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iiIMMUNOINFORMATIC STUDY FOR A PEPTIDE BASED VACCINE AGAINST RABIES LYSSAVIRUS RABV STRAIN PV

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Abstract

Rabies lyssavirus is a bullet-shaped, negative-sense, single-stranded RNA virus of the *Rhabdoviridae* family. There is no treatment for the symptomatic illness. Fatality rate of rabies is close to 100% for non vaccinated people.The Rabies transmitted through the saliva of infected animals. Though the vaccines are commercially available in market,this scientific study was designed to develop a vaccine for rabies in silico analysis. The protein sequence of Rabies virus was retrieved from Uniprot.B-cell and T-cell epitopes were identified and further screened for allergenicity, antigenicity, simulation and the vaccine tertiary structure was designed.

***Key words*:** *Rabies,Dogs,peptide vaccine prediction,Immunoinformatics*.

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Introduction

Viruses in the Rabies serogroup contains 10 viruses, but only Rabies lyssavirus and Australian bat lyssavirus that have been known to cause disease in humans [1]The rabies virus causes fatal neurological symptoms in almost all mammals and is spread through the bite of an infected mammal. Because of the disease, between 40,000 and 70,000 death occur every year worldwide. The RNA genome of the virus encodes five genes . These genes code for nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G) and the viral RNA polymerase (L).[2] The first symptoms of rabies may be similar to the flu, discomfort, fever, or headache. There also may be discomfort, prickling, or an itching sensation at the site of the bite. Symptoms then progress to cerebral dysfunction, anxiety, confusion, and agitation. As the disease progresses, the person may experience delirium, abnormal behavior, hallucinations, hydrophobia (fear of water), and insomnia. The acute period of disease typically ends after 2 to 10 days.[3]

Immunoinformatics approaches are used for the determination of vaccine design [4] .In vaccine design, suitable protein selection is important. Protein which is virulent, highly antigenic, and non-homologous for humans can be used to increase efficacy [5].We design a more effective, safe, and thermodynamically stable epitope base vaccine design for to elicit an innate and adaptive immune response. We used the immunoinformatics approach for vaccine designing, to select nonoverlapping, nonallergic, antigenic epitopes.[6]

Methods

Retrieval of sequence

The protocol for designing a vaccine against *rabies* was initiated by retrieving the protein sequences.Totally 60 sequences had been retrived fromUniProt (https://www.uniprot.org/). [7]

Antigenicity

All 60 rabies virus sequences had been subjected to check the antigenicity using Vaxijen 2.0 server. [8] 8 sequences had been found to be an antigen with a threshold value of 0.5,includes glycoprotein and nucleoprotein sequences.

Allergenicity

The protein was scanned for allergic prediction by analysing it in AllerTOP v. 2.0 (https:// www.ddg.pharm fac.net/ AllerTOP) online server [9]

Prediction of linear B-cell epitopes:

Bepipred test from immune epitope database (http://tools.iedb.org/bcell/result/) [10] was used as linear B-cell epitopes prediction from the desired peptide with a default threshold value of 0.5.

MHC class I and MHC II binding predictions

Analysis of peptide binding to MHC1 and MHC II molecules was assessed by the IEDB MHC prediction tool at http://tools.iedb.org/mhci, http://tools.iedb.org/mhcii/result/ , with selected alleles HLA 01:01,HLA 02:01,HLA B 27:05 and DRB HLA-DRB1*04:01 ,HLA-DRB1*15:01 HLA-DRB1 01:01.Prediction methodisNetMHCpan EL 4.1 based on High Score = good binderBeforeprediction, all epitope lengths were set as 13mers.[11]

Toxigenicity of Selected Peptide

A generic webserver for peptide toxicity predictionToxIBTL was used to check the toxicity of the peptide candidate.https://server.wei-group.net/ToxIBTL/Server.html [12]

Population coverage calculation

Potential MHC I and MHC II and B Cell binders from rabies virus nucleoprotein was evaluated for population coverage against the whole world population with the selected MHC-I and MHC-II interacted

alleles by the IEDB population coverage calculation tool at http://tools.iedb.org/tools/population/iedb_input. [11]

