UNIVERSITI PUTRA MALAYSIA

EXPRESSION, PURIFICATION, AND OLIGOMERIZATION OF PHOSPHOPROTEIN OF NIPAH VIRUS

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By

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Nipah virus (NiV) is a single-stranded, non-segmented negative-sense RNA virus belonging to genus *Henipavirus*. NiV causes fatality to humans and various types of animals. Its genome encodes six major structural proteins: nucleocapsid (N), phospho (P), matrix (M), fusion (F), glyco (G) and large (L) proteins. The P protein is vital for the genome replication and transcription.

The potential diagnostic utility of the purified recombinant NiV P protein was determined in this study. In addition, the oligomeric nature of the P protein was also explored through its multimerization domain. Previously, the NiV *P* gene (2124 bp) was cloned into pTrcHis2-TOPO vector. In the present study, the P protein (120 kDa) was expressed in *Escherichia coli* and the optimal expression conditions such as the cultivation temperature, concentration of IPTG and the time of post-induction were determined in order to express the P protein in soluble form. SDS-PAGE and Western

blot analysis using the anti-His antibody confirmed the protein expression. The optimum cultivation temperature for the recombinant protein expression was at 37 ºC while the optimum induction time was at 9 h with 1 mM IPTG. Solubility analysis showed that about 70% of the P protein was in soluble form at 37 ºC. An immobilized metal affinity chromatography (IMAC) was used to purify the recombinant P protein from the clarified *E. coli* homogenate. The purity of elution after HisTrap purification was 92.67% with a purification factor of 11.58. ELISA and Western blot analysis confirmed that the P protein was antigenic and could be used in serodiagnosis for detecting anti-P antibody of NiV infections.

The oligomerization of P protein plays essential roles in the transcription and replication cycle of NiV. The full length P protein has different phosphorylation status which leads to the existence of more than one protein band in SDS-PAGE and Western blot, it is very difficult to determine the oligomeric nature of the P protein. Thus, the phosphoprotein multimerization domain (PMD) of NiV, which is responsible for the formation of oligomers, was cloned into pTrcHis2-TOPO vector, expressed in *E. coli* and purified with His-Trap column. A stepwise elution protocol was used in order to obtain purer recombinant PMD protein which yielded about 96.73% purity. The purified PMD protein was subjected to chemical cross-linking and dynamic light scattering analyses. The result confirmed that the PMD protein of NiV form tetramers. Circular dichroism analysis revealed that the PMD domain has a high α-helical content, about 55%. This study demonstrated the potential of NiV P protein as a diagnostic reagent for the detection of NiV infections and the oligomeric state of its multimerization domain.

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

PENGEKSPRESAN, PEMULIHAN, DAN OLIGOMERISASI FOSFOPROTEIN VIRUS NIPAH

Oleh

SHAMALA SALVAMANI

Mei 2013

Pengerusi: Tan Wen Siang, PhD

Fakulti Bioteknologi dan Sains Biomolekul

Virus Nipah (NiV) dalam genus *Henipavirus* mempunyai seutas genom RNA negatif yang tidak berbahagi. NiV membawa maut kepada manusia dan pelbagai jenis haiwan. Genom ini mengkod enam protein struktur utama: nucleokapsid (N), fosfo (P), matrik (M), fusion (F), gliko (G) dan besar (L) protein. Protein P memainkan peranan penting dalam replikasi dan transkripsi genom virus tersebut.

Potensi utiliti diagnosis dalam protein P virus Nipah tulen telah ditentukan dalam kajian ini. Selain itu, sifat oligomerik protein P juga dikaji melalui domain penambahan protein tersebut. Sebelum ini, gen NiV *P* (2124 bp) telah diklonkan ke dalam vektor ekspresi, pTrcHis2-TOPO. Dalam kajian ini, protein P (120 kDa) diekspreskan dalam *Escherichia coli* dan keadaan ekspresi yang optimum seperti suhu pengkulturan, kepekatan IPTG dan masa selepas induksi telah ditentukan untuk mengekspres protein P dalam keadaan

mudah larut. SDS-PAGE dan Western blot analisis dengan menggunakan antibodi anti-His mengesahkan ekspresi protein tersebut. Suhu pengkulturan optimum untuk ekspresi protein rekombinan adalah pada 37 ºC manakala masa induksi optimum adalah pada jam ke-9 dengan 1 mM IPTG. Analisis keterlarutan menunjukkan lebih kurang 70% daripada protein P adalah dalam keadaan mudah larut pada 37 ºC. Satu afiniti logam pegun (IMAC) telah digunakan untuk menulen protein P rekombinan daripada homogenat *E. coli* yang dijernihkan. Ketulenan elusi selepas pemulihan HisTrap adalah 92.67% dengan faktor pemulihan sebanyak 11.58. ELISA and Western blot analisis mengesahkan bahawa protein P adalah antigenik and boleh digunakan dalam serodiagnosis untuk mengesan antibodi anti-P daripada jangkitan NiV.

Protein P oligomerisasi memainkan peranan penting dalam transkripsi dan kitaran replikasi NIV. Memandangkan protein P berpanjangan penuh mempunyai status fosforilasi yang berbeza dan menyebabkan kehadiran lebih daripada satu jalur protein dalam SDS-PAGE dan pemblotan Western, ia menjadi sangat sukar untuk menentukan sifat oligomerik protein P. Oleh itu, domain penambahan fosfoprotein (PMD) daripada NiV, yang berperanan dalam pembentukan oligomer, diklonkan ke dalam vektor pTrcHis2-TOPO, diekspreskan dalam *E. coli* dan ditulenkan dengan turus His-Trap. Satu kaedah elusi bertatar telah digunakan bagi mendapatkan protein PMD rekombinan yang lebih tulen dengan ketulenan lebih kurang 96.73%. Protein PMD yang tulen digunakan dalam analisis penghubung silang kimia dan penyerakan cahaya dinamik. Hasil kajian meangesahkan bahawa protein PMD NiV membentuk struktur tetramer. Analisis Circular dichroism (CD) menunjukkan domain PMD mempunyai kandungan α-heliks yang tinggi, lebih kurang 55%. Kajian ini membincangkan potensi protein P virus

Nipah dalam diagnosis reagen untuk mengesan jangkitan NiV dan keadaan oligomerik domain penambahan protein tersebut.

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I certify that a Thesis Examination Commitee has met on 15th August 2013 to conduct the final examination of Shamala Salvamani on her thesis entitled " Expression, Purification, and Oligomerization of Phosphoprotein of Nipah Virus" 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 degree.

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DECLARATION

I declare that the thesis is my original work except for quotations and citations which has been duly acknowledged. I also declare that it has not been previously, and not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

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SHAMALA A/P SALVAMANI

Date: 15 May 2013

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