

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

By

NOOR SUHANA BINTI ADZAHAR

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

March 2006

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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

By

NOOR SUHANA BINTI ADZAHAR

March 2006

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 [³⁵S]-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.

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

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

Oleh

NOOR SUHANA BINTI ADZAHAR

Mac 2006

Pengerusi: Profesor Datin Khatijah Mohd. Yusoff, PhD

Fakulti : Bioteknologi dan Sains Biomolekul

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). Ribonukleoprotein 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 dituliskan diikat pada fasa pepejal, dan kemudiannya ditindakbalaskan bersama setiap domain L yang terlabel dengan radioaktif [³⁵S] 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.

ACKNOWLEDGEMENTS

In the name of Allah, the most Gracious and the most Merciful.

First and foremost, my sincere gratitude and deepest appreciation to my main supervisor, Professor Datin Dr. Khatijah Mohd Yusoff for valuable time she spent on guidance, knowledge, encouragement and support in my research, her generous contributions and supervision, and for the critical review in the completion of this thesis. My appreciation also goes to my co-supervisors, Associate Professor Dr Tan Wen Siang and Associate Professor Dr Zulkeflie Zamrod for their guidance, comments and suggestions on reviewing my research work and progress until completion of this thesis.

I would like also to express my appreciation to our group mentor, Dr Majid Eshaghi, postdoctoral fellow, for his help, guidance, ideas, support and intensive discussions throughout my study.

A very big thank you also goes to my mother, Puan Hamidah Binti Hj. Sulaiman and her friends in Malaysian Agricultural Research and Development Institute (MARDI), Dr Zainuddin Meon and Puan Hamidah Ghazali for kindly reviewed my thesis drafts and threw valuable suggestions and comments to improve the thesis write-up. I would also like to thank Dr Umi Kalsom Abu Bakar and Dr Habibbuddin Hashim for their morale support and advice for me to complete this thesis.

My gratitude also goes to my senior labmates, Dr Wong Sing King, Dr Kho Chiew Ling and Puan Raha Raus, who taught, guided and helped me to conduct the research experiments and for their valuable discussions. I would like to thank my labmates: Rafidah Saadun, Eni Kusumaningtyas, Nazrien Kaman, Zulkifley Othman, Shaherny Zaid, Tang Kah Fai, Taznim Begum, Siti Salwa Hasmoni, Tan Geok Hun, Eddie Chia Suet Lin, Lalita Ambigai, Ong Swee Tin, Noriha Mat Amin, Iswan Budy, Mokrish, Andrew Lee Lian Keat, Tan Yen Peng, Firoozeh Jahanshiri and all my junior, undergraduate students who makes work in the Virology Laboratory of the Department of Microbiology, Faculty of Biotechnology and Biomolecular Sciences enjoyable and inspirable. I wish to thank all the people, although not individually named here, who have contributed throughout my course of study and completion of my thesis.

Last but not least, personal thank to Muhammad Adam Lee and my family: my parents, brother and sister for their encouragement, love and support.

I duly acknowledge the financial support, IRPA grant 01-02-04-003-BTK/ER/006 for this work and the Pascasiswazah scholarship from Ministry of Science, Technology and Innovation, Malaysia.

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:

NORHANI ABDULLAH, PhD

Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

RAJA NOOR ZALIHA RAJA ABDUL RAHMAN, PhD

Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Internal Examiner)

RAHA ABDUL RAHIM, PhD

Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Internal Examiner)

WAN KIEW LIAN, PhD

Associate Professor
Faculty of Science and Technology
Universiti Kebangsaan Malaysia
(External Examiner)

HASANAH MOHD GHAZALI, PhD

Professor / Deputy Dean,
School of Graduate Studies,
Universiti Putra Malaysia.

Date:

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:

KHATIJA H YUSOFF, PhD

Professor

Faculty of Biotechnology and Biomolecular Sciences

Universiti Putra Malaysia

(Chairman)

TAN WEN SIANG, PhD

Associate Professor

Faculty of Biotechnology and Biomolecular Sciences

Universiti Putra Malaysia

(Member)

ZULKEFLIE ZAMROD, PhD

Associate Professor

Faculty of Science and Technology

Universiti Kebangsaan Malaysia

(Member)

AINI IDERIS, PhD

Professor / Dean,

School of Graduate Studies,

Universiti Putra Malaysia.

