Developing a Next-generation Multitudinous Epitope-based Vaccine Against Biotinidase Deficiency; An Immunoinformatics-based Approach

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Abstract

Biotinidase deficiency is a rare genetic disorder brought out by BTD (Biotinidase gene) mutations. Genetic disorders are not curable but there are drugs available for suppressing Biotinidase deficiency. To overcome this problem a vaccine that is much more effective is needed. The goal of the current research is to construct an effective multitudinous epitope-based vaccine. Biotinidase protein was chosen as an objective; various epitopes like T-cells and B-cells were anticipated. Predicted epitopes were shown stability, anti-allergenic, epigenetic, and responsive. Finalist epitopes were significantly antigenic and overlapped. Using In silico Techniques, we predicted the Primary, secondary, and tertiary structures, as also consistency, ligand-receptor association, and MHC class I and II affinity qualities for vaccine designing. The designed vaccine shows high affinity with the human receptors IL-2 alpha and a beta chain. The sequence was then cloned into the plasmid pET-28a. To improve activation in a prokaryotic cell. Docking studies further demonstrated that the forecasted peptides interacted with the HLA-B7 allele. The predicted vaccine could be a promising starting point for vaccine development against genetic disorders. Furthermore, the suggested vaccine must be subjected to in vitro experiment and further confirm to verify its immunogenic and safety profile.

Keywords: Vaccine Designing, B and T-cell Epitopes, Biotinidase Deficiency, Genetic Disorder.

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INTRODUCTION

Biotinidase deficiency is an unusual genetic condition associated with BTD genetic variants. The role of the BTD gene is enzyme creation that produces enzyme biotinidase that supports recycling vitamin H called biotin. If the gene (BTD) is naturally not able to reprocess biotin Biotinidase deficiency will happen [1]. Severe biotinidase deficiency affects one out of every 140,000 individuals. Partial biotinidase insufficiency affects one for every 110,000 persons. There is either a total or partial biotinidase deficiency in around one in 60,000 people. It is an Autosomal recessive pattern inheritance [2] [3].

Biotin produces important five carboxylases (Coenzymes) called Propionyl-CoA carboxylase, pyruvate carboxylase, 3-methylcrotonyl-CoA carboxylase, and acetyl-CoA carboxylases 1,2. These coenzymes involve different metabolic pathways including gluconeogenesis, fatty acid, and amino acid residues metabolism [4] [5] [6] [7].

Several things can lead to a biotin deficiency. It might appear as a severe biotin deficit with neurological and dermatological manifestations. It can be caused by inborn metabolic abnormalities such as holocarboxylase synthetase deficiency or biotinidase deficit. [8]. If we are not treating the Biotinidase deficiency it may cause Developmental delay, vision and hearing problems, hypotonia, Seizures, ataxia, eczema, and alopecia [9].

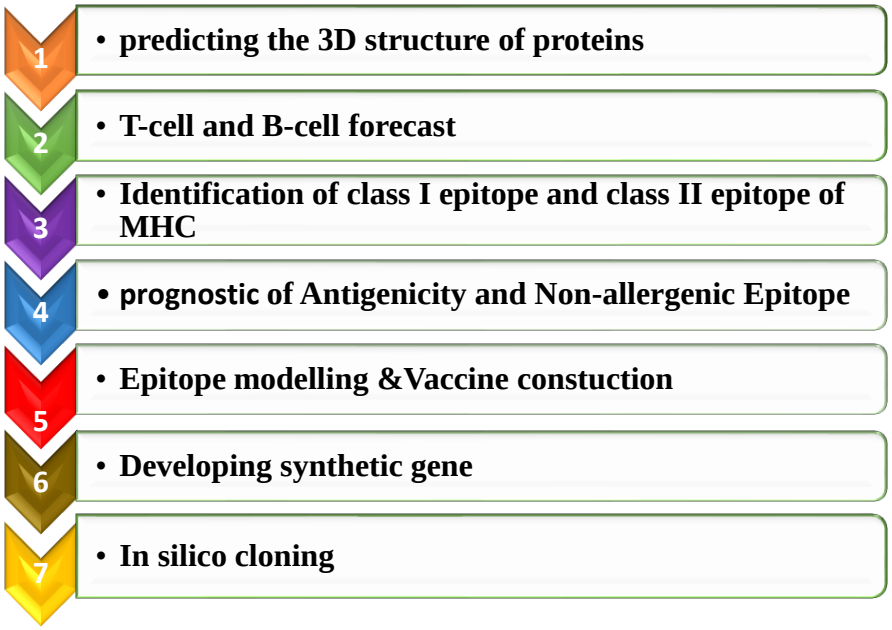
Biotin supplementation is likely to be effective in the treatment of biotinidase deficiency. But still, there are many side effects of using biotin supplements such as digestive upset, skin rashes, kidney problems, and insulin release problems [10]. At present, there is no vaccine against biotinidase deficiency and also there is no significant study for a vaccine designed for a genetic disorder. Few pieces of research shows we can develop a vaccine for the genetic disorder [11]., immuno-informatics is the best method to develop effective vaccines using research methodology. Immuno-informatics progress has enabled the "in silico" design of new molecules and the prediction of their functionality. This strategy aids in the selection of better

molecule tests done in vivo or in vitro. Using the immune-informatics approach, many research groups have created a wide range of in silico molecules. This technique could significantly speed up and simplify the evaluation process. In addition, vaccine development could save money and time [12, 13].

In this current study, we have done molecular modeling for biotinidase protein which is the main cause of Biotinidase deficiency. Then, to speed up the powerful immunological and antigenic responses for vaccine design, we looked into linear, T cell, and discontinuous B cell epitopes. Adjuvant was added in the vaccine construct because it is necessary to add adjuvant for minimizing the usage of antigen and increase the efficient and long-lasting immune responses [14]. In the next step, we have identified physical, chemical, structure, and stability properties, protein transmembrane to molecular docking analysis. Furthermore, the developed vaccine's stability and interaction with immunity responses of humans were determined using docking studies.

