Expression of Hepatitis B viral surface antigen (Pre S1) in E. coli

Shahad Basel Ismail, Ibrahim Abdulla Ahmed, Yaseen Ismael Mamoori*
College of Biotechnology, Al-Nahrain University, Baghdad, Iraq

ABSTRACT

Hepatitis B is the most common liver diseases, which caused by hepatitis B virus (HBV) infection. There are around 257 million people around the world suffer from severe chronic hepatitis B infection. Therefore, it is necessary to develop a vaccine to prevent viral infection. PreS1 is one of the HBV envelope proteins that have been proved to be an effective vaccine. Accordingly, Viral DNA was purified from patients’ serums and amplified by PCR using specific primers. Amplicons of 324 bp bands of PreS1 was observed on gel electrophoresis. The PreS1 was cloned into pTXB21 plasmid to form the recombinant plasmid pTXB1_PreS1 and transformed into DH5α E. coli. Screening of transformants was done using Colony PCR and Sequencing. Alignment of 26 polypeptide sequences showed conservation of this region. The pTXB1_PreS1 was retransformed into T7 Express Competent E. coli and screened using colony PCR. The PreS1 was expressed as a recombinant protein fused to an intein tag with a molecular weight of ~39.5 kD. The PreS1 protein was purified by a single affinity chromatography step and after cleaved from intein tag by Dithiothreitol the obtained protein had a molecular weight of ~11.5 kD. Only one protein band was observed on the SDS-page gel. The PreS1 protein was successfully cloned and expressed in E.coli, which can be used as a vaccine against HBV.

*Corresponding Author
Name: Yaseen Ismael Mamoori
Phone: 
Email: yaseenismaeel@yahoo.com

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INTRODUCTION

Hepatitis B virus (HBV) is a very small virus belong to the Hepadnaviridae family. It is approximate 42-47 nm in diameter as the spherical infectious particle-containing lipid envelope (M, 1998; Liang, 2009). The virus was first discovered in 1965 by Blumberg and his colleagues as a new antigen called it Australia antigen (AuAg) and later on in 1976 he was awarded Nobel Prize in medicine (Gerlich, 2013; Block et al., 2016). HBV has double-stranded DNA genome with only 3.5 kb translated to four overlapping open reading frames (ORFs) which are core ORF (C- ORF), surface ORF (S- ORF), Polymerase ORF (P-ORF) and X-ORF (Lau and Wright, 1993; Gonzalez and Perrillo, 2014).

According to viral genome sequence divergence, the virus was classified into 10 genotypes (A-J). Genotype D is the most common genotype in the middle east (Gerlich, 2013; Lin and Kao, 2015; Paudel and Suvedi, 2019). This virus infects the human liver and leads to acute and chronic liver diseases. During the virus infection, some patients can cure and get rid of the viral infection while the others who cannot recover from the infection during 6 months the infection will develop to be a chronic infection (Johanek, 2019; WHO, 2019).

According to the WHO reports, 20-30% of chronically infected people can be developed to cirrhosis and liver cancer (Karayiannis, 2003; WHO, 2017).
The viral infection can be diagnosed by blood test detecting viral surface antigen (HBsAg), host antiviral antibodies and viral DNA (Song and Kim, 2016; WHO, 2019). According to WHO, there is no particular medication for acute hepatitis B infection; on the other hand, treatment of chronic infection is necessary to reduce the virus progression and liver damage (Lok, 2019; WHO, 2019). Interferon-alpha and polyethylene glycol-conjugated interferon have been used in the HBV treatment (Cornberg et al., 2017; Tsuge et al., 2017; Lok, 2019). Tenofovir and entecavir are two of the most common antiviral drugs that WHO has suggested for oral treatment to stop viral replication and reduce liver damage (WHO, 2019).

In order to prevent HBV infection, people have to get a vaccine especially infants and children (WHO, 2019). The first HBV vaccine was derived from blood serum after that second generation of the vaccine has been developed by producing the viral surface antigens (HBsAg) in yeast cells (Hilleman and Ellis, 1986; Yamaguchi et al., 1998; Sheng et al., 2017). In this study, samples have isolated from blood serum of Iraqis patients who have hepatitis B infection to amplify PreS1 gene and the most dominant sequence was cloned and expressed in E. coli to produce HBV PreS1 polypeptide which can be used as a possible vaccine.

