CHARACTERISATION OF THE CHIMERIC PROTEINS OF VP3 FROM CHICKEN ANEMIA VIRUS WITH NUCLEOPROTEIN FROM THE NEWCASTLE DISEASE VIRUS

By

ZULKEFLEY OTHMAN

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

June 2006

DEDICATED TO……..

My Father,

OTHMAN BIN AWANG

My Mother,

KHOLIJAH BINTI SHAARI

My Wife.

SHAHERNY BINTI ZAID

My Brothers and Sister

ZULRAIDI BIN OTHMAN,

ZULHAFIZ BIN OTHMAN,

ZULIANA BINTI OTHMAN

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

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April 2006

Chairman: Associate Professor Abdul Rahman Omar, PhD

Faculty: Veterinary Medicine

Chicken anemia virus (CAV) causes aplastic anemia, generalized lymphoid atrophy and increased mortality in susceptible chickens. Infections with CAV are considered to be economically significant because of the clinical disease associated with vertical transmission and its potential for inducing immune dysfunction alone or in combination with other pathogens. CAV infection can be detected using several serological methods such as serum neutralization, immunofluorescence assay and enzyme-linked immunosorbent assay (ELISA). With small size and abundant amount in infected cells, VP3 protein of CAV is a good choice for virus detection. Due to its poor solubility, VP3 was unsuitable for immunogenicity studies. This protein was fused to the nucleoprotein (NP) of Newcastle disease virus (NDV), which has the ability to increase its solubility. The VP3 was expressed together with the NP protein in pTrcHis2 vector as full length (pTrcHis2NP+VP3FL) and truncated

(pTrcHis2NP+VP3F1, pTrcHis2NP+VP3F2 and pTrcHis2NP+VP3F3). The chimeric protein of NP and full length VP3 reacted specifically with VP3 monoclonal antibody but not chimeric proteins containing truncated VP3. On the other hand, fusion of NP significantly improved the solubility of truncated but not the full length VP3 protein. The estimated solubility of NP with full-length VP3 and truncated VP3 proteins was 15 and 50 %, respectively. In order to determine the immunogenicity of the expressed VP3 proteins, protein lysates of chimeric protein of NP with full length VP3 was injected into specific-pathogen-free (SPF) chickens and the sera was collected. However, the sera failed to react with whole CAV and VP3 protein when tested using ELISA or Western blot. The sera showed positive reaction with Western blot analysis against purified NP and NDV suggesting that the NP, but not the VP3 protein, was immunogenic and able to induce antibody responses. The inability of the SPF chickens to induce VP3-specific antibody responses was probably due to the small size of VP3 and the low solubility of the protein. In addition, the VP3 protein is relatively unstable based on analysis using ProtParam program. The study also revealed that hydrophobicity graft of all the chimeric proteins were similar with the solubility analysis performed previously. Thus, the expressed chimeric protein of NP and VP3 is not suitable for use as an antigen in the production of antibody for the development of VP3 protein as diagnostic marker. More studies are required before the potential application of the VP3 in the diagnosis of CAV can be ascertained.

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

PENCIRIAN PROTEIN - PROTEIN KIMERA VP3 DARI VIRUS ANEMIA AYAM DENGAN NUKLEOPROTEIN DARI VIRUS PENYAKIT NEWCASTLE

Oleh

ZULKEFLEY OTHMAN

April 2006

Pengerusi: **Profesor Madya Abdul Rahman Omar, PhD**
Fakulti: **Perubatan Veterinar**

Virus anemia ayam (CAV) menyebabkan anemia aplastik, atropi limfoid yang menyeluruh dan peningkatan kematian kepada ayam yang terdedah. Jangkitan dengan CAV memberi kesan yang signifikan kerana penyakit klinikal yang dikaitkan dengan transmisi secara menegak dan keupayaannya untuk meransang kegagalan imuniti dengan sendiri atau dengan kombinasi patogen-patogen lain. Jangkitan CAV boleh dikesan menggunakan beberapa kaedah serologi seperti peneutralan serum, asai imunopendarfluor dan asai imunoperap berkaitan enzim (ELISA). Dengan saiz yang kecil dan jumlah yang banyak di dalam sel yang dijangkiti, VP3 adalah pilihan yang baik untuk pengesanan CAV. Disebabkan oleh kelarutan yang rendah, VP3 tidak sesuai untuk kajian keimunogenan. Protein ini telah digabungkan bersama nukleoprotein (NP) dari virus penyakit Newcastle (NDV) yang berkemampuan untuk meningkatkan kelarutannya. VP3 penuh (pTrcHis2NP+VP3FL) dan terpangkas

(pTrcHis2NP+VP3F1, pTrcHis2NP+VP3F2 dan pTrcHis2NP+VP3F3) telah diekspres bersama protein NP di dalam vektor pTrcHis2. Protein kimera NP dan VP3 penuh bertindakbalas secara spesifik dengan antibodi monoklon VP3 tetapi tidak dengan protein kimera yang mengandungi VP3 terpangkas. Sebaliknya, gabungan dengan NP telah meningkatkan kelarutan VP3 terpangkas secara signifikan tetapi tidak bagi VP3 penuh. Anggaran kelarutan NP dengan VP3 penuh dan VP3 yang terpangkas ialah 15% dan 50%, masing-masing. Bagi menentukan keimunogenan protein VP3 yang diekspres, lisat protein bagi protein kimera NP dengan VP3 penuh telah disuntik ke dalam ayam bebas patogen spesifik (SPF) dan sera dikumpulkan. Walau bagaimanapun, sera tersebut gagal untuk bertindakbalas dengan CAV dan protein VP3 apabila diuji menggunakan ELISA dan penyerapan Western. Sera tersebut ada menunjukkan tindakbalas positif dengan analisis penyerapan Western menggunakan NP tulen dan NDV, menandakan bahawa hanya protein NP dan bukannya protein VP3 yang imunogenik dan mampu untuk meransang tindakbalas antibodi. Kegagalan ayam SPF untuk merangsang tindakbalas antibodi VP3 yang spesifik mungkin kerana saiz VP3 yang terlalu kecil dan kelarutan protein yang rendah. Selain daripada itu, protein VP3 secara relatifnya tidak stabil berdasarkan analisis menggunakan program ProtParam. Kajian juga menunjukkan bahawa graf kehidrofobian bagi semua protein kimera adalah sama dengan analisis kelarutan yang telah dijalankan sebelum ini. Oleh itu, protein kimera NP dan VP3 yang diekspres tidak sesuai digunakan sebagai antigen didalam penghasilan antibodi bagi pembangunan protein VP3 sebagai penanda diagnostik. Lebih banyak kajian diperlukan sebelum penggunaan potensi VP3 dalam diagnosis CAV boleh dipastikan.

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I certify that an Examination Committee has met on 31 March 2006 to conduct the final examination of Zulkefley Bin Othman on his Master of Science thesis entitled "Characterisation of the Chimeric Proteins of VP3 from Chicken Anemia Virus with Nucleoprotein from 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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DECLARATION

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

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ZULKEFLEY BIN OTHMAN

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