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Chiral recognition by fluorescent chemosensors based on *N*-dansyl-amino acid-modified cyclodextrins

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This article is dedicated to the honor and memory of Professor Akihiko Ueno, who passed away on March 23, 2003.

Abstract—Four kinds of *N*-dansyl-amino acid-modified β-cyclodextrins (β-CDs) were prepared as fluorescent chemosensors for chiral discrimination. The use of an amino acid as a spacer improved binding affinities and chiral discrimination abilities of the chemosensors. *N*-dansyl-L-Phe-modified β-CD showed high *d*-selectivity for norbornane derivatives and *N*-dansyl-D-Phe-modified β-CD showed high *l*-selectivity for menthol. Microcalorimetric titration results indicate that the chemosensors selectively accommodate the enantiomer that induces the least unfavorable entropy change on making an inclusion complex.

There has been a great deal of activity devoted to chemosensors for detection of molecules.[1] We have prepared many kinds of chromophore-modified cyclodextrins (CDs) as chemosensors for molecule detection.[2] Colorless neutral molecules can be detected by changes in the intensities of fluorescence, absorption or circular dichroism using chemosensors based on chromophore-modified CDs in aqueous solutions. The mechanism of molecule detection by these chemosensors is shown in Figure 1.[2c,d] The fluorophore-modified CDs can adopt a number of different conformations in aqueous solutions. The conformation equilibrium can be explained by the simplified two-state model shown in parentheses of Figure 1. The 'self-inclusion state', in which the chromophore is located in the interior of the CD cavity, is usually the major conformation, if a spacer is long enough for self-inclusion. An 'induced-fit' conformational change of the chromophore-modified CD occurs in association with accommodation of a guest, which displaces the chromophore from the inside to the outside of the CD cavity, generating the 'non-self-inclusion state' (Fig. 1). The fluorophore-modified CD exhibits a strong fluorescence in the self-inclusion state due to the hydrophobic environment of the CD cavity, while exclusion of the fluorophore from the cavity to the bulk water weakens its fluorescence intensity. The extent of variation in fluorescence intensity upon addition of a guest depends on the affinity of the chemosensor for the guest. Thus, this system can be used as a sensory system for detecting various organic compounds on the basis of molecular recognition.

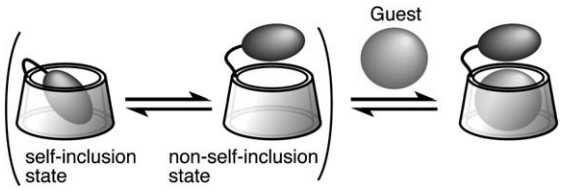

Figure 1. Conformational equilibrium in aqueous solution and guest-induced conformational change of a fluorescent chemosensor.

We previously prepared fluorophore-leucine-CD triad systems as chemosensors for molecule detection (Chart 1).[2c,d] This chemosensor has a leucine unit as a spacer between the CD cavity and the dansyl unit as a fluorophore. The hydrophobic side chain of the amino acid is expected to increase binding affinity due to a hydrophobic cap effect. The chirality of the leucine unit also affects the stability of the self-inclusion complex. The self-inclusion form of **2** having the D-leucine unit is more

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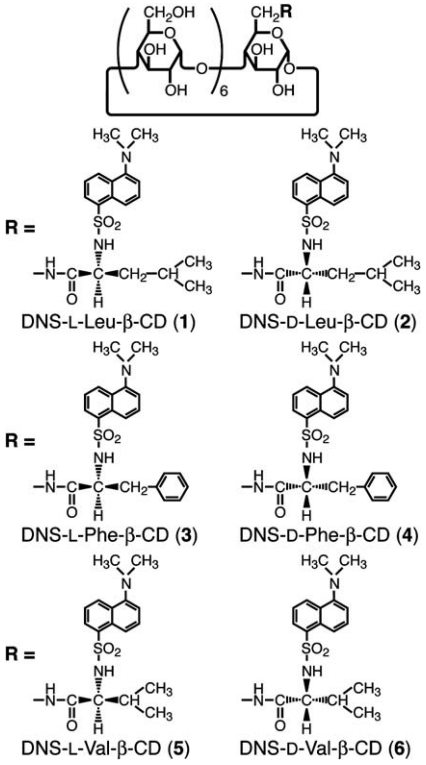

Chart 1. *N*-Dansyl-amino acid-modified β-cyclodextrins.

