Study of Thermal Stability of Riboflavin Synthase of *Eremothecium gossypii* Through Molecular Dynamics Approach

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ABSTRACT

Eremothecium gossypii has the enzymes that able to produce riboflavin naturally. The enzyme that responsible for the final production of riboflavin is riboflavin synthase. Riboflavin synthase catalyzes conversion of two molecules of 6,7-dimethyl-8-ribityllumazine into each one molecule riboflavin and 4-ribitylamino-5-aminouracil. In this study, we determined the interaction of riboflavin synthase isolated from Eremothecium gossypii with 6,7-dimethyl-8-ribityllumazine. We performed a computational approach to see the active sites of riboflavin synthase that play a role in the production of riboflavin. We designed riboflavin synthase isolated from Eremothecium gossypii as a model in PDB format. As a template, the structure of riboflavin synthase isolated from Schizosaccharomyces pombe with 1KZL PDB code was used. The thermal stability of enzyme had been conducted on the molecular dynamics simulation approach at 300K, 310K, 315K, 325K, 335K, and 350K. The results showed that amino acid residues which interact include Thr154, Ile169, Thr172, Val6, and Gly102 at the C-terminal domain and Thr56, Gly68, Ala70, Val109, and His108 at the N-terminal domain. Residue Thr154 was from the C-terminal domain and His108 was from the N-terminal domain, represents two-subunit of the enzyme that acts as an early stage at riboflavin catalysis reaction. These results shown that only one of active sites of the enzyme (N-terminal domain) catalyze riboflavin formation. Molecular dynamics simulation showed the calculation of RMSD values at 300 K and 315 were fluctuated in the range of 22-26 Å from the initial state. At 320 K and 335 K, fluctuation occurred in the range of 29-34 Å. At 350 K, fluctuation occurred in 38-45 Å and the domains structure had separated.

Keywords: Thermal stability, riboflavin, riboflavin synthase, 6,7-dimethyl-8-ribityllumazine, molecular dynamics, Eremothecium gossypii.

INTRODUCTION

Riboflavin is a precursor of coenzymes flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN), which are required for enzymatic oxidation-reduction reaction (Sybesma, et.al., 2004). In general, the production of riboflavin can be achieved by three methods, chemical synthesis, fermentation, and biotransformation of glucose to D-ribose. Environmental and economical studies indicate that the production of riboflavin by fermentation continue to increase, due to cheaper production costs, less generated waste, and lower energy of production (Shrikant, et.al., 2006). Each year, around 3.000 tons of riboflavin is produced in the world, and 2500 tons was produced by fermentation (Choe, et.al., 2005). Some studies have shown that fermentation process using *Eremothecium ashbyii* and *Eremothecium* (*Ashbya*) *gossypii*, produce more riboflavin than other microorganisms, such as *Saccharomyces cerevisiae*, *Candida famata*, and *Bacillus subtilis* (Kato, et.al., 2006). *Eremothecium gossypii* has the enzymes that are naturally able to produce riboflavin. Amount of riboflavin produced is highly dependent on the amount of enzyme and growth conditions. The enzyme responsible for the final production of riboflavin is riboflavin synthase. Riboflavin synthase catalyzes conversion of two molecules of 6,7-dimethyl-8-ribityllumazine to one molecule each of riboflavin and 4-ribitylamino-5-aminouracil. In this study, we determined interaction of riboflavin synthase isolated from *Eremothecium gossypii* and

performed computational approach to examine the active sites of riboflavin synthase that played role in the production of riboflavin.

MATERIAL AND METHODS

The structure of riboflavin synthase isolated from *Schizosaccharomyces pombe* with 1KZL PDB code was used as a template (Gerhardt, et.al., 2002). Sequences of riboflavin synthase of *Eremothecium gossypii* from NCBI (Locus CAC07495) were used as a model and 6-carboxyethyl-7-oxo-8-ribityllumazine was used as a ligand. We designed riboflavin synthase isolated from *Eremothecium gossypii* as a model in PDB format using geno3D Protein Predict and Jigsaw modelers, and simulated using MOE2008.10. The thermal stability of enzyme were simulated using molecular dynamics approach at temperature 300K, 310K, 315K, 320K, 335K, and 350K.

