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An Immunoinformatics Approach to Design Synthetic Peptide Vaccine from *Dendroaspis polylepis polylepis* Dendrotoxin-K(DTX-K)

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Abstract

Dendroaspis polylepis polylepis is the most toxic snake commonly known as black mamba, the black mamba venom contains Dendrotoxin-K which is highly specific and virulently toxic protein. Antigenic peptides of Dendrotoxin toxic protein are most suitable for peptide vaccine development because with single epitope, the immune response can be generated in large population. Analysis shows MHC class II binding peptides of antigenic protein from *Dendroaspis polylepis polylepis* DTX-K are important determinant for protection against several venom toxins. In this assay we predicted the binding affinity of *Dendroaspis polylepis polylepis* DTX-K protein having 79 amino acids, which shows71nonamers. In this analysis, we found the High affinity TAP Transporter peptide regions as, 37-KRKIPSFYY (score-9.550), 45-YKWKAKQCL (Score-8.581) 36-CKRKIPSFY (Score-7.685), 24-AKYCKLPLR (Score-7.669), 42-SFYYKWKAK (Score-6.859), 31-LRIGPCKRK (Score-6.848) 65-NRFKTIEEC (Score-6.698), 25-KYCKLPLRI (Score-6.632), 49-AKQCLPFDY (Score-6.576), 66-RFKTIEECR (Score-6.464), 47-WKAKQCLPF (Score-6.197), 23-AAKYCKLPL (Score-6.166). We also found the SVM based MHCII-IAb peptide regions, 61-GGNANRFKT, 12-TLWAELTPV, 41-PSFYYKWKA, 25-KYCKLPLRI (optimal score is 0.946); MHCII-IAd peptide regions, 2-GHLLLLLGL, 57-SGCGGNAN, 3-HLLLLLGLL, 1-SGHLLLLLG (optimal score is 0.488); MHCII-IAg7 peptide regions 60-CGGNANRFK, 21-SGAAKYCKL, 61-GGNANRFKT, 20-VSGAAKYCK (optimal score is 1.468); and MHCII-RT1.B peptide regions 46-KWKAKQCLP, 24-AKYCKLPLR, 10-LLTLWAELT, 45-YKWKAKQCL (optimal score is 0.569) which represented predicted binders from dendrotoxin. The method integrates prediction of peptide MHC class I binding; proteasomal C terminal cleavage and TAP transport efficiency of the *Dendroaspis polylepis polylepis* DTX-K. Thus a small fragment of antigen can induce immune response against whole antigen. This theme is implemented in designing subunit and synthetic peptide vaccines.

Keywords: *Dendroaspis polylepis polylepis;* Dendrotoxin-K, Antigenic peptides; MHC-Binders; SVM; Nonamers

Introduction

Dendroaspis polylepis polylepis commonly known as black mamba is the aggressive and highly venomous land snake; *Dendroaspis polylepis polylepis* venom contains Dendrotoxin-K (DTX-K), which has ability to kill a mouse within 5 minutes after bite. The dendrotoxin is highly specific and virulently toxic protein of low molecular weight that can spread very rapidly within the bitten tissue, so black mamba venom is the most rapid-acting of all snake venoms. Dendrotoxin inhibits the exogenous process of muscle contraction by means of the sodium potassium pump. Dendrotoxin-K is a selective blocker of voltage-gated potassium channels [1,2].

Strategy

The phenotype of the resistant transgenic plants includes fewer centers of initial virus infection, a delay in symptom development, and low bacterial accumulation. Protoplasts from disease resistant transgenic plants are also resistant, suggesting that the protection is largely operational at the cellular level. Transgenic plants expressing nucleocapsid protein are protected against infection by bacteria but are susceptible to bacterial DNA, indicating that the protection may primarily involve an inhibition of bacterial cell wall. This approach is based on the phenomenon of cross-protection [3], hereby a plant infected with a mild strain of bacteria is protected against a more severe strain of the same bacteria. Plant Proteins are necessary for its production in or on all food commodities. An exemption from the requirement of a tolerance is established for residues of the biological plant pesticide.

MHC class binding peptides

The new paradigm in vaccine design is emerging, following essential discoveries in immunology and development of new MHC Class-I binding peptides prediction tools [4-7]. MHC molecules are cell surface glycoproteins, which take active part in host immune reactions. The involvement of MHC class-I in response to almost all antigens and the variable length of interacting peptides make the study of MHC Class I molecules very interesting. MHC molecules have been well characterized in terms of their role in immune reactions. They bind to some of the peptide fragments generated after proteolytic cleavage of antigen [8]. This binding acts like red flags for antigen specific and to generate immune response against the parent antigen. So a small fragment of antigen can induce immune response against whole antigen. Antigenic peptides are most suitable for subunit vaccine development because with single epitope, the immune response can be generated in large population. MHC peptide complexes will be

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translocated on the surface of antigen presenting cells (APCs). This theme is implemented in designing subunit and synthetic peptide vaccines [9]. One of the important problems in subunit vaccine design is to search antigenic regions in an antigen [10] that can stimulate T cells called T-cell epitopes. In literature, fortunately, a large amount of data about such peptides is available. Pastly and presently, a number of databases have been developed to provide comprehensive information related to T-cell epitopes [11-14].

Materials and Methods

Protein sequence analysis

The antigenic protein sequence of *Dendroaspis polylepis polylepis* DTX-K was analyzed to study the antigenicity [15], solvent accessible regions and MHC class peptide binding, which allows potential drug targets to identify active sites against plant diseases.

Prediction of antigenicity

Prediction of antigenicity program predicts those segments from within bacterial pathogenicity protein that are likely to be antigenic by eliciting an antibody response. Antigenic epitopes are determined using the Gomase [9], Hopp and Woods, Welling, Parker, B-EpiPred Server and Kolaskar and Tongaonkar antigenicity methods [14,16-20].

Prediction of protein secondary structure

The important concepts in secondary structure prediction are identified as: residue conformational propensities, sequence edge effects, moments of hydrophobicity, position of insertions and Deletions in aligned homologous sequence, moments of conservation, auto-correlation, residue ratios, secondary structure feedback effects, and filtering [21,22].

