ORGANIC LETTERS

2006
Vol. 8, No. 2
285−287

Reversible Photoregulation of Helical Structures in Short Peptides under Indoor Lighting/Dark Conditions

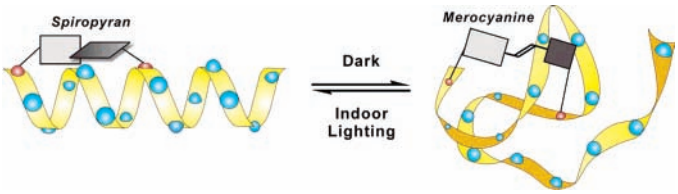
Kazuhisa Fujimoto,* Miwo Amano, Yohei Horibe, and Masahiko Inouye*

Faculty of Pharmaceutical Sciences, University of Toyama, Sugitani 2630, Toyama 930-0194, Japan

fujimoto@ms.toyama-mpu.ac.jp

Received November 1, 2005

ABSTRACT



A photochromic cross-linking agent with a spiropyran skeleton was developed for the reversible photoregulation of helical structures in short peptides. The helical contents of the cross-linked peptides could be regulated by ambient light and dark conditions at room temperature. This switching of the helical contents could be repeated several times without substantial loss of any activity.

In proteins, secondary structures, particularly helices, critically relate to molecular recognition events such as protein−protein interactions. In general, particular amino acid sequences that exist as helices in proteins usually adopt the random-coiled structures in their "isolated" short peptide states.[1] Thus, special attention has been paid to external stabilization of the secondary structures.[2] Recently, we reported the effective stabilization of α-helical structures in short peptides by using acetylenic cross-linking agents.[3] The cross-linked peptides showed a high α-helical content, and the α-helices survived up to a substantially elevated temperature. In addition to such stabilizations, the reversible photoregulation of helical structures is a topic of great interest and may allow the control of biological recognition events in the future.[4]

Woolley et al. recently reported an interesting system for the photoregulation of the helical structures of peptides that involved the use of azobenzene as a photochromic cross-linking agent.[5] Azobenzenes chiefly exist in the *trans* forms in the dark and isomerize to the *cis* forms under visible light irradiation. Both the *trans* and *cis* forms weakly absorb visible light in a relatively wide wavelength region. Hence, intensive irradiation of the *trans* forms by monochromatic visible light is required for the selective, but not predominant, formation of the *cis* forms by using special and expensive instruments. However, such irradiation may not only result in photobleaching of the cross-linked molecules but also influence other biomolecules and tissues in cells when it is applied in vivo.

(1) Sewald, N., Jakubke, H.-D., Eds.; *Peptides: Chemistry and Biology*; Wiley-VCH: Weinheim, 2002; Chapter 3.

(2) Recent papers: (a) Futaki, S.; Kiwada, T.; Sugiura, Y. *J. Am. Chem. Soc.* **2004**, *126*, 15762−15769. (b) Lin, J. C.; Barua, B.; Andersen, N. H. *J. Am. Chem. Soc.* **2004**, *126*, 13679−13684. (c) Verma, A.; Nakade, H.; Simard, J. M.; Rotello, V. M. *J. Am. Chem. Soc.* **2004**, *126*, 10806−10807. (d) Cline, D. J.; Thorpe, C.; Schneider, J. P. *J. Am. Chem. Soc.* **2003**, *125*, 2923−2929. (e) Neidigh, J. W.; Fesinmeyer, R. M.; Andersen, N. H. *Nat. Struct. Biol.* **2002**, *9*, 425−430. (f) Schafmeister, C. E.; Po, J.; Verdine, G. L. *J. Am. Chem. Soc.* **2000**, *122*, 5891−5892.

(3) Fujimoto, K.; Oimoto, N.; Katsuno, K.; Inouye, M. *Chem. Commun.* **2004**, 1280−1281.

(4) For the photoregulation of helical structures on polypeptides by multiple modifications with photochromic compounds as a pendant style, see: (a) Pieroni, O.; Fissi, A.; Angelini, N.; Lenci, F. *Acc. Chem. Res.* **2001**, *34*, 9−17. (b) Inouye, M. In *Organic Photochromic and Thermochromic Compounds*; Crano, J. C., Guglielmetti, R. J., Eds.; Plenum: New York, 1999; Vol. 2, pp 393−402. (c) Goodman, M.; Kossoy, A. *J. Am. Chem. Soc.* **1966**, *88*, 5010−5015.

