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Molecule-Responsive Fluorescent Sensors of α-Helix Peptides Bearing α-Cyclodextrin, Pyrene and Nitrobenzene Units in Their Side Chains

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Abstract

α-Helix peptides bearing one unit of α-cyclodextrin (α-CD), one unit of pyrene and one unit of nitrobenzene (NB) in their side chains have been designed and synthesized as novel molecule-responsive devices. In both the CD-peptides, **α-PR17** and **α-PL17**, the NB unit is separated from the CD unit by two turns of the helix. Two reference peptides (**PR17** and **P17**) have also been synthesized. The circular dichroism studies in the peptide absorption region (200–250 nm) of **α-PR17** and **α-PL17** suggest that the CD-peptides form stable α-helix structures (83–77%), which was destabilized by accommodating guest molecules (e.g., *n*-pentanol) into the CD cavity. It suggests that formation of intramolecular host–guest (CD–NB) complex stabilized the helical structure and exogenous guest molecule excluded the appending NB moiety from inside to outside of the CD cavity, thereby causing destabilization of the helical structure and increasing the random coil content. The ICD spectra of the peptides in the pyrene and nitrobenzene absorption region (250–40 nm) suggest that NB forms inclusion complex with CD. The fluorescence studies revealed that the fluorescence of the pyrene unit is quenched by the NB unit in **α-PR17** and **α-PL17**. The fluorescence intensity increases with increasing guest concentration for the CD-peptides. This guest-responsive enhancement in the fluorescence intensity 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. Using the guest-responsive fluorescence quenching properties of the CD-peptides, we have obtained binding constants for various short chain alkanols. **α-PL17** has higher binding affinity to the guest molecules than its isomer, **α-PR17**, indicating that the location of functional groups on the peptide scaffold is important in molecule detection.

Introduction

Cyclodextrins (CDs) are cyclic oligosaccharides composed of six (α), seven (β), eight (γ) or more glucose units. Since they have a hydrophobic cavity they can form inclusion complexes with various organic molecules (guests) of appropriate size. In the last two decades, a variety of dye-modified β- and γ-CD derivatives have been prepared as sensors for detecting guests in aqueous solution [1–9]. But there are few reports on dye-modified α-CD derivatives that can be used for detecting guest molecules [10, 11]. Therefore, detection of small organic guests remains to be explored in the field of chemosensors. Recently, we have started preparing new host compounds namely β- and γ-CD conjugated peptides as molecule-responsive devices [12–16]. In most of these stimulant-responsive devices, the complex formation with a stimulant molecule changes the location of the attached dye molecule from inside to outside of the cavity, resulting in a marked change in the spectrum of the dye-modified CDs. As we recently reported, the CD-peptides are useful for detecting large-sized compounds such as steroidal compounds. Thus it is interesting to apply the CD-peptide system for detecting small-sized compounds like short chain aliphatic alcohols by introducing α-CD on the peptide scaffold.

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We have attempted to prepare two novel CD-peptides, **α-PR17** and **α-PL17** (Figure 1) that are isomers of each of the others, having three functional units: α-CD, pyrene and nitrobenzene (NB) on the side chain of α-helix peptides. The only difference in their structures is the position of functional groups on the peptide scaffold. In **α-PR17**, NB is located at the right side of the CD unit whereas in **α-PL17**, NB is located at the left side of the CD unit. In both the CD-peptides, NB and CD are separated by two turns of the helix [14, 16]. α-CD is chosen in this study as guest-binding site since it has an appropriately narrow cavity for detecting small compounds. Two reference peptides named **PR17** and **P17** (Figure 1) have been synthesized and used in this study to compare the structural features and molecule sensing ability of **α-PR17** and **α-PL17**. The basis of molecule detection with these CD-peptides is exactly as the same as the system we already have reported [14]. That is, when the NB moiety is included into the CD cavity, it is very close to the pyrene moiety so that pyrene can donate an electron to the NB moiety upon photoirradiation, and thus effective fluoresecence quenching occurs. This state can be regarded as 'quenching on' state. Addition of an exogenous guest will cause displacement of the endogenous guest (NB) from inside of the CD cavity to the outside. Since the excluded NB moiety is apart from the pyrene unit, it is hard for NB to accept electron from pyrene, resulting in less or no fluorescence quenching. This can be regarded as the 'quenching off' state. We can monitor this guest-induced 'on-off' switch of fluorescence quenching process by measuring the fluorescence intensity of the CD-peptides in an aqueous solution.

Experimental

Materials

Rink-Amide resin for the solid phase peptide synthesis was purchased from either Advanced Chemtech or Novabiochem. Amino acid derivatives and the reagents for the peptide synthesis were purchased from either Novabiochem or Watanabe Chemical Co. All other chemicals and solvents for the synthesis or HPLC were of the highest purity available. The mono-6-deoxy-6-amino-α-cyclodextrin [10] and Fmoc-pyrenyl alanine (Fmoc-Pya) [17] were prepared by the methods previously reported.

