UNIVERSITI PUTRA MALAYSIA

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MASTER OF SCIENCE

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SIGNIFICANCE OF L2 LIPASE N-TERMINAL RESIDUE IN MAINTAINING PROTEIN INTEGRITY

By

NUR HANA MD JELAS

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

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SIGNIFICANCE OF L2 LIPASE N-TERMINAL RESIDUE IN MAINTAINING PROTEIN INTEGRITY

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Chairman : Professor Abu Bakar Salleh, PhD

Faculty : Faculty of Biotechnology and Biomolecular Sciences

To understand the function of amino acid residue at lipase N-terminal end, substitution of the second amino acid in L2 lipase, Serine, to Phenylalanine was carried out. Phenylalanine is a very hydrophobic amino acid compared to Serine. Substituting amino acid of a protein with other amino acids that have opposite properties are known to cause major changes towards the characteristics of the protein if the residue holds an important function in the protein. General biochemical characteristics of mutated L2 lipase were studied and compared to the recombinant wild-type to determine the role of L2 lipase N-terminal residue.

Mutated L2 lipase was created by extracting and purifying genomic DNA from thermophilic lipolytic *Bacillus* sp. L2. The purified genomic DNA was used as the template to amplify L2 lipase gene. Two degenerate primers, FT and RT were designed for the gene amplification. During the process, site-directed mutation of S2F was

introduced at the N-terminal of the protein. The mutated gene was ligated into pTrcHis vector and cloned into *Escherichia coli* DH5 alpha.

The recombinant gene was expressed using 0.0 5mM IPTG at the late log phase of cell growth stage, OD_{600nm} ~0.75. The cell was harvested and lysed to obtain the expressed protein. The protein was purified using one step affinity chromatography. Recombinant wild-type L2 lipase worked optimally at 70°C while mutated L2 lipase showed only minute activity when assayed at 70°C. Instead, the mutant was found to be working optimally at 55°C. In addition, thermostability of mutated L2 lipase was also reduced to 45°C, compared to 60°C for wild-type when incubation was carried out for more than 60 min.

The optimum pH for activity and stability of L2 lipase were also changed. Wild-type L2 lipase worked optimally and possessed highest stability at pH 9 while mutated L2 lipase worked optimally at pH 7 and maintained the best stability in solution with pH 10. Despite the decrease in thermostability and changes in optimum pH of the mutated L2 lipase, specific activity of the purified protein was found to be at 2043.14 U/mg. This is much higher compared to the recombinant wild-type which has a specific activity of 458 U/mg.

Based on the nucleotide sequence of the wild-type L2 lipase, no other unintentional mutation had occurred within the newly cloned gene. Hereafter, any difference observed in the characteristics of the mutated L2 lipase which are different compared to the wild-type, it is deemed to be caused by the site-directed mutation of S2F. The effect of S2F

mutation towards the biochemical characteristics of L2 lipase such as characteristics of the cofactor of the enzyme, substrate preferences, positional specificity, and the effect of surfactant and inhibitor towards the activity of the enzyme were studied to determine the potency of the mutation towards the general characteristics of the L2 lipase. It was later found that these properties were the same as the wild-type.

Due to the similarity of the above properties, protein thermal melting analysis was carried out to verify the mutated L2 lipase structure integrity. The analysis confirmed the changes in L2 lipase thermostability. This study supports the theory that the point where the mutation happened has caused alteration of the surface of the proteins. The protein lost the integrity of its surface structure which caused the thermostability of the protein to change. The effect is also confirmed by the altered pH for optimum activity and stability of L2 lipase which occur due to the changes in the surface ionic charges of the protein. The loss however is seen to be compensated by the increase in the specific activity of the protein. Increase in L2 lipase specific activity by the mutation is however inconclusive.

From this study, it was found that the ‘tail’ structure at the N-terminal residues of L2 lipase actually played a very important role in maintaining the integrity of the protein. Site-directed mutation at one site of the amino acids in the N-terminal residues results in a loss of protein integrity which is seen as a decrease in the protein thermostability. Interestingly, the loose protein structure seemed to allow the substrates to have an easier access to the active site of the protein which resulted in an increase in the specific activity of the protein.

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

PERANAN RESIDU N-TERMINAL LIPASE L2 DALAM MENGEKALKAN INTEGRITI PROTIN

Oleh

NUR HANA MD JELAS

Disember 2010

Pengerusi: Profesor Abu Bakar Salleh, PhD

Fakulti : Fakulti Bioteknologi dan Sains Biomolekul

Bagi memahami fungsi residu asid amino di hujung N-terminal lipase, penggantian amino asid yang kedua dalam lipase L2, Serine, kepada Phenylalanine dilakukan. Phenylalanine adalah asid amino yang sangat hidrofobik berbanding Serine. Penggantian asid amino yang bertentangan sifat diketahui dapat menyebabkan perubahan ketara terhdap ciri-ciri protin sekiranya residu tersebut memainkan peranan yang penting dalam struktur tersebut. Ciri-ciri biokimia lipase L2 termutasi dikaji dan dibandingkan dengan lipase L2 rekombinan yang asal untuk menentukan peranan residu pada N-terminal lipase L2.

