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Research Article

Design of a Recombinant Multi-Epitope Subunit Vaccine Targeting Glycoproteins of Herpes Simplex Virus Type 2 using an Immunoinformatics Approach

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Abstract
Herpes simplex virus type 2 (HSV-2) is a major global cause of genital herpes, characterized by its lifelong latency and high transmission rate, yet no licensed vaccine currently exists. This study aimed to design a recombinant multi-epitope subunit vaccine targeting HSV-2 envelope glycoproteins (gB, gC, gD, gH, and gL) using an immunoinformatics-guided approach. A total of 37 epitopes comprising cytotoxic T lymphocyte (CTL), helper T lymphocyte (HTL), and B cell targets were selected based on high antigenicity and confirmed to be non-allergenic and non-toxic. The final construct included adjuvants and immunostimulatory elements to enhance immune recognition. Structural analysis showed a favorable molecular weight of 92.7 kDa, high stability (instability index 39.16), and strong hydrophilicity (GRAVY score -0.367). Validation of the 3D model yielded an ERRAT score of 74.77 and 82.60% of residues within the most favored regions in the Ramachandran plot, indicating a high-quality and reliable conformation. Molecular docking simulations demonstrated strong binding affinities of the vaccine construct with MHC class I, MHC class II, TLR2, and TLR4. Highest binding affinity value of -335.03 kcal/mol for TLR4, supported by the formation of optimal hydrogen bonds and salt bridges indicating potential to elicit robust adaptive and innate immune responses. These findings indicate that the designed vaccine is structurally robust, immunologically promising, and suitable for further *in vitro* and *in vivo* evaluation against HSV-2 infection.

Keywords: HSV-2, glycoprotein, multi-epitope vaccine, immunoinformatics

1. INTRODUCTION

Infection caused by the Herpes simplex virus type 2 (HSV-2) remains a significant challenge in the field of sexually transmitted infections, given its latent nature, its ability to reactivate periodically, and the lack of an effective clinical vaccine [1]. HSV-2 is classified as a member of the subfamily *Alphaherpesvirinae*, which is characterized by its neurotropic properties and high affinity for genital epithelial tissue and the peripheral nervous system. The virus is transmitted chiefly through sexual contact and accounts for the bulk of genital herpes cases on a global scale. Nevertheless, there has been an uptick in the prevalence of HSV-1 infections in the genital region [2]. The most recent epidemiological data published by the World Health Organization indicates that approximately 491 million individuals aged 15 to 49 years, constituting approximately 13% of the global population in that age group, have been infected with HSV-2 in 2023 [3]. HSV-2 possesses a distinctive mechanism for establishing latent infection in dorsal ganglia, enabling the virus to persist in a state of dormancy for extended periods and subsequently reactivate due to various internal factors, including stress, immune system suppression, local trauma, ultraviolet radiation exposure, and hormonal changes, such as menstruation [4].

Despite considerable research over several decades, official authorities have not formally endorsed any vaccine to prevent HSV infection, nor have its immunological mechanisms been definitively elucidated. A promising pre-clinical vaccination study was conducted by Awasthi et al. [5], who designed a trivalent messenger RNA (mRNA)-based vaccine candidate targeting the three main immunogenic glycoproteins of HSV-2, namely gC, gD, and gE. The vaccine was developed using modified mRNA technology encapsulated in lipid nanoparticles (LNP), and its efficacy in preventing the development of genital lesions was evaluated in animal models, namely mice and guinea pigs. Structural analysis of HSV reveals the expression of multiple glycoproteins on