MATERIALS AND METHODS

Schematic Workflow Presentation



1. Selection of Protein

We obtained protein (**Biotinidase)** sequence id: NP_000051 through National Center for Biotechnology Information (NCBI). [15]

2. Primary Structure Prediction

The amino acid linear sequences commonly called protein primary structures were predicted through an online tool program server [16]. physiochemical properties like Molecular weight, PI value, positively and negatively charged residues, hydropathicity, and protein stability were predicted for both biotinidase protein and designed vaccine sequence. Both helical transmembrane segments and the topology of transmembrane proteins are predicted by the prediction server (http://www.enzim.hu/hmmtop) HMMTOP 2.0 is free. This option increases prediction accuracy while epitope insertion investigations and also assists in the interpretation of experimental results [17].

3. Forecasting of Secondary Structures

The SOPMA online web-based servers were used to anticipate the secondary structure. The most important elements are beta sheets and alpha helices of secondary structure and also random coil, protein solvent accessibility were analyzed [18].

4. Forecasting of Tertiary Structures

Biotinidase protein 3D structures were created through homology modeling. The Biotinidase protein was designed using an enhanced design based on numerous templates in ITASSER online web-based server[19]. To examine the stereochemistry of the modeled protein, Biotinidase PDB structure was submitted to SAVESv6.0-Structure Validation Server, and modeled protein structural stability was proven using the Ramachandran plot[20].

5. Epitope identification for B-cells

The free online servers IEDB (Immune-Epitope-Database

and Analysis-Resource) [21] and BCPRED server were used to predict epitopes of B-cell. [22]. The standards were set at a specificity of 75%, and 14 residue-long epitopes were regarded as sufficient to elicit a defensive immune response. Intracellular epitopes were removed, and only certain epitopes exposed on the outside surface were chosen. We identified B-cell epitopes using immunogenicity, flexibility, surface accessibility, hydrophilicity, and linear epitope predictions. Epitopes of Continuous, discontinuous B-cell were prophesied through server EliPro[23]. A range of 0 to 1 with the cut-off of 0.5 is considered an epitope. A non-epitope is indicated by a score of less than 0.5, whereas an epitope is indicated by a value of more than 0.5. the server Disco Tope forecasted epitopes of discontinuous. [24].in this boundary is created to 23% sensitivity,90% specificity, and greater than or equal to 0.5. It shows surface accessibility. Finally observed B-cell epitope clusters 3D structure is viewed by Pepsurf server [25].

6. Epitope prediction for T-cells

In vaccine development, Cytotoxic T-lymphocyte (CTL) are playing a vital role and is capable of recognizing and creating cell-mediated immune responses to particular antigens. MHC class I and II allele was prognosticated through MHC-I and MHC-II Binding Predictions servers sequentially [21].

7. Prediction of Antigenicity

Epitopes were put into the AlgPred software to forecast their allergenicity. [26]. The antigenicity of the epitope was predicted through server VaxiJen [27], It uses the threshold of 0.5 to differentiate antigenic from non-antigenic proteins and it is depending on the physicochemical characteristics. Toxic and non-toxic peptides are predicted by toxinpred [28]. non-allergic,100% conserved, non-toxic, epitopes were only picked up for additional examination.

8. Prediction of IFN (Interferon)$_{-\gamma}$

IFN-γ is reported to create reactions that are inherently safe and can stop virus replication effectively. [29, 30]. Furthermore, it may initiate a diverse range of reactions of the immune-creating HTL (helper T lymphocytes), CTL (cytotoxic T lymphocytes). Prospects for some epitopes to induce IFN-**γ** were determined using the IFN epitope server.

9. Epitope Modelling and Docking

For the construction of a multitudinous epitope vaccination, epitopes with high binding affinities to an established empirically confirmed allele are a good option. Consequently, a docking studies experiment was conducted using a human allele and the screened epitopes. The binding affinity of a ligand and the targeted proteins is displayed via molecular docking. The protein data bank (PDB) was used to obtain HLA-B7(common human allele's X-ray crystallographic structure After doing Docking studies, Multi Epitope Vaccine (MEV) construct investigations were conducted. [31, 32].

10. Development of Multitudinous Vaccine

Developing the Multitudinous vaccine that should be overlapping, hundred percent conserved, antigenic, and non-toxic, powerful association with the typical human genotype, and last but not least there is no resemblance to human proteins. As a result, only epitopes that satisfied the above criteria were chosen to be studied. Construct the vaccine. At first, an adjuvant was added to boost the immune response [33]. After their interaction consistency was validated, linkers GPGPG, AAY, and EAAAK were used to sustain their autonomous antibody response functions.

11. Identification of constructs vaccine Physical and Chemical Properties

The constructs must be substantiality, extremely antigenic,non-allergic, and non-toxic. Physiochemical properties appraise through the protparam tool [16]. AllerTOP V2.0 and VaxiJen 2.0 servers were performed to confirm the multi-subunit vaccine property. The secondary structure was analyzed by the PSIPRED server [34].

12. Prediction of Constructed Vaccine Tertiary Structure

Since the structural model of the vaccine contains a variety of distinct epitopes, a top-notch 3D tertiary structure was created using the RaptorX server. This protein folding method is modeled by distance-based deep learning and also it predicts inter-atom/ inter-residue distance and orientation probability (http://raptorx.uchicago.edu/) [35].

13. Validation and Improvement of the constructed Vaccine

To assess the effectiveness of the carefully developed vaccine structure, Ramachandran plot analysis was carried out using the Saves 6.0 server [20]. Besides, we investigated the vital role of structural flexibility of modeled vaccines using the CABS-Flex 2.0[36]. As well as Server CABS-Flex gives a thorough assessment of the protein's elasticity and consistency [37].

14. Docking analysis

The host produces an effective immune response when an antigen or vaccine interacts well with the particular immune cells. Therefore, interactions between the created vaccine and the body's immune cells were investigated using docking analysis. Alpha, beta, and gamma chains of the IL-2 (interleukin 2 receptor) are extracted for docking analysis. Some immune cells, like lymphocytes, express the interleukin-2 receptor (IL-2R), a heterotrimeric protein that binds to cytokines and reacts to them. They're also

important for long-term cell-mediated immunity [38,39]. HADDOCK server used for Molecular docking analysis between modeled vaccine against human receptor [40]. PDBsum [41] online server is used for further visualization and interaction analysis.

