

Design of a Recombinant Hepatitis B Vaccine Based on Stably Binding HLA-I Peptides

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Abstract

The hepatitis B virus continues to infect many millions of people worldwide. There is a need to develop new vaccines against the virus. Using immunoinformatics approaches, we have predicted epitopes within the hepatitis B virus surface antigen that can be used to develop a new vaccine. The predicted epitopes were then used to construct a synthetic vaccine. The interaction between the synthetic vaccine construct and major histocompatibility complex was also predicted using bioinformatics tools.

Keywords: Hepatitis B; Vaccine; *In silico*; Immune informatics

Introduction

One of the main strategies presently being evaluated for prevention of infectious diseases is the development of subunit vaccines [1]. Epitope based-vaccines offer several advantages over most traditional vaccines. This includes the precise control over activation of the immune response, the specific ability to focus on most relevant and usually highly immunogenic regions of the antigen in addition to production and biosafety advantages. More especially, the epitopes of CD4⁺ T-cells play a key role in the design of epitope based vaccines [2]. Mapping of epitopes *in silico* and combining it with *in vitro* and *in vivo* verification accelerates the process of discovering vaccines by approximately 10-20-fold [3]. Due to the time-consuming and costly nature of experimental screening of large sets of peptides, the *in silico* based methods that allow mapping of CD4⁺ T-cell epitopes on protein antigens are becoming indispensable in development of epitope based vaccines [4]. Although there have been improvements in the performance of methods used to predict the binding of MHC class II to antigenic peptides, evidence from a recent study [5] indicated that state-of-the-art methods are still unsuccessful in predicting CD4⁺ T-cell epitopes. Pan-specific approaches for predicting CD4⁺ T-cell epitopes, which are capable of coping with the extent of HLA class II polymorphism, have the major shortcoming of not fully accounting for the entire allotypic diversity of human ethnic populations [6]. Epitope prediction and construction is not only useful in vaccine development, but also in the prospective engineering of protein therapeutic agents. This helps reduce the risk of undesired immunogenicity and improve the chances of achieving success in the clinical use of such substances [7]. A major complex issue with the design of epitope-based vaccines is that most of the immune response is mounted against a few immunodominant epitopes. This occurs despite the presence of many potential epitopes within an immunogen. There is evidence suggesting that the peptide-MHC kinetic stability plays a central role in controlling MHC class II peptide immunogenicity [8]. This is further supported by other studies demonstrating that not all peptide binders are necessarily immunogenic. This implies that peptide immunogenicity is determined by factors other than binding affinity [9]. In this study, we describe the design of a synthetic peptide vaccine for Hepatitis B based on the stably binding antigenic regions of HBsAg. The molecular complex formed between the recombinant vaccine and MHC molecule is also described.

Materials and Methods

Sequence data

The amino acid sequence of the Hepatitis B Virus surface antigen (HBsAg) was retrieved from the UniProt database with accession number [Q773S4], available at the url: <http://www.uniprot.org/uniprot/Q773S4>. The complete protein had a sequence length of 226 amino acids.

Analysis of protein-MHC stability

The stability of the protein-MHC complex was analysed using the netMHCstab server [10] available at www.cbs.dtu.dk/services/NetMHCstab. The analysis was performed according to the instructions given on the server for entering the format of the protein sequence. The server was set to perform analyses on peptides of 14-mer length. Binding stability threshold was set to six hours for high stability binding and two hours for low stability binding respectively.

