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Syntheses and Molecular Recognition Abilities of 6-O-, 2-O-, and 3-O-Dansyl-γ-Cyclodextrins

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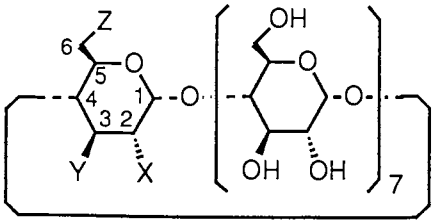
6-O-, 2-O-, and 3-O-Dansyl-γ-CD (γ-1, γ-2, and γ-3) were synthesized as fluorescent sensors by the reaction of γ-CD with dansyl chloride under different conditions. They showed remarkably different molecular recognition abilities for various organic compounds. Molecular size and molecular structure of guests are the important factors which govern the sensitivity in these sensor systems.

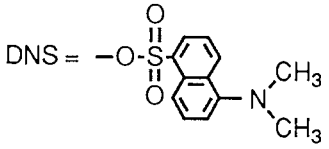
Cyclodextrins (CDs) are macrocyclic oligosaccharides consisting of D-(+)-glucopyranose units. Hexamer, heptamer, and octamer, which are the most common, are often called α-, β-, and γ-CDs, respectively. They can include a variety of organic compounds in their central cavities in aqueous solution. Because CDs are spectroscopically inert, the inclusion phenomena are usually observed by using spectroscopically active guests that exhibit changes in absorption or fluorescence spectra upon complexation with CDs. However, they can be converted into spectroscopically active compounds by modification with chromophores, and spectroscopically inert guests may be detected by spectroscopical changes of the modified CDs. From this point, we have devoted much effort to establish sensory systems which are sensitive to neutral organic compounds in aqueous solution.[1] Recently, we reported dansylglycine-modified β-CD, which decreases its fluorescence intensity upon guest binding and exhibits remarkable molecular recognition ability, as shown by particularly high sensitivities for ursodeoxycholic acid and chenodeoxycholic acid.[2] Dansyl group seems to be a promising chromophore, because its fluorescence is quite sensitive to the polarity of its microenvironment, which is the basic requirement for good sensitivity of our sensory systems, it can be excited by light with the wavelength longer than 350 nm, where many organic compounds have no absorption, its Stokes shift is large, and its fluorescence is free from oxygen quenching, these natures being important from practical point of view. On the other hand, γ-CD is larger than β-CD in molecular size and shows different inclusion behavior in many cases compared with β-CD[3], and it is now desirable to construct dansyl-modified γ-CD systems as novel sensory systems with larger cavities. CDs have three sites which can be modified, that is, C-6 in the primary side and C-2 and C-3 in the secondary side, and it is interesting to know how the geometrical isomers of the modified CDs are different in molecular recognition. We wish to report here the syntheses and molecular recognition abilities of three dansyl-modified-γ-CD derivatives, γ-1, γ-2, and γ-3, which have a dansyl moiety at C-6, C-2, and C-3 positions of γ-CD, respectively, through a rigid sulfonyl linkage.

γ-1 was prepared by the reaction of γ-CD and dansyl chloride in pyridine at room temperature for 2 h, and was purified through HP-20 chromatography, with a yield of 36% based on γ-CD.[4] This is a general

