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Bile Acids-Selective Chemosensors Based on NBD-amine-Modified Cyclodextrins

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Abstract

A new type of fluorescent chemosensor, based on modified cyclodextrins bearing the fluorophore unit NBD-amine, was prepared. One of these new chemosensors, NC0γCD is very sensitive to bile acids, but is not sensitive to other guests (e.g. adamantane and borneol derivatives). The response of the new type of chemosensor to a guest was an increase in the fluorescence intensity and the sensitivity parameter ($\Delta I/I_0$) dose not correlate to the binding affinity of NC0γCD.

Introduction

There has been great interest in the development of new chemical methods to recognize ions and molecules [1–4]. We have prepared many kinds of chromophore-modified cyclodextrins (CDs) as chemosensors for molecule recognition [5–12]. Colorless neutral molecules can be detected by changes in the intensities of the fluorescence, absorption or circular dichroism using chemosensors based on chromophore-modified CDs. The mechanism of these chemosensors is shown in Figure 1. The chromophore-modified CDs can adopt a number of different conformations in aqueous solution. The conformation equilibrium can be explained by the simplified two-state model shown in Figure 2 [9–10]. The 'self-inclusion state,' in which the chromophore is located in the interior of the CD cavity, is usually the major conformation. An 'induced-fit' conformational change of the chromophore-modified CD occurs in association with accommodation of the guest, which displaces the chromophore from the inside to the outside of the CD cavity, generating the 'non-self inclusion state' (Figure 1). Thus, the proportion of the 'non-self-inclusion state' increases with an increase in the guest concentration. In the case of a fluorophore, the fluorescent CD exhibits a strong fluorescence in the self-inclusion state due to the hydrophobic environment of the CD cavity, and exclusion of the fluorophore from the cavity to the bulk water weakens its fluorescence intensity. The extent of the variation in fluorescence intensity depends on the affinity of the chemosensor for a guest. Thus, this system can be used as a sensory system for detecting various organic compounds on the basis of molecular recognition. For example, the guest selectivity of dansyl-L-leucine-modified β-CD (DNS-Leu-β-CD) and dansyl-L-leucine-modified γ-CD (DNS-Leu-γ-CD) are shown in Figure 3.

This chemosensor system is effective for detecting molecules, but it has some defects. (1) A stable self-inclusion state can inhibit accommodation of the guest, thus lowering the sensitivity of the chemosensor. (2) Changing the chromophore or spacer unit can alter the selectivity of the chemosensor, but the effect is not large, because the guest selectivity of the chemosensor mainly depends on the selectivity of the CD itself. (3) For most conventional chemosensors, the detection of a guest is accompanied by a decrease in the fluorescence intensity, even though an increase in the emission intensity in response to guest binding is more effective for chemical sensing systems. DNS-Leu-β-CD responds to not only bile acids but also adamantane and borneol derivatives, while DNS-Leu-γ-CD also responds to both bile acids and borneol derivatives. Therefore these chemosensors are not sufficiently selective to be bile acids-sensitive chemosensors. So, we now propose a new method to overcome the aforementioned defects as shown in Figure 4. If the chromophore is connected to the CD without an alkyl spacer in the linker, the chromophore cannot be self-included and will remain at the entrance of the CD cavity. In this situation, some water is accommodated in the cavity and the chromophore is surrounded by a hydrophilic environment. If a hydrophobic guest then enters the cavity such that the hydrophobic face of the guest interacts with the chromophore, the chromophore will be located in a more hydrophobic environment. When the chromophore is a

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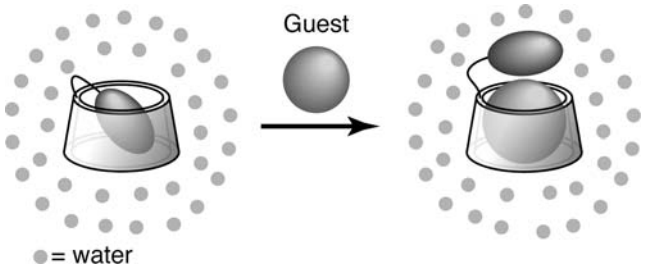

Figure 1. Guest-induced conformational change of a conventional chromophore-modified cyclodextrin.

fluorophore, its fluorescence intensity will be weak in the absence and stronger in the presence of hydrophobic guests. This mechanism is expected to increase selectivity in the chemosensor, because a more limited variety of guests will have the correct shape capable of increasing fluorescence intensity. Furthermore, the fluorophore will act as a hydrophobic cap, increasing the affinity of the chemosensor to guests. Herein, we report a new type of chemosensor that can selectively detect bile acids.

