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Two-Dimensional Correlation Analysis of Peptide Unfolding: Molecular Dynamics Simulations of β Hairpins

Jinhyuk Lee and Seokmin Shin*

School of Chemistry and Molecular Engineering, Seoul National University, Seoul 151-747, Korea

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We have studied the mechanism of formation of a 16-residue β hairpin from the protein GB1 using molecular dynamics simulations in an aqueous environment. Essential dynamics (ED) analysis was performed on the combined trajectories of two separate simulations at five different temperatures. Generalized 2D correlation analysis was performed using the displacements from the ED analysis as a dynamic spectrum. The results of the 2D correlation analysis illustrate the correlated structural changes as the temperature is increased. The asynchronous 2D correlation spectrum reveals the existence of sequential events in the unfolding of the peptide. The order of structural changes suggested by such an analysis support the hydrophobic collapse mechanism of folding for the β-hairpin fragment of protein GB1. The 2D correlation method combined with ED analysis can provide a useful scheme for studying the characteristics of dynamical structural changes which are important in understanding the function of protein and the mechanism of protein folding.

1. Introduction

Knowledge of the time scales and mechanism of formation of basic structural elements such as α helices and β sheets is essential in understanding the folding of larger proteins. α-helix formation has been extensively investigated both experimentally and theoretically.[1−3] In contrast, the formation of β-sheet structures has not been studied in detail. It has been proposed that β turns and β hairpins act as initiation sites in early protein folding events.[4] A β hairpin is the simplest form of antiparallel β-sheet structure and is defined by a loop region flanked by two β strands. Folding of the hairpin was found to be much slower than the rate of α-helix formation. Analysis of the experimental observations suggested that folding of a β hairpin is stabilized by both hydrogen bonding and hydrophobic interactions.[5] This is in contrast to α helices where the stability is the result of local residue interactions, mostly the hydrogen bonds. Although the fragments are usually small, the β-hairpin structures provide suitable systems for investigating fundamental issues in protein folding.

Recent studies suggested that the positioning of the side chain groups in such a way as to promote the formation of a hydrophobic cluster is essential for the folding of hairpin structures. Concerning the detailed pictures of hairpin formation, especially with respect to the relative timing of the formations of the interstrand hydrogen bonds near the turn and the hydrophobic core, two different mechanisms of β-hairpin folding have been proposed. Muñoz et al. studied the kinetics of folding a 16-residue β hairpin from protein GB1 using a nanosecond laser temperature-jump technique.[6] They suggested that the formation of β hairpin from protein GB1 is initiated from the β turn which then "zips up" the remaining native hydrogen bonds. A turn stabilized by interstrand hydrogen bonds positions the aromatic residues so that they are poised to pack into a hydrocarbon cluster. Bonvin and van Gunsteren studied the stability and folding of the 19-residue β-hairpin fragment of the α-amylase inhibitor Tendamistat.[7] Several unfolding and refolding simulations suggested a model for β-hairpin formation in which the turn is formed first, followed by hydrogen bond formation closing the hairpin, and subsequent stabilization by side-chain hydrophobic interactions. Gruebele and co-workers have performed extensive experimental studies on the folding thermodynamics and kinetics of three-stranded antiparallel β sheets.[8,9] It was found that hydrophobic effects contribute significantly to the transition state free energy only at high temperatures, such as under unfolding conditions, in the formation of a β sheet. Mutational phi (Φ_m) values as a function of sequence and temperature unambiguously demonstrated that the local topology at a turn controls the folding rate of the wild-type at physiological temperature. It was also suggested that the relative importance of hydrophobic collapse and hairpin formation can be tuned by mutations and solvent conditions.

Pande and Rokhsar studied the unfolding and refolding pathway of a β-hairpin fragment of protein GB1 using molecular dynamics simulations.[10] They suggested that the high-temperature unfolding of the β hairpin undergoes a series of sudden discrete conformational changes. According to their results, the hydrophobic cluster would form without assistance from the interstrand hydrogen bonds, suggesting that the β hairpin refolds by the "hydrophobic collapse" mechanism. In this model, the hairpin collapses to a compact structure associated with a "molten globule". The driving force toward the compact structure is global and in general nonspecific (a hydrophobic interaction). In the next step, "mistakes" in the compact structure are corrected by forming native hydrogen bonds. The reorientation of the compact structure is relatively slow and resembles the transition from a molten globule state to the native state of the protein. Dinner et al. obtained the free energy surface and conformations involved in the folding of the same β hairpin from multicanonical Monte Carlo simulations.[11] Their results suggested that folding proceeds by a collapse leading to the formation of the hydrophobic assembly; subsequently, the hairpin hydrogen bonds propagate outward in both directions from the hydrophobic core. Ma and Nusssinov studied the

* To whom correspondence should be addressed. Phone: +82-2-880-6639. Fax: +82-2-889-1568. E-mail: sshin@snu.ac.kr.

