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Diastereomeric Molecular Recognition and Binding Behavior of Bile Acids by L/D-Tryptophan-Modified β-Cyclodextrins

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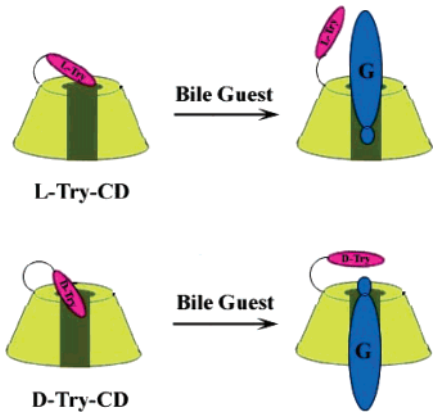

Binding behavior of L- and D-tryptophan-modified β-cyclodextrins (L/D-Trp-β-CD) (**1** and **2**) with four bile acids, i.e., cholate (CA), deoxycholate (DCA), glycocholate (GCA), and taurocholate (TCA), has been investigated by fluorescence, circular dichroism, and 2D-NMR spectroscopies and fluorescence lifetime measurement, as well as isothermal titration microcalorimetry. From the induced circular dichroism (ICD) and 2D NMR spectra, it is deduced that the D-Trp moiety of **2** attached to β-CD is more deeply self-included in the cavity than that of the antipodal L-Trp moiety of **1**, indicating appreciably enantioselective binding of the chiral sidearm by β-CD. Interestingly, the original difference in conformation between **1** and **2** led to quite a large difference in affinity toward DCA, giving 3.3 times higher binding ability for **2** than for **1**. Thermodynamically, the inclusion complexation of **1** and **2** with bile acids was entirely driven by favorable enthalpy change ($\Delta H°$) with accompanying negative entropy change ($\Delta S°$). The stronger binding of bile acids by L/D-Trp-β-CD is attributable to higher enthalpic gains. The combined use of the calorimetric and NMR ROESY spectral examinations revealed the correlation between the thermodynamic parameters and the role of sidearm conformation in modified β-cyclodextrins.

Introduction

Chiral recognition, multipoint recognition, and induced fit are the major concepts of current interest in supramolecular chemistry and biological science.[1,2] Many of natural and artificial receptor systems and their recognition behavior toward a wide variety of guest molecules have already been reported.[3] Among them, native and modified cyclodextrins have been employed as receptor molecules for investigating molecular recognition,[4−8]

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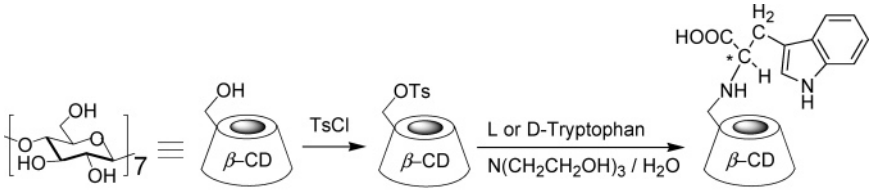
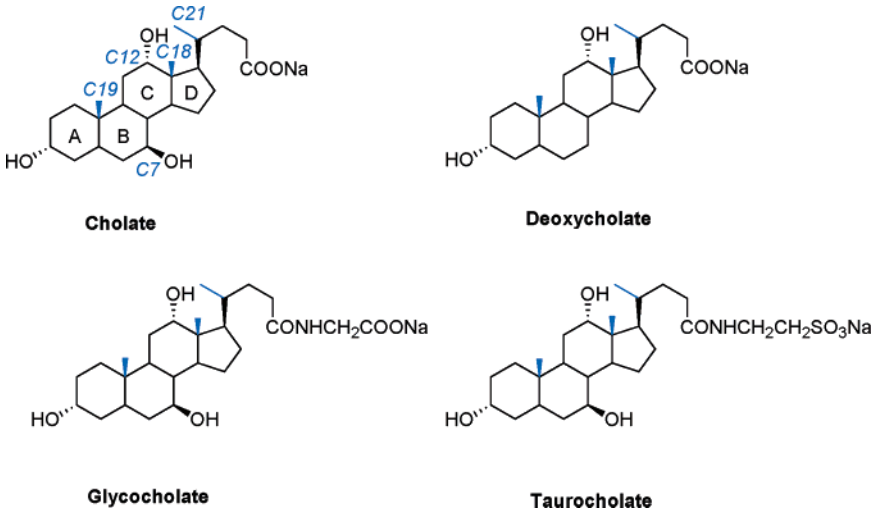
SCHEME 1



1. L-Trp; **2**. D-Trp

CHART 1



mimicking enzymes,[9] and constructing supramolecular assemblies.[10,11] Recently, Kano et al. studied the inclusion mode and thermodynamic parameters upon inclusion complexation of charged porphyrins with CDs, demonstrating that van der Waals interactions and dehydration from CD contribute greatly to the stability of the inclusion complex formation.[4] Tato and co-workers investigated the binding modes of β-CD and its derivatives with steroid guests by ROESY experiments, showing that a steroid molecule enters into the hydrophobic CD cavity from the secondary side, leaving the carboxylate tail outside the cavity.[12] Vecchio et al. reported the self-inclusion analysis of tryptophan-modified cyclodextrin with different flexible chains by 2D NMR, fluorescence, and circular dichroism spectroscopies. 2D NMR showed that an indole aromatic group could be included into the CD cavity to form the intramolecular complex, but the depth of indole included into the cavity was dependent on the length of the chains and the chirality of tryptophan.[12c] Recently, we have reported the molecular recognition behavior of β-CD derivatives, including CD dimers, with chiral alcohols or bile acids through spectral and/or microcalorimetric titrations and discussed the enantioselectivity and thermodynamic quantities for the inclusion complexation.[8,13] However, to the best of our knowledge, the guest binding behavior and thermodynamics of a pair of diastereomeric hosts with enantiomeric sidearms have rarely been investigated,[14] despite their importance in understanding the chiral and multiple recognition mechanisms of CDs. In the present works, we wish to report the self- and guest-inclusion behavior of a pair of diastereomeric L/D-Trp-CD (**1**, **2**) (Scheme 1) toward bile acids by fluorescence and circular dichroism spectroscopy and fluorescence lifetime measurement. The complex stability and host enantioselectivity for a series of bile acids (Chart 1) are discussed from the viewpoint of the induced conformational/microenvironmental changes of CDs and the size/shape fit and hydrophobicity of the guest. Thermodynamically, it is of our special interest to examine the contributions of weak interactions, such as electrostatic, van der Waals, and dipole−dipole, as well as hydrophobic interactions, upon inclusion complexation by CDs.

