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THERMOSTABILITY AND PROTEIN STUDIES OF NEWCASTLE DISEASE VIRUS

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By

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LIST OF ABBREVIATIONS

ND	Newcastle disease
NDV	Newcastle disease virus
2D-PAGE	two-dimensional polyacrylamide gel electrophoresis
ts	temperature-sensitive
RNA	ribonucleic acid
mRNA	messenger RNA
MW	molecular weight
Kbp	kilobase pair
pI	isoelectric point
SDS	sodium dodecyl sulphate
SDS-PAGE	sodium dodecyl sulphate-polyacrylamide gel electrophoresis
Mab	monoclonal antibody
UPM	Universiti Pertanian Malaysia
JPA	Jabatan Perkhidmatan Awam
BSA	bovine serum albumin
HA	hemagglutinating activity
HL	hemolytic activity
NA	neuraminidase activity
NANA	N-acetylneuraminic acid

RBC	red blood cells
DTT	dithiothreitol
CHAPS	cholamidopropyldimethyl-hydroxypropanesulfonate
IEF	isoelectric focusing
TEMED	tetramethylethylenediamine
ncm	nitrocellulose membrane
NBT	nitrobluetetrazolium
BCIP	5-bromo-4-chloro-3-iodolyl-phosphate
EDTA	Ethylenediaminetetraacetic acid disodium salt
R_f	relative mobility
V	volt
Vh	volt-hour
v/v	volume/volume

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THERMOSTABILITY AND PROTEIN STUDIES OF NEWCASTLE DISEASE VIRUS PROTEINS

By

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Chairman: Assoc. Prof. Dr. Khatijah Mohd Yusoff

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The heat stability of four strains of Newcastle disease virus (NDV) belonging to three different pathotypes were studied. The strains were the lentogenic V4 and its heat stable variant V4-UPM, the mesogenic S strain and the velogenic strain AF2240. Analyses of their haemagglutination and neuraminidase activities (which are the functions of the HN protein) and the hemolytic activities at various temperatures showed that strains AF2240, V4 and V4-UPM were heat stable compared to strain S.

There were no differences observed in the mobilities of the various NDV proteins on sodium

dodecyl sulphate-polyacrylamide gel electrophoretic (SDS-PAGE) studies. However, analysis of the various peptides with *Staphylococcus aureus* protease showed that the digested HN proteins of strain V4-UPM was different from the strains V4, AF2240 and S. The peptide analysis was repeated using *Pseudomonas fragi Endoproteinase Asp-N* and *Lysobacter enzymogenes Lys-C* and found to be similar except in strain V4-UPM.

These proteins were further analysed by the two-dimensional polyacrylamide gel electrophoresis (2D-PAGE). The gels were then Western blotted and protein spots were identified using HN,NP and F_1 Mabs and then analysed by the UVP GDS Gel Documentation System (United Kingdom). It was observed that in the heat stable V4 strain the isoelectric point (pI) of the HN protein was in the acidic region, strains V4-UPM and AF2240 in the neutral/weak basic regions and in the thermolabile strain S, the HN protein was shifted to the basic end of the isoelectric focussing run. The pI changes in the NP protein was seen in strain S only. The F protein was at the basic region for all strain except strain V4-UPM. In strain S it was seen that the HN, NP and F proteins were in the basic region and this basic pI could be responsible for the different biological characteristics seen in the thermostable strains compared to the thermosensitive strain.

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KESTABILAN SUHU DAN KAJIAN PROTIN VIRUS NEWCASTLE DISEASE

Oleh

ZURIDAH HASSAN

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Pengerusi: Prof. Madya Dr. Khatijah Bt Mohd Yusoff

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Kajian keatas empat strain virus Newcastle disease (NDV) terdiri daripada tiga jenis patotip telah dijalankan. Strain tersebut adalah strain lentogenik V4 dan varian rintang suhu V4-UPM, strain mesogenik S dan strain velogenik AF2240. Analisis aktiviti haemagglutinasi dan neuraminidase (iaitu fungsi protein HN) dan aktiviti hemolitik pada berlainan suhu menunjukkan strain AF2240, V4 dan V4-UPM rintang kepada suhu berbanding dengan strain S.

