J Incl Phenom Macrocycl Chem (2007) 57:83-87
DOI 10.1007/s10847-006-9215-x

ORIGINAL ARTICLE

New chemosensor for larger guests based on modified cyclodextrin bearing seven hydrophobic chains each with a hydrophilic end group

Hiroshi Ikeda · Takumi Sugiyama · Akihiko Ueno

Received: 15 May 2006 / Accepted: 20 October 2006 / Published online: 23 January 2007

Abstract A new chemosensor for larger guests was prepared. The new chemosensor bears hydrophobic units at the primary hydroxy side and a dansyl unit at the secondary hydroxy side of β-cyclodextrin. Due to the hydrophobic units, the new chemosensor is sensitive to large or slender guests such as SDS and insensitive to 1-adamantanol, which is a good guest for the natural β-CD.

Keywords Cyclodextrin · Chemosensor · Molecular recognition · Fluorescence

Introduction

There has been great interest in the development of new chemical methods for the detection of ions and molecules [1–4]. We have prepared many kinds of chromophore-modified cyclodextrins (CDs) as chemosensors for molecule detection [5–13]. 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 Fig. 1. The 'self-inclusion state', in which the chromophore is located in the interior of the CD cavity, is usually the major conformation in an aqueous solution. An 'induced-fit' conformational change of the chromophore-modified CD, which occurs in association with accommodation of a guest, displaces the chromophore from the inside to the outside of the CD cavity, generating the 'non-self-inclusion state' (Fig. 1). Thus, the proportion of the 'non-self-inclusion state' increases with an increase in the guest concentration. The fluorescent 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 the variation in fluorescence intensity depends on the affinity of the chemosensor for the guest.

The kinds of guests that may be bound by CDs are limited by the shape and size of the CD cavity and modification to enlarge the CD cavity is required for binding of larger guests. Recently, we reported a new water-soluble modified β-CD bearing seven adipic acid units each with a D-glucamine unit at the end to enlarge the hydrophobic cavity without decreasing water solubility [14]. This modified CD shows stronger binding for large guests, such as 1-decanol or cyclododecanol than the natural CD. The binding constants of this modified CD for 1-octanol and cyclooctanol are three times and four times larger than those of β-CD, respectively. We have since introduced a fluorophore to this modified CD to make a new chemosensor for large guests. We here report the synthesis and chemosensing properties of the new chemosensor for large guests.

Experimental

General

Reverse phase HPLC was performed using a HITACHI HPLC system comprising a HITACHI L-7100

H. Ikeda (✉) · T. Sugiyama · A. Ueno
Department of Bioengineering, Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology, 4259-B-44 Nagatsuta-cho, Midori-ku, Yokohama 226-8501, Japan
e-mail: hikeda@bio.titech.ac.jp

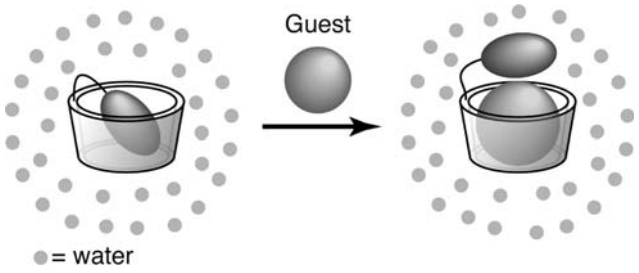


Fig. 1 Guest-induced conformational change of a chromophore-modified cyclodextrin

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 (δ = 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_3 aq.: 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.