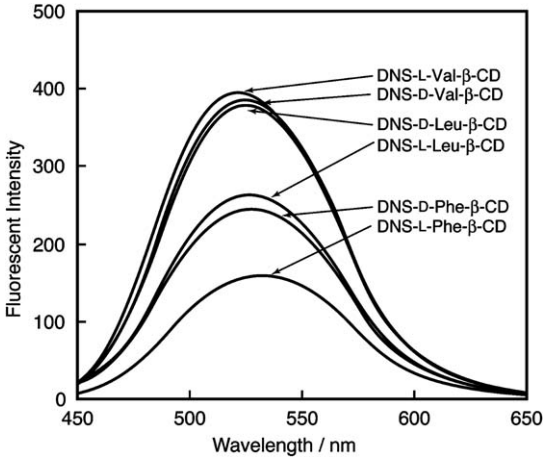

Figure 2. Fluorescence spectra of *N*-dansyl-amino acid-modified β-CDs in aqueous solution; [DNS-AA-β-CD] = 1×10^{-5} M in sodium phosphate buffer (pH 7.0) at 25 °C, λ_{ex} = 345 nm.

stable than that of **1** having the L-leucine unit. The fluorescence lifetime data suggested that the stability of the self-inclusion complex of **2** is 1.7-fold larger than that of **1**. The difference in the stability of the self-inclusion states results in differences in the binding abilities of **1** and **2**, because the guest binding is in competition with the self-inclusion of the dansyl unit. The binding affinity of **2** for guests is about half as that of **1**. The difference in their binding ability is almost inversely proportional to the difference in the stability of the self-inclusion states.

There is interest in whether or not this tendency for the chirality of the spacer to influence the binding affinity is shared by other amino acid spacer units. We are also interested in the ability of the chirality of the spacer to influence enantioselective recognition of chiral guests. Enantioselective chemosensors are currently of great interest to determine enantiomeric excess of samples in drug discovery and catalyst screening.[3]

We selected phenylalanine and valine as spacers and prepared new fluorescent chemosensors. Phenylalanine has a benzyl side chain, which is larger than the isobutyl side chain of leucine, and valine has an isopropyl side chain, which is smaller than the isobutyl side chain of leucine. We here report the fluorescence properties and chiral recognition abilities of dansyl-phenylalanine-appended and dansyl-valine-appended β-CDs (Chart 1).[4]

Figure 2 shows the fluorescence spectra of *N*-dansyl-L-leucine-appended β-CD (**1**), *N*-dansyl-D-leucine-appended β-CD (**2**), *N*-dansyl-L-phenylalanine-appended β-CD (**3**), *N*-dansyl-D-phenylalanine-appended β-CD (**4**), *N*-dansyl-L-valine-appended β-CD (**5**), and *N*-dansyl-D-valine-appended β-CD (**6**) in aqueous solution.

The fluorescence intensity of **4** is much larger than that of **3**. This influence of the spacer chirality on the fluorescence intensity is the same as for *N*-dansyl-leucine-appended β-CD. Since the fluorescence intensity of the dansyl unit is sensitive to its microenvironment, being stronger in a hydrophobic environment than in a hydrophilic environment,[5] this result suggests that the dansyl unit of **4** is located in a more hydrophobic environment or is more deeply accommodated in the CD cavity than that of **3**. The fluorescence intensity of **4** is smaller than that of **2**. This indicates that steric hindrance by the side chain inhibits deeper accommodation of the dansyl unit into the CD cavity and this effect is more pronounced with the benzyl unit of D-phenylalanine than with the isobutyl unit of D-leucine. On the other hand, the fluorescence intensity of **5** is similar to that of **6** and the fluorescence intensities of them are larger than those of **3** and **4**. This indicates that the steric hindrance of the isopropyl side chain of valine scarcely inhibits the self-inclusion of the dansyl unit to the CD cavity.

The binding constants of **3**–**6** for guests listed in Chart 2 were obtained by the usual method (Fig. 3).[2c,d] In all cases the binding affinity of the chemosensor with the L-amino acid spacer is larger than that of the chemosensor with the D-amino acid spacer. It is noteworthy that the binding affinity of **5** is greater than that of **6**, although the fluorescence intensities of **5** and **6** are almost the same. This result indicates that the binding affinity is affected by the chirality of the amino acid spacer. The absolute configuration of the amino acid influences the position of the dansyl unit in the CD cavity and the D-configuration leads to a more stable self-inclusion complex. Although the dansyl units of both **5** and **6** are accommodated deeply enough to be shielded from water and their fluorescence intensities are almost the same, there is a difference between the positions of the dansyl unit in the CD cavity of **5** and **6**, and so the stability of the inclusion complex is not

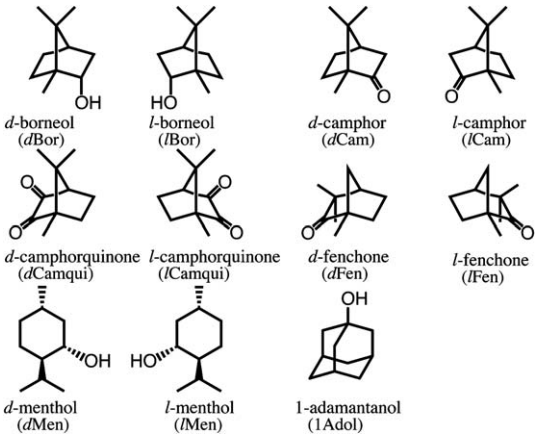

Chart 2. Structures of guests.