Tidak ada perbezaan yang dapat diperhatikan di dalam pergerakan protein NDV didalam kajian elektroforetik sodium dodesil sulfat-gel poliakrilamida (SDS-PAGE).

Walau bagaimanapun, analisis peptida menggunakan protease *Staphyloccocus aureus* menunjukkan protein HN yang terhadam bagi strain V4-UPM berbeza daripada strain V4, AF2240 dan S. Analisis peptida ini telah diulang menggunakan *Pseudomonas fragi Endoproteinase Lys-C* dan *Lysobacter enzymogenes Lys-C* tetapi tidak menunjukkan sebarang perbezaan melainkan strain V4-UPM.

Protein-protein ini telah dianalisis selanjutnya menggunakan kaedah elektroforesis dua dimensi (2D-PAGE). Gel diproses seterusnya dengan kaedah Western blotting dan titik protein dikenal pasti menggunakan Mabs HN, NP dan F dan dianalisis dengan UVP GDS Gel Documentation System (UK). Didapati bahawa bagi strain V4 titik isoelektrik (pI) bagi protein HN berada pada bahagian asid, strain V4-UPM dan AF2240 dibahagian neutral/basik rendah dan pada strain labil suhu S, protein HN telah berubah ke bahagaian basik pemfokusan isoelektrik. Perubahan pada titik pI bagi protein NP hanya dilihat pada strain S sahaja. Protein F adalah di dalam kawasan basik kecuali strain V4-UPM. Bagi strain S, didapati bahawa protein HN, NP dan F berada di kawasan basik dan kemungkinan ini menunjukkan perubahan ciri-ciri biologi yang dilihat bagi strain stabil-suhu dan labil-suhu.

CHAPTER 1

INTRODUCTION

Newcastle disease virus (NDV) is an economically important avian paramyxovirus which causes a highly contagious and fatal disease in poultry known as Newcastle disease (ND). Its genome is a negative-stranded RNA which encodes six major proteins : the nucleocapsid (NP) protein, the phosphoprotein (P), matrix (M) protein, fusion (F) protein, haemagglutinin-neuraminidase (HN) protein and the large (L) protein (Samson, 1988). Several non-structural proteins (36,000 dalton and 33,000 dalton) have also been detected in NDV-infected cells (Iinuma and Simpson, 1974; Moore and Burke, 1974).

The severity of the disease depends on several factors, namely, (i) biological properties of the viral strain, (ii) species and age of the host, (iii) presence of other organisms and (iv) environmental factors. NDV strains can be classified into three major pathotypes :(a) velogenic, with sudden death and very high mortality; (b) mesogenic, with respiratory signs but low mortality and (c) lentogenic, with mild

infections. In addition, some strains are avirulent and cause no disease at all.

Poultry has a unique role in the livestock sector of the countries in the Asia-Pacific region. It is the only livestock species that is widely accepted by people from a variety of cultural background. Although ND in commercial poultry is effectively controlled by vaccination, the use of conventional ND vaccination in village poultry has been an unsatisfactory process. The chickens which are of multi ages, are scattered over the villages and are difficult to catch for formal vaccination. Moreover, many live ND vaccines are heat-labile (Lomniczi, 1975) and are thus not suitable in the Tropics. A new approach is therefore required to control ND in the village poultry.

Through a cloning technique, an immunogenic and heat tolerant vaccine of NDV lentogenic strain V4 designated as V4-UPM was isolated (Ideris et al., 1989) . The thermostabilities of infectivity and haemagglutinin of V4-UPM were found to be greater than those of the parent V4. It took at least 5 h for the HA (haemagglutinating acitivity) titre of strain V4-UPM to decrease by 2 logarithms (base 2) compared to only 2 h for strain V4. In the case of

thermostability of infectivity, the time required for a decrease in titre 2 logarithmic orders (base 10) was within 1 h for strain V4 and 3 h for V4-UPM. This new vaccine is used to coat feed pellets which are readily eaten by the chickens. It is currently being commercialised by a joint venture between Universiti Pertanian Malaysia and a private vaccine company Remee Holdings Sdn. Bhd.

