Association and Guest-induced Dissociation of a Novel α-Helix Peptide Bearing Pyrene and γ-Cyclodextrin in the Side Chains

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A designed α-helix peptide, γ-PR17, which bears γ-cyclodextrin (γ-CD) and pyrene units, exhibits both monomer and excimer emissions, indicating that γ-PR17 forms an association dimer that could be dissociated upon addition of hyodeoxycholic acid as a guest for γ-CD.

Association of two polymer chains is obviously important in nature as seen in DNA double helix or in a leucine-zipper composed of two α-helix polypeptides. In many cases, such polymeric dimers are dissociated by external stimulant molecules. Here we wish to suggest novel strategy for the construction of the host-guest systems, in which peptide dimerization and dissociation can be controlled by external molecules.

Cyclodextrins (CDs) are well-known host compounds and their most fascinating property is that they can accommodate various guest molecules into their truncated cone shaped hydrophobic cavity.[1,2] On the other hand, *de-novo* peptide design and synthesis provide the way to combine multiple functional groups in a constrained molecule. However, it is not easy to construct a molecule with binding site such as seen in enzymes only by using amino acids. The combination of CD and peptide might solve this problem. For producing an example of the peptides having CD binding site and other functional moieties, we have designed and synthesized a novel peptide hybrid γ-PR17, which has a γ-CD as a host and a pyrene moiety as an intramolecular guest (Figure 1). γ-CD and pyrene moieties are in close proximity on the same side of this α-helix peptide so that the pyrene moiety can easily enter into the CD cavity. In the design of the pyrene-γ-CD-peptide triad, alanine that favors α-helical conformation is chosen as a main component of the peptide in order to avoid the undesirable influences of the side chains.[3] In addition to alanine, the peptide contains three lysine/glutamic acid pairs that can act as α-helix stabilizing intramolecular salt bridges.[4]

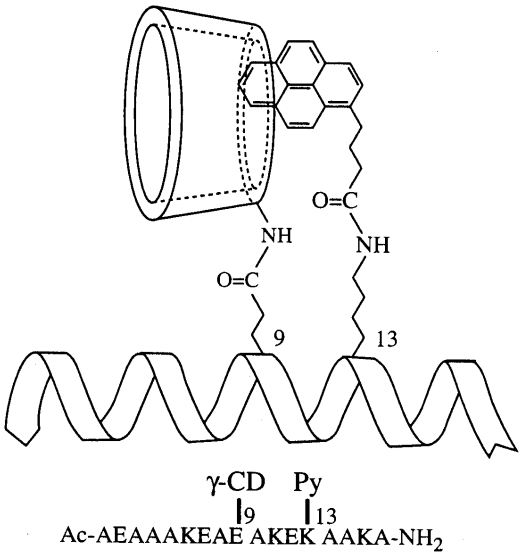

Figure 1. Schematic representation of γ-PR17 and its amino acid sequence.

The 17-peptide was synthesized by solid phase synthesis using Fmoc strategy.[5] The side chains of Glu and Lys except for Glu9 and Lys13 were protected with benzyl and 2-chlorocarbobenzoxyl groups, respectively. The side chain of Glu9 was protected with tertiary butyl (OtBu) and that of Lys13 was protected with *t*-butyloxycarbonyl (*t*-Boc) groups. The synthesized 17-peptide was, first, cleaved from the resin and partially deprotected with trifluoroacetic acid (TFA). The protection groups OtBu and *t*-Boc were removed at this stage. Then 1-pyrenebutyric acid was coupled to the deprotected side chain of Lys13 through an amide bond. 6-Mono-deoxy-6-amino γ-CD was prepared as previously reported[6] and selectively introduced into the deprotected side chain of Glu9. Then remaining all protecting groups were removed by trimethylsilyl trifluoromethanesulfonate (TMSOTf).[7] The product, γ-PR17, was purified with reversed-phase HPLC and identified by matrix assisted laser desorption ionization time-of-flight mass spectrometry (TOF-MS) (γ-PR17 *m/z* 3278.2 [$(M+H)^+$], calcd. 3277.2) and amino acid analysis.