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contributions of three components of a β-hairpin peptide: turn, backbone hydrogen bonding, and side-chain interactions.[12] They examined the structural stability of the β hairpin under systematic perturbations of the turn region, backbone hydrogen bonds, and the hydrophobic core formed by the side-chains. Their results support a side-chain-centric view of the folding of a hairpin structure. For small peptides and proteins, the disruption of the hydrophobic core appears to be one of the major steps in the folding/unfolding process. Studies of the mechanical unfolding of the β hairpin using molecular dynamics by Bryant et al. support the view of a stepwise pathway of unfolding, wherein complete breakdown of backbone hydrogen bonds precedes dissociation of the hydrophobic cluster.[13] Prevost and Ortman performed refolding simulations of a β-hairpin fragment of barnase using a simulated annealing method.[14] They found that interstrand side-chain compactness and backbone hydrogen bonding provide concurrent stabilizing factors for the β-hairpin formation.

Recently, Zagrovic et al. used distributed computing techniques and a supercluster of thousands of computer processors to study folding of the C-terminal β hairpin from protein GB1 in atomistic detail using an implicit solvent model at 300 K.[15] They simulated a total of nearly 38 μs of folding time and obtained eight complete and independent folding trajectories. It was found that partial hydrophobic core formation takes precedence over interstrand hydrogen bonding as the important interaction to initiate folding. They observed that final formation of the complete hydrophobic core occurs cooperatively at the same time that the final hydrogen bonding pattern appears. Zhou et al. studied the folding free energy landscape of the same hairpin by using a highly parallel replica exchange method that combines MD trajectories with a temperature exchange MC process.[16] The simulation results showed that the hydrophobic core and the β-strand hydrogen bond form at roughly the same time. They suggested a folding mechanism that is a blend of the hydrogen-bond-centric and the hydrophobic-centric models.

In the "protein-folding" problem, theoretical and computational studies of simplified models have provided general insights into the specific features of the folding mechanism and the properties of folding free-energy landscapes.[17−20] Predicting the native state structures of proteins from amino acid sequences alone is a long-standing challenge, which has great practical significance as well as considerable scientific interest. Such ab initio structure prediction or protein folding may require the ability to describe the whole folding process at the level of atomic precision, which is still not feasible despite rapid advances in the field.[21] Typical molecular dynamics simulations can examine the trajectories of proteins up to tens of nanoseconds. The time scales of protein folding in the micro- to millisecond range are still not really accessible to direct simulation studies. One fruitful approach is to investigate protein unfolding by performing simulations under strongly denaturing conditions (high temperature and/or pressure). These simulations can provide insights into the early stages of unfolding, which are assumed to reflect the later stages of refolding under native conditions.[22−24] We note that there remain important open questions concerning the relationship between unfolding at high temperatures and the folding process at physiological temperatures.[25,26]

In the previous study,[27] we investigated the mechanism of formation of a 16-residue β hairpin from the protein GB1 using molecular dynamics simulations in an aqueous environment. The analysis of unfolding trajectories at high temperatures suggests a refolding pathway consisting of several transient intermediates. The changes in the interaction energies of residues are related to the structural changes during the unfolding of the hairpin. The electrostatic energies of the residues in the turn region are found to be responsible for the transition between the folded state and the hydrophobic core state. The van der Waals interaction energies of the residues in the hydrophobic core reflect the behavior of the radius of gyration of the core region. The results of the simulations support the hydrophobic collapse mechanism of folding for the β-hairpin fragment of protein GB1.

Two-dimensional (2D) spectroscopy was originally developed in the field of nuclear magnetic resonance, and 2D NMR techniques have been successfully applied to a broad range of applications.[28] From 2D correlation spectroscopy, one can obtain additional useful information which is not readily available from a conventional one-dimensional spectrum. Multidimensional optical spectroscopy is now made possible by the development of ultrafast laser pulses and nonlinear spectroscopic techniques.[29] Recently, Noda and co-workers have proposed perturbation-based 2D correlation spectroscopy, which utilizes an additional external perturbation applied during the spectroscopic measurement to stimulate the system of interest.[30,31] The response of the system to the applied perturbation leads to characteristic variations in the spectrum, which is often called a "dynamic spectrum". A simple scheme of correlation analysis can be applied to a series of perturbation-induced dynamic spectra, collected in some sequential order, yielding desired 2D correlation spectra. The dynamic spectra can be obtained as a function of the quantitative measure of the imposed external perturbation such as temperature, pressure, concentration, stress, or electric field. The type of physical information contained in a dynamic spectrum is determined by the selection of the perturbation method and spectroscopic probe. From the analysis of the 2D correlation spectra, one can obtain detailed information concerning microscopic or molecular level responses of the system to the macroscopic perturbation. General 2D correlation methods can be useful in examining the properties of biological molecules such as proteins undergoing structural rearrangements. Recently, Shultz et al. used 2D infrared correlation analysis to investigate the differences in protein structure upon denaturation for two related enzymes.[32]

The essential dynamics (ED) method has been proven useful in extracting large concerted atomic motions in protein molecules from finite-duration MD trajectories.[33−39] ED analysis provides the average fluctuations, i.e., the amplitude of the correlated motions, describing the overall motion of the protein, as a function of residue number. In the present study, we have studied unfolding of the β hairpin from the protein GB1 by MD simulations in explicit water solvents at several temperatures. Results from the ED analysis on the unfolding trajectories at different temperatures may be considered as "dynamic spectra", which can be subjected to general 2D correlation analysis. Further insights into the detailed mechanism of β-hairpin folding are obtained by examining sequential structural changes in the different parts of the peptide during unfolding induced by raising temperatures.

