Fluorescent Cyclodextrins Responsive to Molecules and Metal Ions. Fluorescence Properties and Inclusion Phenomena of N^{α}-Dansyl-L-lysine-β-cyclodextrin and Monensin-Incorporated N^{α}-Dansyl-L-lysine-β-cyclodextrin

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N^{α}-Dansyl-L-lysine-β-cyclodextrin with a monensin unit (**1**) was prepared as a fluorescent chemosensor for molecule recognition. Its fluorescence properties and inclusion phenomena were examined using N^{α}-dansyl-L-lysine-β-cyclodextrin (**2**) and N^{ϵ}-benzyloxycarbonyl-N^{α}-dansyl-L-lysine-β-cyclodextrin (**3**) as reference compounds. The fluorescence peak intensities of these hosts are in the order **2** > **1** > **3**, suggesting that the dansyl unit of **2** is most deeply included in the β-cyclodextrin cavity. The host **1** exhibits two lifetimes, both in the absence of a guest [18.2 ns (81.8%) and 10.9 ns (18.2%)] and in the presence of 1-adamantanol (0.3 mM) [16.3 ns (17.8%) and 9.4 ns (82.2%)], demonstrating that **1** exists predominantly as a self-inclusion form with a longer lifetime and that it excludes the dansyl moiety to outside the cavity upon guest accommodation. A sodium cation in a solution of **1** enhanced the binding ability of **1** and increased the guest-dependent fluorescence variation. All of these results demonstrate that the monensin moiety of **1** is effective in changing the environment around the cyclodextrin cavity by forming a ring-like conformation with a sodium ion in the center.

Introduction

Signal transduction, from molecular binding to spectral signals, is a current topic in supramolecular chemistry.[1,2] Chemosensors are molecules that are capable of signal transduction and usually have both a receptor part for guest binding and a chromophoric unit for spectral output. Crown ethers,[3,4] calixarenes,[5,6] cyclodextrins (CDs),[7,8] and other synthetic receptors[9,10] are widely used as the receptor parts. We have constructed a variety of CD-based chemosensors. Most of them have a fluorophore and change their fluorescence intensity when a guest is accommodated in the CD cavity,[11−20] whereas others have a dye moiety and change color or absorption intensity with guest binding.[21−25] The mechanism of signal transduction usually involves induced-fit changes in the conformation of the chemosensors, in which the location of the appending chromophore is changed from inside to outside the cavity upon guest accommodation. Dansyl is a typical fluorescence probe; it is very sensitive to the environment and exhibits strong fluorescence in a hydrophobic environment, and its fluorescence intensity weakens in bulk water.[11−19] We previously prepared dansyl-modified β- and γ-CD and observed guest-dependent decreases in fluorescence intensity with exclusion of the dansyl unit from inside to outside the cavity upon guest binding.[11−17] If the system has an environmental moiety other than a fluorophore, the selectivity and binding strength may be modulated. We previously

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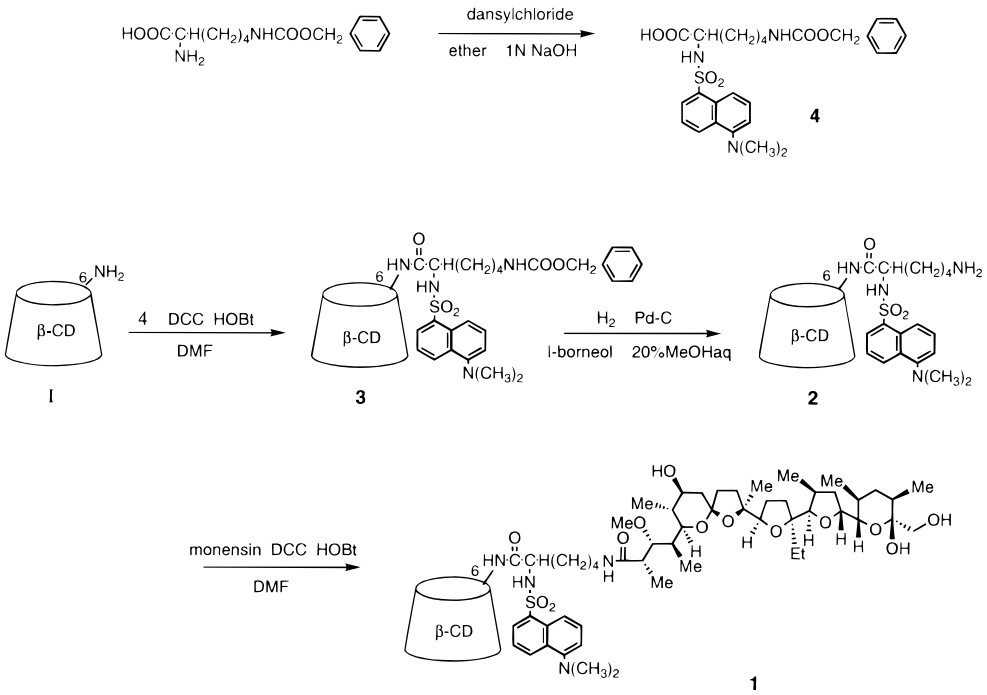
Scheme 1



