Polymer Journal, Vol. 39, No. 1, pp. 16–17 (2007)

SHORT COMMUNICATIONS

Regulation of Self-assembling Process of a Cationic β-Sheet Peptide by Photoisomerization of an Anionic Azobenzene Derivative

Tomoyuki KOGA,[1] Mitsuru USHIROGOCHI,[1] and Nobuyuki HIGASHI[1,†]

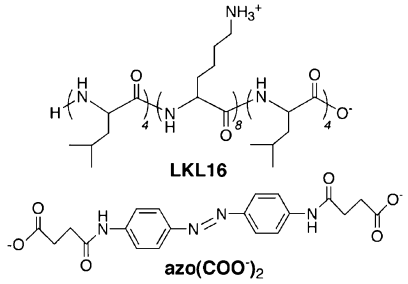
[1]*Department of Molecular Science & Technology, Faculty of Engineering, Doshisha University, Kyotanabe, Kyoto 610-0321, Japan*

(Received September 13, 2006; Accepted October 6, 2006; Published November 16, 2006)

KEY WORDS Nanofiber / Self-assembly / Photoisomerization / β-Sheet / Amphiphilic Peptide / Azobenzene /
[doi:10.1295/polymj.PJ2006110]

Advances in molecular self-assembly are essential to establish practical guidelines to fabricate novel nanostructured materials. Our focus has been on artificial peptides as building blocks for the design of functional materials.[1] The interest in peptides is based on their ability to hierarchically self-organize into well-defined three-dimensional architectures than those which can be generated from conventional synthetic block polymers. In particular, much attention has been focused on the biological β-sheet motif because of their association with neurodegenerative diseases like Alzheimer's, and as a building unit in the design of supramolecular nanofibers.[2–7] Recently, we have reported that the triblock-type amphiphilic peptide, $L_4K_8L_4$ (LKL16), is an excellent β-sheet foldamer and can be hierarchically self-assembled into amyloid-like nanofibers upon partial neutralization of the Lys residues at around pH 9.[6,7] However, it is still difficult to control the spontaneous aggregation processes of peptides. Creating novel and facile methodology to regulate the self-assembling processes by external signals, such as light,[8] is therefore valuable to facilitate a bottom-up nanotechnology.

Here we report a photo-responsive molecular system, in which self-assembling process of the cationic peptide into β-sheet nanofibers is regulated by photoisomerism of the exogenous anionic azobenzene derivative. Azobenzene is well known to change the overall geometry from planar to non-planar and the polarity of azo-linkage by *trans-cis* photoisomerization. In previous studies concerning azobenzene-conjugated peptide molecular systems, such changes in physical properties of azobenzenes have been utilized as a photosensitive switch to regulate conformation,[9] high-order structure,[10] and hydrogen-bonding pattern[11] of peptides. To our knowledge, however, it is the first report that the process of nanofiber formation is controlled by using a polarity change of photochromic additive, which causes slight change of total hydrophobicity of the target peptide.



An azobenzene derivative with two carboxylate anions, azo$(COO^-)_2$, was employed as a control agent for self-assembly of the cationic LKL16. The preparation of LKL16 and its self-assembling properties in water have been described elsewhere.[7] The azo$(COO^-)_2$ was synthesized by reacting the 4,4′-azodianiline with succinic anhydride.

Photoisomerization of the azo$(COO^-)_2$ was first of all investigated in the presence of LKL16 at pH 9.2. In this condition, the azo$(COO^-)_2$ couples with the peptide, which is supported by FT-IR study (described later). The azo$(COO^-)_2$ existed in *trans*-form with an absorption at 370 nm upon dark adaptation. In contrast, UV irradiation of *trans*-azo$(COO^-)_2$ at 365 nm (4W UV lamp) caused a rapid conversion to the *cis*-isomer within 1 min, as evidenced by a *ca.* 60% reduction in the peak intensity at 370 nm and an increase in the absorbance at 270 and 500 nm. Such photoisomerism of the azo$(COO^-)_2$ affected the conformational properties of LKL16. Figure 1 shows the time-dependence of the circular dichroism (CD) spectra of the LKL16 with *trans*- (a) and *cis*-isomers (b) in borate buffer (5 mM) at pH 9.2. The CD spectra initially showed mixed patterns of α-helix and random coil structures with two negative maxima at 220 nm and 200 nm, and then revealed a change typical for a β-sheet structure (single negative maximum at 215 nm) after 4 h in both cases. On the other hand, interestingly, the transition kinetics into β-sheet was quite different between *trans*- and *cis*-isomers, as plotted in Figure 1c. In the presence of *cis*-isomer, the $[\theta]_{215}$ value decreased sigmoidally and the β-sheet formation was found to accelerate at around 2–3 h. Such sigmoidal kinetics is similar to that for the azo$(COO^-)_2$-free LKL16 and authentic amyloids, and can be explained as a nucleation-dependent polymerization model, which has been proposed to explain the mechanisms of amyloid formation by a variety of disease-related peptides.[12,13] This concept has been well established as the fundamental mechanism of crystal growth.[14] Namely, nucleus formation require a step involving association with peptide monomers that is thermodynamically unfavorable, and as a result an induction period has been observed before conformational transition. In contrast, a remarkable acceleration of the α(random)-to-β transition of LKL16 was observed in the presence of *trans*-azo$(COO^-)_2$, and the lag phase was no longer in detectable. These differences in transition kinetics are probably due to the polarity of azobenzene moiety that bound to Lys residues. Since the *cis*-form of azobenzene group exhib-

