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Residual Native Structure in a Thermally Denatured β-Hairpin

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We investigate the thermal denaturation of trpzip2 between 15 and 82 °C using two-dimensional infrared (2D IR) vibrational spectroscopy, dispersed vibrational echo (DVE) spectroscopy, and Fourier transform infrared (FTIR) spectroscopy. The FTIR and DVE spectra of trpzip2 show in the amide I region of the spectrum two resonances, which arise primarily from the interstrand coupling between local amide I oscillators along the peptide backbone. The coupling is seen directly in the 2D IR spectra as the formation of cross-peak ridges. Although small shifts of these frequencies occur on heating the sample, the existence of cross-peak ridges at all temperatures indicates that stable hydrogen bond interactions persist between the two β-strands. These observations indicate a significant amount of native structure in the thermally denatured state of trpzip2.

Small peptides that adopt a single secondary structure are valuable model systems to study factors influencing protein folding and stability.[1,2] Of these, β-hairpins represent a fundamental subunit of extended β-structures that are common in naturally occurring proteins. The small size of hairpin-forming peptides makes them well-suited for folding studies[3−6] because they can be directly modeled with molecular dynamics simulations and experimental spectra are more easily related to the peptide morphology. Additionally, debate about the folding pathway for β-hairpins mirrors the questions posed for folding in globular proteins, which centers on the time ordering, relative importance, and cooperativity between physical events. For example, recent investigations of hairpin peptides have proposed several different initiating events in the folding pathway such as the collapse of the hydrophobic core or the formation of native contacts in the turn region.[7−9] Further restructuring can occur through the formation of native hydrogen bonds or through more efficient packing of the side chains. These events may happen in concert or in a specific sequence. Experimentally it has been difficult to obtain detailed structural information on the time scale of these events that can discriminate between the various pathways. It is especially difficult to identify and characterize non-native ensembles, which are necessary to describe the pathway from the unfolded conformational ensemble to the more compact native state.[6] In this study we have used two femtosecond vibrational spectroscopies, two-dimensional infrared (2D IR) spectroscopy and dispersed vibrational echo (DVE) spectroscopy,[10,11] to investigate changes in the amide I band during the equilibrium thermal denaturation of the trpzip2 hairpin. These methods are particularly sensitive to hydrogen bonding contacts between peptides in antiparallel registry. We find that the interstrand hydrogen bond network of trpzip2 is thermally stable and retains a considerable amount of native contact in the thermally denatured state.

The tryptophan zipper peptides are a family of de novo β-hairpins designed to be especially stable. Much of the stability is attributed to the tryptophan residues that form a tightly folded hydrophobic core.[12] Trpzip2 is a twelve residue peptide in which two β-strands are connected with a type I′ turn using the ENGK sequence. It has been studied using molecular dynamics simulations and a variety of experimental methods.[6,13−17] Equilibrium studies of thermal denaturation using circular dichroism, fluorescence, and IR spectroscopy have shown that the energy landscape of trpzip2 is heterogeneous, with a variety of configurational minima.[14] Melting temperatures from each measurement differed and were interpreted as the transition temperatures for various structural changes. The FTIR data indicated a melting temperature of >60 °C, which was interpreted as the temperature at which backbone hydrogen bonds are broken.[14] Subsequent work characterized IR absorbance changes of the amide I band around 1676 cm^{-1} and gave a melting curve with a much narrower melting transition centered at 60 °C.[15] The 2D IR spectroscopy used in this study builds on the information obtained from the earlier FTIR spectroscopy by adding an extra dimension of resolution and increased sensitivity to vibrational coupling. The results of this work show directly that the two amide I vibrational modes resulting from the native interstrand hydrogen bonds persist up to 82 °C.

The IR spectra of β-strands in antiparallel registry show two amide I vibrational resonances. Strong electrostatic couplings between local amide I oscillators lead to vibrations that are delocalized across the sheet.[18,19] The IR oscillator strength is carried by two modes whose transition moment is oriented roughly perpendicular or parallel to the strands ($\nu_\perp$ and $\nu_\parallel$), and whose splitting is primarily determined by the interstrand coupling.[18] Such features are difficult to discern in traditional IR spectra, which are often broadened by disorder; however, delocalized amide I states can be revealed with 2D IR spectroscopy.[20] 2D IR spectroscopy is a vibrational analogue of multidimensional NMR.[21,22] It uses a sequence of femtosecond mid-infrared pulses to interrogate couplings between molecular vibrations. These are observed as cross-peaks in a Fourier transform 2D IR spectrum. Our prior investigations show

