Communications to the Editor

Photoregulation of Cyclic Peptide Conformation

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The ability to manipulate the specific conformation of peptides and proteins is an essential feature in the development of novel therapeutic agents and biomaterials.[1] To date there are few examples of designed peptides and proteins with the capacity to change conformation as a result of an internal switching mechanism.[2] In this paper we describe a strategy to reversibly change the conformation of a cyclic peptide (**1**) by incorporating a photoresponsive amino acid residue within the peptide.[3]

The cyclic peptide was designed to include an azobenzene-containing amino acid (Aza)[3] as the conformational switch; it was flanked by four alanine residues for flexibility and four residues with high propensities to exist in a turn conformation (Gly-Gly-Pro-Asn).[4] The peptide was designed such that when Aza contained a *trans* azo linkage the peptide portion would exist in an extended conformation, and when isomerized to the *cis* form the peptide would adopt a turn conformation.

The azobenzene-containing amino acid was incorporated into linear peptide **2** by a solid phase methodology on a *p*-alkoxybenzyl alcohol resin[5] with Fmoc as the semipermanent protecting group.[6] Cyclization of peptide **2** (0.5 mM) with BOP (1.1 equiv) and DIEA (2 equiv) at room temperature in DMSO afforded cyclic peptide $\mathbf{1}_{trans}$ in a 48% yield after HPLC purification (Scheme 1).

Peptide $\mathbf{1}_{trans}$ (10 mM in DMSO) was irradiated for 2.5 h with a Hg arc lamp filtered to transmit light from 310 to 410 nm to provide a 93:7 mixture of $\mathbf{1}_{cis}$ and $\mathbf{1}_{trans}$ as judged by ^{1}H NMR, UV, and reverse phase HPLC. Purification of the irradiated material by reverse phase HPLC afforded $\mathbf{1}_{cis}$ in greater than 98% purity. Peptide $\mathbf{1}_{cis}$ thermally reisomerized to the *trans* form with a half-life of approximately 1 week at 25 °C.

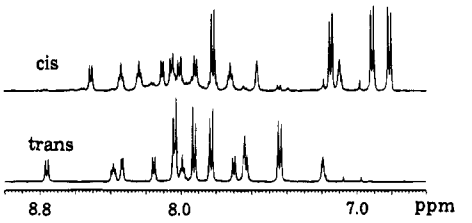


Figure 1. One-dimensional ^{1}H NMR spectra of $\mathbf{1}_{cis}$ and $\mathbf{1}_{trans}$ showing the aromatic/amide portions of the spectra.

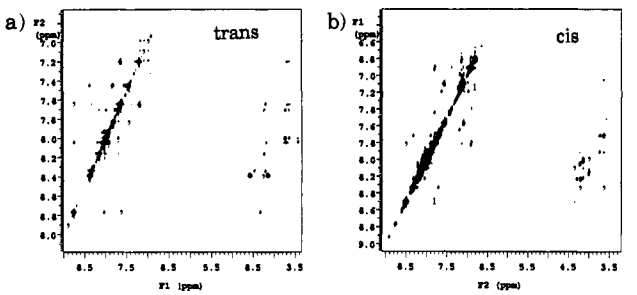
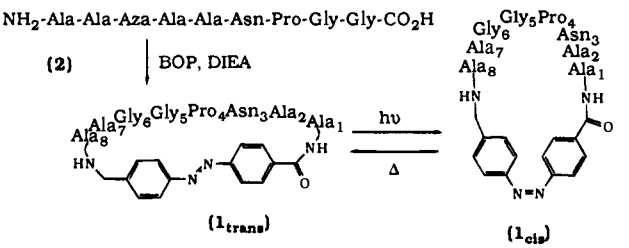


Figure 2. NOESY spectra for $\mathbf{1}_{cis}$ and $\mathbf{1}_{trans}$ showing the cross peaks between the aromatic/amide protons and the α-protons.

Scheme 1. Synthesis and Isomerization of **1**



Inspection of the one-dimensional spectra of the *cis* and *trans* forms of **1** confirmed that a dramatic structural change had taken place upon isomerization (Figure 1). In the aromatic/amide regions of the spectra there was a significant 1.2 ppm upfield shift in the aromatic protons upon isomerization to $\mathbf{1}_{cis}$, due to the close proximity of the aromatic rings in the *cis* form,[7] and there were upfield shifts for the amide protons of Ala_1, Ala_8, and Aza which potentially would lie in the region of the aromatic rings upon isomerization.