RESULT

Homology Model Validation

The final result of multiple sequence alignment of riboflavin synthase from *Schizosaccharomyces pombe* and *Eremothecium gossypii* is shown in Figure 1. The result showed that homologous value was 64%. Modeling using geno3D Protein Predict obtained 3D structure in PDB format, indicates a structural similarity with RMSD deviations between models and template of 0.82 Å. RMSD deviation value is still below the maximum value of 1.70 Å, which indicates that the model and template does not have significant differences. The quality of Ramachandran plots was satisfactory for the models. Number of residues in outlier region are 2.9% for the models and 0.0% for the template. The value is still bellow the maximum value of 15%, which indicates that the model is reliable for performing further studies.

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CLUSTAL 2.0.12 multiple sequence alignment


pdb|model|A      MFTGIVEHIGTVAEYLENDASEAGGNGVSVLIKDAAPILADCHIGDSIACNGICLTVTEF 60
pdb|1KZL|A       MFTGLVEAIG-----VVKDVQGTIDNGFAMKI-EAPQILDDCHTGDSIAVNGTCLTVTDF 54
                 ****:** **     : :*.. : .**.:: * :*. ** *** ***** ** *****:*

pdb|model|A      TADSFKVGIAPETVYRTEVSSWKAGSKINLERAISDDRRYGGHYVQGHVDSVASIVSREH 120
pdb|1KZL|A       DRYHFTVGIAPESLRLTNLGQCKAGDPVNLERAVLSSTRMGGHFVQGHVDTVAEIVEKKQ 114
                     *.******::  *::.. ***. :*****: .. * ***:******:**.**.:::

pdb|model|A      DGNSINFKFKLRDQEYEKYVVEKGFVAIDGVSLTVSKMDPDGCFYISMIAHTQTAVALPL 180
pdb|1KZL|A       DGEAIDFTFRPRDPFVLKYIVYKGYIALDGTSLTITHVD-DSTFSIMMISYTQSKVIMAK 173
                 **::*:*.*: **    **:* **::*:**.*******:* *. * * **::**: * :.

pdb|model|A      KPDGALVNIETDVNG----KLVEKQVAQYLNA--- 208
pdb|1KZL|A       KNVGDLVNVEVDQIGKYTEKLVEAHIADWIKKTQA 208
                 *  * ***:*.*  *    **** ::*::::
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Figure 1. Sequence alignment of riboflavin synthase from *Schizosaccharomyces pombe* (1KZL)and *Eremothecium gossypii* (model); Sign (*) indicates the same amino acid (identity) and sign (*: and.) are homologous region.

Active Sites of Riboflavin Synthase of Eremothecium gossypii

Riboflavin synthase crystal from *Schizosaccharomyces pombe* is used as a template with PDB code 1KZL, determined using X-ray diffraction with a resolution of 2.10 Å. The

structure of riboflavin synthase is in the form of a complex with 6-carboxyethyl-7-oxo-8-ribityllumazine (carboxyethyllumazine). The structure of riboflavin synthase from *S. pombe* has been made with two active site pockets . Only one of the two active sites which catalyze the formation of riboflavin and the other will interact with the solvent (Fischer and Bacher, 2008).. Model of riboflavin synthase of *Eremothecium gossypii* that interact with the substrate carboxyethyllumazine, appeared to have two active sites pocket. Amino acid residues that interact include Thr154, Ile169, Thr172, Val6, and Gly102 at the C-terminal domain and Thr56, Gly68, Ala70, Val109, and His108 at the N-terminal domain (Figure 2). Residue Thr154 was from the C-terminal domain and His108 was from the N-terminal domain, represents two-subunit of the enzyme that acts as an early stage in riboflavin catalysis reaction. The result showed that only one of enzyme active sites (N-terminal domain) catalyzes riboflavin formation (Figure 3).

