DOI 10.5012/bkcs.2010.31.8.2386

On the Structural Stability of a Short Three Stranded β-sheet Peptide (Betanova): Replica Exchange Molecular Dynamics Simulation Study

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Received May 28, 2010, Accepted June 24, 2010

Key Words: Three strands β-sheet peptide, Betanova, REMD, Thermodynamic stability

The folding of proteins into its unique structure has been subject of intensive research both experimentally and theoretically for last several decades. In terms of the folding mechanism, significant progress has been achieved, since the funnel theory was introduced in early 90's. According to this view, the process of finding characteristic unique conformations could be understood statically as progressive evolution of an ensemble of partially folded structures. Nevertheless, much of the proteins folding, including the stability of the native structure and the folding behavior are not fully understood.

The small peptides of simple secondary and/or tertiary structure present opportunities of studying the thermodynamic stabilities and kinetics as well as folding mechanism both theoretically and experimentally. The advantage of studying short peptides is that direct comparisons between experimental and simulation results are feasible due to less demanding computational requirement. Thereby, several peptides with representative secondary structures including α-helix or β-strand have been designed for this purpose. The helix-coil transition is relatively well studied. However, experimental study of β-sheets requires isolated peptides (either designed or naturally occurring) and most β-sheet conformers are aggregated easily under the aqueous environment. The simplest β-sheet is three stranded ones and several sequences of three stranded β-sheet have been designed. Betanova (RGWSVQNGKYTNNGKTTEGR) is a *de novo* designed peptide with 20 residues[1] which has a three stranded anti-parallel β-sheet and has been subject of several studies.[2-8] In fact, it is the first designed three stranded β-sheet. In their original paper, Serrano and coworkers [1] concluded that the stable structure of betanova is an anti-parallel β-sheet with β-sheet population of ~80% based on CD and NMR data. However, subsequent experiment[2] of the same peptide using FT-IR and CD suggested that a significant population of random coil exists along with some trace of β-strand, indicating the peptide may not have a well defined β-sheet conformation. In fact, Serrano and coworker revisited this peptide and revised that the β-sheet population was around 10%.[3] Recently, Serguei *et al.*[5] have studied both the structural and thermodynamic features of the same peptide using CD, FT-IR, and fluorescence resonance energy transfer (FRET). They also found that betanova is largely unstructured with β-sheet population of 22 ~ 26% at 5 ^{o}C. As for simulation studies, Colombo *et al.*[6] was able to fold betanova into "partial formation" of anti-parallel β-sheet at 300 K. Also, Bursulaya and Brooks[7-8] found that the peptide is only marginally stable.

The purpose of the current simulation is to gain more insights on the stability of this seemingly not well defined structure. In the current note, we performed direct folding simulation of betanova starting from an extended conformation. To ensure the successful folding simulation, two requirements need to be satisfied, i.e., the reasonable simulation model and the efficient simulation strategy. In this work, we employed the modified all-atom force field (param99MOD5) under the generalized Born (GB) implicit solvation model with surface area correction (GBSA).[9] The param99MOD5 force field was obtained by systematically adjusting the protein backbone parameters of the original amber ff99 force field.[10] This force field model allows more balanced treatments of α/β propensities and remedies overestimated salt-bride effect by the existing GBSA model. This new parameter set has been successfully applied to folding simulation of various peptides[11-13] including α-helices, β-hairpins, ββα motifs, and flexible three stranded twisted β-sheet. It has been found that not only the final structure of the corresponding peptides but also their folding behaviors can be well described with the param99MOD5 force field.

In regard to an efficient simulation strategy, we used the replica exchange molecular dynamics (REMD) simulation, such that several independent simulation trajectories with different target temperatures are generated simultaneously. Then, the trajectories are exchanged between the two replicas of adjacent temperatures. This enables each replica to explore a wide conformation space efficiently for producing the ensemble of canonical distribution.

