The Effects of Avidin on Inclusion Phenomena and Fluorescent Properties of Biotin-Appended Dansyl-Modified β-Cyclodextrin

Taizo Ikunaga, Hiroshi Ikeda, and Akihiko Ueno*[a]

Abstract: Two biotin-appended dansyl-modified cyclodextrins **3** and **4** were prepared to examine the effects of avidin binding on guest-inclusion phenomena and fluorescence properties of cyclodextrin derivatives. The fluorescence intensities of hosts **3** and **4** are more than three times larger in the presence of avidin than those in the absence of avidin. The fluorescence lifetime measurements indicate that the dansyl moiety is not exposed to bulk water in the presence of avidin. The longer lifetime component, which newly appears in the presence of avidin, is likely to be that of the dansyl moiety located in the hydrophobic region of avidin. The addition of bile acids such as hyodeoxycholic acid resulted in decreases in the fluorescence intensities of **3** and **4** in accordance to the extrusion of the dansyl moiety from the hydrophobic cyclodextrin cavity to bulk water. Similar guest-induced decreases in the fluorescence intensity were observed for **3** in the presence of avidin, but under the same conditions the decreases are smaller for **4**. On the other hand, the binding constants of **3** and **4** became larger in the presence of avidin. All these results demonstrate that avidin perturbs the inclusion phenomena and fluorescence properties of **3** and **4** by providing a hydrophobic environment around the host cavity.

Keywords: avidin · cyclodextrins · host–guest chemistry · supramolecular chemistry

Introduction

Signal transduction of molecule- or metal-binding to spectroscopic changes has recently emerged as an important application of supramolecular chemistry.[1] Many examples of such systems for metal-ion binding have been given with crown ethers,[2] calixarenes,[3] molecular clefts,[4] and chelators,[5] but the examples for small molecules are rather few.[6] We have shown that chromophore-modified CDs (CD: cyclodextrin) can be used for detecting molecules (sensors).[7] With these CDs, the binding of small molecules is transduced to spectroscopic signals, such as a change in fluorescence, absorption, and circular dichroism intensities. The important feature of these systems is that the CD derivatives undergo an induced-fit conformational change associated with guest accommodation, excluding the chromophore from inside to outside the CD cavity. Since the inside of the CD cavity is hydrophobic, while the outside of the cavity is polar bulk water, the induced-fit conformational change results in the remarkable environmental change around the chromophore.

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The other interesting approach of these systems is the new ability to control the environment outside the cavity to change the guest-binding ability and guest selectivity of the system. This has been done, for the first time, with a monensin-dansyl-CD triad system, in which the hydrophobic monensin cap and metal-ion induced promoted guest binding.[8] Proteins can also act as an external environment around the cavity as shown with avidin binding to biotin-appended *N*,*N*-dimethylaminobenzoyl-modified CD.[9] We report here the results of extensive binding studies on avidin with biotin-dansyl-modified CDs.

Results and Discussion

Synthesis: The compounds used in this study were synthesized as shown in Scheme 1. Biotin-modified β-CD **2** was prepared by condensation of **1** and biotin with dicyclohexylcarbodiimide (DCC) in DMF. The reaction of **2** with dansyl chloride in DMF gave biotin-appended dansyl-modified β-CD **3**. On the other hand, treatment of **2** with dansylglycine and benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP) gave biotin-appended dansyl-modified β-CD **4**. We also prepared the reference compound **6**, in which the biotinyl group is replaced by an acetyl group. Reference compound **8** with no CD unit was also prepared.

Absorption spectra: Figure 1 shows absorption spectra of **3**, avidin, tryptophan, and a mixture of avidin and **3**. Here, avidin

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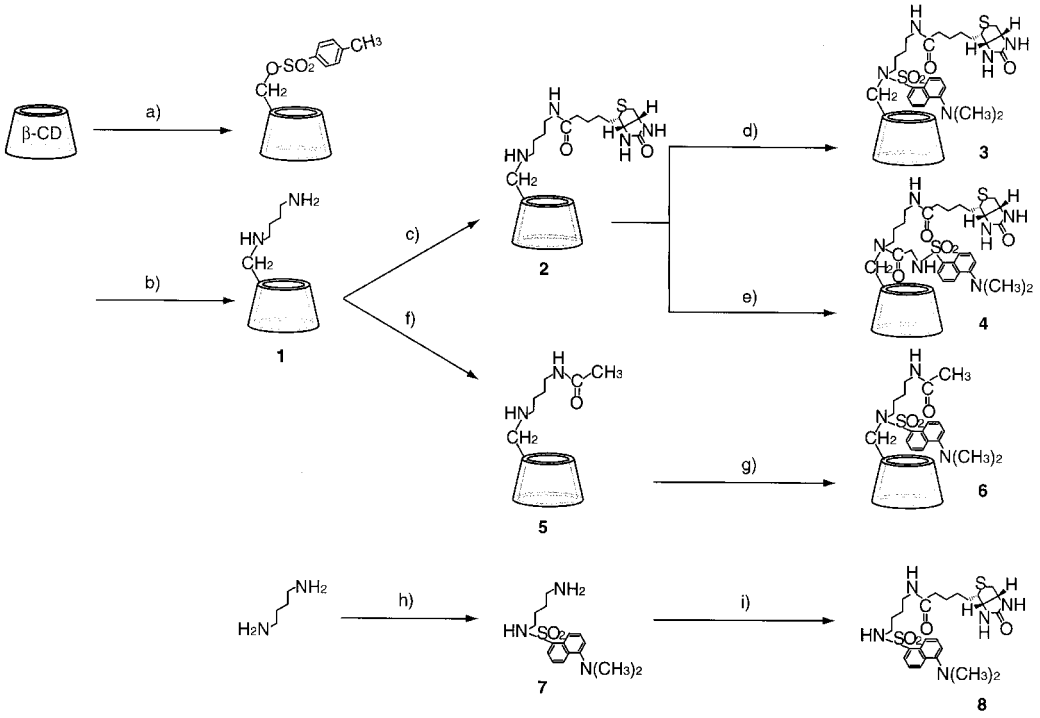