Experimental

General

Reverse phase HPLC was performed using a HITACHI HPLC system comprising a HITACHI L-7100 Intelligent Pump, HITACHI D-7500 Chromato-Integrator and HITACHI L-7400 UV-VIS Detector. ^{1}H NMR spectra were measured on a Varian VXR 500S spectrometer (500 MHz). HDO ($\delta = 4.70$) was used as an internal standard. Matrix assisted laser desorption/ionization and time-of-flight mass spectrometry (MALDI-TOF MS) was performed on a SHIMADZU KRATOS KOMPACT MALDI III mass spectrometer using α-cyano-4-hydroxycinnamic acid as a matrix. Thin-layer chromatography (TLC, *n*-butanol:ethanol:water = 5:4:3, and conc. NH_3aq.:ethyl acetate:2-propanol:water = 1:3:5:4) was carried out with silica gel F_{254} (Merck Co.). Absorption spectra were measured on a SHIMADZU UV–Visible spectrophotometer UV-2550. Fluorescence spectra were measured on a HITACHI fluorescence spectrophotometer F-2500.

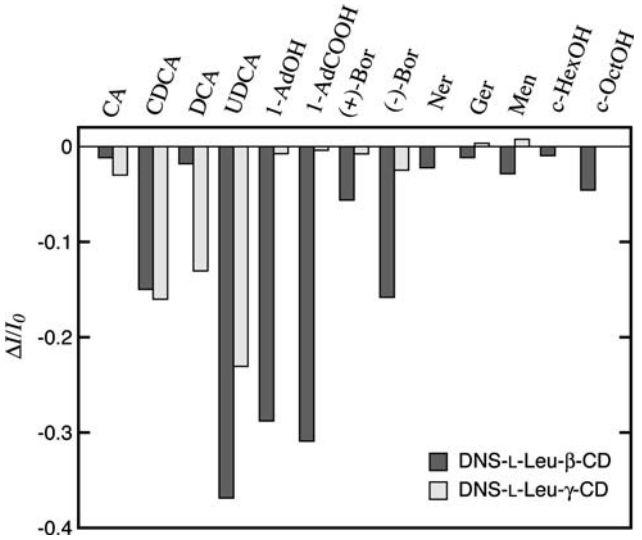

Figure 3. Sensitivity parameters ($\Delta I/I_0$) of dansyl-L-leucine-modified cyclodextrins.

Material

Synthesis of NC0γCD

4-Chloro-7-nitrobenz-2-oxa-1,3-diazole (NBD-Cl, 92.5 mg, 0.464 mmol) was added to a DMF (2.0 mL)/methanol (2.0 mL) solution containing triethylamine (39.8 μL, 0.287 mmol) and mono-6-amino-6-deoxy-γ-cyclodextrin [11] (74.4 mg, 0.0574 mmol). The reaction mixture was stirred at room temperature for 4.5 h. Then, the solution was poured into acetone (200 mL), and the precipitates were dried in vacuo overnight, giving 78.1 mg of crude product. This crude product was purified by reverse phase HPLC, and the final product was obtained as a yellow powder (8.0 mg, 9.6% yield).

Synthesis of NC4γCD

NC4γCD was synthesized by the reaction of mono-6-(4-aminobutylamino)-6-deoxy-γ-cyclodextrin [12] with NBD-Cl in a DMF/methanol solution containing triethylamine in the same manner as described for NC0γCD. The final product was obtained as a yellow powder (6.1 mg, 13.6% yield).