Very little work has been done on the thermostabilities of the NDV proteins. Studies on temperature sensitive (ts) mutants at the molecular level of the mesogenic Beaudette C strain have shown an altered isoelectric point in the P protein of mutant ts172 (Samson et al., 1981) and the HN and M proteins of mutant ts53 (Harper et al., 1983). Sequence analysis of the latter has identified sites in the HN protein which may be important in folding and function (Hughes et al., 1991). These studies suggests that the pathotype of the NDV strain may not be directly related to thermostability since the two temperature sensitive mutants (ts172 and ts53) and the heat stable mutant V4-UPM were from different pathotypes. However, these studies were done in separate experiments. Therefore, the thermostabilities of NDV strains representatives of the three major pathotypes will be determined to

confirm the hypothesis that there is no direct relationship between pathogenicity and thermostability.

Examination of the viral proteins in gel electrophoresis may reveal minute difference in the proteins of the various NDV strains. It is possible to correlate these differences to the thermostability.

This study was undertaken

(1) to determine whether the NDV strains of varying pathotypes have different response to heat based on their biological activities;

(2) to examine these differences at the molecular level using several types of gel electrophoretic techniques; and

(3) to determine the proteins which may be directly involved in thermostability.

CHAPTER II

LITERATURE REVIEW

Newcastle Disease

Newcastle disease (ND) was first reported in 1926 in Jakarta, Indonesia, by Doyle and Kraneveld (Allan, 1971) but the origin and epidemiology of ND may be obscure (Hanson, 1978). In the following year it was recognised in other parts of Asia and later in Europe and America (Allan et al., 1973). In England the disease was centred in Newcastle-upon-Tyne hence the name of the disease (Spradbrow, 1987).

ND is one of the most important viral disease in the poultry industry. It causes severe economic losses due to death of chickens and lowered production of eggs, increased cost of purchasing vaccines and running of eradication and quarantine programmes during an outbreak (Lancaster, 1981; Spradbrow, 1987). In some countries vacccination programmes were adopted as the control measure with

varying successes (Biggs, 1982). The requirement of immunization also varies with each programme depending on the levels of protection needed, the immune status of the birds, the type of field virus occurring in each locality and the relationship between poultry diseases and the administration of the ND vaccines (Lancaster, 1964).

Newcastle disease virus (NDV) which is the causative agent of ND, is a member of the genus paramyxovirus within the family *Paramyxoviridae* which includes the mumps, Sendai and parainfluenza viruses (Compans and Choppin, 1967). Three distinct pathotypes of NDV have been described viz. lentogenic, mesogenic and velogenic (Hanson and Brandly, 1955) and on the basis of tissue tropism, they can be grouped as viscerotropic, pneumotropic and neurotropic (Allan et al., 1973).

The severity of infection is highly strain dependent (Alexander, 1988a). Velogenic strains cause severe disease with high mortality even in adult birds. Mesogenic ones are of moderate virulence, causing mortalities of up to 50% and seriously reducing egg production. The lentogenic strains are of low virulence causing little mortality except in young

chicks but affected egg production (Hanson, 1978; Spradbrow, 1987, Waterson et al., 1967).

The disease can spread either naturally or due to factors associated with the transportation of eggs, birds, carcasses, poultry offal, vaccinating crew and their movements or even by frozen poultry meat or contaminated vaccines (Hanson, 1978; Alexander, 1988a, 1988b).

Viral Structure

NDV is an enveloped, negative stranded RNA virus (Compans and Choppin, 1967). The RNA is complementary to the viral mRNAs and are generated during transcription in infected cells (Davies et al., 1976). The virion contains approximately 67% by weight of protein, 1% RNA, 24% lipid and 7% carbohydrate (Blough and Lawson, 1968; Haslam et al., 1969). Under negative electron-microscopy, the viral particles are pleomorphic in shape with a diameter of 100-500 nm (Hosaka et al., 1966; Kingsbury, 1974, 1990).

The envelope is covered with spikes of glycoprotein, the hemagglutinin-neuraminidase (HN) protein and the fusion (F) protein, of 8 to 12 nm long. The nonglycosylated matrix protein (M) forms a shell on