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UNIVERSITI PUTRA MALAYSIA

MOLECULAR DYNAMICS OF THERMOALKALOPHILIC LIPASES UNFOLDING AT HIGH TEMPERATURES

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By

ROGHAYEH ABEDI KARJIBAN

Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

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Abstract of the Thesis presented to the Senate of Universiti Putra Malaysia
in Fulfilment of the requirements for the degree of Doctor of Philosophy

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Chairman : Associate Professor Mohd Basyaruddin Abdul Rahman, PhD
Faculty : Science

The structure, dynamics and flexibility of thermoalkalophilic lipases of *Bacillus stearothermophilus* L1 (L1 lipase) and *Geobacillus zalihae* strain T1 (T1 lipase) were successfully explored through molecular dynamics simulation (MD) technique. MD simulations at extremely high temperature in explicit solvent were carried out to understand how a thermoalkalophilic lipase starts to unfold at high temperature. The simulations were performed at 400 K and 500 K in addition to a control simulation at 300 K for a total of 12.0 ns. The high stability of both global three-dimensional (3D) structures at control simulation was confirmed by a good correlation between crystallographic experimental and simulated B-factors.

The systematic flexibility and dynamics of both systems were analyzed using the time-averaged root mean square fluctuations (RMSf) and the root mean square deviations (C_α-RMSd). Both systems showed a very similar flexibility and dynamics at 300 K and 400 K while at 500 K, L1 lipase showed more flexibility than T1 lipase. The average RMSf and the C_α-RMSd results for both systems were in a good agreement, indicating that thermostability was correlated with higher flexibility rather than increased rigidity in our model systems.

Both L1 lipase and T1 lipase structures maintained their global 3D structures and did not undergo any significant unfolding process at 400 K, while both structures lost their structures partially at 500 K. The results clearly illustrated that the N-terminal moiety of both model systems showed high flexibility and dynamics during thermal unfolding simulations which preceded and followed by clear structural changes in two specific regions; the small extra domain (consisting of helices α3 and α5, strands β1 and β2, and connecting loops) and the main catalytic domain or core domain (consisting of helices α6- α9 and connecting loops which are located above the active site of the enzyme).

The two domains of both systems interact with each other through a Zn^{2+}-binding coordination with Asp61 and Asp238 from the core domain and His81 and His87 from the small domain via tight interactions. Interestingly, the His81 and His87 were among the highly fluctuated residues at high temperatures while Asp61 and Asp238 did not show any significant fluctuations. The results indicated that these tight interactions

became very weak at high temperatures which presumably contributed to the thermostability of both enzymes.

The results also suggested that the initial steps in the unfolding of a thermoalkalophilic lipase may involve early loss of structure in the small extra domains of these enzymes followed by core opening. Therefore, the N-terminal moiety and the small domain of both enzymes are critical regions to thermostability and they can be a potential target for stability enhancement.

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

DINAMIK MOLEKUL TERHADAP PENGURAIAN LIPASE TERMOALKALOFILIK PADA SUHU TINGGI

Oleh:

ROGHAYEH ABEDI KARJIBAN

September, 2008

Pengerusi: Profesor Madya Mohd Basyaruddin Abdul Rahman, PhD

Fakulti : Sains

Struktur, dinamik dan fleksibiliti bagi lipase termoalkalofilik *Bacillus stearothermophilus* L1 (Lipase L1) dan *Geobacillus zalihae* strain T1 (Lipase T1) telah berjaya diselidiki melalui teknik simulasi dinamik molekul (MD). Simulasi MD pada suhu yang sangat tinggi di dalam pelarut eksplisit telah dijalankan untuk memahami bagaimana lipase termoalkalofilik mula untuk terurai pada suhu tinggi. Simulasi telah dijalankan pada 400 K and 500 K sebagai tambahan kepada simulasi kawalan pada 300 K selama 12.0 ns. Kestabilan tinggi untuk kedua-dua struktur tiga-dimensi global pada simulasi kawalan telah disahkan melalui korelasi yang baik antara kristalografi dan simulasi faktor-B.