Design and synthesis

In the design of **α-PR17** and **α-PL17**, alanine was chosen as the main component of the peptide because of its α-helix stabilizing capability [18]. In addition, α-helix stabilizing three pairs of intramolecular salt bridges (Glu2-Lys6, Glu7-Lys11, and Glu12-Lys16), were introduced into the peptides [19–21]. All the sequences of the peptides and their schematic illustrations are shown in Figure 1. The peptides were synthesized by the stepwise elongation of Fmoc-protected amino acids on a rink-amide resin [22]. The lysine side chains (Lys15 in α-PR17, Lys3 in α-PL17) which are to be coupled with NB butyrate, were protected by *tert*-butyloxycarbonyl (Boc) group while the side chain carboxy group of glutamate (Glu8 in **α-PR17** and Glu10 in **α-PL17**), which is to be coupled with 6-mono-deoxy-6-amino-α-CD was protected by *tert*-butyl Bu^t group. The side chains of other Lys and Glu residues were protected with 2-chlorobenzyloxycarbonyl (ClZ) and benzyl (Bn) groups, respectively. To stabilize α-helix structures of the CD-peptides, *N*-terminal amine and *C*-terminal carbonyl groups were acetylated and amidated, respectively. Then the synthesized peptides were cleaved from the resin by trifluoroacetic acid (TFA) in the presence of *m*-cresol. At this stage, Boc was removed from Lys15 and Lys3 for **α-PR17** and **α-PL17**, respectively, while Bu^t was removed from Glu8 and Glu10 for **α-PR17** and **α-PL17**, respectively. Then, a NB butyrate tail was introduced into the deprotected side chain of the Lys15 in **α-PR17** and Lys3 in **α-PL17**. 6-Mono-deoxy-6-amino-α-CD was coupled with the side chain of the deprotected Glu8 and Glu10 for **α-PR17** and **α-PL17**, respectively. Finally, the remaining protection groups (ClZ and Bn for Lys and Glu, respectively) were removed by treatment of 1 mol dm^{-3} trimethylsilyl trifluoromethane sulfonate (TMSOTf) in TFA solution [23]. The products, CD and pyrene bearing peptides, were purified by HPLC equipped with an ODS column and identified by matrix-assisted laser desorption ionization time of flight mass spectroscopy (MALDI-TOF MS).

Synthesis of **α-PR17**

Fmoc-Ala-Glu(Bn)-Ala-Pya-Ala-Lys(ClZ)-Glu(Bn)-Glu(Bu^t)-Ala-Ala-Lys(ClZ)-Glu(Bn)-Ala-Ala-Lys(Boc)-Lys(ClZ)-Ala-Rink amide resin was synthesized by stepwise elongation of Fmoc-amino acids (3 equiv.) on 4-(2,4-dimethoxyphenyl-fluorenyl-aminomethyl)-phenoxy resin (Rink amide resin) with 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU, 3 equiv.) and 1-hydroxybenzotriazole hydrate (HOBT.H_2O, 3 equiv.) as coupling reagents in *N*-methylpyrrolidone (NMP). The Fmoc group was removed by the treatment with 20% piperidine in NMP solution for 15 minutes. Terminal amino group of the peptide was acetylated by the treatment with acetic anhydride in NMP for 20 minutes. Then the Ac-peptide was cleaved from the resin and partially deprotected with trifluoroacetic acid (TFA) in the presence of *m*-cresol and thioanisole, by stirring for 1.5 h at room temperature. At this stage, Boc and Bu^t were removed from Lys and Glu, respectively. The crude peptide was analyzed by HPLC and identified by MALDI-TOF MS (m/z 2703.5 [$(M + H)^+$], calcd. 2705.2). Ac-peptide (80 mg, ca. 29.57 μmol) and nitrophenyl butyrate succinimide ester (27.15 mg, ca. 88.71 μmol) were dissolved in DMF in the presence of *N*, *N*′-diisopropylethylamine (DIEA 6 equiv.), and the reaction mixture was stirred at room temperature for 6 h. The reaction progress was monitored by analytical HPLC column and the product was identified by MALDI-TOF MS (m/z 2874.4 [$(M + H)^+$], calcd. 2880.4). Then the crude product was purified by gel filtration method using a Sephadex LH-20 column with DMF as eluent. 6-Mono-deoxy-6-amino-α-CD (58.2 mg, ca. 60 μmol) and the NB labeled peptide (60

