
**UNIVERSITI PUTRA MALAYSIA**

# EFFECT OF TRANSITION METAL ION SUBSTITUTION ON THERMOSTABLE T1 LIPASE ACTIVITY

**HARMIZA HARUN**

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# EFFECT OF TRANSITION METAL ION SUBSTITUTION ON THERMOSTABLE T1 LIPASE ACTIVITY

**By**

**HARMIZA HARUN**

**Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirement for the Degree of Master of Science**

**January 2013**

**Especially dedicated to my dearly beloved:**

**Husband,**

**Mohd Hafizuddin Bin Ruslan**

**Son,**

**Muhammad Hasif Danish Bin Mohd Hafizuddin**

**Parents,**

**Hj. Harun Bin Abu**

**Hjh. Zainab Binti Hj. Abdullah**

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

**EFFECT OF TRANSITION METAL ION SUBSTITUTION ON THERMOSTABLE T1 LIPASE ACTIVITY**

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**Chairman: Professor Mohd Basyaruddin Abdul Rahman, PhD**

**Faculty: Science**

This study involved the effect of transition metal ion removal and substitution on lipase activity and thermostability. The role of metal ions in the activity and stability of the enzyme was studied using the holoenzyme, apoenzyme and metal substituted enzyme. T1 lipase from *Geobacillus zalihae* contains a structural zinc ion ($Zn^{2+}$) that apparently contributes to the high temperature tolerance. This $Zn^{2+}$ ion is tightly bound to histidine (H81 and H87) in the catalytic domain and aspartate (D61 and D238) in the extra domain of T1 lipase. Initially, Molecular Dynamics (MD) simulation using YASARA software was performed at 343 K and pH 9 to determine the changes in the protein structure, dynamics and flexibility of T1 lipase affected due to $Zn^{2+}$ ion depletion. Interestingly, D238, H81 and H87 which also the $Zn^{2+}$ ion binding ligand, were found to be highly fluctuated in the absence of $Zn^{2+}$ ion which may have caused weaker intramolecular interaction of zinc ion-binding coordination. The stability of T1 lipase and apo-T1 lipase was estimated based on the Gibbs free energy (ΔG) analysis using FoldX software. Based on the ΔG value calculated, the stability of apo-T1 lipase was reduced to -53 kcal/mol as compared to T1 lipase, -65 kcal/mol. Hence, it is suggested that $Zn^{2+}$ ion contributes to the stability of T1 lipase

at elevated temperature. The role of the $Zn^{2+}$ ion was elucidated by its elimination from the structure of T1 lipase with two metal chelators such as, 2,6-pyridinedicarboxylic acid (2,6-PDCA) and N,N,N',N'-tetrakis-(2-pyridylmethyl) ethylenediamine (TPEN). Among them, the incubation of T1 lipase with TPEN removed almost up to 100 % of the $Zn^{2+}$ ion followed by 83 % removal by 2,6-PDCA. The specific activity of apo-T1 lipase was decreased (84 U/mg) as compared to T1 lipase (104 U/mg), thus confirming the role of $Zn^{2+}$ ion in the stability of T1 lipase. This data is supported by the details obtained in the thermostability study and half-life study of both lipases. T1 lipase has been modified by means of transition metal ion replacement in order to improve its enzymatic activity and protein stability at high temperature. Three potential transition metal ions such as $Mn^{2+}$ ion, $Pd^{2+}$ion, and ruthenium ion ($Ru^{3+}$) has been added in apo-T1 lipase via dialysis at 4 $^{o}$C. $Zn^{2+}$ ion in T1 lipase was successfully replaced by $Mn^{2+}$ ion and $Pd^{2+}$ ion with the ratio of 1 mol of protein to ~1 mol of transition metal ion. The specific activity of $Mn^{2+}$-T1 lipase and $Pd^{2+}$-T1 lipase was enhanced about 1.6 fold and 1.1 fold respectively. However, the substitution of T1 lipase with $Ru^{3+}$ was unsuccessful due to enzyme inactivation. The biochemical and biophysical characterization of T1 lipase and apo-T1 lipase was performed. Both lipases shared the same optimum temperature which is 70 $^{o}$C. However, the removal of $Zn^{2+}$ has reduced the thermostability and half live ($t_{1/2}$) of T1 lipase by 10 $^{o}$C and 20 minutes rescpectively. The fluorescence emission spectra showed the maximum intensity ($\lambda_{max}$) was red-shifted from 433 nm to 511 nm due to the elimination of $Zn^{2+}$ ion. This change ascribed the decrease of the exposure of hydrophobic cavity in apo-T1 lipase. In addition, the changes in the stability and activity of T1 lipase and apo-T1 lipase were investigated during denaturation by urea. An increased in the denaturant concentration has drastically induced inactivation and