PepSySco

Peptide Synthesis Score (PepSySco) predicts the likelihood that they can be synthesized successfully.This Tool provides a score from 0 to 1, with a higher score indicating more likely success at synthesis. http://tools.iedb.org/main/analysis-tools/[11]

Immune Simulation

The primary and secondary immune reactions shows a noteworthy role in factual immune responses. In silico responses of the host immunological system against antigens is represented in (Figure 7). C-IMMSIM Online server available at http://kraken.iac.rm.cnr.it/C-IMMSIM [13]

Secondary Structure Prediction of peptide candidate

The Self-Optimized Prediction method With Alignment (SOPMA) tool is used to predict the secondary structure of a protein. Based on the query SOPMA was utilized to investigate the vaccine secondary structure.https://npsa-prabi.ibcp.fr/cgi-bin/secpred_sopma.pl[14]

3D Modelling of Selected Peptide

(PS) ^{2} – v2 is a neural network based predictor that based on a number of structural features predicts the quality of different parts of a protein model. The quality ranges from 0 for to 1 for a perfect prediction. The predicted scores are the S-score=1/(1+(rmsd/5)2) for each residue.http://ps2.life.nctu.edu.tw/[15]

Vaccine 3D structure validation

For validation of tertiary structure, Galaxy Refine was used. If the score is more than 90, the structure is validated.

Result and Discussion

Retrieval of protein sequence

The protein sequences of Rabies virus were extracted from the UniProt in FASTA format. Total 60 sequneces had been retrived.

Antigenicity

All the 60 sequnces were subjected to test their antigenicity using VaxiJen 2.0 Server with a threshold value of 0.5. Among 60, 8 Protein sequences shows antigenicity(P16285,O92284,P06025,P08667,P08671,P15199,Q08089,Q0GBY1).Except P60205 and p16285,rest all are glycoprotein and Matrix protein sequnces.For further study the nucleoprotein sequences P06025 was selected.

B Cell Epitope Prediction

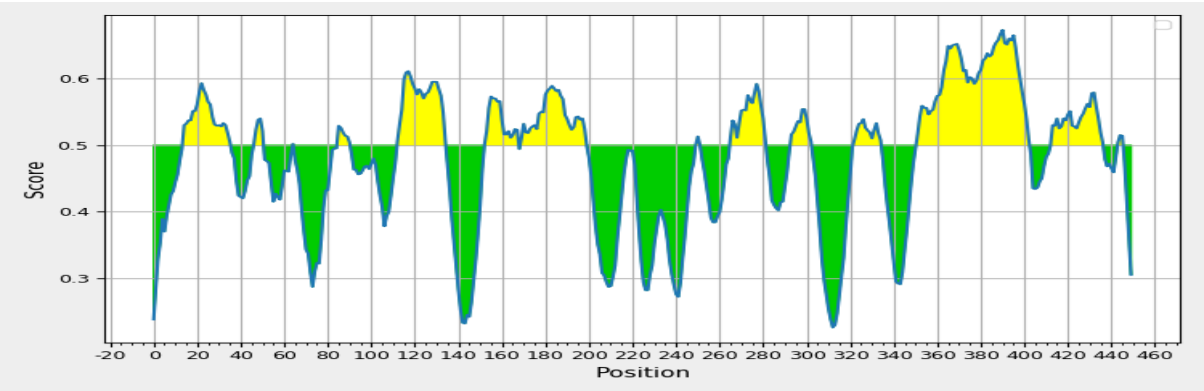


Figure 1:Bepipred epitope prediction

Yellow areas above threshold (red line) are proposed to be a part of B cell epitope while green are not.