15. Computational Codon optimization and cloning

Coding region adaptation is a technique for improving the host's ability to translate outside genes. Following a thorough examination of designed vaccine characteristics and immunological responsiveness, codon modification and computational cloning was done. Codon optimization was performed with an online Java Codon Adaptation Tool (JCAT server). The content GC (guanine and cytosine) as well as (CAI)codon adaptation index [42], were assessed.

RESULTS

1. Sequence and Structural Evaluation

Tool Protparam calculates the Biotinidase protein's physiochemical parameter which shows that it encompasses 543 amino acids(aa), Theoretical pI (isoelectric point) 5.81 which denotes the fact that it is negative, and PI Proteins with a negative charge have an isoelectric point below 7. The instability-index (II) 31.95 determines that the protein is stable. The grand average of hydropathicity (GRAVY) scores -0.032, while the aliphatic score is 86.37, suggesting that the aliphatic side chain holds an equivalent volume.

Secondary structure analysis of biotinidase using the SOPMA server(reference), revealed that the structure contains Beta sheets (26.21%), Helixes (22.18%), and (Random coil 50.86%) (figure:1).

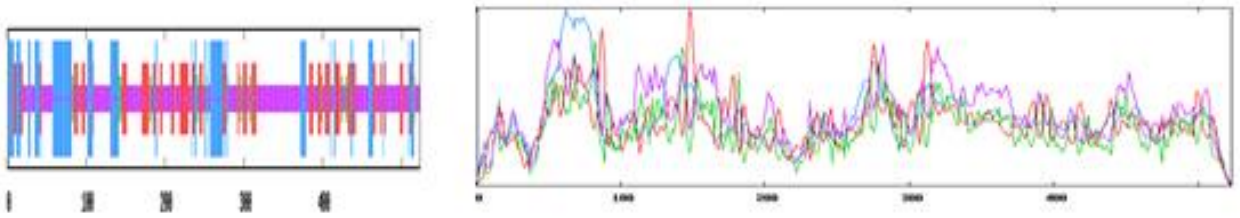


Figure 1: Prediction of the Secondary Structure of Biotinidase Protein

2. Modeling of Biotinidase's 3D Structure

The BTD protein sequences were obtained from NCBI and it contains 543 amino acid sequences. There was no full-length protein template found. We modeled the protein using the threading method. For the analysis, we have used the server I-TASSER server. Tool PROCHECK and ERROT show no error. Figure: 2 (B) shows foremost variants for 90.5 percent of residues fall within the most preferred or allowed region, 6.9 percent in the allowed and 2.2 percent in the generously allowed zone, and only 0.4 percent in the banned region, according to a Ramachandran plot study. For instance, a model with a scoring near 100 percent has great stereochemical quality. [43]. Figure: 2(A) represents the 3-D structure of Biotinidase protein. Modelled protein deposited in the PMDB (Protein Model Database). The deposited PMDB id is PM0084231.

(A) (B)

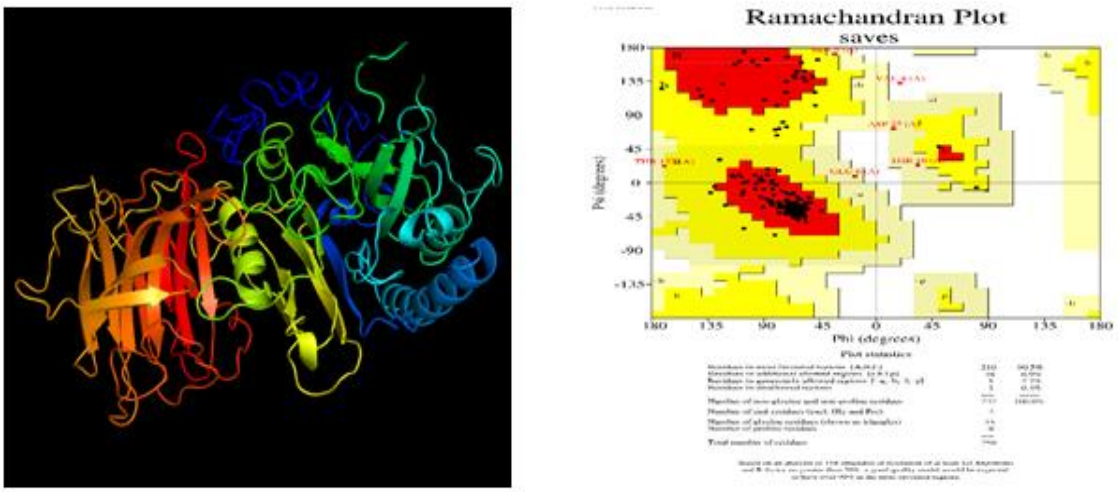


Figure 2: (A) 3-D Structure of Biotinitase (B) Estimate Quality of Protein Structure Using Ramachandran Plot

3. Recognition of B-cell

The B cell epitope plays a crucial role in the acceptance of antibodies, and possible B-cell epitopes can trigger a variety of adaptive immunological responses. The main sequence of Biotinidase was examined utilizing the BCPRED and IEDB server to anticipate B-cell epitopes. Next step, we used the EliPro server to check the Biotinidase protein's predicted tertiary configuration for the existence of B cell continuous and discontinuous epitopes. The results demonstrate that the Biotinidase protein contains both continuous and discontinuous B cell epitopes. There were a total of 48 B-cell epitopes predicted. Only great scored of antigenicity 12 epitopes (Table: 3) have been picked from all predicted epitopes. Among them, "LMNQNLDIY" predicted B cell epitope showing the greatest antigenicity score.

It's also crucial to check the surface accessibility of potential B-cells. By looking at the physicochemical characteristics of amino acids, the prevalence of established B-cell epitopes was discovered. The Kolaskar and Tongaonkar methods were used to analyze the Biotinidase to predict B-cell epitopes. Image: 3 (E). Greater antigenicity markings may have a crucial role in the start of the immune response, according to some theories. To ascertain the surface accessibility of putative B-cell epitopes and hydrophilicity, Parker-hydrophilicity with a threshold value of 0.735 and Emini surface accessibility prediction algorithms with a threshold value of 1.000 were utilized. Figures: 3 (A) and (C) show the visual representations of the results of both tools. The average, minimum, and maximum obtained values by both of these tools were Average: 0.735 Minimum: -6.371 Maximum: 6.143, and Average: 0.979 Minimum: 0.884 Maximum: 1.082 respectively. Because beta-turn is hydrophilic and exposed on the surface, to predict beta-turn, the algorithm Chou and Fasman were used. The defense response is crucially triggered by the beta-turn. The tool's threshold was set to 0.964, and it calculated the following values: 1.009 (average), 0.581 (minimum), and 1.414 (maximum) (maximum). Figure 3(B) displays the results of Chou and Fasman's analysis graphically.

