Biphotonics

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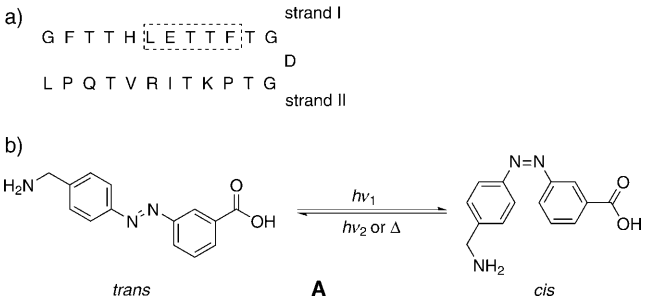
Light-Directed Protein Binding of a Biologically Relevant β-Sheet**

Christian Hoppmann, Sabine Seedorff, Anja Richter, Heinz Fabian, Peter Schmieder, Karola Rück-Braun, and Michael Beyermann**

β-Hairpin structures are frequently involved in protein–protein interactions that control essential processes in cells and are therefore interesting targets for interference. Hairpin-forming peptides that compete with such protein interactions are valuable tools for studying biological processes. Moreover, the incorporation of a photoswitchable unit into appropriate β-hairpin-forming peptide ligands could allow protein interactions in cells to be studied by light-triggered interference. However, β-hairpin structures are rarely studied because of the limited availability and stability of suitable model peptides. This is because such a model peptide has to fulfill at least three requirements: 1) the β-hairpin has to be sufficiently stable as monomer without the tendency to self-aggregate, 2) the photoswitchable unit incorporated must stabilize the biologically active peptide conformation, and 3) disturbing the protein binding site by light-induced isomerization of the photoswitch must not result in intermolecular association or even formation of insoluble fibrils.[1] Herein, we report the first example of a β-hairpin model peptide of a biologically important protein domain that shows considerably different binding affinities for the target protein that are dependent on the isomerization state of the embedded photoswitch.

PDZ domains mediate the formation of a variety of multiprotein complexes in the cell.[2] Besides C-terminal protein sequences, PDZ domains are also able to recognize internal peptide motifs that bind at the same binding pocket as the C-terminal ones. The best example of this type of internal ligand recognition is found in the extended PDZ domain of neuronal nitric oxide synthase (nNOS) which interacts with the PDZ domain from α-1-syntrophin or the second PDZ domain from PSD95.[3] The formation of the PDZ/PDZ heterodimer requires the β-finger structure of nNOS (30 amino acid residues) to bind at the syntrophin PDZ domain, thus mediating the membrane association of nNOS to skeletal muscle and inducing the production of the second messenger nitric oxide (NO) for muscle contraction.[4]

Crucial for binding is the internal recognition motif -LETTF- of the extended PDZ domain of nNOS located in the first strand of the hairpin peptide (Scheme 1), a stable conformation which consists of two antiparallel strands connected by a turn.[5] Previous work was based on cyclic peptides as mimics of the nNOS β-finger (Scheme 2, peptide **1**). Structural calculations revealed a structure of **1** that is in agreement with the original β-finger. Binding studies confirmed binding at the correct site in the protein.[6] Based on these results, a photoswitchable ω-amino acid has been incorporated into the cyclic β-finger peptide **1** replacing the D-Pro-Gly turn element and the two amino acids flanking both ends (Scheme 2b). Photoswitchable ω-amino acids based on either azobenzene[7] or hemithioindigo[8] are good candidates for the modulation of peptide conformations[9] because they undergo ultrafast photoisomerization[10] thus allowing monitoring of conformational transitions in the pico- to femtosecond timescale.[11] For biological applications,[12] azobenzene has been demonstrated to be very effective in that it shows isomerization around the central N=N bond (*trans*⇄*cis*) with high isomerization yields and remarkable changes in geometry. In contrast to the previously reported light-switchable β-hairpins,[9d,e] we embedded the azobenzene-ω-amino acid 3-((4′-aminomethyl)phenylazo) benzoic acid (**A**) as photoswitch because of the high thermal stability of the photostationary state (pss) of the *cis* form (Scheme 1b).[7c] The extended geometry of the *trans* form of the azobenzene was expected to disturb the binding site in the peptide ligand.