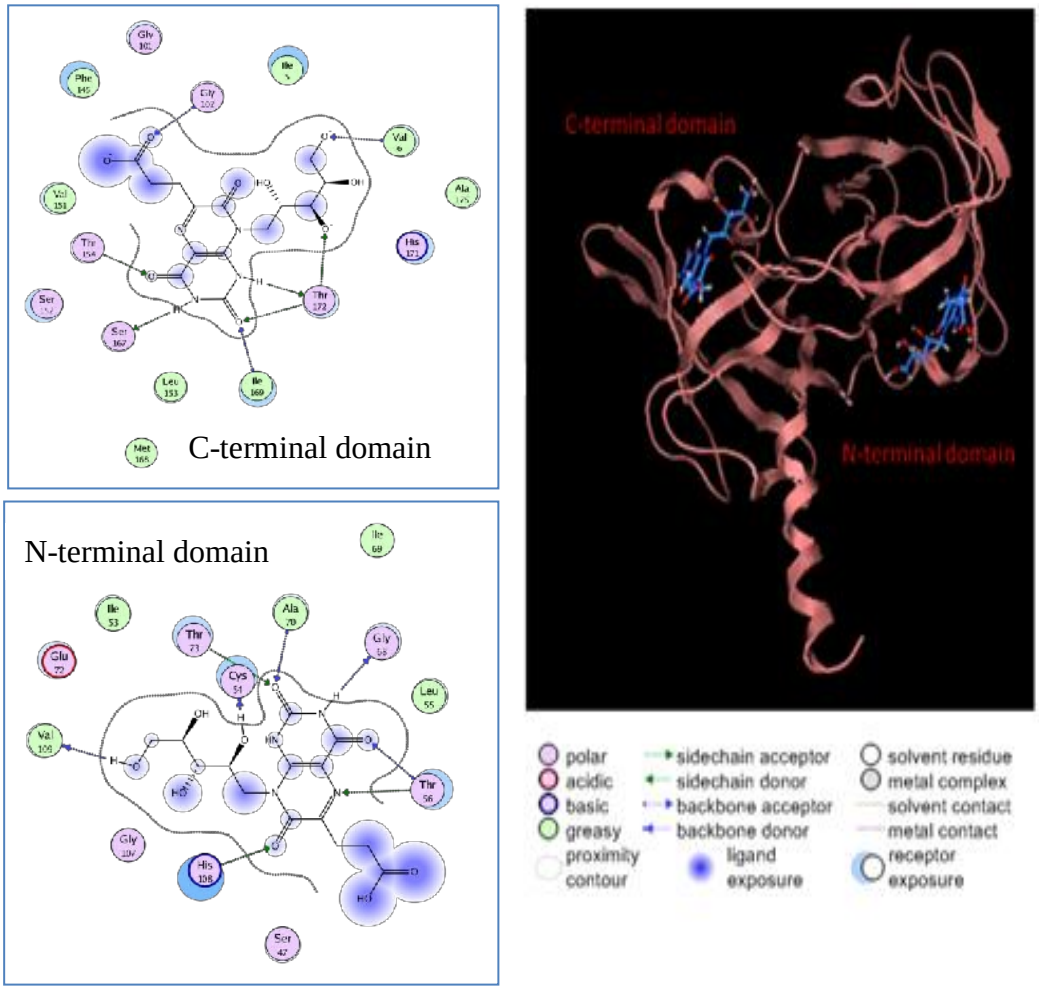

Figure 2. Active sites of riboflavin synthase and amino acid residues that interact in C-terminal domain and N-terminal domain.

