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SITE-DIRECTED MUTAGENESIS OF HELICAL LID OF J15 LIPASE

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SITE-DIRECTED MUTAGENESIS OF HELICAL LID OF J15 LIPASE

BY

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Thesis Submitted to the Department of Cell and Molecular Biology, Faculty of Biotechnology and Biomolecular Sciences, University Putra Malaysia, in Fulfillment of the Requirement for the Bachelor Science (HONS.) Cell and Molecular Biology

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Abstract of thesis presented to the Department of Cell and Molecular Biology in fulfillment of the requirement for the Bachelor Science (HONS.) Cell and Molecular Biology.

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Chair: Dr. Adam Leow Thean Chor, PhD

Faculty: Faculty of Biotechnology and Biomolecular Sciences

Lid of lipases modulate the specificity of the substrate to the catalytic active site of lipase by interfacial activation. To address the role of lid on the substrate specificity, site-directed mutagenesis was used to modify the hydrophobicity of the lid by computer simulation and experimental approach. The lid mutant D99I was constructed by substitution of Asp99 with isoleucine after the structural alignment with *Rhizomucor miehei* lipase, a fungal lipase with different substrate specificity. Lid mutation could alter the substrate specificity in term of chain length specificity. The outcomes of *in silico* molecular docking indicates that the D99I mutant preferred the medium chain-length of substrate instead of long chain-length which

is the preference for J15 lipase based on the binding energies. Gene encoding for J15 lipase was cloned into pEASY E2 vector and expressed in *Escherichia coli* BL21 (DE3). The mutant D99I was generated using Fast Mutagenesis Kit by changing the codon specifies the amino acid. The estimated molecular mass of mutated J15 lipase in soluble protein form was about 40.1 kDa based on the SDS-PAGE analysis. The specific activity of the mutated lipase D99I was 1.66 U/mg, as compared to native J15 lipase with specific activity of 1.95 U/mg. Most of the native J15 lipase and mutated lipase were expressed as insoluble protein due to incorrect folding of protein thus rendered the characterization a difficult task. Further expression optimization or protein purification need to be done in order to collect sufficient protein for characterization studies.

Keywords: Lipase, lid, interfacial activation, site-directed mutagenesis, substrate specificity

Abstrak tesis yang dikemuka kepada Jabatan Biologi Sel dan Molekul sebagai memenuhi keperluan untuk Bacelor Sains (K) Biologi Sel dan Molekul

MUTAGENESIS TERARAH DARIPADA PENUTUP HELIKS LIPASE J15

Oleh

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Pengerusi: Chair: Dr. Adam Leow Thean Chor, PhD

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Penutup lipase memodulasikan kekhususan ikatan substrat kepada tapak aktif lipase dengan pengatktifan antara muka. Untuk mengkaji kepentingan penutup dalam kekhususan ikatan substrat, mutagenesis terarah digunakan untuk mengubahsuaikan kehidrofobian penutup dengan simulasi computer dan kaedah eksperimen. D99I mutan dibina oleh penggantian Asp99 dengan isoleucin selepas pembarisan struktur dengan *Rhizomucor miehei* lipase, satu lipase kulat yang substrat kekhususan ikatan berbeza. Mutasi penutup akan mengubah substrat kekhususan ikatan dalam kekhususan panjangan rantai. Keputusan molekul dok menunjukkan D99I mutan lebih suka substrak yang sederhana panjangan rantai daripada panjang rantai yang disukai oleh lipase J15 berdasaskan tenaga

pengikatan. Lipase J15 gen diklonkan dalam pEASY-E2 vektor dan diekspres oleh *Escherichia coli* BL21 (DE3). D99I mutan dihasilkan oleh Fast Mutagenesis Kit dengan pertukaran kodon yang menspesifikasikan asid amino.Anggaran jisim molekul D99I mutan dalam bentuk protein larut ialah 40.1kDa berdasarkan analisis SDS-PAGE. Keaktifan spesifik D99I mutan ialah 1.66U/mg, sebaliknya lipase J15 ialah 1.95 U/mg. Kebanyakan lipase J15dan lipase mutan telah mengekspreskan sebagai protein tak larut kerana perlipatan yang salah, oleh itu, pencirian protein menjadikan susah. Kelanjuatan pengoptimuman eskpresi atau penceraian protein perlu dijalankan untuk mencukupi protein yang dapat digunakan dalam kajian pencirian protein.

