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CRYSTALLIZATION OF NOVEL ARM LIPASE AND ELUCIDATION OF ITS SPACE-GROWN CRYSTAL STRUCTURE

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CRYSTALLIZATION OF NOVEL *ARM* LIPASE AND ELUCIDATION OF ITS SPACE-GROWN CRYSTAL STRUCTURE

By

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CRYSTALLIZATION OF NOVEL *ARM* LIPASE AND ELUCIDATION OF ITS SPACE-GROWN CRYSTAL STRUCTURE

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March 2013

Chairman: **Professor Raja Noor Zaliha Raja Abdul Rahman, D.Eng**

Faculty: **Biotechnology and Biomolecular Science**

The three-dimensional structure of novel thermostable and organic solvent tolerant ARM lipases was successfully determined by X-ray crystallography technique. The novel ARM lipase was highly purified prior to crystallization and consequently, the crystal structure of ARM lipase was elucidated in order to comprehend its structure-function relationship.

The His-tagged ARM lipase was purified using immobilized metal affinity chromatography followed by anion-exchange chromatography. The highly purified and homogeneous ARM lipase with protein concentration of approximately 2 mg/mL was successfully crystallized by sitting drop, vapour diffusion method using 0.1 M MES monohydrate pH 6.5 and 12% (v/v) polyethylene glycol (PEG) 20000 as precipitant. Optimization of the crystallization conditions was performed by varying the pH and concentration of the precipitant. The optimum crystallization condition

was 2 mg/mL ARM lipase in 0.1 M Tris-HCl, 0.15 M NaCl, pH 8.0 protein solution, crystallized using 0.1 M Tris-HCl, pH 8.0 and 12% (v/v) PEG 20000 as precipitant.

In addition, the crystal growth of ARM lipase was also improved via counter diffusion method and microgravity experiment. Crystals grew in the gel-tube capillaries that are incubated in Protein Crystallization Research Facility on board the International Space Station for over three months in 2011. The space-grown crystal obtained was diffracted and data was collected at synchrotron radiation facility. The data was processed up to 2.3 Å resolution and the crystal belonged to primitive monoclinic P21 space group with the unit cell dimension of a= 55.79 Å, b=143.40 Å, c=63.97 Å, α=γ=90.00° and β= 105.88°.

Crystal structure of ARM lipase showed the typical, canonical alpha-beta hydrolase fold consisting of 13 α-helices and 11 β-strands. The conserved catalytic triad, composed of serine 113, histidine 358 and aspartic acid 317 was found in the hydrophobic active site. Three-dimensional structure features such as zinc- and calcium-binding sites, high percentage of charged, aromatic and proline residues presence in the protein, as well as high percentage of surface-exposed charged, hydrophobic and glycine residues explains the properties of ARM lipase as a thermostable, organic solvent- stable lipase.

In conclusion, the successful crystallization and structure elucidation of novel thermostable, organic solvent-tolerant lipase, ARM gives an understanding of the properties of this enzyme. Information regarding the structural features and

adaptations of this lipase also gives useful insight for the engineering of better, novel lipases with enhanced and desired properties.

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

PENGHABLURAN LIPASE *ARM* DAN PENERANGAN STRUKTUR KRISTAL YANG DIHASILKAN DI ANGKASA

Oleh

NUR DAYANA NISBAR

Mac 2013

Pengerusi: **Profesor Raja Noor Zaliha Raja Abdul Rahman, D.Eng**

Fakulti: **Bioteknologi dan Sains Biomolekul**

Struktur tiga dimensi enzim lipase ARM yang merupakan enzim lipase yang termostabil dan stabil di dalam pelarut organik telah berjaya ditentukan melalui teknik kristalografi sinar X. Penulenan enzim lipase ARM dilakukan sebelum proses penghabluran dan ini diikuti oleh penyelesaian struktur enzim lipase ARM bagi menjelaskan sifat enzim tersebut.

Enzim lipase ARM yang mengandungi tag enam Histidina telah ditulenkan melalui kromatografi turus afiniti dan diikuti oleh kromatografi pertukaran cas anion. Enzim lipase ARM yang homogeniti dan berketulenan tinggi dengan kepekatan protein kira-kira 2 mg/mL telah berjaya dihablurkan melalui kaedah penghabluran resapan wap "sitting drop" menggunakan 0.1 M MES monohidrat pH 6.5 dan 12% (v/v) polietilena glikol (PEG) 20000 sebagai pemendak. Kondisi penghabluran telah dioptimumkan dengan mempelbagaikan pH dan kepekatan pemendak. Kondisi penghabluran yang optimum adalah 2 mg/mL lipase ARM dalam larutan protein, 0.1

M Tris-HCl, 0.15 M NaCl, pH 8.0, dihablurkan menggunakan 0.1 M Tris-HCl, pH 8.0 dan 12% (v/v) polietilena glikol PEG 20000 sebagai pemendak.

Seterusnya, pembentukan hablur enzim lipase ARM yang lebih baik telah dihasilkan di dalam kapilari gel melalui kaedah penghabluran resapan lawan di persekitaran mikrograviti menggunakan 0.1 M MES monohidrat pH 6.5 dan 12% (v/v) polietilena glikol (PEG) 20000 sebagai pemendak. Hablur lipase ARM yang dihasilkan di angkasa dibelau dan data belauan telah dikumpulkan di sebuah fasiliti sinaran sinkrotron di Jepun. Data belauan tersebut diproses pada resolusi maksima 2.3 Å dan hablur tersebut dikategorikan dalam kumpulan ruang primitif monoklinik P21 dengan dimensi unit sel, a= 55.79 Å, b=143.40 Å, c=63.97 Å, α=γ=90.00° dan β= 105.88°.

Struktur hablur enzim lipase ARM menunjukkan organisasi lipatan konikal α/β hidrolase yang mengandungi 13 α-heliks dan 11 β-bebenang. Triad pemangkin terpelihara yang terdiri daripada serina 113, histidina 358 dan asid aspartic 317 didapati di tapak aktif yang berpersekitaran hidrofobik. Struktur tiga dimensi enzim lipase ARM mempamerkan ciri-ciri seperti tapak ikatan logam ion zink dan kalsium, peratusan tinggi asid amino prolina, bercaj dan aromatik di dalam protein serta peratusan tinggi asid amino glisina dan hidrofobik yang berada di permukaan protein. Ciri-ciri struktur enzim lipase ARM ini menjelaskan sifat lipase ARM yang termostabil dan stabil di dalam pelarut organik.

Konklusinya, penghabluran dan elusidasi struktur enzim lipase ARM yang termostabil dan stabil di dalam pelarut organik telah meningkatkan pemahaman penyelidik tentang sifat enzim ini. Informasi berkenaan ciri-ciri struktur tiga dimensi dan adaptasi struktur lipase ini juga memberi manfaat kepada penghasilan lipase yang baharu dan berfungsi dengan lebih baik.

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I certify that a Thesis Examination Committee has met on **22 March 2013** to conduct the final examination of **Nur Dayana Nisbar** on her thesis entitled "**Crystallization of Novel *ARM* lipase and Elucidation of its Space-grown Crystal Structure**" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1988. The Committee recommends that the student be awarded the **Master of Science (with Thesis)** degree.

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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 Master. The members of the Supervisory Committee were as follows:

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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.

NUR DAYANA NISBAR

Date: 22 March 2013

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