Multiscale Approach to Thermodynamics and Dynamics of a β-Hairpin Folding

Jacek Wabik, Dominik Gront, Sebastian Kmiecik, and Andrzej Koliński

Department of Chemistry, University of Warsaw, ul. Pasteura 1, 02-093 Warsaw, Poland
E-mail: {jwabik, dgront, sekmi, kolinski}@chem.uw.edu.pl

Formation of the β-hairpin is the first step along the 2GB1 protein folding pathway. This β-structure is one of the nuclei during this process and controls the rate of the whole protein folding. We present an attempt to improve the Replica Exchange Molecular Dynamics (REMD) by utilising the output structures from the coarse-grained Monte Carlo dynamics as the input for the all-atom REMD. This approach enables effective sampling and can be helpful in elucidating the mechanisms of β-hairpin folding. Thermodynamics and dynamics is analyzed focusing on the number of native contacts during simulations. The energy landscape is analyzed by means of the Histogram Method.

1 Introduction

CABS[1] is a mesoscopic CA-CB side-chain protein model which is used in protein structure prediction and validated during Critical Assessment of Protein Structure Prediction (CASP)[2] experiment. Our results indicate that output β-hairpin structures can be very similar to native structure but they have wrong arrangement of the hydrogen bonds. It can be a problem in some applications eg. docking biologically active substances. Formation of the C-terminal β-hairpin is the first step of the 2GB1 peptide folding[2] so it is important to simulate this process. There were many attempts to elucidate the mechanism of β-hairpin formation[3–5]. There are three main ways how the β-hairpin can fold[6]. The first one is the "zipper mechanism"[3]. In this scenario the β structures are formed and stabilized through the hydrogen bond system. According to second mechanism the first step of folding is the hydrophobic collapse of four residues: Trp3, Tyr5, Phe12, and Val14[4,6]. This "core" stabilizes shape of the β-hairpin from the very beginning facilitating formation of hydrogen bonds. The third mechanism was proposed by Felts et al.[5]. They postulated that the zipping of hydrogen bonds and hydrophobic collapse are the simultaneous events.

2 Methods

We used Replica Exchange Molecular Dynamics method with the replica exchange attempted every 4ps. Simulations and most of the analysis have been conducted using GROMACS package[7]. Bioshell[8,9] and do_dssp[10] programs were also employed. Time of the simulation was 150ns per replica. Ten temperature replicas have been distributed in the range of 285K-325K using algorithm of Patricksson and der Spoel[11]. OPLSAA forcefield and tip4p model for the water were used. The equations of motion were integrated using a leap-frog algorithm with a time step of 2 fs. The non-bonded electrostatic interactions were computed using particle-mesh Ewald method and van der Waals interactions using a simple cut-off. Starting conformations to the all-atom MD simulations were selected from

the β-hairpin dynamics simulation by the CABS model. Beta-hairpin CABS dynamics was performed without using any knowledge about the experimental structure (except the weak bias towards the native secondary structure), starting from random conformations. Resulted CABS trajectory exhibited multiple transitions between near native conformations (RMSD around 1 Angstroms, from which the best ones had 0.7 Angstroms) and fully unfolded (most of the fully unfolded conformers had their RMSD around 5 Angstroms). From the CABS trajectory, we have randomly selected 10 conformations, spanning the most frequent resolutions with RMSD to the native from 5 to 0.7 Angstroms. The selected conformations were subjected to the two-step rebuilding procedure, from C-alpha trace to the backbone atoms by the BBQ method[12], and side chains rebuilding by the SCWRL[13] tool. The worst structures start REMD simulation at high temperatures and the best ones at low temperatures. Input structures were minimized with the steepest decent method and equilibrated for 200ps at constant pressure (1013 hPa) and temperature (285K-325K).

3 Results

Altough we use various structures at the beginning, we obtain the average probabilities of the exchange almost equal for every replica (in range of 14%-17%). One can also notice good overlapping of the histograms of the potential energy for various temperatures, which can indicate that the exchange process was efficient.

