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# Molecular Dynamics Analysis of Thermostable DNA Pol I ITB-1

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One of the thermostable enzymes, which has been widely used in the biotechnological research, is DNA polymerase. The coding sequence of local DNA Pol I gene from a local thermophilic bacterium, namely DNA Pol I ITB-1, has been cloned, sequenced, and overexpressed. However, study on thermostability of this enzyme is very limited. In the present study, thermostability of the protein was evaluated by thermal unfolding simulation at 300, 400, and 500 K. Our simulation revealed that the secondary and tertiary structures of the protein was not significantly affected by thermal perturbation at 300 K, but they were affected and even gradually unfolded by that perturbation at 400 and 500 K. Evaluation of the root mean square fluctuation (RMSF) of individual residues from the simulation at 400 and 500 K revealed the distribution of the thermostability regions in the protein structure. From the RMSF analysis at 400 K, we found that thermostability of the 3'-5' exonuclease domain was lower compared to that of the other domains. Where as from the RMSF analysis at 500 K, we found that in each domain of DNA pol I ITB-1 there was a single extraordinary thermostable α-helix which was likely to be the core of each corresponding domain. Thus our simulation provides a thermostability map of DNA Pol I ITB-1. Such information will be very valuable for the next genetic engineering work in determining a mutation target to modify thermostability of this enzyme.

Key words: thermostable DNA polymerases, molecular dynamics simulation

How thermostable proteins deal with heat? Does the stability come merely from intramolecular interactions among amino acid side chains or is it due to intermolecular interactions with other proteins or with non-protein substances, such as ligands or metal ions? Many efforts have been made to seek possible answers for this question. Jaenicke and Böhm (1998) have summarized almost all of the possible factors responsible for the thermostability of proteins. Among these, are an increase in the number of hydrogen bonds, additional or improved electrostatic interactions caused by salt bridges or networks, optimized hydrophobic interactions, increased compactness or packing densities, increased polarity compared with non-polar surface areas, increased a-helical content and a-helix stability, improvements on the binding of metal ions, improvements of the fixation of the polypeptide chain termini to the protein core, replacement of residues with energetically unfavorable conformations by glycine, truncation of solvent-exposed loops, a higher number of prolines and a-branched amino acids in loops, association to oligomers, a reduction of the content of thermally labile amino acids such as asparagine, glutamine, cysteine, and methionine. This list shows that stabilizing features can originate at all structural levels, from the primary structure to the quaternary structure of proteins.

As outlined above, it is not yet possible to derive rules that govern high protein thermostability. Consequently, the identification of stabilizing interactions needs to be carried out, particularly for new protein samples or genetically engineered ones. In this study, we report on the thermostability of DNA polymerase I from a newly isolate thermophilic bacterium obtained from the Cimanggu Hot Springs, West Java, Indonesia. The gene of the protein has been cloned and over expressed in *Escherichia coli* cells. We named the coding sequence of the gene as DNA Pol I ITB-1 (Pramono 2004). Preliminary biochemical studies revealed that the protein has optimal enzyme activity at 65 °C (Ambarsari 2006), which is lower than the commercially available thermostable DNA polymerase, such as Taq DNA polymerase, that has an optimum enzyme activity of about 80 °C (Peterson 1998). We think that such lower optimum activity is related to the lower thermostability of the protein. Therefore, to improve the optimum temperature for the enzyme activity, it is necessary to improve the thermostability first. Improvement of the thermostability relies on a stability map that provides the information about regions of the protein in terms of their stability level. Using this map, the thermostability can be precisely located by the region that is poorly or highly stabilized. Such information will be very valuable, particularly when one looks for a mutation target to engineer thermostability of a protein e.g., by mutating residues in a poorly stabilized region, there by avoiding false mutation. This kind of information, however, is not generally available, since it requires investigation of intramolecular interactions at the atomic level.

In the present study, we tackled the problem addressed above by performing molecular dynamics (MD) simulation. The advantage of using MD is that it can provide detail information at the atomic level concerning intramolecular interactions during the dynamics of motion of the protein under various conditions. Therefore, the stability map can readily be made based on the level of the strength of intramolecular interactions among interacting residues in the protein. In the current simulation, the stability map of DNA Pol I ITB-1 was obtained from thermal unfolding simulation at various temperatures. By using this strategy, we show that both the thermolabile and the thermostable regions of the protein can be precisely located.

## MATERIALS AND METHODS

**Molecular Dynamics Simulation.** MD simulation was performed using AMBER version 9.0 (Case *et al.* 2005). The

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