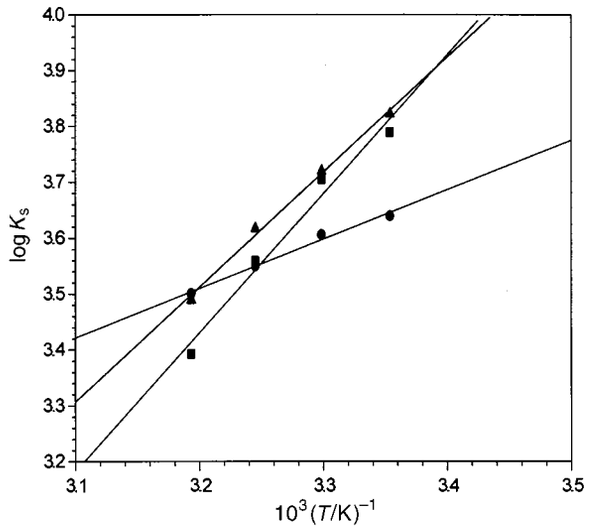


Fig. 5 Typical plots of log K_s *versus* $1/T$ in spectrophotometric titrations of L-Val (▲), L-Pro (■), L-Ile (●), with host compound **1**

Table 2 Thermodynamic parameters (in kcal mol^{-1}) for 1:1 host–guest complexation of some amino acid guest molecules with modified β-cyclodextrins **1** and **2** at 25 °C in aqueous solution (pH = 7.20, 0.10 mol dm^{-3} phosphate buffer)

Host	Guest	$-\Delta G$/kcal mol^{-1}	$\Delta\Delta G$/kcal mol^{-1} [a]	$-\Delta H$/kcal mol^{-1}	$-T\Delta S$/kcal mol^{-1}
1	L-Ala	4.93	0.13	−8.0	−13.0
	D-Ala	4.80		7.3	2.5
	L-Val	5.21		9.6	4.4
	L-Ile	5.17		11.4	6.2
	L-Pro	4.96		4.0	−0.95
	L-Ser	4.74	0.07	−6.3	−11.0
	D-Ser	4.67		−5.1	−10.0
	L-Cys	3.61		15.8	12.1
	L-Glu	5.69		17.1	11.3
	L-Lys	3.49		−7.2	−10.7
	L-Asp	5.47		7.2	1.7
	L-Leu	5.05	0.04	−7.2	−12.2
	D-Leu	5.01		3.2	−1.8
2	L-Ala	4.28	0.11	8.5	4.2
	D-Ala	4.17		4.9	0.72
	L-Val	4.99	0.43	0.92	−4.1
	D-Val	4.56		−10.2	−14.8
	L-Ile	5.33		1.5	−3.7
	L-Pro	4.19		−1.2	−5.3
	L-Ser	4.18	0.33	9.4	5.2
	D-Ser	3.85		12.7	8.3
	L-Cys	3.31		−2.3	−5.6
	L-Asp	4.92		−2.2	−7.7
	L-Leu	5.03	0.31	1.7	−3.4
	D-Leu	4.72		−5.0	−9.7
	L-Lys	3.15		−4.9	−8.0
	L-Met	3.80		−9.5	−13.2
	L-Glu	3.92		−3.2	−7.1

[a] $\Delta\Delta G$ signifies the difference of free energy changes for the complexation behaviour with L- or D-amino acids ($\Delta\Delta G = \Delta G_{(L)} - \Delta G_{(D)}$).

change ($-T\Delta S°$) for inclusion complexation with host compound **1** are highly sensitive to molecular type and the length of the amino acid side chain. Indeed, possessing two carboxylic groups as compared with other amino acids, glutamic and aspartic acids are included most effectively by **1**, giving the highest stability constants (log K_s = 4.04, 4.17). The complex stability constants K_s are roughly 1–1.5 orders of magnitude larger for glutamic acid than other amino acids. The increased complex stability may be attributed to the larger enthalpy change ($\Delta H° = -17.1$ kcal mol^{-1}, 1 cal = 4.184 J). This is because the glutamic and aspartic acids can be taken as zwitter-molecules losing two protons in poorly basic solution to give two CO_2^- groups, and, therefore, the amino group in β-cyclodextrin derivatives might be expected to increase the complex stability through the formation of intermolecular hydrogen bonding with the two CO_2^- groups accommodated in the host **1** cavity, giving the strongest inclusion interaction. On the other hand, cysteine and lysine containing sulfur and nitrogen heteroatoms show poor binding abilities, giving smaller stability constants. One possible explanation for this is that the sulfur and nitrogen heteroatoms in amino acid molecules decrease the hydrophobicity of the side chain because their presence leads to stronger solvation compared with carbon side chains, while the enthalpic and entropic gain are attributed to the stabilities of the complexes as a consequence of compensation. At the same time, the free energy change ($-\Delta G$) increased monotonically with the length of the side chain or size of all amino acids which belong to the same structure series, *i.e.* Val > Pro > Ala > Ser; Pro > Leu > Ile, and is characteristic of the types of guest.

Mono-(6-1-pyridinio-6-deoxy)-β-cyclodextrin 2

As shown in Table 2, the complex stability constants (K_s) for all amino acids examined in the inclusion complexation with host compound **2** show a similar trend. Somewhat unexpectedly, the modified β-cyclodextrin **2** bearing a positively charged group did not lead to greater complex stability *via* electrostatic interactions by ion-pairing between N^+ and the amino acid anion accommodated in the cyclodextrin cavity, and binding constants were 0.5–1 orders of magnitude smaller than with the neutral host **1**. Thermodynamically, the reduced complex stabilities for complexation between **2** and most amino acids are mainly ascribable to the decreased enthalpic gain ($-\Delta H°$), while the entropic gain ($T\Delta S°$) becomes more positive. On the other hand, the smaller entropic loss, or larger positive entropy change ($T\Delta S°$), for **2** than **1**, indicates that the pyridinio group linked to the edge of the cyclodextrin cavity through a shorter chain forms a much more rigid conformation favourable to the complexation from the viewpoint of entropy, but unfavourable to the complexation from the adjusted induced-fit concept, giving lower stability constants.

Chiral recognition

The data listed in Table 2 also show that the modified β-cyclodextrins possessing one chromophoric anilino or one pyridinio moiety as a probe can recognize not only differences between the molecular size and shape of amino acids, but also the chirality of the L- or D-amino acid isomer, giving fairly good enantiomer separation ability. The L or D enantioselectivities calculated from the K_s values are up to 2.1 for valine, and 1.8 for serine, for complexation with host compound **2**. The free energy change ($-\Delta G°$) of complexation with **2** is increased by 0.33 kcal mol^{-1} for L-serine compared with D-serine, and by 0.43 kcal mol^{-1} for L-valine as compared with D-valine. The recognition ability of L- or D-amino acid biological molecules by the host compounds increases in the order **2** > **1**. Thus, the molecular flexibility and the geometric complementarity between the host and the guest play a crucial role in the chiral recognition of amino acid molecules.

Acknowledgements

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