Conformational Switch

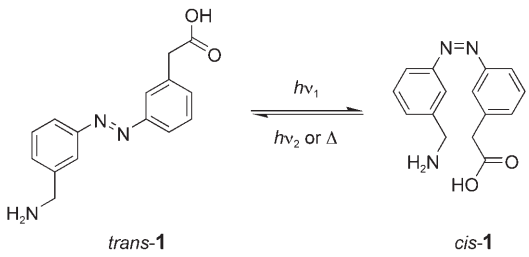
DOI: 10.1002/ange.200602084

A Photoswitchable Miniprotein Based on the Sequence of Avian Pancreatic Polypeptide**

Simon Jurt, Andreas Aemissegger, Peter Güntert, Oliver Zerbe, and Donald Hilvert**

Since the early work of Anfinsen, structural biology has been influenced by the dogma that proteins fold into unique structures dictated by their amino acid sequence.[1] However, the recent observation that prion proteins undergo spontaneous conversion from well-folded and soluble helical entities into insoluble β-sheet-containing aggregates suggests that this view is overly simplistic.[2] It also raises the intriguing possibility that conformational switches might be designed to transform proteins deliberately from one unique structure into another unique structure. Such switches could be quite valuable, for example, for studying folding in the absence of denaturants or for controlling receptor function or enzyme activity.

Photochromic compounds, which undergo large conformational changes when exposed to light of an appropriate wavelength, are interesting in this context as they might permit reversible conformational control. Of the many photochromic systems that have been described, azobenzenes are well suited for this type of application as they have been extensively characterized and are readily synthesized. Irradiation at the wavelength of the $\pi\rightarrow\pi^*$ transition converts the thermodynamically favored *trans* to the *cis* isomer, whereas the reverse process can be achieved either thermally or by irradiation at the wavelength of the $n\rightarrow\pi^*$ transition. Azobenzenes have been introduced into many peptides and proteins, both into side chains[3,4] and into the main chain.[5,6] For example, our research group[7] and Moroder and coworkers[8] have shown that the *meta*-substituted Fmoc-protected linker **1** (Scheme 1) can be inserted into a polypeptide backbone through solid-phase peptide synthesis and, in its *cis* configuration, serve as the β-turn-inducing segment in β-hairpin peptides. Thermally induced isomerization from *cis*-**1** to *trans*-**1** results in unfolding of the hairpin.



trans-**1** *cis*-**1**

Scheme 1. Interconversion of the *trans*- and *cis*-configured azobenzene derivative **1**. The amino acid sequence of the synthesized peptides is ^{1}GPSQPTYPG-**1**-^{13}PVEDLIRFYNDLQQYLNVVTRHRY-NH_2. For simplicity, the amino acids in the sequence are numbered as in wild-type aPP, with amino acid **1** replacing turn residues 10, 11, and 12.

In this study, we have extended our work to larger helical polypeptides. As a model system, we chose a derivative of avian pancreatic polypeptide (aPP). The 36 residue hormone was one of the first peptides for which a crystal structure was available.[9] It consists of a C-terminal α helix (residues 14–31) connected through a β turn (residues 9–13) to an N-terminal type-II polyproline helix, a structural motif known as the PP fold. In the solid state and in solution, two aPP polypeptides further associate to form a symmetric dimer.[10] Despite its small size, aPP thus displays the features of much larger proteins, that is, it a possesses secondary as well as tertiary and quaternary structure.

To allow switching of the aPP peptide from a back-folded (PP-folded) to an extended form, we replaced the β-turn segment between the helices, comprising residues Asp 10–Asp 11–Ala 12, with **1**. The resulting molecule, which exhibits similar helicity to natural aPP (as determined by circular dichroism spectroscopy), is readily interconverted between the *cis* and *trans* forms upon irradiation. At thermodynamic equilibrium, the *trans*/*cis* ratio is 90:10, but the fraction of *cis*-configured peptide can be increased up to about 85% in the photostationary state. Repeated interconversion between the two states ultimately results in peptide precipitation. However, thermal relaxation from the *cis* to the *trans* configuration is relatively slow in aqueous solution at room temperature (half-life > 2 weeks), enabling detailed biophysical characterization of the molecule. Sedimentation equilibrium analysis indicates that, in contrast to wild-type aPP, purified *cis*-**1**-PP is tetrameric in solution. A molecular weight of 16 kDa is determined assuming a single ideal species, which is in good agreement with the expected mass of 16.8 kDa for a tetramer. Detailed structural information on the effect of the linker on the PP fold was obtained by standard 2D NMR spectroscopic methods (Figure 1).[11]