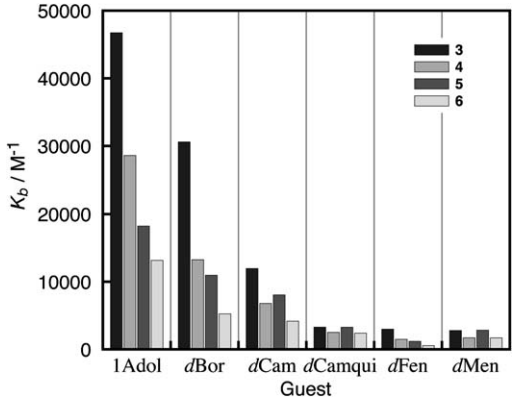

Figure 3. Binding constants of *N*-dansyl-amino acid-modified β-CDs; [DNS-AA-β-CD] = 1×10^{-5} M in sodium phosphate buffer (pH 7.0) at 25 °C.

the same for both. On the other hand, the steric hindrance of the side chain in phenylalanine results in the differences in the depth that dansyl unit inserts into the CD cavity of **3** and **4**. As a result, both the fluorescence intensity and stability of the inclusion complex are different for **3** and **4**.

The abilities of chiral discrimination of **3**–**6** for chiral compounds in Chart 2 were examined. The chemosensor **3** showed good *d*-selectivity for borneol, camphor, camphorquinone, and fenchone, but poor selectivity for menthol (Fig. 4). The chemosensors **4** and **5** showed good *l*-selectivity and *d*-selectivity for menthol, respectively. Only menthol has a cyclohexane skeleton, whereas the other guests have the same norbornane framework. This difference in the framework results in a difference in the selectivity pattern. The chemosensor **3** shows high selectivity for the norbornane derivatives but does not show selectivity for the cyclohexane derivative. Borneol, camphor, camphorquinone, and fenchone have the same framework of norbornane but with a different functional group. Each of the four chemosensors, **3**–**6**, shows a different selectivity pattern for each of the norbornane derivatives. We were able to discriminate these norbornane derivatives by a chemosensor array using **3**–**6**.

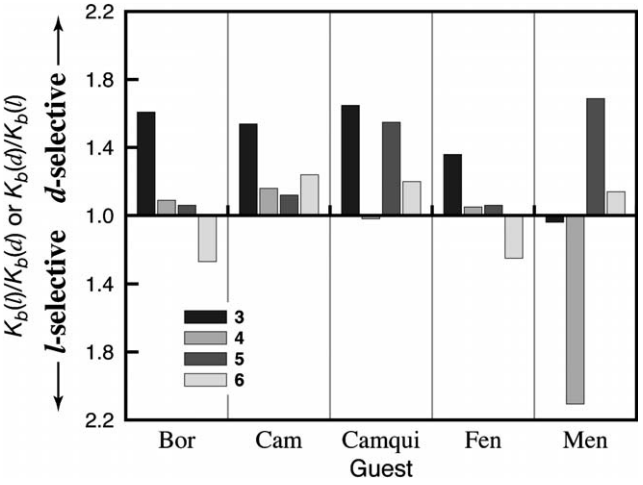

Figure 4. Chiral discrimination abilities of *N*-dansyl-amino acid-modified β-CDs; [DNS-AA-β-CD] = 1×10^{-5} M in sodium phosphate buffer (pH 7.0) at 25 °C.