procedure of sulfonation of CDs in the primary side. Sulfonation of CDs in the secondary side is more troublesome than sulfonation in the primary side, because in organic solvents the primary hydroxyls are more reactive than the secondary ones, and the secondary-side sulfonated compounds are easy to be decomposed through epoxide formation under alkaline conditions. However, Takahashi et al. found that the reaction of α-CD with tosyl chloride in alkaline aqueous solution gave 2-O-tosyl-α-CD, although in the case of β-CD only 6-O-tosyl-β-CD was produced under the same conditions.[5] Fujita et al.[6] studied sulfonation of CDs in alkaline aqueous solution with powdered sulfonyl chlorides, and found that sulfonation in the secondary side of CDs can be achieved by the reaction of CDs with sulfonyl chlorides in alkaline solution where pH of the reaction mixture was allowed to decrease as the reaction proceeds. According to this procedure the epoxide formation could be depressed. We studied sulfonation of CDs in alkaline aqueous solution carefully, and found that pouring solution of CD in pH 10 carbonate buffer into violently stirred solution of sulfonyl chloride in DMF, instead of adding powdered sulfonyl chloride into CD alkaline solution, results in the reaction that proceeds so fast in a way to complete before sulfonyl chloride precipitates from the solution. Under our reaction conditions both of C-2 and C-3 mono-dansylated γ-CDs were produced using dansyl chloride as an agent for sulfonation with a distinguished difference in yield (C-2 and C-3, ca. 4 : 1). No primary dansylated product was found. Because the reaction time was very short (the reaction mixture was brought to slightly acidic to stop the reaction in a few seconds after reaction started), epoxidation of γ-2 and γ-3 seems to be unlikely during the reaction. So γ-2 and γ-3 could be obtained with high yields (38% and 10% for γ-2 and γ-3, respectively). γ-2 and γ-3 were separated from each other by HPLC with an ODS-120A column.[7] If dansyl chloride in DMF was poured into CD buffer solution, dansyl chloride precipitated instantaneously, resulting in slow reaction that gave only primary-side sulfonated product. The ^{1}H-NMR and ^{13}C-NMR spectra of γ-2 and γ–3 are too complicated to allow us to determine their structures, because of loss of C_8 symmetry and the circulating current of the naphthalene ring of dansyl moiety attached. Therefore, these compounds were converted to their corresponding epoxides (γ-4 and γ-5, respectively) by treatment with dilute aqueous K_2CO_3 solution for structural determination. The samples of γ-4 and γ-5 were free of aromatic protons in the 500-MHz NMR (D_2O) and showed one-proton singlet signal at 5.24 ppm and one-proton doublet signal at 5.25 ppm (J_{12}=3.42 Hz), respectively, for the anomeric proton of the glucose epoxide residue. This indicated that γ-4 and γ-5 were the manno-epoxide and allo-epoxide, respectively. So their predecessors, γ-2 and γ-3, are undoubtedly dansyl-modified-γ-CDs substituted at C-2 and C-3 positions, respectively.[8]



γ-1: X = OH Y = OH Z = DNS
γ-2: X = DNS Y = OH Z = OH
γ-3: X = OH Y = DNS Z = OH



γ-1, γ-2, and γ-3 showed marked guest-induced variations in the fluorescence intensity, and this property was used to detect various organic compounds. We define the fluorescence intensity as I_0 for host, alone, and I for a mixture of host and guest, then $\Delta I/I_0$, where ΔI is I_0-I, can be used as a factor reflecting the sensitivity of each sensory system to the guests.[2] Figure 1 shows the $\Delta I/I_0$ values obtained with various organic compounds. These three hosts showed remarkably different sensitivities depending on guest.