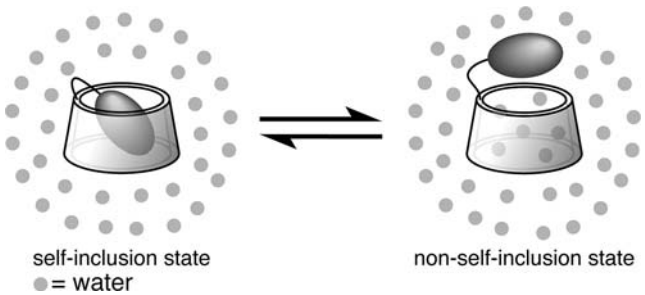

Figure 2. Conformational equilibrium of a modified cyclodextrin in aqueous solution.

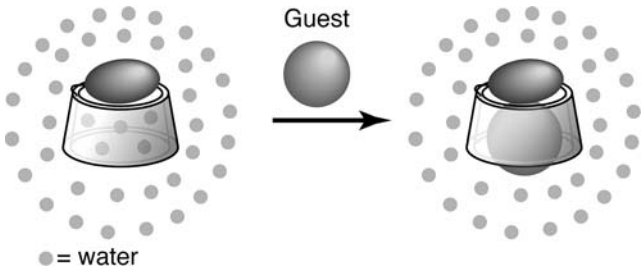

Figure 4. Guest-induced conformational change of a new type of chromophore-modified cyclodextrin.

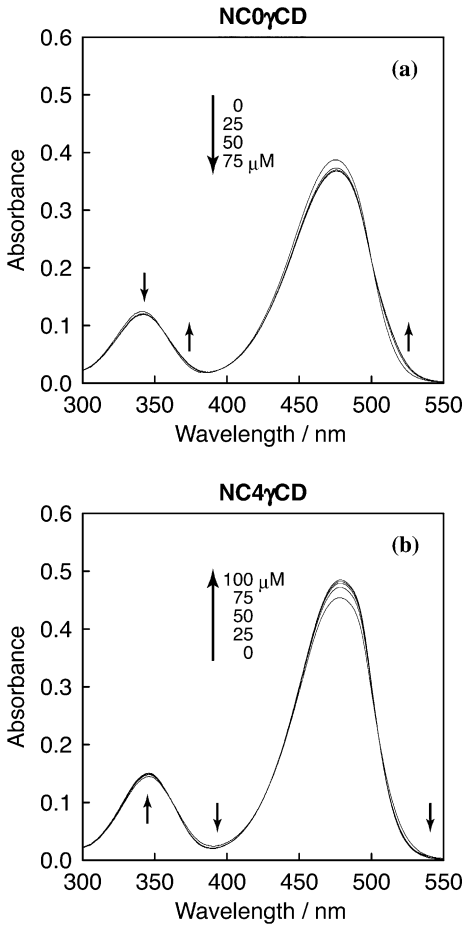

Figure 5. Absorption spectra of (a) NC0γCD (2×10^{-5} M) and (b) NC4γCD (2×10^{-5} M) in the presence of various concentrations of UDCA in phosphate buffer (200 mM, pH 7.0) at 25 °C.

