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HEPATITIS B CORE PARTICLE AS A DISPLAY PLATFORM FOR FOREIGN EPITOPES

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HEPATITIS B CORE PARTICLE AS A DISPLAY PLATFORM FOR FOREIGN EPITOPES

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Chairman: Professor Tan Wen Siang, PhD

Institute: Faculty of Biotechnology and Biomolecular Sciences

Hepatitis B core (HBc) particle is a useful display platform for foreign epitopes due to its high immunogenicity and stability. HBc small and large particles are made up of 180 and 240 subunits of hepatitis B core antigen (HBcAg) with triangulation numbers, T=3 and T=4, respectively. The foreign epitopes are presented preferentially at the amino (N), carboxy (C) terminus or the immunodominant region of HBcAg without disturbing the formation of HBc particles. The effects of N-terminally inserted peptides with various lengths (14, 17, 22 and 25 amino acid residues) were first investigated in this study. A polyhistidine tag was inserted at the N-terminus of HBcAg with or without extraneous peptide linkers. The His-tagged HBc particles were purified with an IMAC-based column. The yield improved with an increase in the length of N-terminal extensions. His-β-L-HBcAg carrying 20 extraneous amino acids at the N-terminus of

HBcAg, was bound by anti-His monoclonal antibody and showed the highest colour intensity in the ELISA. The antigenicity of HBc particles was barely affected by the N-terminal insertion and they were reactive against an anti-HBc monoclonal antibody and human anti-HBc antisera. In addition, they formed virus-like particles with an average diameter of about 30 nm when observed under a transmission electron microscope (TEM).

In order to authenticate the application of His-tagged HBc VLPs as an alternative diagnostic reagent in HBV infections, His-β-L-HBcAg was purified in substantial amount with immobilized metal affinity-expanded bed adsorption chromatography (IMA-EBAC). IMA-EBAC allows a single-step purification of His-tagged HBc particles directly from the unclarified *Escherichia coli* lysate. The duration needed in the protein purification process using IMA-EBAC is shorter (from two to three hours) than sucrose density gradient ultracentrifugation which requires two to three days. The yield of purified His-tagged HBcAg was shown to be 100-fold higher than that obtained with sucrose density gradient ultracentrifugation. An optimal adsorption of His-tagged HBc VLPs to the *Streamline Chelating* adsorbent was achieved at pH 8.0. The majority of the host cell proteins were eliminated by using wash buffers A (pH 8.0) and B (pH 6.0) supplemented with 10% (v/v) glycerol and 50 mM imidazole, respectively. Approximately 56% of the His-tagged HBc particles were recovered from the bacterial homogenate at pH 7.0 by using 500 mM imidazole and 1.5 M NaCl. The purified His-tagged HBc particles remained antigenic and intact throughout the entire protein purification and they also formed HBc particles (30 nm) as verified by TEM. This

implies that the purified His-tagged HBc VLPs can be a potential reagent in the diagnosis of HBV infections.

The C-terminal region of the nucleocapsid (N) protein of Nipah virus, namely $NP_{401-532}$, is vulnerable to bacterial proteases. Since HBc particle is highly stable and antigenic, $NP_{401-532}$ was displayed at the N-terminus ($NP_{401-532}$HBc) and the immunodominant loop ($HBc_{79-80}NP_{401-532}$) of HBc protein. $NP_{401-532}$HBc and $HBc_{79-80}NP_{401-532}$ showed improved stability compared to that of $NP_{401-532}$ when subjected to proteinase K treatment. In responses to the anti-NiV antisera, the antigenicity of the N-terminally inserted $NP_{401-532}$ was enhanced whereas $NP_{401-532}$ presented at the immunodominant loop was only detectable in its denatured form. The N-terminus of HBc protein is therefore preferable to its immunodominant loop with regard to the enhanced stability of the C-terminal domain of NiV N protein.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PARTIKEL TERAS VIRUS HEPATITIS B SEBAGAI PLATFORM PEMAPARAN UNTUK EPITOP ASING