No.	Start	End	Peptide	Length
1	251	252	LT	2
2	266	282	EEEIRRMFEPGQETAVP	17
3	294	303	LSGKSPYSSN	10
4	323	335	RSLNATVIAACAP	13
5	352	403	KGTFERRFFRDEKELQEYEAAELTKTDVALADDGTVNSDDEDYFSGETRSPE	52
6	414	437	GRLKRSHIRRYVSVSSNHQARPNS	24
7	444	446	KTY	3

Table 1 :A list of Bepipred linear epitopes predicted by IEDB analysis

MHC class I and MHC II binding predictions

MHC I alleles

The reference nucleoprotein (NP) strain was analyzed using IEDB MHC-I binding prediction tool based on NetMHCPan with high score is equal to good binder.The list of all epitopes and their correspondent binding MHC1 alleles were shown in Table 1 for the selected HLA alleles

HLA-B*27:05	2	62	74	13	RRYVSVSSNHQAR	RRYVSVSSR	RRYVSVSSNHQAR	0.426342	0.44
HLA-A*01:01	1	198	210	13	STIPNFRFLAGTY	STIPNAGTY	STIPNFRFLAGTY	0.164831	0.64
HLA-A*01:01	1	18	30	13	KPEIIVDQYEYKY	KVDQYEYKY	KPEIIVDQYEYKY	0.143556	0.71
HLA-A*01:01	1	247	259	13	KQINLTAREAILY	KTAREAILY	KQINLTAREAILY	0.142981	0.71
HLA-A*01:01	1	221	233	13	YSAIRVGTVVTAY	YSATVVTAY	YSAIRVGTVVTAY	0.138218	0.72

Table 2: List of HLA alleles MHC I

MHC II alleles

The reference Nucleoprotein (NP) strain was analyzed using IEDB MHC- II binding prediction tool based on NetMHCPan with high score is equal to good binder.The list of all epitopes and their correspondent binding MHC1 alleles were shown in Table

HLA-DRB1*04:01	1	422	436	15	YVSVSSNHQ	RRYVSVSSNHQARPN	0.9765	0.01
HLA-DRB1*04:01	1	420	434	15	YVSVSSNHQ	HIRRYVSVSSNHQAR	0.9772	0.01
HLA-DRB1*04:01	1	421	433	13	YVSVSSNHQ	IRRYVSVSSNHQA	0.9775	0.01
HLA-DRB1*04:01	1	422	434	13	YVSVSSNHQ	RRYVSVSSNHQAR	0.9787	0.01
HLA-DRB1*04:01	1	421	435	15	YVSVSSNHQ	IRRYVSVSSNHQARP	0.9852	0.01
HLA-DRB1*15:01	1	419	431	13	IRRYVSVSS	SHIRRYVSVSSNH	0.9344	0.02
HLA-DRB1*15:01	1	418	430	13	IRRYVSVSS	RSHIRRYVSVSSN	0.9434	0.02
HLA-DRB1*15:01	1	418	432	15	IRRYVSVSS	RSHIRRYVSVSSNHQ	0.9652	0.02
HLA-DRB1*15:01	1	417	431	15	IRRYVSVSS	KRSHIRRYVSVSSNH	0.9534	0.03
HLA-DRB1*04:01	1	423	435	13	YVSVSSNHQ	RYVSVSSNHQARP	0.9343	0.04
HLA-DRB1*04:01	1	419	433	15	YVSVSSNHQ	SHIRRYVSVSSNHQA	0.9569	0.06
HLA-DRB1*15:01	1	363	377	15	LQEYEAAEL	EKELQEYEAAELTKT	0.9192	0.08
HLA-DRB1*15:01	1	419	433	15	IRRYVSVSS	SHIRRYVSVSSNHQA	0.9154	0.09
HLA-DRB1*15:01	1	416	430	15	IRRYVSVSS	LKRSHIRRYVSVSSN	0.9071	0.12
HLA-DRB1*01:01	1	11	25	15	VVSLKPEII	NNQVVSLKPEIIVDQ	0.9596	0.13

Table 3 List of MHC II Peptides

The Sequence P06025.

