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1805-Plat Trpzip-Based Beta-Hairpin Equilibrium and Temperature Jump IR Studies Enhanced by Site-Specific Isotope Labeling

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Beta-hairpins may be the smallest folding units in a protein, and two antiparallel beta-strands connected by a turn make the simplest model system for analysis of the interactions and dynamics of beta-sheets. We have studied site-specific conformational dynamics by use of equilibrium and temperature-jump kinetic IR-spectroscopy with site-specific enhancement via isotopic labelling of the amide with 13C=O in isotopically labeled variants of a modification of Cochran's 12-residue tryptophan zipper peptide, TrpZip2. Equilibrium measurements reflect decreased stability of the hairpin cross-strand H-bonds at the turn and the termini. Spectral analysis of single and doubly labeled species is used to determine specific coupling levels. 13C=O groups introduced at different amide positions lead to distinguishable cross-strand coupling of the labelled residues which is lost on unfolding. These labels have distinct frequency patterns and different thermal behaviors depending on their position in the hairpin and reflect the local structural variation along the strands. Relaxation kinetics upon laser-induced T-jumps of ~10 C have time constants of a few microsec that decrease with increase of the initial temperature of the peptide before the temperature jump. Analysis of the data supports a multistate folding process, consistent with the hydrophobic collapse hypothesis for hairpin folding, but it is not possible to clearly define a folding and unfolding rate.