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Immunoinformatics Approach for Glycoprotein B-based Vaccine Candidate Design Against Infectious Laryngotracheitis Virus

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Abstract

Introduction: Infectious Laryngotracheitis Virus (ILTV) poses a highly contagious upper respiratory tract illness and is regarded as a major concern for the poultry industry worldwide. Considering the emergence of novel virulent variants of ILTVs, the development of novel vaccines is needed to control the disease. This study was carried out based on immunoinformatics and vaccinology strategies to introduce an effective ILTV vaccine candidate targeting glycoprotein B (gB).

Materials and Methods: Both T-cell and B-cell epitopes of the gB protein with the potential to induce immune responses were identified. The highly antigenic and immunogenic PH-1 domain was selected as the potential vaccine candidate. The physiochemical properties and tertiary structure model of the domain were predicted, refined, and validated. The resulting high-quality model was applied for docking analyses with toll-like receptor 2. The affinity of the vaccine candidate to bind with the immune receptor and generate the appropriate immune responses was calculated based on the free energy level.

Results: The results indicated the PH-1 domain of ILTV gB protein to be immunogenic and was effective in stimulating the T helper, T cytotoxic, and B cells. The domain showed high-affinity binding and stability with the TLR2 immune receptor.

Conclusions: Collectively, this research provides an immunogenic candidate for designing a protein-based vaccine against ILTV. The efficacy of the construct should be examined in lab settings and in animal models.

Keywords: Infectious Laryngotracheitis Virus, Glycoprotein B, Vaccine Candidate, Immune Response

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Introduction

Infectious laryngotracheitis (ILT) is an important upper respiratory disease of chickens caused by ILTV; a member of Herpesviridae family, *Alpha herpesvirinae* subfamily, *Gallid alpha herpesvirus 1* species. The respiratory and ocular routes are the natural portal of ILTV entry resulted in severe damage to tracheal, infraorbital sinuses, larynx, and conjunctiva. Clinically, ILT appears in peracute, subacute, and chronic or mild forms. The high morbidity and mortality rate are influenced by the clinical appearance of the disease.[1] The administration of the modified live attenuated ILTV vaccines is suggested to prevent and control the spread of the virus in the endemic areas.[2] However, vaccination may increase the risk of reversion to virulence form of ILTV as well as the creation of carrier birds during bird-to-bird passage in the field due to the latency feature of herpesviruses. Carriers as the source of infection cause further spread of the disease to the susceptible birds.[3] To overcome the problem, developing a new generation of vaccines through genomic-based technologies has been investigated.[3-8]

The subunit vaccine contains the fragment(s) of antigenic proteins which may be an attractive alternative strategy to induce the appropriate immunological response against ILTV as well as prevent chickens from being carriers. The linear dsDNA genomes of ILTV contain 80 open reading frames including a unique long (UL) and unique short (US) flanked by inverted repeat (IR) and terminal repeat (TR) regions, which encode envelop, tegument, capsid, and non-structural proteins.[9,10] The envelopes containing glycoproteins namely gB to gM are encoded by eleven highly conserved open reading frames located in the UL and US regions. In the early stage of ILTV entry, gD binds to 3-O-sulfated heparan sulfate receptor of the host cell surface proteoglycans. Then two regions of gB (UL27) at position 124-130 and 206-213 involve in membrane fusion in assistance with the heterodimer gH/gL, and gG.[11-13] Within the gB structure, the conserved PH-1 and PH-2 domains at positions 104-311 and 313-410 are defined as a specific combination of secondary structures organized into a characteristic three-dimensional (3D) structure or fold.[14]

The ILTV glycoproteins play a critical role in adaptive immunity by eliciting humoral and cell-mediated immune responses in the host.[13,15,16] The generation of an appropriate

immune response is dependent on the interaction between the virus immunogen ligand and the host cell receptor. In response to herpesviruses infection, the Tool Like Receptor (TLR) signaling cascade is activated for recognition of viral glycoproteins by monocyte derived dendritic cells. In this regard, gB-gH/gL complex binds to TLR2 and stimulates the cell surface receptor leading to up-regulation of both inflammatory cytokines and type I interferon.[17,18] Previous studies have been shown that the administration of purified gB,[16] the DNA vector encoding gB,[5] and a recombinant fowl poxvirus construct expressing gB protect the challenged chickens from ILTV infection.[19] Vaccination of chickens with the recombinant Newcastle disease virus expressing ILTV gB conferred significant protection against virulent ILTV and NDV challenges [8]. In order to develop a safe and efficacious vaccine against ILTV, here, a computational workflow using bioinformatics tools was designed. We have explored gB as the highest antigenic protein followed by the prediction of B- and T-cell epitopes with their corresponding major histocompatibility complex (MHC) alleles. The 3D structure was modeled and the binding interaction between the virus peptide and cellular receptor was analyzed by molecular docking.

Materials and Methods

Protein Sequence Data Retrieval

The amino acid sequences of gB of ILTV isolates deposited in GenBank were retrieved from the NCBI database (https://www.ncbi.nlm.nih.gov/protein). Conservation regions were determined using ClustalW in the BioEdit software version 7.2.5. The protein functional information was retrieved from the UniProtKB database (https://www.uniprot.org) with retrieve/IDH9B021. The probability of the antigenicity of the conserved peptides was predicted based on auto cross covariance transformation of protein sequence by VaxiJen server (http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html).