(5) (a) Woolley, G. A. *Acc. Chem. Res.* **2005**, *38*, 486-493. (b) Zhang, Z.; Burns, D. C.; Kumita, J. R.; Smart, O. S.; Woolley, G. A. *Bioconjugate Chem.* **2003**, *14*, 824−829. (c) Kumita, J. R.; Flint, D. G.; Smart, O. S.; Woolley, G. A. *Protein Eng.* **2002**, *15*, 561−569. (d) Flint, D. G.; Kumita, J. R.; Smart, O. S.; Woolley, G. A. *Chem. Biol.* **2002**, *9*, 391−397. (e) Kumita, J. R.; Smart, O. S.; Woolley, G. A. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 3803−3808.

10.1021/ol0526524 CCC: $33.50
Published on Web 12/14/2005

Spiropyrans are another representative class of photochromic molecules that isomerize into merocyanine forms.[6] The following photochromic features of spiropyran skeletons have been noted: (1) only the merocyanine forms strongly absorb visible light, (2) the merocyanines are more stable than the spiropyrans in aqueous media under dark conditions, and (3) the energy barrier for the isomerization of the merocyanines to the spiropyrans is relatively low.[6b] These photochemical characteristics are expected to overcome the drawbacks arising from the use of azobenzenes. Thus, in aqueous media, dark-adapted merocyanines will readily isomerize to spiropyrans on exposure to ambient indoor lighting without specific light sources. This mild operation is indeed advantageous for considering biological applications as mentioned above. We now report a photochromic cross-linking agent containing a spiropyran skeleton and its utilization in the regulation of helical structures in short peptides under indoor lighting and dark conditions at room temperature.

Figure 1A shows the structure of the cross-linking agent

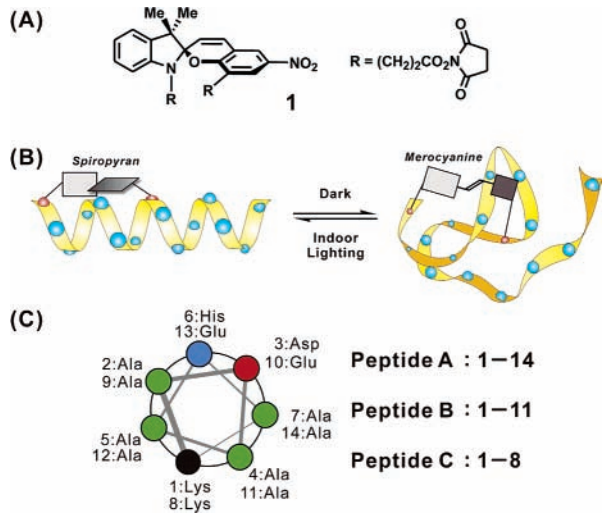
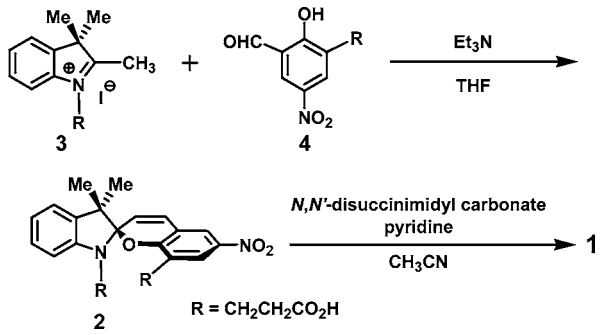


Figure 1. (A) Chemical structure of a photochromic cross-linking agent **1**. (B) A schematic representation for the reversible photoregulation of helical structures on the cross-linked peptides. (C) The sequences of short peptides in this study.