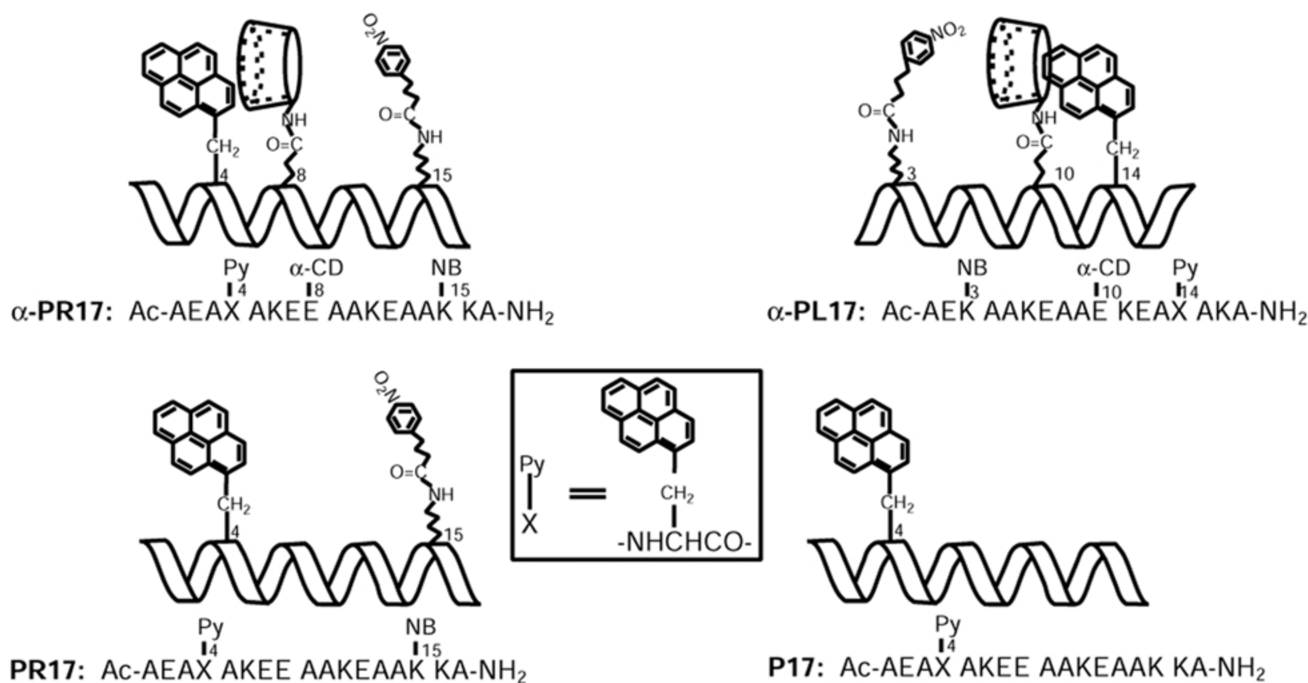

Figure 1. The structures of the CD-peptides, **α-PR17** and **α-PL17**, and their references.

mg; ca. 20 μmol) were dissolved in NMP in the presence of HBTU (3 equiv.), HOBT (3 equiv.) and DIEA (6 equiv.) and the solution was stirred for 12 h. The reaction progress was monitored by HPLC, and the product was identified by MALDI-TOF MS (m/z 3870.1 [(M + Na)$^+$], calcd. 3871.4). The remaining protecting groups, ClZ and Bn, of the crude were removed by TMSOTf: thioanisole (1 : 1 molar ratio) in TFA solution. The final product, **α-PR17**, was purified by HPLC and identified by MALDI-TOF MS (m/z 3074.6 [(M + H)$^+$], calcd. 3075.214) (12 mg, 3.9 μmol, yield 13.1%).

Synthesis of **α-PL17**

This compound was synthesized and purified according to an identical way to **α-PR17**, except for the introduction of functional units at different positions (NB at K3 instead of K15, α-CD at E10 instead of E8, L-Pya at position 14 instead of 4) on the peptide sequence. MALDI-TOF MS (m/z 3076.3 [(M + H)$^+$], calcd. 3075.214) (14 mg; ca. 4.55 μmol, yield 15.3%).

Synthesis of **PR17** *and* **PL17**

This compound was synthesized and purified as previously reported [14]. MALDI-TOF MS (**PR17**: m/z 2120.6 [(M + H)$^+$], calcd. 2120.0) (8 mg, 2.6 μmol, yield 15%); (**P17**: m/z 1928.9 [(M + H)$^+$], calcd. 1929.1) (4 mg; ca. 2 μmol, yield, 30%).

