UNIVERSITI PUTRA MALAYSIA

RATIONAL DESIGN OF ARM LIPASE STABILIZATION

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RATIONAL DESIGN OF ARM LIPASE STABILIZATION

By

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Currently, thermostable lipases are gaining wide industrial and biotechnological interest. The principles of protein folding become growing interest to explore how proteins adapt at high temperatures. However, understanding the mechanism of protein folding is crucial and challenging. To realize the goal, recombinant lipase from newly isolated \textit{Geobacillus} sp. strain ARM has been subjected to protein engineering to enhance thermostability. ARM lipase has comparatively low thermostability compared to other homologous thermostable lipases. A structural model of the target protein (ARM lipase) was built using DS Modeling from three-dimensional structure of a known structure (T1 lipase) with which it had a high sequence homology (91 \%). Consensus approach was used as a strategy to identify the critical point residue in order to increase the thermodynamic stability of ARM lipase. The substitution of Arg157 to Ser was done
using computer software YASARA and the new 3D structure was designated as mutant lipase (R157S lipase). In order to study the thermodynamic stability of ARM and R157S lipases, both lipases were analyzed by molecular dynamics simulations at three different temperatures (50, 60, and 70 °C). The results showed that mutant lipase had reached its folded state at 60 °C, higher than ARM lipase (50 °C). R157S lipase had lower Root Mean Square Deviation (RMSD), Root Mean Square Fluctuation (RMSF), Accessible Surface Area (ASA) and radius of gyration than ARM lipase. It showed that R157S lipase had higher compactness in the structure leading to enhance stability. To validate the computational results, site-directed mutagenesis was used to substitute Arg157 to Ser and the mutation was confirmed by sequencing. The purified ARM and R157S lipases were confirmed to be homogenous as shown by a prominent single band on SDS-PAGE and NATIVE-PAGE. The mutant lipase (R157S lipase) increased the activity and thermal stability from 50 to 60 °C. R157S lipase has a half-life of 24 hours at 60 °C, compared to 13 hours at 50 °C for ARM lipase. The mutant lipase showed increased stability in polar organic solvents especially in DMSO, 2,3-butanediol, methanol, acetonitrile and ethanol. However, the mutation did not affect pH optimum, pH stability, substrate specificities, inhibitors, surfactants and the effect of metal ions. Fluorescence analysis showed that the aromatic Trp residues exposed to solvent were decreased in R157S lipase due to high protein packing and compactness. Thus, the rational design of Arg157 substitution to Ser as inspired from consensus approach increased the protein folding of mutant lipase. This is shown in molecular dynamics simulations and also, subsequently increased of lipase activity, more thermostability, stable in various polar organic solvents and surfactants.
Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Sains Master

PENGUBAHSUAIAN PROTEIN SECARA RASIONAL DALAM MENINGKATKAN KESTABILAN LIPASE ARM

Oleh

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I certify that a Thesis Examination Committee has met on 4 May 2012 to conduct the final examination of Arilla Sri Masayu binti Abd Rahim on her thesis entitled "Rational Design of Arm Lipase Stabilization" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institutions.

ARILLA SRI MASAYU BINTI ABD RAHIM

Date:
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