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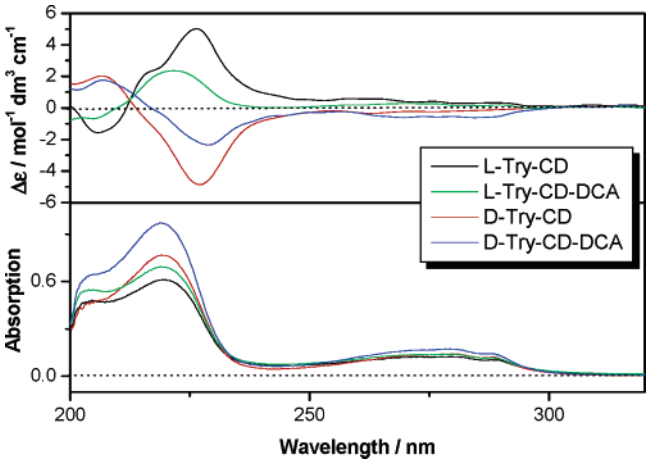

FIGURE 1. Circular dichroism spectra of L/D-Trp-CD **1** and **2** (5.12×10^{-5} M^{-1}) in the presence and absence of DCA (5.1×10^{-4} M^{-1}) in aqueous phosphate buffer solution (pH 7.20).

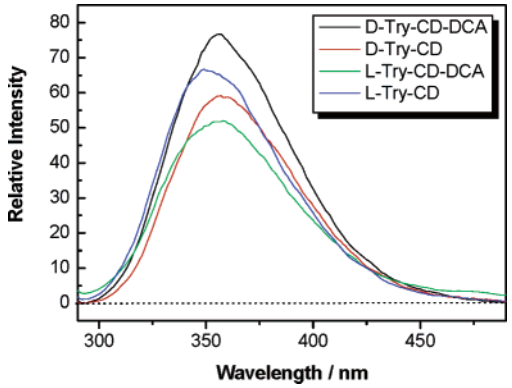

FIGURE 2. Fluorescence spectra of L/D-Trp-CD (1.1×10^{-5} M^{-1}) in the absence and presence of DCA (8.0×10^{-4} M^{-1}) in aqueous phosphate buffer solution (pH 7.20).

Experimental Section

Materials. Reagent grade β-CD was recrystallized twice from water and dried in vacuo at 80 °C for 24 h prior to use. Deoxycholate (DCA), cholate (CA), glycocholate (GCA), and taurocholate (TCA) were purchased from Sigma and used as received. L/D-Tryptophan were purchased from Peptide Institute, Inc. *N*,*N*-Dimethylformamide (DMF) was dried over calcium hydride for 2 days and then distilled under a reduced pressure before use. Mono[6-*O*-(*p*-toluenesulfonyl)]-β-CD (6-OTs-β-CD) was prepared by the reaction of β-CD with *p*-toluenesulfonyl chloride in aqueous alkaline solution.[15] 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 spectral analysis and microcalorimetric titrations.

Measurements. Fluorescence lifetimes were determined by the time-correlated single-photon-counting method with a time resolution of 0.5 ns. A self-oscillating discharge lamp filled with hydrogen gas was employed as a pulsed light source, and the excitation light was made monochromatic by a 10 cm monochromator. The emission from the sample was passed through an appropriate filter (Toshiba UV-35) placed before the detector in order to eliminate scattered excitation light. Maximum counts of up to 10 000 were collected for each measurement. The accumulated signals were then processed, and the lifetimes were determined by deconvolution with nonlinear least-squares fit.

Synthesis of D-Trp-β-CD. D-Tryptophan (1.0 g) and mono-[6-*O*-(*p*-toluene- sulfonyl)]-β-cyclodextrin (2.0 g) were dissolved in water (30 mL) containing triethanolamine (20 mL), and the resulting mixture was heated to reflux for 30 h with stirring under a nitrogen atmosphere. After evaporation of most of the solvent under a reduced pressure, the resulting solution was poured into anhydrous acetone (300 mL) with vigorous stirring, and the resultant mixture was stored in a refrigerator to produce a pale yellow precipitate. The solid product was collected by filtration and then purified by Sephadex C-25 column chromatography with aqueous ammonia as an eluent (1.0 mol dm^{-3}), to give a pale yellow product (0.8 g) in 40% yield: ESI-MS *m/z* (relative intensity) 1343.4 (M^+ + Na, 100%), 1321.3 (M^+ + H, 61%); ^{1}H NMR (D_2O) δ 3.16−3.81 (m, 45H), 4.79−4.89 (m, 7H,), 6.98−7.09 (m, 3H), 7.29 (d, J = 4.2 Hz, 1H), 7.44 (d, J = 3.9 Hz, 1H); FTIR (KBr) ν 3368, 2917, 1626, 1556, 1450, 1391, 1227, 1141, 1076, 1011, 927 cm^{-1}; UV λ_{max}