Oleh

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Disember 2011

Pengerusi: Profesor Tan Wen Siang, PhD

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Partikel teras hepatitis B (HBc) merupakan sebuah platform pemaparan yang amat berguna kepada epitop asing kerana partikel sedemikian berkebolehan tinggi untuk merangsang penghasilan antibodi dalam perumahnya. Partikel teras HBV yang kecil dan besar diperbuat daripada 180 dan 240 salinan protein teras (HBcAg), dengan nombor triangulasi T = 3 dan T = 4. Kebanyakan epitop asing telah dipaparkan pada bahagian amino (N), karboksi (C) atau imunodominan HBcAg tanpa memusnahkan kebolehannya untuk membentuk partikel HBc. Pengaruh beberapa peptida dengan kepanjangan yang berlainan (14, 17, 22 dan 25 residu asid amino) telah dikaji dalam bahagian pertama kajian ini. Tag polihistidin telah dipamerkan tanpa atau bersama dengan sambungan peptida di bahagian amino HBcAg. Partikel HBc yang bertagkan polihistidin telah ditulenkan terus berlandaskan IMAC. Hasil penulenan partikel HBc didapati bertambah dengan kepanjangan peptida yang disambungkan di bahagian

aminonya. His-β-LHBcAg yang mempunyai 20 asid amino berlebihan di bahagian amino HBcAg dikesan oleh antibodi monoklonal anti-His dan menunjukkan intensiti yang paling tinngi dalam ELISA. Keantigenan partikel HBc turut tidak terganggu oleh paparan peptida ketika diuji dengan antibodi monoklonal anti-HBc dan sampel serum pesakit hepatitis B. HBcAg yang ditagkan dengan polihistidin juga membentuk partikel-partikel teras yang seiras dengan virus sebenar (VLPs) dengan diameter kira-kira 30 nm semasa diperhatikan di bawah mikroskop elektron (TEM).

Dalam usaha untuk mengesahkan penggunaan VLPs HBc sebagai reagen diagnostik yang berkesan, His-β-L-HBcAg telah ditulenkan dalam jumlah yang besar dengan mengaplikasikan sistem kromatografi penjerapan molekul melalui logam statik pada lapisan penjerap yang berkembang (IMA-EBAC). IMA-EBAC membolehkan penulenan partikel HBc secara langsung daripada pecahan sel *Escherichia coli* tanpa proses penjernihan. Jangka masa yang diperlukan dalam proses penulenan protein IMA-EBAC adalah lebih singkat (dari dua hingga tiga jam) berbanding dengan ultra-pengemparan ketumpatan sukrosa yang memerlukan dua hingga tiga hari. Hasil penulenan HBcAg yang bertagkan His ditunjukkan 100 kali ganda lebih tinggi daripada yang diperoleh melalui ultra-pengemparan ketumpatan sukrosa. Kadar penjerapan optimum partikel HBc yang bertagkan His pada penjerap Streamline Chelating tercapai pada pH 8.0. Hampir kesemua sisa protein bakteria *E. coli* telah diluruhkan dengan menggunakan larutan penimbal A (pH 8.0) dan B (pH 6.0) yang mengandungi 10% (v/v) gliserol serta 50 mM imidazol masing-masing. Kira-kira 56% partikel teras HBV telah ditulenkan pada pH 7.0 dengan menggunakan 500 mM imidazol dan 1.5 M NaCl.

Partikel HBc bertag-His yang ditulenkan masih antigenik dan utuh selepas proses penulenan IMA-EBAC. Hal ini dapat dibuktikan melalui data yang diperoleh dalam ELISA dan pembentukan partikel HBc (30 nm) seperti yang ditunjukkan melalui TEM. Oleh sebab demikian, partikel teras HBV yang ditagkan dengan polihistidin sememangnya berpotensi tinggi untuk dijadikan reagen dalam diagnosis jangkitan HBV.

Bahagian C nukleokapsid (N) Nipah virus (NiV), yakni $NP_{401-532}$, amatlah sensitif terhadap tindakan protease bakteria. Justeru, $NP_{401-532}$ telah dipamerkan di bahagian amino ($NP_{401-532}$HBc) dan imunodominan ($HBc_{79-80}NP_{401-532}$) HBcAg kerana kestabilan dan keantigenannya yang tinggi. Pemaparan $NP_{401-532}$ di atas partikel teras HBV telah berjaya memperbaiki kestabilannya dalam tindak balasnya dengan proteinase K. Selain itu, keantigenan $NP_{401-532}$ terhadap sampel serum penyakit NiV telah banyak dipertingkatkan melalui pemaparannnya di bahagian amino HBcAg. Walau bagaimanapun, $NP_{401-532}$ yang dipaparkan di bahagian imunodominan HBcAg hanya dapat dikesan apabila dinyahaslikan. Sesungguhnya, $NP_{401-532}$ lebih sesuai dipaparkan di bahagian amino HBcAg berbanding dengan bahagian immunodominannya demi meningkatkan kestabilan dan keantigenannya.

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I certify that a Thesis Examination Committee has met on 19 December 2011 to conduct the final examination of Yap Wei Boon on his Doctor of Philosophy thesis entitled "Hepatitis B Core Particle as a Display Platform For Foreign Epitopes" in accordance with the Universities and University College 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 Doctor of Philosophy.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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DECLARATION

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

YAP WEI BOON

Date: 19 December 2011

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