INTISARI

EKSPRESI DAN PURIFIKASI PROTEIN REKOMBINAN GEN *HBcAg* VIRUS HEPATITIS B PADA *Escherichia coli* BL21(DE3)

Virus hepatitis B (VHB) telah menginfeksi 257 juta orang di seluruh dunia dan menyebabkan sekitar 880 ribu kematian setiap tahunnya. Indonesia merupakan salah satu negara di kawasan Asia Tenggara dengan level endemisitas sedang hingga tinggi, yaitu sekitar 2,5%-10% untuk prevalensi *HBsAg*. Beberapa cara pencegahan dan penanggulangan terhadap penyakit hepatitis B seperti dengan vaksinasi. Salah satu gen struktural yang dapat digunakan untuk pembuatan vaksin protein rekombinan adalah *HBcAg*. Gen *HBcAg* bersifat imunogenik dan dapat memicu antibodi spesifik, sel T helper (Th), dan sel T sitotoksik (CTLs). Penelitian ini menggunkan gen *HBcAg* yang telah dioptimasi kodon, kemudian diujikan secara permodelan komputasi untuk mengetahui karakter fisikokimia, permodelan protein, antigenisitas, alergenisitas serta prediksi epitop. Didapatkan hasil indeks alifatik, titik isoelektrik dan indeks GRAVY sebesar 83,02; 9,93; dan -0,435. Prediksi antigenisitas dan alergenisitas menunjukkan protein HBcAg bersifat antigenik dan non alergen. Hasil permodelan protein menggunakan plot Ramachandran menunjukkan sebaran residu asam amino pada daerah *most favoured regions* sebesar 91,1% dan tidak ada residu asam amino non-glisin pada daerah *disallowed regions*. Dari parameter diatas membuktikan bahwa protein HBcAg memiliki permodelan serta struktur yang baik dan sesuai. Gen *HBcAg* kemudian disisipkan pada vektor plasmid pET 15-b untuk ditransformasikan pada *E.coli* BL21(DE3) menggunakan metode *heat-shock*. Bakteri transforman kemudian diinduksi dengan 0,125-1 mM IPTG untuk selanjutnya dipurifikasi menggunakan kolom Ni-NTA dan dilanjutkan dengan *western blot*. Hasil didapatkan protein rekombinan HBcAg berukuran 21 kDa dengan konsentrasi protein total sebesar 3560 mg/L pada konsentrasi 1 mM IPTG dalam bentuk terlarut. Hasil purifikasi didapatkan konsentrasi protein HBcAg murni sebesar 103 mg/L. Analisis *western blot* menunjukkan bahwa protein terdeteksi dengan menggunakan antibodi primer anti His-tag.

Kata Kunci: *HBcAg*, pET-15b, *E.coli* BL21(DE3), Virus Hepatitis B

ABSTRACT
EXPRESSION AND PURIFICATION OF RECOMBINANT HBcAg PROTEIN OF HEPATITIS B VIRUS IN *Escherichia coli* BL21(DE3)

Hepatitis B Virus (VHB) has infected 257 million people worldwide, causing approximately 880 thousand deaths annually. Indonesia is one of the countries in the Southeast Asian region with a moderate to high level of endemicity, with a prevalence of HBsAg ranging from 2.5% to 10%. Various preventive and control measures against hepatitis B, such as vaccination, have been implemented. One of the structural genes suitable for producing recombinant protein vaccines is *HBcAg*. *HBcAg* gene is immunogenic and capable of eliciting specific antibodies, helper T cells (Th), and cytotoxic T cells (CTLs). This study utilized a codon-optimized HBcAg gene, which was computationally modeled to investigate its physicochemical properties, protein modeling, antigenicity, allergenicity, and epitope prediction. The obtained results include aliphatic index, isoelectric point, and GRAVY index values of 83.02, 9.93, and -0.435, respectively. Antigenicity and allergenicity predictions indicated that HBcAg protein is antigenic and non-allergenic. Protein modeling results, assessed using the Ramachandran plot, showed that 91.1% of amino acid residues fall within the most favored regions, with no non-glycine residues in the disallowed regions. These parameters demonstrate that HBcAg protein possesses good and appropriate structural modeling. The *HBcAg* gene was then inserted into the pET 15-b plasmid vector for transformation into E. coli BL21(DE3) using the heat-shock method. The transformed bacteria were induced with 0.125-1 mM IPTG and purified using a Ni-NTA column, followed by western blot analysis. The obtained recombinant HBcAg protein had a size of 21 kDa, with a total protein concentration of 356 mg/L at 1 mM IPTG solubilization. Purification yielded a pure HBcAg protein concentration of 103 mg/L. Western blot analysis confirmed protein detection using anti-His-tag primary antibodies.

Keywords: *HBcAg*, pET-15b, *E.coli* BL21(DE3), Hepatitis B Virus