reported the preliminary results on a dansyl-modified CD bearing a monensin as an environmental unit.[26] Monensin is an antibacterial hydrophobic substance that can bind sodium cation to form a ring-like conformation with a sodium ion in its center. Consequently, the dansyl-modified CD was expected to act as a unique chemosensor with a hydrophobic cap that was responsive to sodium ion. We report here the details of the syntheses, fluorescence spectra, fluorescence lifetimes, and guest-dependent properties of monensin-incorporated N^{α}-dansyl-L-lysine-β-CD (**1**) using N^{α}-dansyl-L-lysine-β-CD (**2**) and N^{ϵ}-benzyloxycarbonyl-N^{α}-dansyl-L-lysine-β-CD (**3**) as reference compounds.

Experimental Section

Materials. β-CD was a kind gift from Nihon Shokuhin Kako Co. Ltd. All reagents used as guests were purchased from Tokyo Kasi and were used without further purification.

Measurements. Fluorescence decay was measured by a time-correlated single-photon counting method. A self-oscillating flash lamp filled with H_2 was used as a light source. The excitation beam was passed through UV34, U340, and U350 filters (Hoya), and the emission beam was passed through L42 (Hoya) and Y46 (Toshiba) filters and an aqueous solution of $NiSO_4 \cdot 6H_2O$ (500 g/L) in a 1-cm path length cell. Lifetimes were determined by deconvolution with a nonlinear least-squares fitting procedure. Elemental analyses were performed by the Analytical Division of the Research Laboratory of Resources Utilization of Tokyo Institute of Technology. ^{1}H NMR spectra were recorded in D_2O at 25 °C operating at 499.843 MHz for ^{1}H. HDO ($\delta = 4.70$) was used as an internal standard.

***N*$^{\epsilon}$-Benzyloxycarbonyl-*N*$^{\alpha}$-dansyl-L-lysine-β-CD (3).** A solution of N^{ϵ}-benzyloxycarbonyl-N^{α}-dansyl-L-lysine (**4**, 514 mg), 6-amino-6-deoxy-β-CD[8] (1.13 g), dicyclohexylcarbodiimide (DCC, 206 mg), and hydroxybenzotriazole (HOBt, 135 mg) in DMF (20 mL) was stirred at 0 °C for 2 h and at room temperature for 48 h. The solution was then filtered with a membrane filter, and the filtrate was reprecipitated from acetone. The precipitates were collected by a membrane filter, giving a crude product (502 mg). The crude product was dissolved in water, and the aqueous solution was subjected to a HP-20 column. Column chromatography was performed by stepwise elution with water and methanol, and the fractions obtained with 60% methanol contained the desired product, giving a yellow powder after the solvent was removed (yield 56%). Anal. Calcd for $C_{68}H_{100}N_4O_{39}S \cdot 6H_2O$: C, 47.00; H, 6.50; N, 3.22; S, 1.84. Found: C, 47.01; H, 6.06; N, 3.29; S, 1.86. ^{1}H NMR (D_2O) δ 1.10 (m, 2H, γCH_2(Lys)), 1.22 (m, 2H, δCH_2-(Lys)), 1.41 (m, 1H, βCH_2(Lys)), 1.64 (m, 1H, βCH_2(Lys)), 2.78 (s, 6H, $N(CH_3)_2$), 4.89−5.07 (m, 7H, H-1(β-CD)), 5.08 (s, 2H, OCH_2), 7.30 (bs, 2H, Ph), 7.46 (bs, 3H, Ph and 1H, DNS), 7.68 (t, 1H, DNS), 7.77 (t, 1H, DNS), 8.29 (d, 1H, DNS), 8.36 (d, 1H, DNS), 8.58 (d, 1H, DNS).