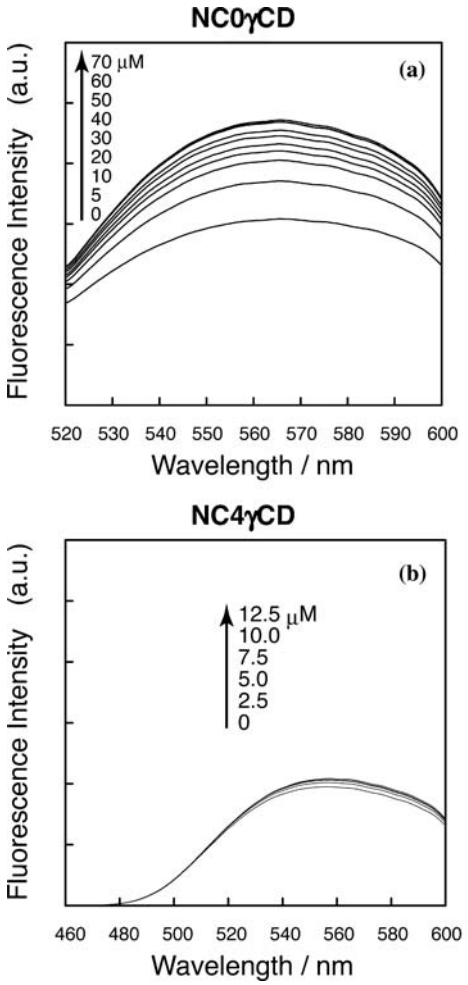

Figure 6. Fluorescence spectra of (a) NC0γCD (5×10^{-6} M) and (b) NC4γCD (5×10^{-6} M) in the presence of various concentrations of UDCA in phosphate buffer (200 mM, pH 7.0) at 25 °C; the excitation wavelength was 500 nm for NC0γCD and 431 nm for NC4γCD.

Results and discussion

Syntheses of NBD-amine-modified CDs

We selected 4-amino-7-nitrobenz-2-oxa-1,3-diazole (NBD-amine) as a fluorophore for our new type of chemosensor (Chart 1). NBD-Cl is a fluorogenic reagent, while not fluorescent itself, it reacts with an amine group to form the fluorescent derivative, NBD-amine [13–15]. NBD-amine displays the interesting property of fluorescing weakly in water and strongly in organic solvents, membranes, or hydrophobic environments. The new type of chemosensor, NC0γCD was synthesized by the reaction of mono-6-amino-6-deoxy-γ-CD with 4-chloro-7-nitrobenz-2-oxa-1,3-diazole (NBD-Cl). The fluorophore unit (NBD-amine) is directly connected to the CD framework of NC0γCD. A reference compound, NC4γCD, which has a butylenediamine linker, was synthesized from 6-(4-aminobutylamino)-6-deoxy-γ-CD. The NBD unit of NC4γCD would be self-included and the guest selectivity of NC4γCD is expected to be similar to that of the conventional CD-based chemosensors.

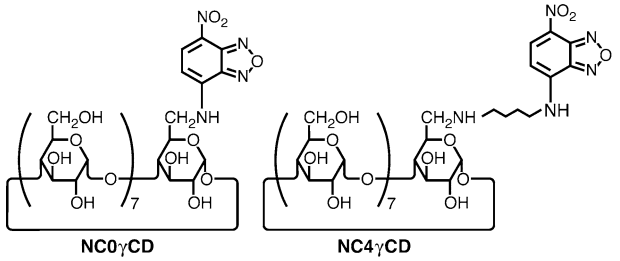

Chart 1. Structures of NBD-amine-modified γ-cyclodextrins.

Absorption and fluorescence spectra of NC0γCD and NC4γCD

The absorption spectra of NC0γCD and NC4γCD in the presence of various amounts of ursodeoxycholic acid (UDCA) were measured in phosphate buffer (pH 7.0) as shown in Figure 5. The peak intensity of NC0γCD was decreased, while that of NC4γCD was increased, by increasing the concentration of UDCA. As the molar absorption coefficients (ϵ) of NBD-amine derivatives rise with increasing solvent polarity [14], this result indicates that the hydrophobic guest increases the

hydrophobicity near the NBD-amine moiety of NC0γCD, whereas it displaces the NBD-amine moiety of NC4γCD from the hydrophobic CD cavity to the bulk water. We observed the isosbestic point for each host (500 nm for NC0γCD, 431 nm for NC4γCD), and the wavelength at the isosbestic point was chosen as the excitation wavelength for fluorescence measurements. The fluorescence spectra of NC0γCD and NC4γCD in phosphate buffer (pH 7.0) have emission bands with peaks at 566 and 556 nm for NC0γCD and NC4γCD, respectively, as shown in Figure 6. This difference in the emission maximum wavelength results from a difference in hydrophobicity near each NBD-amine moiety. It suggests that the NBD-amine moiety of NC4γCD, which has the butylenediamine linker, penetrates more deeply into the hydrophobic CD cavity than that of NC0γCD, which has no linker. However the NBD unit of NC4γCD is not completely shielded from water molecules, because the difference in their emission maximum wavelengths is not large. The fluorescence intensity of NC0γCD rose upon the addition of UDCA, indicating an increase in hydrophobicity near the NBD unit induced by accommodation of the guest (Figure 6a). By contrast, the fluorescence intensity of NC4γCD only slightly changed upon the addition of the guest, indicating the accommodation of the guest into the cavity of NC4γCD cannot significantly change the hydrophobicity around the NBD unit of NC4γCD (Figure 6b).