The results show that the amino acid sequences from 155 to 180 and 345 to 350 are more effective in convincing Burns in peptide structure. The majority of the epitope's components are elastomeric and connect to antibody concentrations or allele frequencies, according to experimental data. As shown in Figure:3 (D) (Karplus), the area between amino acids 180 and 330 sequence positions is highly versatile, according to Karplus and Schulz's flexibility analysis tool. Every predicted epitope's location on the Bioinidase protein's 3-D surface was verified using PepSurf [25].

The Discotope 2.0 server was utilized to ascertain surface availability in regards to residue contact information and to forecast discontinuous epitopes using a novel high propensity amino acid score to increase the specificity and range of B-cell epitopes. (figure: 4(A) & (B)).

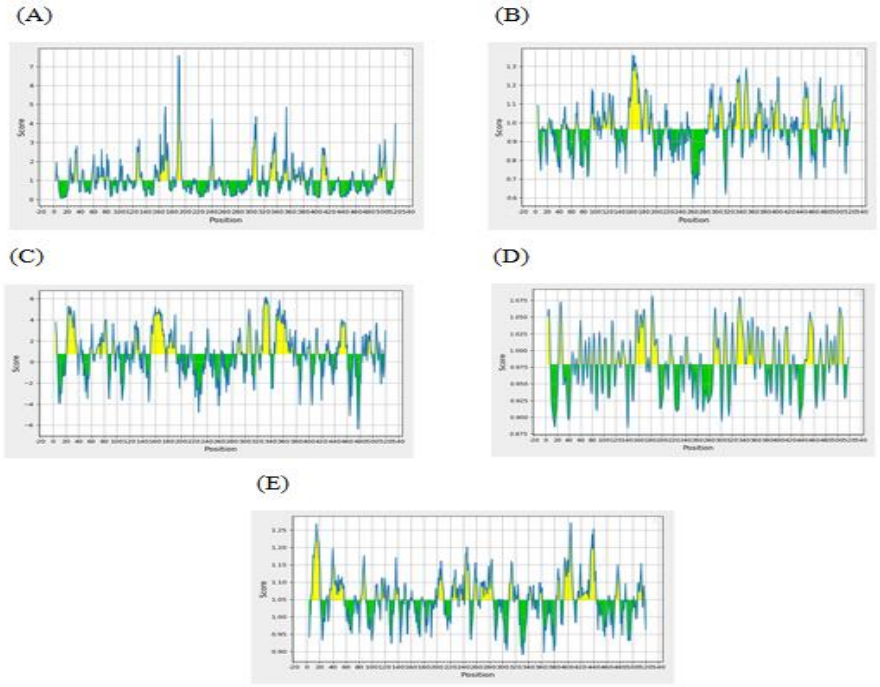


Figure 3: (A) Investigations of Surface Accessibility through Emini Surface Accessibility Scale. (B) Chou and Fasman Beta-turn Prediction is used to Evaluate Beta Turns in Architectural Polyproteins. (C) Parker Hydrophilicity would be used to Predict Hydrophilicity. (D) Examination of Flexibility through Karplus and Schulz Flexibility Scales (E) Antigenic Determinants Were Predicted Using the Kolaskar and Tongaonkar Antigenicity Scales

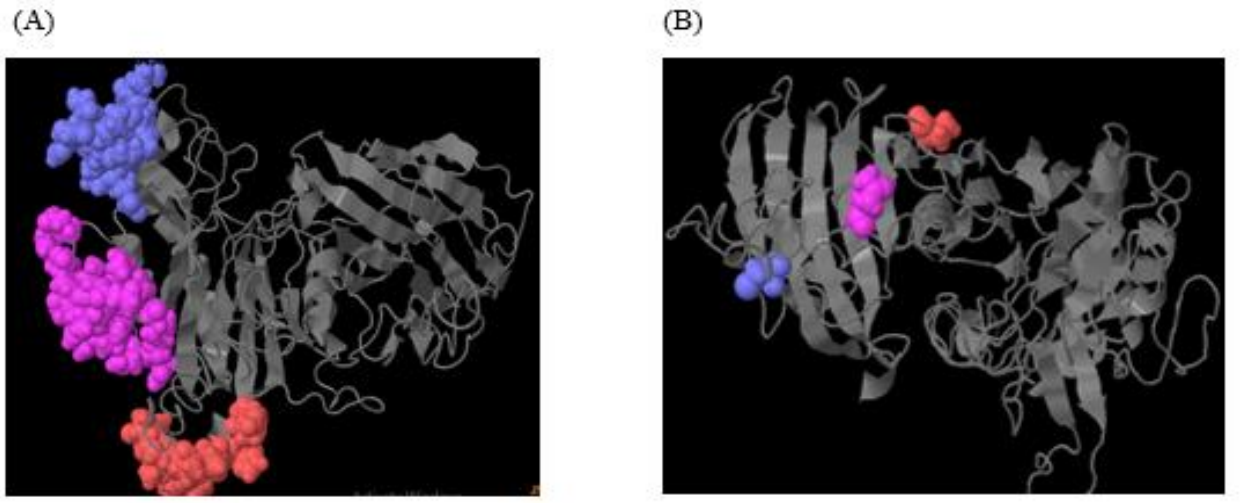


Figure 4: (A) B-cells Predicted Linear Epitopes (B) B-cells Discontinues Epitopes