***N*$^{\alpha}$-Dansyl-L-lysine-β-CD (2).** A solution of **3** (117.3 mg) and (−)-borneol (26 mg) in 20% MeOH aqueous solution was hydrogenated on Pd/C (64 mg) under 3 atm of hydrogen at 80 °C. The solution was then filtered with a membrane and concentrated under reduced pressure. The resultant solution was subjected to column chromatography with CM C-25. After elution with 10 L of water, a 1 N NH_3 solution was passed through the column. The NH_3 solution contained the desired product and was lyophilized after condensation under reduced pressure, giving **2** in 65% yield. Anal. Calcd for $C_{60}H_{94}N_4O_{37}S \cdot 3H_2O$: C, 46.51; H, 6.50; N, 3.62; S, 2.07. Found; C, 46.54; H, 6.44; N, 3.53; S, 1.92. ^{1}H NMR (D_2O) δ 1.45 (m, 2H, γCH_2-(Lys)), 1.60 (m, 2H, δCH_2(Lys)), 1.65 (m, 1H, βCH_2(Lys)), 1.75 (m, 1H, βCH_2(Lys)), 2.91 (s, 6H, $N(CH_3)_2$), 4.85−5.15 (m, 7H, H-1(β-CD)), 7.37 (d, 1H, DNS), 7.44 (t, 1H, DNS), 7.71 (t, 1H, DNS), 8.16 (d, 1H, DNS), 8.50 (d, 2H, DNS).

***N*$^{\epsilon}$-Monensin-*N*$^{\alpha}$-Dansyl-L-lysine-β-CD (1).** Monensin sodium salt was neutralized with 1 N HCl, and the solution was mixed with **2** (83 mg), DCC (55 mg), and HOBt (29 mg) in DMF (13 mL). The mixed solution was stirred at 0 °C for 2 h and at room temperature for 80 h. The resultant solution was concentrated under reduced pressure, and a large amount of acetone was added. The formed precipitates were collected by filtration and dried under reduced pressure, giving the crude product in 89% yield. This product was purified by column chromatography with HP-20 using a water−MeOH mixture under gradient elution, to give the purified product **1** after evaporation. Anal. Calcd for $C_{96}H_{154}N_4O_{47}S \cdot 6H_2O$: C, 51.10; H, 7.42; N, 2.48; S, 1.42. Found: C, 51.01; H, 7.12; N, 2.49; S,

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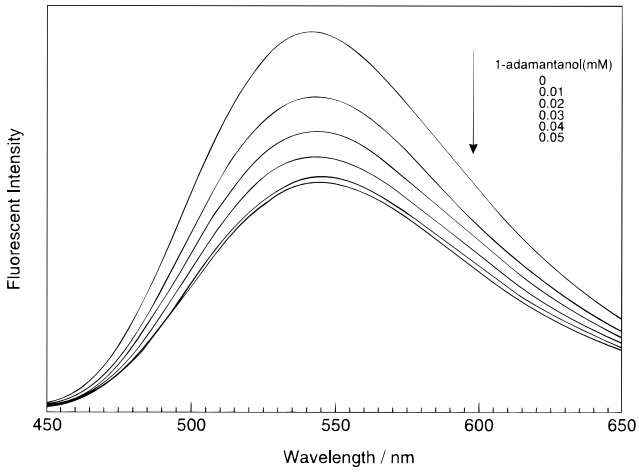

Figure 1. Fluorescence spectra of **1** (2×10^{-6} M) in aqueous solution at 25 °C in the presence of various concentrations of 1-adamantanol; excitation wavelength was 370 nm.

1.46. ^{1}H NMR (D_2O) δ 2.98 (s, 6H, $N(CH_3)_2$), 4.85−5.20 (m, 7H, H-1(β-CD)), 7.40 (d, 1H, DNS), 7.58 (t, 1H, DNS), 7.69 (t, 1H, DNS), 8.32 (d, 2H, DNS), 8.65 (d, 1H, DNS).