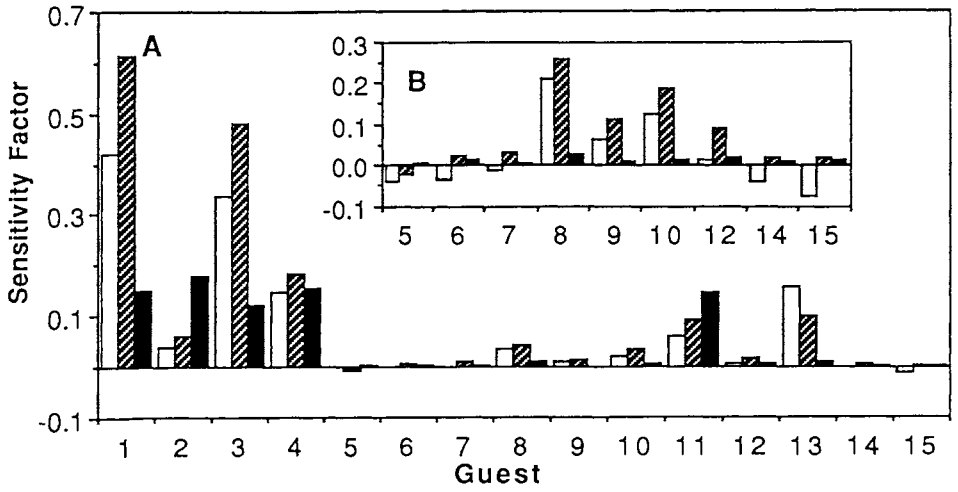


Fig. 1. The sensitivity factor $\Delta I/I_0$ of γ-1, γ-2, and γ-3 for various guests.
□ γ-1, ▨ γ-2, ■ γ-3. [host]=5x10^{-6} M. [guest]=1x10^{-4} M (**A**), [guest]=1x10^{-3} M (**B**). Excitation wavelength: 360 nm for γ-1 and γ-2, 320 nm for γ-3. Fluorescence emission was monitored at 570 nm for γ-1, 560 nm for γ-2, and 520 nm for γ-3. Guest number: **1**, ursodeoxycholic acid; **2**, cholic acid; **3**, chenodeoxycholic acid; **4**, deoxycholic acid; **5**, *l*-menthol; **6**, geraniol; **7**, nerol; **8**, *l*-borneol; **9**, *l*-fenchone; **10**, *d*-camphor; **11**, 1-adamantanecarboxylic acid; **12**, 1-adamantanol; **13**, cyclododecanol; **14**, cyclooctanol; **15**, cyclohexanol.

In most cases, the sensitivities of γ-2 were higher than those of γ-1, and the sensitivities of γ-3 were the lowest (Fig. 1). This distinct difference in molecular recognition behavior between γ-2 and γ-3 suggests that they have different conformations, although they are modified in the same side of γ-CD. This argument may be related with the observation that they exhibit quite different fluorescence spectra with emission maxima at 561 and 517 nm for γ-2 and γ-3, respectively. The emission maximum of γ-1 was observed around 574 nm. Further study is needed to clarify the relationship between the conformations and the emission features. Ursodeoxycholic acid (**1**) and chenodeoxycholic acid (**3**) were detected with remarkably high sensitivities, $\Delta I/I_0$ value reaching 0.61, and 0.48 for γ-2, respectively. The sensitivities for other guests are lower than those for **1** and **3**. It is obvious that the sensitivities are related to molecular sizes of the guests. For example, cyclododecanol (**13**) showed moderately high sensitivity, while sensitivities for cyclooctanol (**14**) and cyclohexanol (**15**) were very low. 1-Adamatanecaboxylic acid (**11**), which is a tricyclic derivative, was detected with higher sensitivity than those for bicyclic derivatives, such as *l*-borneol (**8**), *l*-fenchone (**9**), and *d*-camphor (**10**), and these bicyclic derivatives were detected with higher sensitivities than monocyclic derivative *l*-menthol (**5**). However, molecular structure is another factor which may affect the sensitivities as shown by the much lower sensitivity for 1-adamantanol (**12**) than that for **11**. Furthermore, although deoxycholic acid (**4**) is different from **1** only in the position of one hydroxyl group, it was detected with lower sensitivities than those for **1**, and cholic acid (**2**), which bears one more hydroxyl group than **1**, was detected with very low sensitivities compared with those for **1** when γ-1 and γ-2 were used. Similar trend was observed for 6-dansylglycine-modified-β-CD.[2] But this kind of selectivity for steroidal compounds was not observed for γ-3.

