Construction and molecular analysis of hepatitis B virus core 1-144 + human immunodeficiency virus Gag recombination from Indonesian isolates

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Abstract. The prevalence of hepatitis B virus and human immunodeficiency virus infection is still high. One strategy to develop diagnostic and/or vaccine for the infection is by construction the recombinant protein from both viruses. To study the characterization of hepatitis B core 1-144 + human immunodeficiency virus Gag recombinant from Indonesian isolate, the hepatitis B virus core 1-144 was amplified from genomic HBV DNA isolated from Javanese blood samples. The human immunodeficiency virus Gag gene was amplified from pVLP HIV Gag, an expression plasmid expressing the human immunodeficiency virus Gag. The clones were then fused and sub cloned into an Escherichia coli expression plasmid. The recombinant plasmid was sequencing, and the recombinant gene sequencing results were subjected to bioinformatics analysis. Physicochemical analysis revealed the molecular weight (Mw), estimated half-life, instability index, isoelectric point, aliphatic index, and hydrophilicity of the recombinant protein. The antigenicity and epitope prediction also had been discussed. The results of this study would contribute information about hepatitis B core 1-144 + human immunodeficiency virus Gag recombinant from Indonesian isolate and benefits for further works willing to develop diagnostic and vaccine against the viruses.

1. Introduction
Hepatitis B infection is still a major global health problem affecting an estimated 350 million people in the world with approximately 1 million deaths annually due to complications, such as cirrhosis, liver failure and hepatocellular carcinoma [1]. The hepatitis B virus vaccine has decreased the hepatitis B virus infection, but some populations (five percent of normal people) do not develop protective responses, deemed hepatitis B virus "non-responders". Multiple strategies to improve the immunogenicity of the hepatitis B virus vaccine are currently being pursued, including recombinant vaccines [2]. Moreover, the serological assay based on HBsAg (hepatitis B antigen) testing may add little if any hepatitis B virus risk reduction value when hepatitis B virus nucleic acid testing and anti-HBe (hepatitis B core) screening also apply [3].

Human immunodeficiency virus (HIV) is the causative agent of the Acquired Immunodeficiency Syndrome (AIDS) [4]. HIV/AIDS remains a worldwide disease with significant mortality and morbidity [5]. While the decrease in human immunodeficiency virus incidence is achieved with
Certain coverages of diagnosis, care, and continued treatment, human immunodeficiency virus prevalence is not decreased and sometimes the test-and-treat is accompanied by the increased long-term cost of antiretroviral therapy [6]. Antiretroviral therapy now using as treatment of people living with human immunodeficiency virus but also for post-exposure prophylaxis, treatment as prevention, and, pre-exposure prophylaxis, however still not the best way to halt the ongoing human immunodeficiency virus epidemic [7, 8]. There is a consensus that only a preventive vaccine can contain and the best hope for eliminating the HIV/AIDS pandemic [9]. However, the failure to produce effective human immunodeficiency virus vaccine immunogens and the inability of conventional delivery strategies to elicit the desired immune responses remains a central theme and has ultimately led to a significant roadblock in human immunodeficiency virus vaccine development. Consequently, significant efforts have been applied to generate novel vaccine antigens and delivery agents, which mimic viral structures for optimal immune induction, including that of the virus-like particle formulations [10].

The individuals with human immunodeficiency virus infection are more susceptible to develop coinfections with hepatitis B virus, since the same routes of transmission of these pathogens [11]. The prevalence of chronic hepatitis B virus infection in human immunodeficiency virus-infected individuals is 5-15% and will have a higher level of mortality and morbidity rate than individuals with single infection only [12]. One strategy to develop diagnostic and or vaccine for the infection is by construction the recombinant protein from both viruses [10].

