UNIVERSITI PUTRA MALAYSIA

PURIFICATION OF AND FACTORS AFFECTING 5'-PHOSPHODIESTERASE FROM GERMINATING ADZUKI (*Vigna angularis* L.) BEAN

PUI LIEW PHING

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By

PUI LIEW PHING

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

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia
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PURIFICATION OF AND FACTORS AFFECTING 5'-PHOSPHODIESTERASE FROM GERMINATING ADZUKI (*Vigna angularis* L.) BEAN

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Chair: Prof. Hasanah Mohd. Ghazali, PhD

Faculty: Faculty of Food Science and Technology

5'-Phosphodiesterase (5'-PDE) is an enzyme that hydrolyses RNA to form 5'-inosine monophosphate (5'-IMP) and 5'-guanosine monophosphate (5'-GMP) which function as flavour enhancers. The best producer of 5'-PDE was selected from germinated seeds, namely mung bean (*Vigna radiate*), soybean (*Glycine max*), adzuki bean (*Vigna angularis* L.), chick pea (*Cicer arietinum*), black eye pea (*Vigna unguiculata*) and petai (*Parkia speciosa*). In order to ensure there is no contamination during germination, the effects of different surface sterilizing treatments were examined. Sodium hypochlorite at 0.3% (v/v) concentration was able to inhibit mold growth in adzuki bean, soybean and chickpea. Only 0.1% (v/v) sodium hypochlorite was needed to inhibit mold growth in black eye pea and petai, while mung bean required 0.05% (v/v) sodium hypochlorite to inhibit mold growth. 5'-PDE activity was determined using thymidine 5'-monophosphate p-nitrophenyl ester as substrate at pH 7.0 and 55°C. The formation of nucleotide monophosphates, the products of reaction, was determined at 405 nm. As a strong presence of phosphomonoesterase (PME) will reduce the yield of nucleotide

monophosphates as the enzyme hydrolyzes these products into nucleosides and orthophosphate, PME activity was also determined using p-nitrophenyl phosphate as the substrate at 60°C and pH 5.0. Adzuki bean was found to have the highest 5'-PDE activity when germinated for 15 days.

5'-PDE has an optimum pH and temperature of 8.5 and 80°C, respectively. It was stable between pH 7.0 - 8.5. A higher stability was observed at pH 7.5 compared to pH 8.5, and at 60 and 65°C, the enzyme still possessed good catalytic function even after 4 days of incubation. EDTA, Triton X-100 and sodium dodecyl sulfate (SDS) strongly inhibited 5'-PDE activity (inhibition ranged from 75.2-99.4 %), while Tween 80 and Tween 20 were only slightly inhibitory. The enzyme was activated by Ca^{2+} up to 249%, K^{+}, Mg^{2+} and Li^{+}, when the final concentration of the cations was 10 mM. On the other hand, Na^{+}, Zn^{2+}, Ni^{+}, Hg^{+}, Cu^{2+}, $Pb^{2+,}$ Fe^{2+}, $Al^{3+,}$ Ba^{2+} and Co^{2+} at the same final concentration were inhibitory. 5'-PDE activity increased with an increase in the concentration of Li^{+}, Na^{+} and Mg^{2+}, until it reaches its maximum at 6 mM followed by a decrease in activity at concentrations beyond 6 mM. The presence of Ca^{2+} in the reaction mixture resulted in a similar trend, but the difference is that maximum activation occurred at 4 mM. K^{+}, however, increased the activity of 5'-PDE from 0-10 mM. Al^{3+} decreased the activity of 5'-PDE activity as the concentration of the cation was increased. Thus, it is suggested that 5'-PDE from adzuki bean is a metallo-enzyme.

The yield of 5'-PDE after the four purification steps (acetone precipitation, desalting, anion-exchange chromatography and gel filtration chromatography) was 13.37% with a purification fold of 6.7 and specific enzyme activity of 35.2 µmol p-nitrophenol/min/mg

protein. The molecular weight of 5'-PDE using gel filtration chromatography was estimated at 125 kDa. Native PAGE analysis showed one major protein band, while SDS-PAGE estimated the molecular weight of the enzyme to be 124 kDa and indicated that the active/intact enzyme may be composed of two identical polypeptide chains (subunits) with a molecular weight of 62 kDa each. In conclusion, although 5'-PDE from adzuki bean has a high temperature optimum of 80 ^{o}C, the enzyme is more stable at 60^{o}C, different cations affected the activity of the enzyme differently and the purified 5'-PDE has an estimated molecular weight of 124-125 kDa.