Interestingly, γ-1 exhibits quite different induced-fit behavior from γ-2 and γ-3 for some small molecules, that is **5**, geraniol (**6**), nerol (**7**), cyclooctanol (**14**), and cyclohexanol (**15**), were detected with negative $\Delta I/I_0$ values. This suggests that both dansyl moiety and the guest species are included in the cavity of γ-1. This can be explained in terms of the flexibility of the dansyl moiety of γ-1, arising from the presence of one methylene group between the dansyl moiety and CD framework. So it is easier for γ-1 to form "ternary complex" with small guests. This observation suggests that chromophoric γ-CD systems can exhibit molecular recognition behavior different from that of β-CD systems, because of the larger cavity of γ-CD. All above results suggest that molecular recognition abilities of fluorophore-modified CDs can be modulated by selection of the position of CD hydroxyls to which the fluorophores is attached.

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3) see A. Ueno, F. Moriwaki, T. Osa, F. Hamada, and K. Murai, J. Am. Chem. Soc., **110**, 4323 (1988) and references cited therein.

4) Anal. Found: C, 42.62; H, 6.43; N, 0.86; S, 2.04%. Calcd. for $C_{60}H_{91}NO_{42}S\cdot 9H_2O$: C, 42.58; H, 6.49; N, 0.83; S, 1.89%. ^{1}H-NMR (D_2O): δ 8.66 (1H, d), 8.27 (1H, d), 8.08 (1H, d), 7.70 (2H, m), and 7.44 (1H, d) (aromatic protons of DNS moiety); 2.95 (6H, s, $-N(CH_3)_2$); 4.93-5.21 (8H, m, anomeric protons), 4.38 (1H, m), 3.00-4.17 (majority, m) (protons of CD moiety).

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6) K. Fujita, S. Nagamura, and T. Imoto, Tetrahedron Lett., **25**, 5673 (1984).

7) Anal. of γ-2. Found: C, 44.8; H, 6.21; N, 0.87; S, 2.12%. Calcd. for $C_{60}H_{91}NO_{42}S\cdot 4H_2O$: C, 44.97; H, 6.23; N, 0.87; S, 2.00%. ^{1}H-NMR of γ-2 (D_2O): δ 8.54 (1H, d), 8.22 (1H, d), 8.13 (1H, d), 7.67 (1H,t), 7.60 (1H, t), 7.32 (1H, d) (aromatic protons of dansyl moiety); 2.85 (6H, s, $-N(CH_3)_2$); 5.02-5.22 (8H, m), 4.42 (1H, m), 2.80-4.22 (majority, m) (protons of γ-CD moiety). Anal. of γ-3. Found: C, 42.67; H, 6.42; N, 0.84; S, 1.73%. Calcd. for $C_{60}H_{91}NO_{42}S\cdot 9H_2O$: C, 42.58; H, 6.49; N, 0.83; S, 1.89%. ^{1}H-NMR of γ-3 (D_2O): δ 8.58 (1H, d), 8.41 (1H, d), 8.31 (1H, d), 7.67 (2H, m), and 7.36 (1H, d) (aromatic protons of dansyl moiety); 2.86 (6H, s, $-N(CH_3)_2$); 5.01-5.15 (8H, m, anomeric protons), 3.40-4.12 (majority, m, others) (protons of γ-CD moiety).

8) ^{1}H-NMR of γ-4 (D_2O): δ 5.24 (1H, s, C1'-H), 5.05-5.11 (7H, m, other anomeric protons), 3.76-3.92 (majority, m), 3.51-3.70 (majority, m), 3.44 (1H, d, J_{23}=3.66 Hz, C2'-H). ^{1}H-NMR of γ-5 (D_2O): δ 5.25 (1H, d, J_{12}=3.42 Hz, C1'-H), 4.95-5.04 (7H, m, other anomeric protons), 4.02 (1H, q, J=1.71Hz, 9.52 Hz, C3'-H), 3.44-3.89 (majority, m). This method was first proposed by R. Breslow and A. W. Czarnik to assure the determination of the structure of 2-O-tosyl-β-CD, and K. Fujita et al. used it to determine the structure of 2-O-, and 3-O-tosyl-α-CD. See R. Breslow and A. W. Czarnik, J. Am. Chem. Soc., **105**, 1390 (1983) and Ref.6.

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