Results and Discussion

Synthesis. The synthetic route to **1** is shown in Scheme 1. We prepared N^{ϵ}-benzyloxycarbonyl-N^{α}-dansyl-L-lysine-β-CD (**3**) by reacting N^{ϵ}-benzyloxycarbonyl-N^{α}-dansyl-L-lysine and 6-amino-6-deoxy-β-CD with DCC and HOBt in DMF. N^{α}-Dansyl-L-lysine-β-CD (**2**) was obtained by hydrogenation of **3** on Pd/C in 20% MeOH in the presence of (−)-borneol. An initial attempt to remove the benzyloxycarbonyl (Z) group failed, so we added (−)-borneol to the reaction system with the idea that hydrogenation of **3** might be prevented due to inclusion of the Z moiety in the β-CD cavity. As a result, the presence of (−)-borneol as the guest was effective for excluding the Z moiety from the cavity, and hydrogenation proceeded. Finally, N^{α}-dansyl-L-lysine-β-CD (**2**) was reacted with monensin, DCC, and HOBt in DMF to yield **1**.

Fluorescence Spectra. Figure 1 shows fluorescence spectra of **1** with various concentrations of 1-adamantanol in aqueous solution. The fluorescence peak at around 540 nm showed a decrease in intensity with an increasing concentration of 1-adamantanol, suggesting that the dansyl unit of **1** is excluded from the hydrophobic β-CD cavity to the outside bulk aqueous solution with the accommodation of 1-adamantanol as a guest in the cavity. This guest-dependent fluorescence variation confirms that **1** can be used as a chemosensor for detecting molecules despite the presence of the large monensin residue in **1**.

Figure 2 shows fluorescence spectra of **1**−**3**, and N^{ϵ}-benzyloxycarbonyl-N^{α}-dansyl-L-lysine (**4**) in aqueous solution. The fluorescence peak intensities are in the order **2** > **1** > **3** > **4** with peak maxima at 541, 540, 550, and 570 nm for **1**, **2**, **3**, and **4**, respectively. Because the hydrophobicity around the dansyl moiety should be reflected in the fluorescence intensity, the environment around the dansyl moiety of **2** is the most hydrophobic. This implies that the dansyl moiety of **2** is likely to be included deeply in the hydrophobic CD cavity. The peak wavelength is known to be shifted to a shorter wavelength with increasing hydrophobicity of the environment around a dansyl unit, and the peak wavelengths observed

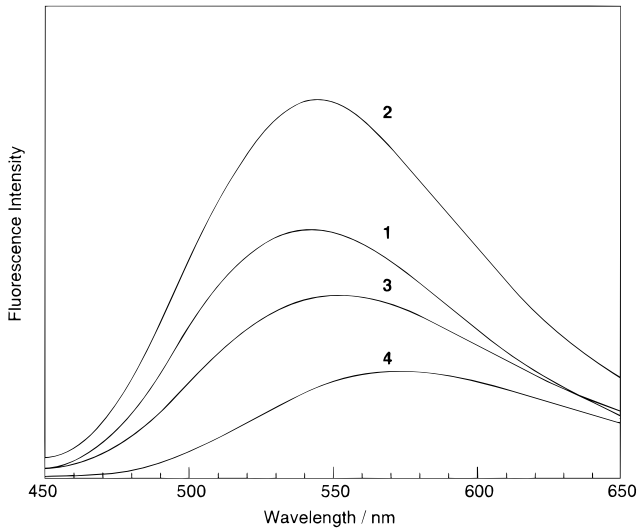

Figure 2. Fluorescence spectra of (1) **1**, (2) **2**, (3) **3**, and (4) N^{ϵ}-benzyloxycarbonyl-N^{α}-dansyl-L-lysine in aqueous solution at 25 °C; excitation wavelength was 370 nm.

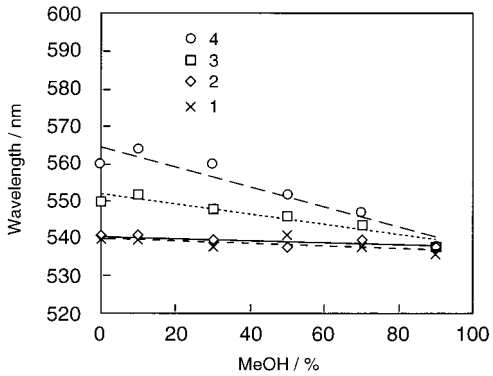

Figure 3. Dependence of emission maxima on solvent polarity at 25 °C; each sample concentration was 2×10^{-6} M; excitation wavelength was 370 nm.

for **1**−**4** are consistent with the relative hydrophobicity around the dansyl units of these compounds. We further examined the effect of solvent polarity on the peak maximum in mixed solutions of water and MeOH (Figure 3). The peak maxima of **1** and **2** were not affected by the MeOH content, but the peak wavelengths of **3** and **4** shifted to shorter values with increasing MeOH content and tended to reach the same value of around 540 nm. This result suggests that the dansyl moieties of **1** and **2** are almost completely included in the cavity in solutions with various contents of MeOH, whereas the dansyl moiety of **3** is more readily excluded from the cavity than those of **1** and **2** in solvents with a high MeOH content.