Scheme 1. a) Sequence of the β-finger peptide of nNOS, the amino acids in the box represent the internal recognition site, b) structure of the photoresponsive unit **A**.

High isomerization yields in the pss of the *cis* form of **2** (90% *cis* content) were achieved after irradiation of the

[*] C. Hoppmann, S. Seedorff, A. Richter, Prof. Dr. K. Rück-Braun
Institut für Chemie, Technische Universität Berlin
Strasse des 17. Juni 135, 10623 Berlin (Germany)
E-mail: krueck@chem.tu-berlin.de
C. Hoppmann, S. Seedorff, Dr. P. Schmieder, Dr. M. Beyermann
Leibniz-Institut für Molekulare Pharmakologie
Robert-Rössle-Strasse 10, 13125 Berlin (Germany)
Fax: (+49) 30-9479-3159
http://www.fmp-berlin.de
E-mail: beyermann@fmp-berlin.de
Dr. H. Fabian
Robert-Koch-Institut
Nordufer 20, 13353 Berlin (Germany)

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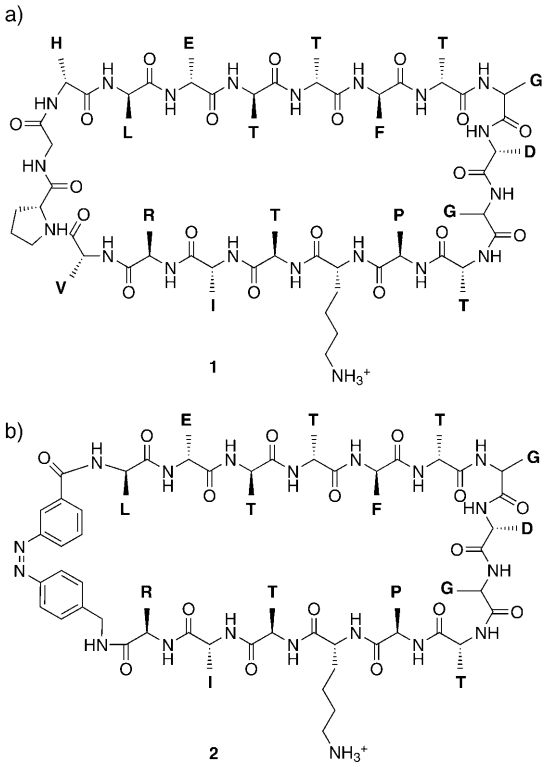

Scheme 2. Structures of synthetic cyclic peptides **1** and **2** related to the β-finger peptide of nNOS a) **1** containing the -Val-D-Pro-Gly-His- motif for the non-switchable form, b) **2** containing **A** as photoswitch. The lysine side chain for immobilization on the sensor chip is depicted.

thermodynamically stable *trans* form at 330 nm in buffer solution at pH 7.5 (Figure 1). The thermal *cis*→*trans* isomerization of **2** in aqueous solution was found to be slow (half-life 25 days). The repeated photochemical interconversion of the two states was achieved without occurrence of association, precipitation, or photobleaching.

Size-exclusion chromatography (SEC) studies under physiological conditions (pH 7.5) have shown that neither photoswitching (up to ten photocycles) nor storage for two weeks at 50 °C in the dark led to association or formation of aggregates of the photoswitchable peptide **2**, which reverts completely into the initial monomeric *trans* form. The separation of the two photoisomers in the pss by SEC is indicative of different hydrodynamic volumes of the two monomeric forms as a result of global changes in their conformations (Figure 2). Thereby, SEC provides an alternative method to reverse phase (RP)-HPLC for determining *cis*:*trans* ratios in that it avoids hydrophobic or ionic interactions of the peptide with the matrix. The *cis* content in the photostationary state determined by SEC is estimated to approximately 75 %.