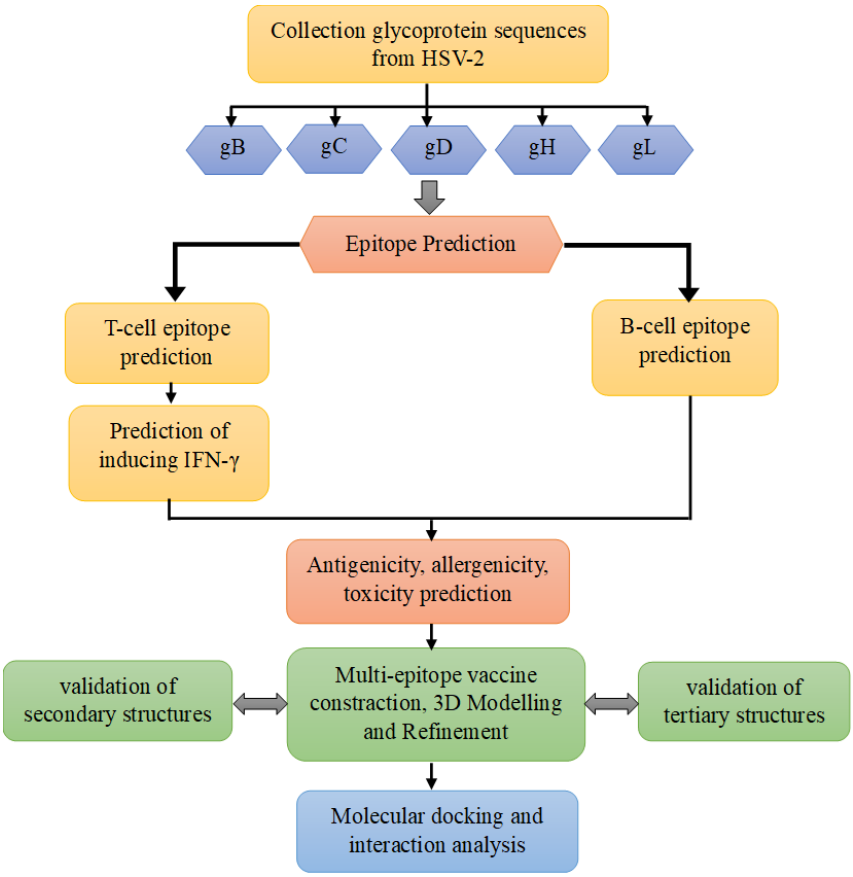

Figure 1. Flowchart for designing of a multi-epitope recombinant vaccine againts HSV-2.

the viral envelope. These glycoproteins mediate the fusion process between the virus and the host cell membrane [6]. A subset of these antigens exhibits high antigenicity and the capacity to elicit a robust adaptive immune response, rendering them optimal candidates for epitope-based vaccine design. Glycoproteins identified as directly involved in the virus's entry into host cells include gB, gC, gD, gH, and gL. These have been identified as key immunogenic components in various experimental vaccine studies [7].

Conventional vaccine development using whole organisms or large proteins faces several obstacles. The time required to develop a conventional vaccine is more than 15 years, which is very costly. On the other hand, conventional vaccines also pose health risks, including an increased risk of allergic reactions and the possibility of antigen mutation [8]. Vaccine design using an immunoinformatics approach, which leverages bioinformatics principles and the immune system, has been extensively studied for various pathogens [9]-[13]. Based on the previous discussion, a comprehensive study is needed to design a multi-epitope vaccine derived from the potential HSV-2 glycoproteins gB, gC, gD, gH, and gL, which are known to play a critical role in the viral fusion process with host cells and exhibit high antigenicity. The primary objective of this study is to develop a recombinant vaccine candidate capable of inducing a potent and specific adaptive immune response against HSV-2. The biological complexity of this virus, including the ability to evade immune control and to cause recurrent infections by latent mechanisms, makes it a priority target for vaccine innovation. Therefore, an immunoinformatics approach to designing multi-epitope vaccines is expected to overcome the limitations of conventional vaccines and provide more efficient, safe, and targeted preventive solutions against HSV-2 infection.

2. MATERIALS AND METHODS

2.1. HSV-2 Glycoprotein Sequence Collection

Amino acid sequence data for HSV-2 glycoproteins were obtained from the National Center for Biotechnology Information (NCBI) GenBank platform. The proteins examined include

Table 1. Prediction of CTL epitopes with MHC I on HSV-2 glycoprotein.

Glycoprotein	Allel	Epitope	IC_{50}	Immunogenicity	IFN-γ	Antigenicity	Allergenicity	Toxicity
gB	HLA-A*02:06	LVLAGLVAA	6.7	0.1160	+	0.6716	-	-
	HLA-A*02:06		9.8					
	HLA-B*35:01		30.9					
	HLA-A*02:06	FVLATGDFV	3.9	0.1925	+	0.6098	-	-
	HLA-A*02:01		46.1					
	HLA-B*15:01	GLVAAFFAF	13.4	0.3227	+	0.4222	-	-
	HLA-A*02:06		32.0					
gC	HLA-B*08:01	FAFRYVLQL	28.8	0.0483	+	0.8998	-	-
	HLA-B*58:01		100.7					
	HLA-A*02:01	GLAVGLWGL	11.1	0.2835	+	0.4931	-	-
	HLA-A*02:06		14.4					
gD	HLA-A*03:01	ALLVVAVGL	170.5	0.1696	+	1.0229	-	-
	HLA-A*68:01		186.9					
	HLA-A*01:01	RTQPRWSYY	109.9	0.0683	+	1.6117	-	-
	HLA-A*03:01		137.3					
	HLA-B*15:01		159.0					
	HLA-B*58:01		326.2					
gH	HLA-A*02:01	ALGYQLAFV	18.3	0.0145	+	1.1896	-	-
	HLA-A*02:06		69.3					
	HLA-A*02:06	VVMGVLVGV	5.0	0.0615	+	0.8118	-	-
	HLA-A*02:01		12.1					
gL	HLA-B*08:01	FLRYHCPGL	13.0	0.0042	+	0.7004	-	-

