



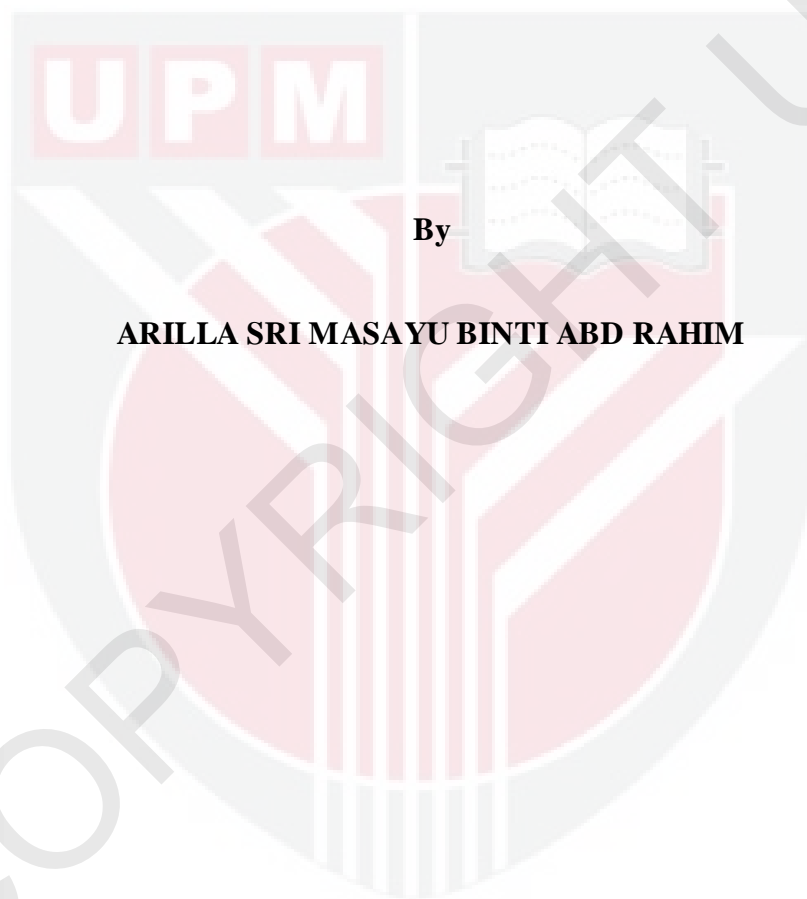
UNIVERSITI PUTRA MALAYSIA

RATIONAL DESIGN OF ARM LIPASE STABILIZATION

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By

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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

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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 termodinamik 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 mempunyai 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 fluoesent 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 meningkatkan aktiviti lipase, kestabilan suhu tinggi, stabil dalam pelarut organik polar dan surfaktan.

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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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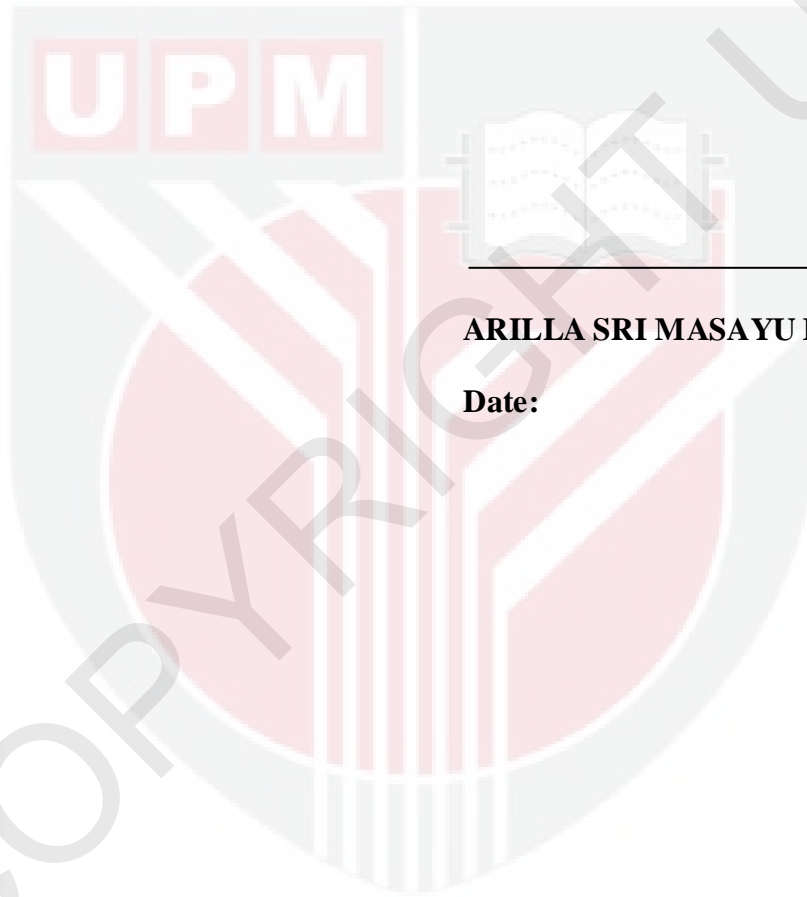
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DECLARATION

