Guest-Induced Diminishment in Fluorescence Quenching and Molecule Sensing Ability of A Novel Cyclodextrin−Peptide Conjugate

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The development in the de novo peptide design[1] provides us the way to construct a molecule with suitable functional groups on α-helix polypeptides. Recently, using the de novo peptide design strategy, we have started preparing various cyclodextrin−peptide hybrids (CD−peptides) to create enzyme mimics[2a] as well as new types of sensors.[2b] CDs are torus-shaped cyclic oligosaccharides and form inclusion complexes with various organic compounds in aqueous solution.[3] On the basis of this property and unique molecular architecture of CD, various chromophore-modified CDs have been constructed and shown to act as chemosensors.[4] Most of the chromophore-modified CDs acting as molecular sensors have single photoreactive moiety or two same moieties. However, in this study, we have synthesized a novel CD−peptide hybrid **1** (Figure 1) that has two different photoreactive moieties, pyrene (electron donor) and nitrobenzene (NB: electron acceptor) on the peptide scaffold. Thus, we report here, for the first time, how it works as a chemosensor when both fluorophore (pyrene) and quencher (NB) are present in a CD-conjugated peptide molecule. To study the conformational change and molecule sensing ability of **1**, we also have synthesized three reference peptides, which have CD and NB units (**2**), pyrene and NB units (**3**) and only one pyrene unit (**4**) in the side chain of the peptides (Figure 1).

Alanine that favors α-helical conformation[5] was chosen as a main component of the peptides to avoid undesirable influences of the side chains. In addition to alanine, the peptide contains three lysine/glutamic acid pairs that can form α-helix-stabilizing intramolecular salt bridges.[6] The CD−peptides were synthesized by stepwise elongation of Fmoc amino acids (Fmoc AA) on the rink amide resin[7] using the same synthetic procedure as previously reported.[2b] The final products, **1**, **2**, **3**, and **4** were purified with reversed phase HPLC and identified by MALDI-TOF MS (**1**: *m*/*z* 3238.5 [(M + H)$^+$], calcd 3236.3; **2**: 3035.9 [(M + H)$^+$], calcd 3036.3; **3**: *m*/*z* 2120.6 [(M + H)$^+$], calcd 2120.00; **4**: *m*/*z* 1928.9 [(M + H)$^+$], calcd 1929.1).

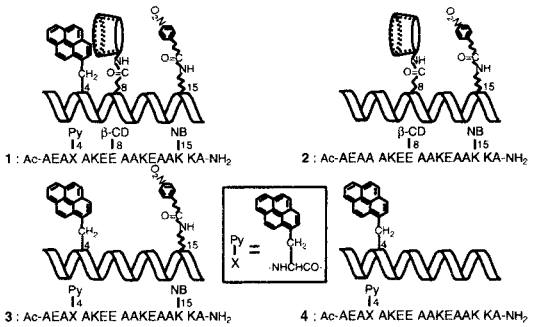

Figure 1. Peptide with pyrene (Py), β-cyclodextrin (β-CD), and *p*-nitrobenzene (NB) unit in the side chain (**1**) and its reference peptides.

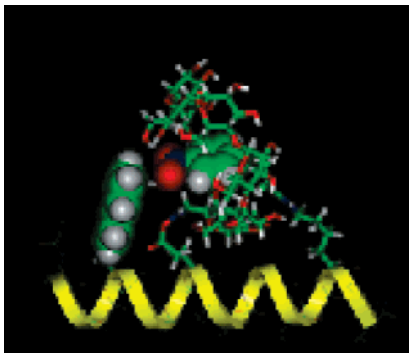

Figure 2. Molecular modeling of **1** derived from energy minimization with consistent valence force field (CVFF) calculation.

To examine the conformation of the polypeptide chain, circular dichroism spectra in the peptide absorption region (200−250 nm) were measured. The circular dichroism studies revealed that **1**, **2**, **3**, and **4** showed a typical α-helix pattern. The α-helix content calculated from the mean residual weight ellipticity at 222 nm, $[\theta]_{222}$[8] was found to be 91, 70, 51, and 52% for **1**, **2**, **3**, and **4** respectively, indicating that the α-helicity of **1** and **2** is higher than that of both **3** and **4**. The difference in the helicity of the peptides gives us an idea that NB moiety may form intramolecular inclusion complex with β-CD and the formation of this intramolecular host−guest complex stabilizes the α-helical conformation of **1** and **2**. The molecular modeling of **1** (Figure 2) has been performed by energy minimization with consistent valence force field (CVFF) calculation with Insight II/Discover software considering that the peptide takes solely α-helix structure. This model provides support that the intramolecular host−guest inclusion complex is structurally reasonable only between CD and NB. The NB unit can easily be incorporated into the β-CD cavity because of its flexible butyrate tail and small size, while the pyrene unit always remains outside because of its limited flexibility and large size to be included into the β-CD cavity.