glycoprotein B (gB), glycoprotein C (gC), glycoprotein D (gD), and the glycoprotein H and L complex (gH-gL), each identified by accession numbers CAB06752. 1, ATB17435.1, AAS01730.1, ATB17565.1, and ATB17544.1, respectively (Figure 1).

2.2. Epitope Prediction

Epitopes recognized by cytotoxic T cells ($CD8^+$) were predicted using NetMHCpan 4.1, which is available in the Immuno Epitope Database (IEDB). Selection was based on binding affinity value (IC_{50} < 500 nM) with a peptide length of 9 amino acids [14]. Immunogenicity prediction was also performed using the T cell class I immunogenicity module of the IEDB. T helper cell ($CD4^+$) epitope prediction was performed using a similar method for MHC class II with a peptide length of 15 amino acids and the same affinity threshold. Candidate epitopes were then tested for their potential to induce interferon-gamma (IFN-γ) production using the IFNepitope platform [15]. Conversely, B cell epitopes are determined based on a default score threshold of 0.500, and the length of the analyzed peptide fragments ranges from 10 to 50 amino acids [16].

2.3. Prediction of Antigenicity, Allergenicity, and Toxicity

The validity of epitopes as vaccine candidates was assessed by antigenicity testing using the VaxiJen v2.0 server, with a default threshold of ≥ 0.4 for viral antigens [13]. Allergenicity potential was tested using the AllerTOP v2.0 platform, while toxicity prediction was performed using ToxinPred, which analyzes toxic potential based on peptide sequences [17][18].

2.4. Multi-epitope Vaccine Construct

Multi-epitope vaccine construction was performed by assembling selected peptide sequences and integrating adjuvant domains, namely the B subunit of cholera toxin (CTB) as a mucosal immune response enhancer [19][20]. Various linkers have been used to combine epitope domains, including EAAK, AAY, GPGPG, KK, and EGGE. The addition of the invasin domain from Yersinia at the C-terminal end is intended to enhance antigen delivery to immune cells [10][21].

2.5. Vaccine 3D Modelling and Refinement

The three-dimensional (3D) structural model of the designed vaccine construct was initially generated using the I-TASSER web server, developed by the Zhang Lab [22]. Following the generation of the preliminary 3D model, structural refinement was performed using the GalaxyWEB Refine server, to enhance the local and global stereochemical quality of the protein structure [23] [24].

2.6. Validation of Secondary and Tertiary Structure

The parameters used to analyze the physical and chemical properties include molecular weight, theoretical isoelectric point (pI), aliphatic index (AI), instability index (II), estimated half-life, and grand average of hydropathicity (GRAVY) using the ExPasy ProtParam web server [25]. On the other hand, ERRAT values, 3D verification tests, and Ramachandran plots were used as tertiary structure parameters. Tertiary structure analysis of the 3D vaccine model was performed using the Structure Analysis and Verification Server (SAVE) web server [26].

2.7. Molecular Docking Prediction and Interaction Analysis

The interaction between the vaccine and immune receptors (MHC class I, MHC class II, TLR2, and TLR4) was analyzed using the HDOCK platform to obtain binding affinity values [9][27]. The PDBsum database was utilized to visualize molecular interactions. This approach enabled the observation of the location of specific bonds and residues involved in the interactions, as well as the types of interactions [28].

3. RESULTS AND DISCUSSION

3.1. Epitope Prediction of HSV-2 Glycoproteins

NetMHCPan 4.1 method used to predict MHC I binding on (http://tools.iedb.org/mhci/). This method utilizes a specific binding prediction on human leukocyte antigen (HLA) in humans. The present study utilizes a set of 10 HLA alleles, selected based on the findings of preceding research, with the majority exhibiting a strong binding affinity to the previously identified epitopes [29]-[31].

Table 2. HTL epitope prediction results with MHC II on HSV-2 glycoprotein.