As shown in Figure 1, *cis*-**1**-PP clearly adopts a back-folded PP structure. The C-terminal α helix, encompassing residues Val 14–Thr 32, exhibits a root-mean-square deviation (RMSD) of 0.67 ± 0.22 Å for all backbone atoms. However, introduction of the azobenzene shifts the position of the turn toward the C terminus, displacing the entire N-terminal

[*] S. Jurt, Priv.-Doz. Dr. O. Zerbe
Institute of Organic Chemistry
University of Zurich
8057 Zurich (Switzerland)
Fax: (+41) 44-635-6882
E-mail: oliver.zerbe@oci.unizh.ch
Dr. A. Aemissegger, Prof. Dr. D. Hilvert
Laboratory of Organic Chemistry
ETH Zurich
8093 Zurich (Switzerland)
Fax: (+41) 44-632-1486
E-mail: hilvert@org.chem.ethz.ch
Priv.-Doz. Dr. P. Güntert
RIKEN Genomic Sciences Center
1-7-22 Suehiro-cho, Tsurumi, Yokohama 230-0045 (Japan)

[**] We thank the ETH Zurich and the University of Zurich for financial support and Joris Beld for operating the analytical ultracentrifuge.

Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

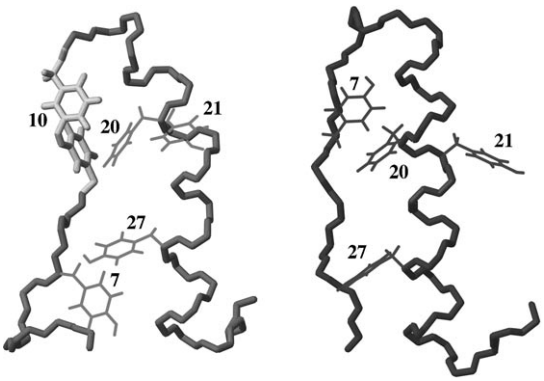


Figure 1. Backbone representation of the monomeric subunits of *cis*-**1**-PP (left) and aPP (right) including the side chains of aromatic residues. In *cis*-**1**-PP, the azobenzene moiety, residue 10, is depicted in pale gray.

segment along the axis of the α helix. The linker itself assumes a relatively extended conformation, despite its *cis* configuration, and forms an aromatic stacking interaction with Phe 20. As a consequence, Tyr 7, which normally contacts Phe 20 in aPP, is able to π stack with Tyr 27, reinforcing the back-fold (Figure 1). Furthermore, we observe a dense network of intermolecular NOEs that define a dimer interface similar to that seen in aPP (Figure 2). As is true for a number of other proteins,[12] aromatic ring-stacking interactions appear to be particularly important in stabilizing this interface. Although the experimental line widths for *cis*-**1**-PP are consistent with the presence of a tetrameric state in solution, we were unable to identify NOEs that allow unambiguous assignment of this higher-order structure. Nonetheless, formation of the tetramer is presumably mediated by the larger exposed hydrophobic surface generated upon replacement of the original Asp–Asp–Ala sequence (Figure 2 D) with the relatively hydrophobic azobenzene (Figure 2 B).

In contrast to *cis*-**1**-PP, *trans*-**1**-PP affords NMR spectra with extremely broad lines, indicating that it is highly aggregated in solution. As a consequence, complete sequential resonance assignment and full structure determination were not possible. Nevertheless, no NOEs were visible in the spectral region in which NOEs between resonances from N- and C-terminal residues were observed in *cis*-**1**-PP, suggesting that *trans*-**1**-PP is not back-folded. Consistent with this inference, other non-back-folded aPP homologues, such as neuropeptide Y,[13,14] are known to form higher-order aggregates at high concentrations.

Receptor binding by peptide hormones related to aPP has been proposed to occur through a mechanism involving initial association of the peptide with the membrane.[13,15] In this model, hydrophobic contacts between residues in the N- and C-terminal regions are replaced by hydrophobic contacts between the C-terminal helix and the membrane, leading to unfolding of the N terminus. As positioning of the N-terminal segment is likely to be the primary difference in the structure between *trans*-**1**-PP and *cis*-**1**-PP, binding of both species to a membrane model such as dodecylphosphocholine (DPC) micelles should result in very similar structures owing to removal of the back-fold in *cis*-**1**-PP. To test this hypothesis, we determined the structures of both *trans*-**1**-PP and *cis*-**1**-PP in the presence of DPC micelles. Strikingly, *trans*-**1**-PP displays line widths that are comparable to *cis*-**1**-PP under these conditions. A superposition of the ensemble of low-energy NMR conformers is depicted in Figure 3. The