Table 1: Final Selected B Cell Epitopes

Peptide	Prediction	Antigenicity	Hydrophobicity	Hydrophilicity	Charge	Mol wt.	Binding score (Kcal/mol) with HLA-B7
MHC class I							
LMNQNLDIY	Non-Toxin	1.51	-0.07	-0.60	1.00	1123.43	-14.62
FTCFDILFF	Non-Toxin	0.85	0.32	-1.33	-0.50	1152.49	-12.01
HTPLESFWY	Non-Toxin	0.72	-0.00	-0.84	-0.50	1179.42	-10.23
ETDPSHSKF	Non-Toxin	0.82	-0.33	0.69	-0.50	1047.20	-10.45
PTFHSEMMY	Non-Toxin	0.63	-0.04	-0.56	-0.50	1142.43	-13.65
HSEMMYDNF	Non-Toxin	0.68	0.17	-0.16	-1.50	1173.40	-9.99
GLHTVHGTY	Non-Toxin	0.68	0.03	-0.82	1.00	984.22	-14.06
STSYIFPLF	Non-Toxin	1.00	-0.19	-1.19	0.00	1074.36	-12.36
LTSGMTLEV	Non-Toxin	1.41	0.09	-0.43	-1.00	950.25	-15.03
QLGWENDHY	Non-Toxin	0.86	0.62	-0.18	-1.50	1161.33	-11.43
VTAALYGRL	Non-Toxin	0.81	0.91	-0.64	1.00	963.27	-9.86
TAALYGRLY	Non-Toxin	0.43	0.4	-0.66	1.00	1190.50	-14.79

4. Recognition of T Cell

The CTLpred service was used to anticipate the cytotoxic T cell epitopes. The CTLpred score is predicted using the selectivity and responsiveness of the relationship among both MHC 1 and CTL. The CTLpred predicted three epitopes with peptide scores of 1, 0.99, and 0.99. The NetMHC server was used to verify the CTL epitope and MHCI molecule's binding affinity. Finally, we chose the peptide with the highest score and the MHC with the greatest binding capacity for additional research.

To back up our findings, we looked at some key features of a few epitopes. Alfred software predicted whether epitopes or allergic or non-allergic. ToxinPred predicts toxicity of selected epitopes as well as evaluates hydrophobicity, hydrophilicity, hydropathicity, and mutations (table: 2). It is based on the SVM (support vector machine) technique.

Table 2: Final Selected T- cell Epitopes

Peptide	Prediction	Mutation	Antigenicity	Hydrophobicity	Hydrophilicity	Binding score (Kcal/mol) with HLA-B7
FVVCIMSGARSKLAL	Non-Toxin	Nil	1.39	1.39	0.51	-15.32
RFVVCIMSGARSKLA	Non-Toxin	Nil	1.01	0.84	-0.19	-12.03
SRFVVCIMSGARSKL	Non-Toxin	Nil	0.98	0.67	-0.13	-11.09
VVCIMSGARSKLALF	Non-Toxin	Nil	0.99	0.56	-0.63	-11.62
KSRFVVCIMSGARSK	Non-Toxin	Nil	1.44	0.77	-0.27	-10.27
EATKWNVNAPPTFHS	Non-Toxin	Nil	0.86	0.22	0.48	-9.48
ATKWNVNAPPTFHSE	Non-Toxin	Nil	0.52	0.54	0.62	-8.83
RGDMFLVANLGTKEP	Non-Toxin	Nil	1.22	0.48	-0.38	-13.11
TKWNVNAPPTFHSEM	Non-Toxin	Nil	0.34	0.78	0.50	-10.77
HSEMMYDNFTLVPVW	Non-Toxin	Nil	1.03	0.80	0.35	-12.16

In response to the release of cytokines like IFN, HTLs help to stimulate CTLs and some other immune cells [44, 45]. As a result, the development of vaccines or immunotherapies needs to find HTL epitopes that cause cytokines.

5. Interaction Studies

As previously stated, the epitopes chosen for a sub-unit vaccine must be 100 percent preserved, antigenic, and overlapping. PEPFOLD servers were used to design the 3-D structure of predicted Peptides. Molecular docking was used to examine the binding correlations of listed epitopes with the HLA-B7 allele. Only 22 epitopes were noticed to bind depths inside the HLA-B7 binding pocket.

6. Vaccine Design

Selected epitopes were tested for inter-interactions before being used to develop a multiple-epitope vaccination. Adjuvant increases the immunogenicity of the vaccine. Since adjuvant alum salt (52 amino acids) was linked via the EAAAK linker, to the N-Terminal. With efficient separation and increased stability, the linker EAAAK decreases protein location connectivity. T cells were combined with the GPGPG linker and B cells with the AAY linker. These linkers are called Junction. There are (figure: 5) 505 amino acid residues in the final vaccine Design.

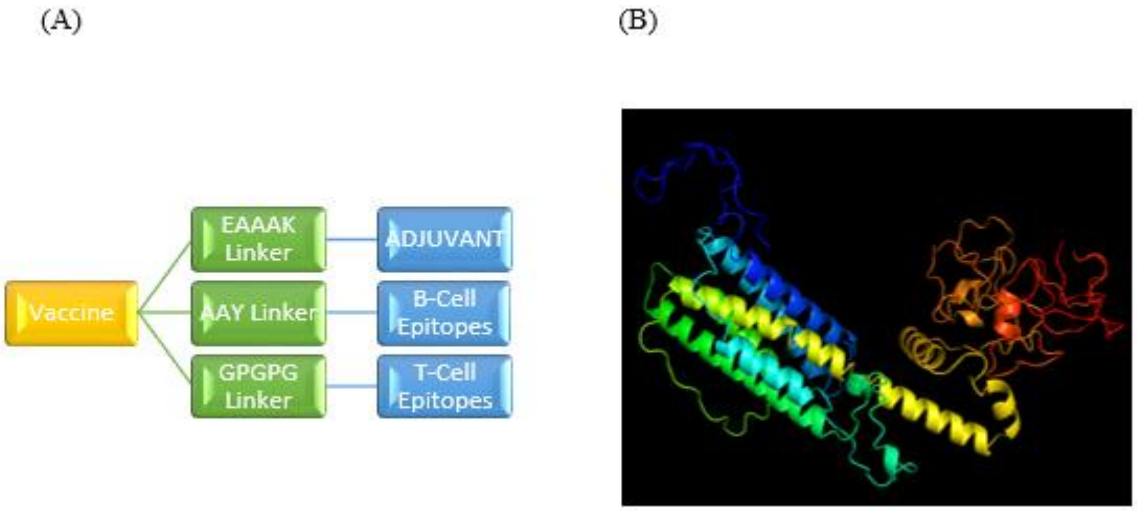


Figure 5: (A) Schematic Diagram of Vaccine Construct (B) 3-D Structure of Final Vaccine Model