The circular dichroism (CD) of the peptide (10 μM in Tris-HCl buffer, pH 7.5) showed a typical pattern of α-helix peptide. The helix content calculated from the molar ellipticity at 222 nm, $[\theta]_{222}$,[8,9] was found to be 81% which is high enough to maintain the proximity of γ-CD and pyrene moiety. Moreover, the intensity of the circular dichroism signals in the absorption region of the amide bond did not change upon addition of hyodeoxycholic acid as a guest. These results indicate that α-helix structure is not affected by the guest inclusion in the CD cavity of the peptide. On the other hand, circular dichroism signals were observed in the longer wavelength region (250–400 nm), and the intensities of the dichroism bands diminished upon guest addition. This result suggests that the circular dichroism bands in this region are induced by the accommodation of the pyrene group into the cyclodextrin cavity.

γ-PR17 (10 μM) exhibits excimer and monomer emissions around 476 and 376 nm, respectively (Figure 2A), and the I_{ex}/I_{mon} value, where I_{ex} and I_{mon} represent fluorescence intensity of excimer and monomer, respectively, decreases with decreasing concentration of γ-PR17 (Figure 2B), eventually exhibiting negligible excimer emission. The excimer emission indicates that γ-PR17 exists as an association dimer or aggre-

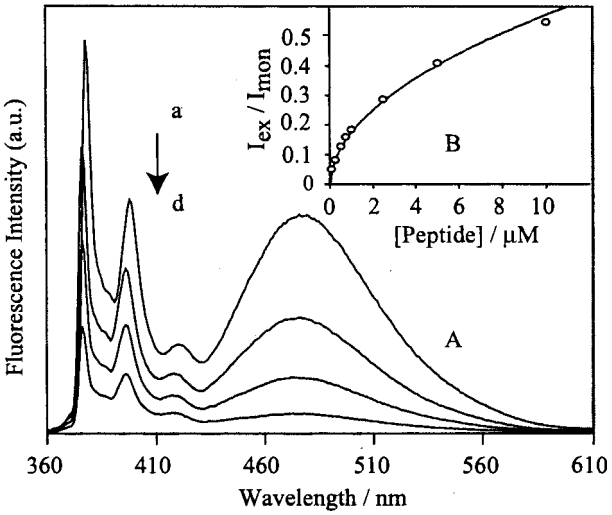

Figure 2. (A) Fluorescence emission spectra of various concentrations of γ-PR17 in 20 mM Tris-HCl buffer (pH 7.5) at 25 °C. Excitation wavelength is 338 nm. [γ-PR17]: (a) 10.0; (b) 5.0; (c) 2.5; (d) 1.0 μM. (B) The plot of peptide concentration vs. I_{ex}/I_{mon}.

gated forms. So, it is reasonable that the I_{ex}/I_{mon} value decreases with decreasing concentration of the peptide (up to 10 nM). The analysis of the concentration dependence of I_{ex}/I_{mon} suggests that γ-PR17 forms an association dimer at a concentration higher than 10 nM. The association constant of the peptide dimer was obtained as 2.8 x 10^7 M^{-1} by the non linear least square curve fitting analysis using the equation of 1:1 stoichiometry (Figure 2B, solid line).

Figure 3 shows the guest-induced variation in the monomer and excimer emission intensities of the peptide. The intensity of monomer increases and that of the excimer decreases with increasing concentration of hyodeoxycholic acid. It indicates that accommodation of the guest in the CD cavity results in the dissociation of the peptide dimer to monomer. It was reported that pyrene-appended γ-CD forms association dimer by accommodating two pyrene moieties in the cavity formed by two γ-CD units and exhibits guest-induced dissociation of the dimer form.[10,11] The guest-induced dissociation of the peptide is supposed to be exactly of this type and represented schematically in Figure 4. The remarkable guest-responsive fluorescence change shows the possibility of this system for application as a molecule sensing system.

