Development of Recombinant Epitope-Based Peptide Vaccine Of Hepatitis B Virus: In Silico Study

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Abstract. Hepatitis B virus was first discovered by Blumberg and colleagues in 1965. The spread of Hepatitis B disease is alarming. The World Health Organization (WHO) in 2002 estimated that one billion living individuals have been infected with Hepatitis B, with 1-2 million deaths attributed to this virus, which attacks liver cells. Therefore, a vaccine is needed to address Hepatitis B virus disease. This study utilizes the Hepatitis B e-antigen in complex with scFv e13 with the code 6CVK. The virus has been inserted with the fusion protein scFv e13 antibody, obtained through the web server https://www.rcsb.org/. B-cell epitopes were identified using the IEDB immune epitope database web server. The results of this study recommend the peptides TLTSGVPSRFKGSGSGTE and SDTSTDTAFGGG as vaccine candidates to combat diseases caused by the Hepatitis B virus. Further studies are recommended to confirm this through in vivo and in vitro approaches.

Keywords: *Hepatitis B, Epitope-based vaccine, peptide, immunoinformatics.*

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1. Introduction

The Hepatitis B virus was first identified by Blumberg and colleagues in 1965, initially known as the Australian antigen. Hepatitis B is a member of the Orthohepadnavirus genus within the Hepadnaviridae family. It is a partially double-stranded virus with circular DNA. The outer strand, which is complete, is referred to as the negative strand, while the inner, incomplete strand is known as the positive strand. The outer strand, consisting of approximately 3200 nucleotides, encodes proteins, while the inner strand plays a role in the replication of the virus. Hepatitis B virus primarily targets liver cells [1].

The spread of Hepatitis B is alarming, with the World Health Organization (WHO) estimating in 2002 that one billion people worldwide have been infected with the virus, resulting in 1-2 million deaths. By 2008, the number of individuals infected had risen to 2 billion, with 350 million developing chronic Hepatitis B infections [2].

One approach to combat this disease is through vaccination, which has been proven to reduce the incidence and mortality rates of infectious diseases [3]. One method for vaccine development is through in silico analysis, which uses computer simulations with specific programming to identify compounds with high potential and selectivity [4]

The creation and development of a vaccine are essential in the fight against Hepatitis B. Advances in bioinformatics and biotechnology aid in vaccine development, enabling the efficient production of synthetic recombinant proteins containing epitopes using modern biotechnology methods [5].

Thus, recombinant epitope-based peptide vaccines are suggested as potential new candidates for effective Hepatitis B vaccines.

2. Method

Sample Collection from Database

The glycoprotein sequence of the Hepatitis B virus was obtained from the National Center for Biotechnology Information (NCBI), USA, and the Protein Data Bank (RCSB PDB), USA.

Domain Screening

Domains within the glycoprotein sequence of the Hepatitis B virus were identified by aligning protein sequences retrieved from NCBI, with 3D structures modeled using the Swiss Model web server (https://swissmodel.expasy.org/) via homology modeling. The resulting 3D models were then visualized using PyMol software.

Immunoinformatics Prediction

This study's predictions were based on identifying Hepatitis B epitopes likely to act as antigens, using the Vaxijen v2.0 web server (https://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html). The selected peptides were those with low scores, below 20%, to reduce the risk of autoimmune responses.

3. Results and Discussion

Determining Candidate Domains

Hepatitis B virus (HBV) is a small DNA virus within the Hepadnaviridae family. The infectious virion, also called the Dane particle, has a spherical, double-shelled structure, approximately 42 nm in diameter [6]. The candidate virus used in this study is the Hepatitis B capsid protein, specifically chain C (SKLCLGWLWGMDIDPYKEFGATVELLSFLPSDFFPSVRDLLDTAAALYRDAL ESPEHASPHHTALRQAILCWGDLMTLATWVGTNLEDPASRDLVVSYVNTNVG LKFRQLLWFHISALTFGRETVLEYLVSFGVWIRTPPAYRPPNAPILSTLPETTVV) as shown in Figure 1.