Lipase L2 ternutasi dihasilkan melalui pengekstrakan dan penulinan DNA genomik daripada *Bacillus* sp. L2 yang mempunyai sifat termofilik dan lipolitik. DNA genomik yang ditulinkan itu digunakan sebagai acuan untuk penggandaan gen lipase L2. Dua primer degenerate, FT dan RT direka untuk membantu proses penggandaan gen. Mutasi terarah S2F pada N-terminal terhasil semasa proses penggandaan gen. Gen termutasi lipase L2 disatukan dengan vektor pTrcHis dan diklonkan ke dalam *Escherichia coli* DH5 alpha.

Ekspresi gen dilakukan menggunakan 0.05 mM IPTG pada fasa log akhir perkembangan sel dengan bacaan OD_{600nm}~0.75. Sel dituai dan dilisiskan untuk memperolehi protin yang diekspreskan. Proses penulinan protin dilakukan menggunakan afiniti kromatografi. Lipase L2 yang asal bekerja pada tahap optima pada suhu 70°C sementara lipase L2 termutasi hanya menunjukkan sedikit aktiviti apabila diuji pada suhu 70°C. Sebaliknya, lipase L2 termutasi mempunyai suhu optima pada 55°C. Selain itu, ketahanan terhadap haba lipase L2 termutasi juga berkurangan sehingga 45°C, berbanding 60°C bagi lipase L2 yang asal apabila enzim dibiarkan selama 60 min pada suhu tersebut.

pH optimum untuk aktiviti dan kestabilan protin juga berubah. Lipase L2 yang asal aktif dah stabil pada pH 9 sementara lipase L2 termutasi aktif pada pH 7 dan stabil pada pH 10. Walaupun pengurangan kestabilan terhadap haba dan perubahan pH optimum berlaku selepas lipase L2 dimutasi, aktiviti spesifik protin yang ditulinkan adalah 2043.14 U/mg. Ini adalah sangat tinggi berbanding lipase L2 yang asal yang mempunyai aktiviti spesifik sebanyak 458 U/mg.

Berdasarkan jujukan nukleotida lipase L2 yang asal, tiada perubahan selain yang dikehendaki telah berlaku dalam gen yang baru diklonkan itu. Oleh itu, setiap perubahan yang dilihat dalam cirri-ciri lipase L2 termutasi berbanding lipase L2 yang asal, dianggap adalah disebabkan oleh mutasi S2F. Kesan mutasi S2F terhadap cirri-ciri biokimia lipase L2 seperti kofaktor enzim, substrat pilihan, kedudukan khusus ikatan ester dalam substrat, dan kesan kehadiran surfaktant dan inhibitor terhadap aktiviti enzim dikaji begi menentukan potensi mutasi tersebut terhadap ciri-ciri lipase L2. Sifat-sifat ini kemudiannya didapati sama dengan lipase yang asal.

Disebabkan persamaan sifat-sifat di atas, analisis pencairan haba protin dilakukan bagi mengkaji integriti struktur lipase L2 termutasi. Analisis tersebut mengeshkan perubahan yang berlaku terhadap kestabilan haba yang dimiliki lipase L2. Kajian ini menyokong teori bahawa titik di mana mutasi tersebut berlaku telah menyebabkan perubahan struktur permukaan protin. Protin termutasi itu telah kehilangan integriti struktur permukaan yang menyebabkan perubahan terhadap kestabilan haba protin. Walaubagaimanapun, kehilangan tersebut dilihat sebagai diganti dengan kenaikan dalam aktiviti spesifik protin. Tetapi penggandaan aktiviti tersebut adalah tidak begitu meyakinkan tanpa kajian kinetik enzim untuk mengesahkan kesahihannya.

Daripada kajian ini, struktur seperti ‘ekor’ pada residu N-terminal lipase L2 sebenarnya memainkan peranan yang sangat penting dalam mengekalkan integriti protin. Mutasi pada titik tertentu pada salah satu asid amino pada residu di N-terminal menyebabkan kehilangan integriti protin yang dapat dilihat sebagai penurunan dalam kestabilan termal protin. Sungguhpun begitu, struktur protin yang lemah dilihat telah membenarkan lebih banyak substrat dapat memasuki tapak aktif protin. Ini dilihat sebagai kenaikan dalam aktiviti spesifik protin.

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I certify that an Examination Committee has met on **8 December 2010** to conduct the final examination of **Nur Hana Md Jelas** on her **Master of Science** thesis entitled "S**ignificance of L2 lipase N-terminal residue in maintaining protein integrity"** 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.

Members of the Examination Committee are as follows:

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

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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 other institutions.

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NUR HANA MD JELAS

Date: 8 December 2010

TABLE OF CONTENTS