Construction of the recombinant vaccine

The sequence of six 14-mer peptides of HBsAg showing high and low binding stability was obtained from the net MHCstab server. In constructing the synthetic vaccine by the *in silico* method, the sequence of the six 14-mer peptides predicted as epitopes were joined together (by copying the epitopes sequence from the netMHCstab server and pasting these sequences close to each other) without any linkers between them. The synthetic vaccine construct was therefore having the following sequence (Table 1)

RFIIFLFILLLCLIIIFLIFILLLCLIFLLKYLW EWASVR
FSWLRFSWLSLLVPFVQWIWMMWYWGPSLYSILYSIVSPFIPLPI

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Received December 16, 2014; Accepted January 16, 2015; Published January 02, 2015

Citation: Ondieki MC, Nyaribari MC, Ejekwumadu NJ, Nyakang'o MJ (2015) Design of a Recombinant Hepatitis B Vaccine Based on Stably Binding HLA-I Peptides. J Biomol Res Ther 3: 120. doi: [10.4172/2167-7956.1000120](http://dx.doi.org/10.4172/2167-7956.1000120)

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HLA	peptide	Identity	Pred Th(hrs)	BindingLevel
HLA-A*24:02	RFIIFLFIILLCLI	Sequence 0.523	2.14	<= WS
HLA-A*24:02	IFLFILLLCLIFLL	Sequence 0.639	3.10	<= WS
HLA-A*24:02	KYLWEWASVRFSWL	Sequence 0.847	8.37	<= HS
HLA-A*24:02	RFSWLSLLVPFVQW	Sequence 0.542	2.26	<= WS
HLA-A*24:02	IWMMWYWGPSLYSI	Sequence 0.768	5.25	<= WS
HLA-A*24:02	LYSIVSPFIPLPI	Sequence 0.578	2.53	<= WS

Table 1: Binding levels of six peptide epitope regions of HBsAg and MHC
 Stability Threshold for highly stable peptides =6.000 hours
 Stability Threshold for weakly stable peptides =2.000 hours
 Number of highly stable bindings=1. Number of weakly stable bindings=5
 WS=Weakly Stable
 HS=Highly Stable

This sequence was predicted to have good binding stabilities with the MHC molecules and therefore considered as a candidate sequence for development of a new vaccine for hepatitis B.

Analysis of Recombinant vaccine-MHC stability

The complete sequence of the designed recombinant vaccine was put into netMHCstab server for analysis of recombinant vaccine-MHC stability. Once again, the server was set to perform analyses on peptides of 12-mer length. Binding stability threshold was set to six hours for high stability binding and two hours for low stability binding respectively.

Homology modelling of the Recombinant construct

Using the amino acid sequence of the recombinant construct, BLASTP [11] was used to identify a suitable template for modelling from the RCSB Protein Databank [12]. Subsequently, the crystal structure of proton-dependent oligopeptide transporter [PDB ID=4LEP] with a sequence identity of 33% was used as a template [13]. Thereafter, the three dimensional structure of the recombinant construct was modelled using the SWISS-MODEL tool [14] available within the ExPASy Bioinformatics resource portal [15]. The structure of the modelled recombinant construct was visualised using the Rasmol version 2.7.2 software [16].

Molecular docking between the recombinant construct and MHC

Docking between the recombinant protein and MHC was comparatively performed using Hex version 6.3 [17] and Patchdock [18]. The three dimensional structure of the recombinant vaccine-MHC complex was also visualised with the Rasmol software.

Results

Binding stability of HBsAg-MHC complex

HBsAg-MHC stability was found in six segments of the surface antigen of the hepatitis B virus. Five segments had low binding stability (threshold of 2 hours) while one had high binding stability (threshold of 6 hours). The most stable segment was bound for 8 hours and 37 minutes while the least stable segment was bound for 2 hours and 14 minutes as can be seen in (Table 1).

Recombinant vaccine-MHC stability

Recombinant vaccine-MHC stability was found to occur in twelve segments of the recombinant construct (2 of them highly stable and 10 weakly stable peptides). The most stable segment was bound for 16 hours and 26 minutes while the least stable segment was bound for 2 hours and 14 minutes as can be seen in (Table 2).

This sequence was taken as the appropriate sequence of the synthetic vaccine since it had a good predicted stability of binding with the MHC molecules.

Modelled structure of the Recombinant protein

Since the synthetic protein was not naturally occurring in nature *per se*, there was no good quality template for modelling found in the PDB database. Even then, a template with a less than 50% sequence identity was selected for the modelling purpose. The selected template was for the proton-dependent oligopeptide transporter [PDB ID=4LEP]. (Figure 1) illustrates the structure of the modelled synthetic vaccine.

