Hindawi Publishing Corporation
Spectroscopy: An International Journal
Volume 27 (2012), Issue 5-6, Pages 557–564
doi:10.1155/2012/102423

Impact of β-Turn Sequence on β-Hairpin Dynamics Studied with Infrared-Detected Temperature Jump

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Abstract. Folding dynamics for β-structure loss and disordered structure gain were studied in a model β-hairpin peptide based on Cochran's tryptophan zipper peptide Trpzip2, but with an altered Thr-Gly (TG) turn sequence, that is, SWTWETGKWTWK, using laser-induced temperature-jump (T-jump) kinetics with IR detection. As has been shown previously, the TG turn sequence reduces the thermodynamic β-hairpin stability as compared to the Asn-Gly sequence used in Trpzip2 (TZ2-NG). In this study, we found that the TG-turn slows down the overall relaxation dynamics as compared to TZ2-NG, which were studied at higher temperatures where the time constants show little difference between relaxation of the β-strand and the disordered conformation. These time constants become equivalent at lower temperatures for TZ2-TG than was seen for TZ2-NG. The correlation of thermodynamic stability and rates of relaxation suggests that the change from NG to TG turn results in a slowing of folding, lower k_f, with less change of the unfolding rate, k_u, assuming two state behavior at higher temperatures.

Keywords: Hairpin peptide, tryptophan, tryptophan zipper, temperature jump kinetics, β-turn, infrared spectroscopy

1. Introduction

The simplest model of a β-sheet is the β-hairpin consisting of a single sequence folded back onto itself to generate two antiparallel strands coupled by a turn. Hairpins have been proposed to act as nucleation sites for protein folding [1–4], and, in particular, β-sheet formation is biomedically relevant due to its role in many diseases, especially amyloid-related ones [5–7]. As compared to sheet structures, β-hairpins are more easily studied and modeled since only two strands interact in a restricted manner, rather than multiple strands as is the case for aggregate or fibril structures.

β-hairpins can be stabilized either by selecting turn residues that tend to bring the strands close enough to enable cross-strand H-bonding, or by interaction, normally hydrophobic, of residues on

opposite strands to stabilize a compact conformation and facilitate turn and H-bond formation [1, 4, 8–10]. The role of the turn in stabilizing the hairpin has been the topic of several equilibrium studies in an attempt to propose mechanisms for the folding [1, 11, 12]. Some of the most studied hairpins are the tryptophan zipper (Trpzip) peptides of Cochran and coworkers [8], which have pairwise Trp-Trp interactions, that can be monitored by a strong exciton-based circular dichroism (CD) arising from the cross-strand contact [13–15]. By contrast, amide I' IR spectra (C=O stretch vibration) sense the peptide backbone conformation and are particularly useful for β-sheet studies [13, 14, 16–23]. Trpzip molecules at low temperatures have characteristic β-structure IR which reverts to a disordered structure spectrum at higher temperatures [16, 17, 22–24]. We have separately reported equilibrium studies of various modifications of the Trpzip2 (TZ2-NG) hairpin sequence Ser-Trp-Thr-Trp-Glu-Asn-Gly-Lys-Trp-Thr-Trp-Lys (SWTWENGKWTWK) [8], varying both the Trps and the turn sequence residues [13, 14, 16, 17, 25, 26]. We and others have also used laser-initiated T-jump spectroscopy to study dynamics of the TZ2-NG sequence and have incorporated isotopic labels to specify sequence dependence of the folding mechanism [17, 27, 28]. In this paper, we compare dynamics for this type of hairpin by modifying the turn sequence (Figure 1), changing from Asn-Gly (TZ2-NG) to Thr-Gly (TZ2-TG), which lowers the hairpin stability, as we have shown separately [25] and slows its relaxation dynamics, as will be shown here.

2. Experimental Section

2.1. Peptide Synthesis and IR Sample Preparation

Peptides TZ2-TG with Thr-Gly turn were synthesized and characterized at UIC using a manual solid state method as described previously [13]. For this study, purified peptides were dissolved directly in D_2O for equilibrium IR measurements without adjusting pH or removing the TFA counterions remaining from the peptide synthesis. Previous studies had varied pH and concentration as well as removed TFA but for the experiments reported here a simpler approach was taken, bypassing lyophilization, which additionally enhanced solubility. For IR studies, TFA containing samples were prepared at low pH and $\sim$20 mg/mL ($\sim$12 mM), which evidenced good solubility and reversible folding.