2. Materials and methods

Our research group (A-IGIC/ A-Infection, Genomics, Immunology & Cancer) performing a molecular epidemiology study of human bloodborne pathogens (including the human immunodeficiency virus and hepatitis B virus in Central Java, Indonesia [13-18]. All blood samples were subjected for serological and molecular assays to find out the human immunodeficiency virus, hepatitis B virus, hepatitis C virus, hepatitis D virus, Torque Teno virus, GB virus C, human T-cell lymphotropic virus, and Toxoplasma gondii (T. gondii) infection status. The human immunodeficiency virus RNA isolated from Javanese human immunodeficiency virus patient negative for other human bloodborne pathogens was used for cloning study. The pVLP HIV Gag, an expression plasmid expressing the human immunodeficiency virus Gag based on VariFlex N-terminal SBP-SET Vector as backbone plasmid (submitted for publication), was used for the present study. Briefly, the HIV Gag gene in the pVLP HIV Gag was amplified with mutagenesis polymerase chain reaction technique to add the additional sequences for fusing with the hepatitis B virus core 1-144 sequence and subcloning into the backbone plasmid using KOD -Plus- Mutagenesis (Toyobo, Osaka, Japan). The HIV Gag gene polymerase chain reaction product was then purified using Zymoclean Gel DNA Recovery (Zymo Research, Irvine, CA).

The hepatitis B virus core 1-144 was amplified from genomic hepatitis B virus DNA isolated from Javanese blood samples negative for other human bloodborne pathogens by nested polymerase chain reaction using MyFi Mix (Bioline, London, UK). The hepatitis B virus core 1-144 polymerase chain reaction product was purified using Zymoclean Gel DNA Recovery (Zymo Research) and then subjected for mutagenesis polymerase chain reaction to add the additional sequences for fusing with the HIV Gag gene and subcloning into the backbone plasmid using KOD -Plus- Mutagenesis (Toyobo). The hepatitis B virus core 1-144 polymerase chain reaction product was purified using Zymoclean Gel DNA Recovery (Zymo Research) and then fused with HIV Gag gene. The recombinant DNA was then subcloned into the Escherichia coli expression backbone plasmid. The recombinant plasmid was then transformed into competent cells, propagated, purified, and sequenced. The recombinant gene sequencing results were subjected to bioinformatics analysis.

The predicted hepatitis B core 1-144 + human immunodeficiency virus Gag protein analysis was performed using CLC Main Workbench 8.0.1 software. The parameters for hydrophobicity plot was set by Kyte-Doolittle, Eisenberg, Engelman, Hopp-Woods, Janin, Rose, Cornette hydrophobicity scale, respectively, with several residues - must be odd as 11. The non-redundant protein sequences
(nr) database with standard database genetic code was used for the blastp protein sequence analysis. Both Welling and Kolaskar-Tongaonkar antigenic scale was used to create the antigenicity plot of the recombinant protein, with a BLOSUM62 matrix with gap cost existence set as 11 and extension as 1 were used. The T Cell Epitope Prediction Tools from Immune Epitope Database (IEDB) Analysis Resource was used to predict the recombinant protein immunogenicity prediction and major histocompatibility complex-binding.

3. Results and discussion

3.1. Protein analysis results

The recombinant protein hepatitis B core 1-144 + human immunodeficiency virus Gag protein in C-terminal had 642 amino acids and 71.885 kDa in weight (Table 1).

Table 1. The hepatitis B core 1-144 + human immunodeficiency virus Gag protein characteristics.

| Protein statistics | Characteristics |
|--------------------|-----------------|
| Sequence information |                |
| Weight             | 71.885 kDa      |
| Isoelectric point  | 8.98            |
| Aliphatic index    | 78.1            |
| Atomic composition |                |
| hydrogen (H)       | 0.500 (n= 5,037)|
| carbon (C)         | 0.315 (n= 3,171)|
| nitrogen (N)       | 0.089 (n= 899)  |
| oxygen (O)         | 0.092 (n= 928)  |
| sulfur (S)         | 0.004 (n= 40)   |
| Count of residues  |                |
| Hydrophobic (A,F,G,I,L,M,P,V,W) | 0.489 (n= 314) |
| Hydrophilic (C,N,Q,S,T,Y)       | 0.262 (n= 168) |
| Other               | 0.249 (n= 160)  |
| Count of charged residues |                |
| Negatively Charged (D & E)       | 0.104 (n= 67)   |
| Positively Charged (R & K)       | 0.118 (n= 76)   |
| Other               | 0.777 (n= 499)  |