Fluorescence Lifetimes. Dansyl-modified CDs that have been reported so far exhibit two lifetimes. The longer lifetime (ca. 17 ns) is attributed to a self-inclusion complex in which the dansyl moiety is included in the CD cavity, and the shorter lifetime (ca. 6 ns) is attributed to a species with the dansyl moiety excluded from the cavity into the water solution.[12,18] In this study, we measured the lifetimes of **1**−**3** in aqueous solution and found two lifetimes for each of these compounds, as shown in Table 1.

Compound **1** had lifetimes of 18.2 ns (81.8%) and 10.9 ns (18.2%) in the absence of a guest. The long-lifetime component is predominant and may be attributed to the

Table 1. Results of Fluorescence Decay of 1−3 Alone and with 1-Adamantanol in Aqueous Solution at 25 °C

host[a]	guest[b]	τ_1, ns (A_1)	τ_2, ns (A_2)	τ_3, ns (A_3)
1		18.2 (0.818)	10.9 (0.182)	
1	1-adamantanol	16.3 (0.178)	9.36 (0.822)	
2		17.0 (0.982)	7.60 (0.083)	
2	1-adamantanol	17.0 (0.266)	7.61 (0.734)	
3		17.4 (0.429)	4.27 (0.571)	
3	1-adamantanol	17.0 (0.096)	4.30 (0.183)	8.21 (0.721)

[a] 50 μM. [b] 300 μM.

self-inclusion species. The shorter-lifetime component is a minor species and may be attributed to the species with the dansyl moiety outside the CD cavity. Therefore, this compound exists as two species in equilibrium with each other within the time scale of the measurements. The value of 10.9 ns is relatively long for the lifetime of the component which is directly exposed to aqueous solution, suggesting that in this component the dansyl is not completely exposed to the water, probably as a result of the effect of the large hydrophobic monensin residue. In the presence of 1-adamantanol (0.3 mM) as a guest, values of 16.3 ns (17.8%) and 9.4 ns (82.2%) were obtained for longer- and shorter-lifetime components. Interestingly, the proportion of the longer-lifetime component decreased while that of the shorter-lifetime component increased. This result corresponds to the scenario in which the dansyl moiety is excluded from inside the cavity upon guest binding. This result indicates that the large monensin residue does not prevent the inside−outside change in the location of the dansyl moiety.

When the solution of **1** contained sodium chloride (1 M), we obtained values of 17.0 ns (75.7%) and 10.8 ns (24.3%), indicating that no significant change in the lifetime was induced by the presence of sodium cation.

Compound **2** also exhibits two lifetimes, 17.0 ns (98.2%) and 7.6 ns (1.8%), in the absence of guest, suggesting that almost all of the dansyl moiety is included in the CD cavity. In the presence of 1-adamantanol, the same lifetimes, but in different proportions (26.6% and 73.4%), were obtained for the longer- and shorter-lifetime components, respectively, suggesting that the induced-fit change in the location of the dansyl moiety also occurs in this case (Figure 4A).

Compound **3** also exhibits two lifetimes, 17.4 ns (42.9%) and 4.3 ns (57.1%), in the absence of a guest. The lifetime of 4.3 ns indicates that the dansyl moiety of this component is completely exposed to the aqueous solution. The fact that this species exists in significant amounts (>50%) suggests that the CD cavity is included competitively by the Z moiety. In the presence of 1-adamantanol, we observed three lifetimes, 17.0 ns (9.5%), 8.2 ns (72.1%), and 4.3 ns (18.3%). The component with a lifetime of 8.2 ns, which is new, is predominant. This new component may be a species with both Z and dansyl moieties outside the CD cavity. Its rather long lifetime (8.2 ns) may reflect the interaction between Z and dansyl moieties outside the cavity (Figure 4B).

