MOLECULAR DYNAMIC SIMULATION OF DNA POLYMERASE I ITB-1 DOMAINS

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INTRODUCTION

DNA polymerases are present in all organisms and involved in genome replication and repair. Much of the biology of DNA polymerases has been elucidated through the study of the first polymerase isolated, DNA polymerase I (Pol I) from *Escherichia coli* (1). DNA Pol I has three discrete structural domains: the N-terminal 5'→ 3' exonuclease domain, the central 3'→5' exonuclease domain and the C-terminal polymerization domain (Kornberg and Baker, 1992). The large C-terminal fragment, the Klenow fragment (KF), of this enzyme includes both the 3'→5' exonuclease and 5'→3'polymerase domains. The crystal structure of representative polymerases have been determinated, showing the right-hand architecture that termed as "palm", "finger" and "thumb" sub domain (Steitz, 1999; Beese *et al*., 1997. A moderate thermostable DNA Pol I from Geobacillus thermoleovorans obtained from Cimanggu mountain crater, West Java, Indonesia, has been cloned and overexpressed in *E. coli* cells. This enzyme named named as DNA Pol I ITB-1 (Akhmaloka *et al*., 2007). Preliminary biochemical studies revealed that the enzyme has optimum temperature at 338K (Laksmi,2006). This study aims to explain the thermostabilitas of Klenow-like DNA Pol I ITB-1 and 3'→5' exonuclease and 5'→3'polymerase domains separately through molecular dynamics (MD) simulation at high temperatures. In the current simulation, the stability map of the Klenow-like DNA Pol I ITB-1 and it domains separately was obtained from thermal unfolding simulation at various temperatures. By this strategy, we will show that thermolabile and thermostable regions of the protein can be precisely located

MATERIAL AND METHOD

MD simulation was performed using AMBER version 9.0 (Case et. al., 2005). Initial coordinate of the klenow-like DNA Pol I ITB-1 and the separated domains of 3'→5' exonuclease and 5'→3'polymerase were obtained from the homology structural prediction of Swiss-model

modeling program (http://swissmodel.expasy.org). The obtained structures were minimized and heated gradually up to the target temperature. All simulations were conducted in implicit solvent with cut off 15. The temperature and pressure were maintained constant. The stability of DNA Pol I ITB-1 and it domains were probed using MD Simulation at 300K and 350K.

RESULT AND DISCUSION

The resulting three dimensional structure of Klenow-like DNA Pol I ITB-1 from homology modeling by Swiss-model modeling showed high identity (99%) with well-characterized crystal structure of Bacillus stearothermophilus DNA polymerase I. Evaluation of Root Mean Square Deviation (RMSD) at 300K showed that the structures of the enzyme and the individual domains ware stable, with RMSD value at around 5 Å for the whole structure and polymerase domain, while 3'-5' exonuclease domain was 3 Å. During simulation at 350 K, the RMSD for the whole enzyme and polymerase domain increased up to 12 Å until 10ns of the simulations. Meanwhile, the RMSD for 3'-5' exonuclease less than 8 Å, suggested that this domain was more stable.

The stability of secondary structures of the Klenow-like DNA Pol I ITB-1 and 3'→5' exonuclease and 5'→3'polymerase domains were evaluated by calculating of solvent accessible surface area (SASA), secondary structure content and root mean square fluctuation (RMSF) as a function of the simulation time. All the simulation at 300K showed that the SASA and secondary structure content were not significantly change, indicated that the tertiary secondary structure of the protein was stable at this temperature. Meanwhile the total SASA value for all simulations ware increased at 350K, suggested that the tertiary structure of the protein are more exposed. Root mean square fluctuation (RMSF) of individual residues from the simulation at 350K revealed that the thermostability was distributed to the whole regions in the protein. RMSF and snapshot analysis at 350K showed that the thumb sub domain was the most unstable region.The simulation at 350K exhibited exchange in secondary structure content for all simulations. At this temperature the composition of α-helix and β-sheet of both 3'→5' exonuclease and 5'→3'polymerase domains were decreased followed by increasing of turn and coil structures. The α-helix and coil structures of the klenow-like DNA Pol I ITB-1 were more fluctuate at 350K compare to the structure at 300K. On the other hand β-sheet structures for both temperatures were decreased and change into turn formed where decreasing of the β-sheet at 300K relative slower than 350K.

CONCLUSIONS

MD simulations have been performed for klenow-like DNA Pol I ITB-1, 3'→5' exonuclease domain and 5'→3'polymerase domain at 300K and 350K. All the parameter showed that the proteins were stable during 300K simulation. The RMSF of individual residues for the whole simulations at 350K revealed that the thermostability was distributed to the whole regions in the protein. Further more the results showed that the thumb subdomain was the most unstable region.

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