We performed the REMD simulation with a modified version of the TINKER molecular modeling package[14] on betanova starting from a fully extended structure. The number of replicas was 14 between temperatures range of 266.0 K ~ 530.0 K; 265, 280 (NMR temperature), 296, 313, 330, 348, 367, 387, 407, 429, 452, 477, 503, 532 K. The overall replica exchange acceptance was around 35%. The total simulation time of each replica was 60 ns with a time step of 2 fs and the replica exchanged was attempted at every 0.4 ps. The bond distance between hydrogen atom and heavy atoms were constrained to its equilibrium value using the SHAKE algorithm.[15] As for the

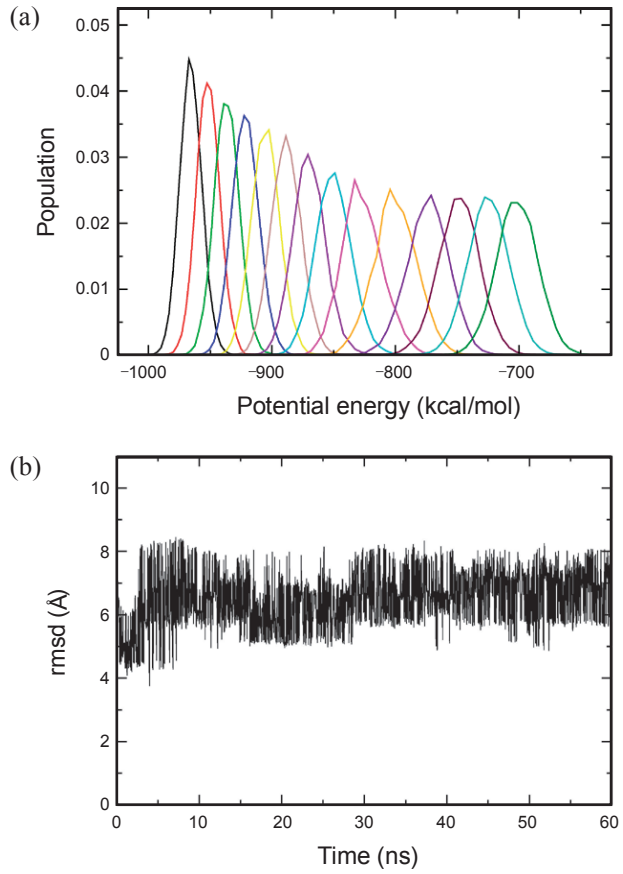

Figure 1. Potential energy distribution of the last 30ns simulation time (a) and time profile of the backbone root mean square deviation (RMSD) of betanova at 280 K (b).

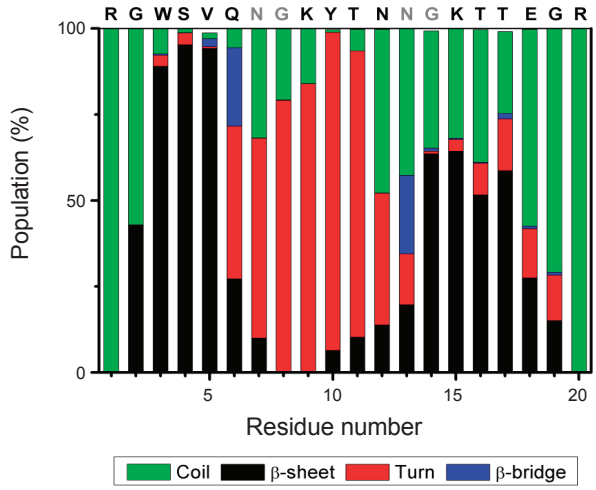

Figure 2. Averaged population of a secondary structure for each residue of betanova after discarding the first 30 ns trajectory. The letter on the top indicates a residue name. α-helix population is less than 1.0%.

temperature control, Berendsen thermostat[16] with coupling constant of 1.0 ps was used. Both the nonbonded interaction and GB solvation cutoff distance were fixed at 24 Å. The trajectories from each replica are saved at every 1.0 ps for further analysis.