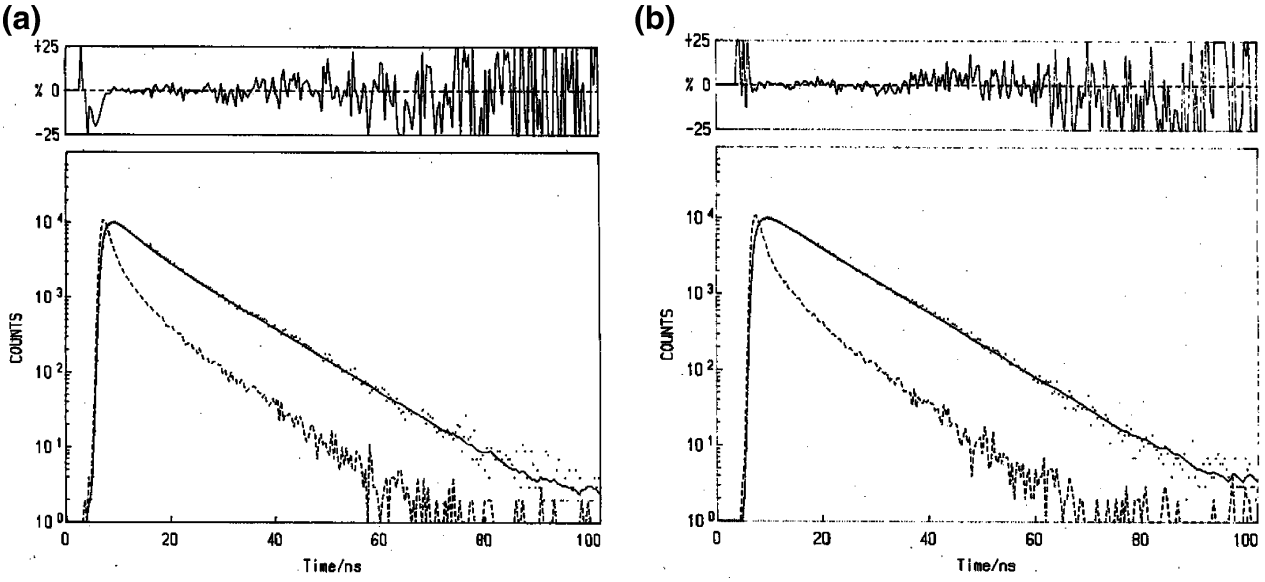

FIGURE 3. (a) Fluorescence lifetime of L-Trp-β-CD **1** (1.196×10^{-5} M^{-1}) in aqueous phosphate buffer solution (pH 7.20). (b) Fluorescence lifetime of D-Trp-β-CD **2** (1.529×10^{-5} M^{-1}) in aqueous phosphate buffer solution (pH 7.20).

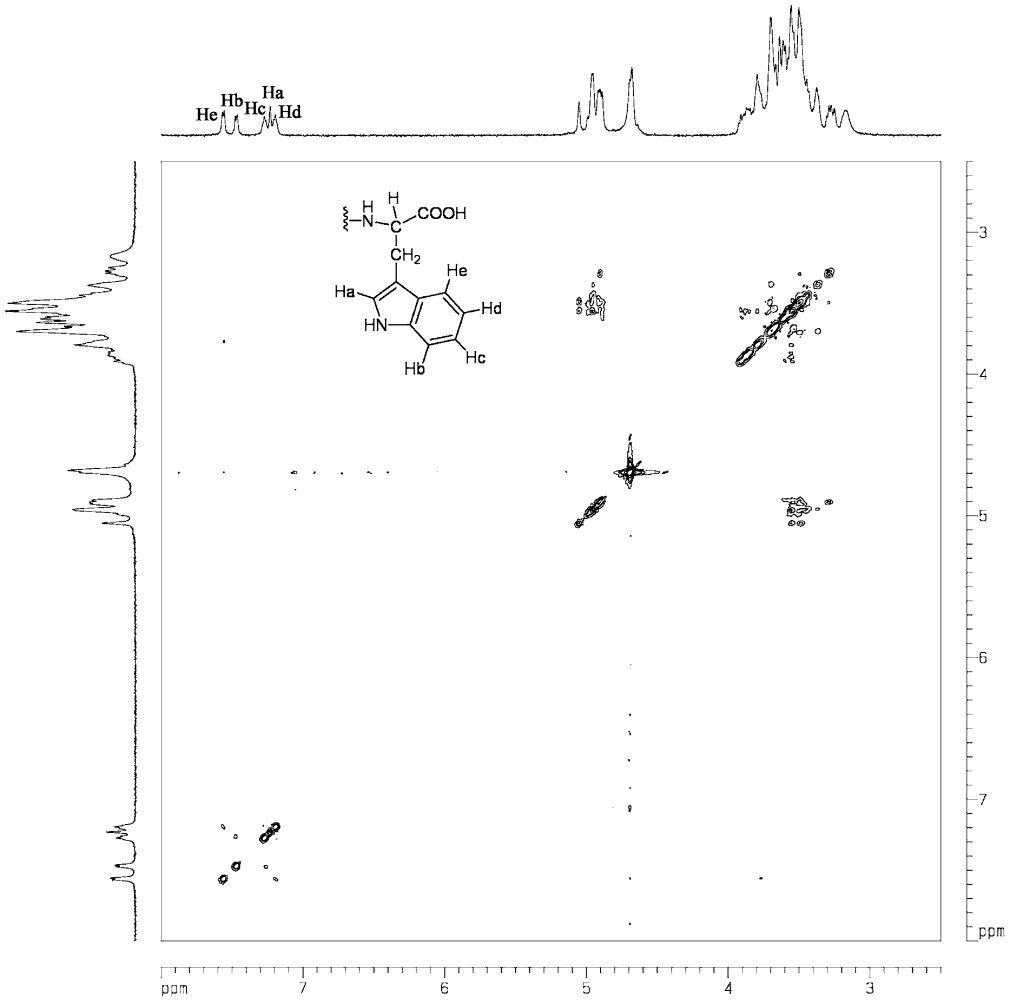

FIGURE 4. ROESY spectrum of L-Trp-β-CD **1** with a mixing time of 200 ms at 298.1 K.

(ϵ) 280 nm (3770 M^{-1} cm^{-1}). Anal. Calcd for $C_{53}H_{80}O_{36}N_2 \cdot 8H_2O$: C, 43.44; H, 6.60; N, 1.91. Found: C, 43.14; H, 6.61; N, 2.24.

Results and Discussion

Circular Dichroism Spectra. The absorption and circular dichroism spectra of L-Trp-β-CD **1** and D-Trp-β-CD **2** are shown in Figure 1. Possessing the enantiomeric aromatic chromophores appended to β-cyclodextrin, the circular dichroism spectra of **1** and **2** are very similar in shape but almost mirror-imaged. Host **1** displays a strong positive Cotton effect (CE) at 226 nm ($\Delta\epsilon$ +5.02 M^{-1} cm^{-1}), the intensity of which was decreased upon addition of DCA guest to $\Delta\epsilon$ = +2.36 M^{-1} cm^{-1} with a small hyprochromic shift to 222 nm. In the case of host **2**, addition of DCA guest led to a decrease of the original negative CE peak intensity at 227 nm ($\Delta\epsilon$ −4.87 M^{-1} cm^{-1}) to −2.34 M^{-1} cm^{-1} with a small bathochromic shift to 229 nm. In our previous report on the inclusion complexation of L-Trp-β-CD with chiral guests, we have shown that the L-Trp residue, originally perching on the edge of the CD cavity or appreciably penetrating into the cavity, is expelled out of the cavity upon guest inclusion.[16] In the present case, L-Trp-β-CD exhibits similar CD signal changes upon addition of DCA, and thus it is reasonable that L-Trp-β-CD experiences similar conformational changes upon complexation of bile acids. The circular dichroism spectrum of D-Trp-β-CD shows an oppositely signed CE peak at 227 nm ($\Delta\epsilon$ −4.87 M^{-1} cm^{-1}), but the CE intensity decreases in a similar manner upon addition of DCA, as exemplified in Figure 1. From these results, it seems reasonable to conclude that the D-Trp moiety is self-included in the CD cavity and driven out upon guest inclusion, as was exactly the case with the antipodal sidearm. However, the CD spectra do not provide further information about the depth of penetration, which prompted us to perform more detailed conformation analysis by 2D NMR (vide infra).