2.2. Infrared Equilibrium Spectra

Infrared spectra in thermal equilibrium were measured in the range of 5°C–85°C with 5°C steps at 4 cm^{-1} resolution using a Bruker Equinox 55 FTIR spectrometer with a HgCdTe detector. The temperature was controlled by a thermostatted water bath (Lauda Ecoline E300) coupled with a home-built cell holder. The peptide solution was sealed between two CaF_2 windows separated by a 100 μm Teflon spacer. Both the reference cell and the sample cell were mounted on a sample shuttle and measured sequentially after equilibration to obtain one spectrum for each temperature so that interference due to long-term baseline drift is avoided. The thermal variations of the FTIR spectra were fit to a standard two-state equilibrium expression to determine the transition temperature, T_m, using a linear low-temperature and a flat high-temperature baseline connection as previously described [16, 25].

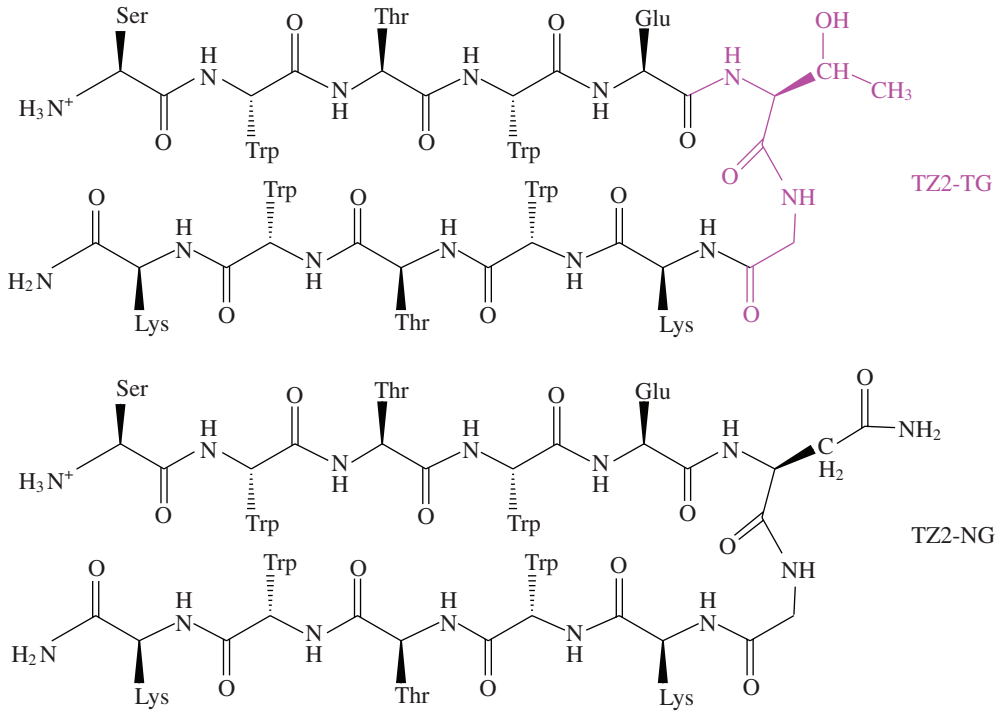

Figure 1: Sequences of Trpzip2 (TZ2) β-hairpin peptides with different turns, Thr-Gly (TZ2-TG), and Asn-Gly (TZ2-NG). Dynamics have been studied for the TG-variant and compared to earlier studies of the NG-variant.

2.3. T-Jump Measurements

T-jump experiments were performed on the home-built instrument which has been described in detail previously [29]. The temperature jump in the sample was obtained by a Raman-shifted 5 ns Nd: YAG laser pulse at 1909 nm. In order to reduce inhomogeneous heating of the peptide sample, the pump pulse was split into two equivalent parts and aligned to impinge on the front and the back side of the sample cell. One part of the excitation pulse was delayed by 5 ns which can reduce the occurrence of cavitation [30]. The rapid heating of the sample by the laser pulse shifted to 1.9 μm was adjusted to yield ΔT $\sim$10°C for various initial temperatures in the range from 5°C to 40°C.