Synthesis

Synthesis of **DNS-AG-CD (4)**

All of the amino groups of heptakis(6-amino-6-deoxy)-*β*-CD (400 mg) were protected by the reaction with (*t*-Boc)$_2$O (1.5 g) under alkaline conditions to produce the compound **2** (450 mg, 69% yield). The crude compound **2** (450 mg) was dissolved in dry DMSO (40 ml) and sodium hydride (60 mg) was added to the solution. After the effervescence stopped, the solution was heated at 50°C. A solution of *N*-dansyl-aminoethyl chloride (156 mg) in dry DMSO (20 ml) was added dropwise to the reaction mixture and the resulting mixture was stirred for 4 h at 50°C. Methanol (10 ml) was added to the reaction mixture and the resulting mixture was poured into cold diethyl ether. The precipitates were collected and dried in vacuo to obtain a yellow solid. The crude product was dissolved in trifluoroacetic acid (30 ml) and the solution was stirred for 2 h. After removing the trifluoroacetic acid, the product was purified by HPLC to obtain **3** (65.0 mg, 2.7% yield). **3** (65 mg) and *N*-adipoyl-D-glucamine (990 mg) were dissolved in water (5 ml) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC, 669 mg) was added to the solution. The resulting mixture was stirred overnight at room temperature while maintaining the pH at 4.5–6.0 by addition of 1 N sodium hydroxide. The product was purified by HPLC to obtain **DNS-AG-CD** (73.0 mg, 44.2% yield). MALDI-TOF MS: *m/z* calcd for $[M+Na]^+$: 3464.5; found, 3463.5.

Synthesis of **DNS-CD (5)**

A solution of *N*-dansyl-aminoethyl chloride (1.38 g) in dry DMSO (40 ml) was added dropwise to a solution of *β*-CD (3.00 g) with sodium hydroxide (0.31 g) in dry DMSO (80 ml). The resulting mixture was stirred overnight. The reaction mixture was poured into acetone. The precipitates were collected and dried in vacuo to obtain a yellow solid. The product was purified by HPLC to obtain **DNS-CD** (559 mg, 15.0% yield). MALDI-TOF MS: *m/z* calcd for $[M+Na]^+$: 1433.4; found, 1433.6.

Results and discussion

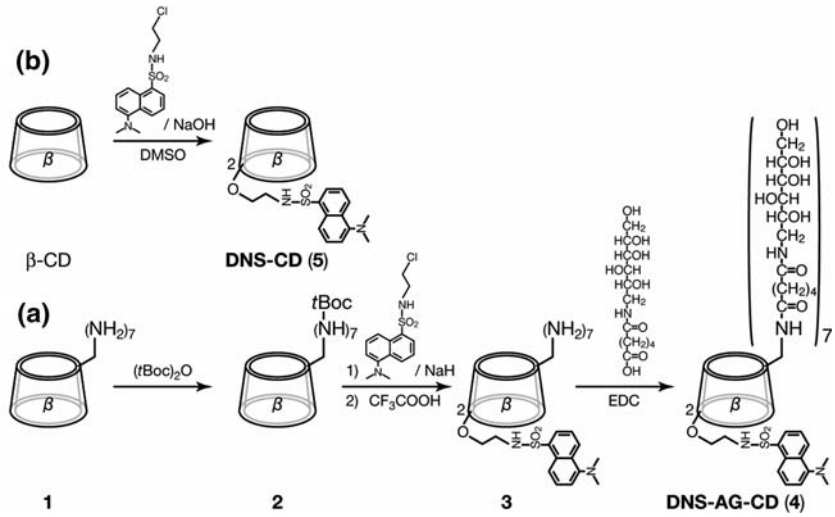
Syntheses of **DNS-AG-CD** and **DNS-CD**

DNS-AG-CD (**4**) was synthesized as shown in Scheme 1. The dansyl unit was introduced as a fluorophore unit at the C2 position of the *β*-CD by alkylation in the presence of sodium hydride in DMSO. After introduction of the fluorophore unit, the *N*-adipoyl-D-glucamine units were coupled to all of the C-6 positions of the *β*-CD. The desired compound **4** was identified by ^{1}H NMR spectra including 2D NMR and MALDI-TOF MS. **DNS-CD** (**5**) was also synthesized as a reference compound. **DNS-CD** (**5**) has the fluorophore unit at the C2 position but has no *N*-adipoyl-D-glucamine unit at the C6 position.