Finding the location in solvent accessible regions

Finding the location in solvent accessible regions in protein, type of plot determines the hydrophobic and hydrophilic scales and it is utilized for prediction. This may be useful in predicting membrane-spanning domains, potential antigenic sites and regions that are likely exposed on the protein surface [1,2,23-42].

Prediction of MHC binding peptide

The MHC peptide binding is predicted using neural network strained on C terminals of known epitopes. In analysis predicted MHC/peptide binding is a log-transformed value related to the IC50 values in nM units. MHC2Pred predicts peptide binders to MHCI and MHCII molecules from protein sequences or sequence alignments using Position Specific Scoring Matrices (PSSMs). Support Vector Machine (SVM) based method for prediction of promiscuous MHC class II binding peptides. The average accuracy of SVM based method for 42 alleles is ~80%. For development of MHC binder, an elegant machine learning technique SVM has been used. SVM has been trained on the binary input of single amino acid sequence. In addition, we predicts those MHCI ligands whose C-terminal end is likely to be the result of proteosomal cleavage [43-45].

Result and Interpretation

A antigenic sequence is 79 residues long as-GEDGYIADGDNCT YICTFNNYCHALCTDKKGDSGACDWWVPYGVVCWCEDLPTP VPIRGSGKCR

Prediction of antigenic peptides

In these methods we found the antigenic determinants by finding the area of greatest local hydrophilicity. The Hopp-Woods scale was designed to predict the locations of antigenic determinants in a protein, assuming that the antigenic determinants would be exposed on the surface of the protein and thus would be located in hydrophilic regions (Figure 1). Its values are derived from the transfer-free energies for amino acid side chains between ethanol and water. Welling antigenicity plot gives value as the log of the quotient between percentage in a sample of known antigenic regions and percentage in average proteins (Figure 2). We also study B-EpiPred Server, Parker, Kolaskar and Tongaonkar antigenicity methods and the predicted antigenic fragments can bind to MHC molecule is the first bottlenecks in vaccine design (Figure 3- 6).

Secondary alignment

The Robson and Garnier method predicted the secondary structure of the *Dendroaspis polylepis polylepis* DTX-K. Each residue is assigned values for alpha helix, beta sheet, turns and coils using a window of 7 residues (Figure 7). Using these information parameters, the likelihood of a given residue assuming each of the four possible conformations alpha, beta, reverse turn, or coils calculated, and the conformation with the largest likelihood is assigned to the residue.

Figure 1: The NMR Solution Structure of Dendrotoxin K from the Venom of *Dendroaspis polylepis polylepis* showing hydrophobicity.

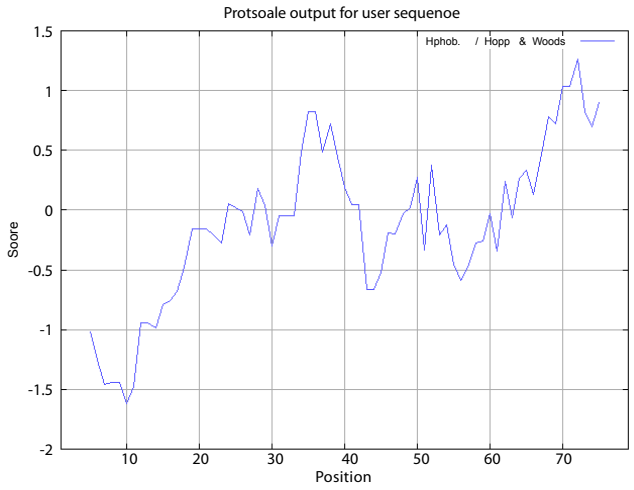

Figure 2: Hydrophobicity plot of Hopp and Woods (1981) of *Dendroaspis polylepis polylepis* DTX-K.

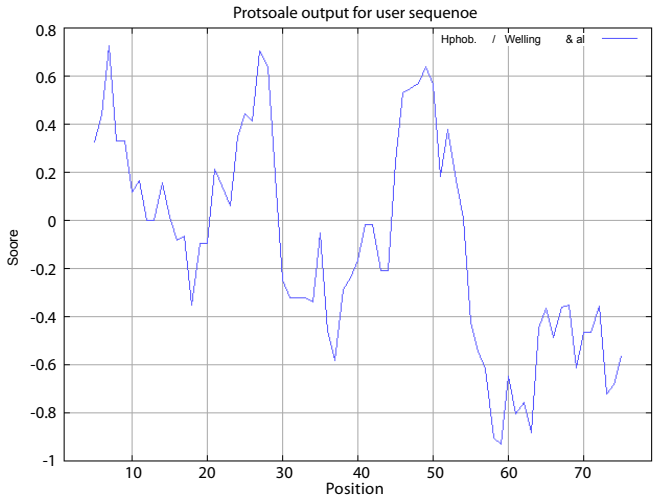

Figure 3: Hydrophobicity plot of Welling et al. (1985) of *Dendroaspis polylepis polylepis* DTX-K.

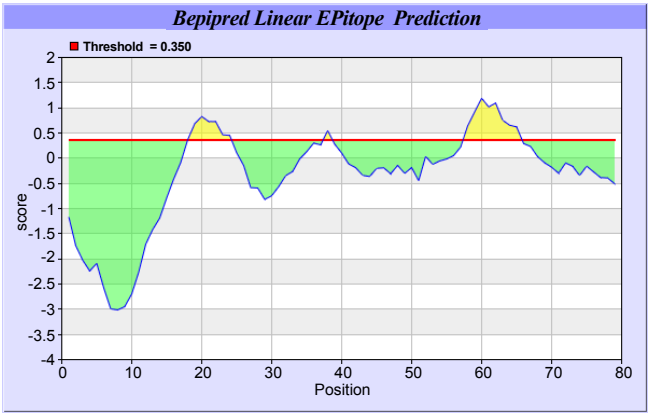

Figure 4: B-cell epitopes are the sites of molecules that are recognized by antibodies of the immune system of the *Dendroaspis polylepis polylepis* DTX-K.