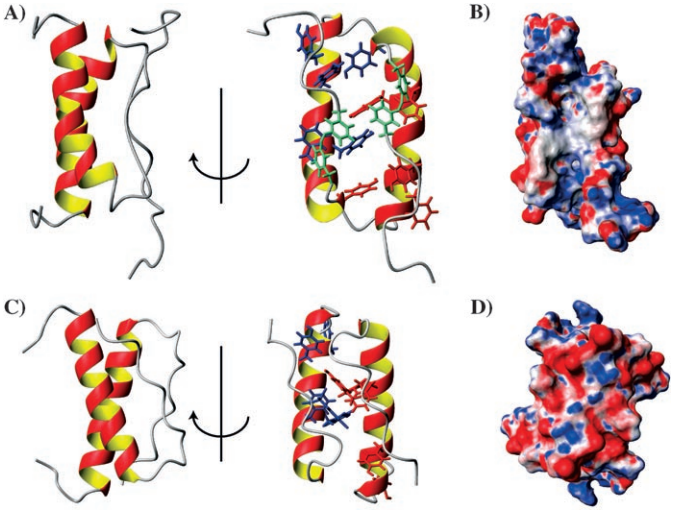


Figure 2. A) Structure of a low-energy NMR conformer of the *cis*-**1**-PP dimer in aqueous solution. The aromatic side chains contributing to the dimer interface are shown in the view at the right. B) Electrostatic surface of the *cis*-**1**-PP dimer with positively and negatively charged surface areas shown in red and blue, respectively. C) Crystal structure of the aPP dimer[9] with the corresponding electrostatic surface (D).

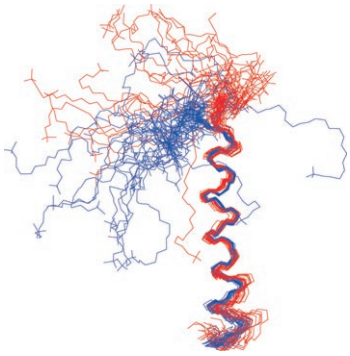


Figure 3. Superposition of the 20 lowest-energy conformers of *trans*-**1**-PP (red) and *cis*-**1**-PP (blue) determined in the presence of DPC micelles. Backbone atoms of residues 14–31 were superimposed in the fit.

structures are highly similar: the C-terminal helix extends over residues 15–28 in both peptides and hydrogen bonds typical for an α helix are populated to approximately the same extent. The N-terminal segment is flexible and no medium- or long-range NOEs were observed. In the absence of DPC micelles, such flexibility, together with exposure of hydrophobic helix residues to solvent, would be expected to promote the formation of aggregates, explaining the broad lines seen for *trans*-**1**-PP in solution.

To conclude, we have demonstrated that a peptide capable of adopting different tertiary structures can be constructed by inserting an azobenzene linker into the polypeptide backbone, and that a simple transformation (irradiation or heating) can be used to switch from one form to the other. This approach thus complements related efforts to control helix conformation through azobenzene-based photo-cross-linkers between appropriately spaced cysteine side chains.[4] Extrapolating from this proof of principle, we envision that incorporation of amino acid **1** into larger polypeptides or proteins will provide a sensitive means of controlling both folding and function in more complex systems.

Experimental Section

Peptides were prepared by solid-phase peptide synthesis on a 0.1-mmol scale with double coupling of all amino acids except the azobenzene residue **1** by using standard 9-fluorenylmethoxycarbonyl (Fmoc) conditions (20% piperidine in *N*,*N*-dimethylformamide (DMF) for Fmoc deprotection, 1-hydroxy-benzotriazole (HOBt)/benzotriazol-1-yl-*N*-tetramethyluronium hexafluorophosphate (HBTU) for activation, *N*,*N*-diisopropylethylamine (DIPEA) as a base, and *N*-methylpyrrolidinone as solvent) and Ac_2O capping on a Rink amide resin. The peptides were cleaved from the resin with a solution of TFA:H_2O:TIPS (93:5:2; TFA = trifluoroacetic acid, TIPS = triisopropylsilane), purified by reverse-phase HPLC, and characterized by electrospray MS ($[M]^+$ *m*/*z* calcd 4187.7, found 4187.0). Although problems with silanes in the cleavage reaction for azobenzene-containing peptides have been reported,[6] good yields were obtained.