Fluorescence spectra of **DNS-AG-CD** and **DNS-CD**

The fluorescence spectra of **DNS-AG-CD** and **DNS-CD** in a phosphate buffer (pH 7.0) have emission bands with peaks at 521 nm and 525 nm, respectively, as shown in Fig. 2. This difference in the emission maximum wavelength results from a difference in hydrophobicity around the dansyl unit. The cavity of **DNS-AG-CD** is more hydrophobic than that of **DNS-CD** due to the *N*-adipoyl-D-glucamine units at the C6 positions. The fluorescence intensities of **DNS-AG-CD** and **DNS-CD** decreased upon addition

Scheme 1 Syntheses of **DNS-AG-CD** (**4**) and **DNS-CD** (**5**)



of ursodeoxycholic acid (UDCA) and the emission maximum wavelengths of the fluorescence spectra were shifted to longer wavelengths with increasing guest concentration. It indicates a positional change of the fluorophore from the inside to the outside of the CD cavity. The extent of the variation in fluorescence intensity of **DNS-AG-CD** is smaller than that of **DNS-CD**. This suggests that some part of the dansyl unit of **DNS-AG-CD** remains accommodated in the CD cavity even after making an inclusion complex with UDCA, because the guest mainly locates near the hydrophobic units at the primary hydroxy side.

Induced circular dichroism of **DNS-AG-CD**

Induced circular dichroism spectrum provides information about the structure of inclusion complex [15–19]. **DNS-AG-CD** shows a negative dichroism band at 330 nm and a positive dichroism band at 270 nm (Fig. 3). This spectrum suggests that the dansyl unit is accommodated in the way where the long axis of the dansyl unit is parallel to the C_7 axis of the β-CD. The intensities of these dichroism bands decreased upon addition of UDCA but the dichroism bands still existed upon addition of UDCA being 10 equivalent to **DNS-AG-CD**. It suggests that some part of the dansyl unit of **DNS-AG-CD** remains accommodated in the CD cavity, in which the guest was accommodated. This result is consistent with the result of fluorescence variation upon addition of the guest.

Sensitivity parameters of **DNS-AG-CD** and **DNS-CD**

The guest selectivities of the chemosensors were evaluated by the sensitivity parameter ($\Delta I/I_0$; where

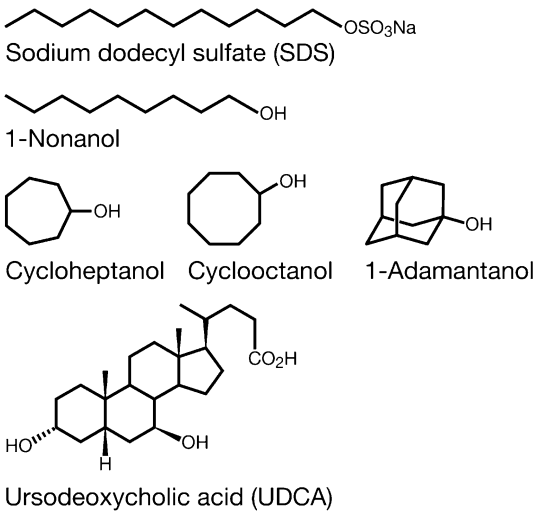


Chart 1 Structures of guests

$\Delta I = I_0 - I$ with I_0 and I being the fluorescence intensities in the absence and presence of a guest, respectively) [9]. The structures of the guests used for evaluation of the guest selectivities of the chemosensors are shown in Chart 1. Figure 4 shows the guest selectivity pattern of **DNS-AG-CD** and **DNS-CD** (1.1×10^{-5} M) for various guests each at a guest concentration of 5×10^{-5} M. **DNS-AG-CD** is more sensitive to each guest than **DNS-CD**, whereas the fluorescence intensity of **DNS-AG-CD** scarcely changed upon addition of 1-adamantanol, which is a good guest for the natural β-CD. These results indicate that the presence of the *N*-adipoyl-D-glucamine units increases the fluorescence variation associated with binding of guests such as SDS, while decreasing it for 1-adamantanol. 1-Adamantanol can be accommodated in both the CD cavity and the hydrophobic site surrounded by the *N*-adipoyl-D-glucamine units. If 1-adamantanol were mainly located in