The recombinant protein constructed in the present study contains amino acid 1-144 of hepatitis B virus core in the N-terminal of the recombinant protein continued by the human immunodeficiency virus Gag protein in C-terminal of the recombinant protein. The predicted recombinant protein had methionine N-terminal half-life in Escherichia coli more than 10 hours, however, shorter than in mammals (30 hours) and in yeast (>20 hours), respectively. The extinction coefficient of the recombinant protein at 280 nm for non-reduced cysteines was 103,580 (absorption at 280 nm 0.1%= 1.441) while for reduced cysteines was 102,560 (absorption at 280 nm 0.1%= 1.427). The alpha helix secondary structures were found dominant in the recombinant protein (n= 27, at position 3-11, 17-22, 35-38, 42-46, 55-71, 81-101, 143-145, 148-151, 157-164, 172-189, 194-214, 216-238, 241-264, 278-282, 293-306, 310-319, 325-335, 338-357, 384-394, 426-429, 437-468, 474-481, 485-494, 503-521, 543-545, 570-575, 613-618, and 630-634, respectively), and only eight beta strand secondary
structures were found (at position 3-11, 26-28, 112-120, 126-137, 405-412, 416-420, 425, and 532-534, respectively).

3.2. Immunogenicity prediction results

The T Cell Epitope Prediction Tools from Immune Epitope Database (IEDB) Analysis Resource was used to predict the immunogenicity and major histocompatibility complex-binding for the recombinant protein. In total, 10 epitopes were found had a high affinity (percentile rank < 0.1) for major histocompatibility complex II-binding (Table 2), consistent with the antigenicity plot.

Table 2. The hepatitis B core 1-144 + human immunodeficiency virus Gag protein major histocompatibility complex-II binding prediction results.

| Allele                    | Position |
|---------------------------|----------|
| HLA-DRB3*01:01            | 24-38    |
| HLA-DRB3*01:01            | 25-39    |
| HLA-DRB3*01:01            | 26-40    |
| HLA-DRB3*01:01            | 27-41    |
| HLA-DRB1*15:01            | 413-427  |
| HLA-DRB1*15:01            | 414-428  |
| HLA-DRB1*15:01            | 415-429  |
| HLA-DRB1*15:01            | 416-430  |
| HLA-DRB1*15:01            | 412-426  |
| HLA-DQA1*01:01/DQB1*05:01| 21-35    |

Protection against human immunodeficiency virus infections seems to be the most challenging foe, with a remarkable, poorly immunogenic and fragile surface glycoprotein and the ability to overpower the cell immune system. Virus-like-particle vaccines for the human immunodeficiency virus have emerged as potent inducers of antibody and helper T cell responses, while replication-deficient viral vectors have yielded potent cytotoxic T cell responses [19]. Virus-like particles are composed of viral structural proteins which self-assemble into non-infectious particles that lack genetic material and resemble native viruses, thus safe and highly immunogenic to induce potent adaptive immune responses [20]. Hepatitis B virus tandem core platform, reported spontaneously assembles into virus-like particles that are immunogenic, confers immunogenicity to proteins incorporated into the major insertion region of core monomers, and reported successful for several infectious diseases [21-23]. In point of the human immunodeficiency virus, the Gag protein could form non-infectious viral particles [24]. In natural human immunodeficiency virus infections, immune responses to Gag are associated with a lower viral load in infected individuals. Priming immune responses to Gag virus-like particles elicits high-magnitude, broad polyfunctional responses, with memory T-cell responses appropriate for virus control [25]. Interestingly, since virus-like particles maintaining or mimicking the symmetry of pathogenic viruses, virus-like particles offer a ready platform for facilitating recognition, uptake, and processing by the immune system, therefore, could be used as diagnostic application [26]. In an attempt to develop diagnostic and or vaccine for hepatitis B virus and human immunodeficiency virus, we constructed the hepatitis B core 1-144 fused with human immunodeficiency virus Gag protein as backbone construction to develop the virus-like particles. Five epitopes in the hepatitis B core 1-144 and five epitopes in the human immunodeficiency virus Gag protein were found had a high affinity for Major histocompatibility complex II-binding with the high antigenic property, therefore, may be
potential as diagnostic or vaccine. However, more study is needing to be optimized the present construction, i.e. by serial mutagenesis to delete the unnecessary region.

4. Conclusions
The present study will contribute information about hepatitis B core 1-144 + human immunodeficiency virus Gag recombination protein, especially for further works willing to develop a diagnostic kit and/or vaccine against both viruses.

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