Steady-State Fluorescence Spectra and Fluorescence Lifetime Measurements. In an aqueous phosphate buffer solution (pH 7.2), L-Trp-β-CD **1** showed much stronger emission at 352 nm (upon excitation at 280 nm) than L-Trp under comparable conditions, clearly indicating that the indolyl moiety of the L-Trp residue is located in more hydrophobic environment rather than in the bulk aqueous solution. This is consistent with the

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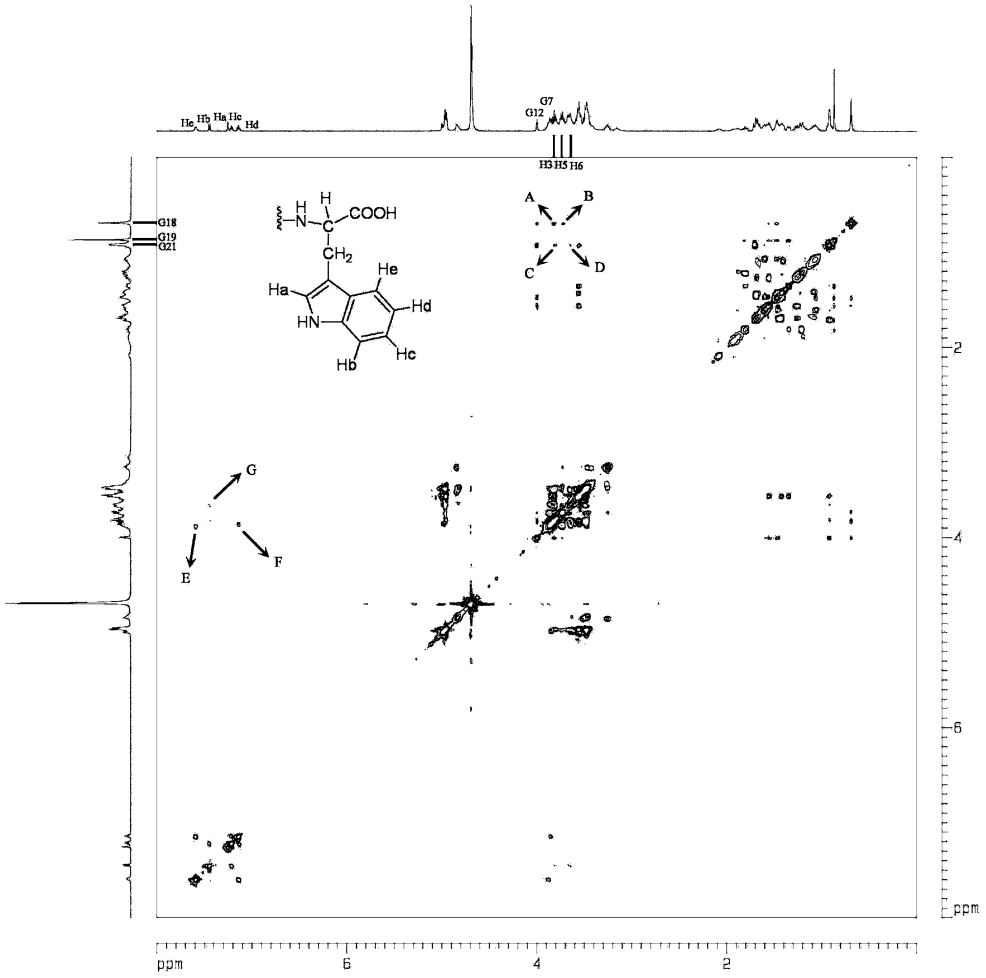

FIGURE 5. ROESY spectrum of the inclusion complex of L-Trp-β-CD **1** and DCA with a mixing time of 200 ms at 298.1 K.

general interpretation of the enhanced fluorescence observed with various aromatic fluorophores included in the cyclodextrin cavity. The fluorescence emission intensity of L-Trp-β-CD decreased (Figure 2) upon addition of bile acids, demonstrating that the guests compete with the tryptophan residue for occupancy of the cyclodextrin cavity and expel the fluorescent residue out of the hydrophobic cavity into the bulk aqueous solution. The observed fluorescence behavior reveals that the indolyl moiety of L-Trp residue is initially accommodated close to/in the cyclodextrin cavity. On the contrary, upon excitation of D-Trp-β-CD at 280 nm, the emission peak intensity is lower than D-Trp under the same conditions, which is not consistent with the general rules, i.e., the fluorescence intensity is enhanced when the fluorophores locate in a hydrophobic environment. To explain this observed result, a control experiment was performed, in which the fluorescence intensity of D-Trp gradually decreases with increasing of natural β-cyclodextrin, indicating that the fluorescence emission intensity of D-Trp is decreased when it accommodates in a more hydrophobic cyclodextrin cavity. Therefore, although opposite spectral changes of L- and D-Trp-β-CD are observed, it is the same that the Trp residues are expelled out of the CD cavity upon complexation of bile guests.