The paper is organized as follows. The theoretical models and computational methods are presented in section 2. Section 3 gives detailed descriptions of the results of the study. The main findings and discussion are presented in section 4.

2. Model and Computational Methods

2.1. Molecular Dynamics and Essential Dynamics Analysis. We simulate unfolding of the β-hairpin structure formed by the 16 C-terminal residues (GEWTYDDATKTFTVTE) of

protein GB1. The three-dimensional structure of the hairpin is stabilized by the native hydrogen bonds and interactions between the side chains of the four residues (Trp43, Tyr45, Phe52, and Val54) forming the hydrophobic core. We have performed simulations with the CHARMM all-H potential.[40] The initial structure was taken from the model conformation of the full Protein G (PDB code: 1GB1). The hairpin was placed in a cubic box with the dimension of 37.712 Å, filled with 1728 TIP3P water molecules to a density of ~1 g/cm^3. After removing the water molecules whose oxygen atoms were within 2.6 Å of any peptide atom, the initial conformation was relaxed by an adapted basis Newton−Raphson method. Bond lengths were constrained through the SHAKE algorithm.[41] We used time steps of 2 fs for simulations at 300 and 400 K. A smaller time step (1 fs) was used to obtain stable trajectories at higher temperatures (500, 600, and 700 K). Each simulation was started with a heating step from 0 K to the simulation temperature, and further simulations were conducted for 1 ns (300 and 500−700 K) or 2 ns (400 K). Two separate trajectories were obtained for each temperature. We collected molecular dynamics trajectories every 5 ps.

The essential dynamics method[34] is based on the diagonalization of the covariance matrix built from atomic fluctuations in an MD trajectory from which overall translation and rotations have been removed:

$$C_{ij} \equiv \langle (x_i - x_{i,0})(x_j - x_{j,0}) \rangle$$

where $x_{i,j}$ are separate Cartesian coordinates of the atoms with corresponding average values denoted by $x_{i,0}$. $\langle\rangle$ represents an average over the whole MD trajectory. The coordinates of the C_α atoms are used for the analysis. Diagonalization of the covariance matrix yields a set of eigenvectors and eigenvalues, which are sorted by the size of the eigenvalue. The eigenvectors indicate directions in the total configuration space, representing correlated displacements of groups of atoms in the system. The corresponding eigenvalues indicate the total mean square fluctuations, i.e., the amplitude of the correlated motions, along these directions. The basic idea of essential dynamics is that only the correlated motions represented by the eigenvectors with large corresponding eigenvalues are important in describing the overall motion of the protein. The essential dynamics method has been found to be useful for revealing functionally significant fluctuations in various protein systems.[35−39]

For each temperature, MD trajectories of two separate simulations are concatenated to form one big trajectory and a covariance matrix is constructed. The resulting eigenvectors now indicate the concerted motions of atoms, which are common to the separate trajectories. The mean square fluctuations reveal structural changes during unfolding trajectories. The essential dynamics analysis was carried out using the WHAT IF modeling program.[42]

2.2. 2D Correlation Analysis. Consider a perturbation-induced change of the spectral intensity observed within the interval of some external variable (e.g., temperature). The dynamic spectrum of a system is defined as the difference between the observed spectrum under perturbation and a reference spectrum. The reference spectrum can be the average spectrum or a spectrum observed at some fixed reference point. 2D correlation is defined as the quantitative comparison of spectral intensity variations observed at two different spectral variables over some finite observation interval between minimum and maximum values of the external perturbation. Let $A(\nu, T)$ be the dynamic spectrum observed at a temperature T. The complex 2D correlation spectrum is decomposed into real and imaginary components, which are called *synchronous* and *asynchronous* correlation intensities, respectively:[31]

$$\Phi(\nu_1,\nu_2) + i\Psi(\nu_1,\nu_2) = (\pi\Delta T)^{-1}\int_0^\infty Y_1(\omega)Y_2^*(\omega)\,\mathrm{d}\omega$$

where $Y_i(\omega)$ represents the Fourier transform of $A(\nu,T)$ in the temperature domain. The synchronous 2D correlation intensity, $\Phi(\nu_1,\nu_2)$, represents the overall similarity between two separate spectral intensity variations for different spectral variables as the value of T is changed. The asynchronous intensity, $\Psi(\nu_1,\nu_2)$, signals the measure of dissimilarity in the spectral variations.