MDADKIVFKV~~NNQVVSL~~*KPEIIVDQ*~~*YEYKY*~~PAIKDLKKPCITLGKAPDLNKAYKSVLS
CMSAAKLDPDDVCSYLAAAMQFFEGTCPEDWTSYGIVIARKGDKITPGSLVEIKRTD
VEGNWALTGGMELTRDPTVPEHASLVGLLLSLYRLSKISGQSTGNYKTNIADRIEQIF
ETAPFVKIVEHHTLMTTHKMCANW*STIPNFRFLAGTY*DMFFSRIEHL*YSAIRVGTVVTAY*
EDCSGLVSFTGFI*KQINLTAREAILY*FFHKNFEEEIRRMFEPGQETAVPHSYFIHFRSLGL
SGKSPYSSNAVGHVFNLIHFVGCYMGQVRSLNATVIAACAPHEMSVLGGYLGEEFF
GKGTFERRFFRD~~EKELQEYEAAELTKT~~DVALADDGTVNSDDEDYFSGETRSPEAVY
TRIIMNGGR~~**LKRSHI*RRYVSVSSNHQAR***~~**PN**SFAEFLNKTYSSDS

Peptide candidate (LKRSHIRRYVSVSSNHQARPN**)** able to bind both T Cell and Bcell,and covered all HLA types are marked as Bold and highlighted with yellow.

Allergenicity

Allergenicity were evaluated by AllerTOP v. 20. AllerTOP calculated the score, showing that the vaccine construct was nontoxic and nonallergic.

PepSySco – Peptide synthesis score

Peptide	
LKRSHIRRYVSVSSNHQARPN	0.93532

Table 4 :Peptide synthesis score

Score provides 0 to 1.More score means more likely to success.Our peptide candidate shows 0.9 score.

Immune simulation

C-ImmSim server was used for immune stimulation. Figure 2indicates that our result immune response was the same as body immune response in the human body. Figure 2A represents the production of IgG and IgM antibodies. Figure 2B shows the high level of antibody production. IFN-γ score was high as shownin Fig. 2C. TH cell population is indicated in Fig. 2D**.**