A lead salt laser diode (Mütek TLS 310) provided CW modes for probing transient transmission changes during the T-jump. Two modes at 1641 cm^{-1} and 1663 cm^{-1} were used to detect the decay of the β-sheet structure and the rise of the random coil structure, respectively. The IR-signal was detected using a photovoltaic HgCdTe detector (20 MHz, Kolmar KMPV11-1-J2) and digitized by a transient recorder board for further data analysis. For each measured temperature and wavelength, one dataset with 2000 corrected transients was acquired as the difference of 2000 collected with the probe laser on and 2000 with the probe laser blocked in order to eliminate background radiation and potential baseline drift.

The observed transients were fitted with a biexponential function:

$$\Delta A(t) = A_0 + A_1 \cdot \exp\left(-\frac{t}{\tau_1}\right) + A_2 \cdot \exp\left(-\frac{t}{\tau_2}\right) \tag{2.1}$$

in order to describe both the dynamics of the TZ2-TG relaxation and the solvent cooling. Fitting details have been reported previously [17, 27].

3. Results and Discussion

Like TZ2-NG, TZ2-TG adopts a β-sheet secondary structure mainly stabilized by Trp-Trp interaction [13]. However, TZ2-TG is less stable, having an apparent T_m of 328 K for the sample conditions used for the T-jump study as compared to 345 K [8] or 352 K [25] for TZ2-NG, depending on conditions, as we reported previously. The role of modifying the turn sequence from Asn-Gly to Thr-Gly in terms of dynamic behavior becomes apparent in the T-jump experiments.

The relaxation of the TZ2-TG peptide sample was probed at two different wavelengths and the relaxation times τ_{obs} were determined after the temperature jump for a series of peptide temperatures. The 1641 cm^{-1} position primarily senses the cross-strand interaction and 1663 cm^{-1} the disordered structure. Four representative transients for $T = 312$–323 K are shown in Figure 2. These data picture the dynamics of the peptide alone since they have been corrected for contributions from the D_2O relaxation. Table 1 summarizes the temperature-dependent relaxation times τ_{obs} measured for TZ2-TG.

The decreased peptide relaxation time of TZ2-TG with increasing temperature is consistent with normal Arrhenius behavior and parallels the data that we have observed on several other (strand-centered) variants of TZ2-NG [17, 27] and helical peptides [31]. Unlike for TZ2-NG and its isotopic variants, the relaxation rates of the β-structure (cross-strand H-bonding) and the disordered structure did not show a clear differentiation for TZ2-TG.

When temperature is varied, the rates (relaxation times) vary in a similar manner for detection at both 1663 cm^{-1} and 1641 cm^{-1}. Due to limitations in signal strength, the 1641 cm^{-1} data at lower temperatures did not allow reliable analysis of the transients, and consequently is not reported.

For two of three of the higher comparable temperature points (above 300 K), the relaxation times for the transients monitored at 1641 cm^{-1} (correlated to β-strand unfolding) and at 1663 cm^{-1} (monitoring disordered fraction) are equivalent within the error, and one could conclude that the two processes have the same rate. Such high-temperature behavior was also seen previously for TZ2-NG, but only above 325 K. Since the TZ2-TG is destabilized, as seen by its lower T_m, the temperature region with equivalent rates seems also to have shifted toward lower temperatures.

Overall, the observed relaxation times of TZ2-TG are slower compared with TZ2-NG [17, 27], although the turn mutation destabilizes the peptide secondary structure [25]. While it is difficult to draw conclusions about Arrhenius behavior from just a few high-temperature data points, the slopes for the relaxation rates obtained when probing at the two wavenumbers are in fact similar and suggest activation energies 2-3 times higher than seen for TZ2-NG, which is consistent with the slower rates we observe. At lower temperatures, the 1663 cm^{-1} data do suggest a deviation from this trend and a possible differentiation between the β-strand and disordered dynamics, but confirmation of this depends on our remeasuring the kinetics with improved S/N.