The computation of the generalized 2D correlation spectra for a discrete set of spectral data can be carried out by a simple matrix manipulation.[43] A synchronous correlation spectrum is obtained as a simple rows cross-product of the following matrix:

$$\mathbf{Z} = (s-1)^{-1}\mathbf{M}\mathbf{M}^{\mathrm{T}}$$

Here $\mathbf{M}$ is the experimental matrix of $w \times s$ where w is the number of the spectral points and s is the number of samples (dynamic spectra). Each column of $\mathbf{M}$ corresponds to one spectrum, whereas the dynamic spectra are the rows of $\mathbf{M}$. An asynchronous correlation spectrum can be obtained if $\mathbf{M}$ is orthogonalized and then the rows cross-product between the original and orthogonal matrix is formed.

The intensity of a synchronous 2D correlation spectrum represents the simultaneous changes of the spectral intensity variations measured at ν_1 and ν_2 during the interval of the external perturbation T. The magnitudes of autopeaks with positive values along the diagonal positions represent the overall extent of spectral intensity variation for a specific spectral variable. In other words, any region of a spectrum with a large change in intensity will show a strong autopeak. The signs of cross-peaks located at the off-diagonal positions become positive if the intensity changes for the two different spectral variables are in the same direction or negative if the intensity changes show opposite trends.

The asynchronous 2D correlation spectrum has no autopeaks and is antisymmetric with respect to the diagonal line. An asynchronous cross-peak develops only if the intensities of two spectral features change out of phase. For the two spectral variables (ν_1,ν_2) with a positive synchronous cross-peak, the sign of the asynchronous cross-peak becomes positive if the intensity change at ν_1 occurs predominantly before ν_2 in sequential order with respect to the variations of the perturbation. The negative value represents the changes at ν_1 after ν_2.

In the present study, the dynamic spectrum $A(\nu, T)$ corresponds to the mean square fluctuations as a function of residue number, obtained from the ED analysis at a specific temperature T. The 2D spectrum $\Phi(\nu_1,\nu_2)$ or $\Psi(\nu_1,\nu_2)$ will represent correlations in the structural changes due to temperature increase (i.e., unfolding) for the two different regions of the peptide denoted by the residue numbers ν_1 and ν_2.

3. Results

We have performed molecular dynamics (MD) simulations of the unfolding of the β-hairpin structure from protein GB1 using the CHARMM all-H potential. At high temperatures such as 600 and 700 K, the trajectories of the simulations usually result in a completely unfolded state within 1 ns, whereas the folded states with nativelike structures are maintained during the simulations at room temperature (300 K). At an intermediate temperature (400 and 500 K), partial unfolding and refolding of the hairpin are observed in typical trajectories. A typical

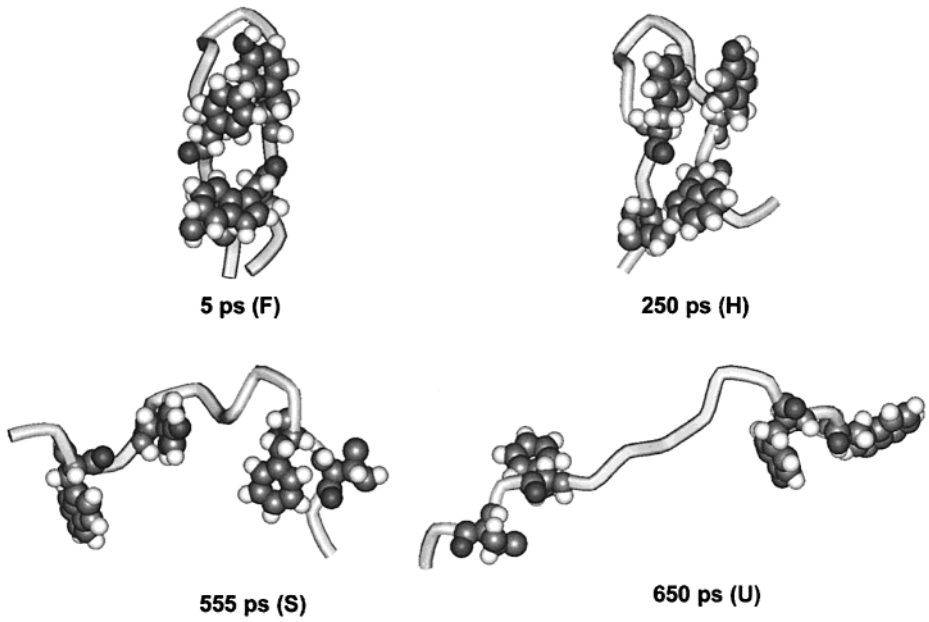

Figure 1. Molecular dynamics snapshots showing the unfolding pathway of the β-hairpin structure from protein GB1. A typical unfolding trajectory at 500 K demonstrates the folded state (F); the hydrophobic core state (H); the partially solvated state (S); and the unfolded state (U). [See refs 10 and 27 for the descriptions of the intermediate states.] The side chains of the hydrophobic core (Trp43, Tyr45, Phe52, and Val54) are shown in space-filling mode.