Glycoprotein	Allel	Epitope	Ic50	IFN-γ	Antigenicity	Allergenicity	Toxicity
gB	HLA-DRB1*04:01	EMIRYMALVSAMERT	14.3	+	0.4776	-	-
	HLA-DRB4*01:01		12.5				
gC	HLA-DRB5*01:01	AVVYLTHASSVRYRR	7.7	+	0.9661	-	-
	HLA-DQA1*05:01		3.0				
	HLA-DRB5*01:01	TAVVYLTHASSVRYR	10.8	+	0.9384	-	-
	HLA-DQA1*05:01		36.8				
gD	HLA-DRB5*01:01	FWVRRRAQMAPKRLR	5.1	+	1.1573	-	-
	HLA-DQA1*05:01		26.5				
	HLA-DQA1*05:01	IAFWVRRRAQMAPKR	13.3	+	1.3319	-	-
	HLA-DRB5*01:01		14.1				
	HLA-DRB5*01:01	RRAQMAPKRLRLPHI	13.7	+	1.1958	-	-
	HLA-DQA1*05:01		33.7				
	HLA-DRB5*01:01	WVRRRAQMAPKRLRL	4.2	+	1.0753	-	-
	HLA-DQA1*05:01		32.7				
gH	HLA-DRB1*01:01	ASSGFAFVNAAHAHD	3.2	+	0.7606	-	-
	HLA-DRB1*04:01		23.8				
	HLA-DRB1*01:01	LASSGFAFVNAAHAH	3.5	+	0.6774	-	-
	HLA-DRB1*04:01		28.4				
	HLA-DRB1*01:01	PGRYVYLSPSASTWP	3.3	+	0.6833	-	-
	HLA-DRB1*04:01		15.1				
gL	HLA-DRB5*01:01	ALYKEIRDALGSRKQ	37.3	+	0.5343	-	-
	HLA-DRB1*04:01		363.6				
	HLA-DRB1*01:01	DVSWRYEAPSVIDYA	136.9	+	1.4597	-	-
	HLA-DRB1*01:01		16.5				
	HLA-DQA1*05:01		163.5				
	HLA-DRB5*01:01		298.8				

Table 2. *Cont.*

Glycoprotein	Allel	Epitope	Ic50	IFN-γ	Antigenicity	Allergenicity	Toxicity
gL	HLA-DQA1*05:01	FGLVVMGAWGAWGGS	78.3	+	0.9451	-	-
	HLA-DRB1*01:01		19.8				
	HLA-DQA1*02:01		43.5				

MHC II binding predictions were performed using the NetMHCPan 4.1 prediction method with specific binding prediction for human HLA (Table 1). This method is an advanced approach developed from a collection of MHC peptide binding affinity data from the IEDB server [32]. The selection of several alleles was driven by their prevalence in interacting with epitopes and their coverage of a substantial global population [33]-[35].

Prediction of B cell epitope was conducted using the Bepipred Linear Epitope Prediction 2.0 method at the following website: https://tools.iedb.org/bcell/ [36]. The threshold score utilized is the default value of 0.500, and the length of the peptide sequence considered is 10 to 50 sequences [16].

Recombinant multi-epitope vaccines represent a novel class of immunotherapeutics characterized by integrating multiple epitopes designed to elicit targeted immune responses within the host organism [12]. Predicting CTL epitopes with MHC I and HTL with MHC II necessitates identifying specific HLA alleles to be targeted (Tables 2 and 3). HLA is part of the major histocompatibility complex (MHC) system in humans that plays a role in presenting epitopes on the cell surface so that they can be recognized by the T cell receptor (TCR) on T cells [13]. The inhibition concentration at 50% (IC_{50}) value was used as an affinity indicator, where peptides with $IC_{50} \leq 50$ nM were categorized as strong binders, while IC_{50} 50–500 nM were categorized as weak binders [14][32]. Conversely, B cell epitopes were predicted using Bepipred Linear Epitope Prediction 2.0, which the threshold score 0.500 was employed due to its ability to optimize the balance between sensitivity and specificity in epitope identification [37].

Subsequently, all epitope candidates from CTL, HTL, and B cells were subjected to a comprehensive evaluation encompassing antigenicity, allergenicity, and toxicity tests. Antigenicity testing was performed using the Kolaskar & Tongaonkar method via VaxiJen, which predicts antigenic potential based on the physicochemical properties of amino acid sequences. A threshold of ≥ 0.400 was used to indicate immunogenic epitopes [15]. Allergenicity testing is performed using AllerTOP, which employs an auto cross covariance (ACC) approach based on machine learning of known allergen

Table 2. Total polyphenol content (TPC) and %I of cinnamon extracts obtained by different methods.