4 Refinement of the Structures

For four replicas we have obtained the stable β-hairpin structures with ca. 5 of 7 native hydrogen bonds. For one additional replica the structure with ca. three native hydrogen bonds have been obtained. The best conformations from the REMD had CRMSD=0.75Å with the six native hydrogen bonds while the best input conformation had 0.7Å CRMSD with only three native hydrogen bonds. Generally, output conformations with stabilized β structures and native-like turn had CRMSD in the the range of 0.6Å to 2.5Å and we can regard them as the native-like.

5 Mechanism of Folding

We have computed radius of gyration (R_g) of hydrophobic core (Trp3, Tyr5, Phe12, Val14) which should stabilize the β structures[6]. This value for native-like conformations was in range of 5.3Å to 5.7Å. All convergent pseudotrajectories had this value set from the beginning so we cannot check the stabilizing effect of the core. However, there were also conformations with a good value of R_g but without any β structures and they were not not very stable. It indicates that in our simulation folding might not undergo according to hydrophobic collapse. It is interesting that the proper pattern of hydrogen bonds in β structures forms starting from the hairpin ends or sometimes starting from the middle of the structure (Fig. 1).

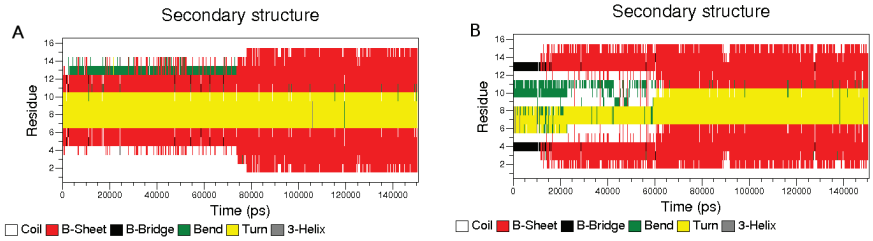

Figure 1. Plots with a secondary structure formed on every residue of β-hairpin for demuxed, continuous trajectories A) pseudotrajectory nr 1 (input CRMSD=1.5Å) B) pseudotrajectory nr 4 (input CRMSD=2.2Å).

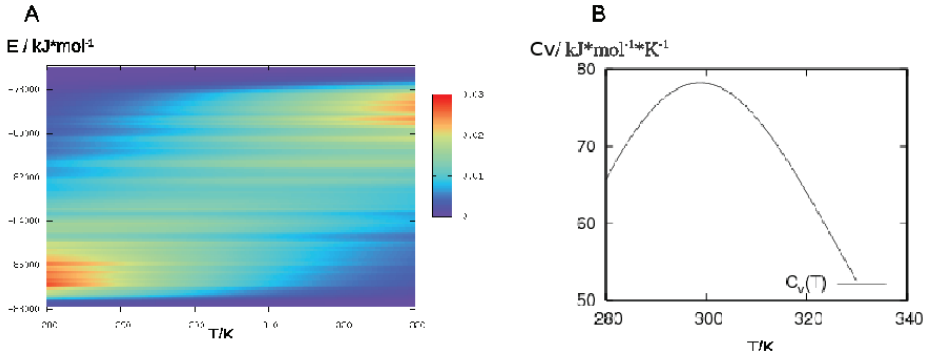

Figure 2. Thermodynamics data A) Plot of density of the states versus T and energy of the system B) Heat capacity versus temperature.

6 Thermodynamic Data

We have computed also some thermodynamic data by the Weighted Histogram Analysis Method (WHAM) technics. Transition temperature corresponding to the maximum of the heat capacity (Fig. 2B) is equal to 298.4K and at this temperature the highest structural mobility and variability was observed. We noticed also that 3- and α-helices constitutes 12% of the population in this temperature, which agrees roughly with the experiment[14] and other simulations. Fig. 2A shows the combined data illustrating the β-hairpin energy landscape and showing cooperative character of the transition with clusters of conformations: native-like and unfolded.

7 Conclusions

We proposed and tested a method that rectifies the conformations generated by coarse-grained Monte Carlo simulation. By REMD we obtained a set of conformations which have proper scheme of native bonds. We also showed that formation of the hydrogen bonds controls the folding mechanism.

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