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

PENULENAN DAN FAKTOR YANG MEMPENGARUHI 5'-PHOSPHODIESTERASE DARIPADA KACANG ADUKI (*Vigna angularis* L.) BERCAMBAH

By

PUI LIEW PHING

Februari 2012

Pengerusi: Prof. Hasanah Mohd. Ghazali, PhD

Fakulti: Fakulti Sains dan Teknologi Makanan

5'-phosphodiesterase (5'-PDE) merupakan satu enzim yang menghidrolisis RNA membentuk 5'-inosina monofosfat (5'-IMP) dan 5'-guanosina monofosfat (5'-GMP), yang juga berfungsi sebagai penambah perisa. Penghasil 5'-PDE terbaik dipilih daripada kekacang yang dibiarkan bercambah, iaitu kacang hijau (*Vigna radiate*), kacang soya (*Glycine max*), kacang adzuki (*Vigna angularis* L.), kacang kuda (*Cicer arietinum*), kacang mata hitam (*Vigna ungiculata*) and petai (*Parkia speciosa*). Untuk memastikan tidak berlakunya pencemaran semasa percambahan, pelbagai rawatan pengsterilan permukaan digunakan. Natrium Hipoklorit pada kepekatan 0.3% mampu untuk menghalang pertumbuhan kulat dalam kacang adzuki, kacang soya dan kacang kuda. Sebaliknya, hanya 0.1% natrium hipoklorit diperlukan untuk menghalang pertumbuhan kulat dalam kacang mata hitam dan petai manakala kacang hijau memerlukan 0.05% natrium hipoklorit untuk menghalang pertumbuhan kulat. Kacang adzuki mengandungi kandungan 5'-PDE tertinggi dan phosphomonoesterase terendah dalam bentuk

bercambah. Paras 5'-phosphodiesterase dalam kacang adzuki berubah semasa percambahan, di mana 15 jam percambahan menghasilkan kandungan 5'-phosphodiesterase yang paling tinggi. Sebaliknya, paras phosphomoesterase adalah paling tinggi di dalam kacang tersebut pada 9 jam percambahan.

5'-PDE mempunyai pH optima dan suhu optima 8.5 dan 80 °C, masing-masing. Ia adalah stabil pada julat pH 7.0 – 8.5. Kestabilan yang lebih tinggi diperhatikan pada pH 7.5 berbanding dengan pH 8.5, dan pada 60 dan 65 ° C, aktiviti enzim masih memiliki fungsi sebagai pemangkin yang baik walaupun selepas 4 hari pengeraman. EDTA, Triton X-100 dan dodecyl natrium sulfat (SDS) menghalang aktiviti 5'-PDE dengan kuat (perencatan adalah antara 75.2-99.4%), manakala Tween 80 dan Tween 20 hanya menghalang activiti dengan sedikit. Ekstrak enzim kasar itu dirangsang oleh Ca^{2+} kepada 249%, K^{+}, Mg^{2+} dan Li^{+}, apabila kepekatan akhir kation adalah 10 mM. Namun, Na^{+}, Zn^{2+}, Ni^{+}, Hg^{+}, Cu^{2+}, $Pb^{2+,}$ Fe^{2+}, $Al^{3+,}$ Ba^{2+} dan Co^{2+} pula merencatkan pada kepekatan yang sama. Aktiviti 5'-PDE meningkat dengan peningkatan kepekatan ion logam Li^{+}, Na^{+} dan Mg^{2+}, sehingga ia mencapai maksimum pada 6 mM diikuti dengan penurunan dalam aktiviti pada kepekatan melebihi 6 mM. Kehadiran Ca^{2+} dalam campuran tindak balas menyebabkan trend yang sama, tetapi perbezaannya adalah pengaktifan maksimum berlaku pada 4 mM. K^{+} pula menunjukkan peningkatan aktiviti 5'-PDE dari 0-10 mM. Al^{3+} mengurangkan aktiviti 5'-PDE dengan peningkatan kepekatan kationnya. Oleh itu, adalah dicadangkan bahawa 5'-PDE dari kacang adzuki merupakan enzim metalo.

Hasil 5'-PDE selepas empat langkah penulenan (pemendakan aseton sejuk 1:1.25, kromatografi penyahgaram, kromatografi penukar anion dan kromatografi penurasan gel)

adalah 13.37% dengan lipatan penulenan sebanyak 6.7 dan aktiviti enzim spesifik sebanyak 35.2 μmol p-nitrophenol/min/mg protein. Berat molekul 5'-PDE asli yang dianggar menggunakan kromatografi penurasan gel adalah 125 kDa. Analisis PAGE natif menunjukkan satu jalur protein utama manakala SDS-PAGE menunjukkan bahawa anggaran berat molekul adalah 124 kDa, yang mungkin terdiri daripada dua rantai polipeptida yang serupa (subunits) dengan berat molekul sebanyak 62 kDa setiap satu. Kesimpulannya, 5'-PDE dengan suhu optimum dan kestabilan suhu yang tinggi telah diperolehi dari kacang adzuki, dan 5'-PDE yang telah ditulenkan mempunyai berat molekul 124 - 125 kDa.

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occasion with deepest respect, who allowed me to pursue my dream in furthering my master. Thank you once again to everyone for the confidence given to me.

I certify that a Thesis Examination Committee has met on 14 February 2012 to conduct the final examination of Pui Liew Phing on her degree entitled "Purification of 5'-phosphodiesterase from germinating adzuki (*Vigna angularis* L.) beans and factors affecting its activity " 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 students be awarded the Master of Science. Members of the Thesis Examination Committee were as follows:

Tan Chin Ping, PhD
Associate Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Chairman)

Foo Hooi Ling, PhD
Associate Professor
Faculty of Biotechnology and Biomolecular Science
Universiti Putra Malaysia

Lasekan Olusegun Olaniyi, PhD
Associate Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia

Othman Omar, PhD
Y. Bhg. Professor
Faculty of Bioscience and Biotechnology
Universiti Kebangsaan Malaysia

_____

Seow Heng Fong , PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

This thesis was 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:

Hasanah Mohd. Ghazali, PhD
Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Chairman)

Abdulkarim Sabo Mohammed, PhD
Associate Professor
Faculty of Food Science and Technology
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 the 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.

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PUI LIEW PHING

Date: 14 February 2012

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