B Cell Epitope Prediction

Linear B cell epitopes of ILTV gB were predicted using a BepiPred-2.0 with the threshold value = 0.50 and a maximum distance of six based on a Random Forest algorithm in Immune Epitope Database (http://tools.iedb.org/bcell/). The antigenicity, surface accessibility, flexibility, and hydrophilicity as important properties for B-cell epitope were screened using the IEDB prediction tools. The antigenicity of the sequences was predicted using Kolaskar and Tongaonker, surface accessibility using Emini, flexibility using Karplus and Schulz, β-turn using Chou and Fasman, and hydrophilicity using Parker methods. Discontinues B cell epitopes were predicted by Ellipro at IEDB, which works by calculating protrusion index (PI) and clustering neighboring residues.

T Cell Epitope Prediction

The potent immune protective T-cell epitopes are bound in a linear form to MHC through interactions between their R group side chains and pockets located on the floor of the molecules. The ability of ILTV gB cytotoxic T-lymphocyte (CTL) epitopes to interact with MHC class I was predicted using the mapping algorithms at http://tools.iedb.org/mhci/. This tool combines predictors of proteasomal C-terminal cleavage which plays a vital role in determining CTL epitope, TAP transport, and MHC binding to produce an overall score for each peptide's intrinsic potential of being a T cell epitope. The binding affinity expressed by half-maximal inhibitory concentration (IC_{50}) value was calculated using the artificial neural network (ANN)-based approaches. The MHC class I alleles having a high binding affinity of IC_{50}<50 nM proposed to be the most potent epitopes. The interaction of the T cell epitopes with MHC class II alleles was predicted using the binding prediction tool at http://tools.iedb.org/mhcii/ available at IEDB.

Physiochemical Properties Prediction

Based on the primary prediction data, the PH-1 domain of ILTV gB was selected for vaccine design. The physiochemical parameters included amino acid composition, theoretical isoelectric point (pI), half-life, aliphatic index, and grand average of hydropathicity (GRAVY) which were assessed using the ProtParam (http://web.expasy.org/protparam/).

Transmembrane Domain Prediction

The transmembrane domain of PH-1 was analyzed by the TMHMM Server (http://www.cbs.dtu.dk/services/TMHMM-2.0/).

Secondary and Structure Prediction

The secondary structure of the peptide was predicted by SOPMA (https://npsa-prabi.ibcp.fr/). The unstructured or disordered regions were predicted by the IUpred2A server (http://iupred2a.elte.hu.) where, scores above 0.5 correspond to disordered residues/regions.

Homology Modeling

The 3D structure homology modeling was carried out using the SWISS-MODEL server (http://expacy.org), which performs template selection, alignment and model building in an automated process. Then the best 3D models were selected according to the Global Model Quality Estimation (GMQE) and QMEAN statistical parameters. The overall stereo-chemical quality was checked according to PROCHECK by plotting a Ramachandran plot (http://mordred.bioc.cam.ac.uk/~rapper/rampage.php). The ProSA-web (https://prosa.services.came.sbg.ac.at/prosa.php) and also ERRAT (http://nihserver.mbi.ucla.edu/ERRATv2) were used for detecting the potential errors in the predicted models and validation of them. The quality of the predicted 3D model was assessed by ProTSAV (http://www.scfbio-iitd.res.in/software/proteomics/protsav.jsp).

Molecular Docking of ILTV gB Protein with TLR2

Interaction between an antigenic protein and the cellular TLR is responsible for the generation of appropriate immune responses. The binding interaction between TLR2 (PDB ID:6NIG) and the viral glycoprotein was predicted by using InterProSurf server (http://curie.utmb.edu/). The server predicts the amino acid residues based on their solvent accessible surface area that propensities most likely to be responsible for protein interaction. The binding pockets were predicted by using CASTp server (http://sts.bioe.uic.edu/castp/). The server provides a quantitative characterization of interior voids and surface pockets of a protein, which are frequently associated with binding. The volume and area of each pocket and void measure analytically using the solvent accessible Richards' surface model with a default value 1.4 Å. Molecular docking was performed using ClusPro 2.0 (https://cluspro.org) and the high ambiguity-driven protein–protein docking (HADDOCK) v2.4-2021.01 (http://haddock.science.uu.nl/) to check the consistency of the prediction. ClusPro server uses energy based filtering and clustering property algorithms for ranking the docked structures. Binding affinity and stability of the docked protein-protein complexes was determined using PRODIGY (http://milou.science.uu.nl/services/PRODIGY), which works based on pair-wise intermolecular contacts within 5.5 Å distance threshold. The binding affinity and stability were calculated by Gibbs free energy (ΔG) and dissociation constant (K_d) at 25.0 °C. Docking validations in HADDOCK are run by ambiguous interaction restraints, which define through the active and passive residues. In the final refinement stage, the complexes are clustered based on the interface pairwise Root Mean Square Deviations (iRMSD) and a minimum number of members to define a cluster. The clusters are then ranked based on the average score. The HADDOCK scoring function consists of a linear combination of standard energies including van der Waals energy (E_{vdW}), electrostatic energy (E_{elec}), desolvation energy (E_{desol}), energy from restraint violations (E_{AIR}), and the buried surface area (BSA). The score was again determined after simulation and water refining that is HADDOCK score = $1.0_{EvdW} + 0.2_{Eelec} + 1.0_{Edesol} + 0.1_{EAIR}$. In addition, the *z*-score represents how many standard deviations the HADDOCK score of a given cluster is separated from the mean of all clusters, that is, the best docking has the highest negative scores and the lower z-score.