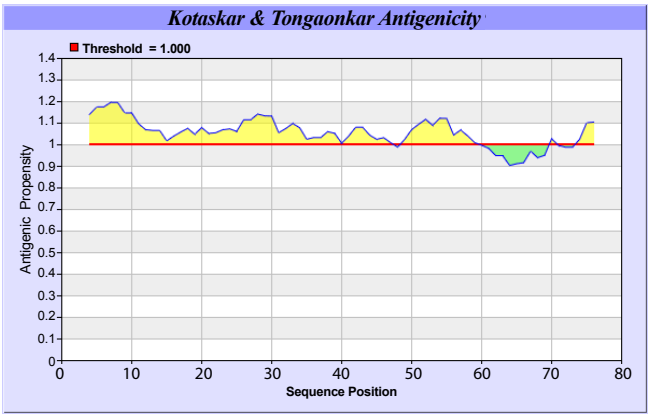

Figure 5: Kolaskar and Tongaonkar antigenicity are the sites of molecules that are recognized by antibodies of the immune system for the *Dendroaspis polylepis polylepis* DTX-K.

Solvent accessible regions

Solvent accessible scales for delineating hydrophobic and hydrophilic characteristics of amino acids and scales are developed for predicting potential antigenic sites of globular proteins, which are likely to be rich in charged and polar residues. It was shown that a *Dendroaspis polylepis polylepis* DTX-K is hydrophobic in nature and contains segments.

Prediction of MHC binding peptides

These MHC binding peptides are sufficient for eliciting the desired immune response. The prediction is based on cascade support vector machine, using sequence and properties of the amino acids. The correlation coefficient of 0.88 was obtained by using jack-knife validation test. In this test, we found the MHCI and MHCII binding regions (Tables 1 and 2). MHC molecules are cell surface glycoproteins, which take active part in host immune reactions and involvement of MHC class-I and MHC II in response to almost all antigens. In this assay we predicted the binding affinity of *Dendroaspis polylepis polylepis* DTX-K having 79 amino acids, which shows different nonamers (Tables 1 and 2). For development of MHC binder prediction method, an elegant machine learning technique support vector machine (SVM) has been used. SVM has been trained on the binary input of single amino acid sequence. In this assay we predicted the binding affinity of *Dendroaspis polylepis polylepis* DTX-K sequence (IsTX) having 79 amino acids, which shows 71nonamers. Small peptide regions found as High affinity TAP Transporter peptide regions as, 37- KRKIPSFYY (score-9.550), 45-YKWKAKQCL (Score-8.581), 36-CKRKIPSFY (Score-7.685), 24-AKYCKLPLR (Score-7.669), 42-SFYYKWKAK (Score-6.859), 31-LRIGPCKRK (Score-6.848), 65-NRFKTIEEC (Score-6.698), 25-KYCKLPLRI (Score-6.632), 49-AKQCLPFDY

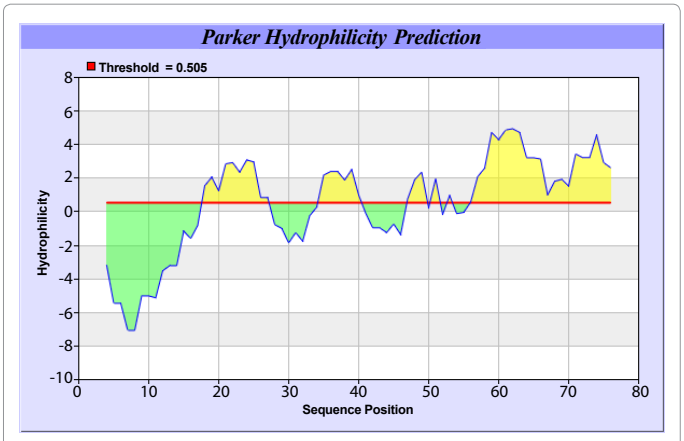

Figure 6: Hydrophobicity plot of HPLC / Parker et al. (1986) of *Dendroaspis polylepis polylepis* DTX-K.

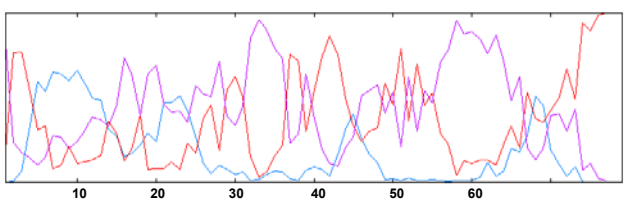

Figure 7: Secondary structure GOR plot of the *Dendroaspis polylepis polylepis* DTX-K.

Peptide Rank	Start Position	Sequence	Score	Predicted Affinity
1	37	KRKIPSFYY	9.550	High
2	45	YKWKAKQCL	8.581	High
3	36	CKRKIPSFY	7.685	High
4	24	AKYCKLPLR	7.669	High
5	42	SFYYKWKAK	6.859	High
6	31	LRIGPCKRK	6.848	High
7	65	NRFKTIEEC	6.698	High
8	25	KYCKLPLRI	6.632	High
9	49	AKQCLPFDY	6.576	High
10	66	RFKTIEECR	6.464	High
11	47	WKAKQCLPF	6.197	High
12	23	AAKYCKLPL	6.166	High

*Optimal Score for given MHC binder in Mouse

Table 1: TAP Peptide binders of *Dendroaspis polylepis polylepis* DTX-K.