Measurements

The peptides were purified by reversed-phase HPLC (RP-HPLC) on a YMC-Pack C4 A-823 column (10 × 250 mm) (YMC Co.) with a linear gradient of acetonitrile/0.1% trifluoroacetic acid (TFA). All the spectroscopic measurements were carried out in 20 mmol dm-3 Tris-HCl buffer solution

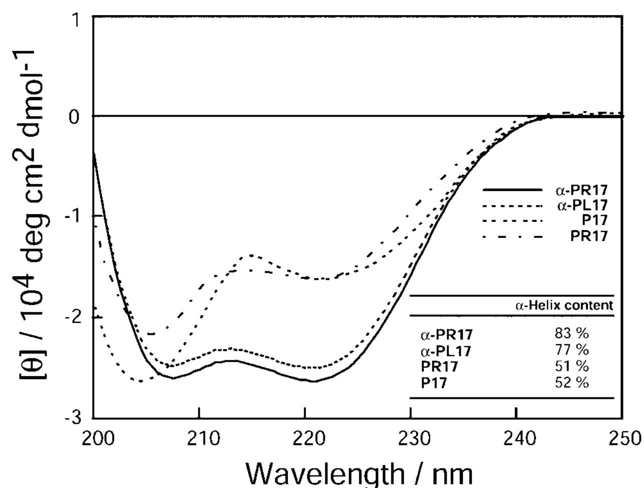

Figure 2. The circular dichroism spectra of **α-PR17** and **α-PL17**, and their reference peptides (**PR17** and **P17**) in the amide absorption region (200–250 nm) in Tris-HCl buffer (20 mmol dm^{-3}, pH 7.5) at 25 °C. [Peptides] = 20–50 μmol dm^{-3}.

(pH 7.5). UV-visible spectra were measured on a Shimadzu UV-1300 or Shimadzu Biospec-1600 in a quartz cuvett with a path length of 1 cm. Fluorescence measurements were carried out on a Shimadzu RF-5300PC equipped with a thermal regulator HAAKE F3. in a quartz cuvett with a path length of 1 cm. Circular dichroism spectra were recorded on a JASCO J-720WI equipped with a thermal regulator JASCO PTC348WI in a quartz cuvett with a path length of 0.1 cm. Mass spectra were measured on a Shimadzu KRATOS KOMPACT MALDI II Spectrometer.

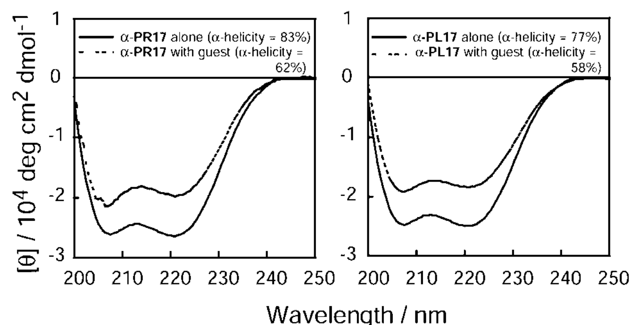

Figure 3. The circular dichroism spectra of **α-PR17** and **α-PL17** in the absence and presence of *n*-pentanol in the amide absorption region (200–250 nm) in Tris-HCl buffer (20 mmol dm^{-3}, pH 7.5) at 25 °C. [Peptide] = 50 μmol dm^{-3}, [*n*-pentanol] = 1 mmol dm^{-3}.

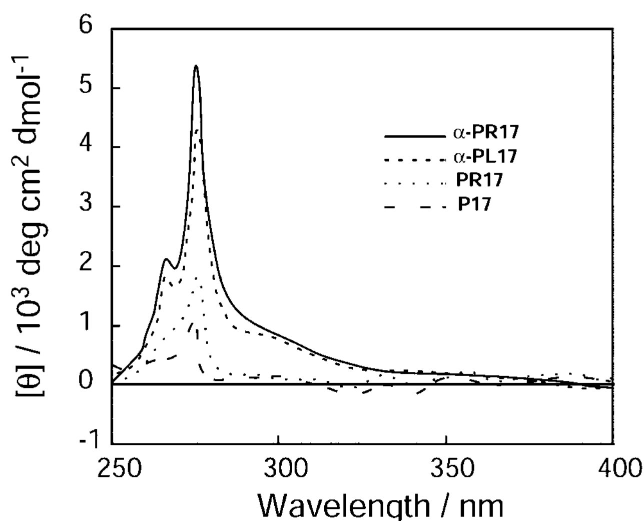

Figure 4. The induced circular dichroism (ICD) spectra of **α-PR17**, **α-PL17**, **PR17** and **P17** in the nitrobenzene and pyrene absorption region (250–400 nm) in Tris-HCl buffer (20 mmol dm^{-3}, pH 7.5) at 25 °C. [Peptide] = 125 μmol dm^{-3}.