Scheme 1. a) TsCl, pyridine, room temperature, 3 h; b) 1,4-diaminobutane, DMF, 65 °C, 1.5 h; c) biotin, DCC, HOBt, DMF, 0 °C, 2 h then RT, 20 h; d) dansyl chloride, DIEA, DMF, RT, 4 h; e) dansylglycine, BOP, HOBt, DIEA, DMF, RT, 2 h; f) acetic anhydride, DIEA, DMF, 0 °C, 30 min; g) dansyl chloride, DIEA, DMF, RT, 2 h; h) dansyl chloride, $CHCl_3$, RT, 2 h; i) biotin, DCC, HOBt DMF, 0 °C, 2 h then RT, 20 h; TsCl = *p*-toluenesulfonyl chloride, DCC = dicyclohexylcarbodiimide, HOBt = 1-hydroxybenzotriazole, BOP = benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate, DIEA = diisopropylethylamine, dansyl = 5-dimethylamino-1-naphthalenesulfonyl.

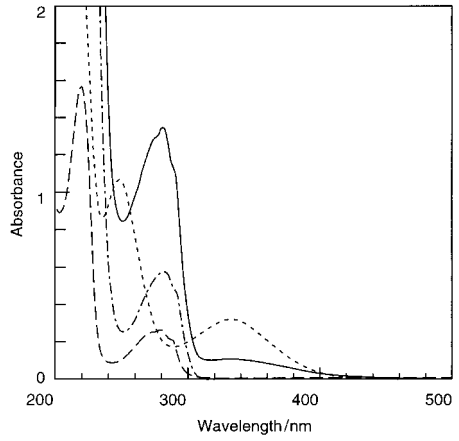


Figure 1. Absorption spectra of avidin (10 μM, ••••), **3** alone (100 μM, - - - -) and in the presence of avidin (25 μM, ——), and tryptophan (100 μM, – – –).

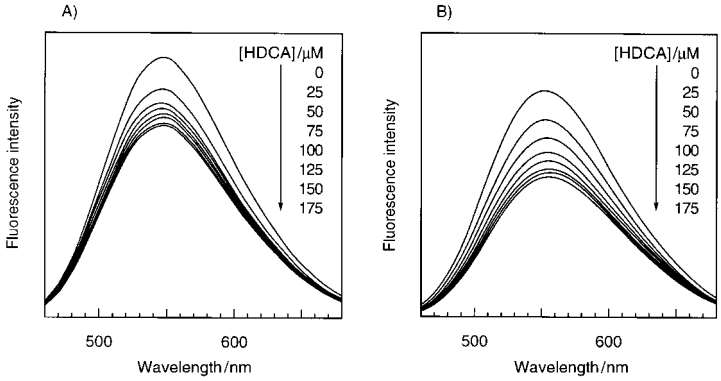


Figure 2. Variations in fluorescence spectra of A) **3** and B) **4** upon addition of hyodeoxycholic acid (HDCA).

is composed of four subunits of the same kind. The absorption peak around 280 nm of avidin is similar to that of tryptophan, suggesting that the peak of avidin is mainly a result of tryptophan residues in the avidin. Compound **3** exhibits a broad peak around 330 nm for the dansyl chromophore.

Fluorescence spectra: Figure 2A and 2B show fluorescence spectra of **3** and **4** (25 μM), respectively, measured by excitation at 360 nm. Both spectra have a peak around 550 nm, and the intensities decrease with increasing concentrations of hyodeoxycholic acid (HDCA) as a guest. Since the fluorescence of the dansyl unit is much stronger in a hydrophobic environment than in water, the results indicate that the dansyl moiety of **3** and **4** is excluded from the hydrophobic CD cavity to bulk water solution.

The change in fluorenscence intensity ΔI, where $\Delta I = I_0 - I$, and I and I_0 are the fluorescence intensities in the presence and absence of guest species, respectively, increases with increasing concentration of HDCA. Figure 3A and 3B show the $\Delta I/I_0$ values of **3** and **4** as a function of concentration of various bile acids (Scheme 2). The dependencies for **3** and **4** display a similar trend, showing remarkable concentration dependencies for HDCA and UDCA, negligible or small concentration dependencies for DCA and cholic acid CA, and moderate concentration dependency for CDCA. These data imply that the order of the sensitivity for the guests of **3** and **4**