†To whom correspondence should be addressed (Tel: +81-774-65-6622, Fax: +81-774-65-6844, E-mail: nhigashi@mail.doshisha.ac.jp).

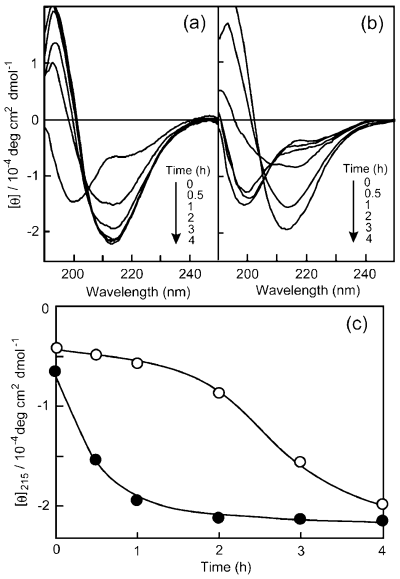


Figure 1. CD spectral changes of LKL16 in the presence of *trans*-azo(COO^-)$_2$ (a) and *cis*-azo(COO^-)$_2$ (b) in borate buffer (containing 5% TFE) at pH 9.2. The peptides were incubated for the time indicated (0–4 h). (c) Time dependences of $[\theta]_{215}$ values at these conditions. (○) *cis*-isomer; (●) *trans*-isomer. [LKL16] = 40 μM. [azo(COO^-)$_2$] = 80 μM.

its a larger dipole moment (3 D) across the azo-linkage compared to the *trans*-form (0–0.5 D), the *trans*-azo(COO^-)$_2$ is more hydrophobic than the *cis*-one.[15] On the other hand, it has been considered for *in vivo* system that hydrophobic defects, derived from hydrophobic amino acid sequences, are important factors to induce the association of many amyloidogenic peptides. Therefore it can be assumed that the hydrophobic *trans*-azo(COO^-)$_2$ triggers a rapid formation of β-sheet nucleus, which leads to a disappearance of the lag phase, by increasing the total hydrophobicity of LKL16 in contrast to the case of relatively hydrophilic *cis*-isomer. In fact, similar phenomenon has been reported for Aβ peptide, at which the addition of the fibrillar seed material to a solution of monomeric Aβ reduces or entirely eliminates the lag phase in conformational change.[13] It should be noted that the acceleration of β-sheet formation by *trans*-azo(COO^-)$_2$ was concentration-dependent, and the lag phase was observed even with the *trans*-isomer when the concentration was very low (0.08 μM).

Atomic force microscopy (AFM) measurements also demonstrate the difference in self-assembling process of LKL16 by the *trans-cis* isomerism of exogenous azo(COO^-)$_2$ (Figure 2). For LKL16/*trans*-azo(COO^-)$_2$ system, the peptide was found to self-assemble into β-sheet nanofiber even at 1 h incubation (Figure 2a), although such nanofiber formation was not observed in LKL16/*cis*-azo(COO^-)$_2$ mixture (Figure 2c). Note that small amount of plate-shape aggregates were only observed in the image (c). At 4 h after incubation, however, the LKL16 formed well-developed and organized nanofibers in both cases of *trans*- and *cis*-isomers (Figure 2b and 2d). These nanofibers possess diameters of *ca.* 5–6 nm (from height analysis) and lengths in excess of 1 μm. Thus, it seems that photoisomerization of azo(COO^-)$_2$ affects only the self-assembling process of the cationic LKL16. In addition, FT-IR spectrum (KBr method) of the nanofiber, which was separated by centrifugation after 24 h, showed characteristic absorption of aromatic ring at 1600 cm^{-1}, demonstrating the complexation of azo(COO^-)$_2$ with LKL16. These results also well correspond