Results

The 67 amino acid sequences of ILTV gB were retrieved from GenBank in FASTA format. Alignment of all retrieved sequences showed high conservancy between the aligned sequences. Two highly conserved regions at 114-321 and 323-420 amino acid position were recognized by identity and similarity of amino acid sequences. Based on the UniProtKB database, the regions are PH-1 and PH-2 domains, respectively. These sequences were screened out using VaxiJen for identification of potential antigenicity at threshold of 0.5. The PH-1 domain was identified as more potent antigenic protein compared to the PH-2 domain; 0.6042 vs 0.5710 overall prediction score.

Table 1. Prediction of the Linear B-cell Epitopes Glycoprotein B Infectious Laryngotracheitis Virus

No.	Start	End	Peptide	Lenght
1	30	71	LSQDSHGIAGIIDPHDTASMDVGKISFSEAIGSGAPKEPQIR	42
2	92	101	RHCHRHADST	10
3	146	163	EYVTRVPIDYHEIVRIDR	18
4	183	183	Y	1
5	185	187	NDE	3
6	197	205	SLLRSTVSK	9
7	210	216	TNFTKRH	7
8	235	241	QARSVYP	7
9	260	280	TKNTTGPRRHSVYRDYRFLEI	21
10	282	296	NYQVRDLETGQIRPP	15
11	315	319	EEKES	5
12	328	328	E	1
13	334	340	RVSYKNS	7
14	354	367	SGKQPFNISRLHLA	14
15	383	396	ARKYSSTHVRSGDI	14
16	414	419	SHGLAE	6
17	421	460	YLEEAQRQNHLPRGRERRQAAGRRTASLQSGPQGDRITTH	40
18	550	555	RMPGDP	6
19	570	587	SSPESQFSANSTENHNLD	18
20	598	610	ILQGRNLIEPCMI	13
21	629	640	TFVRQVNASEIE	12
22	656	656	L	1
23	658	658	F	1
24	665	679	TREELRDTGTLNYDD	15
25	686	686	I	1
26	688	690	NKR	3
27	693	693	D	1
28	696	704	TVIRGDRGD	9
29	790	818	VPPAGTPPRPSRRYYKDEEEVEEDSDEDD	29
30	831	880	LLHKDEQKARRQKARFSAFAKNMRNLFRRKPRTKEDDYPLLEYPSWAEES	50

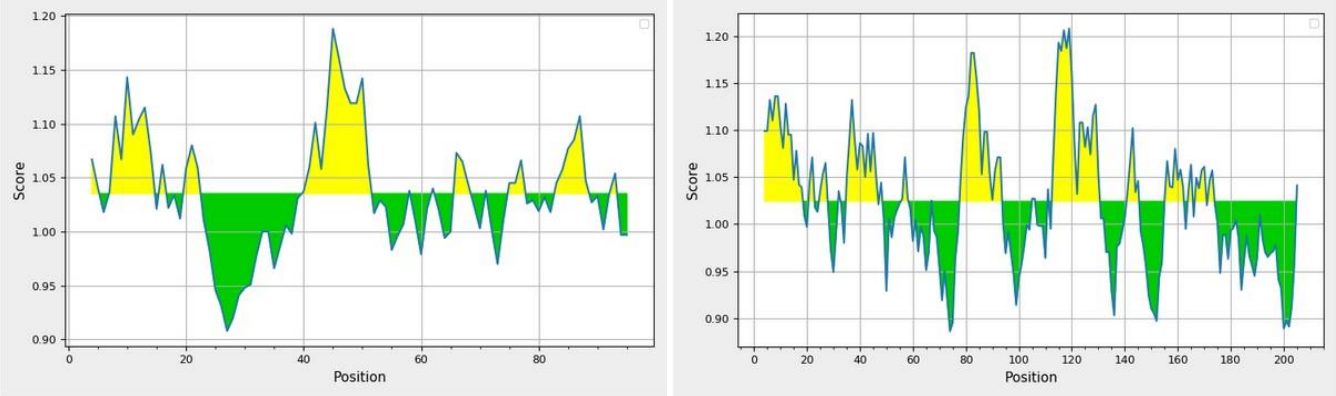

Figure 1. The Average Antigenicity of PH-1 (left) and PH-2 (right) Domains Predicted by Kolaskar and Tongaonkar's Algorithm. Residue colored in yellow showed the highest score and indicates the probability to be a part of the epitope.

B-cell Epitope Prediction

The predict linear B-cell epitopes result have been summarized in Table 1. Out of the 30 predicted epitopes, 14 were identified in the PH-1 and PH-2 domains. On the basis of physicochemical properties of the residues in a protein, the Kolaskar and Tongaonkar's algorithm estimated the average antigenicity 1.024 for PH-1 domain and 1.035 for PH-2 domain, where the threshold value >1.00 was potentiality antigenic. According to the Emini surface accessibility prediction, the highest accessibility was found in regions 181-187 amino acid residues of PH-1 domain with 6.312 score and the threshold value = 1.00, whereas the high accessible region in PH-2 has 4.244 score. The flexibility score of the proteins was not different (0.995 vs 0.991) with the highest score at 147-153 and 181-187 positions in PH-1 and 30-38 position in PH-2. The Chou and Fasman prediction results indicate these proteins have β-turns feature. The Parker hydrophilicity prediction results showed that the PH-1 sequence was more hydrophilic than PH-2, an average 1.60 vs 1.027. The highest picks were found in 57-62, 69-72, 130-160, and 181-187 positions. The PI value of the 12 predicted discontinuous epitopes ranged from 0.543 to 0.987, where the epitopes having PI >0.5 were considered. As a result, the residues with a higher PI score have better solvent accessibility. The highest probability of a discontinuous epitope for ILTV gB was calculated at 98.7% and its position has been shown in Figure 2.