Glycoprotein	Epitope	Peptide Length	Antigenicity	Allergenicity	Toxicity
gB	MVLRKRNKARYSPLHNEDEAGDE	23	0.8889	-	-
	WQEVDEMLRAEYGG	14	0.4746	-	-
	TNLTEYSLSRVDLG	14	0.9529	-	-
	ARKYNATHIKVGQP	14	0.9026	-	-
	RHEIKDSGLLDYTE	14	1.0245	-	-
gC	YATATDAEIGTAPSLEEVMVNVSAPPG	27	0.6369	-	-
	VFDPAQIHTQTQENP	15	0.6066	-	-
gD	HRDSVSFSRRNAS	13	1.1117	-	-
	AQMAPKRLRLPHIRDDDAPPSHQP	24	0.6930	-	-
gH	HDTYWTEQIDPWFLHGLGLARTYWRDTNT	29	0.6023	-	-
	TPDASDPQRGRLAPPGELNLTTA	23	0.8711	-	-
gL	CEGHAREIEPKRLVRTENRRDL	22	0.5897	-	-
	CMRTPADDVSWRYEAPSVIDYA	22	0.6811	-	-
	VFPADTQETT	10	0.5053	-	-

experimental data to classify sequences as allergenic or non-allergenic [17]. Toxicity testing was performed using ToxinPred, to identify toxic properties based on patterns from datasets of toxic and non-toxic proteins and peptides [18].

3.2. Construction of Multi-epitope Vaccine Sequences

The vaccine construction process was undertaken with the objective of incorporating a diverse array of protein sequences, with the intention of enhancing the cellular immune response. The selection of CTB adjuvant was motivated by its specific functionality as an adjuvant for pathogens that disseminate through mucosal surfaces [19][20]. The CTB adjuvant sequence was obtained from NCBI with the GenBank code ACO36766.1. The results of the multi-epitope vaccine sequence for HSV-2 with a length of 845 amino acid sequences are as follows Figure 2.

The construction of the multi-epitope vaccine involved sequential arrangement of selected epitopes along with the strategic incorporation of additional protein sequences to enhance cellular immune responses. An adjuvant, CTB was added due to its capacity to enhance mucosal and systemic immunity through binding to GM1 gangliosides, thereby facilitating antigen delivery to dendritic and other immune cells [19]. CTB has also been shown to induce strong Th1 and Th2 cytokine responses in mucosal pathogens such as HIV [20]. The Pan HLA DR-Binding Epitope (PADRE) was included to promote T-helper cell activation and improve antigen recognition across HLA alleles [38]. Specific linkers were essential to preserve epitope integrity and facilitate antigen processing by antigen-presenting cells (APCs). These included the rigid EAAK linker, AAY, GPGPG, KK, and EGGE [10]. Finally, a C-terminal invasin sequence derived from Yersinia was added to promote antigen internalization [21].

3.3. Vaccine 3D Modelling and Refinement

The creation of a 3D model of this multi-epitope vaccine aims to visualize it as a 3D protein model so that the structure of the aligned epitopes can be understood and evaluated (Figure 3). Three-dimensional protein modeling is also performed to enable further in silico analysis and simulation before entering *in vitro* and *in vivo* [24]. The 3D model of the multi-epitope vaccine was created

MIKLKFGVFFTVLLSSAYAHGTPQNITDLCAEYHNTQIHTLNDKIFSYTESLAGKREMAII
TFKNGATFQVEVPGSQHIDSQKKAIERMKDTLRIAYLTEAKVEKLCVWNNKTPHAIAAI
SMANEAAKAKFVAAWTLKAAAAAYLVLAGLVAAAAYFVLATGDFVAAYGLVAAFF
AFAAYFAFRYVLQLAAYLAVGLWGLAAYALLVVAVGLAAYRTQPRWSYYAAYALGY
QLAFVAAYVVMGVLVGVAAYFLRYHCPGLGPGPGEMIRYMALVSAMERTGPGPGAV
VYLTHASSVRYRRGPGPGTAVVYLTHASSVRYRGPGPGFWVRRRAQMAPKRLRGPGP
GIAFWVRRRAQMAPKRGPGPGRRAQMAPKRLRLPHIGPGPGWVRRRAQMAPKRLRLG
PGPGASSGFAFVNAAHAHDGPGPGLASSGFAFVNAAHAHGPGPGPGRYVYLSPSASTW
PGPGPGALYKEIRDALGSRKQGPGPGDVSWRYEAPSVIDYAGPGPGFGLVVMGAWGA
WGGSKKMVLRKRNKARYSPLHNEDEAGDEKKWQEVDEMLRAEYGGKKTNLTEYSLS
RVDLGKKARKYNATHIKVGQPKKRHEIKDSGLLDYTEKKYATATDAEIGTAPSLEEVM
VNVSAPPGKKVFDPAQIHTQTQENPKKHRDSVSFSRRNASKKAQMAPKRLRLPHIRDD
DAPPSHQPKKHDTYWTEQIDPWFLHGLGLARTYWRDTNTKKTPDASDPQRGRLAPPGE
LNLTTAKKCEGHAREIEPKRLVRTENRRDLKKCMRTPADDVSWRYEAPSVIDYAKKVF
PADTQETTEGGETAKSKKFPSYTATYQF