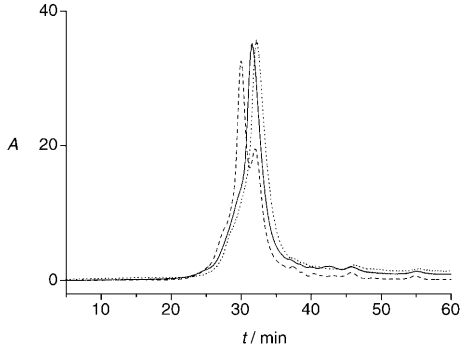

Figure 2. SEC profile (220 nm) of peptide **2** in buffer, pH 7.5 after ten photocycles and two-weeks storage in buffer at 50 °C displaying the pure *trans* form (——), the *cis*/*trans* mixture (-----) and untreated *trans* form (•••••).

Using surface plasmon resonance spectroscopy (SPR) we have investigated the influence of photoisomerization of peptide **2** on binding to syntrophin compared to the binding of the non-switchable peptide **1**.

With the knowledge that strand I of the hairpin contains the internal recognition motif, the ligands were immobilized through the lysine side chain in strand II on a CM5 sensor chip. The pure *trans* form of peptide **2** showed almost no binding (solid line, Figure 3). After photoisomerization, a significant binding of syntrophin ($K_D = 10.6$ μM) was found which is comparable to that of the model peptide **1** ($K_D =$

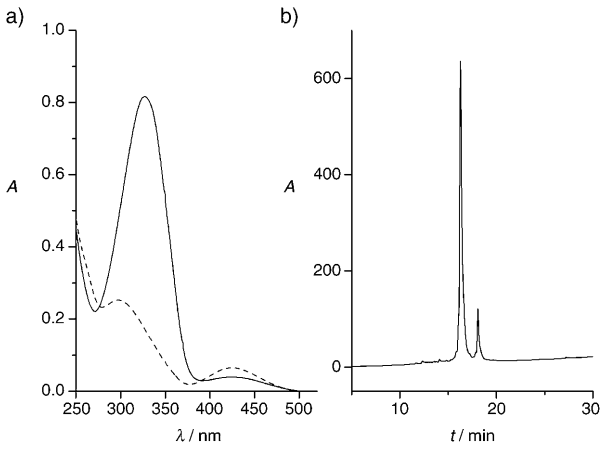

Figure 1. a) UV/Vis spectra in buffer, pH 7.5 of **2** in the pure *trans* form (——) and in the *cis* form in pss (-----); maxima for π–π* and n–π* transitions are at 327 and 424 nm, respectively; b) RP-HPLC profile (220 nm) of the *cis* form in pss (90% *cis* content).

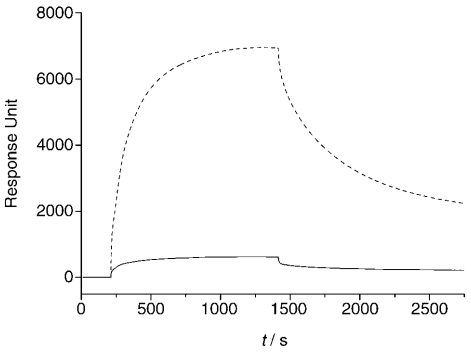

Figure 3. SPR sensorgrams of the interaction of peptide **2** as pure *trans* form (——) and *cis* form in pss (-----) with syntrophin PDZ ($c = 7.74$ μM).

5.4 μM). To rule out any binding arising from the solid phase involved in SPR, we confirmed the different binding behavior of the two isomers of **2** to syntrophin by ITC (isothermal titration calorimetry; Supporting Information). Again, whilst the *trans* form showed no binding, the *cis* form of **2** bound with a remarkable affinity.

