ISOLATION AND CHARACTERISATION OF HEPARIN BINDING PROTEIN IN SLIPPER OYSTER, *Crassostrea iredalei* (FAUSTINO 1932) AND ITS ROLE IN PROPHENOLOXIDASE-ACTIVATING SYSTEM

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MASTER OF SCIENCE
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DEDICATION

This thesis is dedicated to my supervisor, Najiah Musa as she is willing to take me under her supervision since I was an undergraduate student and also dedicated to my co-supervisor Nadirah Musa. Thank you for your patience, ideas and all your thoughts.

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May 2013

Main Supervisor : Associate Professor Najiah Musa @Zakaria, Ph.D.

Co-Supervisor : Nadirah Musa, Ph.D.

Faculty : Fisheries and Aqua-Industry

Recognition proteins play an important role in the immunodefense system of invertebrates. Thus, present study aimed to isolate a recognition protein, *via* Heparin column from Slipper cupped oyster, *Crassostrea iredalei* haemolymph plasma to elucidate its roles in prophenoloxidase-activation systems (proPO) and to characterise it. The protein was isolated using affinity column chromatography with yield of 8% and a single peak. This isolated protein known as heparin-binding protein (HBP). The isolated protein proved to activate proPO system upon treatment with β-1,3 glucan, a pathogen-associated molecular patterns (PAMPs) and show enhancement of phenoloxidase (PO) activity (0.75 unit min^{-1} mg^{-1}) compare to untreated with β-1,3 glucan (0.68 unit min^{-1} mg^{-1}). Moreover, the PO activity increased with the increased concentration of the isolated plasma protein ($r=0.92$, $p<0.05$). The isolated protein possessed a serine protease activity when treated with β-1,3 glucan (0.24 unit min^{-1} mg^{-1}) compare to untreated with β-1,3 glucan (0.180 unit min^{-1} mg^{-1}). However, the protein lacks β-1,3-glucanase activity. The haemolymph of the *C. iredalei* contains HBP with a molecular weight of 35 kDa, as

determined by SDS-PAGE analysis. The isolated protein appear as single band compare to haemolymph plasma that gave three band with molecular weight about >200 kDa, 72 kDa and 35 kDa, respectively. Second dimension electrophoresis showed that the isolated protein present in isoforms. Five different spot with different isoelectric point ranging from 4.6 to 5.5 but same molecular weight had been observed while haemolymph plasma gave 66 spots. Using rabbit antiserum against the isolated protein in immunodiffusion and immunoblotting assays, it produced a single precipitant with partial coalescence pattern and a single band of 35 kDa, respectively. Although the function of this isolated protein is currently unknown, its biochemical properties suggest that it may have a role in the immunoresponse of *C. iredalei*.

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Abstrak tesis yang dikemukakan kepada Senat Universiti Malaysia Terengganu sebagai memenuhi keperluan untuk Ijazah Sarjana Sains

PEMENCILAN DAN PENCIRIAN 'HEPARIN BINDING PROTEIN' DALAM SISTEM PENGAKTIFAN PROPHENOLOXIDASE PADA TIRAM, *Crassostrea iredalei* (FAUSTINO 1932)

ARIEF IZZAIRY ZAMANI

Mei 2013

Penyelia Utama : Profesor Madya Najiah Musa @Zakaria, Ph.D.

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Protein pengecam memainkan peranan penting dalam sistem imunisasi invertebrata. Kajian ini dijalankan bertujuan untuk memencil protein pengecam dari haemolymph plasma tiram, *Crassostrea iredalei* serta untuk menguraikan peranan protein ini dalam sistem *prophenoloxidase-activating* (proPO) serta mencirikanya. Protein ini dipencilkan menggunakan teknik kolum kromatografi dengan penghasilan sebanyak 8%. Protein yang dipencil ini dikenali sebagai Protein pengikat heparin (HBP). Protein ini telah terbukti mengaktifkan sistem proPO apabila diuji dengan molekul asas patogen (PAMPs) yakni β-1,3 glucan dengan kenaikkan aktiviti phenoloxidase (PO) (0.75 unit min^{-1} mg^{-1}) berbanding dengan tanpa ujian β-1,3 glucan (0.68 unit min^{-1} mg^{-1}). Malahan, aktiviti PO meningkat sejajar dengan peningkatan kepekatan protein yang dipencilkan ini ($r=0.92$, $p<0.05$). Protein ini memiliki sifat *serine protease* apabila diuji dengan β-1,3 glucan (0.24 unit min^{-1} mg^{-1}) berbanding tanpa ujian β-1,3 glucan (0.180 unit min^{-1} mg^{-1}). Namun begitu, protein ini tidak memiliki aktiviti β-1,3-glucanase. Haemolymph plasma *C. iredalei* menghasilkan tiga jalur dengan berat molekul sebanyak >200 kDa, 72 kDa and 35 kDa masing-masing. Manakala protein yang dipencilkan menghasilkan hanya satu

jalur sahaja pada 35 kDa. Analisis menggunakan dua dimensi elektrophoresis menunjukkan protein ini hadir dalam bentuk isoform. Lima titik pada isoelektrik point berbeza diperhatikan (4.5 hingga 5.5) manakala heamolymph plasma *C. iredalei* meghasilkan 66 titik pada isoelektrik point berbeza. Menggunakan antiserum dari arnab, protein yang dipencilkan ini diuji dengan ujian *immunodiffusion* dan *immunoblotting*. Protein yang dipencilkan ini menghasilkan satu mendakan dalam ujian *immunodiffusion* manakala dalam ujian *immunoblotting*, menghasilkan satu jalur pada 35kDa. Walaupun fungsi sebenar protein ini masih belum diketahu sepenuhnya, tetapi kajian ini member gambaran bahawa protein ini memaikan lebih dari satu peranan dalam system imunisasi *C. iredalei.*