The stability constant (K_b), standard free energy ($\Delta G°$), enthalpy ($\Delta H°$), and entropy changes ($\Delta S°$) for making inclusion complexes were determined to obtain thermodynamic insights into the factors related to chiral recognition by **3**–**6** (Table 1).[6] Borneol was selected as the guest for a microcalorimetric titration, because the binding constant for borneol with **3**–**6** is large enough to obtain K_b and $\Delta H°$ by curve fitting analysis of the microcalorimetric titration. The formation of the inclusion complexes of both **3** and **4** with the guest gave favorable enthalpy changes but with unfavorable entropy changes. The chemosensor **3** shows a larger favorable enthalpy change upon complexation with the *l*-enantiomer than with the *d*-enantiomer ($\Delta\Delta H° = -5.3$ kJ mol^{-1}). However, this is outweighed by a greater unfavorable entropy change ($\Delta(T\Delta S°) = -6.3$ kJ mol^{-1}) resulting in *d*-selectivity. The favorable enthalpy change on making an inclusion complex of **4** with the *l*-enantiomer ($\Delta\Delta H° = -6.2$ kJ mol^{-1}) was also overruled by its unfavorable entropy change ($\Delta(T\Delta S°) = -7.0$ kJ mol^{-1}) and thus **4** showed *d*-selectivity. Both **3** and **4** selectively bind the guest that results in the smaller unfavorable entropy change for making an inclusion complex. The interaction of **5** and **6** with the *d*-enantiomer gave larger favorable enthalpy change than that with the *l*-enantiomer, but the interaction with the *d*-enantiomer also resulted in an unfavorable entropy change. The difference between the enthalpy or entropy changes upon making an inclusion complex of dansyl-valine-modified β-CD with the *d*-enantiomer and that with the *l*-enantiomer was small and thus the enantioselectivity of dansyl-valine-modified β-CD is poor.

Inoue et al. reported enthalpy–entropy compensation effects in the complexation thermodynamics of a wide variety of guests with CDs or CD derivatives.[6a,b] Large negative enthalpy changes are attributable to the favorable van der Waals interactions, which arise from the precise matching in size and shape between the host and guest, whereas negative entropy changes usually arise from the significantly reduced translational and conformational freedoms of the host and the guest upon

Table 1. Binding constants (K_b), standard free energy ($\Delta G°$), enthalpy ($\Delta H°$), and entropy ($\Delta S°$) changes for 1:1 inclusion complexation of *N*-dansyl-amino acid-modified β-CDs with *d*-borneol and *l*-borneol in sodium phosphate buffer solutions (pH 7.0) at 30 °C

Host	Guest	K_b M^{-1}	$\Delta G°$ kJ mol^{-1}	$\Delta H°$ kJ mol^{-1}	$\Delta S°$ J K^{-1} mol^{-1}
3	*d*-borneol	23940	−25.41	−26.66	−4.184
	l-borneol	15770	−24.36	−31.97	−25.09
4	*d*-borneol	9726	−23.14	−32.44	−30.73
	l-borneol	7063	−22.34	−38.66	−53.92
5	*d*-borneol	5519	−21.72	−29.58	−26.01
	l-borneol	5345	−21.63	−27.59	−19.70
6	*d*-borneol	3800	−20.77	−24.40	−12.04
	l-borneol	3347	−20.45	−23.05	−8.615

complexation. Most of the enthalpic gain is canceled out by the entropic loss.

Our results suggest that there is a hydrophobic interaction between the guest and the DNS unit, of which the position is affected by steric hindrance of the side chain of the amino acid. A previous study indicates that the side chain of the amino acid is located near the rim of the entrance of the CD cavity.[2c,d] The steric hindrance of the side chain of the amino acid with the rim of the entrance of the CD cavity reduces the translational and conformational freedom of the DNS unit. The benzyl unit of phenylalanine, being bulkier, is more influential in the enantiomeric discrimination of borneol than the smaller isopropyl unit of valine.

In conclusion, the use of an amino acid as a spacer is effective for chiral discrimination of chemosensors.

Acknowledgments

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4. **3**: Anal. Calcd for $C_{63}H_{91}O_{37}N_3S·7H_2O$: C, 46.12; H, 6.45; N, 2.56; S, 1.96. Found: C, 45.95; H, 6.25; N, 2.59; S, 1.94. MALDI-TOF MS: *m*/*z*: calcd for $[M+Na]^+$: 1537.5; Found, 1537.0. **4**: Anal. Calcd for $C_{63}H_{91}O_{37}N_3S·8H_2O$: C, 45.62; H, 6.50; N, 2.53; S, 1.93. Found: C, 45.72; H, 6.20; N, 2.57; S, 1.94. MALDI-TOF MS: *m*/*z*: calcd for $[M+Na]^+$: 1537.5; Found, 1537.0. **5**: Anal. Calcd for $C_{59}H_{91}O_{37}N_3S·8H_2O$: C, 44.00; H, 6.70; N, 2.61; S, 1.99. Found: C, 44.00; H, 6.41; N, 2.63; S, 1.97. MALDI-TOF MS: *m*/*z*: calcd for $[M+Na]^+$: 1489.4; Found, 1489.2. **6**: Anal. Calcd for $C_{59}H_{91}O_{37}N_3S·7H_2O$: C, 44.49; H, 6.65; N, 2.64; S, 2.01. Found: C, 44.33; H, 6.50; N, 2.67; S, 1.93. MALDI-TOF MS: *m*/*z*: calcd for $[M+Na]^+$: 1489.4; Found, 1489.2.
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