



**UNIVERSITI PUTRA MALAYSIA**

**REDUCTIVE ALKYLATION OF *CANDIDA RUGOSA* LIPASE :  
STRUCTURAL APPROACHES**

**BIMO ARIO TEJO**

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DOCTOR OF PHILOSOPHY  
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**by**

**BIMO ARIO TEJO**

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**September 2004**

**Chairman : Professor Abu Bakar Salleh, Ph.D.**

**Faculty : Biotechnology and Biomolecular Sciences**

The properties of alkylated lipase are successfully explored through experimental and molecular modelling methods. Alkylation was done using aldehydes with different degree of modification to represent different levels of hydrophobicity which is important for enzymes to work in nonaqueous environment.

Far ultraviolet circular dichroism (CD) spectroscopy of the lipase in aqueous solvent shows that increasing the degree of modification from 49% to 86% resulted in loss in secondary structure which is attributed to the enzyme unfolding. The secondary structure elements of the CD spectra of native and modified lipase were analysed using the CDPro software and the K2D program. Both methods yield the same results in that the ratio of  $\alpha$ -helical



structure is lost. This result explains why alkylated lipases have much lower activity in an aqueous environment.

Molecular modelling simulations were performed to study the structural and dynamical changes of the lipase upon different levels of modification. Simulations were run for 1 ns (300 K) with five different initial velocities to obtain better conformational sampling. Two solvent systems were used: TIP3P water model and carbon tetrachloride (CCl<sub>4</sub>) solvent model in periodic boundary condition (PBC). Generally, lipases simulated in water are less deviated in term of root mean square deviations (rmsd) compared to lipases simulated in CCl<sub>4</sub>.

Lid movements are essential for lipase function, both in water and water-lipid environments. Analyses of lid dynamics were done using time-correlation function and second-order Legendre polynomial function. Lipase in water and CCl<sub>4</sub> shows different properties of dynamics. Without alkylation, the time correlation function of lipase in water shows one slow exponential decay with a correlation time of  $\tau = 92.8$  ps. In contrast, for simulations in CCl<sub>4</sub> the lid has a more complex dynamics. Exponential fit of open CRL in CCl<sub>4</sub> results in two different  $\tau$  values: a fast motion  $\tau_1 = 5.6$  ps and a slow motion  $\tau_2 = 163.8$  ps.



Upon alkylation, different levels of modification show different properties of lid motions. In CCl<sub>4</sub>, lid region is highly stabilised upon 95% alkylation with slow motion mode of  $\tau_1 = 4.1$  ps and  $\tau_2 = 577.8$  ps. Slow motion effect of lid region is also observed at 63% with  $\tau_1 = 2.9$  ps and  $\tau_2 = 209.2$  ps and 43% modification with  $\tau_1 = 3.4$  ps and  $\tau_2 = 117.9$  ps. In water, 43% and 95% modification show similar motion with unmodified lipase, with one slow exponential decay of  $\tau = 142.8$  ps and 133.6 ps, respectively. However, 63% modification shows more complex dynamics with different  $\tau$  values which mimics the dynamics properties in CCl<sub>4</sub>.

A novel lid-locking mechanism which stabilises the opening form of lid region has been observed during simulations of unmodified CRL in CCl<sub>4</sub>, *i.e.* a salt bridge between Lys85 and Asp284. This salt bridge is highly stabilised on unmodified lipase with a distance of 3.3 Å compared with lipase simulated in water with a distance of 15.25 Å. Alkylation at 43% causes the salt bridge to be deformed in CCl<sub>4</sub> with a distance of 6.03 Å; however, 63% modification stabilises the salt bridge with a distance of 3.88 and 95% modification shows the most stabilising effect with a distance of 3.19 Å.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia  
sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**PENGALKILAN REDUKSI LIPASE *CANDIDA RUGOSA*:  
PENDEKATAN STRUKTUR**

Oleh

**BIMO ARIO TEJO**

**September 2004**

**Pengerusi : Profesor Abu Bakar Salleh, Ph.D**

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Sifat-sifat lipase teralkil telah berjaya dikaji dengan kaedah uji kaji dan dinamik molekul. Pengalkilan dilakukan dengan aldehida pada beberapa peringkat pengubahsuaian yang berbeza untuk menggambarkan perbezaan hidrofobisiti yang penting bagi enzim untuk bekerja dalam persekitaran tanpa air.

Spektroskop "circular dichroism" sinar ultraungu jauh menunjukkan bahawa kenaikan peringkat pengubahsuaian dari 43% kepada 86% menyebabkan kehilangan struktur kedua yang berhubung kait dengan struktur enzim yang lebih terbuka. Unsur-unsur struktur kedua spektrum CD bagi lipase semula jadi dan terubah suai telah dianalisis menggunakan program CDPro dan K2D. Kedua kaedah itu menghasilkan keputusan yang sama bahawa nisbah struktur  $\alpha$ -helix telah menurun. Keputusan ini memberi

penjelasan mengapa enzim lipase terubah suai memiliki aktiviti rendah di persekitaran yang mengandungi air.

