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Folding of Two Helical Peptide with Free Energy Methods and Molecular Dynamics

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Studying the folding dynamics of a protein using Molecular Dynamics might require an extra criterium when selecting the studied protein. We present a study of the free energy landscape a two-helix protein using the free-energy protein forcefield PFF01. The free energy landscape of this protein is very simple, suggesting it as candidate for all-atom molecular dynamics simulations. In independent simulations we find the formation of the correct secondary structure and several folding events into the tertiary structure.

1 Introduction

The investigation of protein folding mechanism is one of the most important problems of biophysical chemistry. Many β -hairpin systems have been investigated both experimentally and theoretically of small two-helix peptides that are known to fold experimentally into well-defined tertiary structure. Since two-helix proteins constitute a minimal model, in which to investigate the interplay of hydrophobic collapse, secondary structure formation and the formation of native contacts, the identification of such systems may be helpful to elucidate the protein folding mechanism.

In this work we investigate the folding of 1WQE, which exhibits a parallel two-helix bundle. It folds reproducibly with free-energy forcefield into a stable tertiary structure, with very simple free-energy funnel. We demonstrate through molecular dynamics simulation that the lack of competing metastable conformations makes these protein an ideal candidate for folding studies to elucidate the interplay of secondary and tertiary structure formation.

2 Method

An all-atom (except apolar CH_n groups) free-energy protein forcefield^{1,2} (PFF01) parametrizes the internal free energy of the protein (excluding backbone entropy).

The elimination of high energy barriers in the free energy surface is the basis of the basin hopping technique³, also known as Monte Carlo with minimization.

Starting from the same unfolded conformation as above, we performed all-atom implicit water molecular dynamics simulation using AMBER99 forcefield. We generated five trajectories with 50 ns total simulation time each, three at 300 K and two at 325 K, after independent minimization and equilibration.

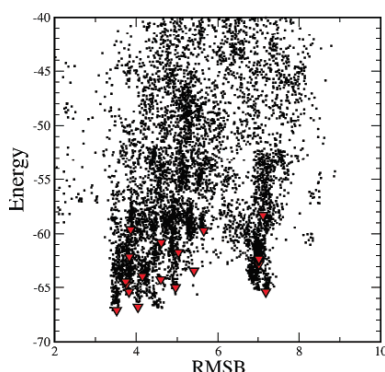


Figure 1. Plot of the energy vs the RMSB in all accepted conformations in the free-energy simulations, the triangles show the best conformations of the 20 simulations. There are only two structural clusters in the free-energy landscape with characteristic RMSB deviations of around 3 and 6 Å to the experimental model.

3 Results

Figure 1 shows energy versus RMSB for all accepted configurations at the end of basin hopping cycles (from all simulations). The triangles indicate the final configurations of the individual simulations. We clearly see two broad funnels of conformations, which terminate into low-energy structures with 3.4 Å and about 7.0 Å RMSB deviation to the native conformation, respectively. The configuration corresponding to the non-native funnel is inconsistent with the formation of the correct number of native disulfide bridges of this peptide.

There is only one, very broad folding funnel consistent with the native disulfide bridge topology. For this reason, the protein studied here may be ideal example to follow the kinetics of protein folding with molecular dynamics or replica exchange methods.

The internal free-energy estimate does not contain backbone entropy; stabilization of one particular conformation with respect to all others does not mean that this conformation is stable with respect to the unfolded ensemble. To settle this question kinetic or thermodynamic simulations must be performed.

We have therefore performed all-atom implicit water molecular dynamics simulations for this protein as described in the methods section. The results for the deviation of the actual conformation from the native structure and the two helices are shown in Figure 2A. The simulations equilibrate quickly into a rapidly fluctuating ensemble with an average overall rmsd deviation between 5 and 8 Å. When we analyze the rmsd deviation of the helical segments however (Helix 1: 1-11, Helix 2: 15-21), we find that the entire simulation is dominated with conformations that are within 1-2 Å of the respective fragment of the protein.

We have also analyzed the helix propensity as a function of time for each amino acid as a function of time, as measured by DSSP. Figure 2B demonstrates a very strong helical content for both segments, but the propensity of helix formation may be forcefield dependent. We also analyze the sulfur-sulfur distance between CYS8-CYS18 and CYS4-22 as a function of time (lower panel in Figure 2A). These distances fluctuate strongly, but on

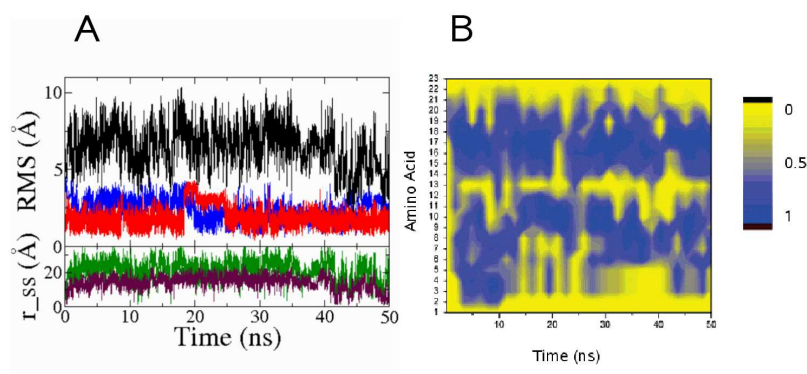


Figure 2. **A:** Analysis of the molecular dynamics trajectories as a function of simulation time. The top panel shows the rmsd of the actual conformation to the native conformation (black) and for the helical fragments only (red: helix 1-11, blue: helix 15-21). The lower panel always shows the deviation of the sulfur-sulfur distance for a potential disulfide bridge (at 2 Å distance) for the amino acids forming the first (green, CYS8-CYS18) and the second disulfide bridge (brown, CYS4-CYS22). **B:** Time average over a 100-ps moving window of the helix propensity of each amino acid in the molecular dynamics simulations. Blue: maximal propensity, yellow: no helical content. In the native conformation the first helix spans amino acids 1-11, and the second helix spans amino acids 14-21, respectively.

occasion, however, some of the sulfur atoms approach each other to within 3-4 Å, i.e., close enough for a disulfide bridge to form. On isolated instances, folding events occur in which both pairs of sulfur atoms approach one another, while both helices are preformed. In those occurrences (which last several ps), the simulations attain all-atom RMSDs to native smaller than 3.5 Å. The intrahelix rmsd vary between 2.1 and 2.5 Å for helix 1 and between 0.8 and 1.0 Å for helix 2 in this time frame.

4 Concluding Remarks

According to our MD simulations, secondary structure formation precedes hydrophobic collapse. The next step would be to substitute the cysteine residues by hydrophobic residues leading to hydrophobic collapse of the already formed helical ensemble into a well-defined tertiary structure without need of disulfide bridges for the peptide to be stable and help to guide the design of stable hydrophobic cores for such small proteins, which would have implications for important challenges in protein design, e.g., for zinc-finger design.

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