FACTORS IN MECHANICAL STABILITY OF PROTEIN L : A STEERED MOLECULAR DYNAMICS STUDY

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ABSTRACT

The purpose of this study is to investigate mechanical stability factors of protein L. The structure of protein L has an adjacent parallel β strand between C-terminal and N-terminal. Previous studies showed that protein L has mechanical resistance to external force because of the hydrogen bond in the adjacent β strand. In this study, we use Steered Molecular Dynamics (SMD) simulation with explicit solvent to examine mechanical stability of protein L. Constant Velocity Molecular Dynamics (CVMD) and Constant Force Molecular Dynamics (CFMD) have been applied in this simulation. To verify this study, we also pull the protein L on the opposite direction and different initial coordinate. Simulation results showed four hydrogen bond in parallel β strand has important role to mechanical stability factor of protein L. In addition we have obtain cooperative hydrogen bonds breaking mechanism in the parallel β strand. This results is in agreement with previous studies that mechanical resistance of protein L is determine by an existence of adjacent parallel β strands.

Keywords : *Steered molecular dynamics, protein L, cooperative mechanism, mechanical stability*

INTRODUCTION

Protein L is one of the proteins supporting immune system isolated from *Peptostreptococcus magnus* [1,2]. Protein L has a caracteristic of resistance to external as a result of the existence of hydrogen bond in its β parallel strand as reported by Brockwell [3]. The development of Atomic Force Microscope (AFM) has help us to understand about mechanical stability. However analysis with AFM did not give us much information in the atomic level [4]. On the other hand S*teered Molecular Dynamics* (SMD) giving more detail mollecular data information to the atomic level [5] in addition it also gives a brief information about the mechanical stability factors of Protein L.

MATERIALS AND METHODS

Initial coordinates of protein L were taken from PDB with accession code 1HZ6 [6]. SMD were perfomed using NAMD version 2.6 [7] with the CHARMm22 force field [8]. Cut off was set to 15 Å with switching function at 13 Å and the k value was set to 6 kkal/mol/A^2. Visualization of molecullar structure were conducted using VMD version 1.8.6 [9].

RESULT AND DISCUSSION

Constant Force Molecular Dynamics resulting force-extension data which the profile shown in Figure 1. Pulling to the C termini and N termini gives a similar pattern. At the extension around 19 Å there is siginificant force increasment, while in this extension begin parallel β strand position shifting. At the extension around 25 Å begin the sliding of parallel β strand and at 133 Å is the initiation of sliding proccess and unfolded protein were happen over this extension.

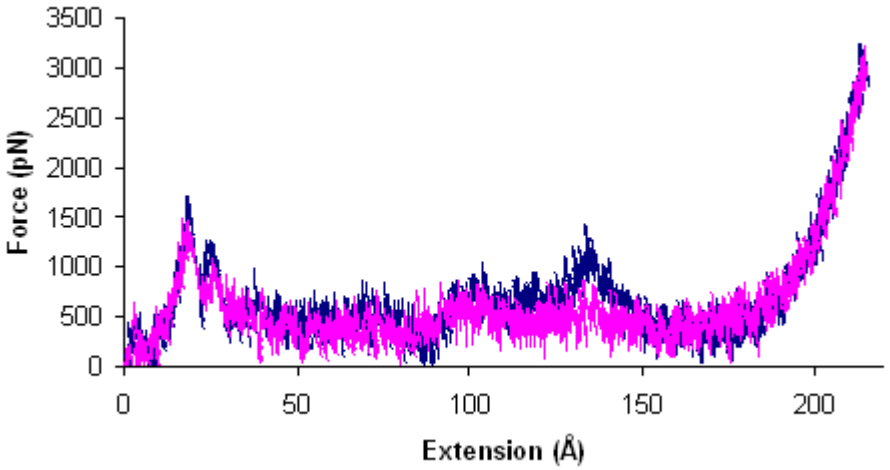


Figure 1. Force-extension profile of pulling protein L in constant velocity. Pulling of C-termini is shown blue line and pulling of N-termini is shown by pink line.

End to End Distance-Time profile is shown in Figure 2. At simulation time around 6.7 ns (N-termini pulling) and 8.7 ns (C-termini pulling) occur the initiation of β parallel shifting. This initiation holds until time 10.8 ns (N-termini pulling) and 11.9 ns (C-termini pulling). After this initiation stage, protein L has totally unfolded.