MATERIALS AND METHODS

Samples

Blood serum was obtained from 50 patients who showed positive for HBV test (Hospital of the digestive system and liver diseases, Baghdad).

PreS1 gene amplification and recombinant vector construction

Viral DNA was extracted and purified from blood serum using Viral Nucleic Acid Extraction Kit II (Geneaid, Taiwan). The PreS1 gene was amplified by PCR using specific primer pair PreS1-F: 3’-GGTGGTCATATGGGGCAGAATCTTTCCACCA-5’ containing NdeI recognition site and PreS1-R: GGTGGTGCTCTTCCGCAGGCCTGAGGATGAG containing SapI recognition site. PreS1 amplicon as well as pTXB1 Vector (New England Biolabs, USA) were digested with NdeI and SapI restriction endonucleases and ligated together using T4 DNA ligase (New England Biolabs, USA). The recombinant vector pTXB1_PreS1 was transformed into DH5α E. coli (New England Biolabs, USA). Primary screening for real transformants was conducted using colony PCR and confirmed by sequencing.

Expression of PreS1 polypeptide

The pTXB1_PreS1 vector had been used to transform T7 Express Competent E. coli (New England Biolabs, USA) by heat shocking. The colonies were also screened by colony PCR to select the target colonies, which contain the PreS1 gene. The selected colonies were cultured in 50 ml Luria broth containing 100 μg/ml ampicillin overnight at 37°C and 250 rpm. Next day, 250 ml of culture was inoculated and incubated under the same culture conditions until the OD₆₀₀ reached 0.5-0.8. After that, IPTG as gene expression inducer had been added into the culture and returned into the incubator for 2-4 h incubation. Three IPTG concentration have been used 0.1, 0.4 and 0.8 mM. The bacteria were harvested by centrifugation at 5000 xg for 15 min at 4°C. The pellet was resuspended in 100 ml of ice-cold Column Buffer (20 mM Tris-HCl, pH 8.5; 500 mM NaCl) and cells were lysed by sonication. The cell lysate was clarified by centrifuged at 15,000 xg for 30 min at 4°C.

Pre S1 polypeptide purification

The protein was purified on 10 ml chitin column (New England Biolabs, USA) after washing the column with 10 column volume (CV) of column buffer. The clarified cell lysate loaded onto the chitin column at a flow rate of 0.5 ml/min. Then, the column washed with 20 CV of the column buffer after that the column flushed with 3 CV of the Cleavage Buffer (column buffer containing 50 mM DTT). The column left overnight at room temperature next day the released PreS1 protein was recovered and stored at -20°C.

Analysis of Pre S1 polypeptide on SDS-PAGE

The protein samples were mixed with 3X SDS Sample Buffer (New England Biolabs, USA), boiled for 5 min then 15 μl of the supernatant loaded into each well of SDS-PAGE. The gel was stained with a staining solution containing 0.1% Coomassie Brilliant Blue R-250 overnight with gentle agitation. Next day, the gel washed with distilled water and transferred into destaining solution and incubated at room temperature with gentle shaking. The destaining solution was changed several times until the gel background was clear. Then, the gel was documented using a Bio-Rad gel documentation system.

RESULTS

Amplification of PreS1 gene

DNA was purified from blood samples and PreS1 gene was amplified using the specific primers (PreS1-F+PreS1-R). DNA bands with around 324 bp were amplified Figure 1.

Both PCR product (PreS1) and the Plasmid pTXB1
Figure 1: Amplification of Hepatitis B PreS1 gene analyzed on 1% agarose gel at 5 V/cm for around 90 min. Lane M: DNA marker ladder (100bp, Bioneer, Taiwan), A: lanes 1-14; B: lanes 1-12; C: lanes 1-12 amplified PreS1 with 324 bp.

Figure 2: Cloning vector map of PreS1 gene and pTXB1 plasmid. A: pTXB1 plasmid (6706 bp), B: Integration of PreS1 gene into pTXB1 to form pTXB1_PreS1 recombinant vector (6982 bp).

were digested with NdeI and SapI restriction endonuclease and purified. After that, the digested plasmid and the preS1 were ligated to form the recombinant plasmid pTXB1_PreS1 as shown in Figure 2.