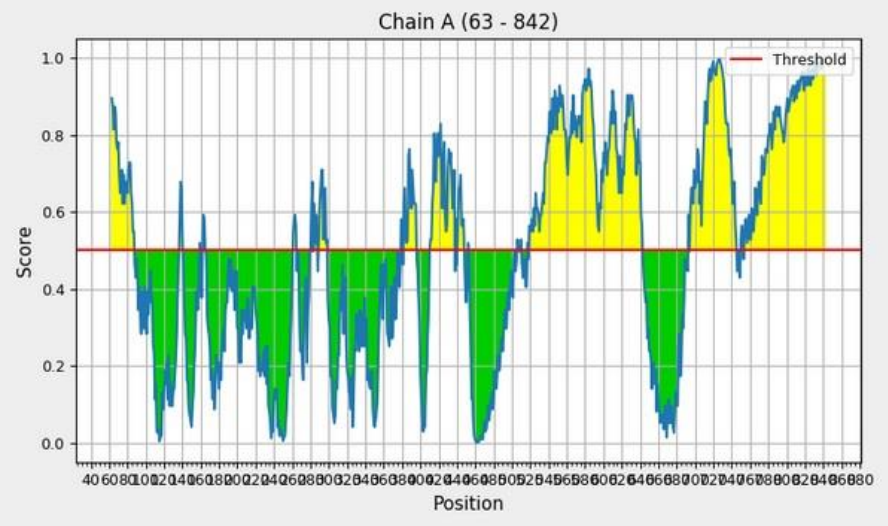

Figure 2. The Highest Probability of a Discontinuous Epitope for Glycoprotein B Infectious Laryngotracheitis Virus.

T-cell Epitope Prediction

The PH-1 and PH-2 domains of ILTV gB were subjected to predict the MHC I binding epitopes through the proteasome cleavage and TAP transport. From the predicted epitopes for PH-1, five epitopes had the IC_{50}<50 nM include ^{138}DTVEISPFY, ^{34}YVTRVPIDY, ^{58}ATYHKNFMF, ^{23}ARSVYPY, and ^{121}LQARSVYPY. Among these, the CTL epitope at 138-146 position showed the lowest IC_{50} (2.55) with the highest binding affinity and e-value score that revealed their potentials in initiating immune responses. All predicted CTL epitopes for PH-2 have an IC_{50} value greater than 100 nM. Due to the low affinity of PH-2 for binding with MHC class

I alleles, we chose the PH-1 domain for further analyses.

Physiochemical Properties Prediction

The physicochemical analysis of the PH-1 domain of ILTV gB with molecular weight 24416.48 showed a 7.85 pI value, which indicates the peptide is slightly basic. The *in vivo* estimated half-life in *E. coli* was >10 hours. The estimated aliphatic index was 66.49 which indicates the protein is thermostable. The negative value of GRAVY (-0.571) indicated the protein is hydrophilic and can interact with water molecules.

Secondary and Structure Prediction

The secondary structure feature indicated that 49.52% of PH-1 consists of coils, 19.71% helix, 25.48% extended stranded, and 5.29% beta turn. No disorder residues by loops/coils in PH-1 was predicted (Figure 3).