Candidate selection was performed using the website https://www.rcsb.org/ with a focus on the Hepatitis B e-antigen in complex with scFv e13, identified by code 6CVK. The virus was modified with a scFv e13 antibody fusion protein (AELVLTQTPSSVSAAVGGTVTINCQASQSISSRLGWYQQKPGQPPKLLIYGAS TLTSGVPSRFKGSGSGTEFTLTISGVQRDDAATYYCLGSDTSTDTAFGGGTEVV VKGGSSRSSSSGGGGSGGGGQEQLVESGGRLVPPGGSLTLTCTVSGIDLSSNAI SWVRQAPGKGLEYIGIIYGGSIPYYSRWAKGRFTISKTSTTVALKMSTLTASDT ATYFCARGKSDGDGYAAYRLDPWGLGTLVTISSLVPRGSHHHHHH), as shown in Figure 2. Antibodies in the scFv format (single-chain variable fragment) consist of variable regions of the heavy (VH) and light (VL) chains connected by a flexible peptide linker [7].

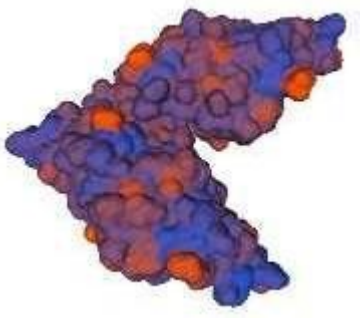

Fig. 1. Protein structure of Hepatitis B capsid protein

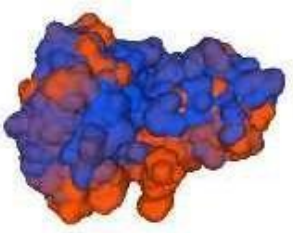

Fig. 2. Protein structure of Hepatitis B e-antigen in complex with scFv e13

Epitope Candidate Prediction

In the epitope candidate selection stage, the website http://tools.iedb.org/main/ was used to input sequences intended as vaccine candidates. Peptides selected as vaccine candidates had scores above the threshold of 0.350, indicated as positive predictions in yellow and negative predictions in green, as shown in Figures 3. B-cell epitopes recognized by the immune system were identified with a sequence length of more than 10 residues, as listed in Table 1. From the initial sequences, nine peptide candidates were identified.

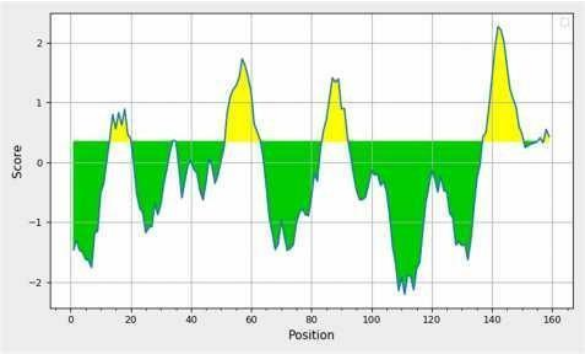

Fig.3. Epitope prediction graph showing positive predictions in yellow and negative predictions in green for potential vaccine candidates based on sequence scores.

TABLE 1. LIST OF CANDIDATE EPITOPES AND THEIR SEQUENCES

Candidate Epitope	Sequence
Peptide 1	LESPEHASPHHT
Peptide 2	RTPPAYRPPNAPIL
Peptide 3	TPSSVSAAVG
Peptide 4	YQQKPGQPPK
Peptide 5	TLTSGVPSRFKGSGSGTE
Peptide 6	SDTSTDTAFGGG
Peptide 7	EVVVKGGSSRSSSSGGGGSGGGGQEQLVE
Peptide 8	GRLVPPGGSL

Peptide 9	GKSDGDGYAAY

Antigenicity Test

An antigenicity test was conducted using the VaxiJen web server (https://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html). VaxiJen is a server used to predict the alignment of protective antigens derived from bacterial, viral, and tumor origins. The server's predictive model has been tested with internal cross-validation on a training set and external validation on a test set, achieving predictive accuracy between 70% and 89% [8]. The antigenicity test aimed to determine whether the peptide candidates could bind to antibodies, given that antigens are only detectable when the virus replicates [9]. Using the *in silico* approach, antigenicity was assessed for each peptide, as shown in Table 2. Four peptide candidates were identified as antigens.