Prediction method	Rank	Sequence	ResidueNo.	Peptide Score
ALLELE I-Ab	1	GGNANRFKT	61	0.946
ALLELE I-Ab	2	TLWAELTPV	12	0.918
ALLELE I-Ab	3	PSFYYKWKA	41	0.687
ALLELE I-Ab	4	KYCKLPLRI	25	0.639
ALLELE I-Ad	1	GHLLLLLGL	2	0.488
ALLELE I-Ad	2	YSGCGGNAN	57	0.484
ALLELE I-Ad	3	HLLLLLGLL	3	0.467
ALLELE I-Ad	4	SGHLLLLLG	1	0.396
ALLELE I-Ag7	1	CGGNANRFK	60	1.468
ALLELE I-Ag7	2	SGAAKYCKL	21	1.467
ALLELE I-Ag7	3	GGNANRFKT	61	1.369
ALLELE I-Ag7	4	VSGAAKYCK	20	1.208
ALLELE RT1.B	1	KWKAKQCLP	46	0.569
ALLELE RT1.B	2	AKYCKLPLR	24	0.344
ALLELE RT1.B	3	LLTLWAELT	10	0.257
ALLELE RT1.B	4	YKWKAKQCL	45	0.248

*Optimal Score for given MHC II peptide binder in Mouse

Table 2: Peptide binders to MHCII molecules of *Dendroaspis polylepis polylepis* DTX-K.

(Score-6.576), 66-RFKTIEECR (Score-6.464), 47-WKAKQCLPF (Score-6.197), 23-AAKYCKLPL (Score-6.166). We also found the SVM based MHCII-IAb peptide regions, 61-GGNANRFKT, 12-TLWAELTPV, 41-PSFYYKWKA, 25-KYCKLPLRI (optimal score is 0.946); MHCII-IAd peptide regions, 2-GHLLLLLGL, 57-SGCGGNAN, 3-HLLLLLGLL, 1-SGHLLLLLG (optimal score is 0.488); MHCII-IAg7 peptide regions 60-CGGNANRFK, 21-SGAAKYCKL, 61-GGNANRFKT, 20-VSGAAKYCK (optimal score is 1.468); and MHCII-RT1.B peptide regions 46-KWKAKQCLP, 24-AKYCKLPLR, 10-LLTLWAELT, 45-YKWKAKQCL (optimal score is 0.569) which represented predicted binders from *Dendroaspis polylepis polylepis* DTX-K. (Table 2). The predicted binding affinity is normalized by the 1% fractil. The MHC peptide binding is predicted using neural networks trained on C terminals of known epitopes. In analysis predicted MHC/peptide binding is a log-transformed value related to the IC50 values in nM units. These MHC binding peptides are sufficient for eliciting the desired immune response. Predicted MHC binding regions in an antigen sequence and there are directly associated with immune reactions, in analysis we found the MHCI and MHCII binding region.

Discussion and Conclusion

Gomase method [9], B-EpiPred Server, Hopp and Woods, Welling, Parker, Kolaskar and Tongaonkar antigenicity scales were designed to predict the locations of antigenic determinants in *Dendroaspis polylepis polylepis* DTX-K. Nucleocapsid shows beta sheets regions, which are high antigenic response than helical region of this peptide and shows highly antigenicity (Figure 1-5). We also found the Sweet hydrophobicity, Kyte & Doolittle hydrophobicity, Abraham & Leo, Bull & Breese hydrophobicity, Guy, Miyazawa hydrophobicity, Roseman hydrophobicity, Cowan HPLC pH7.5 hydrophobicity, Rose hydrophobicity, Eisenberg hydrophobicity, Manavalan hydrophobicity, Black hydrophobicity, Fauchere hydrophobicity, Janin hydrophobicity, Rao & Argos hydrophobicity, Wolfenden hydrophobicity, Wilson HPLC hydrophobicity, Cowan HPLC pH-3.4, Tanford hydrophobicity, Rf mobility hydrophobicity and Chothia hydrophobicity scales, Theses scales are essentially a hydrophilic index, with a polar residues assigned negative values (Figures 7-28). In this assay we predicted the binding affinity of *Dendroaspis polylepis polylepis* DTX-K having 79 amino acids, which shows 71nonamers.Small peptide regions found as, 37-KRKIPSFYY (score-9.550), 45-YKWKAKQCL (Score-8.581) 36-CKRKIPSFY (Score-7.685), 24-AKYCKLPLR

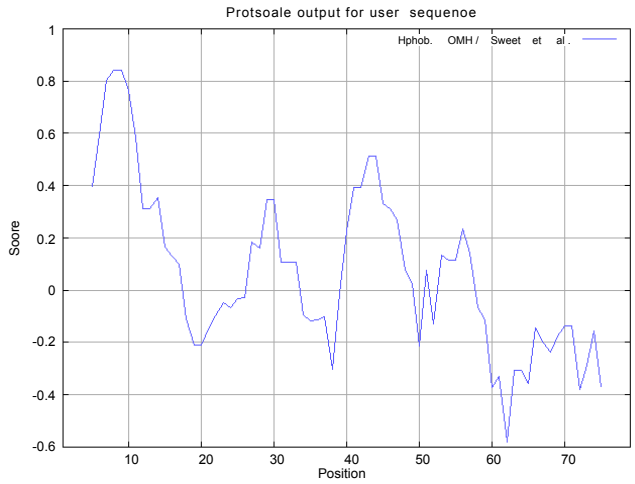

Figure 8: Hydrophobicity Sweet plot of OMH for the *Dendroaspis polylepis polylepis* DTX-K.

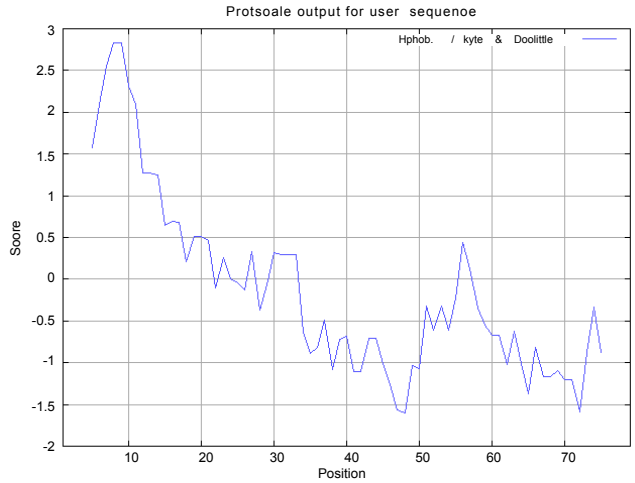

Figure 9: Hydrophobicity plot of Kyte and Doolittle (1982) for the *Dendroaspis polylepis polylepis* DTX-K.