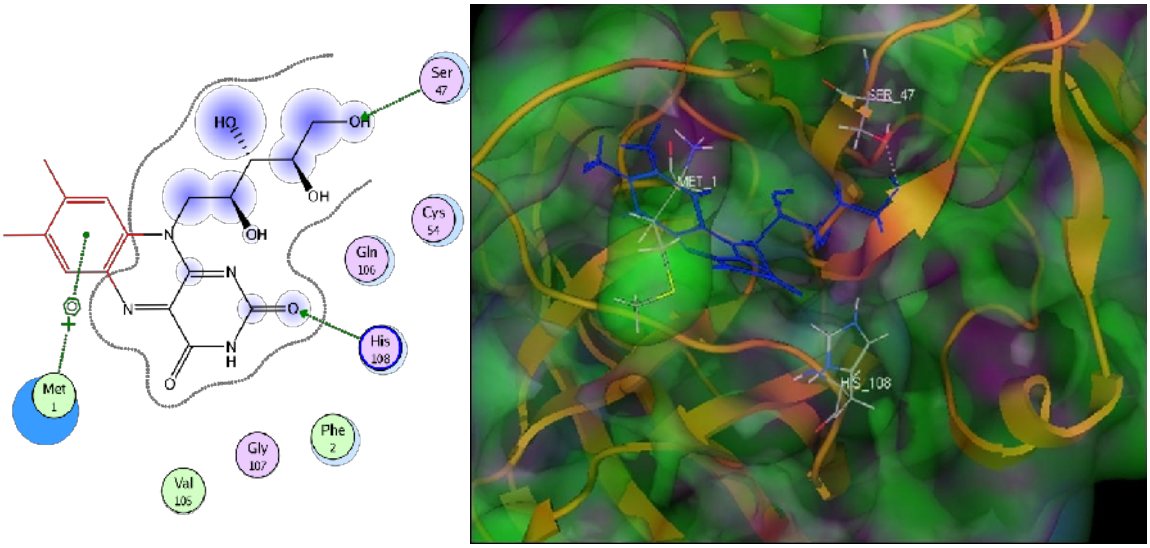

Figure 3. Active sites of the enzyme at N-terminal domain that catalyze riboflavin formation.

Thermal Stability of Riboflavin synthase of Eremothecium gossypii

The results of RMSD values versus the simulation time at 300 K showed that the value are fluctuated in the range of 22-26 Å from the initial state . RMSD value changes very

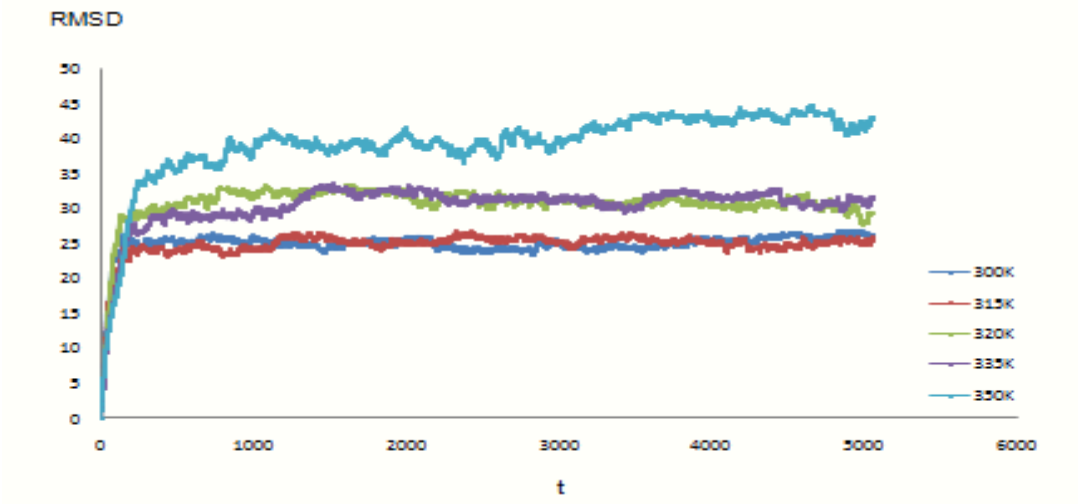

Figure 4. RMSD curves (Å) at 300 K, 315 K, 320 K, 335 K and 350 K versus the simulation time (t).