The time-correlated single-photon-counting technique provides us with direct information about the environment around the indolyl moiety of the L/D-Trp residue. Typical fluorescence decay profiles of hosts **1** and **2** are showed in the Figure 3. Multicomponent decay profiles were analyzed by using the following equation:

$$F(t) = \sum_{i=1}^{n} A_i \exp(-t/\tau_i) \quad (n = 1, 2, 3, \text{etc.})$$

The fluorescence decay curves obtained for L/D-Trp-β-CD in the presence and absence of bile acids could not be fitted to a single-exponential function but were nicely analyzed by a linear combination of two exponential functions. The fluorescence lifetimes (τ) and relative quantum yields (Φ) for L/D-Trp-β-CD in the presence and absence of bile acids in aqueous phosphate buffer are summarized in Table 1. The two-component decay ob-

TABLE 1. Fluorescence Lifetimes and Relative Quantum Yields of L/D-Trp-β-CD 1 and 2 in the Presence and Absence of Bile Acids

host	guest	τ_1 (ns)	Φ_S	τ_2 (ns)	Φ_L
1		2.8	0.53	9.4	0.47
1	cholate	2.8	0.55	9.1	0.45
1	deoxycholate	2.2	0.59	6.9	0.41
2		3.4	0.35	9.3	0.65
2	cholate	3.3	0.52	8.2	0.48
2	deoxycholate	3.2	0.50	7.9	0.50

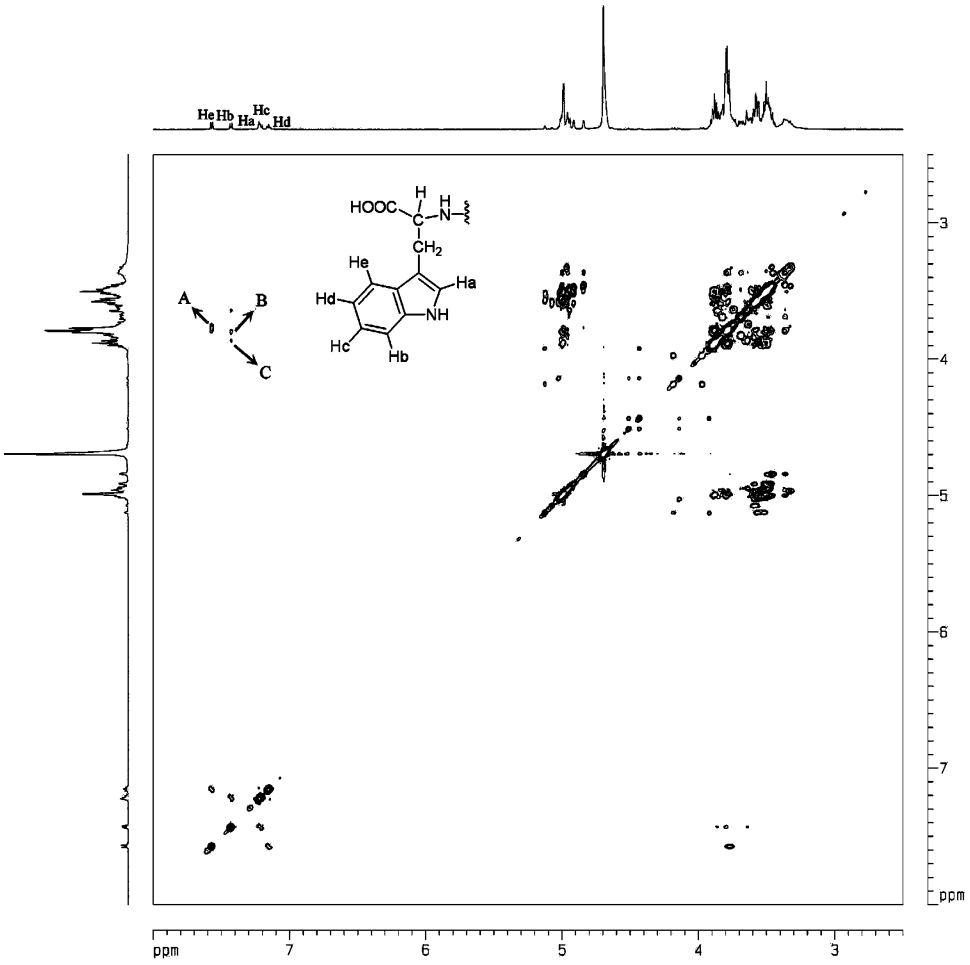

FIGURE 6. ROESY spectrum of D-Trp-β-CD **2** with a mixing time of 200 ms at 298.1 K.

served for Trp-β-CD in the presence and absence of guest molecules indicates that the Trp's indolyl moiety is located in two sorts of environment of distinctly different hydrophobicity and also that the interconversion of the two species is much slower than the fluorescence decay, which occurs on the nanosecond time scale. Thus, the shorter lifetimes (τ_S) and the longer lifetimes (τ_L) are reasonably assigned to those indolyl moieties that are exposed to the bulk aqueous solution and located in a hydrophobic environment, respectively. As can be seen from Table 1, the relative quantum yield of long-lived species of D-Trp-CD ($\Phi_L = 0.65$) is obviously higher than that of L-Trp-β-CD ($\Phi_L = 0.48$), indicating that the proportion of long-lived species (self-included component) of D-Trp-β-CD is higher than that of L-Trp-β-CD. Thus, the guest added to the host solution competes with the self-included indolyl moiety for CD cavity and expels the indolyl moiety from the cavity, resulting in the increased contribution of the τ_S component.

Binding Model of Hosts with Bile Acids. Before analyzing the molecular binding ability and selectivity in further detail, we examine the binding mode of L/D-Trp-β-CD with bile acid guests and the complex structure by using the results of 2D NMR experiments, which are essential for seriously discussing the binding behavior on the molecular basis. As can be seen from Chart 1, the bile acids possess a steroid skeleton composed of four hydrophobic rings (A−D) and a hydrophilic side chain. Previous investigations revealed that different binding modes are possible for the inclusion complexation of bile acids with CD.[17,18] Most native and monomodified CDs include the bile acids into the CD cavity from the CD's secondary side, with some exceptions. For an instance, CD dimers with a variety of tethers can either include two guests from the secondary side to give 1:2 inclusion complexes or cooperatively bind one guest to yield a 1:1 sandwich complex.[19]

Two-Dimensional NMR Experiments. To obtain information about the binding mode of L/D-Trp-β-CD with bile acids, the ROESY spectra of L/D-Trp-β-CD complex with DCA were measured in D_2O. It is well-known that only H3, H5, and H6 of CDs can give cross-peaks for analyzing host−guest interactions, as H2 and H4 are not facing to the inner cavity and H1 is affected by D_2O. As shown in Figure 4, there is no appreciable cross-peak between the indolyl moiety of L-Trp-β-CD and H3 or H5 of CD even in the absence of guest, indicating that the indolyl group is not deeply included into the CD cavity. Combining this fact with the result of circular dichroism and fluorescence spectral studies mentioned above, we

