CHEMISTRY LETTERS, pp. 167-170, 1994.

Synthesis of a Cyclodextrin Heterodimer Having α- and β-Cyclodextrin Units and Its Cooperative and Site-Specific Binding

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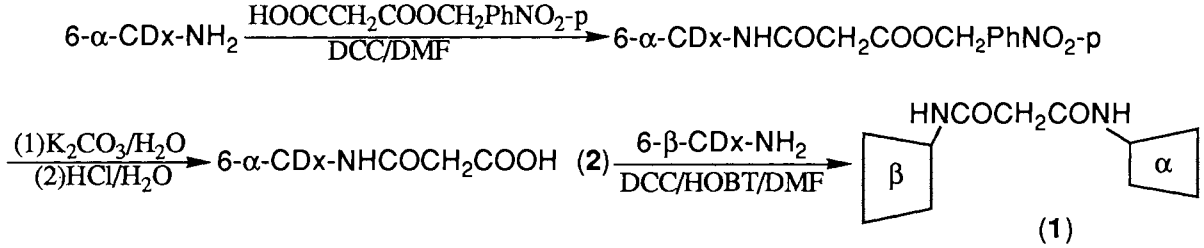
A cyclodextrin heterodimer, which has α- and β-cyclodextrin units as two different receptor sites, was prepared. It showed cooperative and site-specific binding to isoamyl *p*-dimethylaminobenzoate with the alkyl group included in the β-cyclodextrin cavity while dimethylaminobezene moiety partially included in the α-cyclodextrin cavity. This binding mode was substantiated by the fact that the TICT emission of this guest is greatly enhanced by the cyclodextrin heterodimer.

Recently, due to its promising future as a kind of sophisticated artificial receptors, much attention has been paid to cyclodextrin (CDx) dimers,[1] which have two CDx units in their structures. But the dimers so far prepared were limited to those that have the same kind of CDx, mostly to the dimers of β-CDx. The synthetic strategy of CDx dimers so far reported is not suitable to synthesize dimers having different CDx units. We report here the first example of preparation of CDx dimer having different CDx units, α- and β-CDx, and its



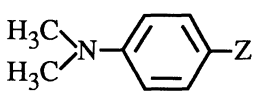
a) α-CDx, α-cyclodextrin; β-CDx, β-cyclodextrin; DCC, N, N'-dicyclohexylcarbodiimide; HOBT, 1-hydroxybenzotriazole.

Scheme 1.

cooperative and site-specific binding to isoamyl *p*-dimethylaminobenzoate (DMBA). Such heterodimers may be useful to explore the sophisticated enzyme mimics or supramolecular assemblies by formation of structurally well-defined complexes.

The synthesis of **1** includes condensation of 6-deoxy-6-amino-α-CDx and malonic acid mono-*p*-nitrobenzyl ester, hydrolysis of the product and the condensation of the resulting acid **2** and 6-deoxy-6-amino-β-CDx. The reactions proceeded with fairly good yields, ca. 85% for **2** on the basis of 6-deoxy-6-amino-α-CDx and ca. 70% for the condensation of **2** and 6-deoxy-6-amino-β-CDx. The IR absorption of **1** has the expected bands of the amide bonds, 1642 cm^{-1} (s) and 1545 cm^{-1} (m). Its ^{1}H-NMR in 500 Hz exhibits anomeric protons centered at 4.95 ppm, and other CDx protons in the field of 3.3-3.9 ppm. Its ^{13}C-NMR spectrum shows the expected two carbonyl carbons (172.14, 172.12 ppm), two groups of anomeric carbons (centered at 104.66 and 104.25 ppm, respectively), and two peaks (43.13, 43.06 ppm) in the upper field corresponding to the C(6) carbons of CDx moieties linked to the nitrogen atoms of the amide bonds. TLC analysis on silica plate showed one spot, and elemental analysis was satisfactory.[2)]

The complex formation between **1** and isoamyl *p*-dimethylaminobenzoate (DMBA) has been studied. The emission spectra of an aqueous solution containing $2.50X10^{-5}$ M DMBA as a function of concentration of **1** are shown in Fig. 1. In aqueous solution DMBA has a single "normal planar" (NP) emission peak at 375 nm. The emission quantum yield is extremely low. With addition of **1** the NP emission increases steadily with a slight blue shift. The more significant change in the spectra is the appearance of the "twisted intramolecular charge transfer" (TICT) emission at the longer wavelength region. This fluorescence change indicated formation of the complex between **1** and DMBA. A computer simulation using fluorescent intensity at fixed wavelength as a function of concentration of **1** proved that experimental data fit to 1:1-type Benesi-Hildebrand equation very well. This is a strong evidence for the formation of an 1:1 complex. The binding constants calculated by this method are independent of the wavelength and are the same within the experimental errors, for example being $(1.07\pm0.09)X10^{4}$ M^{-1} at 360 nm and $(1.14\pm0.08)X10^{4}$ M^{-1} at 500 nm.



