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Supramolecular Chemistry of Cyclodextrin-Peptide Hybrids: Azobenzene-Tagged Peptides

AKIHIKO UENO[1]*, TOMOKO SHIMIZU[1], HISAKAZU MIHARA[1], KEITA HAMASAKI[1] and K. PITCHUMANI[2]

[1]*Department of Bioengineering, Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology, Yokohama, Japan;* [2]*Department of Chemistry, School of Chemistry, M.K. University, Madurai-625 021, Tamil Nadu, India*

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Abstract

AC17, which is composed of 17 amino acids and has an azobenzene moiety but has no cyclodextrin (CD) unit in the side chain, exhibits 54% helix content. However, ACα17, which has both *trans*-azobenzene and α-CD, shows 82% helix content. This result suggests that the helix structure is stabilized by host (CD)-guest (azobenzene) bridge in the side chain of the peptide. The helix content changed by *trans-cis* photoisomerization as shown by 64% helix content for ACα17 in its *cis* form. This result suggests that *cis*-azobenzene unit is excluded from the α-CD cavity, thus resulting in the smaller helix content. The helix contents for ACβ17, which has both azobenzene and β-CD, are 94% in the *cis* form and 87% in the *trans* form, suggesting that the *cis* form is included in the β-CD cavity. Azobenzene-tagged CD-peptide hybrids with histidine unit were also prepared and photoregulation of catalytic activity in ester hydrolysis was examined.

Introduction

Cyclodextrins (CDs) form inclusion complexes with a variety of organic compounds in aqueous solution [1]. Base on this property, they have been used as molecular capsules to dissolve many hydrophobic compounds in water. CDs have been converted into talented compounds such as catalysts and sensors by modification with appropriate functional units [2–4], and recently cyclodextrin-peptide hybrids have been prepared as new entities in which α-helix peptide and CD act as scaffold and guest binding site, respectively [5–7]. In such CD-peptide hybrids, CD and functional units are placed at three-dimensionally appropriate sites and effective sensors and catalysis systems have been constructed. In this study, azobenzene unit was used as a functional unit, which undergoes trans-cis photoisomerization and changes the peptide conformation [8] Furthermore photoregulation of catalytic reaction was attempted by introducing catalytic group in the side chain. Azobenzene photoisomerization is reversible as shown in Chart 1 and this on-off switching phenomenon has been used to phtotoregulate complexation [9, 10] and catalytic reactions [11, 12] of CD-related systems.

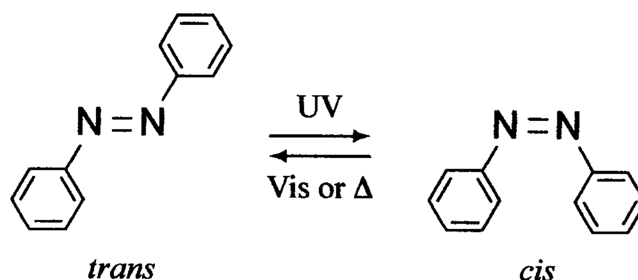


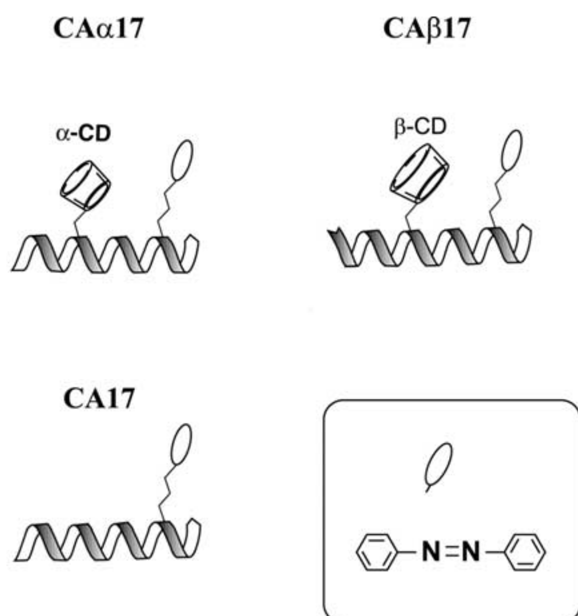
Chart 1.