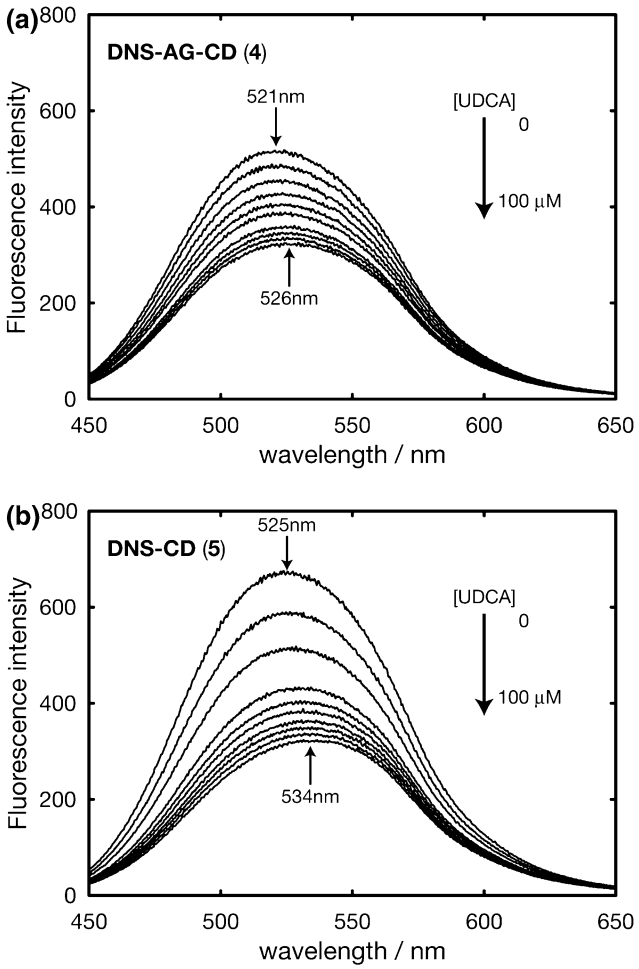


Fig. 2 Fluorescence spectra of (**a**) **DNS-AG-CD** (**4**) (1.1×10^{-5} M) and (**b**) **DNS-CD** (**5**) (1.1×10^{-5} M) in the presence of various concentrations of UDCA in phosphate buffer (200 mM, pH 7.0) at 25°C; the excitation wavelength was 347 nm

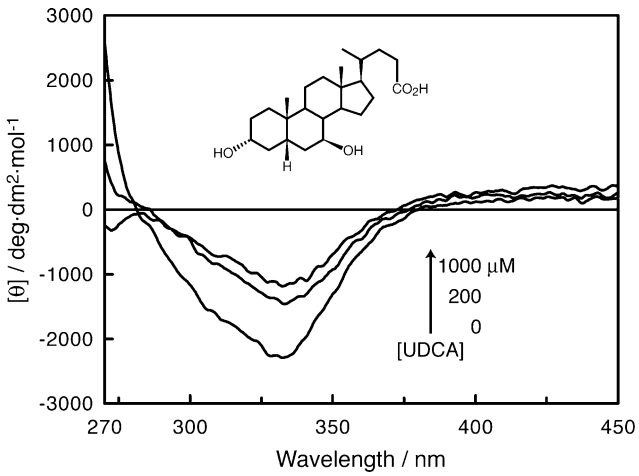


Fig. 3 CD spectra of **DNS-AG-CD** (1.1×10^{-4} M) alone and in the presence of UDCA in phosphate buffer (200 mM, pH 7.0) at 25°C