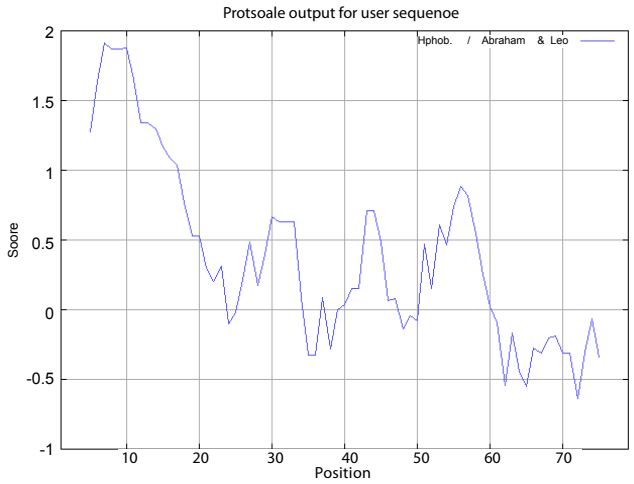

Figure 10: Hydrophobicity plot of Abraham and Leo (1987) for the *Dendroaspis polylepis polylepis* DTX-K.

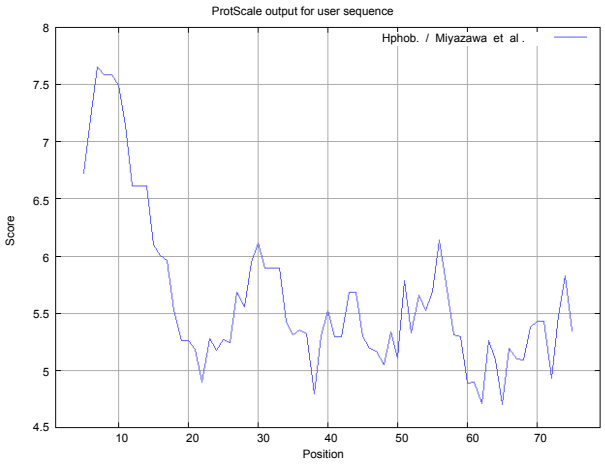

Figure 13: Hydrophobicity plot of Miyazawa, et al (1985) for the *Dendroaspis polylepis polylepis* DTX-K.

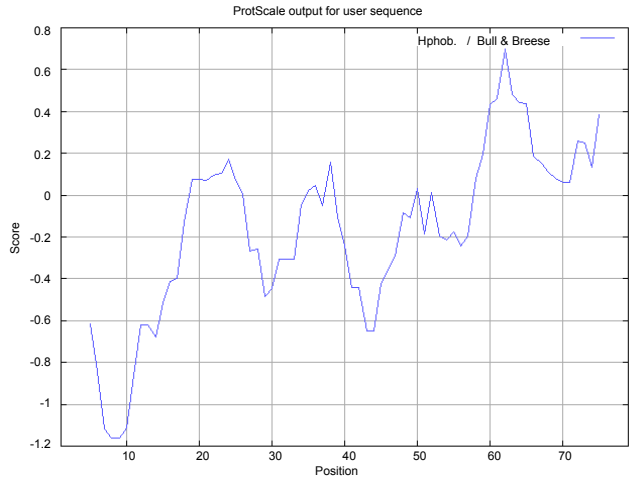

Figure 11: Hydrophobicity plot of Bull and Breese (1974) for the *Dendroaspis polylepis polylepis* DTX-K.

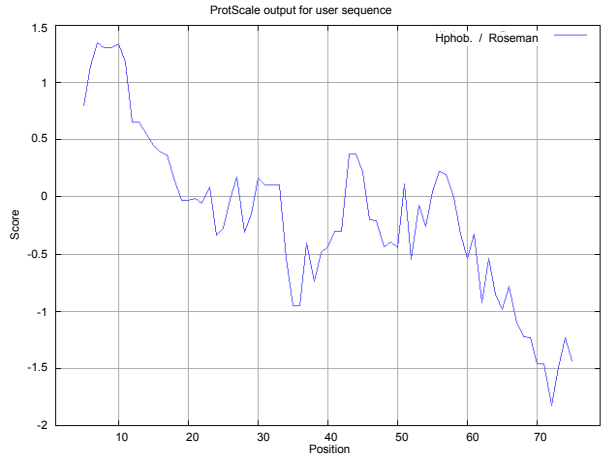

Figure 14: Hydrophobicity plot of Roseman (1988) for the *Dendroaspis polylepis polylepis* DTX-K.

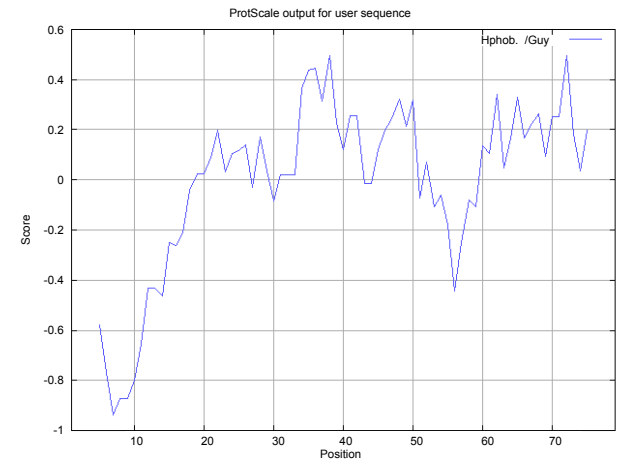

Figure 12: Hydrophobicity plot of Guy (1985) for the *Dendroaspis polylepis polylepis* DTX-K.

