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Two-Dimensional Infrared (2DIR) Spectroscopy of the Peptide Beta3s Folding

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Abstract

Probing underlying free energy landscape, pathways, and mechanism is the key for understanding protein folding in theory and experiment. Recently time-resolved two-dimensional infrared (2DIR) with femtosecond laser pulses, has emerged as a promising tool for investigating the protein folding dynamics on faster timescales than possible by NMR. We have employed molecular dynamics simulations to compute 2DIR spectra of the folding process of a peptide, Beta3s. Simulated non-chiral and chiral 2DIR signals illustrate the variation of the spectra as the peptide conformation evolves along the free energy landscape. Chiral spectra show stronger changes than the non-chiral signals because cross peaks caused by the formation of the β -sheet are clearly resolved. Chirality-induced 2DIR may be used to detect the folding of β -sheet proteins with high spectral and temporal resolution.

Keywords

Protein Folding; Free Energy Landscape; Multidimensional Spectroscopy; Nonchiral signal; Chiral signal

The energy landscape theory provides an essential framework for understanding protein folding and has been widely used to interpret the folding process.^{1–3} The theory assumes a funnel-like shape of the surface which is sufficiently biased to direct the folding so that the native state can be reached on the experimental time scale. Monitoring conformational changes of proteins is important for understanding the details of energy landscape and uncovering the mechanism of folding process. There are very limited effective probes that can detect structural evolution on the time scales of folding dynamics. Commonly used

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Supporting Information **Available:** List of Native Contact of Peptide Beta3s and a one-dimensional free energy profile with respect to RMSD from the native state structure. This material is available free of charge via the Internet <http://pubs.acs.org>.

spectroscopic techniques such as NMR, due to limited millisecond time resolution, may not directly trace the faster dynamical process. Two-dimensional infrared (2DIR) spectroscopy provides a promising avenue to investigate protein folding dynamics down to 100fs resolution.⁴⁻⁶ In 2DIR experiments, the amide I band, associated with the peptide bond carbonyl stretch, is by far the most studied because of its sensitivity to hydrogen bonding, dipole-dipole interactions, and geometry of the peptide backbone, thus providing a good indicator of secondary structure. The cross peaks in 2DIR spectra provide signatures of intra- and intermolecular couplings, providing additional structural information not available in linear spectroscopy. Here we report simulations of 2DIR spectra of the folding of Beta3s on the free energy landscape. Beta3s is a 20 amino acid peptide (Thr1-Trp2-Ile3-Gln4-Asn5-Gly6-Ser7-Thr8-Lys9-Trp10-Tyr11-Gln12-Asn13-Gly14-Ser15-Thr16-Lys17-Ile18-Tyr19-Thr20), and NMR experiments have shown that it folds into a single structured compact form, the three-stranded antiparallel β -sheet conformation with turns at Gly14-Ser15 and Gly6-Ser7, in equilibrium solution.⁷

We have applied a molecular dynamics (MD) protocol to simulate the folding dynamics of the peptide. We used the CHARMM c35b5 package⁸ with the TOPH19/PARAM19 parameter set⁹ to perform all MD simulations and part of the analysis. The folding simulations were performed with an implicit solvent model.¹⁰ Honig et al have suggested that the van der Waals and hydrophobic interactions are the primary factors which determine the stability of β -sheet proteins.¹¹ Also, recent studies^{12,13} implies that the contacts between side chains are responsible for driving the folding of peptide with antiparallel β -sheets, suggesting that the use of an implicit solvent does not significantly influence the folding process. The nonbonded interaction list was extended to cut off as 7.5 Å, and all covalent bond lengths including hydrogen atoms were fixed with the SHAKE algorithm.¹⁴ From the native structure, 20 trajectories were simulated at 330K for 500 ns. The total 10- μ s folding simulation trajectories provided sufficient data to build up the free energy landscape which is a function of RMSD (root mean square deviation) and Q (fraction of native contacts of C_α atoms). The native contacts are listed in Table 1 in the Supporting Information, and a contact was defined as formed when its pair distance falls within a range $r_{\text{native}} \pm 2$ Å, where r_{native} is the distance of that contact in the native structure. Along the dominant folding pathway, 100 locations were selected on the energy landscape. The distance between any two adjacent locations was moderate, so that these locations can well characterize the entire pathway.