7. Evaluation of Multi Epitope-based Vaccine

Protparam was used to determine the expected vaccine's physicochemical characteristics. it contains 437 amino acids with 47753.40 mol.wt which is highly antigenicity. The designed vaccine is negative since PI (Isoelectric point) is 8.56. The chemical formula denotes $C_{2099}H_{3269}N_{587}O_{629}S_{30}$. GRAVY value of sequence 0.373 points that nature hydrophobicity. The designed vaccine was classified as stable because the instability index was 32.60. An aliphatic index score of 71.26 indicates commensurate volume. The total time it takes for a BTD to vanish after metabolizing in a cell was determined by calculating it to be > 10 hours for Escherichia coli, > for yeast for 20 hours, and mammalian reticulocytes for 30 hours.

The vaccine construct's toxicity, antigenicity, and allergenicity were assessed. 0.7721 is vaccine antigenicity with an o.5% threshold. Considering, non-toxic and non-allergenic according to the findings.

8. Predicted Vaccine Structure Analysis

The secondary structure was forecasted by the server PSIPRED. 141 amino acids make up the alpha helix, accounting for 32.26 percent of the total 437 amino acids, 104 amino acids make up the beta-strands, accounting for 23.79 percent, and 192 amino acids make up the random coils, accounting for 43.93 percent of the total vaccine construct.

The RaptorX online server results established the vaccine's tertiary structure. Galaxy web server was used to fine-tune the structure. Figure: 6(A) shows darker colors have a higher probability. As per plot Ramachandran representation, the improved structure showed 97.9 percent of amino acids in the favorable region, 1.7 percent in the permitted region, and 0.3 percent in the outer region. RMSD is 0.428, poor rotamers are 0.3%, MolProbity is 1.657, and clash score is 12.6, according to more analysis. ERRATA and PROCHEAK also showed 0 errors. Finally, Figure: 6 (B) establishes a refined superior quality vaccine design.

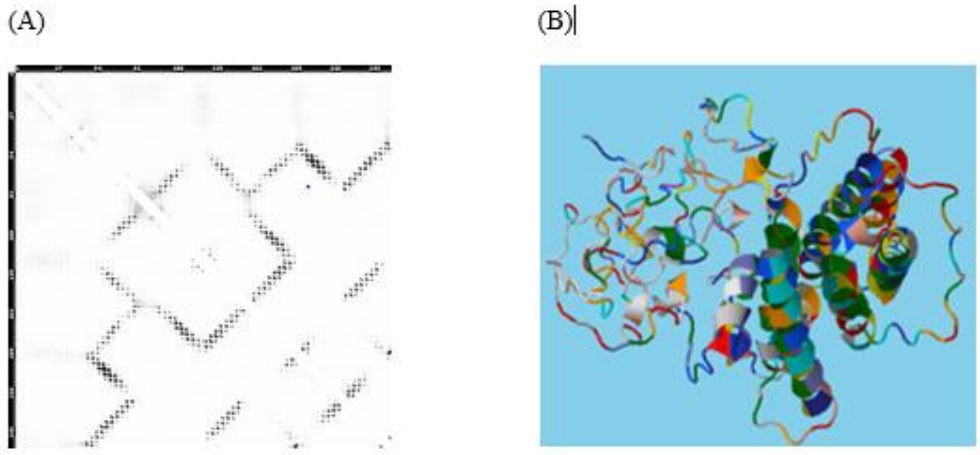


Figure 6: (A) Probability Score Matrix (B) Galaxy Web Server Refine the Structure

9. Molecular Docking

To activate an immune response, an acceptable correlation in both the antigen molecule and the immunological receptor are necessary. After pathogen recognition, IL-2 (alpha and beta chain) can effectively induce an autoimmune reaction. Human immune receptor Interleukin 2 receptor (IL-2) alpha chain and beta chain were selected for molecular docking analysis. Docking analysis was carried out through the HADDOCK server. The docking scores of the designed vaccine with (IL-2) alpha chain was 236.3 Kcal/mol and (IL-2) beta chain was -242.6 Kcal/mol. The molecular docking analysis reveals a positive contact between the vaccine and the human receptor. (Figure: 7, 8) showing a graphical representation of interaction.

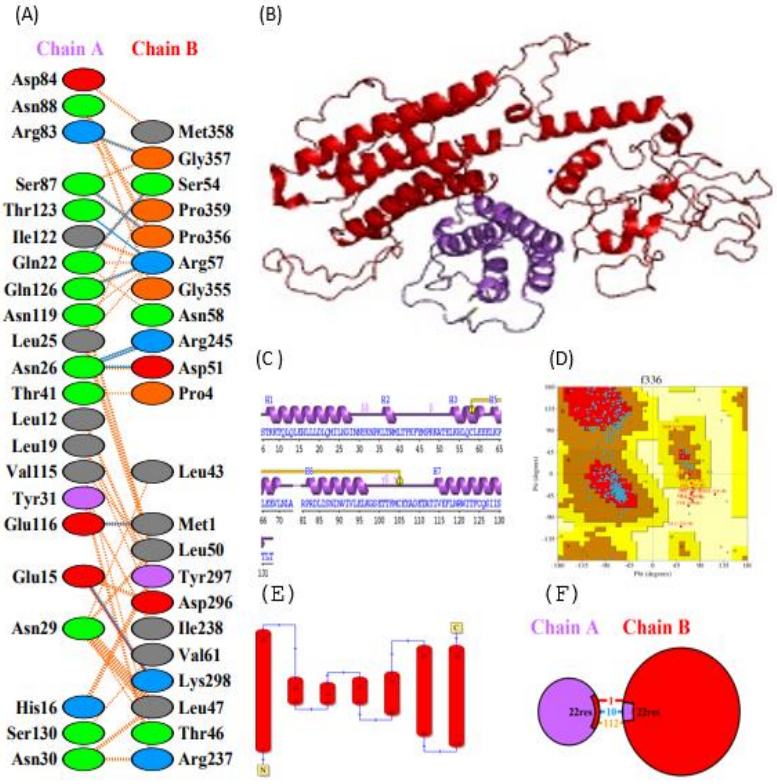


Figure 7: (A) Designed Vaccine with Receptor Interleukin 2 Receptor (IL-2) Alpha Chain Residues that Interact (B) Docked 3-D Structure Representation Vaccine - (IL-2) Alpha Chain. Red Colour Displayed (IL-2) Alpha Chain and Purple Color Displayed Vaccine Construct (C) Vaccine Secondary Structure (D) Vaccine Constructs Ramachandran Plot. (E) Topology (F) IL2 Alpha Chain- Designed Vaccine Interaction