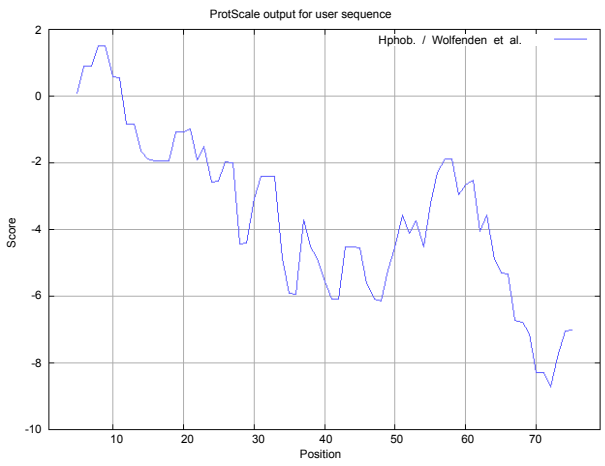

Figure 15: Hydrophobicity plot of Wolfenden et al. (1981) for the *Dendroaspis polylepis polylepis* DTX-K.

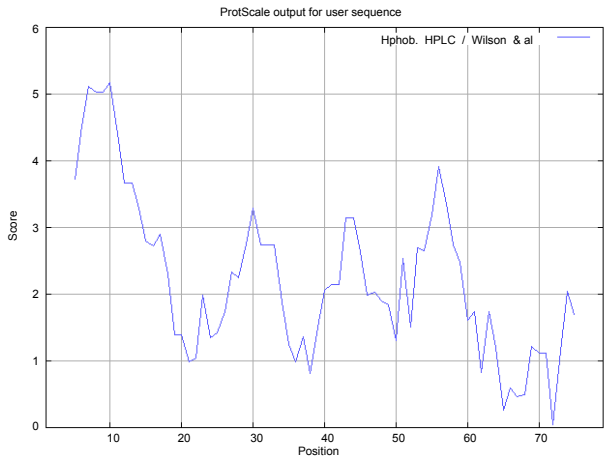

Figure 16: Hydrophobicity Wilson et al. (1981) plot of HPLC for the *Dendroaspis polylepis polylepis* DTX-K.

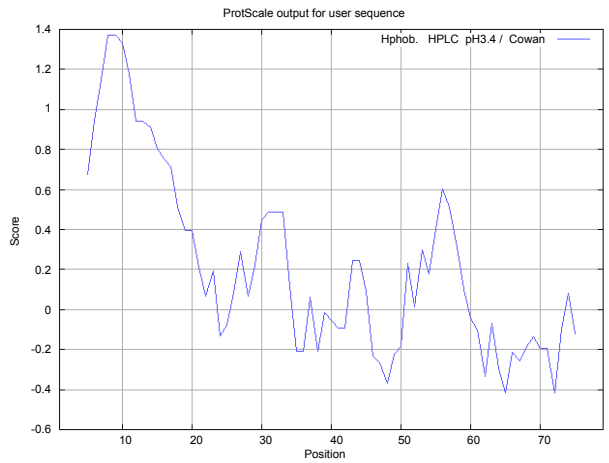

Figure 17: Hydrophobicity Cowan (1990) plot of HPLC pH3.4 for the *Dendroaspis polylepis polylepis* DTX-K.

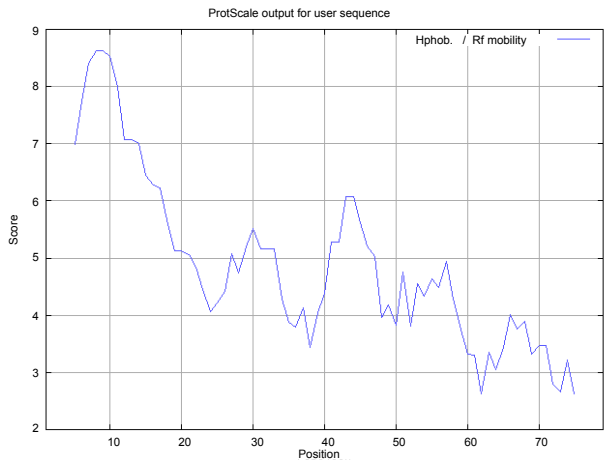

Figure 18: Hydrophobicity plot of Rf mobility for the *Dendroaspis polylepis polylepis* DTX-K.

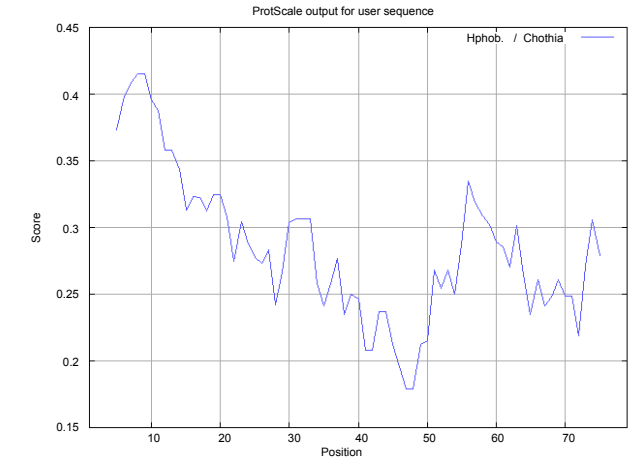

Figure 19: Hydrophobicity plot of Chothia (1976) for the *Dendroaspis polylepis polylepis* DTX-K.

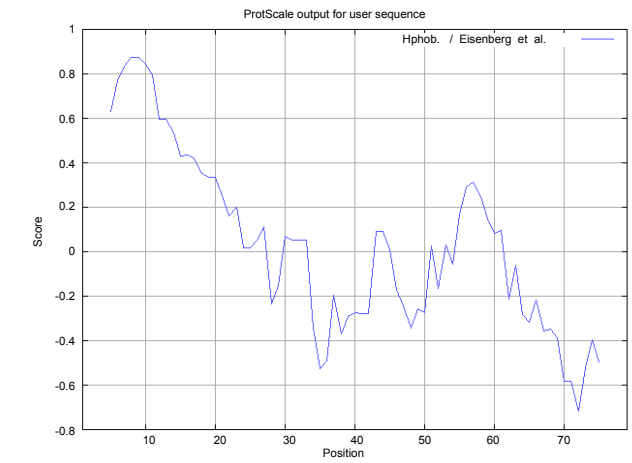

Figure 20: Hydrophobicity plot of Eisenberg et al. (1984) for the *Dendroaspis polylepis polylepis* DTX-K.