Interaction between the synthetic peptide and MHC-I

(Figure 2) illustrates the structure of the complex formed between the synthetic vaccine and MHC-1. The synthetic vaccine can be seen bound at the open groove on the top of the MHC molecule, which is the actual site of binding for peptides within MHC molecules.

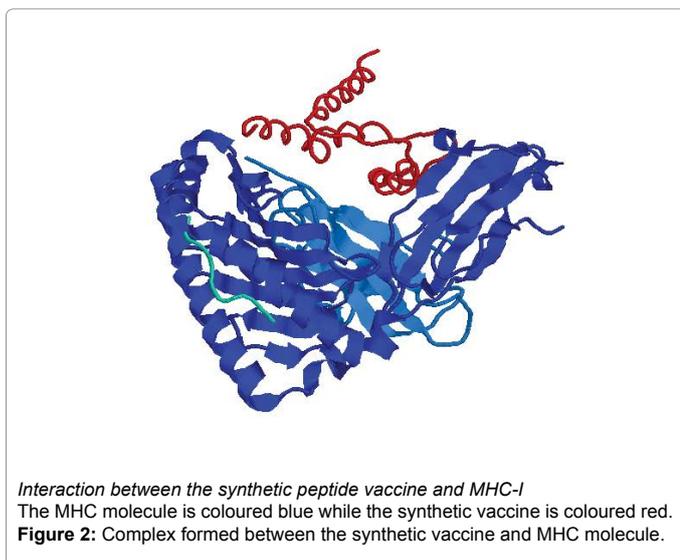
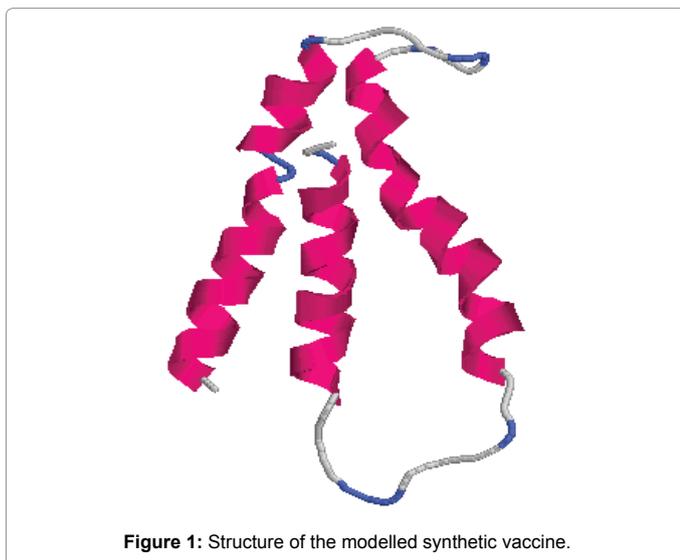
Discussion

Increasingly sophisticated technological advances in molecular biology and immunology in the last few years has had a very big impact in the identification of possible vaccines candidates for a number of diseases [19]. With our understanding of the virulence factors of infectious agents combined with the advent of biotechnology, a great revolution has been witnessed in the number of diseases which can be controlled by vaccination, including the new methods being exploited to create vaccines [20]. In this paper, we report the use of new approaches for identification of stably binding peptides of hepatitis B virus, which can be exploited in the future design of new vaccines. Generation of the three dimensional model for interaction of synthetic hepatitis B virus surface antigen and MHC molecules from this work helps to give an insight into the interaction of the two molecules. The Laboratory experimentation for efficient identification of MHC-binding peptides and T-cell epitopes is complemented by computational models. In our work, the results show the multiple binding stabilities between the