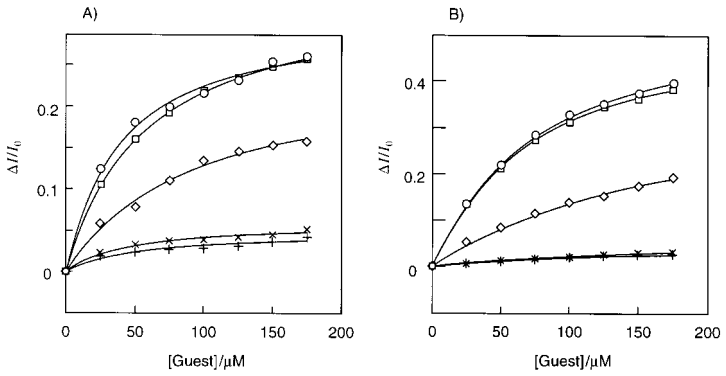
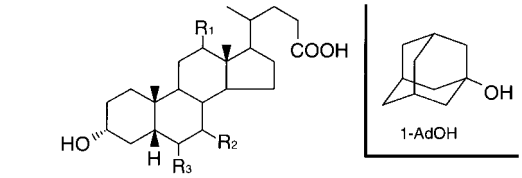


Figure 3. The $\Delta I/I_0$ values of A) **3** and B) **4** as a function of various guests; ○: HDCA, □: UDCA, ◇: CDCA, x: DCA, +: CA (see Scheme 2).



	HDCA	UDCA	CDCA	DCA	CA
R_1	–H	–H	–H	···OH	···OH
R_2	–H	◀OH	···OH	–H	···OH
R_3	···OH	–H	–H	–H	–H

HDCA : Hyodeoxycholic acid
UDCA : Ursodeoxycholic acid
CDCA : Chenodeoxycholic acid
DCA : Deoxycholic acid
CA : Cholic acid
1-AdOH : 1-Adamantanol

Scheme 2. Various bile acids.

is CA,DCA ≪ CDCA ≪ UDCA,HDCA. Noteworthy, HDCA, UDCA, CDCA, and DCA are isomeric steroids with only one hydroxy group located at different positions or stereochemically inverted with respect to the same carbon.

Figure 4 shows avidin-induced enhancement of dansyl fluorescence of **3** and **4**. The fluorescence intensities of **3** and **4** are enhanced 3.6 and 2.8 times, respectively, by the

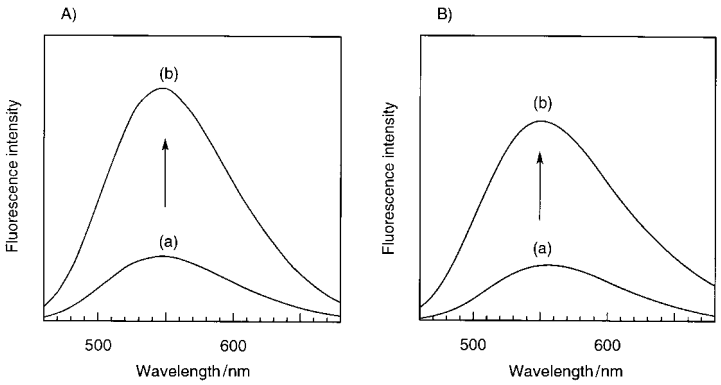


Figure 4. Fluorescence spectra of **3** (A, 10 μM) and **4** (B, 10 μM) in the absence (a), and presence of avidin (b) (10 μM).

presence of an equimolar amount of avidin (10 μM), suggesting that avidin provides a hydrophobic environment around the dansyl moieties of **3** and **4**. Figure 5 shows the plausible structure of **3**:avidin complex. This structure contains two

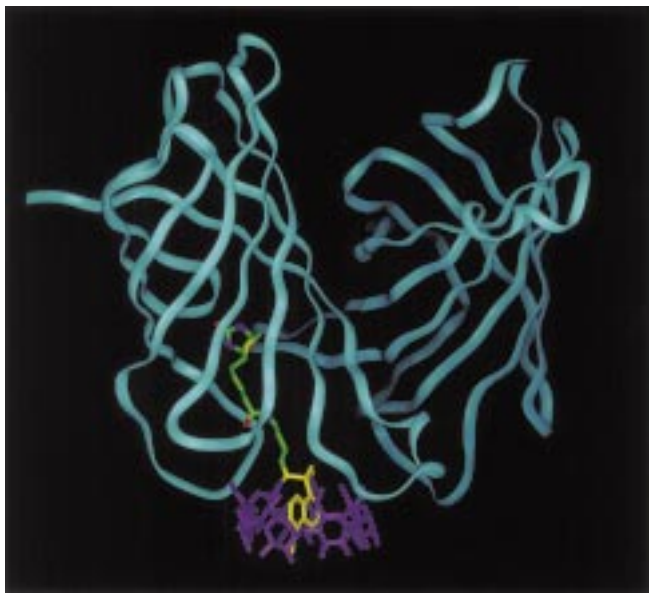


Figure 5. The plausible structure of **3**-(avidin subunit) complex. The figure contains two avidin subunits; **3** is bound to one of them. The structure of avidin and biotinyl unit is taken from the reported crystal structure.[10]

subunits of avidin and one molecule of **3**. The interaction of the protein and biotin part of **3** is based on the X-ray crystallographic data; the CD unit is located around the border of the protein. Although avidin provides a hydrophobic environment, the amino acids positioned near the rim of the CD are serine, glutamic acid, and valine, and not those with an aromatic side chain such as phenylalanine or tryptophan.

