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Full Length Research Paper

Prediction of antigenic epitopes and MHC binders of neurotoxin alpha-KTx 3.8 from *Mesobuthus tamulus* sindicus

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The potassium channel inhibitor alpha-KTx 3.8, a 38-residue peptide was isolated from the venom of *Mesobuthus tamulus* sindicus. In this assay we have predicted the binding affinity of alpha-KTx 3.8 having 38 amino acids, which shows 30 nonamers. Peptide fragments of the neurotoxin can be used to select nonamers for use in rational vaccine design and to increase the understanding of roles of the immune system in neurotoxin studies. Small segment '4-INVKCRGSPQCIQPCR-19'of neurotoxin protein called the antigenic epitopes is sufficient for eliciting the desired immune response. We also found the SVM based MHCII-IAb peptide regions, 26- GKCMNGKCH, 20- DAGMRFGKC, 1- GVPINVKCR, 19-RDAGMRFGK, (optimal score is 0.388); MHCII-IAd peptide regions, 20- DAGMRFGKC, 14- CIQPCRDAG, 10- GSPQCIQPC, 25- FGKCMNGKC, (optimal score is 0.386); MHCII-IAg7 peptide regions, 18-CRDAGMRFG, 17- PCRDAGMRF, 14- CIQPCRDAG, 3- PINVKCRGS, (optimal score is 1.341); and MHCII-RT1.B peptide regions, 16- QPCRDAGMR, 29- MNGKCHCTP, 8- CRGSPQCIQ, 7- KCRGSPQCI, (optimal score is -0.039) which represented predicted binders from neurotoxin protein. CTL epitope with their (ANN/SVM) scores were predicted to be 1- GVPINVKCR (0.81/0.87220559). This theme is implemented in designing subunit and synthetic peptide vaccines. We have predicted a successful immunization.

Key words: Potassium channel inhibitor alpha-KTx 3.8 nonamers, subunit vaccine, antigenic epitope, CTL-prediction of alpha-KTx 3.8, MHC-binders.

INTRODUCTION

Mesobuthus tamulus sindicus belongs to the family *Buthidae*, which is medium to large-sized scorpion; the adults are usually 65-90 mm long (max. 94+ mm). The primary structures of four low molecular mass peptides (Bs 6, 8, 10 and 14) from scorpion Buthus sindicus were elucidated via combination of Edman degradation and matrix-assisted laser desorption ionization mass spectrometry. Bs 8 and 14 are cysteine-rich, thermostable peptides composed of 35-36 residues with molecular weights of 3.7 and 3.4 kDa, respectively. These peptides show close sequence homologies (55-78%) with other scorpion chlorotoxin-like short-chain neurotoxins (SCNs) containing four intramolecular disulfide bridges. Despite the sequence variation between these two peptides (37% heterogeneity) their general structural organization is very similar as shown by their clearly related circular dichorism spectra. Furthermore, Bs6 is a minor component, composed of 38 residues (4.1 kDa) containing six half-cystine residues and having close sequence identities (40-80%) with charybdotoxin-like SCNs containing three disulfide bridges. The non-cysteinic, basic and thermo-labile Bs10 is composed of 34 amino acid residues (3.7 kDa), and belongs to a new class of peptides, with no sequence resemblance to any other so far reported sequence isolated from scorpions. Surprisingly, Bs10 shows some limited sequence analogy with oocyte zinc finger proteins (Ali et al., 1998).