Analytical HPLC showed the presence of both the *trans* (90%) and *cis* (10%) isomers at thermodynamic equilibrium. After irradiation at 320 nm (Philips PL-S 9W/12 compact fluorescent tube), the *cis* content increased to 85%. Both isomers were obtained in greater than 99% purity by preparative HPLC (linear gradient of 5–60% MeCN in H_2O and 0.1% TFA, flow rate 10 mL min^{-1}, Macherey-Nagel Nucleosil 100–7 C18 21 × 250 mm^2 column). UV spectroscopy did not show significant isomerization under the conditions used for NMR measurements.

Sedimentation equilibrium experiments were performed with purified *cis*-**1**-PP on a Beckman Optima XL-A ultracentrifuge with an AnTi60 rotor and two channel Epon charcoal centerpieces at 4 °C and rotor speeds of 30, 35, 40, and 42 krpm in 20 mM acetate buffer solution (pH 4.1) with 80 mM NaCl. The partial specific volume was calculated from the amino acid composition, substituting the azobenzene linker by two phenylalanine residues. Runs were analyzed by using the Ultrascan II software package.[16]

Structure elucidation of the peptides in solution was performed on approximately 2 mM samples at 22 °C in 20 mM acetate buffer solution (pH 4.1) in $H_2O/^2H_2O$ (9:1) or at 37 °C in the presence of 300 mM dodecylphosphocholine micelles and 50 mM *N*-[2-morpholinoethane]sulfonic acid (MES) buffer solution (pH 6.0). Structural data were measured on Bruker DRX-600 and AV-700 instruments. Sequential resonance assignments were obtained by standard procedures by using clean-TOCSY spectra[17] with mixing times of 12 and 40 ms and a NOESY spectrum[18] recorded with 70 ms mixing time and a zero-quantum suppression filter.[19] Spectra were processed with the Bruker TOPSPIN software and transferred into the XEASY program[20] for data analysis. Structures were calculated by using a simulated-annealing protocol based on molecular dynamics in torsion-angle space as implemented in the program CYANA.[21] NOESY cross peaks for the homodimeric model of *cis*-**1**-PP were assigned by a combination of manual[20] and automated approaches[21] with a modified version of the program CYANA 2.1. The modified version takes the homodimer symmetry explicitly into account for the network anchoring of the NOE assignments and ensures an identical conformation of the two monomers by imposing dihedral-angle difference restraints for all corresponding torsion angles. This maintains a symmetric relative orientation of the two monomers by applying distance difference restraints between symmetry related intermolecular Cα–Cα distances.

The final input (including only meaningful restraints) for the structure calculations consisted of 940 upper distance limits in the case of *cis*-**1**-PP in the absence of DPC. Structure calculations were started from 100 starting conformers with random torsion-angle values and used 20000 torsion-angle dynamics steps per conformer. The 20 CYANA conformers with the lowest final target function values were subjected to restrained energy minimization in explicit water against the AMBER force field[22] by using the program OPALp.[23] The computed structures contained no consistent violation of experimental observables and displayed overall good geometric features as judged by the program PROCHECK.[24] Graphics were prepared with the program MOLMOL.[25] The coordinates of the structures have been deposited in the Protein Data Bank under access codes 2H4B (*cis*-**1**-PP), 2H3S (*cis*-**1**-PP bound to DPC micelles), and 2H3T (*trans*-**1**-PP bound to DPC micelles).

Received: May 24, 2006
Published online: August 25, 2006

Keywords: azobenzene · peptides · photochromic compounds · protein folding · tertiary structures