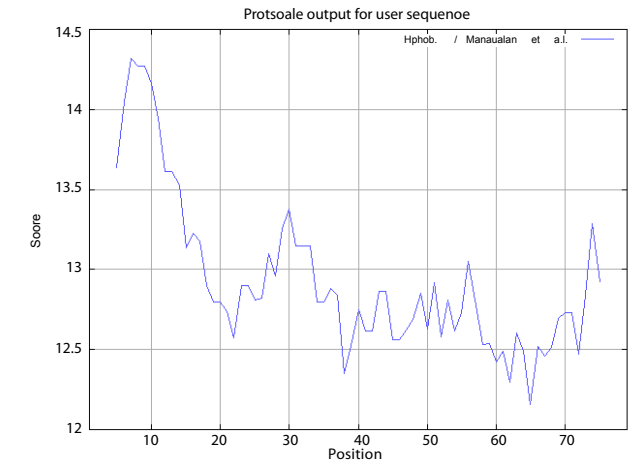

Figure 21: Hydrophobicity plot of Manavalan, et al (1978) for the *Dendroaspis polylepis polylepis* DTX-K.

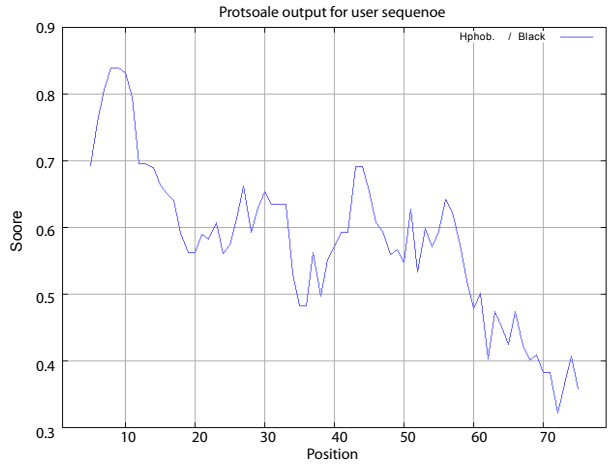

Figure 22: Hydrophobicity plot of Black (1991) for the *Dendroaspis polylepis polylepis* DTX-K.

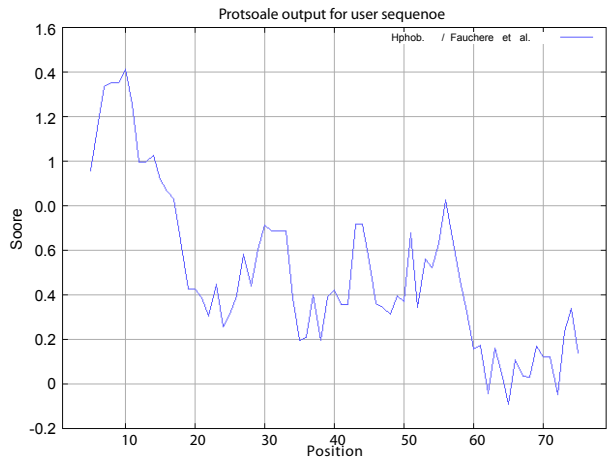

Figure 23: Hydrophobicity plot of Fauchere, et al (1983) for the *Dendroaspis polylepis polylepis* DTX-K.

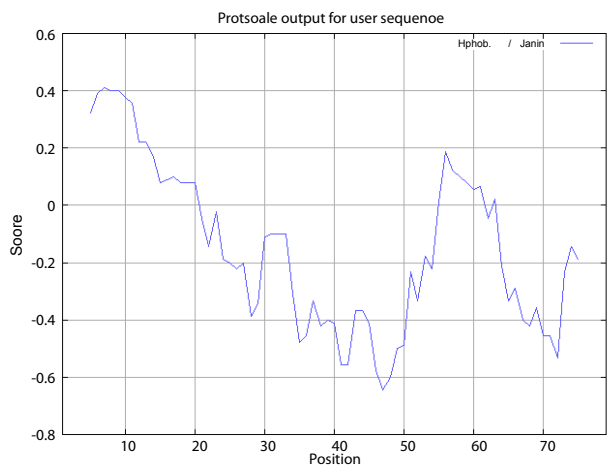

Figure 24: Hydrophobicity plot of Janin (1979) for the *Dendrotoxin-K*.

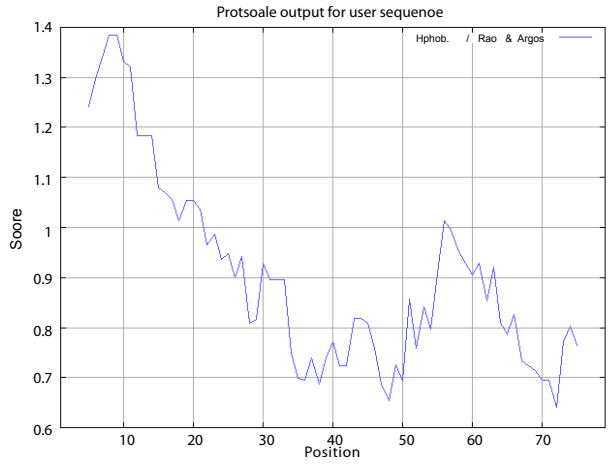

Figure 25: Hydrophobicity plot of Rao and Argos (1986) for the *Dendroaspis polylepis polylepis* DTX-K.