The recombinant vector was transformed into E. coli. Consequence, many transformant colonies were obtained and the transformants were screened using colony PCR followed by sequencing. Then, the sequence was aligned with the references sequence (Data not shown). Protein alignment of the obtained sequences was also conducted as shown in Figure 3. In this figure, two positions (40 and 79) were the most variable between tested samples.

Expression and analysis of PreS1 polypeptide on SDS-PAGE

The expression of the PreS1 polypeptide was induced using three different inducer concentrations introduced into the bacterial medium. After that, the bacteria that contain the recombinant vector was lysed and analyzed on SDS-PAGE as shown in Figure 4.
DISCUSSION

Many people worldwide are suffering from liver problems caused by Hepatitis B Virus infection (Lavanchy, 2004; Schweitzer et al., 1965). Hepatitis B surface antigen (HBsAg) can be produced as a diagnostic tool to detect virus infection and could be also used as a vaccine (Elghanam et al., 2012; Bian et al., 2017). However, this antigen can stimulate antibody production necessary for the clearance of Hepatitis B virus (Dienstag, 1982; Milich, 1987).

PreS1 region is a part of large (L) viral surface protein and this region exists in mature HBV virions and mediates the interaction of the virus with the entry receptors of the hepatocyte (Pontisso et al., 1989; Klingmüller and Schaller, 1993; Ganem and Prince, 2004). PreS1 polypeptide also plays essential roles in the assembly and infectivity of the hepatitis B virus (Persing et al., 1986; Bruss and Ganem, 1991). All these characteristics made this polypeptide a target for Hepatitis therapy.

This study was conducted to clone and express the most common PreS1 DNA sequence of the tested sera samples from Iraqi patients. Around 1493 sequences of the HBV genotype D were aligned (https://hbvdb.lyon.inserm.fr/HBVdb/) using the Sanpgene program and the most consensus sequence was chosen to be used as a template to design the forward and reverse primer pair. The PreS1 gene sequences were amplified in certain samples where the viral DNA concentration was enough to be amplified by using PCR as shown in Figure 1.

From the alignment shown in Figure 3, threonine
Figure 4: SDS-PAGE analysis of PreS1 polypeptide at 10V/cm for about 3 h: Photo A: optimization of IPTG inducer concentration. PhotoB: Purification of PreS1 polypeptide using affinity chromatography

at the position 40 were >65% in the reference sequence when compared to protein of the samples as well as threonine at position 79 was >55% in the samples when compared to alanine in the reference gene. The other variances are barely found. Consequently constructing four PreS1 polypeptide variants and include them simultaneously in the same vaccine with HbVs antigen could protect the patients from tolerance of HBVs antigen and might help in the clearance of the virus (Bian et al., 2017).

The amplified gene was cloned into pTXB1 plasmid which has many properties made it a very suitable plasmid for cloning and expression in E. coli. These include the relatively small size (6706 bp) and the ampicillin resistance as a selective marker as well as the T7 strong promoter. Furthermore, the desired protein is fused to an intein protein that contains the chitin binding domain (CBD) in which the protein can be purified easily by a single step using thiol groups such as Dithiothreitol (Pezza et al., 2004; Morassutti et al., 2005).

Prokaryotic expression systems particularly E. coli have many benefits such as they can be grown in inexpensive media and easy handling and high level expression of recombinant proteins (Mr and Sambrook, 2012). The capability of recombinant protein production by E. coli is several times higher than eukaryotic systems such as S. cerevisiae (Müller et al., 2006). Additionally, the continuous development of metabolic engineering tactics decreased the gap of glycoengineering in E. coli, which make it a valuable host for the production of the human therapeutic protein (Pandhal and Wright, 2010; Harding and Feldman, 2019).

CONCLUSIONS

In conclusion, The PreS1 polypeptide is conserved among samples tested with little variations in 40 and 79 positions of amino acid sequences. Furthermore, this polypeptide was expressed successfully as a fused protein and purified to homogeneity as PreS1 polypeptide. This polypeptide could be used as a candidate vaccine to eliminate chronic hepatitis disease.

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Conflict of Interest
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