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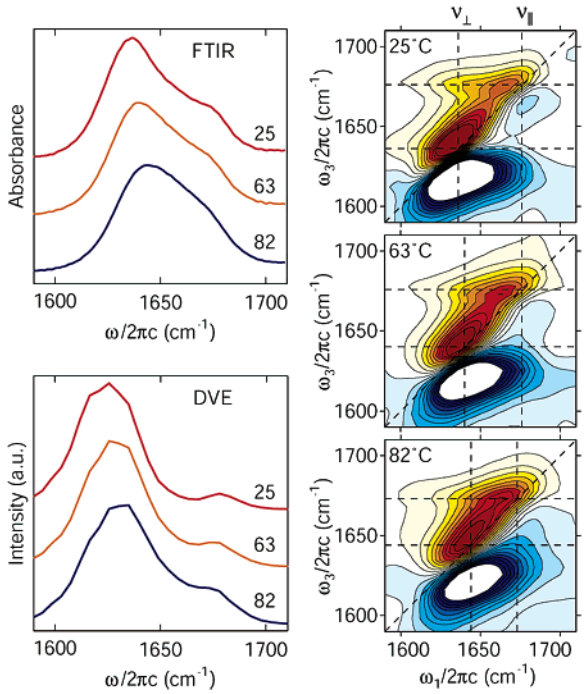

Figure 1. FTIR, DVE, and 2D IR spectra of trpzip2 are shown at three representative temperatures: 25, 63, and 82 °C. FTIR and DVE spectra are offset for clarity. For 2D IR spectra, 20 equally spaced contour levels are drawn to ±60% of peak intensity.

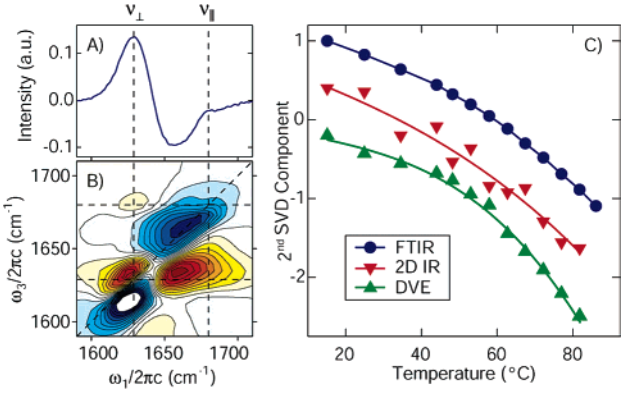

Figure 2. Second component spectra, $c_N^{(2)}(\omega)$, calculated from the SVD analysis of the FTIR (A) and 2D IR (B) data. The dashed guidelines intersect the axes at 1629 and 1680 cm^{-1}. (C) Melting curves from each experiment are plotted as the amplitude of the second SVD component at each temperature. The solid lines are guides for the eye. For clarity, the 2D IR and DVE melting curves are offset by −0.6 and −1.2, respectively.

that cross-peaks are observed between the $\nu_\perp$ and $\nu_\parallel$ modes of antiparallel β-sheets, even when the FTIR spectrum is congested.[10,11] For proteins, the cross-peaks elongate and interfere with the diagonal peaks to become ridges, yielding characteristic "Z"-shaped contours in the 2D IR line shape.[10,11,23] By using 2D IR spectroscopy to investigate trpzip2, it is possible to characterize the changes in interstrand amide I couplings that would arise from complete unfolding of the hairpin.

Trpzip2 was synthesized as a C-terminal amide peptide using established Fmoc solid-phase synthesis. The peptide was HPLC purified and then further treated by lyophilization against 50 mM HCl to remove residual trifluoroacetic acid. The sample was dissolved in D_2O, heated to 60 °C for 30 min for hydrogen−deuterium exchange, and lyophilized before being used in the experiments. For all experiments, the sample was dissolved in a 50 mM, pH 7.0, potassium deuterium phosphate buffer to a final concentration of about 4 mM. For spectral measurements, the sample was placed between CaF_2 windows separated with a 50 μm Teflon spacer. The filled sample cell was heated to 80 °C and then cooled to room temperature before any measurements were taken. The experimental methods used to acquire 2D IR and DVE spectra are described elsewhere.[10] Spectra were collected over temperatures ranging from 15 to 86 °C. Higher temperatures were not accessed because of trpzip2's propensity to aggregate. Under the described conditions, there was no spectral evidence for the formation of trpzip2 aggregates. The sample conditions in this study closely matched those outlined in previous investigations where there also was no evidence of aggregation.[14,15]

Representative FTIR spectra are shown in Figure 1. In the room-temperature spectrum, the amide I absorption maximum is at 1636 cm^{-1}, with a shoulder at higher frequencies indicating a second peak at 1676 cm^{-1}. The overall changes in the FTIR spectrum with temperature were analyzed by singular value decomposition (SVD). The second component spectrum, representing the spectral changes over the temperature range, is displayed in Figure 2a, with the corresponding melting curve shown in Figure 2c. The loss of the two-mode structure is seen in the second component spectrum as two positive-going peaks at 1629 and 1680 cm^{-1}, representing the $\nu_\perp$ and $\nu_\parallel$ modes at 1636 and 1676 cm^{-1}. The broad negative peak at 1655 cm^{-1} is attributed to increasing random coil-like geometry. Although there is no indication of a sharp transition temperature, these results suggest that interstrand couplings are being lost during thermal denaturation. Indeed the loss of amplitude in the shoulder was previously observed and interpreted as loss of the high-frequency component common to antiparallel β-sheets.[15]