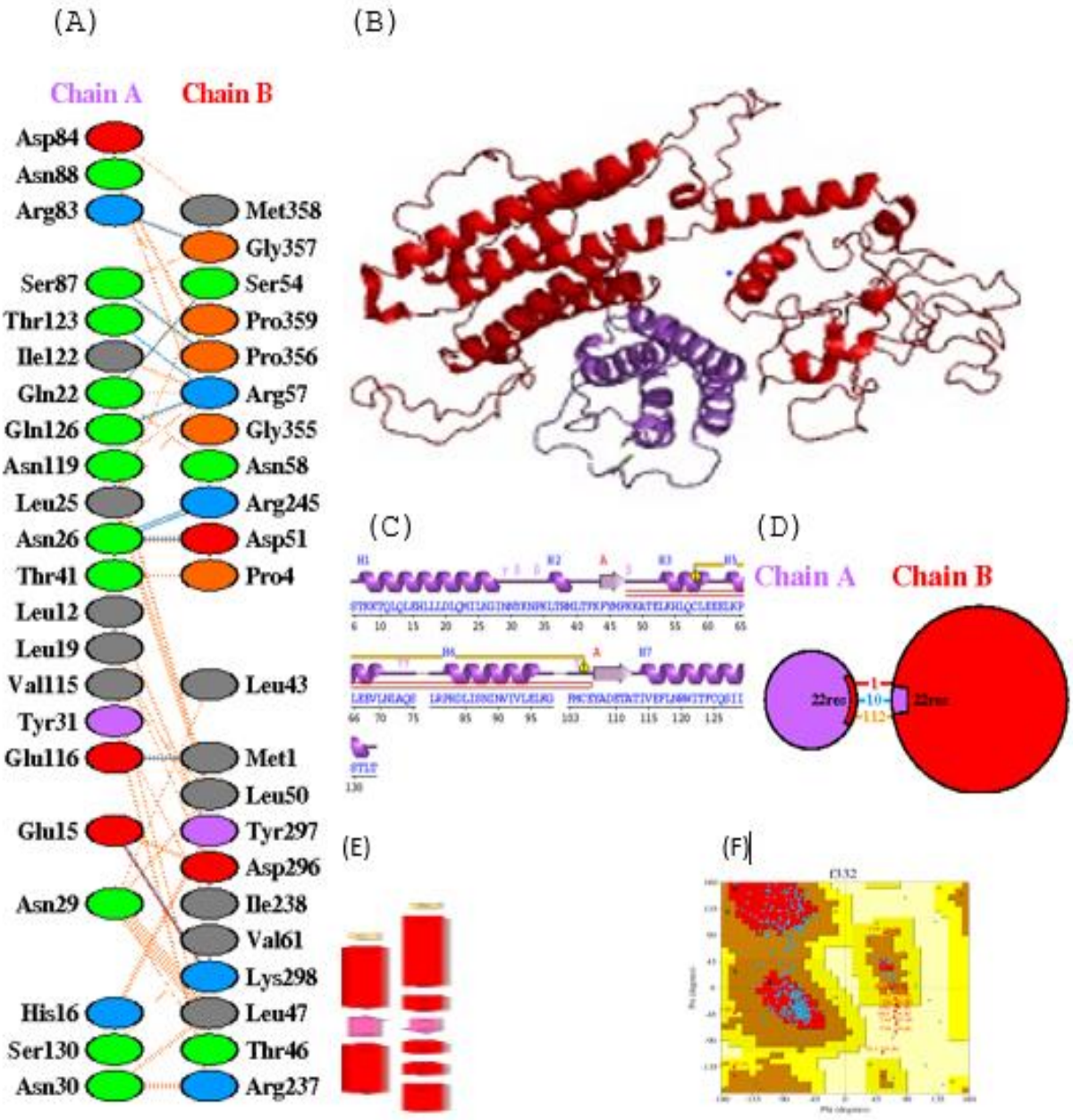


Figure 8: (A) Designed Vaccine with Receptor Interleukin 2 Receptor (IL-2) Beta Chain Residues that Interact (B) Docked 3-D Structure Representation Vaccine - (IL-2) Beta Chain. Red Colour Displayed (IL-2) Beta Chain and Purple Color Displayed Vaccine Construct (C) Vaccine Secondary Structure (D) Vaccine Constructs Ramachandran Plot. (E) Topology (F) IL2 Beta Chain -Designed vaccine Interaction

10. Computational approach to Cloning

In silico cloning was utilized to establish vaccine expression in the often employed E. coli host. The designed vaccine codons were first adapted to the E. coli expression platform's codon usage. The codons were optimized using the JCAT Online server. The server showed improved DNA. Content GC and CAI(Codon Adaptation Index) value 54.99 and 0.971 respectively.It is proven Probability of positive protein expression and dependability are both high. We added the ECORI restriction enzyme at beginning of the codon region and BamHI restriction enzyme at the end of the codon for the cloning process. Cloned Multitudinous epitope-based vaccine inserted into vector pET28a (+). Figure: 9 red colored part showing cloned vaccine sequence

