Molecular recognition of fluorescent dyes with novel triethylenetetraamine-tethered bis(β-cyclodextrin) and its copper(II) complex: enhanced binding and selectivity by tether ligation

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Abstract

In order to explore the possibility of external modification of the binding ability through metal ligation, the inclusion complexation behavior of newly synthesized bis(β-cyclodextrin) (**4**) with an oligo(ethylenediamine) tether has been investigated with representative fluorescent dyes, 6-(*p*-toluidino)-2-naphthalenesulfonate (TNS) and 8-anilino-1-naphthalenesulfonate (ANS), and found to give enhanced binding and selectivity for the Cu(II)-ligated host (**5**). © 2000 Elsevier Science Ltd. All rights reserved.

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It is well-documented that tethering two cyclodextrin moieties with a short linker group greatly enhances the original molecular binding ability of the parent cyclodextrin through the cooperative binding of one guest molecule in the closely located two cyclodextrin cavities.[1] Hence, a number of dimeric cyclodextrins with structural diversities have been designed, synthesized, and applied to various fields of science and technology, being employed as molecular receptors[2,3] and enzyme mimics,[4] and have received much attention in the past decade. In such studies, diverse bifunctional groups, e.g. alkanedioates, disulfides, dipyridinio and imidazole, have been used as the linker (or spacer) tethering the two cyclodextrins. Somewhat unexpectedly, cyclodextrin dimers tethered with oligo(ethylenediamine) units have rarely been synthesized and therefore their molecular recognition behavior has not been extensively investigated except for the study of Tabushi et al.[5] However, there is an inherent advantage for the oligo(ethylenediamine) tether incorporated in a bis(cyclodextrin), since the tether group can ligate to transition metal ions, thus enabling us to modify, and potentially switch, the original binding ability through the metal ligation.

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In this communication, we wish to report the preliminary results of exploratory work to control the molecular recognition behavior of bis(cyclodextrin) through metal ligation to an oligo(ethylenediamine) tether. As a model bis(cyclodextrin) system, 6,6′-triethylenetetraamine-bridged bis(β-cyclodextrin) (**4**) and its copper(II) complex (**5**) were synthesized from β-cyclodextrin (**1**), according to the route shown in Scheme 1. In this synthetic route, β-cyclodextrin 6-mono-tosylate (**2**), prepared by the reaction of **1** with *p*-toluenesulfonyl chloride in aqueous alkaline solution,[6] was converted to mono(6-triethylenetetraamino-6-deoxy)-β-cyclodextrin (**3**) in 72% yield on heating in excess triethylenetetramine at 70°C for 7 h.[7] Subsequent reaction of this intermediate with a slight excess of the 6-tosylate (**2**) in *N*,*N*-dimethyl formamide at 80°C for 3 days, followed by being poured into acetone, gave the crude bis(cyclodextrin) **4** as a precipitate. The crude product was purified on a column of Sephadex G-25 with water as the eluent to give pure **4** in 34% yield.[8]
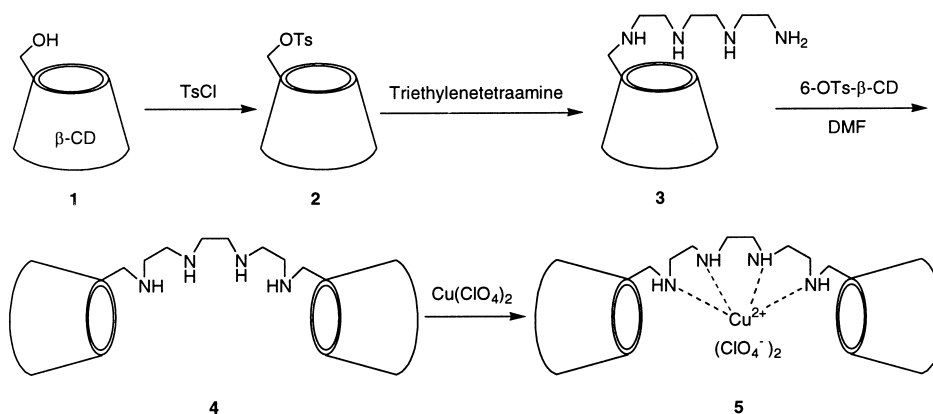
Scheme 1.