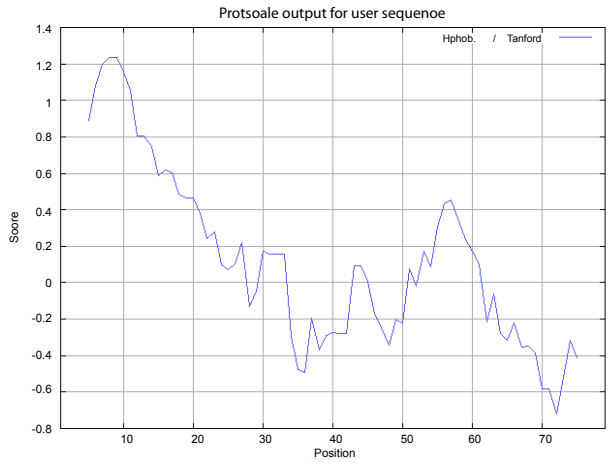

Figure 26: Hydrophobicity plot of Tanford (1962) for the *Dendroaspis polylepis polylepis* DTX-K.

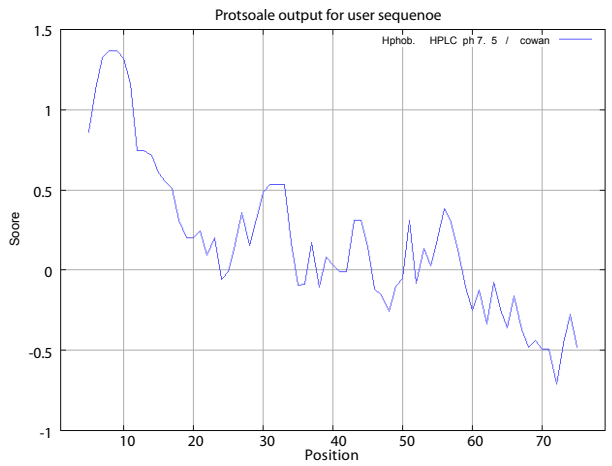

Figure 27: Hydrophobicity Cowan (1990) plot of HPLC pH7.5 for the *Dendroaspis polylepis polylepis* DTX-K.

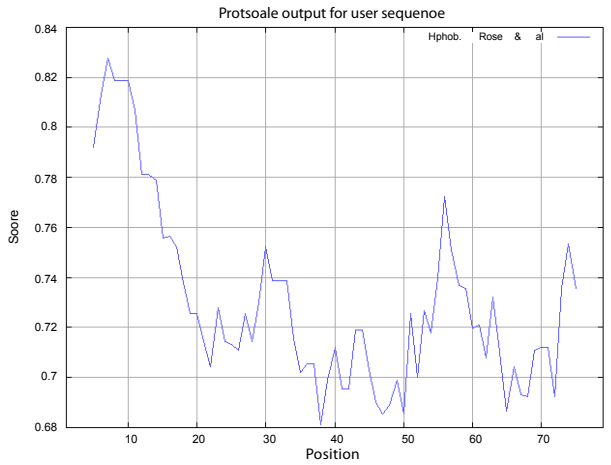

Figure 28: Hydrophobicity plot of Rose et al. (1985) for the *Dendroaspis polylepis polylepis* DTX-K.

(Score-7.669), 42-SFYYKWKAK (Score-6.859), 31-LRIGPCKRK (Score-6.848) 65-NRFKTIEEC (Score-6.698), 25-KYCKLPLRI (Score-6.632), 49-AKQCLPFDY (Score-6.576), 66-RFKTIEECR (Score-6.464), 47-WKAKQCLPF (Score-6.197), 23-AAKYCKLPL (Score-6.166). Adducts of MHC and peptide complexes are the ligands for T cell receptors (TCR) (Table 1). MHC molecules are cell surface glycoproteins, which take active part in host immune reactions and involvement of MHC class-I and MHC II in response to almost all antigens (Table 2). Kolaskar and Tongaonkar antigenicity are the sites of molecules that are recognized by antibodies of the immune system for the *Dendroaspis polylepis polylepis* DTX-K, analysis shows epitopes present in the *Dendroaspis polylepis polylepis* DTX-K the desired immune response. The region of maximal hydrophilicity is likely to be an antigenic site, having hydrophobic characteristics, because C-terminal regions of *Dendroaspis polylepis polylepis* DTX-K is solvent accessible and unstructured, antibodies against those regions are also likely to recognize the native protein. For the prediction of antigenic determinant site of *Dendroaspis polylepis polylepis* DTX-K, we got eighteen antigenic determinant sites in the sequence. The SVM based MHCII-IAb peptide regions, 61-GGNANRFKT, 12-TLWAELTPV, 41-PSFYYKWKA, 25-KYCKLPLRI (optimal score is 0.946); MHCII-IAd peptide regions, 2-GHLLLLLGL, 57-SGCGGNAN, 3-HLLLLLGLL, 1-SGHLLLLLG (optimal score is 0.488); MHCII-IAg7 peptide regions 60-CGGNANRFK, 21-SGAAKYCKL, 61-GGNANRFKT, 20-VSGAAKYCK (optimal score is 1.468); and MHCII-RT1.B peptide regions 46-KWKAKQCLP, 24-AKYCKLPLR, 10-LLTLWAELT, 45-YKWKAKQCL (optimal score is 0.569) which represented predicted binders from *Dendroaspis polylepis polylepis* DTX-K (Table 2). Which is a larger percentage of their atoms are directly involved in binding as compared to larger molecules.

Future Perspectives

This method will be useful in cellular immunology, Vaccine design, immunodiagnostics, immunotherapeutics and molecular understanding of autoimmune susceptibility. *Dendroaspis polylepis polylepis* DTX-K sequence involved multiple antigenic components to direct and empower the immune system to protect the host from the dendrotoxin. MHC molecules are cell surface proteins, which take active part in host immune reactions and involvement of MHC class in response to almost all antigens and it give effects on specific sites. Predicted MHC binding regions acts like red flags for antigen specific and generate immune response against the parent antigen. So, a small fragment of antigen can induce immune response against whole antigen. The method integrates prediction of peptide MHC class binding; proteosomal C terminal cleavage and TAP transport efficiency. This theme is implemented in designing subunit and synthetic peptide vaccines.

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