Color Representation: Adjuvant, PADRE, CTL Epitop, HTL Epitop, B Cell Epitop, Invasin

Figure 2. Multi-epitope vaccine sequence for HSV-2.

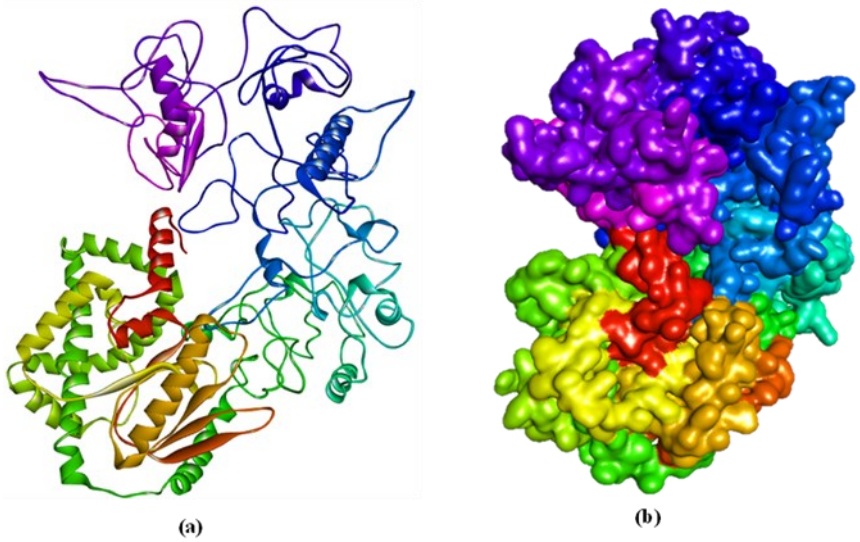

Figure 3. 3D model of a multi-epitope recombinant HSV-2 vaccine with (a) cartoon mode and (b) surface mode created on the I-TASSER web server.

using the Zhang Lab Prediction Iterative Threading Assembly Refinement (I-TASSER) web server (https://zhanggroup.org/I-TASSER/) [22]. The three -dimensional results of the vaccine model are then refined to improve the low resolution of the model to a higher resolution that is more similar to the original protein structure [23].

3.4. Validation of Vaccine Structure Protein

The secondary structure of the 3D vaccine model was subjected to both physical and chemical analysis. This was done in order to determine the characteristics of the designed protein. The determination of these characteristics was based on the arrangement, composition, and conformation of amino acid residues (Table 4).

The HSV-2 vaccine candidate protein has a molecular weight of approximately 92.7 kDa, which is below the 110 kDa threshold considered optimal for downstream processing, including purification, and is thus expected to retain favorable antigenic properties [39]. The pI of 9.94 indicates that the protein carries a net positive charge under physiological pH, a characteristic relevant for predicting solubility and biological interactions [40]. The AI of 71.15 suggests high thermostability, supporting the structural integrity of the protein during formulation and storage [41]. Furthermore, the II of 39.16, which is below the threshold of 40, classifies the vaccine construct as stable based on its amino acid composition [42]. The predicted half-life of the protein is approximately 30 h in mammalian cells and 10 h in *E. coli*, indicating moderate resistance to proteolytic degradation. A negative GRAVY score of –0.367 reflects the hydrophilic nature of the protein, which is advantageous for solubility and suitable for systemic administration via injection [39][43].