Figure 6 shows the fluorescence intensity as a function of the concentration of dansyl-incorporated compounds. The

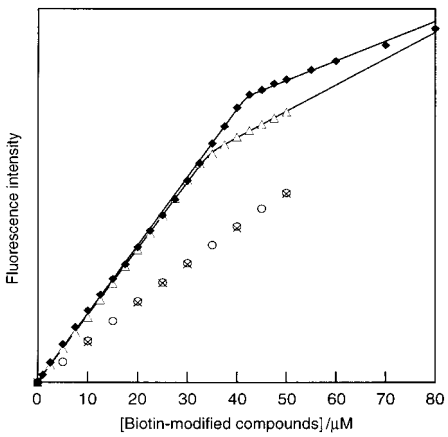


Figure 6. The fluorescence intensities of the solutions with avidin (10 μM) (◆, ○), with a mixture of avidin (10 μM) and biotin (10 μM) (△), and with neither avidin nor biotin (x) as a function of various dansyl derivatives added. ◆: **3**; x: **6**; △: **3**; ○: **6**.

fluorescence intensity of **3** increases linearly with increasing concentration of **3** until a concentration of 40 μM is reached in the solution containing 10 μM avidin, then the slope becomes gentler at higher concentration.

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This result is consistent with the fact that avidin is composed of four subunits with one binding pocket for each biotin. When the solution contains 10 μM avidin and 10 μM biotin, the change in slope occurs around 30 μM of **3**; this is also consistent with four biotin units being included in four binding pockets of avidin. Compound **6**, the dansyl derivative without a biotin unit, increases the fluorescence intensity linearly with increasing concentration of these compounds both in the absence and presence of avidin, and no slope change was observed around 40 μM, demonstrating that the biotin unit is a prerequisite for the slope change. Notably, the fluorescence intensities of **3** are larger than those of other dansyl derivatives over the entire range of concentrations; this reflects the effect of the hydrophobic environment of avidin on the dansyl fluorescence.

Guest-induced variations in fluorescence spectra: Figure 7 shows the changes in the fluorescence spectra of **3** and **4** (10 μM) induced by HDCA in the presence of avidin (10 μM).

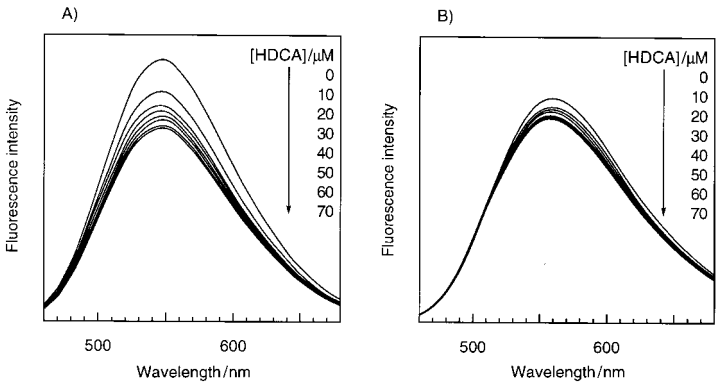

Figure 7. Fluorescence spectra of A) **3** and B) **4** in the presence of avidin (10 μM) at different concentrations of HDCA.

The fluorescence intensity around 550 nm decreases with increasing concentration of HDCA. This result suggests that the dansyl moiety is excluded from the hydrophobic CD cavity to make way for the accommodation of HDCA in the CD cavity. Notably, the extent of the variation of guest-induced fluorescence for **4** is much smaller than that without avidin (Figure 2). This observation might be related to the fact that in the presence of avidin the dansyl moiety excluded from the cavity is not totally exposed to the bulk water solution.

Figure 8 shows the $\Delta I/I_0$ values of **3** and **4** in the presence of avidin as a function of guest concentration. The order of the $\Delta I/I_0$ for **3** is CA < DCA < CDCA < UDCA < HDCA and that for **4** is similar except for CA. When we compare these data with those of Figure 3, we found that in the presence of avidin a better separation of the binding curves is obtained for HDCA and UDCA. An enhanced separation of the curves in the presence of avidin was also observed for DCA and CA. The results demonstrate that the avidin environment promotes the separation of the binding curves of **3** and **4**, although the detailed mechanism for this is not clear.

Binding constants: Table 1 shows binding constants (K) of **3** and **4** for bile acids and 1-adamantanol (1-AdOH) in the

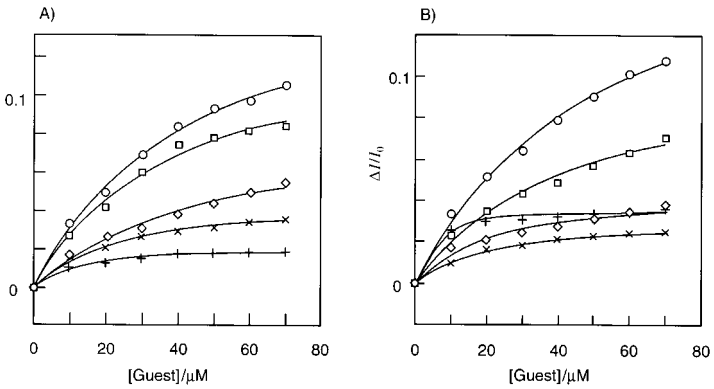

Figure 8. The $\Delta I/I_0$ values of A) **3** (10 μM) and B) **4** (10 μM) in the presence of avidin (10 μM) as a function of the concentration of various guests; ○: HDCA, □: UDCA, ◇: CDCA, x: DCA, +: CA.