Sensitivity parameters of NC0γCD and NC4γCD

The guest selectivity of the chemosensor was evaluated by the sensitivity parameter ($\Delta I/I_0$; $\Delta I = I - I_0$ where I_0 and I denote the fluorescence intensities in the absence and presence of a guest, respectively). The sensitivity parameters of NC0γCD and NC4γCD for various guests (Chart 2) are shown in Figure 7. NC0γCD is relatively sensitive to each bile acid but has no response to other guests, and NC4γCD is not sensitive to any of the guests. The response of NC0γCD to the guests is an increase in the fluorescence intensity in all cases. These results suggest that the hydrophobic face of the bile acid can interact with the NBD unit, increasing hydrophobicity of the chromophore's environment. However, the γ-CD cavity is too large for other guests, such as adamantane derivatives, to increase hydrophobicity around the NBD unit, as the water molecules cannot be completely excluded from the cavity. The difference in the sensitivity parameters between each bile acid is not large, although the binding affinities of the native γ-CD are different for these bile acids. It indicates that the variation in the fluorescence intensity does not correlate to the binding affinity of NC0γCD. The structure of the host-guest complex has a greater influence on the fluorescence intensity than the binding affinity does.

Maximum variation and binding constants of the chemosensors for guests

The plot of $\Delta I/I_0$ *versus* the guest concentration can be fitted to an equation for the 1:1 host–guest complex [9]. The binding constant (K_b) and $\Delta I_{max}/I_0$ can be obtained from this curve fitting analysis [9]. ΔI_{max} is the fluorescence spectra variation for the addition of an infinite quantity of the guest and the $\Delta I_{max}/I_0$ value provides information about the environment around the fluorophore in the complex. The $\Delta I_{max}/I_0$ values of NC0γCD are shown in Table 1. The $\Delta I_{max}/I_0$ of NC0γCD for each guest differs considerably in contrast with conventional chemosensors where $\Delta I_{max}/I_0$ is broadly similar for each guest. For example, the $\Delta I_{max}/I_0$ values for bile acids are large positive values, whereas those for the adamantane and borneol derivatives are small positive values. This

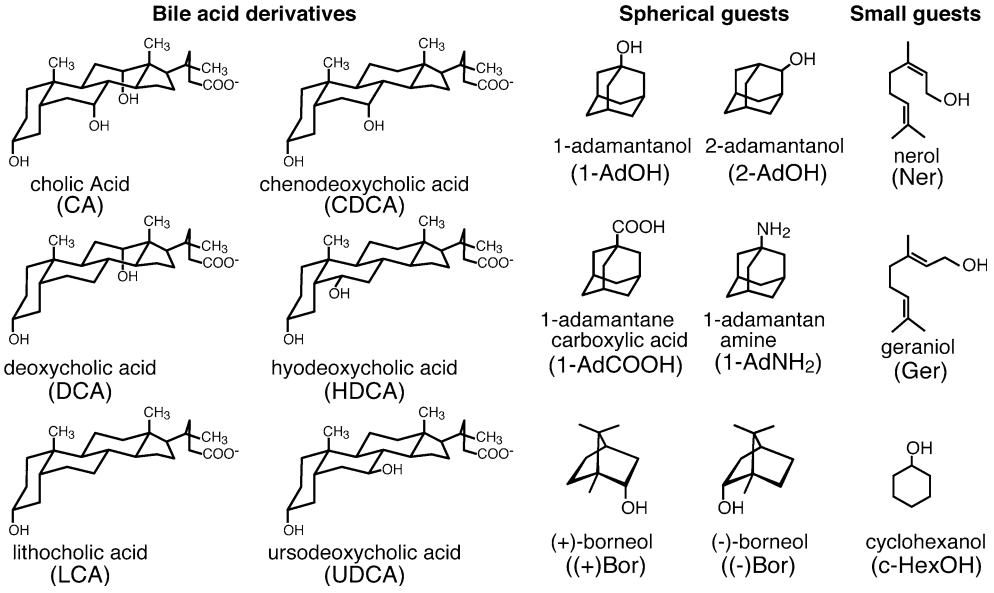

Chart 2. Structures of guests.