Experimental

The CD-peptide hybrids were prepared by Fmoc method and the details in the synthesis will be shown elsewhere. Azobenzene was introduced as *p*-(phenylazo)benzoyl unit in the side chain of lysine by an amide bond. CD unit was introduced by the reaction of 6-deoxy-6-amino-CD and the carboxyl group of glutamic acid side chain of the peptides through an amide bond. CAα17, CAβ17, CA17 and C17 were used to examine the effect of photoisomerization on the peptide conformation. CAβ1, CAβ2, NAβ1, and NAβ2 were prepared to examine photoregulation in hydrolysis of ester (*p*-nitrophenyl acetate). Trans-cis photoisomerization was performed by xenon lamp using Corning 7–37 filter to isolate the light of 310 nm < λ < 390 nm. The peptide concentration was 50 μM for absorption and circular dichroism measurements.

Catalytic activities of CAβ1, CAβ2, NAβ1 and NAβ2 (50 μM) were measured at 400 nm in 50 mM phosphate buffer (pH 8.0) at 25 °C using 3 mM *p*-nitrophenyl acetate and the rate of hydrolysis was estimated by the absorption change min^{-1}.

* Author for correspondence.



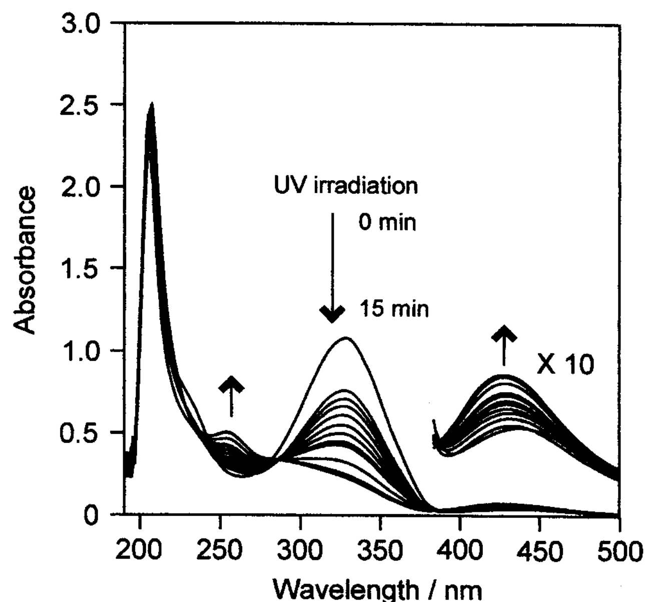


Figure 2. Absorption spectral changes of CAα17 (50 μM) associated with trans-cis photoisomerization in 20mM Tris-HCl buffer (pH 7.4) at 25 °C.

amino acid sequences

CAα17	Ac-AEAAAREE(8, α-CD)AAREAAK(15, Az)RA-NH2
CAβ17	Ac-AEAAAREE(8, β-CD)AAREAAK(15, Az)RA-NH2
CA17	Ac-AEAAAREA(8)AAREAAK(15, Az)RA-NH2
C17	Ac-AEAAAREA(8)AAREAAA(15)RA-NH2

Figure 1. Structures of designed peptides.

Results and discussion

The CD-peptide hybrids, which are composed of 17 amino units, were prepared by Fmoc method (Figure 1). CAα17 has both α-CD unit and azobenzene moiety in the side chain of peptide with the azobenzene moiety in the C-terminal side of the peptide with respect to α-CD. On the othert hand, CAβ17 has β-CD unit in place of α-CD of CAα17. CA17 has no CD unit and has an azobenzene moiety in the corresponding position of CAα17 and CAβ17. CA has neither azobenzene moiety nor CD unit and acts as the reference peptide. In CAα17 and CAβ17, CD unit is separated from azobenzene moiety by two helix turns. All these peptides were designed to form three salt bridges between carboxylate of glutamic acid and amino cation of arginine to stabilize the α-helix.

Absorption spectra of azobenzene-tagged peptides (1 mM) were measured in 20 mM Tris-HCl buffer (pH 7.4) (Figure 2). We observed that the absorption around 330 nm decreases by UV light (310 nm $< \lambda <$ 390) irradiation. When the absorption around the wavelength is assumed to be 0 for cis-azobenzenene, the cis contents at the photostationary state are 82, 86, and 79% for CAα17, CAβ17, and CA17, respectively.