unfolding trajectory at 500 K demonstrating discrete unfolding steps is illustrated in Figure 1. As discussed in the previous study, the behavior of the order parameters such as the number of backbone hydrogen bonds (N_{HB}) and the radius of gyration of the hydrophobic core (R_{core}) is consistent with the four-state discrete unfolding mechanism proposed by Pande and Rokhsar.[10]

Using the combined trajectories of the two separate simulations, essential dynamics analysis was performed to examine the changes in the dynamical structures of the peptide at different temperatures. As in the previous studies,[38,39] only a few eigenvectors are found to represent the essential motions in the peptide. Figure 2 shows the displacements as a function of residue number for the first eigenvector with the largest eigenvalue, obtained from the simulations at different temperatures. It is noted that we assigned the residue numbers 1 through 16 to the 16 residues of the peptide starting Gly41. At 300 K, the region around the hydrophobic cluster (Trp43, Tyr45, Phe52, and Val54: residue numbers 3, 5, 12, and 14) shows much smaller displacements. At higher temperatures, increased displacements represent the unfolding of the peptide structure.

The generalized 2D correlation analysis has been performed using the displacements from the ED analysis as input data for generating the synchronous and asynchronous plots. These plots represent the correlated structural changes as the temperature is increased. The analysis was done with the averaged displacements over the temperature range as the reference spectrum. Figure 3 shows the synchronous 2D correlation spectrum of the unfolding processes of the peptide. The synchronous plots have all positive values, indicating that the mean square fluctuations of the residues become larger as the temperature is increased. Figure 3 also displays the magnitudes of the autopeaks along the diagonal of the synchronous spectrum. The residues around the peptide ends show larger structural changes as the temperature is increased.

The asynchronous 2D correlation plot (Figure 4) suggests the existence of sequential events in the unfolding of the peptide. It is noted that the asynchronous spectrum reveals different behavior for the three regions of the peptide. We denote these regions as **R1** (residue numbers 1−4); **R2** (residue numbers 5−9); and **R3** (residue numbers 10−16). In Figure 5, we plot the values of the asynchronous spectrum $\psi(n_1,n_2)$ for the three residues ($n_1 = 3, 7, 12$) representing the three regions. The positive values of the off-diagonal elements between the region **R2** and the other regions, ψ(**R2**, **R1**) > 0, ψ(**R2**, **R3**) > 0, indicate that the structural changes in **R2** precede those in **R1** and **R3** as the temperature is raised (i.e., as the peptide unfolds). Most of the cross-peaks between **R1** and **R3** have small values, ψ(**R1**, **R3**) $\approx$ 0, suggesting that the changes in these regions occur simultaneously with respect to the temperature variation.

It is assumed that the folding sequence of the peptide follows the reverse order of the temperature-induced unfolding process. Therefore the folding of the peptide is thought to occur in the order **R1**, **R3** $\Rightarrow$ **R2.** This is consistent with the "hydrophobic collapse" mechanism of hairpin folding where the formation of the hydrophobic core is followed by the formation of hydrogen bonds in the β-turn region. The region **R2** corresponds mostly to the turn region (residue numbers 6−11), and the hydrophobic core residues are in **R1** and **R3** with the exception of Tyr45. The cross-peaks between the three residues of the hydrophobic core (Trp43, Phe52, and Val54) have values close to zero. This implies that the movements of these residues are correlated during the folding process.

We have also performed the 2D analysis using the displacements at 300 K as the reference spectrum. Figures 6 and 7 show the synchronous and asynchronous spectra in this case. The general features of the 2D correlation spectra are the same as the results discussed above. The intensities of the synchronous and asynchronous plots are enhanced when compared to the case with the averaged displacements as the reference spectrum.

4. Concluding Remarks

The characteristics and dynamics of correlated structural changes are important in understanding the function of protein and the mechanism of protein folding. We have presented a theoretical scheme based on the ED and 2D correlation analysis for studying such structural changes. ED analysis on the molecular dynamics simulation trajectories illustrate concerted