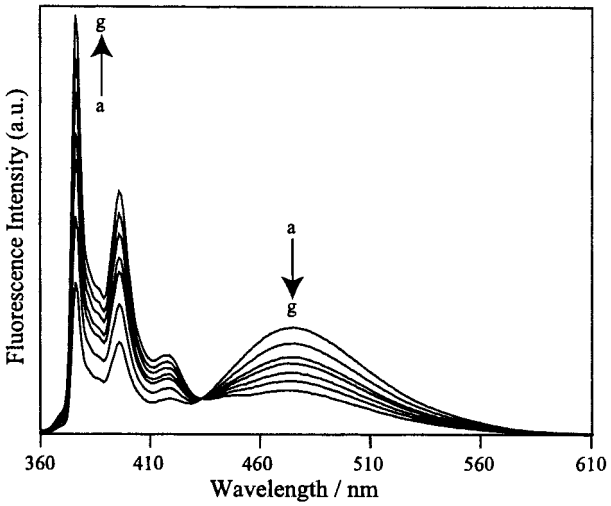

Figure 3. Fluorescence emission spectra of γ-PR17 (10 μM) in 20 mM Tris-HCl buffer (pH 7.5) at 25 °C in the presence and absence of hyodeoxycholic acid as a guest molecule. Excitation wavelength is 338 nm. [Hyodeoxycholic acid]: (a) 0.0; (b) 26.6; (c) 53.3; (d) 66.7; (e) 93.3; (f) 120.0; (g) 160.0 μM.

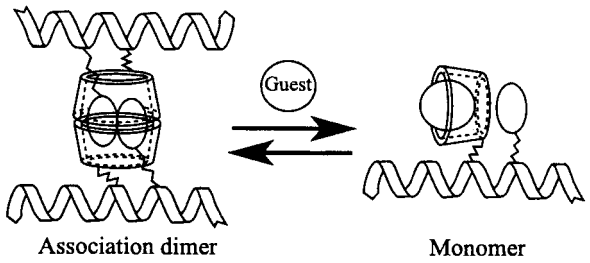

Figure 4. Schematic representation of the guest-induced dissociation of the peptide.

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References

1 G. Wenz, *Angew. Chem., Int. Ed. Engl.*, **33**, 803 (1994).
2 A. Ueno, *Supramolecular Sci.*, **3**, 31 (1996).
3 P. Y. Chou and G. D. Fasman, *Annu. Rev. Biochem.*, **47**, 258 (1978).
4 S. Dao-pin, U. Sauer, H. Nicholson, and B. W. Matthews, *Biochemistry*, **30**, 7142 (1991).
5 E. Atherton, R. C. Sheppard, "*Solid Phase Synthesis: A Practical Approach*," IRL Press, Oxford (1989).
6 F. Hamada, K. Murai, A. Ueno, I. Suzuki, and T. Osa, *Bull. Chem. Soc. Jpn.*, **61**, 3758 (1988).
7 N. Fujii, A. Otaka, O. Ikemura, K. Akaji, S. Funakushi, Y. Hayashi, Y. Kuroda, and H. Yajima, *J. Chem. Soc., Chem. Commun.*, **1987**, 274.
8 J. M. Scholtz, H. Qian, E. J. York, J. M. Stewart, and R. L. Baldwin, *Biopolymers*, **31**, 1463 (1991).
9 Helix content determind from $[\theta]_{222}$ value is not so much affected by the induced circular dichroism of pyrene moiety in the petide chain.
(a) H. Mihara, Y. Tanaka, T. Fujimoto, and N. Nishino, *J. Chem. Soc. Perkin Trans. 2*, **1995**, 1915. (b) H. Mihara, J. Hayashida, H. Hasegawa, H. Ogawa, T. Fujimoto, and N. Nishino, *J. Chem. Soc. Perkin Trans. 2*, **1997**, 517.
10 A. Ueno, I. Suzuki, and T. Osa, *Anal. Chem.*, **62**, 2461 (1990).
11 A. Ueno, I. Suzuki, and T. Osa, *J. Am. Chem. Soc.*, **111**,