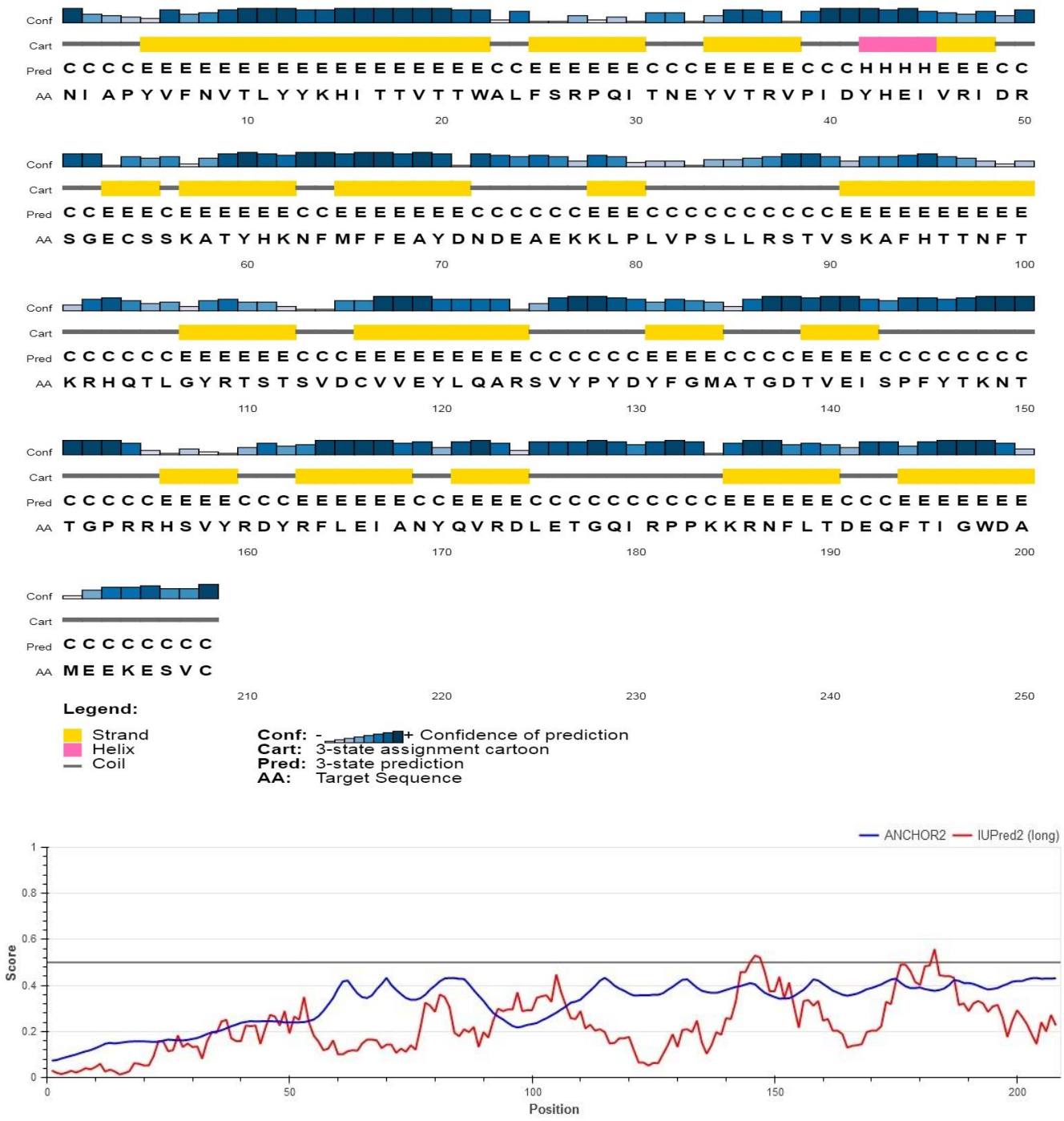

Figure 3. Graphical Representation of Secondary Structure of PH-1 Domain Glycoprotein B Infectious Laryngotracheitis Virus Comprise Coils, Beta Strands, and Alpha-Helices **(up)**, None of Positions Are Predicted as Disordered Identified by ANCHOR2. Amino Acids Were Considered Disordered When the Red Line Is Above the Grey Dashed Line, That Is the Confidence Score is Higher Than 0.5 **(down)**.

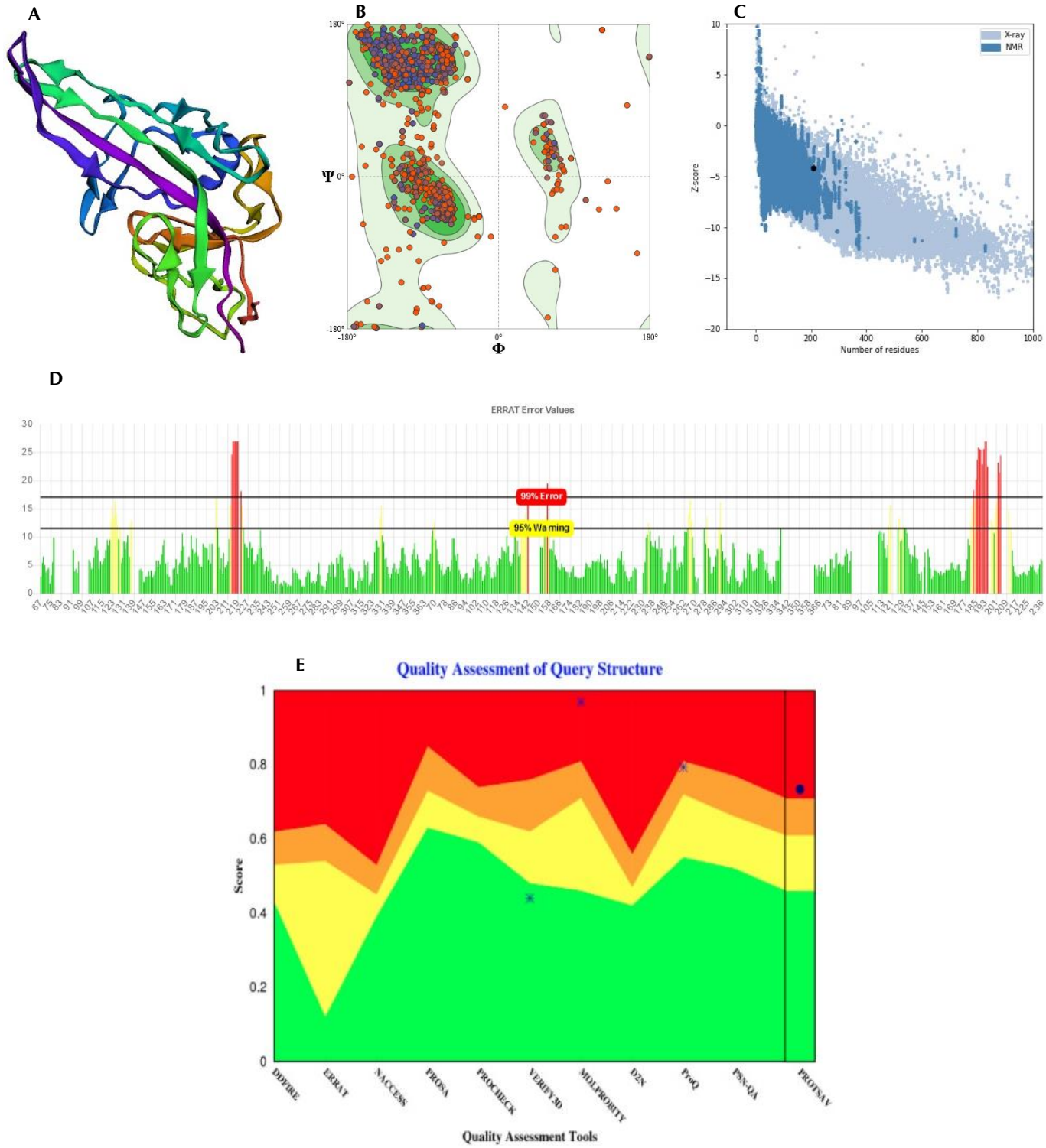

Figure 4. Protein Modelling, Refinement and Validation of PH-1 domain. **A)** the final 3D model of PH-1 domain, **B)** validation of the 3D structure by Ramachandran (higher percentage residues in allowed regions signify better accuracy) and **C)** ProSA giving a Z-score of −4.15, **D)** assessment of ERRAT showed an overall quality factor >92% indicates a high resolution and good model structure (red-colored regions are erroneous), **E)** ProTSAV quality assessment predicted the 3D structure to be in green region within the range of 0–2 E RMSD.