The half lifetimes of these peptides measured at 35 °C were 82, 44 and 83 h for CAα17, CAβ17 and CA17, respectively.

We prepared several peptides bearing CD and dansyl units in their side chains as chemosensors for molecule detection [5]. The results indicated that intramolecular host-guest complexation (host-guest bridge) occurring between dansyl and CD units in the side chain enhances helix content of the peptides. This stabilization effect by host-guest bridge formed in the side chain was more remarkably shown in the peptide bearing γ-CD, and dansyl moiety and cholic acid unit [13]. In this case, strong host-guest complexation occurs between γ-CD and cholic acid with the helix content of 78% while the reference peptide without cholic acid unit exhibits only 46% as the helix content.

Figure 3 shows circular dichroism spectra of the azobenzene-tagged CDs and the reference peptide. The helices of azobenzene-tagged CD-peptide hybrids are likely to be stabilized by the intramolecular host-guest bridge between CD and the azobenzene moiety. For example, AC17, which has an azobenzene moiety without CD, exhibits 54% as the helix content. However, ACα17, which has both trans-azobenzene and α-CD, shows 82% helix content. Furthermore, ACβ17, which has β-CD instead of α-CD of ACα17, exists as the peptide with 87% helix content. This helix content changed by trans-cis photoisomerization as shown by 64% helix content for CAα17 in its cis form. This result suggests that cis-azobenzene unit is excluded from the α-CD cavity, thus resulting smaller helix content. The unexpected result is 94% helix content for ACβ17 in the cis form, suggesting that the cis form is included in the β-CD cavity in this case.

Figure 4 shows circular dichroism spectra of CAα17 and CAβ17 in 20 mM Tris-HCl buffer at 25 °C. Trans-CAα17 exhibits a peak around 325 nm and trough around 450 nm. On the other hand, trans-CAβ exhibits similar dichroism