The triethylenetetramine bridge in **4** is expected to function as a polydentate ligand, forming coordination complexes with transition metal ions. Hence, we have prepared a copper(II) complex of **4** as an excellent example of a bridged bis(cyclodextrin) with metal-ligated tether. To a dilute aqueous solution of a slight excess of copper(II) perchlorate was added bis(cyclodextrin) **4** portionwise in an ice-water bath. Several drops of chloroform were added further, and the resultant solution was kept at 5°C for 2 days. Then the precipitate formed was collected by filtration, washed successively with a small amount of ethanol and ether, and then dried in vacuo to give the bis(cyclodextrin) Cu(II) complex **5** in 42% yield.[8] The IR spectrum of **5** clearly indicated the presence of perchlorate, showing vibration bands at 1114.6 and 939.4 cm^{-1}. In addition, a weak absorption at 624 cm^{-1}, which may be assigned to a weakly coordinated perchlorate, was also observed, indicating that the Cu(II) ion is coordinated with the triethylenetetramine-bridged bis(cyclodextrin) **4** to form the metallobis(cyclodextrin) **5**.

The molecular recognition behavior of the cyclodextrin dimers **4** and **5** was studied by spectrofluorometric titrations. 6-(*p*-Toluidino)-2-naphthalenesulfonate (TNS) and 8-anilino-1-naphthalenesulfonate (ANS) were chosen as guest molecules, since these two water-soluble dyes barely fluoresce in aqueous solutions but give intense fluorescence in a hydrophobic environment such as the cyclodextrin cavity. Upon gradual addition of bis(cyclodextrin) **4** or **5**, the fluorescence intensity of both guests increased dramatically, accompanying significant hypsochromic shifts of

the fluorescence peak. Fig. 1 illustrates the typical fluorescence spectral changes of ANS upon gradual addition of **4** (0–0.45 mM). As can be seen from Fig. 1, the original fluorescence maximum of ANS at 522 nm in aqueous solution is shifted to 476 nm in the presence of added **4**. This pronounced hypsochromic shift may be attributed to the cooperative binding of two hydrophobic cyclodextrins, since the hypsochromic shift observed upon addition of natural β-cyclodextrin is less, giving an ultimate fluorescence maximum of 515 nm. From the fluorescence intensity changes induced by adding bis(cyclodextrin)s, we can determine complex stability constants (K_S) by using the non-linear least-squares method.[9] A representative result of the curve-fitting analysis of the spectral changes, assuming 1:1 stoichiometry, is shown in Fig. 1 (inset), where no serious diversions from the theoretical values are found, confirming the formation of a 1:1 complex. The complex stability constants obtained for all of the host–guest combinations are listed in Table 1.

The K_S values for the inclusion complexation of native β-cyclodextrin (**1**) with ANS and TNS have been reported as 103 and 3670, respectively.[9] Possessing two hydrophobic cavities, β-cyclodextrin dimers **4** and **5** form much more stable inclusion complexes with ANS and TNS through the cooperative binding by two β-cyclodextrin units. As can be seen from Table 1, the K_S values for **4** and **5** are 4–13 times higher than the corresponding values for **1**. Although both the monomeric (**1**) and dimeric cyclodextrins (**4,5**) give lower K_S values for ANS rather than TNS, the effect of cooperative binding by dimeric cyclodextrins is more remarkable for ANS, showing the larger enhancement upon complexation with **5**. This may be attributed to the shape of the guest. The present results indicate that the linear guest (TNS) can be well embedded in the cavity of the monomeric host (**1**), and the second cavity added in the dimeric hosts (**4**, **5**) merely enhances the K_S value by 4–6 times, while the bent guest (ANS) is only poorly accommodated in the cavity of **1** and therefore the contribution of the second cavity in **4** and **5** is much more pronounced to give an enhancement of K_S by a factor of 10–13.
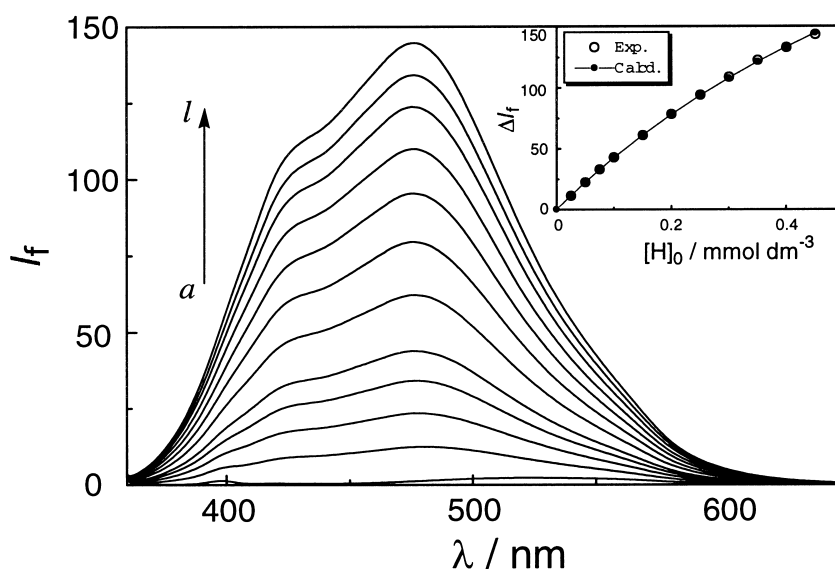