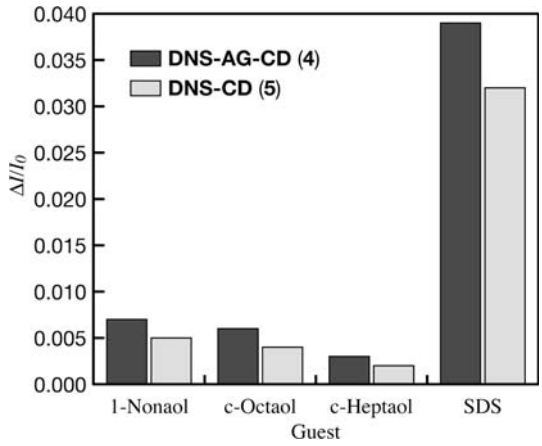


Fig. 4 Sensitivity parameters ($\Delta I/I_0$) of **DNS-AG-CD** (**4**) (1.1×10^{-5} M) and **DNS-CD** (**5**) (1.1×10^{-5} M) for various guests (5×10^{-5} M). The excitation wavelength was 347 nm and the emission wavelength was 525 nm

the hydrophobic site surrounded by the *N*-adipoyl-D-glucamine units, the dansyl unit would not be excluded to the water solution and so the fluorescence intensity would not change. This means that the introduction of the *N*-adipoyl-D-glucamine units changes the guest selectivity and that the new chemosensor is sensitive to large or slender guests and not sensitive to 1-adamantanol. This selectivity is different from the sensitivity of the CD itself and the response of the new chemosensor to the guests is not consistent with its binding affinity for the guests.

Binding constants of **DNS-AG-CD** and **DNS-CD**

The plot of $\Delta I/I_0$ versus the guest concentration can be fitted to an equation for a 1:1 host–guest complex [9]. The binding constant (K_b) can be obtained from this curve fitting analysis (Table 1). The introduction of the hydrophobic units at the C6 position is expected to increase the binding affinity of the CD. Although the binding constant of **DNS-AG-CD** for UDCA is only half as many as that of **DNS-CD** in Table 1, this value

Table 1 Apparent binding constants (K_b) of **DNS-AG-CD** (**4**) and **DNS-CD** (**5**) for the guests shown in Chart 1

Guest	**DNS-AG-CD** K_b/M^{-1}	**DNS-CD** K_b/M^{-1}
1-Nonanol	460	780
Cyclooctanol	170	120
Cycloheptanol	110	80
SDS	1,200	1100
UDCA	11,300	27,500

would not be accurate for binding affinity. ICD spectra change suggested that some part of the dansyl unit of **DNS-AG-CD** still remains accommodated in the CD cavity, even when UDCA was accommodated. The guest that mainly locates near the hydrophobic units at the primary hydroxy side cannot be correctly counted by the variation in fluorescence intensity, because the accommodation of the guest at the hydrophobic units slightly changes the fluorescence intensity. Although the introduction of the hydrophobic unit at the primary hydroxy side could expand the hydrophobic CD cavity, it is difficult to evaluate this effect only by the fluorescence variation of the pendant unit.

Conclusion

We prepared a new chemosensor bearing *N*-adipoyl-D-glucamine units to enlarge the CD cavity at the primary hydroxy side and a dansyl unit for the fluorophore at the secondary hydroxy side of CD. The *N*-adipoyl-D-glucamine units improve the guest-response for large or slender guests and decrease it for 1-adamantanol.

Acknowledgments This article is dedicated to the honor and memory of Professor Akihiko Ueno, who passed away on March 23, 2003. We are grateful to Nihon Shokuhin Kako Co., Ltd. for the generous supply of cyclodextrins. This work was supported by a Grant-in-Aid for Science Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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