Tertiary structure validation was carried out to assess the structural accuracy and reliability of the 3D vaccine model through evaluations of geometric consistency, stereochemistry, and conformational stability [26]. The HSV-2 vaccine model yielded an ERRAT score of 74.77, well above the acceptable threshold of 50, indicating reliable non-bonded atomic interactions with deviations within the 3–4 Å range, typical of high-quality protein structures [44][45]. Verify 3D analysis, which compares the compatibility of the protein's 3D model with its amino acid sequence environment, showed that 80.12% of the residues scored acceptably, surpassing the 80% cut-off and confirming structural validity [46]. These validation results confirm that the HSV-2 multi-epitope vaccine model possesses a structurally sound and biologically plausible conformation, appropriate for further experimental development. In contrast, the Ramachandran plot results indicate that the HSV-2 vaccine shows 82.6% of residues in the most favored regions, 14.0% in the additional allowed regions, 1.0% in the generously allowed regions, and 2.4% in the disallowed region.

The analysis results indicate that 82.6% of HSV-2 residues are located in the most favored regions, suggesting that the structural model quality is satisfactory with resolution estimates ranging from 3–4 Å [47]. Concurrently, the proportion of residues in disallowed regions was 2.6% and 2.4%, respectively, which remained well below the 15% threshold. These findings suggest that both protein models possess valid structural conformations and are suitable for further analysis [48]. In light of the findings, it can be posited that the two vaccine structural models under consideration possess adequate quality and are deemed appropriate for subsequent phases of vaccine development.

3.5. Molecular Docking and Interaction Analysis

By performing molecular docking simulations on a selection of immune cells, the capacity of vaccine candidates to activate diverse components of the immune system can be ascertained [9]. The magnitude of the binding affinity value is directly proportional to the strength of the bond formed between the vaccine protein and the target protein [27] Furthermore, an increase in the number of hydrogen bonds and salt bridges formed, as well as a decrease in the average distance between them, has been shown to result in increased protein stability [28].

Molecular docking simulations were utilized to assess the potential interactions between HSV-2 vaccine candidates and various immune system target proteins, including MHC class I, MHC class II, TLR2, and TLR4. The simulation results indicate that the HSV-2 vaccine has a binding affinity value of -280.77 kcal/mol toward MHC class I, reflecting high affinity and thermodynamic stability of the interaction (Table 5). This value aligns with the findings of previous studies by Kar et al. [9] and Alharbi et al. [12], who reported that binding affinity values above -200 kcal/mol are considered strong and stable. The HSV-2 vaccine establishes an average of 9 hydrogen bonds with MHC class I, with a mean distance of 2.80 Å. According to Jeffrey's classification [49], this falls within the category of moderate electrostatic bonds, with a range of 2.5–3.2 Å indicating moderate bond strength. Furthermore, the formation of three salt bridges has been demonstrated to enhance the stability of the interaction between the vaccine and

Table 4. Results of secondary structure validation parameters of vaccine design.

Vaccine	Molecular weight (kDa)	Theoretical isoelectric point (pI)	Aliphatic index (AI)	Instability index (II)	Estimated half-life	(GRAVY)
HSV-2	92.7	9.94	71.15	39.16	• 30 h in mammals and *in vitro* • >10 h in *E. coli* and *in vivo*	-0,367

Table 5. Results of molecular docking simulation of HSV-2 with target proteins.

Target Protein	Binding Affinity (kcal/mol)	Interaction	Bonds	Average Distance (Å)
MHCI	-280.77	28–29 residue 3 salt bridges 9 hydrogen bond 224 nobonded contact	***Hydrogen Bonds*** Asn86–Gln346 (3.11 Å), Arg131–Arg233 (3.03 Å), Gln141–Tyr236 (2.85 Å), Arg145–Tyr236 (2.95 Å), Arg145–Tyr236 (2.55 Å), Gln155–Asp619 (2.81 Å), Thr163–Gln822 (2.79 Å), Thr163–Lyr832 (2.21 Å), Trp167–Glu826 (2.92 Å) ***Salt Bridges*** Arg65–Glu611 (2.90 Å), Glu148–Arg233 (3.87 Å), Glu166–Lys832 (3.69 Å)	2.80 3.48
MHCII	-266.05	30–45 residue 2 salt bridges 12 hydrogen bond 278 nobonded contact	***Hydrogen Bonds*** Lys38–Gln188 (2.93 Å), Thr41–Gln188 (3.30 Å), Glu47–Trp234 (3.24 Å), Glu55–Arg184 (2.40 Å), Glu98–Lys834 (2.90 Å), Arg100–Tyr620 (1.89 Å), Pro102–Arg233 (2.71 Å), Pro102–Arg233 (1.47 Å), Asp152–Ser235 (3.21 Å), Thr154–Gln231 (2.68 Å), Ser167–Glu829 (2.66 Å), Trp188–Ala831 (2.78 Å) ***Salt Bridges*** Glu55–Arg184 (2.40 Å), Glu98–Lys834 (2.90 Å)	2.68 2.65
TLR2	-277.01	28–33 residue 3 salt bridges 11 hydrogen bond 201 nobonded contact	***Hydrogen Bonds*** Lys37–Pro690 (2.98 Å), Ser42–Arg609 (2.76 Å), Asp58–Arg594 (2.92 Å), Ser60–Arg594 (3.11 Å), Asn61–Tyr596 (2.42 Å), Ser85–Glu632 (2.82 Å), Tyr11–Thr629 (2.81 Å), Met159–Arg387 (3.23 Å), Thr161–Arg389 (3.21 Å), Asp185–Arg389 (2.66 Å), Pro320–His432 (3.20 Å) ***Salt Bridges*** Asp58–Arg594 (2.92 Å), Asp185–Arg387 (3.86 Å), Arg321–Asp433 (1.23 Å)	2.92 2.67