Figure 1. Fluorescence spectral changes of ANS (9.8 μM) upon addition of 6,6′-triethylenetetraamine-bridged bis(β-cyclodextrin) **4** in aqueous solution at 25°C; the concentration of **4** was from 0 to 0.45 mM (from *a* to *l*); excitation at 350 nm. Inset: curve-fitting analyses for the above inclusion complexation

Table 1

Fluorescence maxima (λ^F_{max}) and complex stability constants (K_S) upon complexation of fluorescent dyes ANS and TNS with natural β-cyclodextrin **1** and bis(β-cyclodextrin)s **4** and **5**

Host	Guest	λ^F_{max}/nm[a]	K_S / M^{-1}	Ref.
1	ANS	515	103	*b,c*
	TNS	483	3670	*b,c*
4	ANS	475	1040 ± 40	*c*
	TNS	455	13900 ± 200	*c*
5	ANS	475	1350 ± 70	*c*
	TNS	450	23000 ± 500	*c*

[a] Ultimate fluorescence maximum obtained upon addition of large excess of host, while the λ^F_{max} of ANS and TNS were 522 and 491 nm, respectively. [b] Ref. 9. [c] This work.

It is also interesting to note that the coordination of the ligand tether of **4** to Cu(II) further enhances the binding ability of **5** by a factor of 1.3–1.7 for ANS and TNS, respectively. This further enhancement is attributable to conformational fixation by metal ligation, electrostatic interaction with the ligated Cu(II), and/or ligation of the anilino-nitrogen of ANS/TNS to Cu(II) in **5**. If one of the latter two mechanisms is operative, the metallobis(cyclodextrin) **5** is a host with ternary recognition (two hydrophobic and one electrostatic/coordination) sites. Thus, the TNS/ANS selectivity is enhanced from 13 for **4** to 17 for **5**, which could be ascribed to the triple recognition of the guest by **5**.

Endeavors to synthesize a series of oligo(ethylenediamine)-bridged bis(cyclodextrin)s and their transition metal complexes are currently in progress in order to elucidate their molecular binding abilities and detailed complexation mechanisms.

Acknowledgements

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8. β-CD dimer **2**: anal. calcd for $C_{90}H_{154}O_{68}N_4 \cdot 8H_2O$: C, 42.82; H, 6.79; N, 2.22. Found: C, 42.83; H, 6.58; N, 2.45. ^{13}C NMR(D_2O): 104.78, 102.89, 86.26, 84.03, 76.10, 75.05, 74.85, 63.22, 54.43, 51.79, 47.13. FAB-MS (NaI): m/z 2403 ($M+Na^+ - 8H_2O$). β-CD dimer **3**: anal. calcd for $C_{90}H_{154}O_{68}N_4 \cdot Cu \cdot 2ClO_4 \cdot 4H_2O$: C, 39.82; H, 6.02; N, 2.06. Found: C, 39.79; H, 5.82; N: 2.07

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