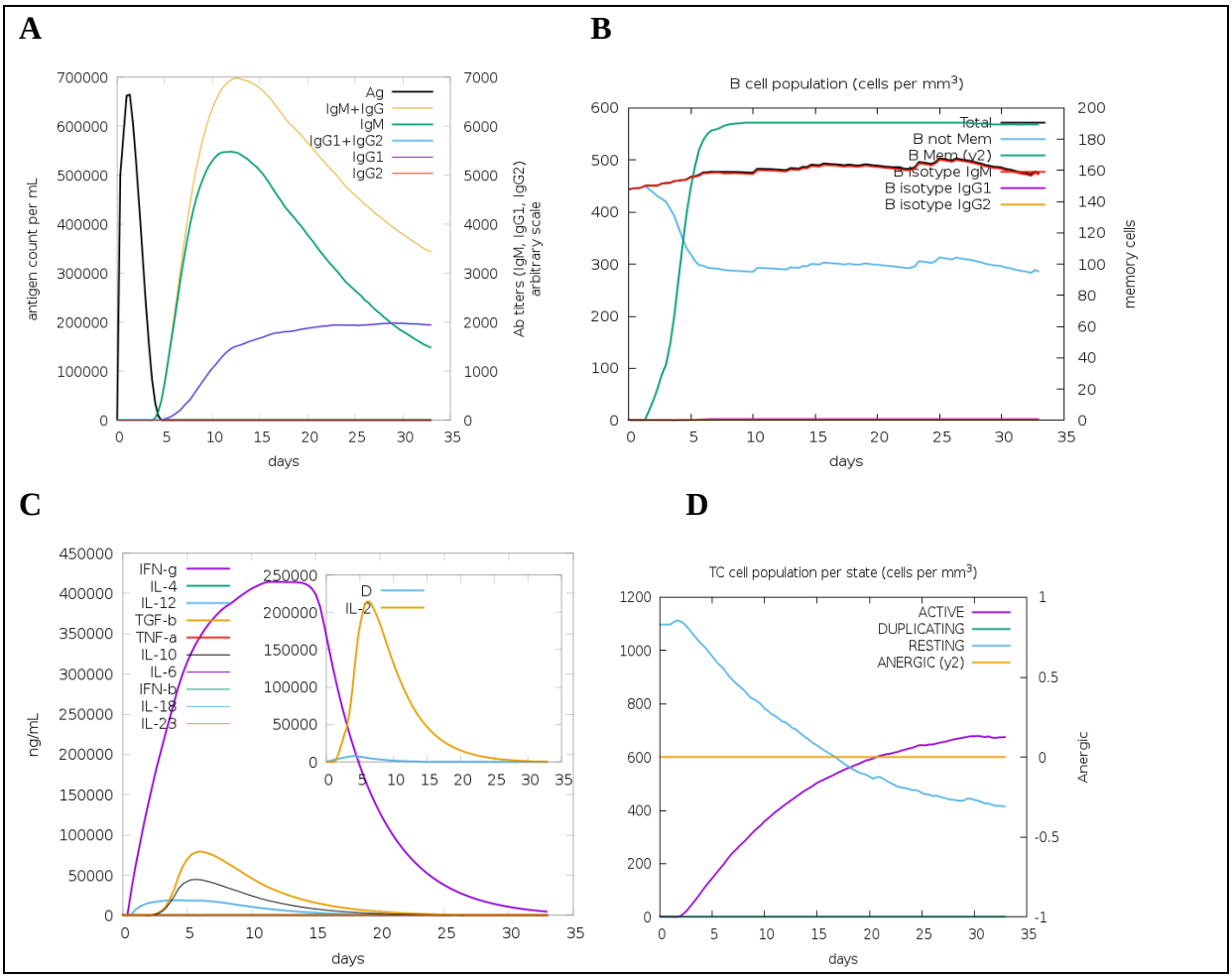
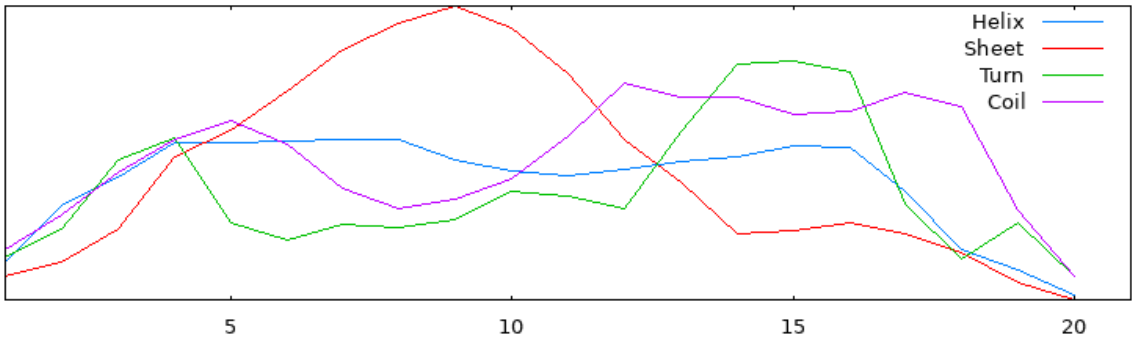


Figure 2: Vaccine immune simulation through C-ImmSim server

Analysis of secondary structure

The secondary structure of the vaccine sequence was predicated on employing the online server SOPMA, which exhibits 14.29% helix, 35.7% beta-sheets, and 28.57% loops (coil) 28.57.




```
  10       20
     |       |
LKRSHIRRYVSVSSNHQARPN
hhhttceeeeeecctttcccct
```

Figure 3:Secondary Structure prediction using SOPMA

3D Modelling of the Vaccine Candidate

(PS) [2] – v2 Protein structure prediction server is used to construct the 3D structure of the peptide candidate.