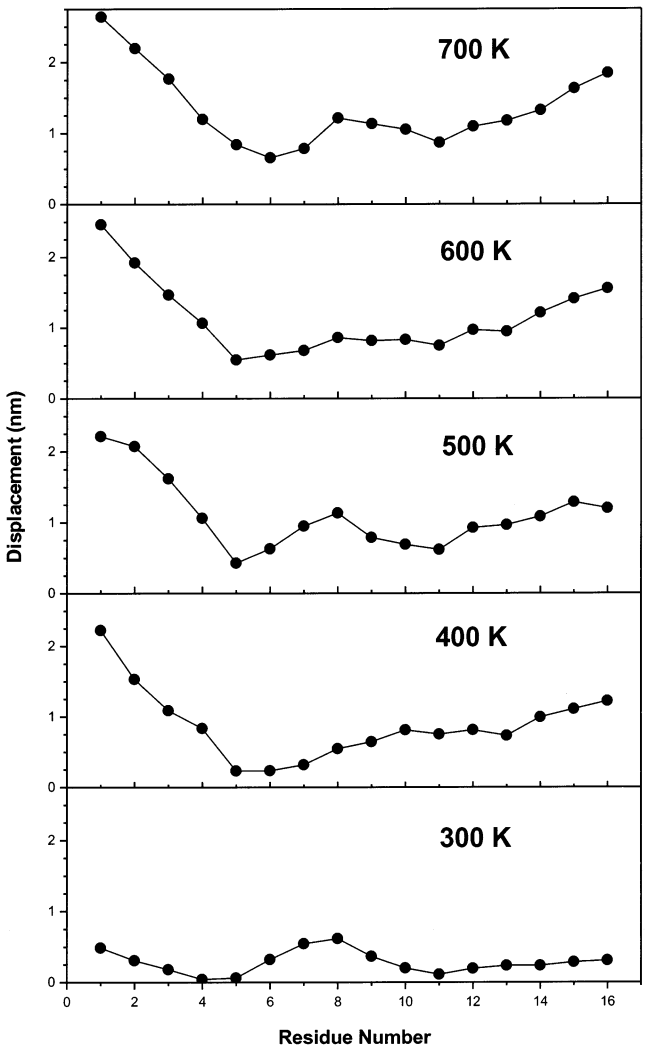

Figure 2. Absolute value of the components of the first eigenvector with the largest eigenvalue as a function of residue number, obtained from the essential dynamics (ED) analysis on the combined trajectories of the two separate simulations at five different temperatures. Residue numbers 1−16 are assigned to the 16 residues of the β-hairpin structure (GEWTYDDATKTFTVTE) starting Gly41.

motions of the residues in a protein. The results of the ED analysis for the simulations at different temperatures can be used as dynamic spectra for the generalized 2D correlation analysis. The resulting 2D correlation spectra can provide information on the order of structural changes for the different parts of the protein as the temperature is varied. Balsera et al. criticized the use of principal component analysis such as ED analysis as sampling errors due to finite simulation times could preclude the identification of slow modes.[44] In our studies, the simulation times are sufficient enough to observe the complete unfolding of the peptide at high temperatures. Therefore, it is reasonable to assume that the ED analysis can capture important conformational changes involved in the (un)folding of the peptide.

We have studied the mechanism of a β-hairpin formation by unfolding simulations at high temperatures. The analysis of trajectories obtained from molecular dynamics simulations in explicit aqueous solution suggests a refolding pathway consisting of several transient intermediates. ED analysis was performed on the combined trajectories of the two separate simulations at five different temperatures. Generalized 2D correlation analysis was performed using the displacements from the ED analysis as input data. The results of the 2D correlation analysis illustrate the correlated structural changes as the temperature is increased.

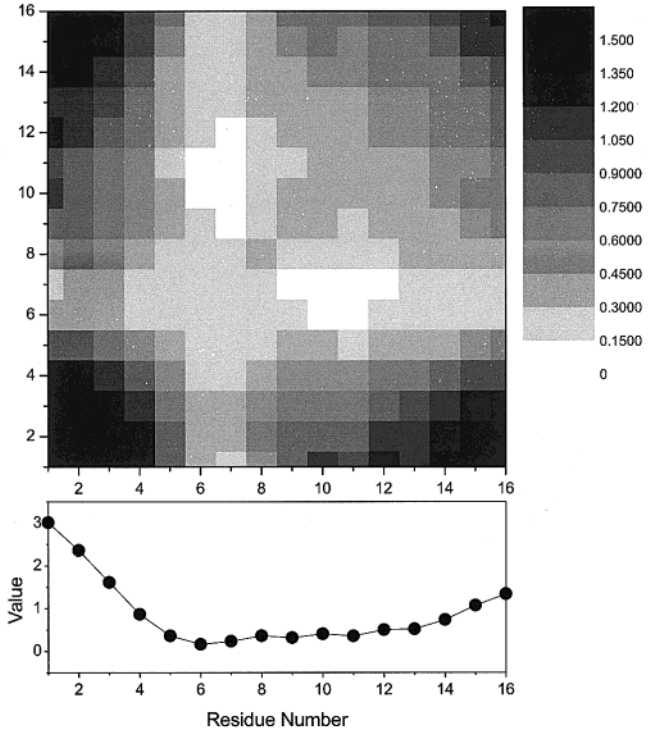

Figure 3. Synchronous 2D correlation plot of the unfolding processes of the β hairpin. The generalized 2D correlation analysis was done using the displacements from the ED analysis as input data with the averaged displacements over the temperature range as the reference spectrum. Also shown is the graph for the magnitudes of the autopeaks along the diagonal of the synchronous spectrum.