HLA	peptide	Identity	Pred Th(hrs)	BindingLevel
HLA-A*02:01	FIIFLFILLLCL	Sequence 0.526	2.16	<= WS
HLA-A*02:01	IIFLFILLLCLI	Sequence 0.523	2.14	<= WS
HLA-A*02:01	ILLLCLIIFLFI	Sequence 0.736	4.53	<= WS
HLA-A*02:01	LLLCLIIFLFIL	Sequence 0.775	5.44	<= WS
HLA-A*02:01	LLCLIIFLFILL	Sequence 0.596	2.68	<= WS
HLA-A*02:01	IIFLFILLLCLI	Sequence 0.523	2.14	<= WS
HLA-A*02:01	FLFILLLCLIFL	Sequence 0.856	8.91	<= HS
HLA-A*02:01	LLLCLIFLLKYL	Sequence 0.704	3.94	<= WS
HLA-A*02:01	FLLKYLWEWASV	Sequence 0.918	16.26	<= HS
HLA-A*02:01	LLVPFVQWIWMM	Sequence 0.612	2.82	<= WS
HLA-A*02:01	MMWYWGPSLYSI	Sequence 0.685	3.67	<= WS
HLA-A*02:01	ILYSIVSPFIPL	Sequence 0.770	5.30	<= WS

Table 2: Binding levels of twelve peptide epitope regions of the synthetic construct

Stability Threshold for highly stable peptides =6.000 hours
 Stability Threshold for weakly stable peptides =2.000 hours
 Number of highly stable bindings=2. Number of weakly stable bindings=10
 WS=Weakly Stable
 HS=Highly Stable



synthetic construct and MHC. To gain maximum benefit, the use of *in silico* models must be treated as experiments analogous to standard laboratory procedures. They must also be performed according to strict standards, requiring careful selection of data for model building, and adequate testing and validation. A wide range of web-based databases is available, including MHC-binding prediction programs [21]. The rational design of peptide vaccines aimed at T-cell immunity requires that the first step of identifying immunodominant peptides be accomplished. Specificities in each of the processing stages that precede antigen presentation influence the selection of T-cell epitopes along a protein sequence. The most selective of these processing stages is the binding of the peptides to the major histocompatibility complex molecules. Many of the predictive algorithms for peptide-MHC binding focus on this stage [22]. The systematic computational sequence-based predictions and experimental testing of epitopes for candidate vaccines is made possible in part by the availability of complete genome data of infectious microorganisms [23]. The combined search for epitopes in antigenic proteins using predictive algorithms and *in vitro* screening methods has given rise to the new area of *immuno-informatics*. This area of science allows for an expanded number of proteins that can be screened for development of vaccines at the same time narrowing the search to only those regions in antigenic proteins that are very likely to induce an immune response. It is estimated that, as the predictive algorithms improve with time, it will soon be possible to forego the numerous *in vitro* screening steps and move directly from genome sequence to vaccine design [24]. It is suggested that in the near future, the closer merge between docking algorithms and protein interface prediction software, structural databases, and techniques for sequence analysis should help bring about improved protein network interaction predictions. It will also help produce more accurate structural models regarding the fundamental molecular interactions within the cell [25]. This method of predicting epitopes on microbial antigens has previously been used and tested previously in combination with *in vitro* experiments with promising results [7]. It is believed that future *in vitro* experiments with the synthetic vaccine that we have proposed will yield considerable progress in the search for a better vaccine for the hepatitis B virus.

Conclusion

In this work, we have demonstrated the use of immunoinformatic approaches that can be used to develop new vaccines for the hepatitis B

virus. We recommend that further research (especially *in vitro* studies) is required to validate the use of the epitopes we have predicted for designing a new vaccine for hepatitis B virus. The sequence of the synthetic vaccine we have proposed can be chemically synthesized and used in such future *in vitro* studies in order to validate the acceptability of this sequence as a possible candidate for a vaccine against hepatitis B virus. Such attempts in validating *in silico* peptide sequences have been made with promising results. The approach we have used in this work will greatly facilitate future attempts to design synthetic vaccines and understand the molecular interaction between the vaccines and the immune system.

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