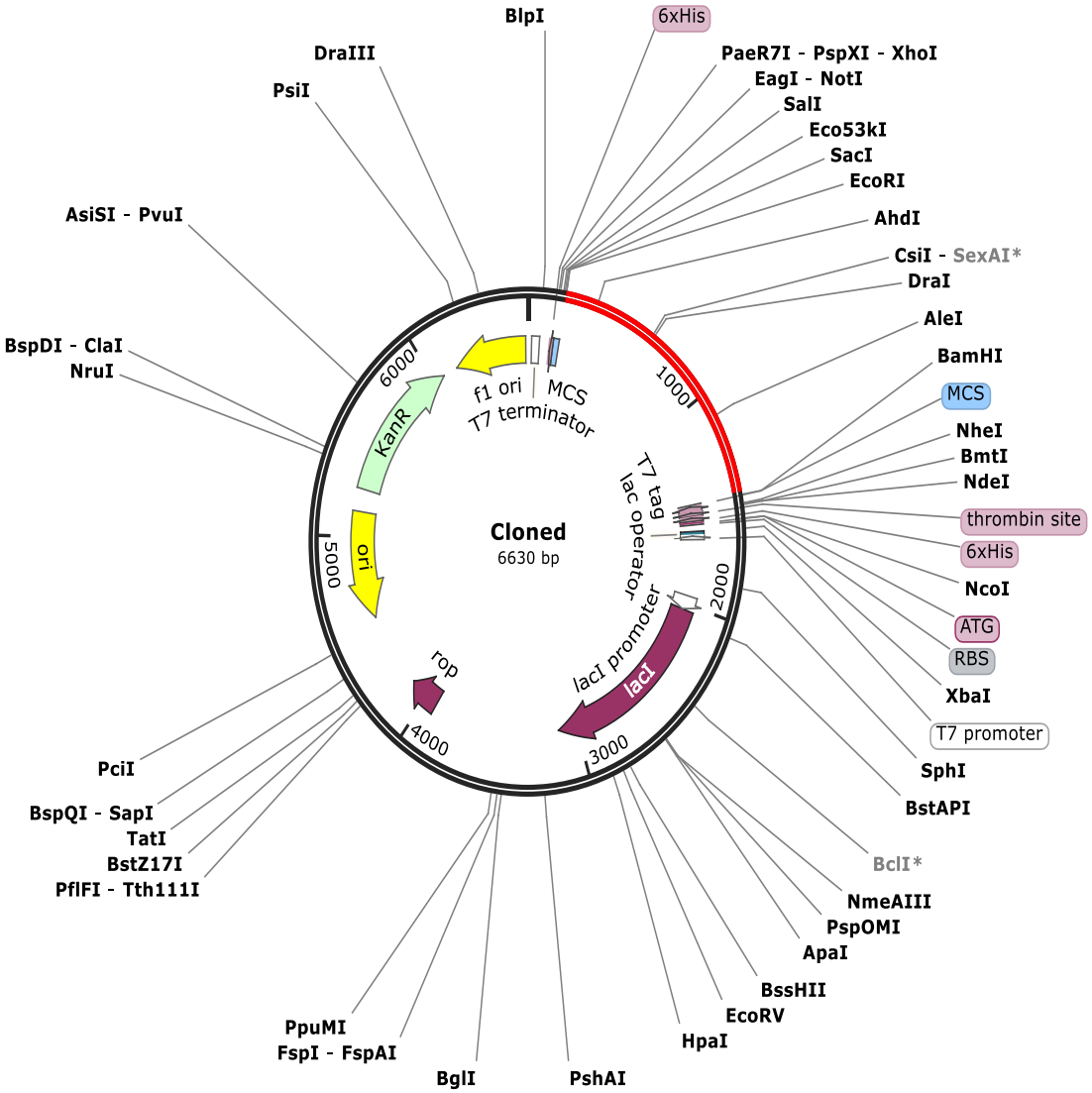


Figure 9: In Silico Cloned Sequence, E. Coli Expression System Nucleotide Sequence Optimised for Vaccination Codons

DISCUSSION

Vaccination does have several beneficial influences on people's better life at a minimal price and is the most effective way to prevent communicable diseases all over the world. Vaccine development is time-consuming, expensive and also it needs more manpower. However Computational approach can help to reduce the above drawbacks. Computational biology is a field that deals with the computer analysis of various biological data. With the help of this, we can predict Datasets on immunology.

In the current study, we have designed a vaccine based on B-cell and T-cell epitopes through various *in silico* techniques. To date, various studies are conducted on Genetic disorders but still, there are no proper reports on Vaccine development. Based on the cancer vaccinology technique we have performed this analysis. Similar studies are also going on for Alzheimer's disease for vaccine development. We have retrieved the BTD protein sequence from NCBI and predicted 3-D structure for the same. Next step we checked the antigenicity of protein. Higher antigenicity was observed and hence it was chosen to design the vaccine. Biotinidase protein Primary, secondary structure analysis was performed and followed by B-cell and T-cell epitopes prediction To validate the epitopes, molecular docking was performed on the HLA-B7 against antigenic B-cell epitopes and T-cell epitopes. Above all, adjuvant (alumni salt) was added N-terminal of the vaccine sequence linked with the EAAAK linker for increasing immunogenicity. Next, The AAY linker links B-cell epitopes. GPGPG linker connected to a T-cell epitope. When imported to the human body this linker is mainly used for epitopes to maintain their functionality. So epitopes can work properly. The constructed vaccine is the best model to

boost immune response due to its great immunogenic, non-toxic, non-allergic and highly antigenic properties. Modeled vaccine docked with Human immune receptor Interleukin 2 receptor (IL-2) alpha chain and beta chain and the result displayed the best docking score. Designed vaccine hosted into vector pET28a (+) for additional "in vivo" and "in vitro" research.

CONCLUSION

We designed a vaccine for Biotinidase deficiency using a computational approach. To find surface-exposed peptides, we used a reverse vaccinology technique. To generate a prospective and safe vaccine that could induce immune responses, *in silico* techniques and immunoinformatics approaches are used. A significant attempt to create a multitudinous epitope-based vaccine against biotinidase deficiency was made in this study. Effective vaccine candidates were identified that are non-toxic to B and T cells, antigenic, and have overlapping epitopes. In the future, this preparatory work might deliver huge effectiveness in vaccine development for genetic disorders. Current research is only on in silico approaches. The designed vaccine is safe and stable. Although this work needs to conduct more experimental-based research to prove the vaccine's efficiency. So far, there have not been many immune informatics studies on genetic disorders. Without a doubt, this work will help us understand genetic disorders better using immunoinformatics approaches.

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