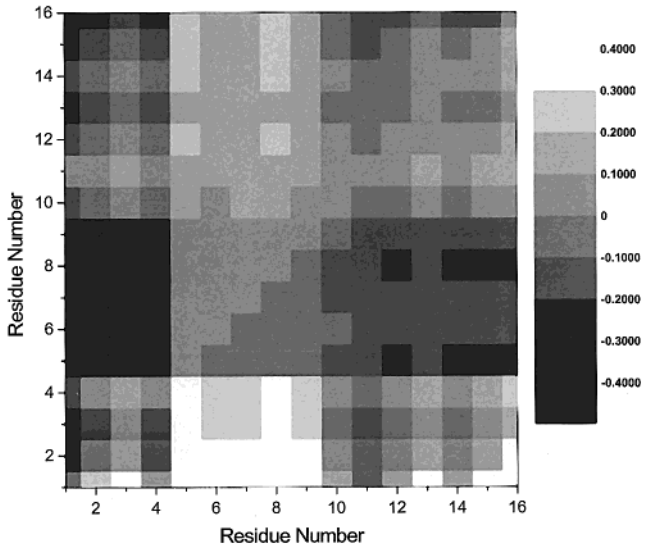

Figure 4. Asynchronous 2D correlation plot of the unfolding processes of the β-hairpin. The generalized 2D correlation analysis was done using the displacements from the ED analysis as input data with the averaged displacements over the temperature range as the reference spectrum.

The asynchronous 2D correlation plot suggests the existence of sequential events in the unfolding of the peptide. The order of structural changes suggested by such analysis is consistent with the hydrophobic collapse mechanism of folding for the β hairpin from the protein GB1, whereby the formation of a hydrophobic assembly is followed by a final tune-up for the secondary structure through the formation of hydrogen bonds in the turn region.

As discussed in the Introduction, the β-hairpin structures from different proteins seemed to follow different mechanisms of

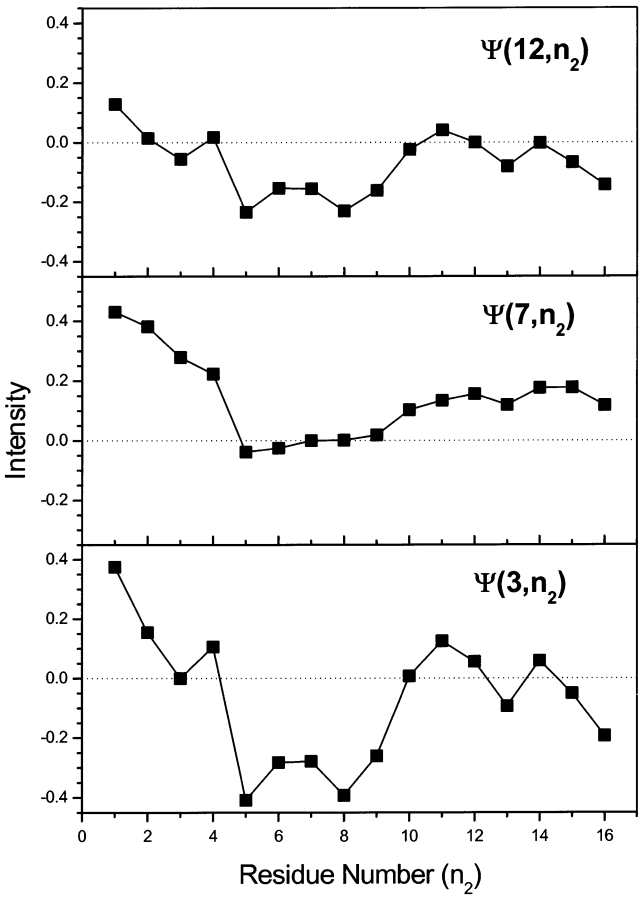

Figure 5. Parts of the asynchronous 2D correlation plot shown in Figure 4. We plot the values of the asynchronous spectrum $\psi(n_1,n_2)$ for the three residues ($n_1 = 3$, 7, and 12) as a function of the second residue number n_2.

folding. The present study and other studies suggest that the β-hairpin fragment of protein GB1 folds by the hydrophobic collapse mechanism. The folding of the 19-residue β-hairpin fragment of the α-amylase inhibitor Tendamistat was found to follow a different mechanism in which the turn is formed first, followed by hydrogen bond formation closing the hairpin, and subsequent stabilization by side-chain hydrophobic interactions.[7] In the case of a β-hairpin fragment of barnase, it was found that all of the observed increases in compactness due to the hydrophobic interactions occur simultaneously with the formation of a backbone hydrogen bond.[14] In a theoretical study on the thermodynamics and kinetics of off-lattice models for the β-hairpin fragment, Klimov and Thirumalai suggested that the basic mechanisms of folding depend on the intrinsic rigidity of the hairpin, which is determined by the location of the hydrophobic cluster.[45] In the previous study,[27] it was argued that the positions of the large (hydrophobic) side-chains could be related with the different folding mechanisms exhibited by the three hairpins discussed above. Simulation studies on these different hairpin fragments are currently underway in order to provide valuable information about the stability and formation of β-hairpin structures.