TABLE2. ANTIGENICITY RESULT OF CANDIDATE PEPTIDES

Peptide Candidate	Sequence	Probability
Peptide 1	LESPEHASPHHT	probable non-antigen
Peptide 2	RTPPAYRPPNAPIL	probable non-antigen
Peptide 3	TPSSVSAAVG	probable antigen
Peptide 4	YQQKPGQPPK	probable antigen
Peptide 5	TLTSGVPSRFKGSGSGTE	probable antigen
Peptide 6	SDTSTDTAFGGG	probable antigen
Peptide 7	EVVVKGGSSRSSSSGGG-GSGGGGQEQLVE	probable non-antigen
Peptide 8	GRLVPPGGSL	probable non-antigen
Peptide 9	GKSDGDGYAAY	probable non-antigen

Allergenicity Test

At this stage, we determined whether the selected peptides would cause an allergic reaction, using the AllerTOP web server (https://www.ddg-pharmfac.net/AllerTOP/). AllerTOP is a free server used to predict allergens *in silico* based on the primary physicochemical properties of proteins [10]. Among the four selected peptides, only two were found to be non-allergenic, as shown in Table 3. In developing a vaccine, it is crucial to avoid adverse effects, including those causing allergies, to ensure vaccine safety [11].

TABLE 3. ALLERGENICITY RESULT OF CANDIDATE PEPTIDES

Peptide Candidate	Sequence	Result
Peptide 1	TPSSVSAAVG	probable allergen
Peptide 2	YQQKPGQPPK	probable allergen
Peptide 3	TLTSGVPSRFKGSGSGTE	probable non-allergen
Peptide 4	SDTSTDTAFGGG	probable non-allergen

Toxicity Test

Of the four peptide candidates, two passed the toxicity test. The toxicity test was conducted using the ToxinPred web server https://webs.iiitd.edu.in/raghava/toxinpred/protein.php, which identifies toxic regions in a specific protein sequence. The server first generates overlapping peptide

sequences and then predicts the toxicity of each peptide. This enables the easy identification of highly toxic regions within a protein [12]. To ensure vaccine safety, it is essential to confirm that no toxic properties are present in the vaccine candidate peptides, as shown in Table 4.

TABLE 4. TOXICITY RESULT OF CANDIDATE PEPTIDES

Peptide Candidate	Sequence	Result
Peptide 1	TLTSGVPSRFKGSGSGTE	non-toxic
Peptide 2	SDTSTDTAFGGG	non-toxic

Stability Test

The final stage in the vaccine design modeling process was to conduct stability tests on the peptide candidates. Stability testing was performed using the ProtParam web server (https://web.expasy.org/protparam/) to assess the stability of the selected peptides. As a result, two stable peptides were identified as suitable for vaccine development, as shown in Table 5.

TABLE 5. STABILITY TEST RESULT OF CANDIDATE PEPTIDES

Peptide Candidate	Sequence	Result
Peptide 1	TLTSGVPSRFKGSGSGTE	stable
Peptide 2	SDTSTDTAFGGG	stable

The *in silico* analysis in this study identified two peptide sequences, TLTSGVPSRFKGSGSGTE and SDTSTDTAFGGG, as potential vaccine candidates for Hepatitis B. These peptides demonstrated strong antigenicity, low allergenicity, non-toxic properties, and high stability, making them suitable for further development. The use of bioinformatics tools effectively streamlined the candidate selection process, focusing on peptides that meet multiple safety and efficacy criteria essential for vaccine development. However, further in vivo and in vitro studies are necessary to validate these findings and confirm the immunogenic potential of these peptides in biological systems.

4. Conclusion

This study successfully identified two promising epitope-based peptide candidates for a Hepatitis B vaccine using an *in silico* approach. The peptides TLTSGVPSRFKGSGSGTE and SDTSTDTAFGGG showed favorable properties, including antigenicity and stability, and minimal adverse effects, indicating their suitability for vaccine development. This research underscores the potential of computational methods in accelerating vaccine discovery, although laboratory-based validation remains essential for future application.

5. Conflict of Interest

The authors declared no conflict of interest.

6. Acknowledgment

None declared

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