unfolding of both lipases. In the absence of $Zn^{2+}$ ion, the maximal fluorescence emission spectrum of T1 lipase was red shifted to a maximum value of 355 nm. The effect of transition metal ion chelation and addition on the structure of T1 lipase was observed using Circular Dichroism (CD) based on the changes in the secondary structure components, such as, α-helix, β-sheet, turn and random coil. Fascinatingly, the addition of $Mn^{2+}$ ion and $Pd^{2+}$ ion into the apoenzyme increased the thermal denaturation ($T_m$) point based on the CD analysis. In conclusion, experimental analysis performed in this study highlighted the understanding of holding the catalytic and extra domains together with a metal ion can stabilize this lipase; changing the metal ion can increase this stability. This work sets the foundation for the design of T1 lipase as a metal ion biosensor.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

**KESAN PENUKAR GANTIAN ION LOGAM PERALIHAN TERHADAP AKTIVITI T1 LIPASE TERMOSTABIL**

Oleh

**HARMIZA HARUN**

**January 2013**

**Pengerusi: Professor Mohd Basyaruddin Abdul Rahman, PhD**

**Fakulti: Sains**

Kajian ini meliputi kesan penyingkiran dan penukargantian unsur-unsur peralihan logam ion terhadap aktiviti, kestabilan terma dan struktur konformasi lipase T1. Lipase T1 yang berasal daripada *Geobacillus zalihae* mempunyai satu struktur ion zink ($Zn^{2+}$) yang diperlukan untuk lipase ini bertoleransi terhadap suhu yang tinggi. Fungsi ion logam dalam aktiviti dan kestabilan enzim ini telah dikaji dengan menggunakan holoenzim, apoenzim dan enzim dengan ion logam yang telah digantikan. Ion $Zn^{2+}$ ini terikat dengan kuat kepada histidine (H81 dan H87) di dalam domain katalitik dan asid aspartik (D61 dan D238) di dalam domain tambahan. Pada mulanya, simulasi molekul dinamik (MD) telah dilakukan dengan menggunakan perisian YASARA pada suhu 343 K dan pH 9 untuk menentukan perubahan yang memberi kesan terhadap dinamik dan fleksibiliti protein lipase T1. Tanpa kehadiran ion $Zn^{2+}$, fluktuasi tinggi yang berlaku pada ligand $Zn^{2+}$ iaitu D238, H81 dan H87 mungkin mengakibatkan interaksi intramolekul yang lemah pada koordinasi ini. Kestabilan lipase T1 dan apo-lipase T1 telah diketahui berdasarkan analisis tenaga bebas Gibbs ($\Delta G$) dengan menggunakan perisian FoldX. Merujuk kepada nilai $\Delta G$ yang telah dikira, kestabilan apo-lipase T1 telah berkurang kepada -53 kcal/mol jika

dibandingkan dengan tenaga lipase T1 yang bernilai -65 kcal/mol. Oleh itu, ion $Zn^{2+}$ telah diramalkan menjadi faktor utama dalam menentukan kestabilan lipase T1 pada suhu tinggi. Fungsi ion $Zn^{2+}$ telah dikenalpasti melalui penyisihannya daripada struktur lipase T1 dengan menggunakan dua agen penyingkiran logam seperti 2,6-pyridinedicarboxylic acid (2,6-PDCA) dan N,N,N',N'-tetrakis-(2-pyridylmethyl) ethylenediamine (TPEN). Inkubasi lipase T1 dengan TPEN telah menyebabkan hampir 100 % ion $Zn^{2+}$ tersisih diikuti dengan pengurangan sebanyak 83 % ion $Zn^{2+}$ oleh 2,6-PDCA. Aktiviti spesifik bagi apo-lipase T1 telah berkurang kepada 84 U/mg jika dibandingkan dengan lipase T1 (104 U/mg). Dengan ini, peranan ion $Zn^{2+}$ di dalam memastikan sifat kestabilan terma lipase T1 telah disahkan. Data ini telah disokong oleh perincian yang telah dicapai daripada analisa kestabilan terma dan kestabilan suhu bagi kedua-dua lipase tersebut. Lipase T1 telah dimodifikasi melalui penukargantian logam ion peralihan bagi membaikpulih atau meningkatkan aktiviti enzim dan stabiliti protein pada suhu tinggi. Tiga logam peralihan yang berpotensi seperti ion $Mn^{2+}$, ion $Pd^{2+}$ dan ion $Ru^{3+}$ telah dipilih untuk dimasukkan ke dalam apo-lipase T1 melalui kaedah dialisis pada suhu 4 $^{o}$C. Ion $Zn^{2+}$ telah berjaya digantikan dengan ion $Mn^{2+}$ dan ion $Pd^{2+}$ dengan nisbah 1 mol protein kepada 1 mol logam ion peralihan. Lipase T1-$Mn^{2+}$ dan lipase T1-$Pd^{2+}$ menunjukkan peningkatan aktiviti spesifik sebanyak 1.6 dan 1.1 kali ganda masing-masing, berbanding lipase T1. Manakala, Ion $Ru^{3+}$ tidak berjaya ditukarganti ke dalam struktur lipase T1 disebabkan oleh penyahaktifan enzim. Pencirian sifat biokimia dan biofizik bagi lipase T1dan apoenzim (Lipase T1 tanpa ion $Zn^{2+}$) telah dikaji. Kedua-dua lipase mempunyai suhu optima yang sama, iaitu 70 $^{o}$C. Walaubagaimanapun, penyingkiran ion $Zn^{2+}$ telah menurunkan kestabilan terma dan $t_{1/2}$ bagi lipase T1 sebanyak 10 $^{o}$C dan 20 minit masing-masing. Seperti yang ditunjukkan oleh pembebasan spektra fluorescence,

