Ultrafast Unzipping of a Beta-Hairpin Peptide

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Abstract. Light induced switching of a beta-hairpin structure is investigated by femtosecond IR spectroscopy. While the unzipping process comprises ultrafast kinetics and is finished within 1 ns, the folding into the hairpin structure is a much slower process.

1. Introduction

Protein misfolding is the cause of severe human diseases, such as Alzheimer's disease, Creutzfeld-Jacob or Parkinson's disease, where conformational transitions of peptides or proteins take an active role in forming stable, often fibrillar, arrangements in the human brain leading to the pathological symptoms. A general feature of the disastrous conformational changes is a transition from native and "healthy" structures to β-extended precursors of insoluble fibrils. We have now succeeded in designing, synthesizing and investigating a special β-hairpin model [1], where the introduction of a femtosecond light switch in a β-hairpin peptide allowed the observation of ultrafast structural changes - orders of magnitude faster than in previous experiments on β-structures. We show here that an optical trigger starts a series of picosecond conformational transitions of the β-structure (see Fig. 1) which are finished extremely fast on the subnanosecond time scale.

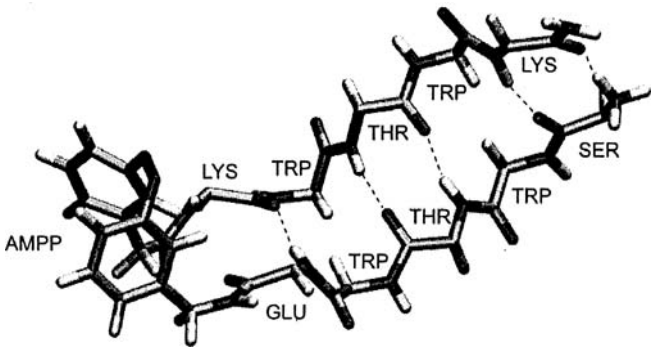

Fig. 1. Structue of the β-hairpin model system in the *cis* form of the AMPP.

The study of the ultrafast β-hairpin folding and unfolding was enabled by the construction of a peptide model system where the absorption of light allows the reversible switching between two different structures. We follow here the line of other successful investigations on light triggered chromopeptides, where different types of switching molecules were used [2, 3]. The new molecular system was constructed in such a way that the *cis*-conformation of the azobenzene switch, the 3-(3-aminomethyl-phenylazo)phenyl-acetic acid (AMPP), mimics the turn within a β-hairpin. It was confirmed by NMR that the peptide acquires a β-hairpin structure when the switch is in the *cis* form [1]. The hairpin structure is stabilized by hydrogen bonds between the two peptide β-strands and by hydrophobic interactions between tryptophanes attached to these strands (see Fig. 1). It is interesting to note, that a differently folded structure results, if the switch is in the *trans* conformation. Here coupling between the N- and C-terminal parts of the peptide is reduced by the *trans*-geometry of the azobenzene switch.

2. Experimental

The experiments are performed by the pump and probe method using femtosecond pump pulses exciting the nπ*- transition of the azobenzene at 404 nm and probing infrared pulses [4, 5] in the spectral range of the amide region (1750 - 1550 cm^{-1}). The excitation induced absorption changes are recorded by a 32-element MCT-detector array. The sample solution (the β-hairpin molecules dissolved in deuterated methanol) is used in the *cis*-rich photostationary state.

The known properties of the amide I absorption band are used for qualitative interpretation of the experimental observations: when a non-interacting amide group is placed in non-polar solvents, the amide I frequency is close to 1700 cm^{-1}. Increased polarity of the solvent reduces the absorption frequency into the 1650 cm^{-1} range. Hydrogen bonding and strong dipole interactions to other amide groups may lead to absorption at even lower frequencies. These qualitative interpretations are supported by quantum mechanical calculations of the molecular constituents of the hairpin model system considering results on the molecular structure obtained by molecular dynamics simulations.

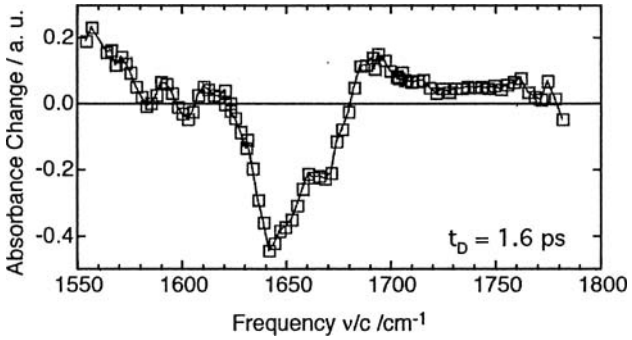

Fig. 2. Transient absorption changes induced by pumping the β-hairpin model (*cis* form of the AMPP chromophore) with light at 404 nm. Pronounced absorption changes are found, indicative for a strongly disturbed molecular structure.

3. Results and Discussion

Results from the time resolved experiments are shown in Fig. 2. Immediately after excitation of the azobenzene chromophore, it undergoes internal conversion to the ground state. At that time we observe a characteristic change of the infrared absorption spectrum. Further absorption curves yield indications for a strong heating of the molecule (broad absorption increase and bleach of groundstate absorption band), disruption of hydrogen bonds and desolvation of the amide group (absorption reduction around 1630 cm^{-1}, and absorption increase at 1680 cm^{-1}). During the next few picoseconds the absorption decrease in the 1630 cm^{-1} range is intensified. At the same time the molecular temperature is reduced. After about 10 ps the model system has acquired an open form with reduced hydrogen bonding and desolvated amide groups. One may interpret the peptide structure reached at that time as a (molden) intermediate state, from where the different folding routes towards new configurations can be accessed. After 150 ps the first steps in that direction are completed: the desolvated amide groups are resolvated (absorption decreases at 1680 cm^{-1}). Within the next 600 ps we observe a strong absorption increase at 1660 cm^{-1} leading to a difference spectrum which strongly resembles the difference spectrum in the photostationary state. Thus the molecule has essentially finished the reactions from the β-hairpin to the newly folded structure.

In conclusion, we presented a β-hairpin model system, where the incorporation of the azobenzene switch AMPP allows the ultrafast unzipping of the β-hairpin structure. Preliminary experiments on the inverse reaction from *trans* to *cis*, show that the light triggered formation of the hairpin structure also contains ultrafast components, but is not finished within 1 ns. Much slower reaction dynamics are present which extend into the microsecond range.

References

1 S.-L. Dong, M. Löweneck, T.E. Schrader, W.J. Schreier, W. Zinth, L. Moroder and C. Renner, in *Chem. Eur. J.* Vol.12, 1114, 2006.

2 S. Spörlein, H. Carstens, H. Satzger, C. Renner, R. Behrendt, L. Moroder, P. Tavan, W. Zinth, and J. Wachtveitl, in *Proc. Natl. Acad. Sci. USA*, Vol.99, 7998, 2002.

3 J. Bredenbeck, J. Helbing, A. Sieg, T. Schrader, W. Zinth, C. Renner, R. Behrendt, L. Moroder, J. Wachtveitl and P. Hamm, in *Proc. Natl. Acad. Sci. USA*, Vol.100, 6452, 2003.

4 E. Riedle, M. Beutter, S. Lochbrunner, J. Piel, S. Schenkl, S. Spörlein, and W. Zinth, in *Appl. Phys.* Vol.B 71, 457-465 (2000).

5 T. Schrader, A. Sieg, F. Koller, W. Schreier, Q. An, W. Zinth and P. Gilch, in *Chem. Phys. Lett.* Vol.392, 358, 2004.