Table 1. Binding constants K and $\Delta I_{max}/I_0$ values of **3** and **4**.[a]

Guest	Avidin [μM]	**3**		**4**	
		K [M^{-1}]	$\Delta I_{max}/I_0$ [%]	K [M^{-1}]	$\Delta I_{max}/I_0$ [%]
HDCA	0	43300	29.1	16700	54.6
UDCA	0	27400	31.6	18700	51.1
CDCA	0	15200	22.8	6980	35.5
DCA	0	[b]	[b]	[b]	[b]
CA	0	[b]	[b]	[b]	[b]
1-AdOH	0	30700	19.2	9730	39.2
HDCA	10	28900	16.3	22200	18.3
UDCA	10	35900	12.5	27600	10.7
CDCA	10	21800	9.1	17600	4.5
DCA	10	[b]	[b]	[b]	[b]
CA	10	[b]	[b]	[b]	[b]
1-AdOH	10	69700	10.9	63700	4.2

[a] Measured at 25 °C in phosphate buffer (pH 7.0). The concentrations of **3** and **4** were 10 μM. Errors of the curve-fitting analyses were within 10%. [b] Values were not determined.

absence and presence of avidin together with the values of $\Delta I_{max}/I_0$, which were estimated for the complexes from the curve-fitting analysis used to obtain the binding constants.

The binding constant of **3** for HDCA is 43300 M^{-1} in the absence of avidin which is much larger than the value of 28900 M^{-1} obtained in the presence of avidin. On the other hand, the binding constant of **4** for HDCA is 16700 and 22200 M^{-1} in the absence and presence of avidin, respectively. The binding constants of **3** for bile acids and 1-adamantanol are always larger than those of **4**. However, the changes of the binding constants induced by avidin are more complicated. The other important result is the avidin-induced decrease in the $\Delta I_{max}/I_0$ value as shown by the decrease of **3** from 29.1% to 16.3%, and that of **4** from 54.6% to 18.3% for HDCA. The results may be related with the hydrophobic nature of the avidin part located near the CD ring. This implies that the dansyl moiety excluded from the CD cavity by guest accommodation is not totally exposed to bulk water but exposed to or included in the hydrophobic environment of avidin. Most serious effects of this kind are seen for CDCA and 1-adamantanol, when **4** is the host. These guests exhibit decreases in the value of $\Delta I_{max}/I_0$ from 35.5% and 39.2% to 4.5% and 4.2%, respectively. In spite of these features, the binding becomes stronger in the presence of avidin with the exception of the case for HDCA, as shown by about six times

Table 2. Fluorescence lifetimes and the ratios of compounds.[a]

		3		4		6	
HDCA [μM]	Avidin [μM]	τ_1 (A_1) [ns]	τ_2 (A_2) [ns]	τ_1 (A_1) [ns]	τ_2 (A_2) [ns]	τ_1 (A_1) [ns]	τ_2 (A_2) [ns]
0	0	7.50	14.5	7.10	14.5	5.00	10.2
		(0.613)	(0.387)	(0.695)	(0.305)	(0.400)	(0.600)
150	0	7.90	13.8	5.20	14.8	5.20	10.7
		(0.789)	(0.211)	(0.839)	(0.161)	(0.635)	(0.365)
0	10	12.6	22.1	12.3	22.8	4.90	12.5
		(0.927)	(0.073)	(0.893)	(0.107)	(0.308)	(0.692)
60	10	12.4	20.0	9.80	18.9	5.40	11.8
		(0.638)	(0.362)	(0.638)	(0.362)	(0.640)	(0.360)

[a] The concentrations of **3**, **4**, and, **6** were 10 μM. Compound **8** exhibits a single lifetime of 3.50 ns.

larger binding constant for 1-adamantanol. These results suggest that avidin acts as a hydrophobic cap that strengthens the guest binding.

Lifetimes: Table 2 shows the result of lifetime measurements. Both **3** and **4** exhibit two lifetimes, about 7 ns for the shorter one and about 14.5 ns for the longer lifetime. Upon addition of HDCA, the component ratio of the longer lifetime decreases from 38.7% to 21.1% for **3**, and from 30.5% to 16.1% for **4**. This result is consistent with the exclusion of the dansyl moiety from the hydrophobic CD cavity to bulk water solution upon guest accommodation.

However, the shorter lifetime of about 7 ns disappears in the presence of avidin. Hosts **3** and **4** have values of 12–13 ns for the shorter and 22–23 ns for the longer lifetime component. The disappearance of the component at approximately 7 ns suggests that avidin forces the dansyl moiety into the CD cavity, in such a way that it probably acts as a large heavy stone on the cavity, and consequently results in the enforced accommodation of the dansyl unit in the hydrophobic CD cavity. The newly observed lifetime of about 22 ns indicates that the dansyl moiety is located in a very hydrophobic environment, even if it is excluded from the CD cavity. Upon addition of HDCA, **3** and **4** have values of 9.8–12.4 ns for the shorter and 18.9–20.0 ns for the longer lifetime component. On the other hand, the ratio of longer lifetime component increases from 7.3% to 36.2% for **3** and from 10.7% to 36.2% for **4**, reflecting the guest-induced locational change from the inside of the CD cavity to the outside of the CD cavity. From this viewpoint, the longer lifetime components should be from the species in which the dansyl moiety is excluded from the CD cavity, this trend being opposite to the case without avidin. These features are summarized in Figure 9. It is interesting to compare these results with those of **6**, which has no biotin unit and therefore cannot bind avidin. Compound **6** shows two lifetimes of 5 ns for the shorter and 10.2 ns for the longer lifetime component; these values are shorter than those of **3** and **4**. The ratio of the longer lifetime component decreases from 60.0% to 36.5% upon addition of HDCA (150 μM); this reflects that the dansyl moiety is excluded from the hydrophobic CD cavity to the polar bulk water solution. The case is essentially the same in the presence of avidin. All these results indicate that avidin acts as a hydrophobic environment for **3** and **4**.