It is interesting to note that recent extensive folding simulations[15] and the folding free energy calculations[16] proposed the following folding mechanism for the hairpin fragment of protein GB1: after the early collapse of the peptide, it forms a partially folded state with two or three nonspecific hydrogen bonds before it finally folds into the native structure. It was suggested that the final backbone hydrogen bonding pattern tends to form in tandem with complete hydrophobic core formation.[15] Using an

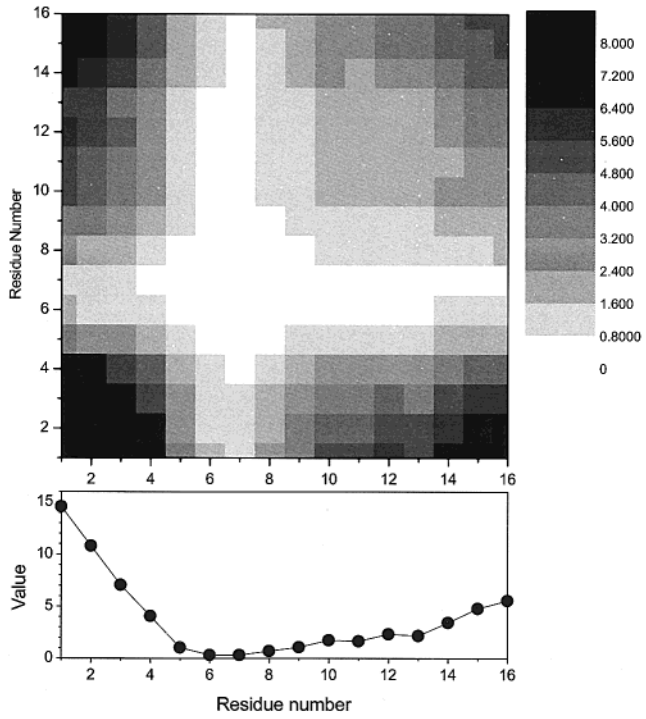

Figure 6. Synchronous 2D correlation plot of the unfolding processes of the β hairpin. The generalized 2D correlation analysis was done using the displacements from the ED analysis as input data with the displacements at 300 K as the reference spectrum. Also shown is the graph for the magnitudes of the autopeaks along the diagonal of the synchronous spectrum.

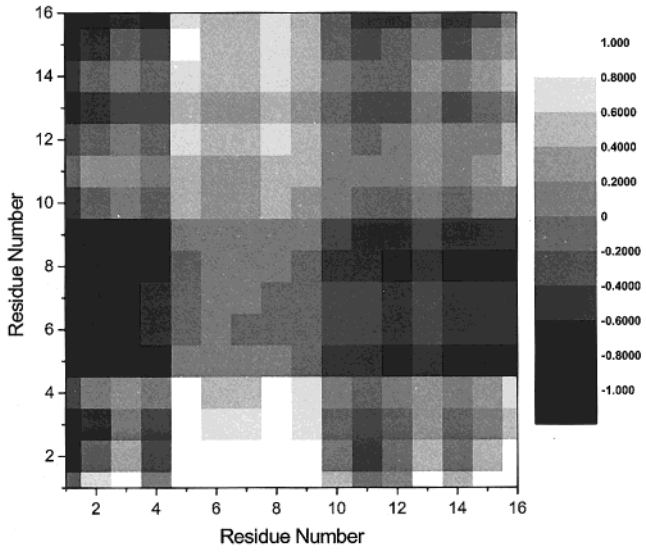

Figure 7. Asynchronous 2D correlation plot of the unfolding processes of the β hairpin. The generalized 2D correlation analysis was done using the displacements from the ED analysis as input data with the displacements at 300 K as the reference spectrum.

analytical solvable model Hamiltonian for the (un)folding kinetics of a β hairpin, Guo et al. indicated that there is a close competition between local hairpin topology and hydrophobic clusters.[46] Experimental studies on the folding mechanism of a three-stranded antiparallel β sheet demonstrated that the relative importance of hydrophobic collapse and turn formation to the transition state free energy depends on the external conditions. The fact that the hydrophobic collapse mechanism has been proposed by most of the unfolding simulations studies including the present one may be related with the finding that hydrophobic contributions become dominant at higher temperatures. It can

be concluded that the detailed folding mechanism of a β hairpin is determined by close interplay between several factors such as hydrophobic clusters, hydrogen bonding, and propensity for turn formation. One needs to be careful to single out one of the factors as the dominant mechanism with general applicability.

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