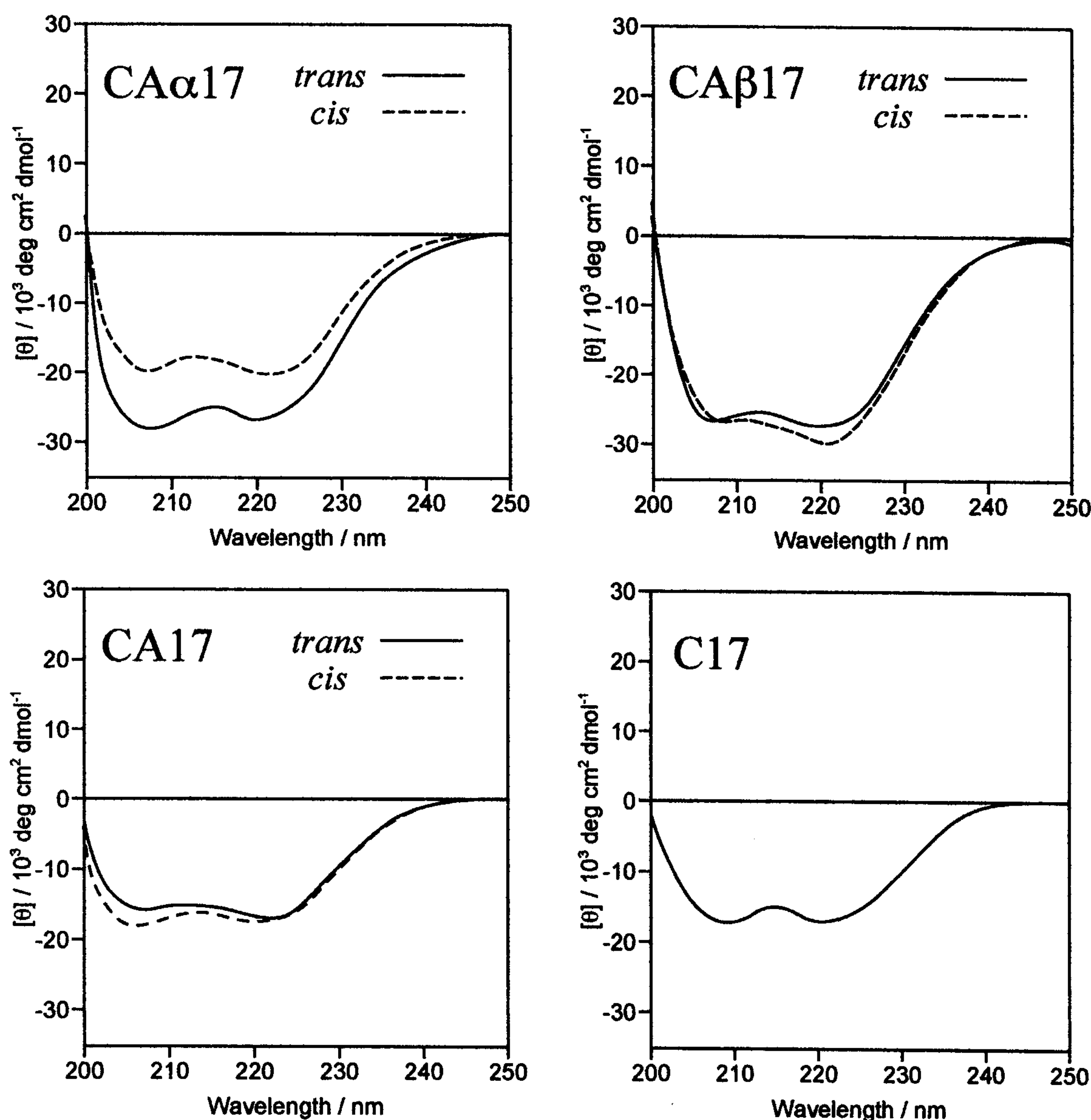


Figure 3. Circular dichroism spectra of designed peptides (10 μM) in 20 mM Tris-HCl buffer (pH 7.4) at 25 °C.

bands to those of trans-CAα17. It is noted that remarkable dichroism bands were observed even after photoisomerization. These phenomena suggest that cis-azobenzene is excluded from the α-CD cavity in the case of CAα17 while it is still bound to β-CD unit in the case of CAβ17.

Photoregulation of catalytic activity was attempted by using histidine-incorporated azobenzene-tagged CD-peptides CAβ1, CAβ2, NAβ1, and NAβ2 (Figure 5). CAβ1 and CAβ2 have an azobenzene moiety in the C-terminal side of the peptides. In CAβ1 and NAβ1, the CD unit and azobenzene moiety are separated by one-pitch of α-helix while in CAβ2 and NAβ2 they are separated by two pitches of α-helix. In these systems, hydrolysis of p-nitrophenyl acetate may proceed with imidazole of histidine as a catalytic group. The cis contents of these peptides are about 85, 81, 83, and 84% for CAβ1, CAβ2, NAβ1, and NAβ2, respectively. The half lifetimes at 35 °C are 46, 116, 44, and 39 h for CAβ1, CAβ2, NAβ1, and NAβ2, respectively. The α-helix contents of the cis form are almost the same as those of the trans one, as shown by 53, 61, 51, and 51% for CAβ1, CAβ2, NAβ1, and NAβ2, respectively and there exhists no indication to support the host-guest bridge formation in their cis forms. However, we observed the circular dichroism bands for the peptides after photoirradiation. So, it is not clear why host-guest bridge effects is not remarkable in this case. The rates of hydrolysis, which were measured by monitoring the absorption intensity at 400 nm of p-nitrophenolate ion, are shown in Figure 6. In any case, cis form gives larger rate than the trans one. The simple explanation for this phenomenon is that cis-azobenzene is excluded from the cavity and then the cavity is available for substrate to be included and attacked by the imidazole catalytic group. However, as previously shown, the cis-form seems to be capable of binding β-CD unit, and therefore further work is needed for this photo-induced rate acceleration.