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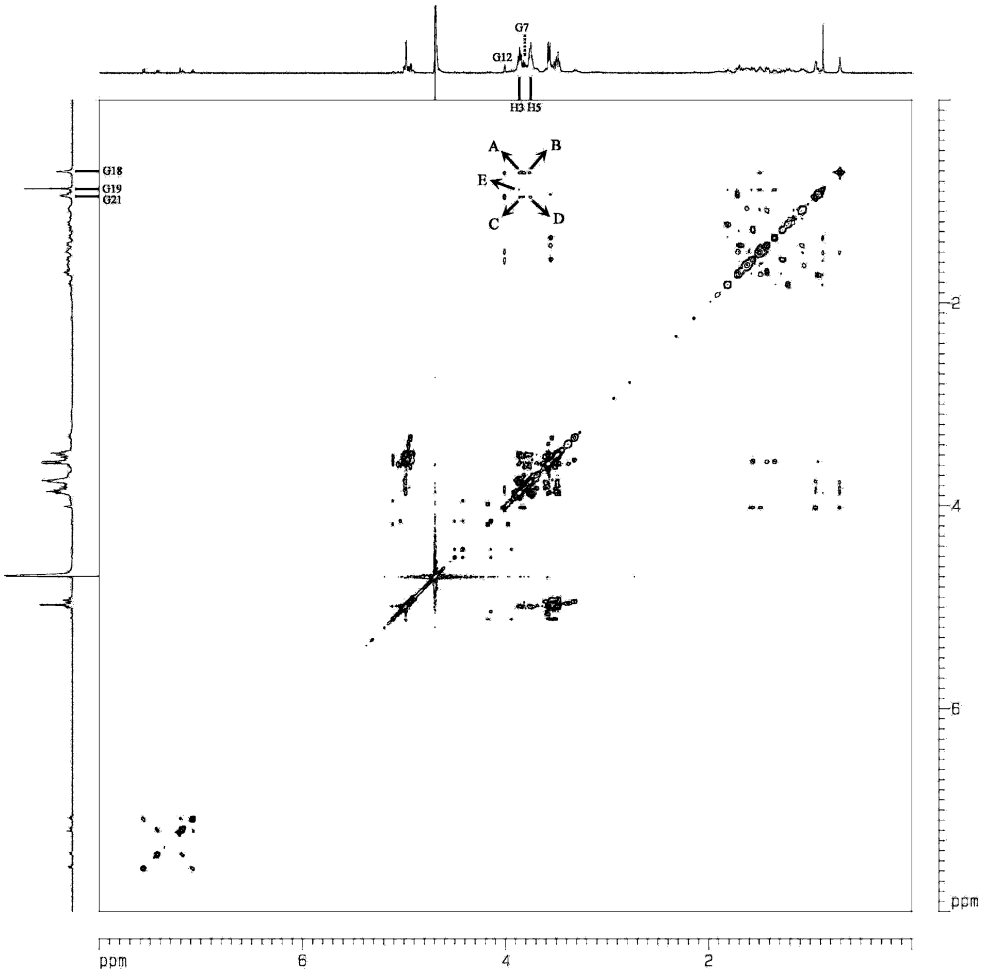

FIGURE 7. ROESY spectrum of the inclusion complex of D-Trp-β-CD **2** and DCA with a mixing time of 200 ms at 298.1 K.

can conclude that the L-Trp residue is only shallowly included or perching on the rim of the CD cavity. However, the 2D NMR examinations of an equimolar mixture of L-Trp-β-CD and DCA under identical conditions exhibited clear correlation peaks between CD's inner protons and DCA protons as shown in Figure 5; see the NOE cross-peaks A and B between G18 protons of DCA and the H3, H5 of CD, respectively. On the other hand, the weak cross-peak C is attributable to the interaction of G21 protons of DCA's carboxylic tail with CD's H3, revealing that the D-ring of DCA is close to the wide end of CD cavity. In the aromatic region, there are clear correlations between indolyl H_e/H_d protons of L-Trp and DCA's G7 protons, which are denoted as E and F, respectively. The weaker cross-peak G shows the interactions of the proton H_b of the indoly group with the H6 protons of CD. From the above information, it is deduced that the D-ring of DCA and the side chain is co-included in the same cavity from the primary side of CD **1**.

In sharp contrast to L-Trp-β-CD **1**, diastereomeric host D-Trp-β-CD **2** shows unequivocal correlations between the aromatic indolyl residues and the CD's inner protons as shown in Figure 6. Thus, the cross-peak A presents the correlations between proton H_e of indoly and protons H5/6 of CD, and the cross-peaks B and C are assigned to the correlations between protons H_b and H5/6 and H3, respectively. The results indicate that the D-Trp residue attached to β-CD is more deeply self-included than the corresponding L-Trp residue. Upon formation of the DCA−**2** complex, the methyl protons at G18 and G21 of DCA gave strong cross-peaks with the H-3 and H5 protons of CD, which are marked with A−D in Figure 7, and a weak cross-peak E indicating the interaction of between G19 and H-3 proton of CD. These data indicate that the carboxylate side chain and D-ring of DCA penetrate into the CD cavity from the primary side deeply or from the secondary side shallowly. From the fact that no correlation between the D-Trp protons of **2** and the ring B or C protons of DCA was observed, we can exclude the former possibility, i.e., deep penetration from the primary side. Therefore, we can conclude that the inclusion from the secondary side is the dominant mode of penetration for D-Trp-β-CD complex with DCA.

Microcalorimetric Titration. The thermodynamic parameters were determined at atmospheric pressure in aqueous phosphate buffer solution (pH 7.20) at 25 °C by using a Microcal VP-ITC titration microcalorimeter, which allows us to determine simultaneously the enthalpy and equilibrium constant from a single titration curve. The instrument is calibrated chemically as we described before.[19] All solutions were degassed and thermostated using a ThermoVac accessory before each titration run, and the titrations were performed below the critical micelle concentration (cmc) of the bile acid salts.