Homology Modeling

The 3D structure of PH-1 domain had a high sequence identity with varicella-zoster virus gB (PDB accession no. 6vlk.A), which was used as the template for homology modeling. The GMQE score for the built model was 0.76, where the number between 0 and 1 reflected the expected accuracy of the model and coverage of the target. The QMEAN Z score >-0.4 provides an estimation of the degree of nativeness of the structural features observed in the model on a global scale. The score −3.27 for the PH-1 model

denotes a relatively high accuracy rate. In the Ramachandran plot, 92.59% of the amino acid residues were within the most favored regions; 5.52% in allowed regions; and 1.89% in outliers regions. The negative Z-score of ProSA (-4.15) showed high matching between the 3D structures of the modeled PH-1 and the template. ERRAT recognizes the correct and incorrect regions based on characteristic atomic interactions and provides an overall quality factor for the model. The overall quality factor of the modeled 3D structure estimated by ERRAT was 92.588 indicating that the model had high resolution, where an ERRAT score >80.00 denotes higher quality of a model. Based on the ProTSAV score value, the predicted PH-1 protein was stable and had RMSD values in the range of high accuracy model structure (Figure 4). The VERIFY 3D assesses a 3D structure by checking its compatibility with its amino acid sequence with a measure of the 3D-1D score for each residue. The results showed that 82.92% of residues had an average 3D-1D score ≥0.2, indicating that the structure was favorable and reliable.

Molecular Docking

To identify the binding sites on PH-1 and TLR2 surfaces, a clustering algorithm to identify surface regions with residues of high interface propensities was determined. The InterproSurf prediction result showed the 60-66, 98-102, 22-28, 103-109, 31, 33-37, 192, and 193 residues of PH-1 and the 684-688, 690, and 722-725 residues of TLR2 were involved in binding. The Leu24, Ser26, Met65, Phe25, 64, and 66 residues of PH-1 and Trp684, Ile685 and 686, Asp687, and Ile689 residues of TLR2 located at TIR domain are the most interacting amino acids upon complex formation. The metric of binding site was predicted by the calculation of solvent-accessible surface area/volume. The CASTp prediction result revealed that the Asn1, Tyr129, Arg8, Ser206, and Cys208 residues of PH-1 were involved in the hydrogen bond interactions (Figure 5). The molecular surface area of the binding pocket

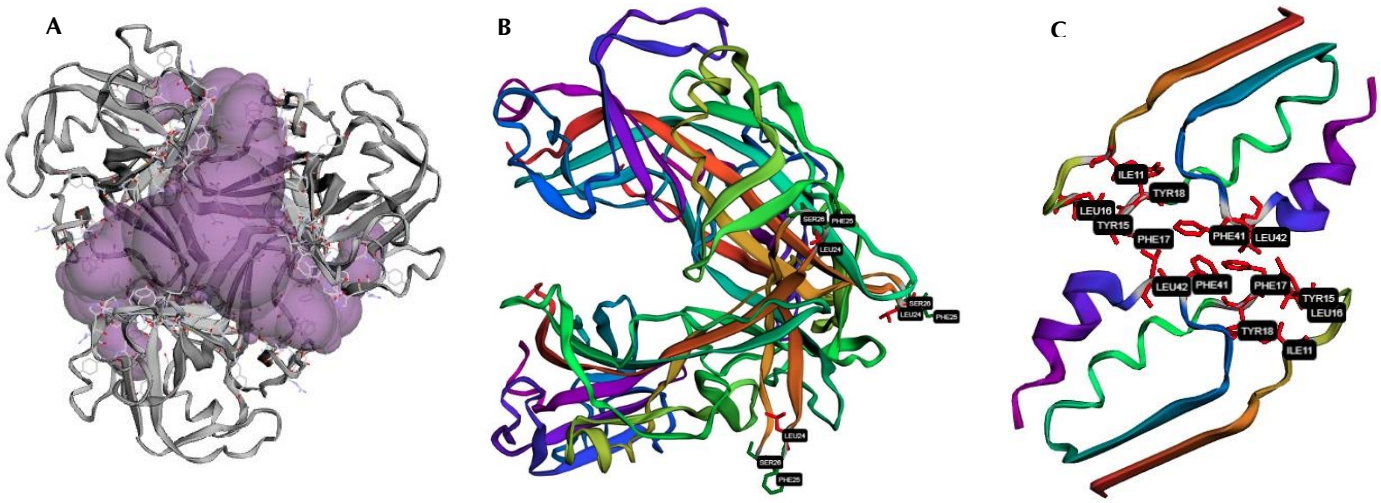

Figure 5. Hydrogen Bond Interactions and Binding Sites Upon Complex Formation Between PH-1 and TLR2 **(A)**. The Most Interacting Amino Acids are Shown for PH-1 **(B)** and TLR2 Surface Receptor **(C)**.