Guest-Dependent Fluorescence Intensities of 1−3. The results of lifetime measurements indicate that three

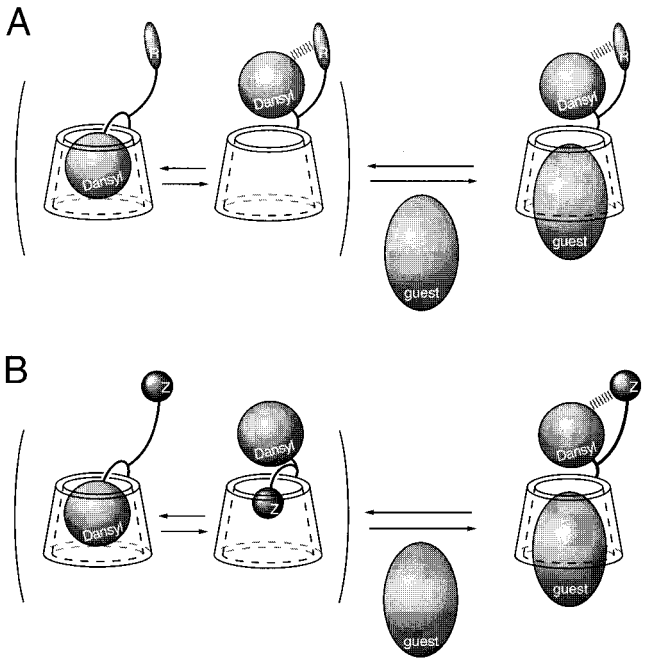

Figure 4. Conformational equilibrium in aqueous solution and guest-dependent conformational changes in **1**, **2**, and **3**.

dansyl-modified β-CDs, **1**, **2**, and **3**, undergo conformational changes with guest accommodation in their CD cavity. Therefore, it would be interesting to compare the changes in their fluorescence intensities upon guest binding. Figure 5 shows the changes in the fluorescence intensities of **1**, **2**, and **3** (2 μM) upon guest addition (10 μM). We used four steroid compounds (**5**−**8**), three adamantane derivatives (**9**−**11**), nine monoterpenes (**12**−**19**), and two cycloalkanols (**20**, **21**) (Chart 1). Steroids **5**−**7** are isomers with one hydroxyl group at C-7 stereochemically inverted for **5** and **6** and one hydroxyl group at C-12 for **7**. Figure 5 shows the sensitivity parameter $\Delta I/I_0$, where I_0 and I are the fluorescence intensities in the absence and presence of guest, respectively, and $\Delta I = I_0 - I$. The order of the sensitivity parameters of **1**−**3** for **5** is **2** > **1** > **3**, indicating that **2** is most responsive to **5**. The same order was observed for all of the steroid compounds, and the sensitivity parameters of each host for **1**−**3** are in the order **5** > **6** > **7**, which indicates that the hosts **1**−**3** discriminate the steroid isomers distinctly. Cholic acid has one more hydroxyl group than **5**, **6**, and **7** and exhibits small sensitivity values similar to those of **7**. All of the guests other than the steroids give sensitivity values in the order **1** > **2** > **3**. This remarkable sensitivity of **1** suggests that the monensin residue of **1** is very effective in enhancing the guest-dependent fluorescence variation due to the capping effect of the hydrophobic monensin residue. The rather depressed sensitivity of **1** for steroids may be explained in terms of steric hindrance between the monensin residue and the steroids in complexes where large steroid molecules penetrate the cavity.

Effects of Metal Ions on Guest-Dependent Fluorescence Variation. Monensin is an antibacterial compound that forms a ring-like conformation with a sodium cation bound in the center. The monensin residue of **1** may take a flexible conformation in the absence of sodium ion, but it is expected to take a rigid ring-like conformation in the presence of sodium cation. We examined the

