INTERACTIONS OF THE L, P AND NP PROTEINS OF THE NEWCASTLE DISEASE VIRUS

By

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

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Chairman : Professor Datin Khatijah Mohd. Yusoff, PhD

Faculty : Biotechnology and Biomolecular Sciences

The large (L) and phospho- (P) proteins together with the nucleocapsid (NP) protein of Newcastle disease virus (NDV) are involved in the transcription and replication of the viral genome. The L protein interacts with the P protein to form the active RNA dependent RNA polymerase complex which then acts on the ribonucleoprotein (NP:RNA) comprising the single stranded negative RNA genome which is tightly bound to the NP protein. Amino acid sequence alignment of the L proteins of several non-segmented negative stranded RNA viruses revealed six highly conserved domains described as Domains I to VI which were proposed to specify the essential activities common to the polymerases of these virus. In this study, the individual domains of the L gene of NDV strain AF2240 were cloned separately into pCITE2b plasmid expression vector. An *in vitro* protein binding assay was used to determine the conserved domains on L protein that interact with the purified NP protein. The full length purified NP protein was immobilized on a solid phase and then interacted with radiolabelled [^{35}S]-L domains synthesized in rabbit reticulocyte lysates. The interaction affinity was

quantitated by measuring the radioactivity that was retained on the solid phase. Domain III, which is located between amino acids 631-861, was shown to be highly interactive with the NP protein. In addition, Domains II (amino acid 502 to 607), IV (amino acid 904 to 1071) and V (amino acid 1488 to 1597) showed weak interaction with the NP protein. On the other hand, the interactions between *in vitro* translated L protein domains with the P protein were determined by the immunoprecipitation method. In this approach, the L-P complexes which formed in the mixture were captured with anti-*myc* monoclonal antibody conjugated to protein G agarose. These complexes were precipitated and analysed by autoradiography. Domain V was observed to exhibit the strongest binding with P whereas Domains III and IV showed weaker binding capacities. In conclusion, the core domains of L comprising Domains III, IV and V were interacted with both P and NP proteins which are involved in transcription and replication, but their levels of interactions differed.

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TINDAK BALAS PROTEIN L, P DAN NP VIRUS PENYAKIT SAMPAR AYAM (NDV)

Oleh

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Mac 2006

Pengerusi: Profesor Datin Khatijah Mohd. Yusoff, PhD

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Protein besar (L) dan fosfo (P), bersama dengan protein nukleokapsid (NP) virus Newcastle disease (penyakit sampar ayam: NDV) adalah terlibat dalam proses transkripsi dan replikasi genom virus. Protein L bertindak balas dengan protein P untuk membentuk kompleks polimerase RNA tergantung pada RNA yang aktif yang akan bertindak balas ke atas ribonukleoprotein (NP:RNA). Ribonukleprotein ini terdiri daripada genom RNA negatif bebenang tunggal yang terikat bersama protein NP. Penjajaran jujukan amino asid protein L beberapa virus RNA bebenang negatif yang tidak berserpihan menunjukkan enam domain terpelihara yang dihuraikan sebagai Domain I hingga VI. Domain-domain tersebut telah dicadangkan terlibat dalam penentuan aktiviti perlu yang lazim bagi enzim polimerase dalam golongan virus ini. Dalam kajian ini, setiap domain gen L strain AF2240 NDV masing-masing diklonkan secara berasingan ke dalam plasmid vektor pengekspresan, pCITE2b. Suatu asai pengikatan protein secara *in vitro* telah digunakan untuk menentukan domain terpelihara

pada protein L yang bertindak balas dengan protein NP tulen. Protein NP lengkap yang telah ditulenkan diikat pada fasa pepejal, dan kemudiannya ditindakbalaskan bersama setiap domain L yang terlabel dengan radioaktif [^{35}S] melalui sintesis di dalam lisat retikulosit arnab. Afiniti tindak balas dikira melalui penentuan aktiviti radioaktif yang tertinggal pada fasa pepejal selepas asai dijalankan. Domain III yang terletak antara asid amino 631 - 861, menunjukkan tindak balas terhadap protein NP yang tertinggi. Justeru, Domain II (asid amino 502 hingga 607), IV (asid amino 904 hingga 1071) dan V (asid amino 1488 hingga 1597) menunjukkan tindak balas yang lemah. Manakala, tindak balas antara domain protein L yang ditranslasikan secara *in vitro* dengan protein P ditentukan melalui kaedah pemendakan imuno. Dalam kaedah tersebut, kompleks L-P yang terbentuk dalam campuran tersebut dijerap oleh antibodi monoklon anti-*myc* yang terkonjugat kepada agarosa protein G. Kompleks-kompleks ini dimendak dan dianalisiskan melalui autoradiografi. Domain V didapati menunjukkan ikatan kepada protein P yang tertinggi manakala Domain III dan IV menunjukkan kapasiti untuk mengikat yang lebih lemah. Kesimpulannya, domain utama L yang terdiri daripada Domain III, IV dan V bertindak balas dengan kedua-dua protein P dan NP yang terlibat di dalam transkripsi dan replikasi, tetapi tahap tindak balas tersebut adalah berbeza.

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I certify that an Examination Committee has met on 28th March 2006 to conduct the final examination of Noor Suhana Binti Adzahar on her Master of Science thesis entitled "Interactions of the L, P and NP Proteins of The Newcastle Disease Virus" 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 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 are as follows:

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DECLARATION

I hereby declare that the thesis is based on my original work except for equations and citations, which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

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NOOR SUHANA BINTI ADZAHAR

Date:

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