Remarkable Molecular Recognition of Dansyl-modified Cyclodextrin Dimer

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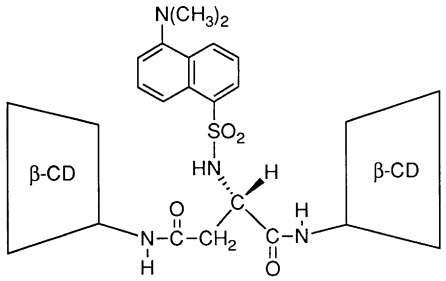
Dansyl-modified β-cyclodextrin dimer changes fluorescence intensity depending on the molecular species in aqueous solution and exhibits remarkable molecular recognition for steroidal compounds.

Cyclodextrins (CDs) are typical hosts, which form inclusion complexes with various organic molecules in aqueous solution.[1] Although CDs have limited binding ability when compared with antibodies in biological systems, recently CD dimers have been shown to exhibit strong binding ability by cooperation of two CD units with the binding constants comparable to those of antibodies.[2]

On the other hand, much effort has been devoted to construct various molecule-responsive indicators based on chromophore-modified CDs.[3] We wish to report here the synthesis of a fluorescent CD dimer as a new indicator with two binding sites and preliminary results of its molecular recognition ability that involves remarkable recognition observed for steroidal compounds.

The synthesis of dansyl-modified β-CD dimer **1** was performed by the reaction of dansyl-L-aspartic acid (0.83 mmol) and 6-deoxy-6-amino-β-CD[4] (2.24 mmol) in N,N-dimethyl-formamide (13 ml) with dicyclohexylcarbodiimide (1.83 mmol) and hydroxybenztriazole (1.83 mmol). The crude product was purified by column chromatography (DIAION HP-20, H_2O) and HPLC (ODS-2101-D, MeOH / H_2O = 19 / 81), the yield being 16%. Compound **1** was characterized by elemental analysis, electrospraying ionization type liquid chromatograph mass spectrometry (ESI LC/MS), and ^{1}H-NMR.[5]



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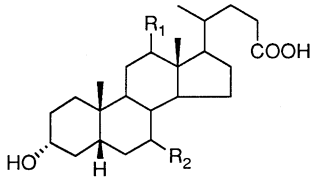
An aqueous solution of **1** exhibits a fluorescent peak at 528 nm, and the intensity was reduced by addition of guest species. The value of $\Delta I/I^0$ ($\Delta I = I - I^0$) was used as a sensitivity parameter for the solution of **1** (2 μM), where I^0 and I are fluorescence intensities of **1**, alone and in the presence of guest, respectively. Figure 1 shows the variations of $\Delta I/I^0$ as a function of concentration of steroidal compounds such as ursodeoxycholic acid (UDCA), chenodeoxycholic acid (CDCA), deoxycholic acid (DCA), and cholic acid (CA). It is surprising that these steroids exhibit very different concentration dependencies.[6] The resultant sensitivity values at 0.3 mM of the guests are presented in Figure 2.



	UDCA	CDCA	DCA	CA
R_1	—H	—H	···OH	···OH
R_2	—OH	···OH	—H	···OH

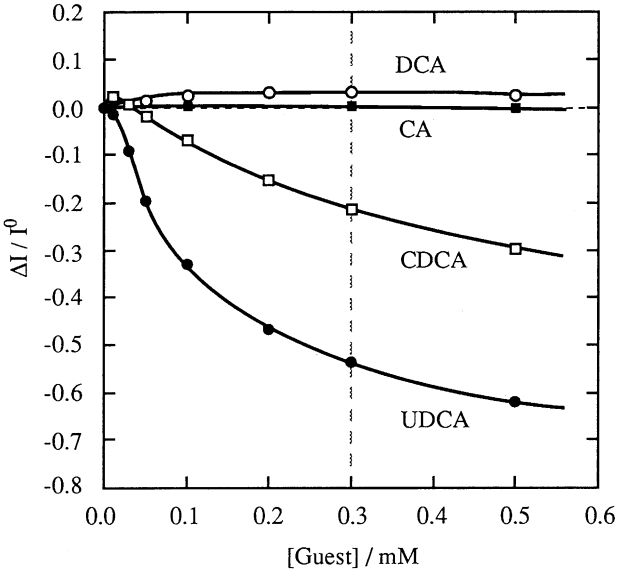


Figure 1. Variations of fluorescence sensitivity value of **1** (2 μM) as a function of concentration of four steroidal compounds in aqueous solution at 25°C. λ_{EX} : 340nm, λ_{EM} : 528nm.

In spite of the fact that the former three compounds are geometrical isomers with one hydroxy group at opposite orientation or different position, they exhibit markedly different $\Delta I/I^0$ values, with negative values -0.54 and -0.21 for UDCA and CDCA, respectively, and with a positive value +0.03 for DCA. On the other hand, CA exhibits negligible sensitivity. The fluorescence diminishment observed suggests that the

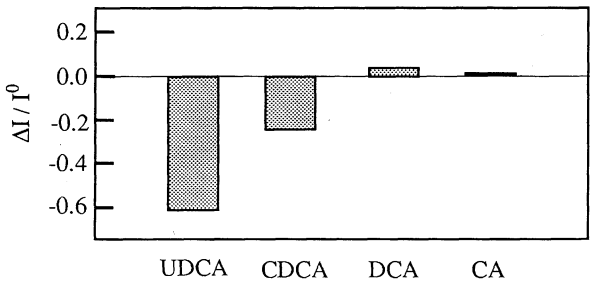


Figure 2. Molecular recognition of **1** (2 μM) for four kinds of steroids (0.3 mM) in aqueous solution at 25 °C.

dansyl moiety included in the one β–CD cavity of the CD dimer is excluded from the hydrophobic cavity to bulk water environment. Since this dimer is not symmetrical, there remains a problem which cavity of the two CD units accommodates the dansyl moiety. We have obtained binding constants for UDCA (6900 M^{-1}) and for CDCA (1300 M^{-1}) according to the least square curve-fitting analysis using a Benesi-Hildebrand type equation[7] for 1:1 complex formation. The formation of the 1:1 host-guest complexes suggests that cooperation of the two CD units operates in this system for binding a guest molecule although the binding strengths are not large. On the other hand, the fluorescence enhancement observed for DCA may be tightly related to the structural feature of the complex. We are now undergoing the structural studies of the complexes of the present systems. In spite of the ambiguous structural features of the complexes, the present results demonstrate that the fluorescent indicators, which discriminate very small differences among guest species, can be constructed by using CD dimers.

References and Notes

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5 The structure of **1** was fully characterized by the spectroscopic analysis. The selected data are as follows. Anal. Found : C, 40.1; H, 6.04; N, 1.73; S, 1.22. Calcd for $C_{100}H_{156}N_4O_{72}S \cdot 22H_2O$: C, 40.1; H, 6.74; N, 1.87; S, 1.07. ESI LC/MS (*m/z*) 1297, $[M-2H]^{2-}$ and 864, $[M-3H]^{3-}$. ^1H NMR (D_2O) δ 8.57 (d, 1H), 8.26 - 8.22 (br, 2H), 7.73 (t, 1H), 7.49 (t, 1H), 7.38 (d, 1H), 5.12 - 4.82 (br m, 14H), 4.4 - 3.0 (br m, 84H), 2.90 (s, 6H).

6 When dansyl-L-aspartic acid was used in place of **1**, no change in the dansyl fluorescence intensity was induced by the presence of the steroidal compounds used here.

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