Penyerupaan model dinamik molekul telah dijalankan untuk mengkaji struktur dan dinamik enzim lipase pada peringkat pengubahsuaian yang berbeza. Penyerupaan model telah dijalankan selama 1 ns (300 K) dengan lima kelajuan awal yang berbeza untuk mendapatkan contoh-contoh struktur molekul yang lebih baik. Dua sistem pelarut telah digunakan: model molekul air TIP3P dan model pelarut karbon tetraklorida ( $\text{CCl}_4$ ) dalam keadaan berkala bersempadan (PBC). Secara amnya, lipase yang menjalani penyerupaan dalam pelarut air adalah kurang menyimpang dari segi "root mean square deviation" (rmsd) berbanding dengan lipase yang menjalani penyerupaan dalam pelarut  $\text{CCl}_4$ .

Pergerakan kawasan penutup adalah penting untuk fungsi lipase samada di dalam air atau dalam persekitaran yang mengandungi air-lipid. Analisis dinamik kawasan penutup telah dilakukan dengan menggunakan fungsi pertalian masa dan fungsi polinomial Legendre urutan kedua. Lipase dalam air dan  $\text{CCl}_4$  menunjukkan sifat-sifat dinamik yang berbeza. Tanpa pengalkilan, fungsi pertalian masa lipase dalam air menunjukkan satu pereputan eksponen yang lambat dengan pertalian masa  $\tau = 92.8$  ps. Sebaliknya, kawasan penutup menunjukkan dinamik yang lebih rumit ketika



dilakukan penyerupaan dalam pelarut  $\text{CCl}_4$ . Kepadanan eksponen CRL terbuka dalam pelarut  $\text{CCl}_4$  menunjukkan dua pertalian masa yang berbeza: satu pergerakan cepat  $\tau_1 = 5.6$  ps dan satu pergerakan lambat  $\tau_2 = 163.8$  ps.

Dengan pengalkilan, beberapa peringkat ubah suai yang berbeza menunjukkan sifat-sifat dinamik kawasan penutup yang berbeza. Dalam  $\text{CCl}_4$ , kawasan penutup sangat stabil pada pengalkilan 95% dengan pergerakan lambat  $\tau_1 = 4.1$  ps dan  $\tau_2 = 577.8$  ps. Pergerakan lambat kawasan penutup juga dilihat pada pengalkilan 63% dengan  $\tau_1 = 2.9$  ps dan  $\tau_2 = 209.2$  ps dan pada pengalkilan 43% dengan  $\tau_1 = 3.4$  ps dan  $\tau_2 = 117.9$  ps. Dalam air, pengubahsuaian 43% dan 95% menunjukkan pergerakan yang serupa untuk lipase semula jadi, dengan satu pertalian masa yang lambat, masing-masing 142.8 ps dan 133.6 ps. Tetapi, pengubahsuaian 63% menunjukkan dinamik yang lebih rumit dengan dua nilai  $\tau$  berbeza yang menyerupai sifat-sifat dinamikanya dalam  $\text{CCl}_4$ .

Mekanisma baru tentang penguncian kawasan penutup yang menstabilkan bentuk terbuka lipase telah dilihat dalam penyerupaan lipase semula jadi dalam  $\text{CCl}_4$ , iaitu satu jambatan garam Lys85 dengan Asp284. Jambatan garam ini sangat stabil pada lipase semula jadi dalam  $\text{CCl}_4$  dengan jarak 3.3 Å berbanding lipase dalam air dengan jarak 15.25 Å. Pengalkilan 43% menyebabkan jambatan garam menjadi cacat dalam  $\text{CCl}_4$  dengan jarak 6.03

Å; tetapi pengubahsuaian 63% menstabilkan jambatan garam tersebut dengan jarak 3.88 Å dan pengubahsuaian 95% menunjukkan kesan penstabilan terbaik dengan jarak 3.19 Å.

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I certify that an Examination Committee met on 9<sup>th</sup> September 2004 to conduct the final examination of Bimo Ario Tejo on his Doctor of Philosophy thesis entitled "Reductive Alkylation of *Candida rugosa* Lipase: Structural Approaches" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree.

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## DECLARATION

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



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**BIMO ARIO TEJO**

Date :



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## LIST OF ABBREVIATIONS

BPTI	bovine pancreatic trypsin inhibitor
CD	circular dichroism
CRL	<i>Candida rugosa</i> lipase
DHFR	dihydrofolate reductase
GCL	<i>Geothricum candidum</i> lipase
HF	Hartree-Fock
MD	molecular dynamics
NMR	nuclear magnetic resonance
PDB	Protein Data Bank
PEG	polyethylene glycol
PME	Particle Mesh Ewald
RESP	restrained electrostatic potential
rmsd	root mean square deviation(s)
SASA	solvent accessible surface area
TNBS	trinitrobenzenesulfonate