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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TABLE OF CONTENTS

	Page
ABSTRACT	ii
ABSTRAK	iv
ACKNOWLEDGEMENTS	vii
APPROVAL	viii
DECLARATION	x
LIST OF TABLES	xiv
LIST OF FIGURES	xv
LIST OF ABBREVIATIONS	xx
CHAPTER	
1 INTRODUCTION	1
2 LITERATURE REVIEW	
2.1 The three-dimensional structure of proteins	
2.1.1 A journey from amino acid to secondary structure	4
2.1.2 Importance and determinants of secondary structure	6
2.1.3 Tertiary and quaternary structures	9
2.1.4 The structure of enzyme-substrate complexes	11
2.1.5 Factors enhancing protein stability	12
2.2 Lipases	
2.2.1 Structure and function of lipases	15
2.2.2 Properties of lipases	19
2.2.3 Thermostable lipases	20
2.3 Protein engineering	
2.3.1 Stabilization through protein engineering	22
2.3.2 Choice of mutation in selected lipases by rational design	24
2.3.3 Critical point residues (hot and bad residues)	27
2.4 Computational modeling	
2.4.1 Methods and applications	29
2.4.2 Modeling protein structures	29
2.4.3 Molecular dynamics simulations	31
3 MATERIALS AND METHODS	
3.1 Materials	35
3.2 Bacterial sources	39
3.3 Computational works	
3.3.1 Prediction of lipase 3D structure	39
3.3.2 Prediction of mutant 3D structure	40
3.3.3 Molecular dynamics simulations	40
3.3.4 VORONOIA analysis	41

3.4	Experimental works	
3.4.1	Extraction of recombinant plasmid	41
3.4.2	Site-directed mutagenesis	41
3.4.3	DNA sequencing	42
3.4.4	Transformation into <i>E.coli</i> Top10	43
3.4.5	Expression of recombinant ARM and mutant lipases	43
3.4.6	Lipase assay	44
3.4.7	Standard curve of oleic acid	44
3.4.8	Purification of recombinant ARM and mutant lipases	45
3.4.9	Characterization of purified ARM and mutant lipases	
3.4.9.1	Determination of protein concentration	45
3.4.9.2	Effect of temperature on activity and stability	46
3.4.9.3	Effect of pH on lipase activity and stability	46
3.4.9.4	Effect of organic solvent on lipase stability	46
3.4.9.5	Effect of surfactants on lipase stability	47
3.4.9.6	Effect of metal ions on lipase activity	47
3.4.9.7	Effect of inhibitors on lipase activity	48
3.4.9.8	Specificity of lipase towards natural oils	48
3.4.9.9	Screening chain length using <i>p</i> NP substrates	49
3.4.9.10	Regioselectivity study using TLC plate	49
3.4.9.11	Fluorescence study	50
4	RESULTS AND DISCUSSIONS	
4.1	Computational results	
4.1.1	Prediction of ARM lipase structure: Comparative Modeling	51
4.1.2	Prediction of critical point by consensus approach	59
4.1.3	Molecular Dynamic Simulations	67
4.1.3.1	Root Mean Square Deviation (RMSD)	68
4.1.3.2	Root Mean Square Fluctuation (RMSF)	73
4.1.3.3	Solvent Accessible Surface Area (SASA)	81
4.1.3.4	Radius of Gyration (R_g)	84
4.1.3.5	Hydrogen Bonds	86
4.2	Experimental results	
4.2.1	Site-directed mutagenesis (R157S)	90
4.2.2	Sequencing of the mutated ARM lipase (R157S)	93
4.2.3	Purification of the ARM and mutant lipases	94
4.2.4	Characterization study of ARM and mutant lipases	
4.2.4.1	Effect of temperature on lipase activity and stability	101
4.2.4.2	Effect of pH on lipase activity and stability	106
4.2.4.3	Effect of organic solvents on lipase stability	109
4.2.4.4	Effect of surfactants on lipase activity	113
4.2.4.5	Effect of metal ions on lipase activity	117
4.2.4.6	Effect of inhibitors on lipase activity	121
4.2.4.7	Effect of substrate specificity on lipase activity	124
4.2.4.8	Fluorescence study	130
4.3	Integration of computational and experimental works	134

5 SUMMARY, CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH	138
REFERENCES	141
APPENDICES	155
BIODATA OF STUDENT	168
LIST OF PUBLICATION/CONFERENCE	169