Kelenturan sistematik dan dinamik untuk kedua-dua sistem telah dianalisis menggunakan purata masa "fluktuasi puncamin kuasa dua" (RMSf) dan "sisihan min kuasa dua"(C_α-RMSd). Kedua-dua sistem menunjukkan persamaan dari segi kelenturan dan dinamik pada 300 K dan 400 K manakala pada 500 K, lipase L1 menunjukkan lebih lentur daripada lipase T1. Keputusan purata RMSf dan C_α-RMSd untuk kedua-dua sistem adalah dalam persetujuan yang baik, menunjukkan bahawa kestabilan terma berkait rapat dengan kelenturan tinggi berbanding dengan peningkatan ketegaran untuk model sistem ini.

Kedua-dua struktur lipase L1 dan lipase T1 mengekalkan struktur global tiga dimensi dan tidak melalui mana-mana proses peleraian yang ketara pada 400 K, manakala kedua-dua lipase kehilangan separa struktur asal masing-masing pada 500 K. Keputusan ini jelas menunjukkan bahawa moieti N-terminal untuk kedua-dua sistem mempunyai kelenturan dan dinamik yang tinggi semasa simulasi peleraian terma sebelum preubahan struktur. Ini diikuti dengan perubahan struktur yang jelas di dalam dua kawasan; di dalam domain kecil tambahan (terdiri daripada heliks α3 dan α5, jalur β1 dan β2 dan gelung penyambung) dan domain pemangkinan utama ataupun domain teras (terdiri daripada heliks α6-α9 dan lengkok bersambung yang terletak di atas tapak aktif enzim tersebut).

Dua domain tersebut bagi kedua-dua sistem bersaling tindak antara satu sama lain melalui koordinasi ikatan Zn^{2+} bersama Asp61 dan Asp238 daripada domain teras dan His81 dan His87 daripada domain kecil melalui saling tindahan kuat. Yang menariknya, His81 dan His87 adalah antara residu berkelenturan tinggi pada suhu tinggi manakala

Asp61 dan Asp238 tidak menunjukkan kelenturan yang ketara. Keputusan itu menandakan interaksi yang rapat menjadi terlalu lemah pada suhu tinggi yang mungkin menyumbang kepada kestabilan terma kedua-dua enzim.

Keputusan ini juga mencadangkan bahawa langkah awal dalam penguraian sesuatu lipase termoalkafilik mungkin melibatkan kehilangan struktur awal di dalam domain kecil tambahan dalam enzim tersebut dan diikuti dengan pembukaan teras. Oleh itu, moieti N-terminal dan domain kecil bagi kedua-dua enzim adalah kawasan kritikal untuk kestabilan terma dan berpotensi menjadi sasaran utama bagi meninggikan kestabilan kedua-dua enzim tersebut.

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I certify that an Examination Committee has met on 15 September 2008 to conduct the final examination of Roghayeh Abedi Karjiban on her Doctor of Philosophy thesis entitled "Molecular Dynamics Study of Thermoalkalophilic Lipases Unfolding at High Temperatures" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanain Malaysia (Higher Degree) Regulations 1981. The committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee are as follows:

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Date: 13 November 2008

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.

ROGHAYEH ABEDI KARJIBAN

Date:

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

3D	three-dimensional
BPTI	bovine pancreatic trypsin inhibitor
CD	circular dichroism
C_α-RMSd	backbone atom root mean square deviation
CI2	chemotrypsin inhibitor 2
C_p	heat capacity
ΔG	Gibbs free energy change
ΔH	enthalpy change
ΔS	entropy change
D	denatured state
DSSP	define secondary structure of the proteins
E	energy
F_i	force exerted on atom i
Hz	Hertz
i	atom
LGA	local-global alignment
m_i	mass of atom i
MD	molecular dynamics
N	number of atoms
NMR	nuclear magnetic resonance
NPT	isothermal-isobaric ensemble
*o*GNM	Gaussian network model
PBC	periodic boundary condition
PDB	protein data bank
PME	particle mesh Ewald
POME	palm oil mill effluent
PU	partially unfolded state
q	atomic charge
RESP	restrained electrostatic potential
r_i	position of atom i
r_c	cut-off radius
R_g	radius of gyration
RMS	root mean square
RMSd	root mean square deviation
RMSf	root mean square fluctuation
SASA	solvent accessible surface area
SGB	surface generalized Born
T	temperature
U	unfolded state
V	potential energy function

CHAPTER 1

INTRODUCTION

Comparative structural studies of enzymes have resulted in new insights into protein stability, protein folding and unfolding, and structure-function relationships (Karlstrom, 2006). In addition to the basic knowledge gained, this research area is important for a more detailed understanding and treatment of several diseases related to protein stability (Dobson, 2006), development of rational protein engineering, and biotechnological use of enzymes (Egorova & Antranikian, 2005; Eisenthal *et al.*, 2006).