In aqueous solution the model peptide **1** adopts an antiparallel β-sheet structure.[6] The correlation between the binding affinities of either **2** in the *cis* form in the pss or **1** lead us to assume similar structural features of the binding site. The IR spectrum of the *trans* form of **2** (solid line in Figure 4a) is characterized by a broad and featureless amide I

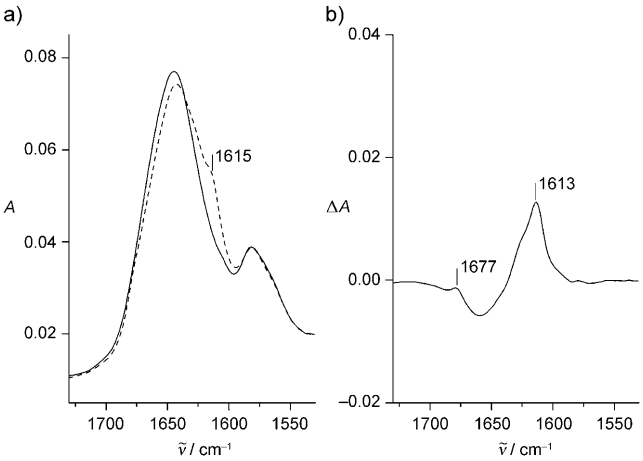

Figure 4. FTIR spectra of **2** in D_2O buffer, pH 7.5; a) pure *trans* form (——) and *cis* form in pss (-----); b) *cis-trans*-difference spectra.

band contour centered at approximately 1645 cm^{-1}, typical of an unordered peptide structure.[13] Photoisomerization to the *cis*-azobenzene induced the formation of secondary structure, as indicated by a band component at approximately 1615 cm^{-1} (dashed line, Figure 4a). The strong low-frequency band at 1613 cm^{-1} and a weaker band at 1677 cm^{-1} of the IR difference spectrum (Figure 4b) indicate that some amide C=O groups are involved in an antiparallel β-type structure[13,14] that is induced by the *trans*→*cis* photoisomerization of the photoswitch in the peptide.

Both forms of peptide **2** have been investigated by NMR spectroscopy (Figure 5). The region of the amide and aromatic protons of the *trans* form and *cis* form are shown. Most importantly, the sharp lines in the spectra show that neither conformer forms aggregates, that is, the peptide is monomeric in both photoisomeric forms even at the relatively high concentrations required for NMR spectroscopy.

The light-triggered modulation of the binding behavior of a β-hairpin peptide to its target protein has been successfully demonstrated. The interaction was studied between α-1-syntrophin and a β-hairpin peptide derived from the PDZ domain binding site of the neuronal NO synthase. By incorporating an azobenzene-ω-amino acid as a photoswitch in a peptide ring of appropriate size, a cyclic light-directed ligand was developed. Its *trans* form shows no binding while the *cis* form features overall binding comparable to the

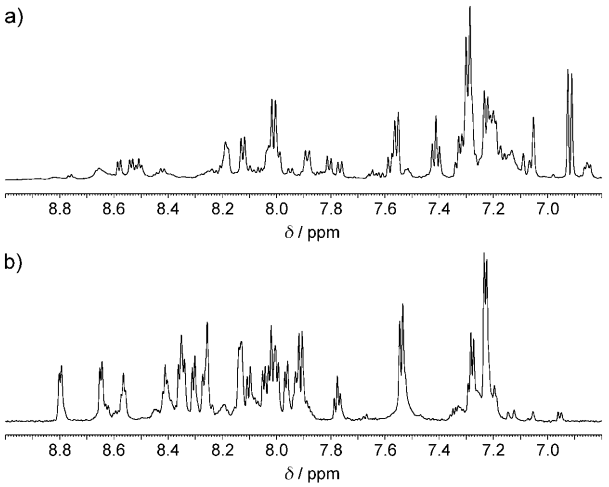

Figure 5. ^1H NMR spectra of peptide **2** in D_2O/H_2O (1:9), pH 6 recorded at 600 MHz; a) *cis* form in pss and b) *trans* form, containing traces of the *cis* form.

corresponding non-switchable model peptide that adopts a structure similar to the β-hairpin in the native protein in aqueous solution. The interaction of a biologically important β-sheet with a protein domain has been modulated by a light-induced conformational change without destabilizing the system. Therefore, the peptide may serve as a suitable model for a light-triggered β-sheet for use in cells, however for intracellular applications peptides may be subject to reduction[15] and proteolytic degradation.

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