was 6697.573Å^2 with a volume of 17500.182Å^3. Three residues including Ser645, Cis672, Pro681, and Val704 of TLR2 have stronger interactions with PH-1 based on the hydrophobic interaction. The molecular surface area of the binding pocket was 480.633Å^2 with a volume of 258.057Å^3. Molecular docking demonstrated a significant binding of the ILTV protein and TLR2 immune receptor (Figure 6). The ClusPro approach consists of three steps including the rigid body docking, clustering of lowest energy structure, and structural refinement. The best docked complex was selected based on the lowest energy score and larger cluster size. Among the 30 generated poses, the PH-1 was appropriately docked into the TLR2 in cluster 0 (the first pose) with the lowest energy score (-40.105 kcal/mol) and the cluster size 13. The binding interactions of the complex depend on the

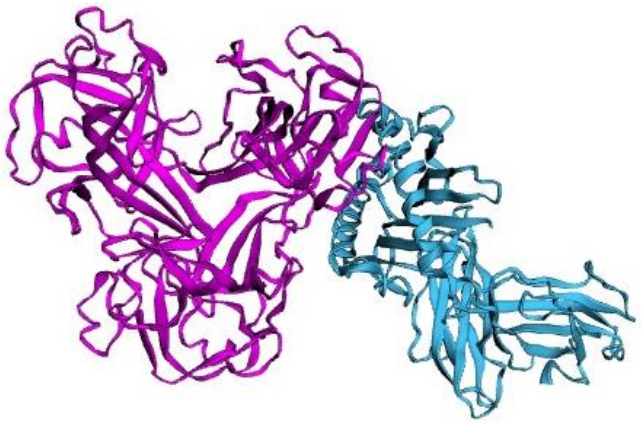

Figure 6. Molecular Docking for PH-1 Infectious Laryngotracheitis Virus and TLR2. Magenta color represents the vaccine candidate and the blue color represents the receptor in the protein complex.

binding free energy was evaluated. The predicted ΔG and K_d values were -8.7 kcal/mol and K_d 4.2E-07 M, respectively. The negative K_d value indicates high stability and strong binding affinity between PH-1 and TIR/TLR2.

In HADDOCK docking, the active site residues predicted by InterproSurf were selected owing to participate in the interaction between PH-1 and TLR2. HADDOCK clustered 165 structures of PH-1 and TLR2 binding in nine clusters, which represents 82% of the water-refined models generated. The statistics of the clusters are shown in Table 2. According to the HADDOCK scoring, the cluster four with the lowest score (-78.7±2.4), the more negative z-score (-1.7), and the iRMSD value of 0.5 Å was considered the most reliable of all generated models. The ClusPro and HADDOCK analysis showed that PH-1 has a supreme binding affinity with TLR2, which can be useful in the generation of appropriate immune responses against ILTV in birds.

Table 2. Docking Calculation Details of ILTV PH-1 and TLR2 Complex by HADDOCK

No	PH-1-TLR2 structure cluster	HADDOCK score	Cluster size	RMSD (E)	E_{vdW} (kcal/mol)	E_{elec} (kcal/mol)	E_{desol} (kcal/mol)	E_{AIR} (kcal/mol)	BSA (E^2)	z-score
1	4	-78.7±2.4	11	0.5±0.1	-46.4±5.4	-254.4±23.2	16.0±4.4	26.7±16.8	1887.2±122.1	-1.7
2	1	-69.9±202	98	0.3±0.1	-39.3±5.9	-263.6±31.9	17.6±4.0	43.2±2.0	1700.1±101.8	-1.2
3	7	-58.4±11.4	5	0.3±0.1	-27.5±7.5	-223.24±25.5	11.5±4.8	22.4±14.3	1342.1±238.7	-0.4
⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮
9	8	-28.6±4.1	4	0.4±0.1	-12.1±5.7	-153.8±3.7	9.9±4.6	44.0±4.0	848.8±136.1	1.5

Discussion

Since the first report of ILT in 1925, severe outbreaks of the disease have been observed worldwide where poultry productions are concentrated.[20] The attenuated live vaccines prevent chickens from ILTV outbreaks but possible reversion to virulence of the virus vaccine is a serious issue for the poultry industry.[3,21,22] The development of genetically engineered ILTV strain as well as subunit viral-vectored vaccine have been suggested to overcome this problem. In this trend, the primary focus is relying on ILTV glycoproteins because of their critical roles in attachment, fusion, and entry. The ability of gB on eliciting neutralizing antibodies make the protein as an appropriate antigenic target for vaccine development against ILTV.[13,19] This work, therefore, focused on the *in silico* abilities of gB protein to induce appropriate immune responses against ILTV infection.

To determine which parts of the protein selectively stimulate antigen specific B- and T-cells and induce the appropriate immunological response against ILTV, the gB potential immunological profile was evaluated using bioinformatics tools. Initially, a set of 67 ILTV gB sequences were retrieved from the NCBI database and subjected to multiple sequence alignment. The potent antigenic sequences were determined based on the immunogenic properties. The PH-1 domain was found to be the most antigenic than PH-2 with the highest probability by VaxiJen. By comparing the immunoinformatics analyses as well as the linear B-cell epitopes prediction results, the 181-187 antigenic region in PH-1 had maximal hydrophilicity, high-predicted flexibility, and accessibility, and can be bind to MHC molecule.