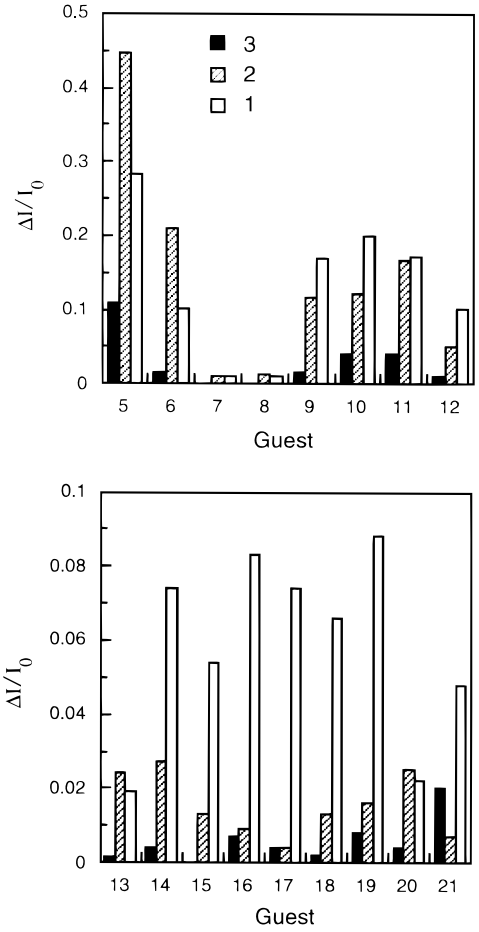

Figure 5. Sensitivity factors of **1**, **2**, and **3** for various guests at 25 °C in aqueous solution: [**1**−**3**] = 2 × 10^{-6} M, [guest] = (left) 10^{-5} M, (right) 10^{-4} M. λ_{ex} = 370 nm, λ_{em} = 543 nm.

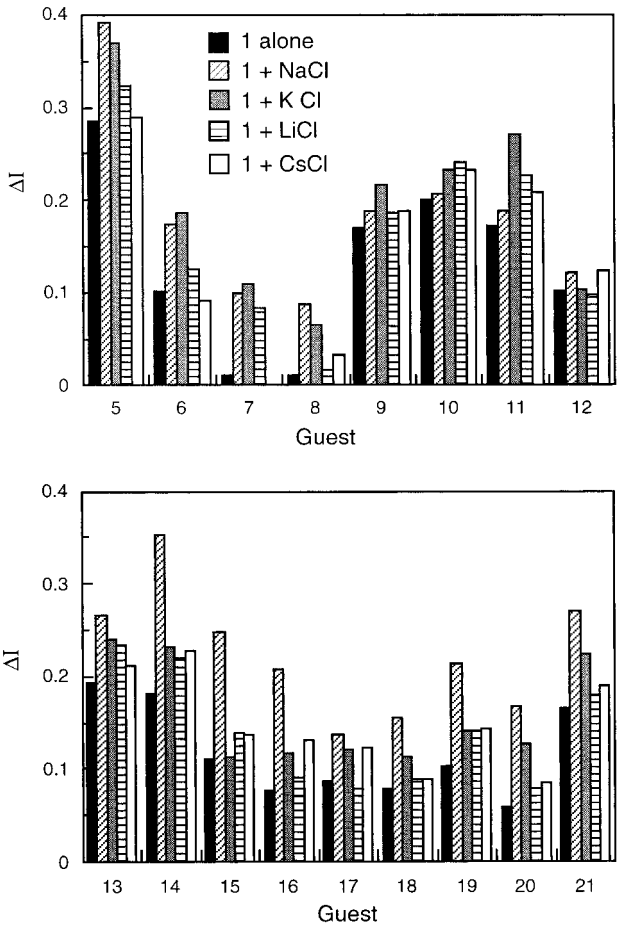

Figure 6. Δ*I* values of **1** in the absence and presence of alkali metal cations at 25 °C in aqueous solution: [**1**−**3**] = 2 × 10^{-6} M, [guest] = (left) 10^{-5} M, (right) 10^{-4} M, [metal cation] = 1 M. λ_{ex} = 370 nm, λ_{em} = 543 nm.

effects of alkali metal cations on guest binding and fluorescence variation in **1**. Figure 6 shows the fluorescence variation of **1** for various guests (10 mM for **5**−**12**; 100 mM for **13**−**21**) in the absence and presence of alkali metal cations such as Na^+, K^+, Li^+, and Cs^+ (1.0 M). In most cases, fluorescence variation was enhanced by metal ions, particularly by sodium ion for **13**−**21**. The enhancement for **5** was in the order $Na^+ > K^+ > Li^+ > Cs^+$, which is consistent with the expected order for monensin itself. Although there are some complicated features for **5**−**12**, the guest-dependent fluorescence variation is in the order $Na^+ > K^+ > Li^+$ for **13**−**21**, with the variation with Cs^+ deviating from this trend. It should be noted that ionic strength is not a factor in these systems, because there was almost no sodium ion-induced enhancement of $\Delta I/I_0$