penyisihan ion $Zn^{2+}$ telah menyebabkan intensiti maksima ($\lambda_{max}$) teralih dari 433 nm ke 511 nm. Perubahan ini menunjukkan pengurangan bagi keterbukaan bahagian hidrofobik apoenzim. Tambahan lagi, perubahan bagi kestabilan dan aktiviti lipase T1 and apo- lipase T1 telah dikenalpasti semasa denaturasi oleh urea. Peningkatan kepekatan agen denturasi telah menyahaktif dan membuka lipatan kedua-dua lipase dengan mendadak. Ketiadaan ion $Zn^{2+}$ telah menyebabkan spektra pembebasan pendaflour maksima bagi lipase T1 teralih kepada nilai yang tertinggi iaitu 355 nm. Kesan penyisihan dan penambahan logam ion peralihan kepada struktur lipase T1 dapat dilihat melalui perubahan dalam komponen struktur sekunder seperti, α-helix, β-sheet, lekukan beta (β-turn) dan gelung rawak. Analisa Circular Dichroism (CD) telah menunjukkan peningkatan titik denaturasi termal ($T_m$) dan kestabilan ($\Delta G$) apoenzim yang disebabkan oleh penambahan ion $Mn^{2+}$ dan ion $Pd^{2+}$. Kesimpulannya, analisa eksperimen yang telah dijalankan di dalam kajian ini dapat memperluaskan lagi pemahaman terhadap penyatuan domain katalitik dan ekstra dengan ion logam dapat menstabilkan lipase; pertukaran ion logam boleh meningkatkan kestabilan. Kajian ini menjadi asas kepada penciptaan lipase T1 sebagain biosensor ion logam.

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I certify that an Examination Committee met on 4 January 2013 to conduct the final examination of Harmiza Harun on her Master of Science thesis entitled "The Effect of Transition Metal Ions Substitution on The Activity of Thermostable T1 Lipase" In accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulation 1981. The Committee recommends that the candidate be awarded the Master of Science. Members of the Examination Committee are as follows:

**Nor Azowa Ibrahim, PhD**
Lecturer
Faculty of Science
Universiti Putra Malaysia
(Chairman)

**Tan Wee Tee, PhD**
Associate Professor
Faculty of Science
Universiti Putra Malaysia
(Internal Examiner)

**Mohd. Yunus Abd. Shukor, PhD**
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Internal Examiner)

**Awang Ahmad Sallehin Awang Husaini, PhD**
Associate Professor
Faculty of Resource Science Technology
Universiti Malaysia Sarawak
(External Examiner)

_____
**SEOW HENG FONG**
Professor/Deputy Dean
School of Graduate Studies
University Putra Malaysia

Date:

This thesis 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:

**Mohd Basyaruddin Abdul Rahman, PhD**
Professor
Faculty of Science
Universiti Putra Malaysia
(Chairman)

**Abu Bakar Salleh, PhD**
Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)

**Adam Leow Thean Chor, PhD**
Senior Lecturer
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)

**Romas J. Kazlauskas, PhD**
Professor
Biochemistry, Molecular Biology and Biophysics
University of Minnesota
(Member)



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**BUJANG BIN KIM HUAT, PhD**
Professor/Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

## 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 Universiti Putra Malaysia or other institutions.

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**HARMIZA HARUN**

Date: 4 January 2013

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