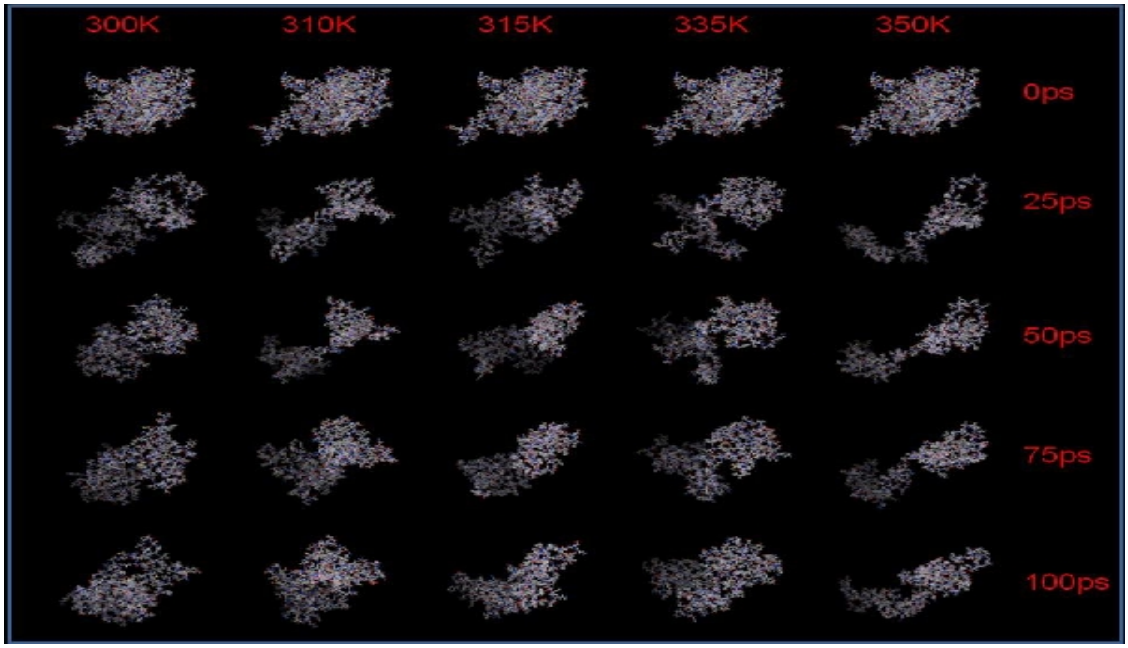

Figure 5. Conformational changes of the riboflavin synthase structure in the range of 0-100ps at temperature 300 K, 310 K, 315 K, 335 K and 350 K

sharply in the range 0-100 ps (Figure 4), indicates the occurrence of a cooperative unfolding process. Visualization results (Figure 5), indicate that the enzyme structure changes take place very rapidly, this was because the structural changes occurred followed the pattern of structural change in catalyzing formation of riboflavin from 6.7-dimethyl-8-ribityllumazine. The stability of the enzyme structure will be obtained after 100 ps with a globular shape. This form will be retained until the end of simulation. Enzyme conformational changes at 315 K does not vary much with conformational changes at 300 K, the range of 0-100ps RMSD value reaches 22 Å and fluctuating between 22-26 Å from the initial state. At 320 K and 335 K, after 100ps, the fluctuation of RMSD values increased to 29-34 Å. These results indicate the occurrence of different conformational change in unfolding process at 300 K and 315 K, which describe the instability of the structure. At 350 K, the domains structure had separated.

DISCUSSION

Thermal stability is defined as the resistance of a protein to maintain the structure in such a way as a response to high temperature so that the protein can still perform its function. RMSD values showed that the enzyme structure changes take place very rapidly for all temperature, and visualization results showed that the structural changes very sharply in the range of 0-100ps. Visualization of the results shows that the conformational changes the enzyme structure at 300 K and 315 K are most likely to catalyze the formation of riboflavin, because the N-terminal domains are not separate. From the results of dynamics simulation of the enzyme and substrate, shows that the separation of the domains will release the substrates faster thus catalyzing the process would not be possible (data not shown).

CONCLUSION

Amino acid residues of riboflavin synthase of *Eremothecium gossypii* that interact to catalyze the formation of riboflavin include: Thr154, Ile169, Thr172, Val6, and Gly102 at the C-terminal domain and the Thr56, Gly68, Ala70, Val109, and His108 in the N-terminal. Only one of active sites of the enzyme that catalyze riboflavin formation that is N-terminal domain. RMSD value at 300 K and 315 K increased to 23 Å from initial state and the simulation time in the range of 0-100ps with fluctuations around 23-27 Å and had a similar structure that

conformational changes. RMSD value at 320-350 K fluctuation occurred in 29-34Å with a conformational change towards the unstable structure.

ACKNOWLEDGEMENT

The researchers are grateful to Ridla Bakri PhD, Chairman of Department of Chemistry, Faculty of Science, University of Indonesia for his support of this research.

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