In each run, a phosphate buffer solution of host charged in a 250 μL syringe was sequentially injected

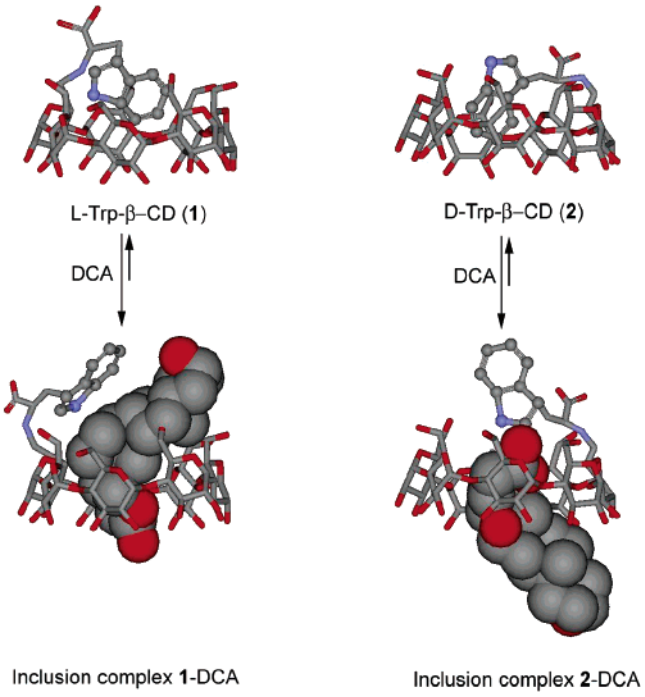

FIGURE 8. Plausible initial conformations of L/D-Trp-β-CD and their inclusion complex structures with DCA guests elucidated from the ROSEY experiments. The molecular modeling was studied by using the DS viewerPro 6.0 software. The initial structure of β-CD was taken from the crystal structure data in the literature[20] and its energy was optimized by the MM2 force field.

with stirring at 300 rpm into a buffer solution of bile acid guest in the calorimeter's sample cell. The sample volume was 1.4227 mL in all experiments. Each titration experiment was composed of 25 successive injections (10 μL per injection). The concentration of bile acid solution used was in a range of 0.10−0.52 mM, which is well below the cmc.[19] 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 acid. 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 Figure 9 for the complexation of DCA with modified D-Trp-β-CD **2**.

TABLE 2. Complex Stability Constant (K_S) and Standard Enthalpy ($\Delta H°$) and Entropy Changes ($T\Delta S°$) for 1:1 Inclusion Complexation of Bile Acids Guests with L-Trp-β-CD 1 and D-Trp-β-CD 2 in Phosphate Buffer Solution (pH 7.20) at T = 298.15 K

guests	hosts	K_S (M^{-1})	$-\Delta H°$ (kJ mol^{-1})	$T\Delta S°$ (kJ mol^{-1})
CA	β-CD[a]	4070 ± 80	23.0 ± 0.5	−2.4
	L-Trp-β-CD (**1**)	2020 ± 20	23.2 ± 0.1	−4.3
	D-Trp-β-CD (**2**)	6680 ± 130	37.9 ± 0.3	−14.5
DCA	β-CD[a]	4840 ± 20	25.8 ± 0.0	−4.8
	L-Trp-β-CD (**1**)	2310 ± 30	32.1 ± 0.2	−12.9
	D-Trp-β-CD (**2**)	6770 ± 190	46.0 ± 0.3	−24.1
GCA	β-CD[a]	2350 ± 70	23.0 ± 0.1	−3.7
	L-Trp-β-CD (**1**)	1110 ± 20	23.4 ± 0.2	−6.0
	D-Trp-β-CD (**2**)	1760 ± 40	24.9 ± 0.3	−6.4
TCA	β-CD[a]	2290 ± 10	23.8 ± 0.1	−4.6
	L-Trp-β-CD (**1**)	1060 ± 20	23.1 ± 0.2	−5.8
	D-Trp-β-CD (**2**)	1470 ± 10	24.3 ± 0.1	−6.2

[a] Reference 8b.

Molecular Binding Ability and Molecular Recognition. The microcalorimetric titrations of native and L/D-Trp-modified β-CD with a series of bile acids, i.e., CA, DCA, GCA, and TCA, showed typical titration curves, which can be nicely analyzed by assuming the 1:1 complex stoichiometry. A representative Job's plot for the inclusion complexation of host **1** with deoxycholate, shown in Figure 10, confirms the formation of 1:1 host−guest complex of bile acid guest with **1**. As shown in Table 2, modified β-CD **1** and **2** exhibited appreciably smaller binding abilities, particularly for GCA and TCA guests, than those of native β-CD. This seems reasonable, since GCA and TCA, possessing a strongly hydrophilic and hydrated sulfonate tail, are not expected to deeply

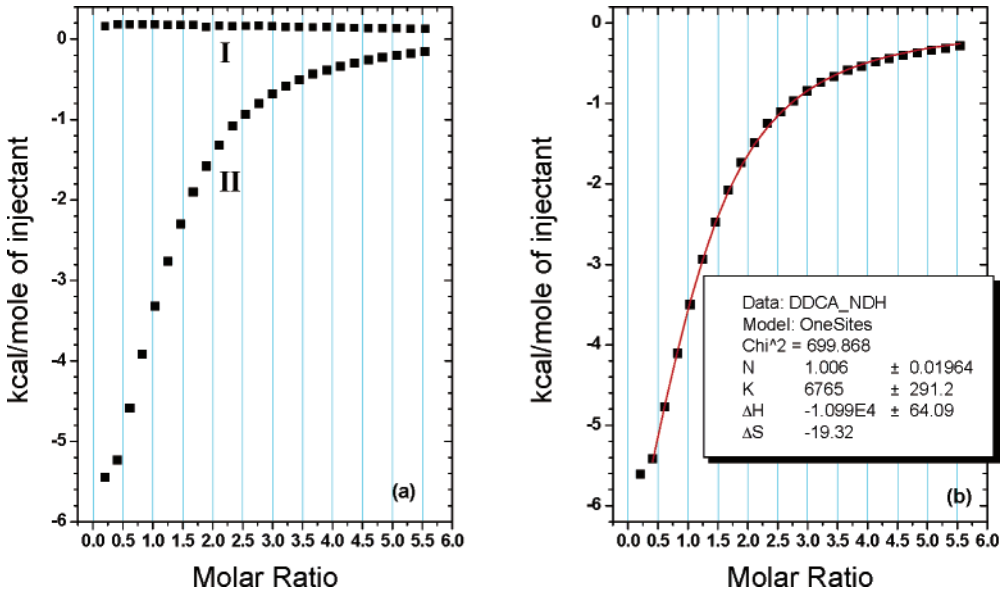

FIGURE 9. (a) Heat effects of dilution (I) and of complexation (II) of **2** with DCA for each injection during titration microcalorimetric experiment. (b) "Net" heat effect obtained by subtracting the heat of dilution from the heat of reaction, which was analyzed by computer simulation using the "one set of binding sites" model.