1 that consists of a photochromic spiropyran core and succinimidyl esters for bridging with two amino side chains on Lys residues. The molecular structure of **1** possesses the right balance of rigidity in the spiripyran core and flexibility in the alkyl spacers, and this may allow it to effectively and intramolecularly react with the amino groups on the side chains.[3] Isomerization of the spiropyran to the merocyanine is accompanied by a drastic structural change that influences the helical contents of the cross-linked peptides (Figure 1B). Short peptides **A**−**C** that possess a common sequence from the *i* to the *i* + 7 positions were prepared using a peptide synthesizer (Figure 1C). These sequences contain a cross-linking site on Lys residues at the *i* and *i* + 7 positions (black circle in Figure 1C). Anionic (Asp) and cationic (His) residues are also included at the *i* + 2 (red circle) and *i* + 5 (blue circle) positions, respectively; this will contribute to the partial stabilization of the helices by forming a salt bridge. The positions of these charged residues were chosen such that there would be no interaction with the charged merocyanine on the cross-linked peptides, and the net influence for the structural change in the spiropyran could be assessed for the helical contents. Considering biological applications, the cross-linking site was placed on the N-terminal side of the target peptides. We expected the cross-linked moiety at that position to behave as a "*trigger*" for regulating the entire structure of longer peptides with the help of the dipole moment resulting from the photogenerated local helix on the terminal side (see below).[7]

The photochromic cross-linking agent **1** was synthesized according to Scheme 1. Spiropyran **2** was prepared from the

Scheme 1. Synthetic Route for **1**



known indole **3**[8] and salicylaldehyde **4**[9] and treated with *N*,*N*′-disuccinimidyl carbonate to afford **1**. The cross-linking reaction of peptides **A**−**C** with **1** was performed as described previously.[3] Purification of each of the cross-linked peptides by reverse-phase HPLC yielded two peaks corresponding to the target product. These were identified as regioisomers on the basis of the ESI-MS and HPLC analyses (Figure S1 in Supporting Information). Despite the fact that the orientation of the zwitter ion on the merocyanine of one isomer is opposite to that of the other, both of the cross-linked regioisomers exhibited similar behavior in all of the following experiments.[10] This finding indicates the absence of electrostatic interactions between the polar residues (Asp and His) and the charged merocyanine on the cross-linked peptides as expected. Thus, each of the later fractions of the cross-linked peptides in the HPLC chart was used in the following experiments.

(6) (a) Bertlson, R. C. In *Organic Photochromic and Thermochromic Compounds*; Crano, J. C., Guglielmetti, R. J., Eds.; Plenum: New York, 1999; Vol. 1, pp 11−83. (b) Inouye, M. *Coord. Chem. Rev.* **1996**, *148*, 265−283. (c) Guglielmetti, R. In *Photochromism Molecules and Systems*; Dürr, H., Bouas-Laurent, H., Eds.; Elsevier: Amsterdam, 1990; Chapter 8.

(7) Huang, C.-H.; He, S.; DeGrado, W. F.; McCafferty, D. G.; Gai, F. *J. Am. Chem. Soc.* **2002**, *124*, 12674−12675 and references therein.

(8) Li, X.; Wang, Y.; Matsuura, T.; Meng, J. *Heterocycles* **1999**, *51*, 2639−2651.

(9) Inouye, M.; Noguchi, Y.; Isagawa, K. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1163−1166.

(10) Which is which is not determined.

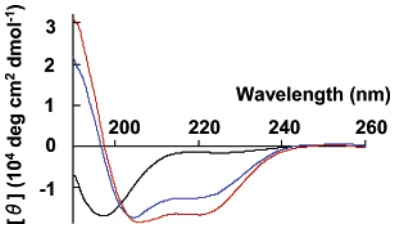


Figure 2. CD spectra of the native peptide A (black) and the cross-linked peptide **A·1** under indoor lighting (red) and dark (blue) conditions in 100 mM phosphate buffer (pH 6.6) at 25 °C using a 1 mm cell.

Figure 2 shows the CD spectra of native peptide **A** and its cross-linked **A·1** at 25 °C. The helical contents of the peptide were estimated on the basis of the mean residue ellipticity at 222 nm.[11] The native **A** almost takes a random-coiled structure (12% helix) in a phosphate buffer, while the helical content of **A·1** increased up to 62% in the spiropyran state after exposure to indoor lighting. On the other hand, the value decreased to 48% at the spiropyran-merocyanine equilibrium state in the dark for ca. 3 h.[12] Subsequent exposure of **A·1** again to ambient indoor lighting for 20 min restored the helical content to nearly the same level as that in the previous spiropyran state. This switching behavior could be repeated more than four times under the same conditions as mentioned above without substantial loss of the photoresponsive ability (Figure 3). Although spiropyrans

