UNIVERSITI PUTRA MALAYSIA

CRYSTALLOGRAPHIC ANALYSIS OF GROUND AND SPACE-GROWN THERMOSTABLE T1 LIPASE CRYSTAL

SAYANGKU NOR ARIATI BT MOHAMAD ARIS

FBSB 2013 17

CRYSTALLOGRAPHIC ANALYSIS OF GROUND- AND SPACE-GROWN THERMOSTABLE T1 LIPASE CRYSTAL

SAYANGKU NOR ARIATI BT MOHAMAD ARIS

MASTER OF SCIENCE
UNIVERSITI PUTRA MALAYSIA

2013

CRYSTALLOGRAPHIC ANALYSIS OF GROUND- AND SPACE-GROWN THERMOSTABLE T1 LIPASE CRYSTAL

By

SAYANGKU NOR ARIATI BT MOHAMAD ARIS

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Master of Science

May 2013

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

CRYSTALLOGRAPHIC ANALYSIS OF GROUND- AND SPACE-GROWN THERMOSTABLE T1 LIPASE CRYSTAL

By

SAYANGKU NOR ARIATI BT MOHAMAD ARIS

May 2013

Chair: Professor Raja Noor Zaliha Raja Abd Rahman, D.Eng.

Faculty: Biotechnology and Biomolecular Sciences

X-ray crystallography is a major tool to provide three dimensional structures. High resolution protein crystal data is important in revealing a highly accurate structure at atomic level. This is essential in order to understand the properties and function of the protein. In crystallization process, convection and sedimentation is thought to be detrimental for growth of high quality protein crystals. Both aspects can be avoided by either working in gelled systems, working in systems of small dimensions, or in the absence of gravity. In this research, crystallization by using counter diffusion method in space was performed with the aim to obtain high resolution diffracting crystals with better internal order to improve the accuracy of the structure.

Recombinant T1 Lipase from *Geobacillus zalihae* was successfully purified to homogeneity via two step affinity chromatography followed by ion exchange chromatography with 8.9 % yield. Purified T1 lipase was then crystallized in microbatch and vapour diffusion methods. The best crystal growth was obtained in Formulation 21 (Crystal Screen 2) with optimum growth temperature of 20 °C. The crystallization set up condition was applied to counter diffusion method for space experiment as well as ground control. Crystallization of T1 lipase under microgravity condition experiment was done in collaboration with JAXA (Japanese Aerospace Exploration Agency) under the JAXA-UPM Protein Crystal Growth (PCG) #2 Flight 2 program. The synchrotron diffraction data set were collected to 1.3 Å and 1.1 Å resolutions and belonged to monoclinic *C2* Space group for ground grown and space grown crystal, respectively.

The major difference between the two crystal growth systems is the lack of convection and sedimentation in microgravity environment resulted in the growth of much higher quality crystals of T1 lipase. The structural analysis of T1 lipase was performed using molecular replacement method with final R factor 0.134 (R_{free} 0.162) for ground-grown crystal and 0.129 (R_{free} 0.150) for space-grown crystal, respectively. The structure of T1 lipase crystal contains two molecules per asymmetric unit with 387 amino acids each. Overall topology showed of α/β hydrolase fold containing catalytic triad active site and covered by α-helix 6 and α-helix 7 as a 'lid'. The structure contained zinc and calcium binding site which was important for the structural stabilization. Comparative crystallographic analysis revealed that the space-grown crystal structure has improved as compared to ground-

grown crystal. This study has shown that crystallization in counter diffusion method using microgravity environment improved the internal order of crystals thus gave a more precise three dimensional structure.

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

ANALISIS KRISTALOGRAFI TERHADAP LIPASE TERMOSTABIL T1 DI BUMI DAN ANGKASA

Oleh

SAYANGKU NOR ARIATI BT MOHAMAD ARIS

Mei 2013

Pengerusi: Professor Raja Noor Zaliha Raja Abd Rahman, D.Eng.

Fakulti: Bioteknologi dan Sains Biomolekul

Kristalografi sinar-X adalah alat utama dalam memberikan struktur tiga dimensi. Data dari hablur protein yang beresolusi tinggi adalah penting untuk mendedahkan struktur yang lebih tepat pada tahap atom. Ini adalah penting dalam memahami ciri-ciri dan fungsi protein. Perolakan dan pemendapan dikatakan memudaratkan dalam pertumbuhan hablur berkualiti tinggi. Kedua-dua aspek tersebut boleh diatasi samada dengan melakukan proses penghabluran di dalam sistem gel, di dalam dimensi yang kecil atau keadaan ketiadaan graviti. Di dalam kajian ini, penghabluran menggunakan kaedah resapan lawan di angkasa telah dijalankan bertujuan untuk memperolehi hablur yang mempunyai susunan dalaman yang lebih teratur untuk mendapatkan ketepatan struktur.