Chart 1

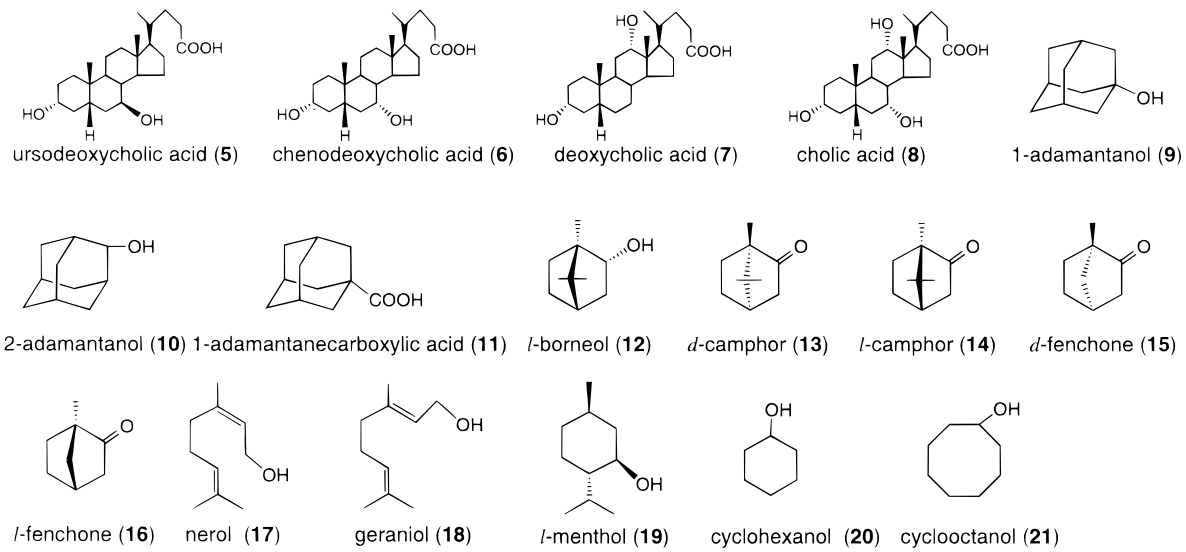

Table 2. Binding Constants of 1 and 2 in Aqueous Solution at 25 °C[a,b]

	K (M^{-1})		
guest	**1**	**1** + Na^+	**2**
9	38200	43000	26400
10	46400	64900	27400
11	17200	42400	23400
12	15100	19700	12500
13	6340	7320	7020
14	6570	13200	5650
15	5790	10400	4900
16	5940	7780	4000
17	5370	10300	2390
18	6320	11900	5590
19	3250	4890	2030
20	2250	15800	1420
21	3310	9010	2150

[a] The values of **1** in the presence of sodium cation were obtained in 1 M NaCl solution. [b] The values of **2** in 1 M NaCl solution were 26100, 23400, 2090, and 1910 M^{-1} for guests **10**, **11**, **17**, and **21**, respectively, suggesting that the binding constants of **2** are not appreciably affected by the presence of NaCl.

when we examined fluorescence variation using **2** in place of **1**. This result confirms that the monensin unit of **1** in the presence of sodium ion takes a conformation (a ring-like conformation with a sodium ion in the center) different from that with **1** alone. The enhancement of ΔI may be related to an increase in binding constants, and we actually obtained larger binding constants in the presence of sodium ion, as shown in Table 2. This increased binding suggests that the monensin residue acts as a hydrophobic cap, especially when it takes a ring-like conformation.

Binding Constants. Table 2 shows the binding constants (K) of **1** and **2** obtained on the basis of a 1:1 stoichiometry for selected guests. The binding constants of **1** are larger than those of **2** except for **13**, which is completely consistent with the sensitivity factors shown in Figure 5. Another feature is the effect of the sodium ion. The binding constants for **1** were dramatically increased in the presence of sodium ion (1 M), which is consistent with the data shown in Figure 6.

In conclusion, the monensin residue in **1** was shown to be responsive to sodium cation and to increase the binding ability and the degree of guest-dependent fluorescence variation. It is likely that the ring-like conformation with a sodium ion in its center acts as an effective hydrophobic cap. These results demonstrate that an environmental unit incorporated into a simple fluorescent host may become a key factor that governs the sensitivity of chemosensors.

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