The structures of $\mathbf{1}_{cis}$ and $\mathbf{1}_{trans}$ were elucidated by two-dimensional NMR techniques in DMSO-d_6. Stereospecific sequential assignments were made using double quantum filtered COSY (DQF-COSY)[8] and NOESY[9] experiments (Figure 2). Strong NOESY cross peaks were observed between the backbone NH of Asn_3 and the αH of Pro_4, indicating a *trans* proline amide bond in both $\mathbf{1}_{cis}$ and $\mathbf{1}_{trans}$. Also sequential NOEs from Ala_1 to Asn_3 and Gly_7 to Aza, followed by the lack of NOEs from Pro_4 to Gly_5, were consistent with a turn conformation in $\mathbf{1}_{cis}$.

Interproton distances obtained from NOESY experiments[10] and dihedral angles from coupling constants were used as constraints in molecular dynamics simulation at 600 K for 1000

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(1) (a) Hruby, V. J. *Pept.: Chem., Struct. Biol., Proc. Am. Pept. Symp., 13th* **1994**, 3-17. (b) Marshall, G. R. *Tetrahedron* **1993**, *49*, 3547-3558. (c) Freidinger, R. M.; Bock, M. G.; Clineschmidt, B. V.; DiPardo, R. M.; Erb, J. M.; Evans, B. E.; Gilbert, K. F.; Gould, N. P.; Hobbs, D. W. *Perspect. Med. Chem.* **1993**, 179-192. (d) Tirrell, J. G.; Fournier, M. J.; Mason, T. L.; Tirrell, D. A. *Chem. Eng. News* **1994**, *72* (*51*), 40-51.

(2) Light switch: (a) Willner, I.; Willner, B. In *Bioorganic Photochemistry—Biological Applications of Photochemical Switches*; Morrison, H., Ed.; Wiley: New York, 1993; Vol. 2, pp 1-110. (b) Pieroni, O.; Fissi, A.; Houben, J. L.; Ciardelli, F. *J. Am. Chem. Soc.* **1985**, *107*, 2990-2991. (c) Ciardelli, F.; Fabbri, D.; Pieroni, O.; Fissi, A. *J. Am. Chem. Soc.* **1989**, *111*, 3470-3472. (d) Willner, I.; Rubin, S.; Riklin, A. *J. Am. Chem. Soc.* **1991**, *113*, 3321-3325. (e) Willner, I.; Rubin, S. *J. Am. Chem. Soc.* **1992**, *114*, 3150-3151. (f) Willner, I.; Rubin, S.; Cohen, Y. *J. Am. Chem. Soc.* **1994**, *115*, 4937-4938. (g) Zahavy, E.; Rubin, S.; Willner, I. *J. Chem. Soc., Chem. Commun.* **1993**, 1753-1755. (h) Ueda, T.; Murayama, K.; Yamamoto, T.; Kimura, S.; Imanishi, Y. *J. Chem. Soc., Perkin Trans. 1* **1994**, 225-230. Redox switch: (i) Dado, G. P.; Gellman, S. H. *J. Am. Chem. Soc.* **1993**, *115*, 12609-12610. pH switch: (j) Subbarao, N. K.; Parente, R. A.; Szoka, F. C.; Nadasdi, L.; Pongracz, K. *Biochemistry* **1987**, *26*, 2964-2972.

(3) Ulysse, L.; Chmielewski, J. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2145-2146.

(4) Chou, P. Y.; Fasman, G. D. *Annu. Rev. Biochem.* **1978**, *47*, 251-276.

(5) Wang, S. S. *J. Am. Chem. Soc.* **1973**, *95*, 1328-1333.

(6) Atherton, E.; Sheppard, R. C. In *The Peptides*; Gross, E., Meienhofer, J., Eds.; Academic Press: New York, 1987; Vol. 9, pp 1-38.

(7) Chambers, E. M.; Hawthorn, I. S. *J. Chem. Soc., Chem. Commun.* **1994**, 1631-1632.

(8) Rance, M.; Sorensen, O. W.; Bodenhausen, G.; Wagner, G.; Ernst, R. R.; Wuthrich, K. *Biochem. Biophys. Res. Commun.* **1983**, *117*, 479-485.

(9) Macura, S.; Ernst, R. R. *Mol. Phys.* **1980**, *41*, 95-117.

(10) Pardi, A.; Billeta, M.; Wuthrich, K. *J. Mol. Biol.* **1984**, *180*, 741-751.