T1 lipase rekombinan dari *Geobacillus zalihae* telah berjaya ditulenkan melalui dua kaedah kromatografi affiniti diikuti dengan kromatografi pertukaran ion dengan peratus perolehan 8.9 %. T1 lipase tulen kemudiannya dihablurkan dengan kaedah 'microbatch' dan difusi wap. Pertumbuhan hablur yang terbaik diperolehi dari formulasi 21 (Crystal Screen 2) dengan suhu optimum pada 20°C. Kondisi terbaik penghabluran diaplikasikan kepada kaedah resapan lawan untuk eksperimen di angkasa dan eksperimen di bumi sebagai kawalan. Kajian penghablurlan T1 lipase di bawah persekitaran mikrograviti telah dijalankan dengan kolaborasi pihak JAXA (Agensi Angkasa Jepun) di bawah program JAXA-UPM Pertumbuhan Penghabluran Protein (PCG) #2 penerbangan kedua. Set data pembelauan oleh Synchrotron dikumpulkan sehingga resolusi 1.3 Å dan 1.1 Å dan tergolong dalam kumpulan ruang *C2* monoklinik untuk hablur yang tumbuh di bumi dan angkasa setiap satunya.

Perbezaan utama antara dua sistem pertumbuhan hablur adalah pengurangan perolakan dan pemendapan pada keadaan persekitaran kekurangan mikrograviti menghasilkan hablur T1 lipase yang lebih berkualiti tinggi. Hablur yang tumbuh di angkasa mempamerkan darjah ketidak aturan yang lebih rendah berbanding dengan hablur yang tumbuh di bumi dan data-data pembelauannya juga mempunyai nilai faktor-B yang lebih rendah. Analisis struktur T1 lipase dilakukan dengan menggunakan kaedah penggantian molekul dengan nilai akhir faktor-R 0.134 (R_{free} 0.162) untuk hablur yang tumbuh di bumi dan 0.129 (R_{free} 0.150) untuk hablur yang tumbuh di angkasa. Struktur T1 lipase mengandungi dua molekul di setiap unit asimetri dengan mempunyai 387 asid amino setiap satunya. Topologi keseluruhan menunjukkan lipatan α/β hidrolase mengandungi triad katalitik sebagai tapak aktif

diliputi dengan α-helix 6 dan α-helix 7 sebagai penutup. Struktur juga mengandungi tapak mengikat untuk zink dan kalsium yang mana ianya penting untuk penstabilan struktur. Analisis perbandingan kristalografi menunjukkan bahawa struktur hablur yang tumbuh di angkasa adalah lebih baik berbanding dengan yang tumbuh di bumi. Kajian ini menunjukkan penghabluran menggunakan kaedah resapan lawan dengan menggunakan keadaan mikrograviti telah memperbaiki susunan dalaman hablur dan telah menghasilkan struktur tiga dimensi protein yang lebih tepat.

ACKNOWLEDGEMENTS

First and foremost, all praises to Allah for the strengths and His blessings in completing this thesis. Special appreciation goes to my supervisor, Prof. Dr. Raja Noor Zaliha Raja Abd. Rahman, for her supervision and constant support. Her endless encouragement and personal guidance have provided a good basis for the present thesis.

I am deeply grateful to my co-supervisors, Prof. Mahiran Basri and Dr. Adam Leow Thean Chor for their invaluable guidance, encouragement, help and patient through the course of this thesis. Special thanks to Dr. Mohd Shukuri Mohamad Ali for his contributions in helpful discussions and his knowledge. Special thanks are also extended to Dr Koji Inaka, Prof. Yoshiki Higuchi and the staffs of the JAXA for their help and cooperation in data collection.

Sincere thanks to all my friends especially Ana, Maya, Dayana, Miza, Fiza, Dina, Arilla, Menega, Azmir and other past and present members of the Enzyme and Microbial Technology Lab (EMTECH). I will always be grateful for their support, the get-togethers, the friendly working atmosphere, and for all of the other helps that they gave me along the way. I am also very thankful to Nischal and Mei Yuan, who supported me in the good times and cheered me up in the bad times. Thanks for the friendship and memories.

I am eternally grateful to my beloved parents Mr. Mohamad Aris Mat Lazim and Mrs. Hatimah Bakar for their endless love, prayers and encouragement. I strongly believe that their prayers played very important role in my life. To those who

indirectly contributed in this research, your kindness means a lot to me. Thank you very much.

I certify that a Thesis Examination Committee has met on 30th May 2013 to conduct the final examination of Sayangku Nor Ariati Bt Mohamad Aris on her thesis entitled "Crystallographic Analysis of Ground and Space Thermostable T1 lipase Crystal" 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.

Members of the Examination Committee were as follows:

Norazizah Shafee, Ph.D.
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

Mohd Arif Syed, Ph.D.
Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Internal Examiner)

Sieo Chin Chin, Ph.D.
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Internal Examiner)

Mohammed Razip Samian, Ph.D.
Professor
School of Biological Sciences
Universiti Sains Malaysia
(External Examiner)

NORITAH OMAR, PhD
Assoc. Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 16 August 2013

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Masters of Science. The members of the Supervisory Committee were as follows:

Raja Noor Zaliha Raja Abd. Rahman, PhD
Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

Mahiran Basri, PhD
Professor
Faculty of Science
Universiti Putra Malaysia
(Member)

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

_____
BUJANG BIN KIM HUAT, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

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 institution.

_____

SAYANGKU NOR ARIATI BT MOHAMAD ARIS

Date : 30 May 2013

TABLE OF CONTENTS