Biocatalysts are catalysts of biological origin, which are very important, because they are highly specific, highly active under mild conditions and biodegradable (Polastro, 1989; Benkovic & Ballesteros, 1997). Thus, there is a strong tendency to replace conventional chemical reactions and develops new processes using this novel type of catalysts (Illanes, 2000). Despite the obvious advantages; biocatalysts are fragile molecules. Therefore, biocatalyst stability and stabilization is a central issue of biotechnology nowadays, especially at high temperatures (Adamczak & Krishna, 2004).

Protein stability is a key factor to determine the economic feasibility of applying an enzyme in an industrial process (Eijsink *et al.*, 2004). Thermostability allows a higher operation temperature, which is clearly advantageous because of a higher reactivity,

higher stability, higher process yield, lower viscosity and fewer contamination problems (Fields, 2001). There are many types of potentially stabilizing interactions in thermoenzymes. Identification and understanding of specific factors contributing to the thermostability of these organisms has been a longstanding challenge (Mozhaev, 1993; Querol *et al.*, 1996; Kumar *et al.*, 2000; Vieille & Zeikus, 2001; Beck *et al.,* 2006). However, no single preferred mechanism for stabilization of thermoenzymes has appeared (Fitter & Herble, 2000).

Computer simulation techniques have become very important tools for understanding and exploring the physical basis of the structure and function of biomacromolecules. In a theoretical study the objective is to create a simplified model of a real physical system in order to reproduce known structural changes and dynamics behaviour of the system under study. The application of computer simulation in the structural and dynamics studies of proteins and understanding the mechanisms of protein folding and unfolding at atomic details has been the subject of many research for several years (Karplus & Sali, 1995; Colombo, 2004; Daggett, 2006).

Molecular dynamics (MD) is a powerful computer simulation technique to investigate dynamics properties of a protein in atomic detail. Both simplified and all-atom level MD simulation are common computational methods in this area (Brooks, 1998). All-atom level MD simulations with high resolution in time and space are more preferred (Daggett, 2006). It gives a detailed comparison between energetic and structural properties of a protein at various temperatures and provides a large amount of information which is not

directly accessible from laboratory experiment (Mark & van Gunsteren, 1992; van Gunsteren & Mark, 1992; Beck & Daggett, 2004).

It has been shown that a small set of five to ten MD simulations are sufficient to capture the average properties of proteins (Day & Daggett, 2005). The early view of unfolded proteins as extended polypeptide chains has changed into a more complex picture. The current view of an unfolded state is an ensemble of partially folded conformers of the protein, where the extent of unfolding depends on the denaturing conditions (Floriano *et al.*, 2007). Proteins begin to unfold because of the increased intramolecular motions caused by increasing temperature (Liu & Wang, 2003). In order to simulate unfolding of a protein, we performed the MD simulations at high temperatures for two reasons. First, there is a large difference between the experimental timescale for folding that can be achieved with available computer power. Secondly, unfolding occurs from the best characterized state of a protein, the native state (Fersht & Daggett, 2002).

The overall unfolding pathway does not change due to high temperatures and use of high temperatures will just speed up the kinetics (Day *et al.*, 2002; Marianyaganam & Jackson, 2004; Daggett, 2006). Since the overall pathway of unfolding is independent of temperature, high-temperature MD simulations give an accelerated but still relevant view of the unfolding process (Larios *et al.*, 2004). Some of the well studied systems include barnase (Caflisch & Karplus, 1995), chymotrypsin inhibitor 2 (Li & Daggett, 1996), src SH3 domain (Tsai *et al.*, 1999), *Staphylococcal* protein A (Alonso & Daggett, 2000), EcDHFR (Sham *et al.*, 2002), and SNase (Smolin & Winter, 2006).