DMBA, Z= $-CO_2CH_2CH_2CH(CH_3)_2$
DMBE, Z= $-CO_2CH_2CH_3$
DMBN, Z= $-CN$
DMBB, Z= $-CO_2CH_2CH_2O(CH_2)_3CH_3$

The 1:1 stoichiometry and large binding constant of the complex formed between DMBA and **1** strongly suggests the cooperative binding of **1** with its two CDx cavities. To substantiate this further, we have studied the binding of DMBA with α-CDx and β-CDx. The fluorescence changes of DMBA induced by α-CDx or β-CDx can not be analyzed by a simple 1:1 stoichiometry model. The complicated

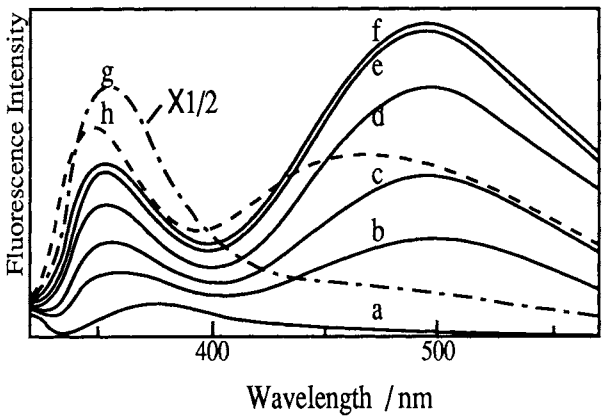


Fig. 1. Fluorescence emission spectra of DMBA in aqueous solution at 25 °C, in the presence of a, 0; b, 0.0552 mM; c, 0.110 mM; d, 0.331 mM; e, 1.10 mM; f, 1.65 mM of **1**, and g, 5 mM of β-CDx; h, 10 mM of α-CDx. Excitation wavelength is 290 nm.

behavior for complex formation between DMBA and α-CDx or β-CDx is reasonable since DMBA is a rather large molecule compared to CDx cavity. In contrast to DMBA, the complex formation between ethyl *p*-dimethylaminobenzoate (DMBE), which has an only small side chain, and α-CDx or β-CDx can be analyzed using a simple 1:1 model, giving 883 M^{-1} and 129 M^{-1} as the binding constants for β-CDx and α-CDx, respectively.

From the examination of CPK model there are two possible ways in which DMBA can be cooperatively bound by **1**, A-type complex : with the dimethylaminobenzene moiety partially included in the α-CDx cavity and the alkyl group included in the β-CD cavity; and B-type complex : with the isopropyl group partially included in the α-CDx cavity, while the aromatic moiety included in the β-CDx cavity. If DMBA has different fluorescent properties in these two different complex states, these two structures can be distinguished. We found that in aqueous solution DMBA, as well as other *p*-substituted dimethylaminobenzene alkoxycarbonyl derivatives checked in this study, exhibits a very different fluorescence spectrum in the presence of α-CDx from that in the presence of β-CDx. α-CDx strongly increases the TICT emission, but β-CDx had little effect on the TICT emission, although it numerically increases the NP emission (Fig. 1). Similar observation of *p*-dimethylaminobenzonitrile (DMBN) with α-CDx and β-CDx was reported.[3,4] It was explained that DMBN can only be partially included in α-CDx cavity because it is larger in size than α-CDx cavity. So in α-CDx complex DMBN is likely to be in a microenvironment with a polarity lower than water but not hydrophobic one, resulting in a strong TICT emission. In β-CDx complex, DMBN can be deeply included in the β-CDx cavity, and the hydrophobic microenvironment around DMBA was suggested to lead to very strong NP, but weak TICT emission. This explanation is based on the fact that TICT emission is strong in polar organic solvents, but week in nonpolar, or highly polar solvents such as water, and is consistent with our observation with *p*-substituted dimethylaminobenzene alkoxycarbonyl derivatives. So for DMBA-**1** complex the A-type complex would have a strong TICT emission, while the B-type complex would have a strong NP emission but a weak TICT emission. Due to their different fluorescence behavior of these two kinds

complexes, if DMBA is not site-specifically bound by **1**, the binding constants obtained using a simple 1:1 model must be dependent on the wavelength. But we have found that binding constant is independent of the wavelength at which it was calculated. This suggests that DMBA is specifically bound by **1** to form the A-type complex.

The further evidence of site-specific binding of DMBA by **1** comes from the study of complex formation between butoxyethyl *p*-dimethylaminobenzoate (DMBB) and **1**. Since DMBB has no branched alkyl chain, it is expected that the side chain of DMBB may enter the cavities of α -CDx as well as β-CDx. We found although the change of fluorescence intensity of DMBB as a function of concentration of **1** can be perfectly analyzed by a 1:1 complex model, the binding constants obtained is a function of the wavelength, for example, with binding constants of $(2.21\pm0.09)\times10^3$ M^{-1} and $(4.18\pm0.09)\times10^3$ M^{-1} for calculation at the NP and TICT peaks, respectively. This indicated that DMBB does not form a well-defined 1:1 complex with **1**. Furthermore, in aqueous solution containing **1,** the TICT emission of DMBB is much weak than that of DMBA, while its NP emission is much stronger than that of DMBA. This can be explained by the formation of two kinds of cooperative binding complex between **1** and DMBB.

Further studies to clarify the structure of the DMBA-**1** complex are underway.

References

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2) R_f 0.15 (butanol : ethanol : water = 5 : 4: 3) and R_f 0.29 (aqueous ammonia : water : ethyl acetate : 2-propanol = 1 : 4 : 3 : 5). Elemental analysis (calculated for $C_{81}H_{132}N_2O_{65}\cdot5H_2O$) C 42.95 (42.97), H 6.43 (6.32), N 1.31 (1.24).

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(Received October 6, 1993)