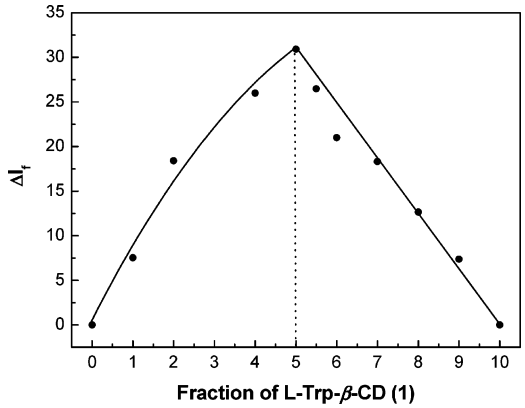

FIGURE 10. Job's plot for the complexation of **1** with deoxycholate in phosphate buffer solution (pH 7.20) at 25 °C ([**1**] +[deoxycholate] = 1.0×10^{-4} M).

penetrate into the CD cavity by removing the originally included L/D-Trp group out of the hydrophobic cavity. In contrast, DCA and CA, possessing a less hydrophilic/hydrated carboxylate tail, showed comparable or even stronger binding and higher selectivities for host's chirality than TCA and GCA. Indeed, 2D NMR examinations of complexes of host **1** and **2** with DCA revealed that the L/D-Trp group is expelled out of the CD cavity by DCA upon inclusion complexation. The diastereomeric host pair, L-Trp-β-CD **1** and D-Trp-β-CD **2**, display distinctly different affinities toward DCA and CA. Thus, the modification of native β-CD with L-Trp leads to decreased K_S values by a factor of 2, whereas that with D-Trp gives ca. 1.5 times enhanced K_S values, endowing a host selectivity of up to 3.3 for CA guest. The differential enthalpy and entropy changes were calculated from the data shown in Table 2: $\Delta H°_1 - \Delta H°_{CD} = -0.2$ kJ/mol and $T\Delta S°_1 - T\Delta S°_{CD} = -1.9$ kJ/mol for CA, and $\Delta H°_1 - \Delta H°_{CD} = -6.3$ kJ/mol and $T\Delta S°_1 - T\Delta S°_{CD} = -8.2$ kJ/mol for DCA. These values clearly indicate that the decreased affinities caused by introducing L-Trp are absolutely entropic (and never enthalpic) in origin. This large entropic loss may be attributed at least in part to the conformational changes and increased hydration upon extrusion of the Trp group originally perching above the cavity, which is suggested by the ICD studies.

For complexation of D-Trp-β-CD **2**, the situation is just the opposite. Thus, the enthalpic gains ($\Delta H°_2 - \Delta H°_{CD} = -14.9$ kJ/mol for CA; $\Delta H°_2 - \Delta H°_{CD} = -20.2$ kJ/mol for DCA) exceed the entropic loss ($T\Delta S°_2 - T\Delta S°_{CD} = -12.1$ kJ/mol for CA; $T\Delta S°_2 - T\Delta S°_{CD} = -19.3$ kJ/mol for DCA). This result may indicate that upon strong inclusion of CA or DCA the D-Trp moiety of **2** is excluded from the cavity but not completely driven out of the cavity so as to keep some van der Waals interactions, avoiding heavy hydration outside the cavity.

Diastereomeric Host Recognition. It is well-known that native and modified β-CDs exhibit moderate enantioselectivities for chiral guests.[21] However, the diastereomeric host selectivity has rarely been discussed for modified CDs with enantiomeric sidearms. In the present study, we synthesized both L- and D-Trp-modified β-CDs, which are suitable for such an examination to compare the diastereomeric host selectivity. As can be seen from Table 2, both CA and DCA favor D- rather than L-Trp-β-CD. The differential thermodynamic parameters ($\Delta H°_2 - \Delta H°_1 = -14.7$ kJ/mol and $T\Delta S°_2 - T\Delta S°_1 = -10.2$ kJ/mol for CA; $\Delta H°_2 - \Delta H°_1 = -13.9$ kJ/mol and $T\Delta S°_2 - T\Delta S°_1 = -11.2$ kJ/mol for DCA) clearly indicate that the high preference of the guests for D-Trp-modified β-CD **2** is enthalpic in origin, most probably arising from the van der Waals, electrostatic, hydrophobic, and/or hydrogen bond interactions.

This idea is reinforced by the 2D ROSEY studies, from which the plausible inclusion complex structures of L- and D-Trp-β-CDs with DCA, shown in Figure 8, are drawn. DCA enters into the CD cavity of **1** from the primary side but cannot form a stable complex because of the steric hindrance of L-Trp. In sharp contrast, in the **2**-DCA complex case, the steroidal D-ring of DCA smoothly penetrates into the hydrophobic cavity of **2** from the secondary side of CD. The expelled Trp group is still located near the CD cavity, increasing the hydrophobicity of the cavity and enhancing the van der Waals interactions between host and substrate. In this context, it is interesting to note that positively charged ammonio-β-CDs show higher affinities toward negatively charged DCA at pH 7.2 than those for native β-CD,[22] through the additional electrostatic interactions in the oppositely charged host−guest complexation.

Conclusion

A diastereomeric pair of β-cyclodextrins modified with enantiomeric L- and L/D-Trp residues as chiral spectral probes have been synthesized, and their binding ability and selectivity were determined for a series of bile acids of varying size, shape, and hydrophobicity. The fluorescence lifetime measurements clearly demonstrate the coexistence of long- and short-lived species, assignable to the Trp residues located inside and outside of the cavity, respectively. Upon gradual addition of a guest, part of the Trp group, which is originally self-included in the cyclodextrin cavity in the absence of guest, is expelled out of the cavity. Experimentally, CA and DCA give the most stable complex with D-Trp-β-CD (**2**), and CA exhibits the highest host diastereoselectivity ratio of **2/1**, as high as 3.3. Thermodynamically, the enhanced molecular recognition ability originates from the enthalpic gain with accompanying small entropic loss. From the ICD and ROESY experiments, it is inferred that the initial conformation, particularly the penetration depth of the sidearm, is significantly different for each diastereomeric host, which appears to be one of the major origins of the different binding behavior exhibited by L- and D-Trp-modified β-CD, as well as the highly diastereomeric host selectivities exhibited by CA and DCA for L- and D-Trp-β-CD. The differential thermodynamic parameters determined for these host−guest combinations also support the above conclusions.

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