The simulation protocol of amide I linear absorption and 2DIR spectra has been discussed in detail elsewhere. The map of reference 16 was used to model the fluctuating amide I frequencies while the anharmonicity was fixed to -16 cm⁻¹. The nearest-neighbor couplings were given by the Torii and Tasumi dihedral angles map,¹⁷ while all other couplings were calculated using the transition dipole coupling formula.¹⁷ All simulations were performed in a homogeneous dephasing of 5.5 cm⁻¹ and impulsive pulses. Static(inhomogeneous) averaging was performed. The absorptive (k_I+k_{II}) 2DIR spectra were simulated for five locations (L1, L25, L50, L75, L100) on the free energy landscape. At each location, the spectrum was averaged over 150 conformations. Each conformation was explicitly solvated in TIP3P¹⁸ water and equilibrated for 10 ps prior to calculating the fluctuating parameters in the excitonic Hamiltonian. Spectra were simulated for the non-chiral (i.e. xxyy) and chirality-induced (CI) (i.e. xxxy) polarization configurations, where $ijkl$ indicates the pulse polarization in chronological order.

Figure 1 shows the free energy landscape of Beta3s folding plotted as a function of the RMSD and Q (the one-dimensional one is in the Supporting Information). The free energy profile was obtained by $F = -\log(P)$, where P is the statistical population obtained from the 10- μ s MD simulated data. At L1, the peptide has an extended structure. At L25, the termini have random-coil structures, yet residues 8-13 formed a short α -helix. At L50, the short α -

helix reopened and the orientations of three strands formed. Between L50 and L75, we witnessed the formation of two β strands. L100 was the folded state which consisted of three antiparallel β sheets. This folding scenario is consistent with a previous study.¹⁹

The transition dipole couplings are displayed in Figure 2 to demonstrate the structural changes that occur during folding. At L1, the coupling is weak and is dominated by nearest-neighbor couplings. This is indicative of a random coil. At L25, a short α -helix forms from residues 8 to 13 has formed as seen by the strong positive nearest neighbor coupling and the strong negative 1-3 coupling in this region.^{20–22} When the peptide arrives at L50, the helical structure opens and the couplings between residues 8-13 decrease. From L75, as the orientations of three strands form and the peptide folds to the native state, the couplings between residues 1-5 and 8-12 and the couplings between residues 8-12 and 16-20 are enhanced, clearly indicating the formation of the antiparallel β -sheet structure. These couplings strengthen as the peptide evolves from L75 to the stable folded state at L100. The couplings J_{ij} (Fig. 2) will now be used, as input to the system Hamiltonian, to calculate the infrared absorption, vibrational circular dichroism, and non-chiral and chiral 2DIR spectra. As we will show, these couplings manifest themselves as cross peaks in the 2DIR spectra.

Figure 3(a) shows the infrared absorption spectra of the five locations along the folding pathway. They all have one major peak, which red-shifts from 1645 cm^{-1} at L1 to 1630 cm^{-1} at L100. This redshift is consistent with previous studies on similar systems.^{23–28}

Figure 3(b-f) shows the real part of the absorptive 2D $xxyy$ spectra. All spectra are dominated by an inhomogeneously (diagonally) broadened peak. The inhomogeneous distribution and the homogeneous dephasing which were used to compute the spectra largely determined the peak shape. Compared to L1, the diagonal L100 peak is red-shifted around 10 cm^{-1} (from 1645 cm^{-1} to 1630 cm^{-1}), consistent with the above linear absorption spectrum. The similarity of the 2DIR $xxyy$ spectra indicates that the $xxyy$ spectra are not very sensitive to protein secondary structure. This motivates the study of exploring more significant probes such as chiral 2DIR spectra.