Figure 1 shows the potential energy distributions of a total of 14 replicas and the backbone root mean square deviation

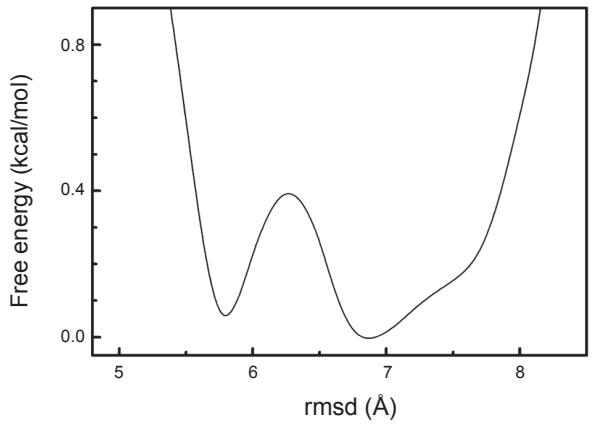

Figure 3. Free energy profile of the backbone root mean square deviation (RMSD) at 280 K. The difference of free energy value between two minima is less than 0.5 kcal/mol.

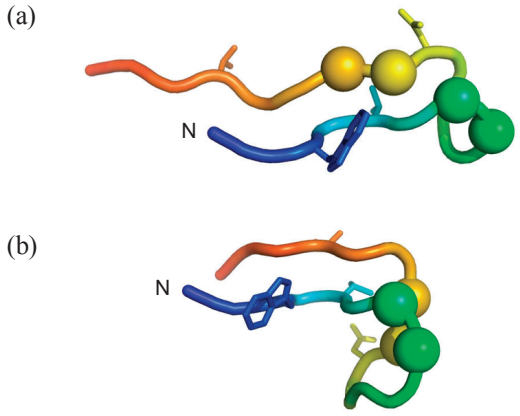

Figure 4. Two representative structures from one dimensional free energy profile (Figure 3). The most populated structure is one with rmsd = 6.7 Å (a) and the second most populated structure is one with rmsd = 5.8 Å (b). The "N" letter indicates N-terminal. Sphere form represents Cα of two turn parts (residue number 7, 8, 13, and 14). The hydrophobic core residues (Trp3, Val5, Asn12, and Thr17) are described by a stick form.

(RMSD) of our simulated trajectory with respect to the NMR native structure[1] at 280 K as a function of the REMD simulation time. The RMSD reaches around 6 Å in about 10 ns and remains steady with a typical steady state fluctuation after 30 ns. To characterize its secondary structure, we discarded the initial 30 ns of simulation trajectory and performed a secondary structure analysis using the STRIDE program.[17] The residue-by-residue secondary structure populations are represented in Figure 2. One can notice immediately that only the extended strand and turn regions can be observed. Residues 6-12 were assigned to a single broad turn (loop) region. The resulting secondary structure of somewhat extended conformations with a single turn clearly contradicts to the NMR native structure. To further investigate the native structure of betanova, we constructed a free energy surface in RMSD space using trajectories of the last 30 ns of REMD simulation time (Figure 3). Essentially, there are one major well around 6.7 Å and a minor well at RMSD of more than 5.8 Å. The two conformations corresponding to each well are shown in Figure 4. In the original design of

betanova, there were two turn regions. In Figure 4, these turn residues were represented as spheres, and the hydrophobic cores was represented as stick model. The predicted structure at the lowest free energy basin is in line with the structural information resulting from our secondary structure analysis. All of our simulation revealed that betanova has only a partial β-sheet in its native state. As shown in Figure 3, the free energy barrier between the two free energy minima is less than 0.5 kcal/mol, indicating that the structural transition between these local minima is rather easy at room temperature. Therefore, the overall shape of the betanova structure may be largely unstructured and somewhat flexible, which is in agreement with a recent experiment by Kuznetsov *et al.*[5] More importantly, the total β-sheet population from the current simulation was 35%, which is comparable to the experimental value.

We have studied a designed 20-residue peptide (betanova) with the REMD simulation protocol using all-atom level parm 99MOD5/GBSA force field that we have developed earlier. Originally, this peptide was known to form a three stranded anti-parallel β-sheet, but recent experimental studies suggest that this might be not the case. Starting from a linear structure, we determined the free energy minimum predicted structure of betanova based on the computation of free energy profile. The resulting lowest free energy structure, however, is predicted to be rather a distorted β-strand like conformation with a single broad turn. Also, the β-strand contents at 280 K from the current simulation conforms to most recent experimental result (22 ~ 26%). The current simulation study supports these recent experimental findings. We anticipate that further detailed structural studies are needed with more elaborated models to resolve the structure and stability issues of betanova.

Acknowledgments. This work is supported by the Pusan National University Research Fund.

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