Results and discussion

Structural analysis of peptide backbones in the CD-peptides

For the elucidation of the structural features of the CD-peptides and side chain configuration, circular dichroism spectra were measured. All the experiments were carried out in a buffer solution (20 mmol dm^{-3}, Tris-HCl, pH 7.5) at 25 °C, and the CD-peptide concentrations were 50 μmol dm^{-3} and 125 μmol dm^{-3} for measurement in the peptide bond region (200–250) and NB and pyrene absorption region (250–400 nm), respectively. Figure 2 shows the circular dichroism spectra (intensity unit: mean residual weight ellipticity) of amide bond region (200–250 nm) for the peptides **α-PR17**, **α-PL17**, **PR17**, and **P17**. All of them exhibited circular dichroism spectra of a typical α-helical pattern. The CD-peptides also exhibit circular dichroism in NB and pyrene absorption region (250–400 nm) with much smaller molar ellipticities than those of the amide region (200–250 nm) [23–25]. However, the α-helix contents of **α-PR17**, **α-PL17**, **PR17** and **P17** were estimated from the values of their mean residual weight ellipticities at 222 nm, $[\theta]_{222}$ [26]. The values were 83%, 77%, 51% and 52% for **α-PR17**, **α-PL17**, **PR17** and **P17** respectively. These results show that the helix contents of **α-PR17** and **α-PL17** are enough to maintain the appropriate location and proximity of α-CD and NB moieties, thus enabling these two moieties to form an intramolecular inclusion complex. Upon the addition of *n*-pentanol (1 mmol dm^{-3}) as an exogenous guest molecule, a significant decrease in the amide region (200–250 nm) of the circular dichroism spectra was observed for both **α-PR17** and **α-PL17** (Figure 3). That is, the helicity of **α-PR17** decreased from 83% to 62% and that of **α-PL17** decreased from 77% to 58%. On the other hand, no change in the helicity has been observed for the reference compounds **PR17** and **PL17**. This result is consistent with the result we observed previously [14], indicating that the inclusion of *n*-pentanol into the CD cavity may cause a displacement of the NB moiety from inside of the cavity to outside and destabilize the conformation of the CD-peptides, **α-PR17** and **α-PL17**, from α-helix to random coil. The NB can enter into the α-CD cavity because of its small size whereas the pyrene unit always remains outside of the CD cavity because of its large size to be accommodated inside of the α-CD cavity. No change in the spectra for reference peptides is reasonable since there is no CD and/ NB unit in those peptides. Figure 4 shows the induced circular dichroism (ICD) spectra (intensity unit: molar ellipticity) in the NB and pyrene absorption region (250–500 nm) of the CD-peptides and its reference peptides (125 μmol dm^{-3}) in the absence of guest molecule, *n*-pentanol. The ICD spectra of **α-PR17** decreased from 5330 deg cm^2 dmol^{-1} to 4300 deg cm^2 dmol^{-1} and that of **α-PL17** decreased from 4270 deg cm^2 dmol^{-1} to 3680 deg cm^2 dmol^{-1} (Figure 5) upon addition of *n*-pentanol. On the other hand, **PR17** and **P17** exhibit smaller ICD bands (1820 deg cm^2 dmol^{-1} for **PR17** and 1010 deg cm^2 dmol^{-1} for **P17**) compared to **α-PR17** and **α-PL17** at the absorption region of 250–400 nm (Figure 4), which might be due to the absorption of pyrene and NB moiety, and no change in their ICD spectra has been observed upon addition of *n*-pentanol. These observations suggest that the circular dichroism signals of **α-PR17** and **α-PL17** are mostly generated by the formation of an inclusion complex between NB and α-CD on the α-helix peptide and

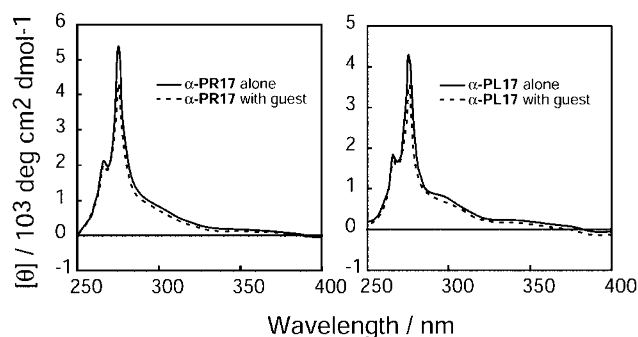

Figure 5. The ICD spectra of **α-PR17** and **α-PL17** alone and in the presence of *n*-pentanol in the nitrobenzene and pyrene absorption region (250–400 nm) in Tris-HCl buffer (20 mmol dm^{-3}, pH 7.5) at 25 °C. [Peptide] = 125 μmol dm^{-3}, [*n*-pentanol] = 5 mmol dm^{-3}.

