

Site-Specific Folding Dynamics of Peptides Studied by Time-Resolved Infrared-Spectroscopy

C. Krejtschi¹, O. Ridderbusch¹, R. Huang², T.A. Keiderling², K. Hauser¹

¹*Institut für Biophysik, University of Frankfurt, Max-von-Laue-Str. 1, 60438 Frankfurt, Germany*

²*Department of Chemistry, University of Illinois at Chicago, 845 W. Taylor St. Chicago, Illinois 60607-7061, USA*

Peptides with well-defined secondary structure are ideal model systems to study protein folding mechanisms by infrared spectroscopy. IR-techniques provide both, the necessary time resolution as well as the structural sensitivity. The amide I' region, mainly the C=O stretching vibrations of the polypeptide backbone, is a marker band for secondary structure. However, vibrational transitions of individual amide groups are not resolved. Isotopic labeling of individual amide ¹³C=O groups induce site-specific frequency shifts and thus enhance localized structural information. We initiate thermal unfolding by a nanosecond laser-excited temperature jump (~10 °C) and probe amide I' absorption changes at single wavelengths. The alpha-helix to random coil transition of polyglutamic acid has been studied under reversible folding/refolding pHconditions [1]. The observed relaxation kinetics indicate a 2-state folding process and time constants for folding and unfolding could be extracted using complementary equilibrium measurements. Site-specific dynamics have been monitored for the thermal unfolding of an isotopically labeled β-hairpin peptide, a 12-mer tryptophan zipper peptide whose conformation is stabilized by a hydrophobic core formed by four tryptophan residues. Various single and crossstrand ¹³C=O isotopically labeled peptide variants have been studied by probing the relaxation kinetics at individual amide I' components. Differences in kinetic behavior have been found for the loss of beta-strand and the gain of disordered structure. The isotope-edited kinetics show variations in local structural stability of the hairpin backbone. Our data supports a multistate dynamic behavior that prevents clear determination of folding and unfolding time constants. Nonetheless, the site-specific kinetics are consistent with a hydrophobic collapse hypothesis for hairpin folding [2].

[1] C. Krejtschi, R. Huang, T.A. Keiderling, K. Hauser, *Vibrational Spectroscopy* (2008), in press.

[2] K. Hauser, C. Krejtschi, R. Huang, L. Wu, T.A. Keiderling, *J. Am. Chem. Soc.* 130 (2008) 2984-2992.