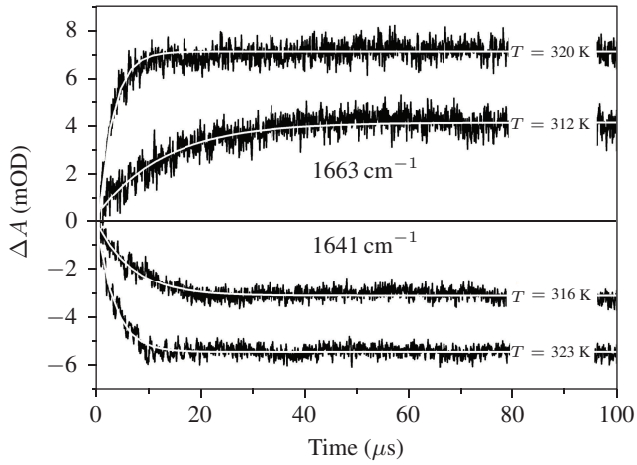

Figure 2: Representative transients of TZ2-TG after the T-jump. The traces evidence different curvature and signal amplitude indicating temperature dependence in the dynamic of unfolding/folding of TZ2-TG.

Table 1: Observed relaxation times τ_{obs} for TZ2-TG initiated by a T-jump of ΔT = 10°C and probed at two different wavelengths 1641 cm^{-1} (correlated to β-structure decay) and 1663 cm^{-1} (monitoring disordered fraction). The temperatures refer to the final peptide temperature after the T-jump.

TZ2-TG	T (K)	τ_{obs} (μs)
1641 cm^{-1}	308	17.1 ± 1.1
	316	6.8 ± 0.2
	323	3.3 ± 0.1
1663 cm^{-1}	290	47.3 ± 14.9
	297	21.6 ± 0.9
	306	16.1 ± 0.5
	312	11.5 ± 0.4
	320	2.8 ± 0.7

The meaning of this rate change from TZ2-NG to TZ2-TG must lie in the role of Asn in the turn. Tight turns in protein hairpin structures often involve Asn. Asn-Gly alone is not sufficient to nucleate a turn, but in combination with hydrophobic interaction between the strands, as in TZ2-NG, it is quite stable. The loss of stability on change to a TG turn would imply a reduction in the folding equilibrium constant. If we were to assume this to be an approximate two-state process at high temperatures and assume negligible impact on the unfolding rate constant, k_u, then the folding, k_f, would be reduced for TZ2-TG as compared to TZ2-NG. For simple unimolecular relaxation kinetics,

$$k_{\text{obs}} = k_f + k_u, \qquad K_{\text{eq}} = \frac{k_f}{k_u}, \tag{3.1}$$

where $k_{\text{obs}}(= 1/\tau_{\text{obs}})$ is the observed relaxation rate and K_{eq} is the equilibrium constant. In such a situation, the reduction in K_{eq} would lead to a reduction in k_{obs}, which is just what we see experimentally. However, under different conditions, if the mechanism becomes more complex, and not two state, such a simple analysis will not be valid.

In the low-temperature range, where we could clearly conclude that the previously studied TZ2-NG variants exhibited multistate behavior [17, 27], we cannot obtain enough data for TZ2-TG to draw similar conclusions. However, we also expect to find a differentiation of relaxation times for loss of β-strand and rise of disordered structure. Direct comparison of the low-temperature time constants between TZ2-NG and TZ2-TG might provide a better understanding of the slower overall dynamics of a hairpin whose thermal stability is destabilized by the turn.

4. Conclusions

In the current study, we report on the conformational dynamics of the β-hairpin peptide TZ2-TG and the influence of its turn residues on stability. Although the TG-turn residues have destabilizing impact as compared to an NG-turn, as seen in the decreased thermodynamic transition temperature, the conformational dynamics are unexpectedly slower. We attribute that to a modification of the multistate folding mechanism caused by the altered turn.

Abbreviations

FT-IR: Fourier Transform Infrared
NG: Asparagine-Glycine
TFA: Trifluoroacetic Acid
TG: Threonine-Glycine
TZ: Tryptophan zipper

Acknowledgments

This work was funded in part by grants from the National Science Foundation (CHE07-18543 to T. A. Keiderling) and the Deutsche Forschungsgemeinschaft (HA-5142/2-1 to K. Hauser) and in part by a research award from the Alexander von Humboldt Stiftung (to T. A. Keiderling).

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