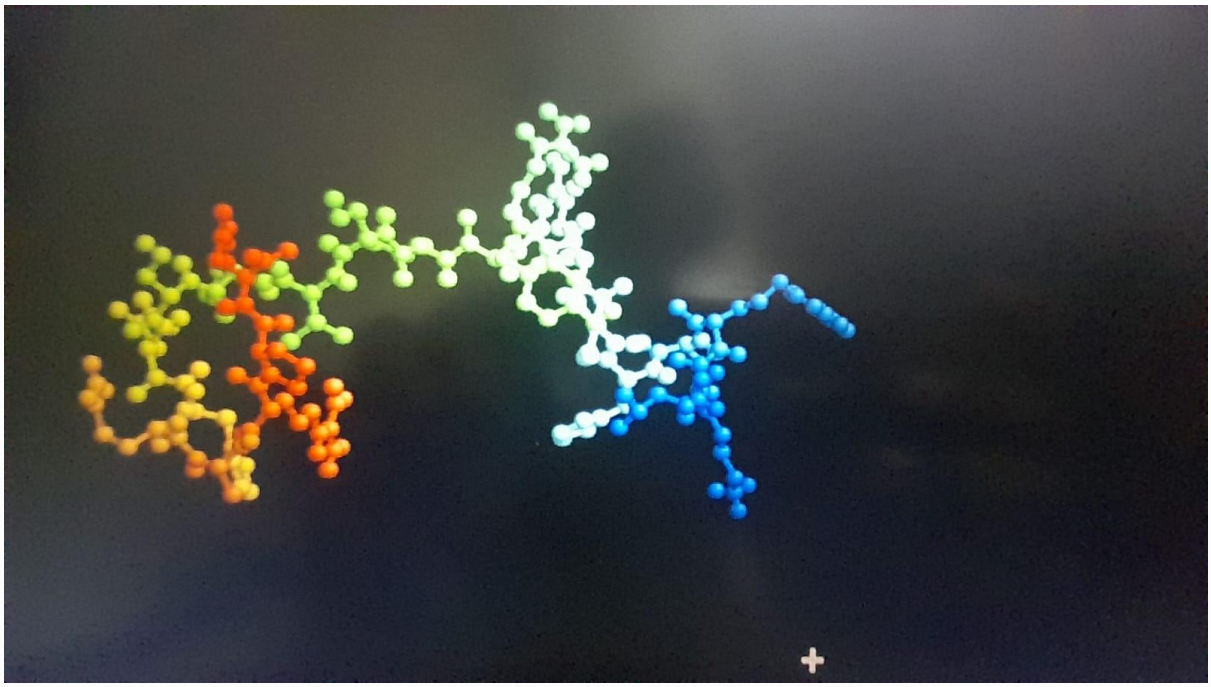


Figure 4: 3D Modelling of selected vaccine peptide .In ball& Stick Model.

Refinement of tertiary structure

Using Galaxy Refine, the vaccine tertiary structure was refined to further modify its structure quality.Vaccine 3D structure validation For validation of tertiary structure, Galaxy Refine was used. If the score is more than 90, the structure is validated.

Sequnce LOGO for the Selected Vaccine Peptide Candidate.

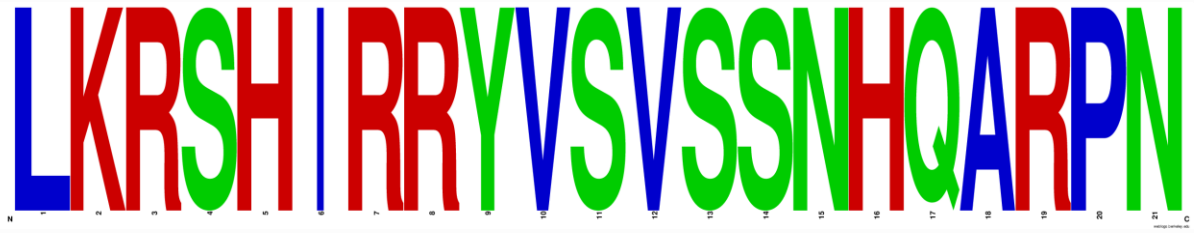


Figure 5 : Sequence Logo

The whole height of each stack shows the sequence conservation at that position measured in bits), while the height of symbols within the stack reflects the relative frequency of the corresponding amino or nucleic acid at that position[17]

CONCLUSION

Vaccination plays a vibrant role in immune system stimulationand also prevents the outbreak of various pathogen-borne contagious illnesses.Vaccines predicted for the different pathogens are used all over the world and are supposed to be the best way treatment of various disorders. The vaccine is hoped to evoke and give a broader immune protection to human against the rabies virus. Firstly, a sequence-based analysis was done to identify the best peptide, which is more likely to initiate immune response. The predicted peptide sequence for the vaccine was established to allow an experimental validation in the future.

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