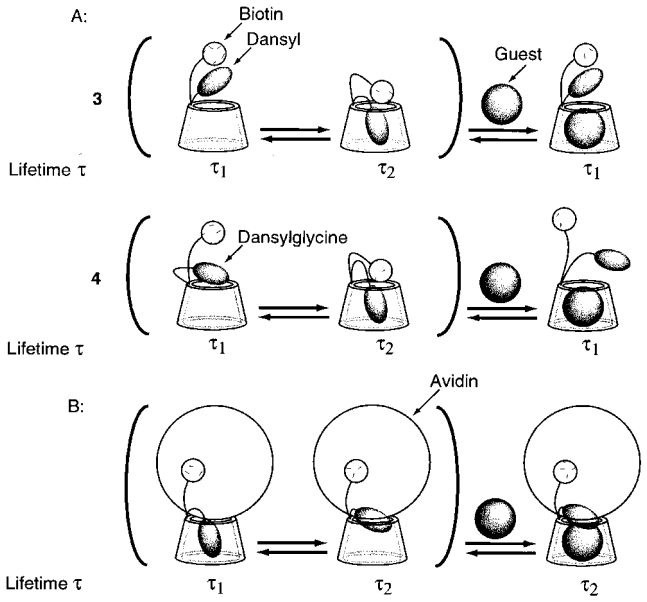

Figure 9. Schematic representation for A) the dansyl-modified CDs alone and B) in the presence of avidin.

Conclusion

In conclusion, avidin changes the fluorescence properties of dansyl-modified CDs **3** and **4**. The fluorescence intensities are enhanced more than three times by avidin. The avidin forces the dansyl moiety of **3** and **4** into the CD cavity, so that the dansyl moiety is then excluded into the hydrophobic environment of avidin rather than into the bulk water associated with guest binding. The guest binding becomes stronger as a result of the promoted stability of the formed complexes. These results demonstrate that avidin acts as an effective hydrophobic cap in the ternary complex of [avidin]–[CD host]–[guest].

Experimental Section

Thin-layer chromatography (TLC) was carried out on silica gel 60 F_{254} (Merck) coated plates. Preparative layer chromatography (PLC) was performed on silica gel 60 F_{254} (layer thickness 2 mm, Merck) PLC plates. Absorption and fluorescence spectra were acquired with a Shimadzu UV-3100 spectrometer and a Hitachi 850 fluorescence spectrometer, respectively. Mass spectra were recorded with Shimadzu MALDI III (TOF-MS). Fluorescence decay was measured by a time-correlated single-photon

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counting method on a Horiba NAES-550 system. A self-oscillating flash lamp filled with H_2 was used as a light source. The excitation beam was passed through filters U340 (Hoya), and emission beam through the filter of Y44 (Hoya) to isolate the light with wavelengths longer than 440 nm. ^{1}H NMR spectra were recorded on a Varian VXR-500S FT-NMR spectrometer. HDO ($\delta = 4.70$) and [D_6]dimethyl sulfoxide ([D_6]DMSO: $\delta = 2.50$) were used as internal standards, and [D_4]3-(trimethylsilyl)propionic acid sodium salt (TSP: $\delta = 0$) and tetramethylsilane (TMS: $\delta = 0$) as external standards. Elemental analyses were performed by the Analytical Division in Research Laboratory of Resources Utilization of Tokyo Institute of Technology. Analyses and collection of samples with HPLC were performed on a Hitachi Intelligent Pump 7100, Hitachi D-7500 Chromato-Integrator, and Hitachi L-7400 UV-Vis Detector. Phosphate buffer (pH 7.0) with an ionic strength of 100 mM was used for fluorescence measurements.

β-Cyclodextrin was a kind gift from Nihon Shokuhin Kako Ltd. Avidin was purchased from Sigma. All other reagents including bile acids and other guest compounds were purchased from Tokyo Kasei except for 1-adamantanol (Merck). Deuterium oxide, with an isotopic purity of 99.95%, and [D_6]DMSO with an isotopic purity of 99.95%, were purchased from Merck.

6-Deoxy-6-(4-aminobutylamino)-β-cyclodextrin (1): A solution of 6-*O*-tosyl-β-CD (5 g, 3.88 mmol) in DMF (30 mL) was added to a mixture of 1,4-diaminobutane (40 g) and DMF (5 mL) and the resulting mixture was stirred at 65 °C for 1.5 h under argon. The reaction mixture was concentrated under reduced pressure and then poured into acetone (2 L). The precipitates formed were collected on a glass filter and dried under reduced pressure. The crude product was dissolved in water and purified by column chromatography on CM Sephadex C-25. After removal of *p*-toluenesulfonic acid and other impurities by elution with water (5 L), the fractions containing the product were obtained with a gradient elution of ammonia solution from 0 to 1M. The fractions containing the product were concentrated under reduced pressure. Lyophilization of the product gave a white powder (4.18 g, yield 88%). TLC: $R_f = 0.04$ (28% NH_3, ethyl acetate, 2-propanol, water 1:3:5:4); ^{1}H NMR (500 MHz, [D_6]DMSO): $\delta = 1.47$–1.58 (4H, m, CH_2), 2.55 (2H, t, CH_2NH_2), 2.73 (1H, dd, H-6a (CD)), 2.83 (2H, t, C6-NHCH_2), 3.00 (1H, dd, H-6b (CD)), 3.37 (1H, t, H-4 (CD)), 3.35–3.91 (39H, m, CD), 4.99–5.02 (7H, m, H-1 (CD)); anal. calcd for $C_{46}H_{80}N_2O_{34} \cdot 2H_2O$: C 44.52, H 6.82, N 2.29; found: C 44.58, H 6.84, N 2.21.