Representative temperature-dependent 2D IR spectra of trpzip2 are shown in Figure 1, and results of SVD analysis are shown in Figure 2b,c. At 25 °C, two peaks are observed along the diagonal axis ($\omega_1 = \omega_3$) corresponding to the $\nu_\perp$ and $\nu_\parallel$ resonances. (In 2D IR spectra, each resonance is composed of a vertically displaced, oppositely signed doublet.) Cross-peaks between the two resonances appear in the off-diagonal region of the spectrum and are shaped here due to overlap with the main diagonal peaks. In the off-diagonal region centered at $\omega_1 = \nu_\perp$ and $\omega_3 = \nu_\parallel$, the cross-peaks appear as a ridge stretching in the ω_1 direction at constant ω_3. The ridges indicate a distinct two-mode structure characteristic of the splitting induced by vibrational couplings and consistent with the cross-strand interactions of a hairpin with antiparallel symmetry.[11,18] With increasing temperature, the diagonal peaks shift to band center, and some of the ridge structure is lost (Figure 2b). However, at 82 °C there are still two distinct maxima in the positive diagonal peaks. The splitting decreases with increasing temperature, but the cross-peak ridge is observed at all temperatures. This indicates that antiparallel structure and considerable native hydrogen bonding contacts remain in trpzip2 at high temperature.

Similar observations are also apparent in the DVE spectra (Figures 1 and 2c). DVE measurements are related to the absolute value of the 2D IR surface projected onto the ω_3 axis.[10] For β-sheets, the projection along cross-peak ridges that originate in the interstrand couplings leads to distinct features in the spectrum. The DVE spectra of trpzip2 display two resolvable peaks in the amide I′ region of the spectrum (Figure 1). At higher temperatures, the peaks shift toward band center, but retain a spacing of about 30 cm^{-1} at 82 °C. This result provides further evidence of residual hydrogen bond interactions between the two strands in the denatured state.

The melting curves for each of the infrared spectroscopy techniques in this study follow the same trends, including a broad melting transition and an approximate melting temperature of 60 °C. Similar trends were observed in previously reported FTIR data.[14,15] Wang et al. specifically analyzed the high frequency (~1676 cm^{-1}) amide I transition in the FTIR spectrum, assigning it to the $\nu_{||}$ component of the β-sheet doublet and interpreting the loss of the peak as the loss of β-sheet structure. This high frequency mode is directly observable in the 2D IR and DVE spectra without the need for band decomposition or subtraction methods. The added resolution, along with the direct observation of coupling, confirms the peak assignment, while contributing much more detail to the structural interpretation. Specifically, the cross-peak ridge observed in the 82 °C 2D IR spectrum demonstrates that the symmetry of the antiparallel contacts between strands is preserved and that the proximity of the strands for these contacts remains similar to that at low temperatures. Because both the 2D IR and the DVE spectra are able to explicitly resolve the high-frequency component at 82 °C, we conclude that there is a more significant population of molecules with intact interstrand hydrogen bonds than was previously recognized.

The results of our IR measurements point to a picture in which the peptide remains compact, with interstrand hydrogen-bond contacts persisting, even at high temperatures. Our high temperature spectra are consistent with fraying of the ends of the hairpin and an intact turn region much like the frayed or F state recently reported in the Bolhuis study of the GB1 hairpin.[7] We also can not rule out increased amplitude of fluctuations around the native geometry, a view consistent with the mean structure hypothesis proposed by Zagrovic et al.[6] More specific conclusions will be possible following careful simulations of the amide I vibrational couplings and spectroscopy, drawing on the results of previous molecular dynamics simulations.[6,14,16,17] This work has shown that the details obtained from the 2D IR and DVE spectra add another experimental observable that can help researchers construct a more accurate model of β-hairpin folding. By incorporating these methods into a transient experiment,[24] it will be possible to resolve events such as the formation of the turn region and the formation of interstrand hydrogen bonds. The results may be combined with those obtained from time-resolved fluorescence experiments that probe the collapse and restructuring of the hydrophobic core to more accurately test recent simulations of β-hairpin folding.[7−9]

The gradual changes in the trpzip2 melting curves over the temperature range measured here indicate a thermally stable hydrogen bond network. This observation, combined with the earlier observation of a compact hydrophobic core at high temperatures, leads to the conclusion that temperature-dependent studies on β-hairpins, either at equilibrium or as temperature-jump experiments, may not be probing large changes in peptide conformation, at least without the presence of additional chemical denaturant. Such considerations must be taken into account when the purpose is to obtain detailed information on folding from an extended state. Ongoing investigations are needed to understand how this observation holds for other systems and how it varies depending on the stability of the system. De novo systems designed for stability may inherently fold differently when compared to naturally occurring systems where the dynamics are shaped through evolutionary mechanisms.

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