Date:

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.

NOOR SUHANA BINTI ADZAHAR

Date:

TABLE OF CONTENTS

| | |
|--|----------|
| ABSTRACT | ii |
| ABSTRAK | iv |
| ACKNOWLEDGEMENTS | vi |
| APPROVAL | viii |
| DECLARATION | x |
| LIST OF TABLES | xiii |
| LIST OF FIGURES | xiv |
| LIST OF ABBREVIATIONS | xvi |
| LIST OF AMINO ACID ABBREVIATIONS | xx |
| | |
| CHAPTER | |
| | |
| 1 INTRODUCTION | 1 |
| | |
| 2 LITERATURE REVIEW | |
| 2.1 Newcastle disease virus (NDV) | 4 |
| 2.2 Viral morphology and genome structure | 6 |
| 2.3 Viral transcription and replication | 7 |
| 2.4 Ribonucleoprotein complex (RNP) | 12 |
| 2.4.1 Large (L) protein | 13 |
| 2.4.2 Phosphoprotein (P) | 15 |
| 2.4.3 Nucleocapsid (NP) protein | 17 |
| 2.5 Domains in the L protein | 18 |
| 2.6 Interactions between viral proteins | 21 |
| | |
| 3 MATERIALS AND METHODS | |
| 3.1 Materials | 24 |
| 3.1.1 Viruses | 24 |
| 3.1.2 Bacterial strains | 24 |
| 3.1.3 Plasmid vectors | 24 |
| 3.2 General procedures | 25 |
| 3.3 Propagation of NDV | 26 |
| 3.4 Isolation of ribonucleic acid (RNA) | 27 |
| 3.5 Multiple sequence alignment of the L protein | 28 |
| 3.6 Preparation of L gene segments | 29 |
| 3.6.1 Extraction of plasmid DNA | 29 |
| 3.6.2 Reverse transcription polymerase chain reaction (RT-PCR) | 30 |
| 3.6.3 DNA cloning | 33 |
| 3.6.4 Transformation of DNA | 35 |
| 3.6.5 Screening for positive clones | 37 |
| 3.6.6 <i>In vitro</i> transcription and translation | 38 |

| | | | |
|----------|-------|--|-----|
| | 3.6.7 | TCA precipitation | 39 |
| 3.7 | | Protein purification | 40 |
| | 3.7.1 | Time course expression | 41 |
| | 3.7.2 | Lysis of bacterial cells and solubility test | 42 |
| | 3.7.3 | Ammonium sulphate precipitation | 43 |
| | 3.7.4 | Purification using Probond™ column | 44 |
| 3.8 | | Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) | 44 |
| 3.9 | | Western blot analysis | 45 |
| 3.10 | | The Bradford assay | 47 |
| 3.11 | | Protein binding assay | 47 |
| 3.12 | | Immunoprecipitation | 48 |
| 4 | | RESULTS | |
| | 4.1 | Sequence alignment of L protein with other negative stranded RNA viruses | 49 |
| | 4.2 | Construction of plasmid encoding L domains | 57 |
| | | 4.2.1 Amplification of L gene domains | 57 |
| | | 4.2.2 Construction of recombinant plasmids | 59 |
| | 4.3 | <i>In vitro</i> transcription and translation of <i>LI</i> to <i>VI</i> | 59 |
| | 4.4 | Cloning of the full length L gene | 63 |
| | 4.5 | Expression and purification of NP protein | 69 |
| | 4.6 | Expression and purification of P protein | 72 |
| | 4.7 | Interaction between the viral proteins | 75 |
| | | 4.7.1 L and P proteins | 75 |
| | | 4.7.2 L and NP proteins | 78 |
| 5 | | DISCUSSION | |
| | 5.1 | The L protein conserved domain | 80 |
| | 5.2 | Cloning and expression of the full length NDV L protein | 83 |
| | 5.3 | Interaction studies between L and P proteins | 84 |
| | 5.4 | Interaction studies between L-NP | 89 |
| | 5.5 | Conclusion | 90 |
| | | REFERENCES | 93 |
| | | APPENDICES | 103 |
| | | BIODATA OF THE AUTHOR | 111 |