6-Deoxy-6-(4-*N*-biotinylaminobutylamino)-β-CD (2): A mixture of **1** (600 mg, 498 μmol), biotin (122 mg, 498 μmol), DCC (123 mg, 548 μmol), HOBt (74 mg, 548 μmol) in DMF (6 mL) was stirred for 2 h at 0 °C, and then overnight at room temperature. After filtration, the filtrate was added dropwise to acetone (600 mL). The precipitates formed were collected on a glass filter and dried under reduced pressure. The crude product was dissolved in water, and purified by column chromatography on CM Sephadex C-25. The column chromatography was performed with a gradient elution of ammonia solution from 0 to 1M, and the fractions containing the desired product were concentrated under reduced pressure and then lyophilized to give a white powder (602 mg, yield 85%). TLC: $R_f = 0.28$ (28% NH_3, ethyl acetate, 2-propanol, water 1:3:5:4); ^{1}H NMR (500 MHz, [D_6]DMSO): $\delta = 1.38$–1.81 (10H, m, CH_2), 2.18 (2H, t, $COCH_2$), 2.69 (1H, dd, SCH_2), 2.95 (1H, dd, SCH_2), 3.20 (1H, m, SCH), 2.80–3.20 (6H, m, CH_2NHCO, H-6 (CD), C6-NHCH_2), 3.30–3.95 (40H, m, CD), 4.23 (1H, m, CHNH), 4.41 (1H, m, CHNH), 4.90–5.00 (7H, m, H-1 (CD)); anal. calcd for $C_{56}H_{94}N_4O_{36}S_1 \cdot 8H_2O$: C 42.69, H 7.04, N 3.56, S 2.03; found: C 42.61, H 7.06, N 3.56, S 2.00.

6-Deoxy-6-(4-*N*-biotinylamino-1-*N*-dansylbutylamino)-β-CD (3): A mixture of **2** (100 mg, 70 μmol), diisopropylethylamine (DIEA) (122 mL, 699 μmol), and dansyl chloride (75 mg, 279 μmol) in DMF was stirred at room temperature for 4 h. The solution was poured into acetone (200 mL). The precipitates formed were collected on a glass filter and dried under reduced pressure. The crude product was dissolved in water and purified by column chromatography on CM Sephadex C-25 and QAE Sephadex C-25. The fractions containing the desired product were concentrated under reduced pressure and lyophilized. The crude product was dissolved in water and purified by HPLC and the fractions containing the desired compound were collected and again subjected to QAE Sephadex C-25. The eluted solution (1 L) was collected and concentrated under reduced pressure. The lyophilization of the residue gave a white powder (64 mg, yield 55%). TLC: $R_f = 0.36$ (*n*-butanol, ethanol, water 5:4:3); $R_f = 0.44$ (28% NH_3, ethyl acetate, 2-propanol, water 1:3:5:4); ^{1}H NMR (500 MHz, [D_6]DMSO): $\delta =$ 2.95 (6H, s, $N(CH_3)_2$), 7.39 (1H, d, DNS), 7.74 (2H, m, DNS), 8.16 (1H, d, DNS), 8.27 (1H, d, DNS), 8.60 (1H, d, DNS); anal. calcd for $C_{68}H_{105}N_5O_{38}S_2 \cdot 8H_2O$: C 45.15, H 6.74, N 3.87, S 3.54; found: C 45.12, H 6.66, N 3.90, S 3.85.

6-Deoxy-6-[4-*N*-biotinylamino-1-*N*-(*N*-dansylglycinyl)butylamino]-β-CD (4): A mixture of **2** (150 mg, 105 μmol), dansylglycine (49 mg, 157 μmol), BOP (70 mg, 157 μmol), HOBt (24 mg, 157 μmol), diisopropylethylamine (27 mL, 157 mmol) in DMF was stirred at room temperature for 2 h. The solution was poured into acetone (400 mL) and the precipitates formed are collected on a glass filter. The crude product was dissolved in water, and then purified by column chromatography on CM Sephadex C-25 and then on QAE Sephadex C-25. The eluted solution was concentrated under reduced pressure and subsequently was treated with 1N NH_3 solution (50 mL) for 3 h to hydrolyze the ester linkages formed by the side reaction. The solution was concentrated under reduced pressure and subjected to QAE Sephadex C-25, and desired product was eluted with water (1 L). The solution was concentrated under reduced pressure and then lyophilized to give a white powder (25 mg, yield 14%). TLC: $R_f = 0.44$ (28% NH_3, ethyl acetate, 2-propanol, water 1:3:5:4); ^{1}H NMR (500 MHz, [D_6]DMSO): $\delta =$ 2.84 (6H, s, $N(CH_3)_2$), 7.26 (1H, d, DNS), 7.62 (2H, m, DNS), 8.15 (1H, d, DNS), 8.32 (1H, d, DNS), 8.47 (1H, d, DNS); anal. calcd for $C_{70}H_{108}N_6O_{39}S_2 \cdot 8H_2O$: C 43.56, H 6.48, N 4.35 S 3.32; found: C 43.52, H 6.50, N 4.36, S 3.55.