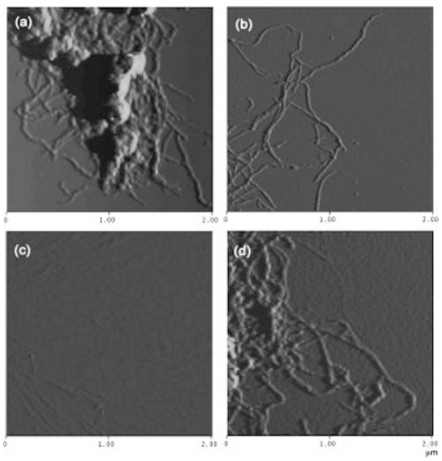


Figure 2. Amplitude AFM images ($2 \times 2\,\mu m^2$) of LKL16 in the presence of *trans*-azo(COO^-)$_2$ (a and b) and *cis*-azo(COO^-)$_2$ (c and d) obtained after incubation for 1 h (a and c) and 4 h (b and d) at pH 9.2.

to the results of CD studies.

In this communication, we have reported on a novel method to regulate the self-assembling process of peptide into β-sheet nanofibers by *trans-cis* isomerism of photochromic additive. The ability to control the peptide self-assembly should be useful for the design of bio-related nanomaterials. Furthermore, our approach is also interesting as an amyloid model to understand a nucleation-dependent polymerization mechanism for fibril formation.

Acknowledgment. This work was supported in part by a grant-in-aid for scientific research (No. 17710104) from the Japan Society for the Promotion of Science, and by the Academic Frontier Research Project on "New Frontier of Biomedical Engineering Research" of Doshisha University & Ministry of Education, Culture, Sports, Science and Technology.

REFERENCES

1. N. Higashi, T. Koga, M.-a Morikawa, and M. Niwa, in "Handbook of Surface and Interfaces of Materials," H. S. Nalwa, Ed., Academic Press, CA, 2001, vol. 5, p 167.
2. J. D. Sipe, *Crit. Rev. Clin. Lab. Sci.*, **31**, 325 (1994).
3. J. W. Kelly, *Curr. Opin. Struc. Biol.*, **6**, 11 (1996).
4. Y. Takahashi, A. Ueno, and H. Mihara, *ChemBioChem*, **3**, 637 (2002).
5. H. Yokoi, T. Kinoshita, and S. Zhang, *Proc. Natl. Acad. Sci. U.S.A.*, **102**, 8414 (2005).
6. T. Koga, M. Matsuoka, and N. Higashi, *J. Am. Chem. Soc.*, **127**, 17596 (2005).
7. T. Koga, M. Higuchi, T. Kinoshita, and N. Higashi, *Chem. Eur. J.*, **12**, 1360 (2006).
8. C. J. Bosques and B. Imperial, *J. Am. Chem. Soc.*, **125**, 7530 (2003).
9. O. Pieroni, J. L. Hoben, A. Fissi, P. Constantino, and P. Ciardelli, *J. Am. Chem. Soc.*, **102**, 5913 (1980).
10. M. Higuchi and T. Kinoshita, *J. Photochem. Photobiol., B*, **42**, 143 (1998).
11. M. S. Vollmer, T. D. Clark, C. Steinem, and M. R. Ghadiri, *Angew. Chem., Int. Ed.*, **38**, 1598 (1999).
12. A. Lomakin, D. S. Chung, G. B. Benedek, D. A. Kirschner, and D. B. Teplow, *Proc. Natl. Acad. Sci. U.S.A.*, **93**, 1125 (1996).
13. J. D. Harper and P. T. Lansbury, *Annu. Rev. Biochem.*, **66**, 385 (1997).
14. D. M. Blow, N. E. Chayen, L. F. Lloyd, and E. Saridakis, *Protein Sci.*, **3**, 1638 (1994).
15. H. Rau, in "Photochromism. Molecules and Systems," H. Durr and H. Bouas-Laurent, Ed., Elsevier, Amsterdam, 1990, p 165.