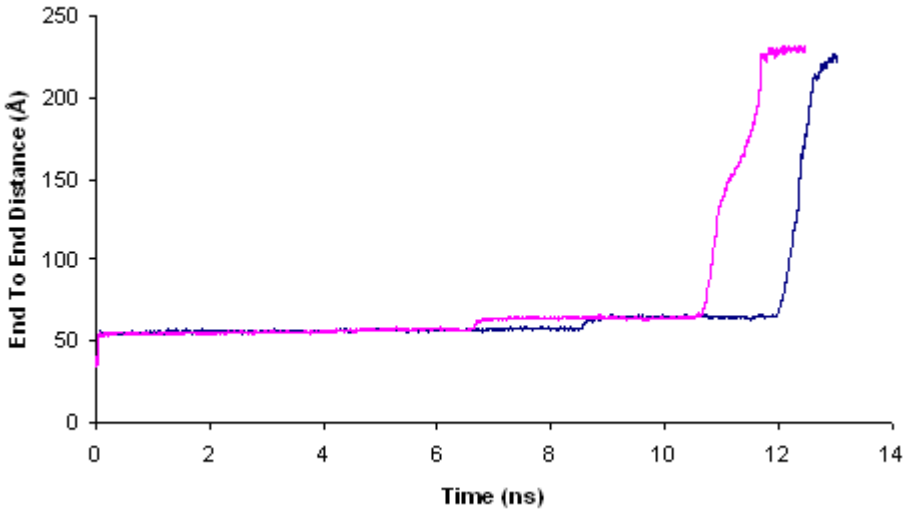
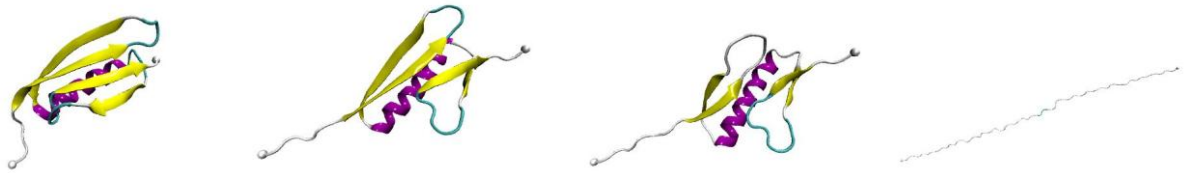


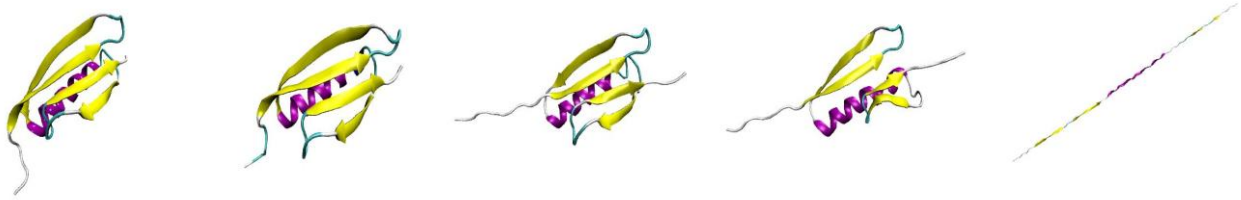
Figure 2. End to end distance-time profile on Pulling with force constant. Pulling of C-termini is shown by blue line and pulling of N-termini is shown by pink link.

Trajectory analysis were conducted to interprete data resulted form the simulation. From all of the simulation have been performed in constant velocity and constant force pulling, were obtained mechanical unfolding snapshots of protein L shown in Figure 3. From this analysis, the mechanical unfolding of protein L according to two-state transition mechanism. It is clearly shown that the parallel β strand of protein L play an important role to its mechanical stability. When the parallel β strand is was in close range, it seems like there is a constrain that make protein L difficult to unfold mechanically. However, when the parallel β strand was not in close range, protein L is easily to unfold mechanically.



0 ns 8.7 ns 12 ns 13 ns

a. Constant Force Molecular Dynamics

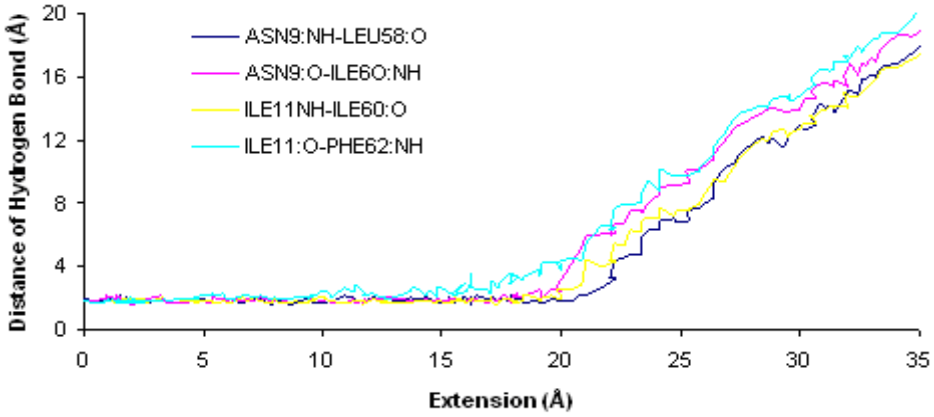


0 Å 19 Å 25 Å 133 Å 220 Å

b. Constant Velocity Molecular Dynamics

Figure 3. Trajectory Snapshots during the Simulation

The stability of parallel β strand is determine by the existence of four hydrogen bond, between residues ASN9:NH-LEU58:O, ASN9:O-ILE60:NH, ILE11:NH-ILE60:O dan ILE11:O-PHE62:NH. Distances plot of the four hydrogen bond is shown by Figure 4. This plot shows that the distance increasing significantly in narrow range extension. This data shows that there is cooperative mechanism on breaking of the four hydrogen bond.



Gambar 4. Distance of hydrogen bond versus simulation time.

CONCLUSION

From simulations result and analysis performed, can be concluded that mechanical unfolding of protein L has to pass disruption stage of parallel β strand which stablity factor of protein L. There are four hydrogen bond play an important role to the mechanical stability of protein L in its parallel β strand between residues ASN9:NH-LEU58:O, ASN9:O-ILE60:NH, ILE11:NH-ILE60:O and ILE11:O-PHE62:NH, in which the mechanical unfolding of protein L occurd through cooperative hydrogen bond breaking mechanism.

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