6-Deoxy-6-(4-*N*-acetylamino-1-*N*-dansylbutylamino)-β-CD (6): A mixture of **1** (200 mg, 160 μmol) and diisopropylethylamine (224 mL, 1.33 mmol) in DMF (3 mL) was stirred at 0 °C. Acetic anhydride (15.7 μL, 166 μmol) in DMF (2 mL) was added to this solution. After 30 min, the solution was poured into acetone (400 mL), and the precipitates formed were collected on a glass filter. The precipitates were dissolved in water, and purified by column chromatography on CM Sephadex C-25 by elution with water (1 L) and by a gradient elution of ammonia solution from 0 to 1M. The eluted solution was concentrated under reduced pressure and then lyophilized to give a white powder of 6-deoxy-6-(4-*N*-acetylaminobutylamino)-β-CD (**5**, 173 mg, yield 84%).

A mixture of **5** (100 mg, 80 μmol), diisopropylethylamine (54 mL, 321 μmol), and dansyl chloride (22 mg, 80 μmol) in DMF (2 mL) was stirred at room temperature for 2 h. The reaction mixture was added dropwise to acetone (22 mL) and the precipitates formed were collected on a glass filter. The precipitates were dissolved in water and purified by column chromatography on CM Sephadex C-25 and QAE Sephadex C-25. The product was eluted with water (1 L), and the eluted solution was concentrated under the reduced pressure and lyophilized to give a white powder (53 mg, yield 45%). TLC: $R_f = 0.42$ (*n*-butanol, ethanol, water 5:4:3); ^{1}H NMR (500 MHz, [D_6]DMSO): $\delta = 1.82$ (3H, s, $COCH_3$), 2.91 (6H, s, $N(CH_3)_2$), 7.38 (1H, d, DNS), 7.74 (2H, m, DNS), 8.15 (1H, d, DNS), 8.27 (1H, d, DNS), 8.60 (1H, d, DNS); anal. calcd for $C_{60}H_{93}N_3O_{37}S_1 \cdot 15H_2O$: C 41.16, H 7.08, N 2.40, S 1.83; found: C 41.23, H 7.05, N 2.41, S 1.85.

***N*-Dansylbutylamine (7)**: A mixture of 1,4-butylenediamine (1.31 g, 14.8 mmol) and dansyl chloride (200 mg, 741 μmol) in chloroform (5 mL) was stirred for 2 h. The solution was concentrated under reduced pressure and subjected to silica gel column chromatography with chloroform (500 mL), and then with a 50:50 methanol/chloroform solution. The fractions containing the product were filtered and the filtrate was concentrated under reduced pressure to give a yellow powder (267 mg, yield 70%). TLC: $R_f = 0.49$ (*n*-butanol, ethanol, water 5:4:3). ^{1}H NMR (500 MHz, [D_6]DMSO): $\delta = 1.51$–1.58 (4H, m, CH_2), 2.67 (2H, m, CH_2NH_2), 2.89 (2H, m, SO_2NHCH_2), 2.94 (6H, s, $N(CH_3)_2$), 7.37 (1H, d, DNS), 7.69–7.74 (2H, m, DNS), 8.21 (1H, d, DNS), 8.42 (1H, d, DNS), 8.57 (1H, d, DNS); anal. calcd for $C_{16}H_{23}N_3O_2S_1$: C 59.79, H 7.21, N 13.07, S 9.97; found: C 59.80, H 7.18, N 13.08, S 9.98.

4-*N*-Biotinyl-1-*N*-dansylbutylamine (8): A mixture of *N*-dansylbutylamine (70 mg, 218 μmol), biotin (59 mg, 240 μmol), DCC (49 mg, 240 μmol), and HOBt (32 mg, 240 μmol) in DMF (91 mL) was stirred at 0 °C for 2 h, and then at room temperature overnight. The reaction mixture was dried under reduced pressure and dissolved in a small amount of methanol. The solution was developed on PLC with a 2:8 methanol/chloroform solution. The PLC spots that were identified to contain the desired product by MS were scratched off and washed with methanol. The solution of the compound in methanol was concentrated under reduced pressure and added dropwise to chloroform (100 mL). After the insoluble solid was

removed by filtration, the solution was concentrated and subjected to silica gel chromatography. After the elution with 2:8 methanol/chloroform, the eluted solution was concentrated under reduced pressure to give a yellow powder (12 mg, yield 10%). TLC: $R_f = 0.57$ (chloroform, methanol 4:1); ^{1}H NMR (500 MHz, [D$_6$]DMSO): $\delta = 1.32 - 1.80$ (10H, m, CH_2), 2.11 (2H, t, $NHCOCH_2$), 2.69 (1H, dd, SCH_2), 2.87 – 3.30 (6H, m, $NHCH_2$, $CONHCH_2$, SCH_2, SCH), 2.95 (6H, s, $N(CH_3)_2$), 4.24 (1H, m, NHCH), 4.44 (1H, m, NHCH), 7.37 (1H, d, DNS), 7.69 – 7.75 (2H, m, DNS), 8.20 (1H, d, DNS), 8.41 (1H, d, DNS), 8.57 (1H, d, DNS); anal. calcd for $C_{26}H_{37}N_5O_4S_2 \cdot 3H_2O$: C 51.89, H 7.20, N 11.64, S 10.66; found: C 51.95, H 7.25, N 11.53, S 10.44.

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