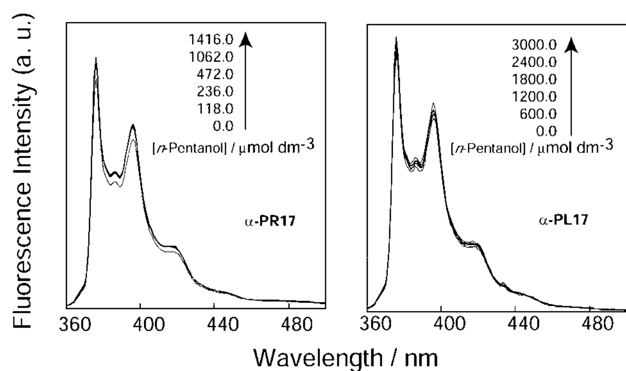

Figure 6. Fluorescence spectra of **α-PR17** and **α-PL17** in the presence and absence of *n*-pentanol in Tris-HCl buffer (20 mmol dm^{-3}, pH 7.5) at 25 °C. [Peptide] = 5 μmol dm^{-3}). Excitation wavelength is 345 nm.

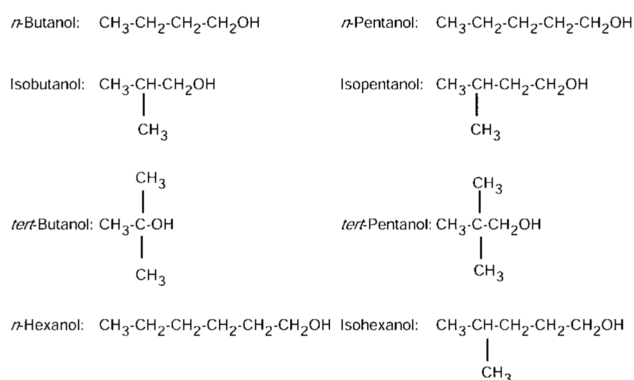

Figure 7. The structures of guest compounds.

exclusion of the NB group from the α-CD cavity results in decrease of the circular dichroism intensities. This result is consistent with the result we have reported that NB forms an inclusion complex with β-CD in aqueous solution [14].

Study of fluorescence quenching

Figures 6 shows the fluorescence spectra of the CD-peptides measured by excitation at 345 nm at 25 °C. Both of the CD-peptides, **α-PR17** and **α-PL17**, exhibit normal fluorescence with a peak at 376 nm and 396 nm. The fluorescence

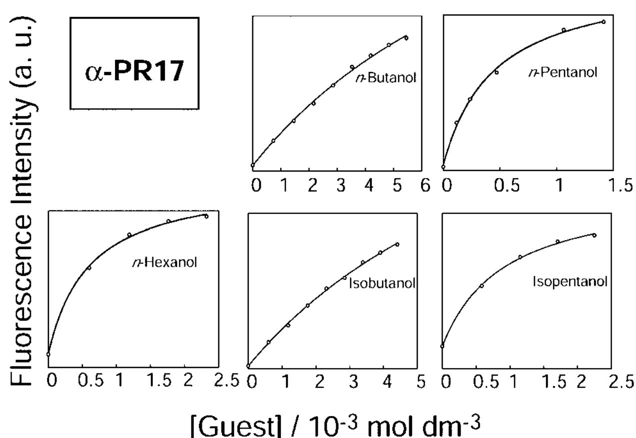

Figure 8. The plot of fluorescence intensity of **α-PR17** (5 μmol dm^{-3}) as a function of the guest concentration.

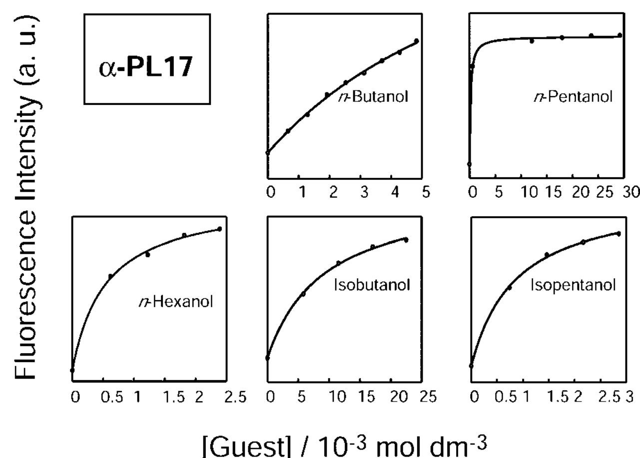

Figure 9. The plot of fluorescence intensity of **α-PL17** (5 μmol dm^{-3}) as a function of the guest concentration.

