UNIVERSITI PUTRA MALAYSIA

RATIONAL DESIGN OF ARM LIPASE STABILIZATION

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FBSB 2012 58

RATIONAL DESIGN OF ARM LIPASE STABILIZATION

By

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**Thesis Submitted to the School of Graduate Studies,
Universiti Putra Malaysia, in Fulfillment of the Requirements
for the degree of Master of Science
May 2012**

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

RATIONAL DESIGN OF ARM LIPASE STABILIZATION

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May 2012

Chairman : Professor Dato' Abu Bakar Salleh, PhD

Faculty : Biotechnology and Biomolecular Sciences

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 *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

ARILLA SRI MASAYU BINTI ABD RAHIM

Mei 2012

Pengerusi: Profesor Dato' Abu Bakar Salleh, PhD

Fakulti: Bioteknologi dan Sains Biomolekul

Kini, lipase yang thermostabil telah mempunyai kepentingan yang tinggi dan meluas di dalam industri mahupun di dalam bidang bioteknologi. Hal ini disebabkan kepentingan pelipatan protein dalam struktur protein dan telah menjadi faktor utama bagi protein untuk beradaptasi pada suhu tinggi. Namun begitu, ilmu pengetahuan mengenai mekanisma pelipatan struktur protein masih menjadi persoalan. Bagi memahami mekanisma ini dengan lebih mendalam, lipase rekombinan daripada pencilan bakteria *Geobacillus* sp. strain ARM telah digunakan dengan menggunakan kaedah kejuruteraan protein untuk meningkatkan kestabilan ARM lipase pada suhu tinggi. ARM lipase mempunyai kestabilan suhu jauh lebih rendah jika dibandingkan dengan kebanyakan lipase thermostabil yang homolog. Satu model protein yang dikenali sebagai protein

sasaran (Lipase ARM) telah dibina menggunakan program komputer DS Modeling di mana menggunakan struktur protein tiga dimensi yang sedia ada (Lipase T1) dengan peratus jujukan homologi yang tinggi (91 %). Susunan jujukan pelbagai telah digunakan sebagai strategi untuk mengenal pasti asid amino yang kritikal di samping meningkatkan kestabilan thermodinamik lipase ARM. Penukaran asid amino Arg157 kepada Ser telah dilakukan dengan menggunakan program komputer YASARA dan struktur protein 3D baru telah dibina yang dikenali sebagai mutan lipase R157S. Lipase ARM dan R157S dianalisa melalui simulasi dinamik molekul pada tiga suhu berbeza (50, 60, and 70 °C). Keputusan simulasi dinamik molekul telah menunjukkan bahawa lipase R157S mempunyai tahap kestabilan pada suhu 60 °C, di mana lebih tinggi dari ARM lipase (50 °C). Lipase R157S menunjukkan nilai keputusan RMSD, RMSF, ASA dan jejari putaran lebih rendah daripada lipase ARM. Ini menunjukkan bahawa pelipatan protein bagi lipase R157S telah meningkat disebabkan oleh peningkatan kepadatan struktur protein yang membawa kepada kestabilan protein. Bagi menyokong hasil keputusan dari simulasi komputer, kaedah mutagenesis telah digunakan dengan menukarkan residu Arg157 kepada Ser dan mengesahkannya melalui jujukan asid amino. Ketulenan lipase ARM dan R157S disahkan melalui penghasilan satu jalur pada SDS-PAGE dan NATIVE-PAGE. Lipase mutan (R157S) telah meningkatkan lipase aktiviti dan kestabilan suhu daripada 50 kepada 60 °C. Lipase R157S telah menunjukkan separuh hayat masa (24 jam) lebih panjang pada suhu 60 °C, di mana lebih stabil berbanding lipase ARM yang menpunyai separuh hayat 13 jam pada suhu 50 °C. Di samping itu, lipase mutan telah menunjukkan kestabilan dalam pelarut organik polar terutama di dalam pelarut DMSO, 2,3-butanediol, methanol, acetonitrile dan ethanol. Namun begitu, perubahan titik mutasi tidak menunjukkan perubahan pada optimum pH, kestabilan pH,

kespesifikan substrat, perencat dan ion besi. Kajian analisis fluoresent menunjukkan penurunan residu Trp yang bersifat aromatik pada lipase R157S adalah disebabkan peningkatan pelipatan struktur protein. Justeru itu, penukaran Arg157 kepada Ser secara rational melalui susunan jujukan pelbagai telah meningkatkan pelipatan struktur lipase mutan. Hal ini telah dibuktikan melalui simulasi dinamik molekul dan juga peningkatkan aktiviti lipase, kestabilan suhu tinggi, stabil dalam pelarut organik polar dan surfaktan.

ACKNOWLEDGEMENTS

All praises do to Allah, Lord of the universe for strength, wisdom and grace in times when human abilities fail. Only by his grace and mercy this thesis can be completed.

Hereby, I would like to express my utmost gratitude and appreciation to my supervisors Professor Dato' Dr. Abu Bakar Salleh, Professor Dr. Raja Noor Zaliha Raja Abd. Rahman, Professor Dr. Mahiran Basri and Dr Adam Leow Thean Chor for their support, guidance, and patience. They always helped me in every way possible. I am also grateful to Professor Dr. Mohd Basyaruddin Abdul Rahman, Dr Mohd Syukuri Ali, Dr Wong Chee Fah and Dr Bimo Ario Tejo for their unlimited guidance, advice and suggestion.

Special thanks are also extended to all my seniors and friends in Enzyme and Microbial Technology Research group for their contributions, knowledge and friendship. Special thanks are also extended to the staff of the Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia for their help and cooperation.

I wish to express my deepest gratitude to my parents, Abd Rahim bin Mohd Yusof and Wasilah binti Tulus for their prayers, continuous moral support and unending encouragement. Last but not least, I wish especially to acknowledge my beloved husband, Bani Afnan Azizi bin Baniyamin for his unbelievable love, support, patience and understanding for every situation I am in. Without my family, I would never be here.

Sincerest thanks.

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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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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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.

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ARILLA SRI MASAYU BINTI ABD RAHIM

Date:

TABLE OF CONTENTS