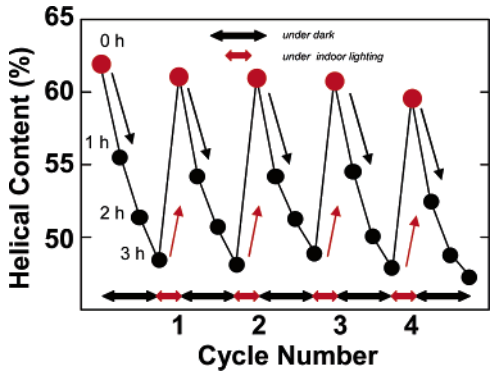


Figure 3. Switching behavior for the helical contents of the dark-adapted **A·1** under the same conditions described in text. Red circles correspond to the helical contents after exposure of **A·1** to indoor lighting for 20 min.

are well-known to suffer from fast and significant decomposition upon intensive irradiation by visible light, the present experimental conditions avoid such problems of the spiropyran moiety on the cross-linked peptides.

To demonstrate the above-mentioned "trigger" effect of the cross-linked moiety on the N-terminal side of the cross-linked peptides, the helical contents of the peptides were plotted as a function of their lengths (Figure 4). Shorter

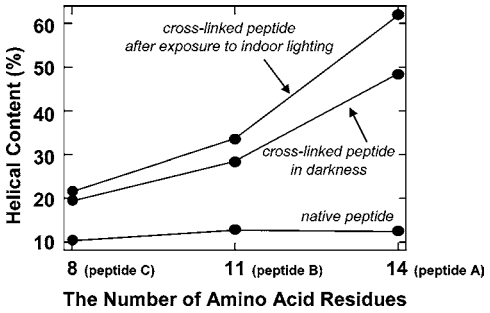


Figure 4. Helical contents of the native and the cross-linked peptides against the peptide lengths.

peptides **B** and **C** consist of 11 and 8 amino acid residues, respectively, and the common sequence from the i to $i + 7$ positions were in peptide **A**. The helical contents of the native **A**−**C**, ca. 10% helix, are almost independent of their lengths. On the other hand, the helical contents of the cross-linked peptides remarkably increased with the increasing number of amino acid residues after exposure to indoor lighting. Noteworthy is that the switching efficiency, i.e., the difference in the helical contents under indoor lighting and dark conditions, was enhanced in a consecutive manner. Thus, in the case of **C·1**, the difference was negligible, whereas that of **A·1** increased to 14%. These data imply that the cross-linked terminal is capable of communicating with the remote residues through the dipole moment of the local helix, thereby inducing a change in the entire helicity.

We developed a new photochromic cross-linking agent **1** containing a spiropyran skeleton for regulating the helical structures in short peptides. The helical contents of the cross-linked peptides could be reversibly regulated under ambient indoor lighting and dark conditions at room temperature. The photoregulation of the local helix on the terminal side could provide total structural regulation of the target peptides. This trigger effect may have a significant applicability in the photochemical control of the peptide−protein and peptide−DNA interactions separately; an approach of this nature is currently being developed.

Supporting Information Available: Experimental procedures, HPLC profiles and UV/vis spectra of **A·1**, and ^{1}H NMR spectra of **1** and **2** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

OL0526524

(11) For the conversion of ellipticity to % helix, see ref 2f. During the photochromic process of the cross-linked peptides, the CD spectral changes were accompanied by an isodichroic point at 203 nm, and this implied that the chromophoric spiropyran and merocyanine portions do not interfere with the CD spectra.

(12) The ratio of [merocyanine form]/[spiropyran form] in **A·1** was estimated to be 1.13 and 0.01 under dark and indoor lighting conditions, respectively (Figure S2 in Supporting Information). Each of the concentrations was determined as follows: (1) a control compound was prepared from the reaction of **1** with 2 equiv of Gly, (2) the molar coefficient at 528 nm of this control compound in the merocyanine form was calibrated on the basis of the [merocyanine]/[spiropyran] ratio; this ratio was determined on the basis of the integrations of the ^{1}H NMR spectrum under the same dark conditions, and (3) the coefficient of the control compound was assumed to be the same as that of the cross-linked peptide.