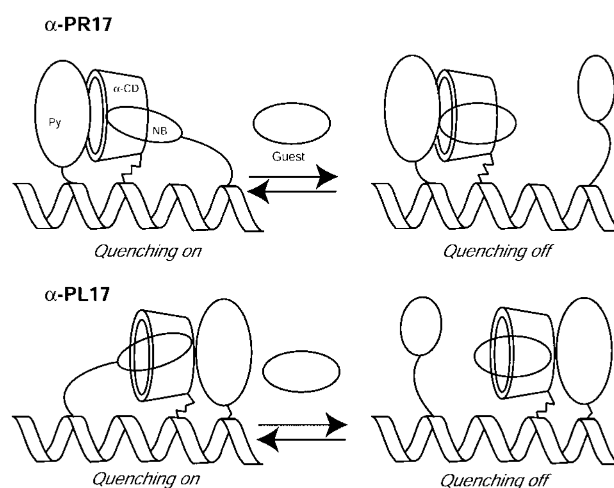

Figure 10. The schematic representation of the guest-induced structural change in the side chain of **α-PR17** and **α-PL17**.

emission of the CD-peptides increases with increasing the concentration of *n*-pentanol. This guest-induced enhancement in fluorescence intensity can be explained in terms of increased distance between pyrene and NB units. The NB unit that is included into the α-CD cavity is thought to be close to the pyrene unit so that fluorescence quenching occurs. Addition of an external guest into the aqueous solution causes exclusion of the NB moiety from inside to outside of the cavity and subsequently causes diminishment in fluorescence quenching process. So, the fluorescence intensity increases in the presence of an external stimulant molecule.

As described above, the fluorescence emission of the CD-peptides changes upon guest addition. It is interesting to determine binding constants of the CD-peptides for various guest molecules. In order to avoid peptide aggregation, the CD-peptide solutions were diluted to 5 μmol dm^{-3}. At this concentration, **α-PR17** and **α-PL17** are expected not to get aggregated since no concentration dependence of the CD-peptides has been observed in the range of 1–200 μmol. A series of short chain alcohols (Figure 7) have been chosen as guests for these two CD-peptides. Both of the CD-peptides increased their fluorescence intensity upon

Table 1. Binding constants of the peptides with short chain alcohols

CD-peptides	*n*-Butanol	Isobutanol	*tert*-Butanol	*n*-Pentanol	Isopentanol	*tert*-Pentanol	*n*-Hexanol	Isohexanol
α-PR17	90 ± 17	106 ± 16	–[a]	2233 ± 287	1087 ± 126	–[a]	1691 ± 227	–[a]
α-PL17	113 ± 17	108 ± 12	–[a]	5226 ± 537	1157 ± 120	–[a]	1844 ± 298	–[a]

[a] The binding constants are too small to be estimated correctly.

addition of the guest molecules, and the binding constants of CD-peptides for guest molecules were obtained from guest-induced variations of the emission intensity at 376 nm by the least-squares curve fitting analysis [27, 28] performed using the following Benesi–Hildebrand type equation [29] [Equation (1)] which holds under the conditions of a large excess of the guest and 1 : 1 host–guest stoichiometry,

$$I_{\text{obs}} = (I_{\text{host}} + I_{\text{com}}K_{\text{b}}[\text{guest}]_0)/(1 + K_{\text{b}}[\text{guest}]_0), \quad (1)$$

where I is the fluorescence intensity, I_{obs} for the sample, I_{host} for the host alone and I_{com} for the complex. K_{b} is the binding constant of the complex formation and $[\text{guest}]_0$ is the initial total concentration of the guest. Figures 8 and 9 show the fluorescence intensity at 376 nm of the CD-peptides as a function of guest concentration. The solid curves were obtained by the curve fitting analysis as described above. Binding constants of **α-PR17** and **α-PL17** for various guest molecules were summarized in Table 1. For three series of alcohols the order of binding constants is *normal* > *iso* > *tert*. The results are consistent with the conclusion drawn by the examination of the molecular models (CPK) that *n*-alkanols are easy to be included in the α-CD cavity in comparison with the branched alkanols [10]. Between two isomers of the CD-peptides, **α-PR17** showed the smaller binding constants for each of the alcohols. This result is consistent with the extremely high helix content of this CD-peptide (83%), indicating that **α-PR17** maintains the most stable intramolecular inclusion complex between α-CD and NB moieties. The remarkable difference between **α-PR17** and **α-PL17** in the guest binding is related to the location of the functional units along the α-helix peptide. Namely, placement of the NB group at the *C*-terminal side of the CD-peptide is favorable for the formation of intramolecular inclusion complex between the NB and the CD units than at the *N*-terminal side, and consequently the tight intramolecular complex formation stabilizes α-helix formation of the CD-peptide [13]. These CD-peptide hybrids bind short chain compounds with different binding constants, even if the differences between those compounds are a single carbon molecule. These results demonstrate this system is applicable to sense the small sized molecules. The guest-induced diminishment in fluorescence quenching is presented schematically in Figure 10.

Conclusion

We have succeeded in constructing two novel CD-peptide isomers that can be used for detecting small guests like linear chain alcohols. Branched compounds are less effective in changing the fluorescence intensity, indicating that the α-CD cavity prefers the linear-shaped molecules. **α-PR17** showed the highest α-helix content, indicating that the location of the side chain functional group is an important factor to maintain α-helix conformation of the peptide. The CD-peptide systems are responsive to the external stimulant molecules and their fluorescence intensity increased with increasing guest concentration. This phenomenon can be explained as guest responsive diminishment in fluorescence quenching. These results demonstrate the applicability of this CD-peptide system to the construction of small-sized molecule responsive devices or materials.