0002-7863/95/1517-8466$09.00/0

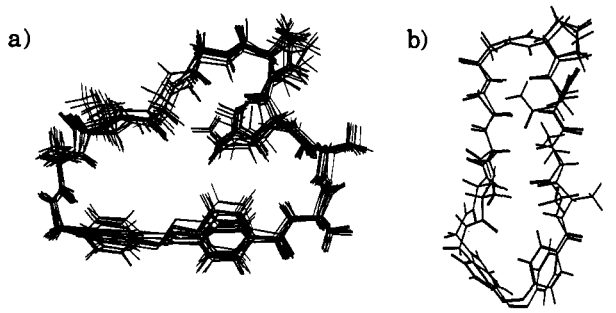

Figure 3. Superposition of structures generated from restrained molecular dynamics simulation and energy minimization for (a) $\mathbf{1}_{trans}$ and (b) $\mathbf{1}_{cis}$.

ps with sampling every 10 ps using the AMBER force field.[11] The 100 conformers obtained from the dynamics simulation were subjected to a restrained minimization for refinement, and a search for the conformers in agreement with the NMR data was carried out based on the dihedral angles and interproton distances; 25 structures were obtained with an average RMS deviation of 1.09 Å for $\mathbf{1}_{trans}$ (Figure 3a), and five structures were obtained with an average RMS deviation of 1.10 Å for $\mathbf{1}_{cis}$ (Figure 3b).[12]

Careful inspection of the peptide portion of $\mathbf{1}_{trans}$ showed a β-strand extending from residue Ala_2 to Gly_6, interrupted by a bend at Pro_4, with bends at residues Ala_1, Ala_7, and Ala_8 which are adjacent to Aza. The only hydrogen bond which existed in the *trans* conformer was one between the side chain NH of Asn_3 and the carbonyl of Gly_5. Interestingly, inspection of the peptide portion of $\mathbf{1}_{cis}$ showed that a type II β-turn existed from residue Gly_6 to Asn_3, with a hydrogen bond between the carbonyl of Gly_6 and the backbone NH of Asn_3, and an antiparallel β-sheet extending from the residues adjacent to Aza up to the β-turn, with hydrogen bonds between the NH of Gly_6 and the backbone carbonyl of Asn_3, and the NH of Ala_8 and the carbonyl of Ala_1. D_2O exchange experiments with $\mathbf{1}_{cis}$ demonstrated that the NH of Gly_6 and the backbone NH of Asn_3 did not exchange immediately upon addition of D_2O (data not shown), which is consistent with the hydrogen bonds predicted by our model and the presence of a β-turn.

We have demonstrated that an azobenzene moiety within **1** photoregulated the conformation of the peptide, and it templated a β-turn conformation when in the *cis* form. The effects of the photochemical isomerization of the azobenzene upon the conformation of the cyclic peptide were also reversible upon heating. As many biologically active regions of proteins[13] and peptide hormones[14] contain β-turns, this methodology provides a powerful means for obtaining biological activity on demand by simple irradiation. Further work is underway to incorporate this novel scaffolding into a number of biologically active systems.

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Supporting Information Available: Synthetic details, one-dimensional NMR data, DQF-COSY and NOESY data, NMR assignments, and constraints used in molecular dynamics (17 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

JA9517025

(11) Weiner, S. J.; Kollmann, P. A.; Case, D. A.; Singh, W. C.; Ghio, C.; Alagona, G.; Profeta, S., Jr.; Weiner, P. *J. Am Chem. Soc.* **1984**, *106*, 765.

(12) The structures obtained from restrained molecular dynamics simulation and energy minimization were screened using the dihedral angles (±5°) and the NOE distances (±0.3 Å) obtained from the NMR experiments.

(13) (a) Pflugrath, J. W.; Wiegand, G.; Huber, R.; Vertesy, L. *J. Mol. Biol.* **1986**, *189*, 383−386. (b) Kline, A. D.; Braun, W.; Wuthrich, K. *J. Mol. Biol.* **1986**, *189*, 367−382. (c) Wang, J.; Yan, Y.; Garrett, T. P. J.; Liu, J.; Rodgers, D. W.; Garlick, R. L.; Tarr, G. E.; Husain, Y.; Reinherz, E. L.; Harrison, S. C. *Nature* **1990**, *348*, 411. (d) Ryu, S. E.; Kwong, P. D.; Trunch, A.; Porter, T. G.; Arthos, J.; Rosenberg, M.; Dai, X.; Xuong, N. H.; Axel, R.; Sweet, R. W.; Hendrickson, W. A. *Nature* **1990**, *348*, 419.

(14) Rose, G. D.; Gierasch, L. M.; Smith, J. A. *Adv. Protein Chem.* **1985**, *37*, 1−109.