Towards a Molecular Movie: 
Real Time Observation of Hydrogen Bond Breaking by 
Transient 2D-IR Spectroscopy in a Cyclic Peptide

Christoph Kolano, Jan Helbing and Peter Hamm
Physikalisch-Chemisches Institut, Universität Zürich, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland
phamm@pci.unizh.ch

Wolfram Sander
Lehrstuhl für Organische Chemie II, Ruhr-Universität Bochum, Universitätsstr. 150, D-44801 Bochum, Germany

Abstract: Transient two-dimensional infrared spectroscopy (T2D–IR) has been used to observe in real time the non-equilibrium structural dynamics of intramolecular hydrogen bond breaking in a small cyclic disulfide-bridged peptide.

©2006 Optical Society of America

OCIS codes: (000.1570) Chemistry; (300.6340) Spectroscopy, infrared; (300.6420) Spectroscopy, nonlinear; (300.6530) Spectroscopy, ultrafast

1. Introduction

The understanding of processes in living organisms at the molecular level is one of the most challenging questions currently investigated in biological research. Understanding peptides at the atomic level means determining the unique and highly organized functional three dimensional structure in which peptides fold as well as the mechanism of the folding process. The mechanism of folding is strongly influenced by non-covalent interactions, most importantly hydrogen bonding, which plays a decisive role in defining the three-dimensional structure of proteins and peptides. This comparatively weak interaction keeps peptide chains (e.g. collagen helix) together, stabilizes secondary structures (e.g. α-helix and β-sheet) and changes the direction of a peptide chain (β-turn).

Scheme 1. Band assignement and proposed opening of cyclo(Boc-Cys-Pro-Aib-Cys-OMe).

Our view of structural properties and hydrogen bonds in biomolecules is mainly based on the static architecture, probed by X-ray and neutron crystal structure analysis and nuclear magnetic resonance (NMR). Changes in these architectures on ultrafast timescales are, however, very difficult or impossible to observe by these techniques. Ultrafast two-dimensional infrared spectroscopy (2D-IR) and transient two-dimensional IR spectroscopy (T2D-IR), on the other hand, are promising spectroscopic tools to monitor peptide dynamics in real time. 2D-IR spectroscopy is a technique ideally suited for fast dynamical processes, offering means to resolve distributions and dynamics of fast interconverting structures in equilibrium. Transient 2D-IR can be understood as an extension of the 2D-IR experiment to the non-equilibrium regime, which allows to take full advantage of the high time resolution. Substantial changes can be found in the transient 2D-IR spectra at times when conventional pump-probe spectra show only minor time dependence. [1] Here we use T2D-IR to investigated the opening of a beta-turn, formed by the small cyclic disulfide-bridged peptide cyclo(Boc-Cys-Pro-Aib-Cys-OMe). [2] This beta turn is stabilized by a hydrogen bond, the breaking of which could be monitored and characterized in real time directly.
2. Results and Discussion

The stationary FTIR absorption spectrum of the peptide cyclo(Boc-Cys-Pro-Aib-Cys-OMe) in CD$_3$CN at ambient temperature consists of four well resolved bands in the amide I region (plus ester protection group) and is shown in Figure 1a. Since a disulfide bridge (–S–S–) constitutes a weak covalent bond (bond dissociation energy 64.5 kcal/mol), the disulfide bridge in the peptide serves as a predetermined “breaking point”, which can be cleaved by UV light ($\lambda_{\text{exc.}}$=266 nm) generating non-equilibrium conditions.[3] Figure 1b shows the magic angle difference infrared absorption spectra at different time delays after excitation of the peptide in CD$_3$CN. The pulsed photolysis resulted in the instantaneous bleaching of the amide I bands of the starting conformation and the formation of a new set of amide I bands. To extract the relevant times scales involved in the dynamics, the magic angle signals after excitation were analyzed using SVD. The corresponding time traces have been fitted simultaneously by a function containing three exponentials, yielding three global time constants. The first time constant (approx. 20 ps) reflects the cooling of the molecule as excess energy is dissipated to the solvent. The second time constant (approx. 200 ps) is assigned to the conformational change of the entire backbone, which is accompanie by the weakening of the intramolecular hydrogen bond (see Scheme 1). The last time constant (approx. ns time scale) is dependent on the concentration of the solution and not present in the most dilute samples. It is caused by thiyl radicals that diffuse apart and undergo a diffusion controlled intermolecular reaction with other radicals or unreacted peptide.

Fig. 1. (a) FTIR absorption spectrum of the amide I region of the peptide in CD$_3$CN at ambient temperature. Band assignments correspond to the highlighted regions in the peptide in the previous scheme. (b) Magic angle difference infrared absorption spectra at different time delays after excitation $\lambda_{\text{exc.}}$=266 nm of the peptide in CD$_3$CN (spectral resolution 4 cm$^{-1}$, concentration 200 mM, degassed). Bands appearing on irradiation are pointing upward; bands disappearing are pointing downward.

To obtain structural informations, we extended our investigations to the second dimension. Two-dimensional infrared (2D-IR) spectroscopy allows for the detection of the coupling between certain vibrational modes of different peptide units in a similar way as 2D-NMR spectroscopy measures couplings between spin states. The 2D-IR experiment is performed under equilibrium conditions and gives rise to crosspeaks in the off-diagonal region that arise from coupling between vibrational modes that are in close proximity or connected to each other. The equilibrium 2D-IR spectrum of cyclo(Boc-Cys-Pro-Aib-Cys-OMe) in CD$_3$CN at ambient temperature is shown in Figure 2a. In addition to the complex set of crosspeaks that arises from the coupling of the nearest neighbours, we observe a crosspeak located at 1640 cm$^{-1}$ caused by the intramolecular hydrogen bond between the C=O of Cys1 and the NH group of the peptide bond between Aib and Cys4. To investigate the breaking of the intramolecular hydrogen bond, we extended our investigations to the non-equilibrium. Figure 2b shows a series of T2D-IR spectra at different delay times between the UV pump pulse and the IR pulse sequence. After UV excitation we observed a
constant bleaching of the crosspeak at 1640 cm\(^{-1}\), which was caused by the intramolecular hydrogen bond. This result indicates that the initial structure of the backbone, including the intramolecular hydrogen bond, is falling apart on cleavage of the disulfide bridge (creating non-equilibrium conditions). The time of disappearance of the cross peak is in perfect agreement with the extracted times scales from the pump-probe experiments and confirm the proposed opening of cyclo(Boc-Cys-Pro-Aib-Cys-OMe) (Scheme 1 and Fig. 1).

3. Conclusion

Hydrogen bonds play an important role in defining the three-dimensional structure of proteins and peptides, but their dynamics is difficult to track with conventional techniques. We have presented the first real time observation of the breaking of an intramolecular hydrogen bond in a small cyclic disulfide-bridged peptide upon ultrafast cleavage of the disulfide bridge using transient 2D infrared spectroscopy. The breaking of the intramolecular hydrogen bond and therefore the collapse of the initial \(\beta\)-turn structure is implicated by continuous bleaching of the corresponding transient cross-peak and takes place on a time scale of a few hundred picoseconds. Our spectra demonstrate that T2D-IR spectroscopy is a powerful tool to probe non-covalent interactions in biomolecules and to monitor essential structural changes in real time.

4. References