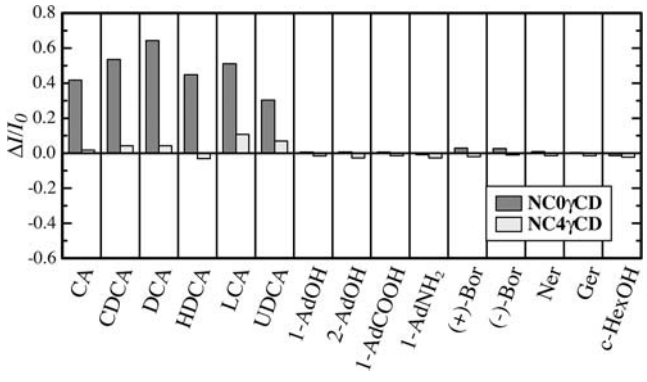

Figure 7. Sensitivity parameters ($\Delta I/I_0$) of NC0γCD (5×10^{-6} M) and NC4γCD (5×10^{-6} M) for various guests (each at 1×10^{-5} M). The excitation wavelength was 500 nm for NC0γCD and 431 nm for NC4γCD. The emission wavelength was 566 nm for NC0γCD and 556 nm for NC4γCD.

observation suggests that the environment around the fluorophore in the NC0γCD/guest complex is different for each guest. This difference in the fluorophore environment gives rise to the observed differences in sensitivity parameters for various guests. The $\Delta I_{max}/I_0$ values of NC0γCD for cholic acid (CA) is three timer larger than that for UDCA, whereas the binding constant for CA is one-third of that for UDCA. Therefore, the sensitivity parameter for CA is similar to that for UDCA. The sensitivity parameter of the new chemosensor depends on not only the binding affinity for the guest, but also the structure of the inclusion complex, as this strongly affects the environment around the fluorophore. Whereas the sensitivity parameter of the conventional chemosensors in general depends on only the binding affinity for the guest.

The binding constants for complexes of bile acids with NC0γCD are considerably larger than those with the natural γ-CD (Table 1). This finding suggests that the NBD unit also acts as a hydrophobic cap for guest binding rather than a binding inhibitor.

Table 1. $\Delta I_{max}/I_0$ and binding constants (K_b) of NC0γCD for the guests shown in Chart 2

Guest	$\Delta I_{max}/I_0$	K_b/M^{-1}
CA	1.59	41,000
CDCA	1.01	150,000
DCA	1.07	220,000
HDCA	0.67	310,000
LCA	0.54	3,700,000
UDCA	0.58	150,000
1-AdOH	0.37	1500
2-AdOH	0.37	1600
1-AdCOOH	0.61	870
1-$AdNH_2$	[a]	[a]
(+)-Bor	0.45	7200
(−)-Bor	0.44	6500
Ner	0.44	2200
Ger	0.31	1100
c-HexOH	[a]	[a]

[a]The accurate value could not determined due to small changes in fluorescence.

Conclusion

A new type of fluorescent chemosensor was produced by connecting the fluorophore unit (NBD-amine) to the γ-CD framework without an alkyl spacer in the linker. The chromophore is not self-included, instead remaining at the entrance of the γ-CD cavity and thus the fluorescence intensity of the new chemosensor increases upon the addition of guests. The new chemosensor is very sensitive to bile acids, but is not sensitive to other guests. The sensitivity parameter of the new chemosensor depends on not only the binding affinity for the guest, but also the structure of the inclusion complex, which strongly affects the environment around the fluorophore.

Acknowledgments

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