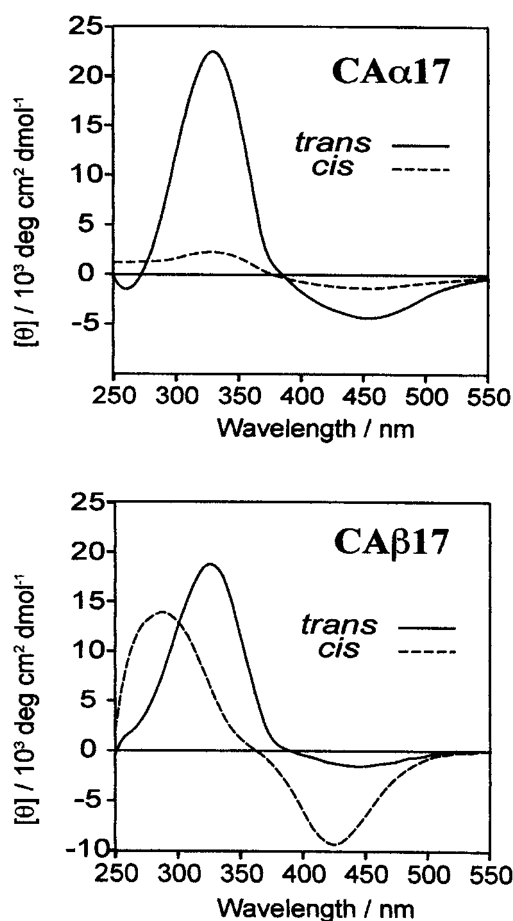


Figure 4. Circular dichroism spectra of CAα17 and CAβ17 (50 μM) before and after photoirradiation in 20 mM Tris-HCl buffer (pH7.4) at 25 °C.

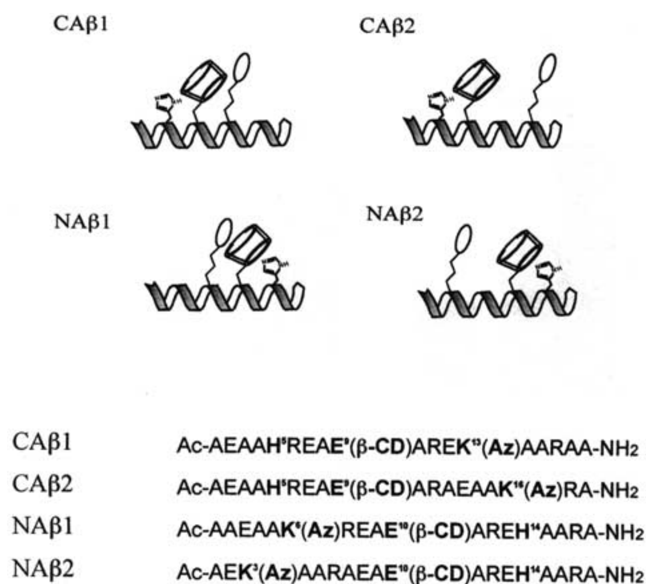


Figure 5. Structures of peptides designed as photoregulated catalytic systems.

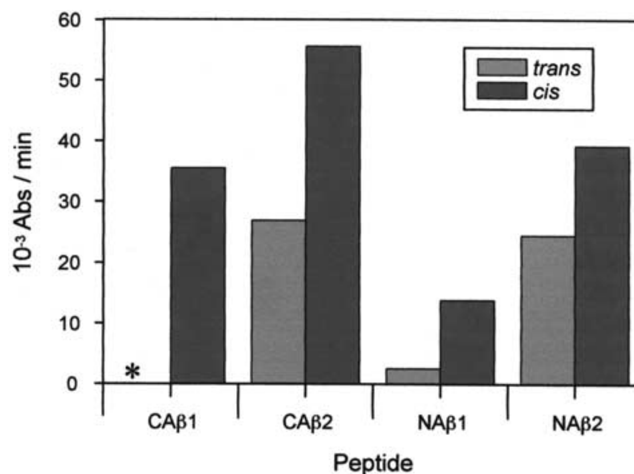


Figure 6. Catalytic activity of the designed peptides. Rate of hydrolysis were determined by the change of the absorbance at 400 nm of *p*-nitrophenolate anion (product) in 50 mM phosphate buffer (pH 8.0) at 25 °C. [*p*-nitophenyl acetate] = 3 mM; [peptides] = 50 μM.

Conclusion

It was found that intramolecular host-guest bridge is important to stabilize the α-helix. This was typically shown in CAα17, which forms inclusion complex between azobenzene moiety and α-CD unit. In this case, the intramolecular host-guest bridge is cancelled by exclusion of the cis-azobenzenene moiety from the α-CD cavity. In the case of CAβ17, there was an indication that the cis-azobenzene moiety binds CD unit. When histidine is incorporated into the CD-peptide hybrids, it was shown that ester hydrolysis is accelerated by photoirradiation.

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