Table 5. Results of molecular docking simulation of HSV-2 with target proteins.

Target Protein	Binding Affinity (kcal/mol)	Interaction	Bonds	Average Distance (Å)
TLR4	-335.03	30–40 residue 3 salt bridges 13 hydrogen bond 191 nobonded contact	***Hydrogen Bonds*** Asn156–His392 (2.74 Å), Asn156–His392 (3.33 Å), Ser360–Ala404 (2.53 Å), Lys362–His326 (2.64 Å), Arg382–Met406 (2.52 Å), Asp428–Lys409 (3.02 Å), Tyr451–Lys409 (2.68 Å), Asp453–Lys409 (2.64 Å), His552–Ser421 (3.35 Å), Thr553–Ser421 (2.91 Å), Thr553–Ser421 (2.91 Å), Ser580–Ser421 (2.96 Å), Lys582–Ser420 (2.85 Å) ***Salt Bridges*** Asp294–Arg373 (3.38 Å), Asp428–Lys409 (3.02 Å), Asp453–Lys409 (2.64 Å)	2.85 3.01

MHC class I [50].

The HSV-2 vaccine candidate demonstrated a binding affinity of -266.05 kcal/mol when interacting with MHC class II. This interaction resulted in the formation of 12 hydrogen bonds, with an average distance of 2.80 Å. The formation of two salt bridges was also observed, indicating the presence of bond stability and a strong interaction capacity [51]. In the course of interactions with TLR2, a binding affinity value of -277.01 kcal/mol was obtained, accompanied by the formation of 11 hydrogen bonds at an average distance of 2.95 Å, as well as three salt bridges that serve to enhance the stability of the complex further [52]. For TLR4, the binding affinity value reached -335.03 kcal/mol, with the formation of 13 hydrogen bonds and an average distance of 2.85 Å. The vaccine also formed three salt bridges, with an interaction distance of approximately 3.01 Å, which falls into the category of moderate electrostatic bonds [53] [54]. The results of the present study indicate that the HSV-2 vaccine has significant potential to induce adaptive and innate immune responses through the activation of various molecular pathways of the immune system.

4. CONCLUSIONS

The design of a recombinant multi-epitope glycoprotein-based herpes vaccine for HSV-2 consists of 10 CTL, 13 HTL, and 14 BCL epitopes combined with adjuvants CTB, PADRE, and invasin sequences. These components are linked using specific linkers to maintain structural stability and immunogenic efficacy. Structural analysis showed a favorable molecular weight of 92.7 kDa, high stability (instability index 39.16), and strong hydrophilicity (GRAVY score -0.367). Validation of the 3D model yielded an ERRAT score of 74.77 and 82.6% of residues within the most favored regions in the Ramachandran plot, indicating a high -quality and reliable conformation. Molecular interaction simulations indicate high binding affinity and stable interactions with MHC I, MHC II, TLR2, and TLR4 immune receptors, with the highest binding affinity value of -335.03 kcal/mol for TLR4, supported by the formation of optimal hydrogen bonds and salt bridges. In consideration of the aforementioned results, it is evident that the

HSV-2 vaccine candidate exhibits considerable promise for advancement in the domains of *in vitro* and *in vivo* testing as a potential genital herpes vaccine.

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Conflicts of Interest

The authors declare no conflict of interest.

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