The vibrational circular dichroism (VCD) and CI 2DIR spectra are shown in Figure 4. The VCD spectra (Figure 4(a)) for all locations have a positive couplet (+ peak followed by - peak with increasing frequency). The positive peak is red-shifted by about 10 cm^{-1} from L1 to L100 as the β -sheet structure forms. The intensity of the positive peak increases slightly from L1 to L25. From L50 to L100, the intensities of both peaks slightly increases, as the planar anti-parallel β -sheet forms. The VCD spectrum at L100 agrees well with the ab-initio simulated VCD spectrum of a three-stranded planar antiparallel β -sheet,³⁰ which has a different spectrum when compared with the twisted β -sheets normally observed in proteins. While a negative couplet at L1 is expected due to the presence of a random coil state,^{31,32} the under-stabilization of PPII conformations³³ in MD simulations of unfolded peptides^{34,35} may cause L1 to display a positive couplet as noted in a recent study.³³ Our simulations indicate that only 2% of conformations exist in the PPII state at L1. Newer force fields,^{34,35} which have been optimized to accurately calculate properties for unfolded peptides, may be used to acquire more accurate VCD spectra.

In the 2DIR $xxxx$ spectra (Figure 4(b-f)), there is a dominant diagonal peak at L1, L25, and L50 which splits into two peaks at L75 and L100. The cross peak labeled peak 'A' at $(\omega_1, \omega_3) = (-1670, 1640)\text{ cm}^{-1}$ slightly enhance from L1 to L25, indicating the helical structure tends to strengthen the chiral signals. Interestingly, this cross peak gradually fades when the antiparallel β -sheet structure forms, and basically disappear at L100.

A new cross peak at $(-1630, 1650)\text{ cm}^{-1}$ clearly appears in the $xxxx$ spectrum of the folded state (peak 'B', Figure 4(f)). This feature begins to occur at L75 as the β -sheet forms, and is

well resolved at L100 when the antiparallel β -sheet structure forms. This cross peak is caused by the coupling between the low and high frequency β -sheet eigen-states, and is well resolved because it appears as a positive feature near the negative diagonal feature. This cross peak, which has also been observed in simulated chiral 2DIR spectra of the A β 42 monomer, can be a good indicator of β -sheets.

Our results have demonstrated how conformational evolution on the folding free energy landscape can be monitored by the 2DIR spectroscopy. The simulated linear absorption spectra, non-chiral and chirality-induced 2DIR spectra illustrate the conformational changes during folding. In our simulations, the chiral 2DIR spectra displayed a very strong feature indicating the formation of the folded antiparallel β -sheet. Whereas in the non-chiral signals, the formation of the β -sheet is seen as similar pattern in the spectra, the chiral signals display very interesting spectra features. The appearance of the β -sheet cross peak at $(-1630, 1650) \text{ cm}^{-1}$ in the chiral spectra is better resolved than the cross peak in the non-chiral spectra due to the opposite signs of the overlapping cross peak and diagonal feature. Comparing the chiral and nonchiral 2DIR spectra, we have found that the chiral signals are more sensitive when the structural changes. However, these signals are roughly 1000 times weaker than their non-chiral counterparts and have not yet been observed in experiment. In the chiral 2DIR experiments, a typical box-car or pump-probe geometry may be used. A highly sensitive detector could be achieved by upconverting the signal to the visible and detecting using a charge-coupled device.³⁶ Nevertheless, chiral 2DIR spectroscopy is a promising technique for studying fast protein folding with improved structural and temporal resolution.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

(a) The native structure from the model 1 of the NMR structures.⁷ (b) Free energy profile of Beta3s folding as a function of RMSD(Å) from the native structure and the fraction of native contacts. Five locations on the folding pathway are indicated. (c) The corresponding structures of the five locations.

Figure 2.
Average dipole-dipole coupling among the different amide I vibrational modes at L1, L25, L50, L75, and L100.

Figure 3.

(a) Linear absorption spectra of the locations L1, L25, L50, L75, and L100. (b-f) Non-chiral(xxyy) 2DIR signals of the same locations. The 2D spectra were plotted by using arcsinh scale.²⁹

Figure 4.

(a)VCD spectra and (b-f) Chiral 2DIR spectra of the locations L1, L25, L50, L75, and L100. The 2D spectra were plotted by using arcsinh scale²⁹.