[1] C. B. Anfinsen, *Science* **1973**, *181*, 223.
[2] B. Caughey, *Trends Biochem. Sci.* **2001**, *26*, 235; S. B. Prusiner, M. R. Scott, S. J. DeArmond, F. E. Cohen, *Cell* **1998**, *93*, 337.
[3] O. Pieroni, J. L. Houben, A. Fissi, P. Costantino, F. Ciardelli, *J. Am. Chem. Soc.* **1980**, *102*, 5913; D. Liu, J. Karanicolas, C. Yu, Z. H. Zhang, G. A. Woolley, *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2677; J. R. Kumita, O. S. Smart, G. A. Woolley, *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 3803.
[4] G. A. Woolley, *Acc. Chem. Res.* **2005**, *38*, 486; L. Guerrero, O. S. Smart, C. J. Weston, D. C. Burns, G. A. Woolley, R. K. Allemann, *Angew. Chem.* **2005**, *117*, 7956; *Angew. Chem. Int. Ed.* **2005**, *44*, 7778; G. A. Woolley, A. S. I. Jaikaran, M. Berezovski, J. P. Calarco, S. N. Krylov, O. S. Smart, J. R. Kumita, *Biochemistry* **2006**, *45*, 6075.
[5] L. Ulysse, J. Cubillos, J. Chmielewski, *J. Am. Chem. Soc.* **1995**, *117*, 8466; C. Renner, R. Behrendt, S. Spörlein, J. Wachtveitl, L. Moroder, *Biopolymers* **2000**, *54*, 489; C. Renner, J. Cramer, R. Behrendt, L. Moroder, *Biopolymers* **2000**, *54*, 501.
[6] C. Renner, U. Kusebauch, M. Löweneck, A. G. Milbradt, L. Moroder, *J. Pept. Res.* **2005**, *65*, 4.
[7] A. Aemissegger, V. Kräutler, W. F. van Gunsteren, D. Hilvert, *J. Am. Chem. Soc.* **2005**, *127*, 2929; V. Kräutler, A. Aemissegger, P. H. Hünenberger, D. Hilvert, T. Hansson, W. F. van Gunsteren, *J. Am. Chem. Soc.* **2005**, *127*, 4935.
[8] S. L. Dong, M. Löweneck, T. E. Schrader, W. J. Schreier, W. Zinth, L. Moroder, C. Renner, *Chem. Eur. J.* **2006**, *12*, 1114.
[9] T. L. Blundell, J. E. Pitts, S. P. Tickle, C. W. Wu, *Proc. Natl. Acad. Sci. USA* **1981**, *78*, 4175.
[10] X. A. Li, M. J. Sutcliffe, T. W. Schwartz, C. M. Dobson, *Biochemistry* **1992**, *31*, 1245.
[11] K. Wüthrich, *NMR of Proteins and Nucleic Acids*, 1st ed., Wiley, New York, **1986**.
[12] S. K. Burley, G. A. Petsko, *Science* **1985**, *229*, 23; D. A. Dougherty, *Science* **1996**, *271*, 163; E. A. Meyer, R. K. Castellano, F. Diederich, *Angew. Chem.* **2003**, *115*, 1244; *Angew. Chem. Int. Ed.* **2003**, *42*, 1210.
[13] R. Bader, A. Bettio, A. G. Beck-Sickinger, O. Zerbe, *J. Mol. Biol.* **2001**, *305*, 307.

[14] S. A. Monks, G. Karagianis, G. J. Howlett, R. S. Norton, *J. Biomol. NMR* **1996**, *8*, 379.
[15] R. Bader, O. Zerbe, *ChemBioChem* **2005**, *6*, 1520; M. Lerch, V. Gafner, R. Bader, B. Christen, G. Folkers, O. Zerbe, *J. Mol. Biol.* **2002**, *322*, 1117; M. Lerch, M. Mayrhofer, O. Zerbe, *J. Mol. Biol.* **2004**, *339*, 1153; M. Lerch, H. Kamimori, G. Folkers, M.-I. Aguilar, A. G. Beck-Sickinger, O. Zerbe, *Biochemistry* **2005**, *44*, 9255.
[16] B. Demeler, Ultrascan II 6.2, The University of Texas, San Antonio, http://www.ultrascan.uthscsa.edu/
[17] C. Griesinger, G. Otting, K. Wüthrich, R. R. Ernst, *J. Am. Chem. Soc.* **1988**, *110*, 7870.
[18] S. Macura, R. R. Ernst, *Mol. Phys.* **1980**, *41*, 95.
[19] G. Otting, *J. Magn. Reson.* **1990**, *86*, 496.
[20] C. Bartels, T.-h. Xia, M. Billeter, P. Güntert, K. Wüthrich, *J. Biomol. NMR* **1995**, *6*, 1.
[21] P. Güntert, *Methods Mol. Biol.* **2004**, *278*, 353.
[22] W. D. Cornell, P. Cieplak, C. I. Bayly, I. R. Gould, K. M. Merz, D. M. Ferguson, D. C. Spellmeyer, T. Fox, J. W. Caldwell, P. A. Kollman, *J. Am. Chem. Soc.* **1995**, *117*, 5179.
[23] R. Koradi, M. Billeter, P. Güntert, *Comput. Phys. Commun.* **2000**, *124*, 139.
[24] R. A. Laskowski, J. A. C. Rullmann, M. W. MacArthur, R. Kaptein, J. M. Thornton, *J. Biomol. NMR* **1996**, *8*, 477.
[25] R. Koradi, M. Billeter, K. Wüthrich, *J. Mol. Graphics* **1996**, *14*, 51.