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References

1. T. Aoyagi, A. Nakamura, H. Ikeda, T. Ikeda, H. Mihara, and A. Ueno: *Anal. Chem.* **69**, 659 (1997).
2. H. Ikeda, M. Nakamura, N. Ise, N. Oguma, A. Nakamura, T. Ikeda, F. Toda, and A. Ueno: *J. Am. Chem. Soc.* **118**, 10980 (1996).
3. A. Ueno, I. Suzuki, and T. Osa: *Anal. Chem.* **62**, 2461 (1990).
4. A. Ueno, T. Kuwabara, A. Nakamura, A. Ueno, and F. Toda: *Nature* **356**, 136 (1992).
5. A. Ueno, I. Suzuki, and T. Osa: *J. Am. Chem. Soc.* **111**, 6391 (1989).
6. T. Kuwabara, H. Nakajima, M. Nanasawa, and A. Ueno: *Anal. Chem.* **71**, 2844 (1999).
7. Q. X. Guo, Z. Z. Li, T. Ren, X. Q. Zhu, and Y. C. Liu: *J. Inclusion Phenom. Mol. Recog.* **17**, 149 (1994).
8. A. Ueno, F. Moriwaki, T. Osa, F. Hamada, and K. Murai: *Tetrahedron* **43**, 1571 (1987).
9. A. Ueno, F. Moriwaki, T. Osa, F. Hamada, and K. Murai: *J. Am. Chem. Soc.* **110**, 4323, (1988).
10. K. Hamasaki, A. Ueno, and F. Toda: *J. Chem. Soc., Chem. Commun.* 331 (1993).
11. K. Hamasaki, A. Ueno, F. Toda, I. Suzuki, and T. Osa: *Bull. Chem. Soc. Jpn.* **67**, 516, (1994).
12. M. A. Hossain, K. Hamasaki, H. Mihara, and A. Ueno: *Chem. Lett.* 252 (2000).
13. M. A. Hossain, S. Matsumura, T. Kanai, K. Hamasaki, H. Mihara, and A. Ueno: *J. Chem. Soc., Perkin Trans. 2* 1527 (2000).
14. M. A. Hossain, K. Hamasaki, Keiko Takahashi, H. Mihara, and A. Ueno: *J. Am. Chem. Soc.* **123**, 7435 (2001).
15. T. Toyoda, S. Matsumura, H. Mihara, and A. Ueno: *Macromol. Rapid. Commun.* **21**, 485 (2000).
16. S. Matsumura, S. Sakamoto, A. Ueno, and H. Mihara: *Chem. Eur. J.* **6**, 1781, (2000).
17. H. Mihara, Y. Tanaka, T. Fujimoto, and N. Nishino: *J. Chem. Soc., Perkin Trans. 2* 1133, (1995).
18. P. Y. Chou, and G. D. Fasman: *Annu. Rev. Biochem.* **47**, 251 (1978).
19. S. Dao-pin, U. Sauer, H. Nicholson, and B. W. Matthews: *Biochemistry* **30**, 7148 (1991).

20. S. Marqusee, and R. L. Baldwin: *Proc. Natl. Acad. Sci. USA* **84**, 8898 (1987).
21. J. W. Bryson, S. F. Betz, H. S. Lu, D. J. Suich, H. X. Zhou, K. T. O'Neil, and W. F. DeGrado: *Science* **270**, 935 (1995).
22. E. Atherton, and R. C. Sheppard: *Solid Phase Synthesis: A Practical Approach*. IRL Press, Oxford (1998).
23. N. Fujii, A. Otaka, O. Ikemura, K. Akaji, S. Funakushi, Y. Hayashi, Y. Kuroda, and H. Yajima: *J. Chem. Soc., Chem. Commun.* 274 (1987).
24. H. Mihara, Y. Tanaka, T. Fujimoto, and N. Nishino: *J. Chem. Soc., Perkin Trans. 2* 1915 (1995).
25. H. Mihara, J. Hayashida, H. Hasagawa, H. Ogawa, T. Fujimoto, and N. Nishino: *J. Chem. Soc., Perkin Trans. 2* 517 (1997).
26. J. M. Scholtz, H. Qian, E. J. York, J. M. Stewart, and R. L. Baldwin: *Biopolymers* **31**, 1463 (1991).
27. S. Hamai: *J. Phys. Chem.* **93**, 2047 (1989).
28. A. Nakamura, K. Saitoh, and F. Toda: *Chem. Phys. Lett.* **187**, 110 (1991).
29. H. A. Benesei, and J. H. Hildebrand: *J. Am. Chem. Soc.* **71**, 2703 (1949).