Interestingly, α-helix content of **1** and **2** decreased from 91 to 59% and 70 to 55% respectively, upon addition of lithocholic acid ([LCA] = 200 μM) as an exogenous guest molecule, whereas, no change in the helicity has been observed when 200 μM of the same guest is added to the solution of **3** and **4**. These results demonstrate that LCA excludes the appending NB moiety from inside to outside of the β-CD cavity, thereby causing destabilization of the helical structure and increasing the random coil content of the CD−peptides **1** and **2**. On the other hand,

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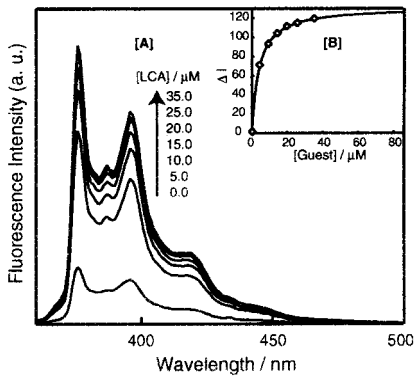

Figure 3. (A) Fluorescence spectra of **1** (1 μM) in the presence and absence of LCA in Tris-HCl buffer (20 mM, pH 7.5) at 25 °C. The excitation wavelength is 345 nm. (B) The plot of the fluorescence intensity (ΔI) of **1** (1 μM) as a function of guest concentrations.

helicity remains unchanged for **3** and **4** even after addition of excess LCA to their aqueous solutions. This result is consistent with the fact that **3** contains no host (CD) and **4** contains neither host nor endogenous guest (NB), and thereby, there is no possibility of host−guest interaction in their side chains. Since CDs are composed of chiral glucose units, the circular dichroism is expected to be induced on the absorption bands of the achiral guest molecules, which are included in the cavity of chiral β-CD. We thus investigated induced circular dichroism spectra (ICD) of **2** to confirm intramolecular host−guest complex formation between CD and NB. The CD−peptide **2** exhibits the ICD spectra in the NB absorption region (250−400 nm) with a sharp positive ICD bands at around 270 nm ($[\theta]_{270} = 3800$ deg cm^2 dmol^{-1}), and the addition of LCA caused diminishment of the circular dichroism intensities. These observations are unambiguous proofs[9] for the formation of an inclusion complex between NB and β-CD in the α-helix side chain. The diminishment of the circular dichroism intensity is associated with the exclusion of the NB unit from inside to outside of the β-CD cavity by accommodating a LCA molecule.

The typical fluorescence spectra of **1**, **3**, and **4** exhibit monomer emission of pyrene with the peaks at 376 and 396 nm. The fluorescence intensity of **4** (2 μM) is 19 and 25 times higher (at 376 nm) than that of **3** and **1** (2 μM), respectively, indicating that most effective quenching occurs between pyrene and NB in **1**. The NB moiety included in the CD cavity in aqueous solution may be thought to be in close to the pyrene moiety so that the effective quenching occurs in this system. Fluorescence spectra of **1** alone (1 μM) and in the presence of LCA (up to 35 μM) in aqueous solution are shown in Figure 3A. The spectral intensity dramatically increases with increasing concentration of LCA, whereas no remarkable change in the spectra has been observed when LCA is added to **3** and **4** (1 μM). Hence, the guest-responsive enhancement in the fluorescence intensity of **1** can be explained in terms of increased distance between the pyrene and NB moieties, which is caused by exclusion of the NB moiety from the CD cavity by guest accommodation. This finding is consistent with the result previously reported by Shinkai et al.[10] where they showed that metal ions can be detected by fluorescence enhancement with a calixarene system bearing the same pair of the pendants in an organic solvent.

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Table 1. Binding Constants and Sensitivity Values of 1 for Various Guests at 25 °C

guest	binding constant (K_b/M^{-1})	sensitivity value ($\Delta I/I^0$)
lithocholic acid (LCA)	281000	3.35
hyodeoxycholic acid (HDCA)	97600	1.44
ursodeoxycholic acid (UDCA)	59900	0.89
chenodeoxycholic acid (CDCA)	23000	0.77
deoxycholic acid (DCA)	n.d.[a]	n.d.[a]
cholic acid (CA)	n.d.[a]	n.d.[a]

[a] The value could not be determined due to the small change in the spectrum.

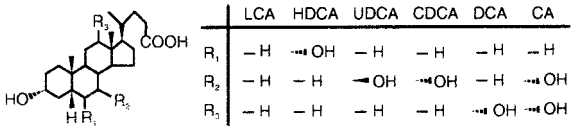

Figure 4. Structures of guest compounds.

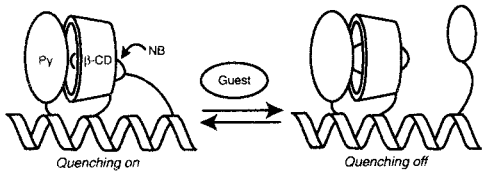

Figure 5. Schematic representation for guest-induced structural change in the side chain of **1**.

The plots of ΔI ($\Delta I = I - I^0$ when I^0 and I denote the fluorescence intensities in the absence and presence of guest, respectively) against the guest concentration (Figure 3B) gave binding constant by the curve-fitting analysis based on the equation of 1:1 stoichiometry and the results are shown in Table 1. The binding constants of **1** for various guests (Figure 4) examined are in the order of LCA > HDCA> UDCA> CDCA. The binding constant of **1** for DCA and CA could not be determined since no remarkable change in the fluorescence intensity has been observed.

The sensitivity factors of the CD−peptide **1** (1μM) for various guests were evaluated using fluorescence-intensity variation ($\Delta I/I^0$) at a guest concentration of 20 μM (Table 1). The CD−peptide is sensitive to the guests in the order of LCA> HDCA> UDCA> CDCA which is correlated with the binding constants. The guest-induced enhancement in the fluorescence intensity is presented schematically in Figure 5.

In conclusion, we have succeeded in constructing a system that undergoes guest-induced diminishment in fluorescence quenching and can act as a molecule responsive device.

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Supporting Information Available: Details for synthesis and purification of peptides (**1**-**4**) and ICD spectra of **2** in the absence and presence of LCA (PDF). This material is available free of charge via the Internet at http: pubs.acs.org.

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