Designing an effective vaccine that generates cell-mediated responses is an important factor in protection against ILTV.[15,23] To gain promising responses, only peptides with IC_{50}<50 nM having a high binding affinity with the alleles were considered in MHC-I binding prediction. The five potent 9-mer epitopes predicted by CTL for PH-1, while the predicted epitopes for PH-2 had an intermediate or low affinity. Taking into consideration the high binding affinity to MHC class I and class II, the PH-1 was chosen as ILTV vaccine antigen could provide the overall immunogenicity. The knowledge of physiochemical properties, secondary and tertiary structures of the target protein is essential in vaccine development. The physiochemical parameters indicate that PH-1 is a hydrophobic and thermally stable extracellular protein, hence it can be easily separated and purified. Because PH-1 has not any transmembrane domain, the full interaction between the protein and antigen-presenting cells may occur to initiate T- and B- cell priming and strong immune responses. Secondary structure analysis indicated that PH-1 is dominated by the random coil structure that plays an important role in the high flexibility of the protein and implies to most protein-forming antigenic epitopes. No disordered residues or regions was found in PH-1 and the disordered regions were located outside this domain in the range of amino acids 400-440 and 790-820 of gB protein. Intrinsically disordered peptides are poor immunodominants and fail to contribute to immune protection. It has been suggested that they may elicit weak immune responses by interfering with the maturation and differentiation B-cells into memory or antibody producing cells.[24] Disordered proteins are no less likely than ordered proteins to be recognized by antibodies and their interactions have relatively low affinity.[25] Therefore, we can expect that an appropriate antibody response against PH-1 will be induced.

The 3D model of the PH-1 domain had a favorable and reliable structure based on the overall quality parameters.

When the accuracy of the model was confirmed by the GMQE and QMEA, the built model exported to the RAMPAGE server, which estimates stereochemical quality through the Ramachandran plot. The plot visualizes energetically allowed and disallowed backbone torsional angles; ψ and φ; of an amino acid and is calculated based on van der Waal radius of the side chain. In the 3D model of PH-1, 92.59% of the amino acid residues were within the most favored regions. Since more than 90% of residues are in the allowed regions, it can be concluded that this model had a high quality. The structure of the built model was validated by ProSA, which calculates an overall quality score for PH-1 structure in the context of the template protein structure. The calculated negative Z-score for PH-1 falls inside the range characteristic of native proteins and so the structure did not contain errors. Finally, the other scores indicated the high resolution and reliably of the PH-1 model.

The potent binding affinity of viral glycoproteins towards the TLR cellular receptor is necessary for effective transportation of vaccine antigen to the host cells. TLR2 signaling has been involved in mediating innate responses to herpes viruses leading to the activation of the key transcription factor NF-κB, production of inflammatory cytokines, and initiation of the adaptive immune response through MyD88 protein signaling cascade.[18,26] This interaction is mediated by the Toll/interleukin-1 receptor (TIR) domain, which plays a key role in activating IL-1R pathways and innate immune signaling.[27] The TIR domain is located at 639-782 position of TLR2 and has three highly conserved regions among the different members of TLR family. Based on the protein-protein interaction analysis, the amino acid residues of TLR2 involved in hydrogen bonding and hydrophobic interactions with PH-1 are located at box 1 and box 2 TIR domain. The residues around the protein binding pocket and also the minimal energy levels determine the specificity of protein-protein interaction. The Ser and the hydrophobic residues, Phe, Leu, and Ile showed the stronger hydrophobic interactions and construct hydrogen bonds at binding TLR2 with PH-1. The low binding free energy and dissociation rate constant scores further confirmed the potent binding affinity between PH-1 and the receptor.

TLR2 plays a key role in herpesvirus innate immunity by orchestrating antigen-specific adaptive immune responses. Initiate dimerization of the TIR domain and its conformational change leads to the recruitment of MyD88 adaptor protein to form a post-receptor signaling complex. Activate downstream signaling pathways leads to the induction of immune responses by producing cytokines and other inflammatory mediators essential for adaptive immune responses.[27] The possible interaction of the cellular TLR2 with the viral PH-1 protein were examined. Molecular docking using the Cluspro and HADDOCK servers demonstrated that the amino acid residues in the active site of PH-1 possess a strong binding affinity to the cellular TLR2 receptor. The electrostatic energy formed on the HADDOCK confirmed the accuracy of the stable interaction between these molecules (about -254.4 kcal/mol with an iRMSD value of 0.5). The more negative HADDOCK score and z-score can guarantee the PH-1-TLR2 complex binding and stability. Such efficient interaction is crucial for immune activation of dendritic cells for subsequent antigen processing as well as induction pro-inflammatory responses. One of the reasons for the immunopathological manifestations of ILTV can be the interaction between the virus protein and the cellular receptor. Immunopathology of ILTV has been reported to be caused due to the binding of viral glycoprotein and the innate immune receptor. The up-regulation of IL-12p40, CXCLi2, IL-1β, IFN-γ, IL-13, and IL-10 cytokines correspond to establish adaptive immune response has been detected during the early and late stages of ILTV infection.[28] Induction of a strong cellular immune response, which is significantly involved in the protection against ILTV, requires the coordinated expression of cytokines. It is possible that the establishment of the stable protein-protein interactions between PH-1 and TLR2 leads to the induction of immune responses by releasing proinflammatory cytokines.

Conclusion

The results from the current study revealed that the PH-1 domain of gB can be an immunogenic target for the generation of ILTV recombinant vaccines. Activation of dendritic cells for the antigen presentation to T-cells via MHC molecules, release proinflammatory cytokines following the activation of TLR signaling. Finally, the host immune responses to the protein needed more experimental studies.

Authors' Contributions

All authors contributed equally to this study.

Conflict of Interest Disclosures

The authors declare that they have no conflicts interest.

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