Kata Kunci: Lipase, penutup, pengaktifan antara muka, mutagenesis terarah, kekhusasan ikatan substrat

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LIST OF ABBREVIATIONS

bp	base pair
˚C	degree celcius
DNA	deoxyribonucleic acid
kb	kilobase pair
KDa	kilodalton
M	molar
mg	milligram
ml	milliliter
mM	millimolar
MW	molecular weigtht
PCR	polymerase chain reaction
PDB	protein data bank
rpm	rational per minute
SDS-PAGE	sodium dodecyl sulphate polyacrylamide gel electrophoresis
µl	microliter
w/v	weight per volume

CHAPTER 1

INTRODUCTION

Lipases (triacylglycerol acylhydrolases EC 3.1.1.3) catalyze the hydrolysis of the ester bonds in triglycerides to form fatty acids and glycerol (Sharma *et al*., 2001). Besides, lipases also involve in catalytic biotransformation reactions such as alcoholysis, acidolysis, esterification, and transesterification, for various industrial purposes. Compared to lipases origin from the flora and fauna, microbial lipases particular yeast, fungal and bacterial lipases are commonly used in the industries because the microorganism IS easily cultured on the simple, cheap media and the lipase easily extracted from the microbes (Gupta *et al.,* 2004). In this study, J15 lipase gene was amplified from the genome of the *Photobacterium* strain J15, a Gram-negative bacteria that lives in marine environment.

Substrate specificity of the lipase is an important consideration in the industrial application as substrate specificity describes how good an enzyme is at catalyzing the conversion of a substrate to product. The molecular properties of the lipase, substrate structure and factors affecting binding of the enzyme to the substrate are variables that manipulate the substrate specificity. Through the protein engineering, we can manipulate the substrate specificity by controlling the molecular properties of J15 lipase through site-directed mutation on lid to enhance the efficiency of the lipase acts as versatile biocatalysts and can have better cost performance.

The lid, a mobile amphipatic structure that covers the active site could neglect the access of the substrate to the catalytic active site of lipase, thus, the activity and substrate specificity of the lipase are manipulated (Dugi *et al.,* 1995). In the closed conformational state, their hydrophobic part is pointed towards the protein core while hydrophilic side directed toward the solvent. Lipases are activated in the aqueous medium by interfacial activation when the lid is displaced with the presence of lipid interface (Brzozowski *et al.,* 1991). However, the lipases are in inactive form when the lipases make contact against with the soluble substrate in water. Thus, with the presence of lid, the active site is covered when in the water solution, making it inaccessible to the substrate, whereas the lid will in open conformation when in the insoluble solution, thus exposing the substrate-recognition region for binding (Cygler and Schrag, 1999; Ericsson *et al.,* 2008; Grochulski *et al.,* 1993; Miled *et al.,* 2003). The present study mutate helical lid on the J15 lipase by site-directed mutagenesis hoping to change the substrate specificity of the J15 lipase.

The purpose of this study is to define the role of lid on the substrate specificity. Furthermore, this research was performed with the following specific objectives:

1. To model the 3D structure of J15 lipase.
2. To find possible point of mutation *in silico.*
3. To modify the hydrophobicity of the lid of J15 lipase through site-directed mutagenesis.
4. To characterize the expression of the J15 lipases and its mutant for the verification of *in silico* result.

CHAPTER 6

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