The new paradigm in vaccine design is emerging, following essential discoveries in immunology and deve-

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lopment of new MHC Class-1 binding peptides prediction tools. MHC molecules are cell surface glycoproteins, which take active part in host immune reactions. The involvement of MHC class-1 in response to almost all antigens and the variable length of interacting peptides make the study of MHC Class 1 molecules very interesting. MHC molecules have been well characterized in terms of their role in immune reactions (Singh and Raghava, 2002; Bhasin et al., 2003 and Cui et al., 2006). They bind to some of the peptide fragments generated after proteolytic cleavage of antigen (Kumar et al., 2007). 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. Potassium channel toxin alpha-KTx 3.8 peptides are most suitable for subunit vaccine development because with single epitope, the immune response can be generated in large population. TAP is a transporter associated with MHC class 1 restricted antigen processing. The TAP is heterodimeric transporter belong to the family of ABC transporter, that uses the energy provided by ATP to translocate the peptides across the membrane (Bhasin and Raghava, 2004a). The subset of this transported peptide will bind MHC class 1 molecules and stabilize them. These MHC-peptide complexes will be translocated on the surface of antigen presenting cells (APCs). This theme is implemented in designing subunit and synthetic peptide vaccines (Gomase et al., 2007). One of the important problems in subunit vaccine design is to search antigenic regions in an antigen that can stimulate T cells called T-cell epitopes (Schirle et al., 2001). In literature, fortunately, a large amount of data about such peptides is available. In the past and presently, a number of databases have been developed to provide comprehensive information related to T-cell epitopes (Rammensee et al., 1999; Blythe et al., 2002; Schonbach et al., 2002 and Korber et al., 2001). Cytotoxic T lymphocyte (CTL) epitopes are potential candidates for subunit vaccine design for various diseases. Most of the existing T cell epitope prediction methods are indirect methods that predict MHC class 1 binders instead of CTL epitopes. In this study, a systematic attempt has been made to develop a direct method for predicting CTL epitopes from a neurotoxin alpha-KTx 3.8 protein sequences. This method is based on quantitative matrix (QM) and machine learning techniques such as Support Vector Machine (SVM) and Artificial Neural Network (ANN). This method has been trained and tested on non-redundant dataset of T cell epitopes and non-epitopes that includes 1137 experimentally proven MHC class 1 restricted T cell epitopes (Bhasin and Raghava, 2004b).

MATERIALS AND METHODS

Protein sequence analysis

Here we have analyzed the neurotoxin protein sequence (alpha-KTx 3.8) of *M. tamulus* sindicus.

Prediction of antigenicity

This program predicts those segments from within neurotoxin protein sequence that are likely to be antigenic by eliciting an antibody response. Antigenic epitopes is determined using Gomase method, B-EpiPred Server, Hopp and Woods, Welling, Parker, Kolaskar and Tongaonkar antigenicity methods. Predictions are based on a table that reflects the occurrence of amino acid residues in experimentally known segmental epitopes (Gomase, 2006; Hopp and Woods, 1981; Welling et al., 1985; Jens Erik et al., 2006; Parker et al., 1986 and Kolaskar and Tongaonkar, 1990).

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 (Garnier, 1978; Robson and Garnier, 1993).

Finding the location in solvent accessible regions

For setting the solvent accessible regions in protein, type of plot determine the hydrophobic scale 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 (Sweet and Eisenberg, 1983; Kyte and Doolittle, 1982; Abraham and Leo, 1987; Bull and Breese, 1974; Guy, 1985; Miyazawa and Jernigen, 1985; Roseman, 1988; Wolfenden, et al., 1981; Wilson, et al., 1981; Aboderin, 1971; Chothia, 1976; Eisenberg et al., 1984; Manavalan and Ponnuswamy, 1978; Black and Mould, 1991; Fauchere and Pliska, 1983; Janin, 1979; Rao and Argos, 1986; Tanford, 1962; Cowan and Whittaker, 1990; Rose et al., 1985; Wilkins et al., 1999; Eisenberg et al., 1984).

Prediction of MHC Binding peptide and CTL epitope

MHC2Pred predicts peptide binders to MHCI and MHCII molecules from protein sequences or sequence alignments using Position Specific Scoring Matrices (PSSMs). In addition, we predict those MHCI ligands which C-terminal end is likely to be the result of proteosomal cleavage (Nielsen et al., 2005; Kesmir et al., 2002). 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. 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 2 binding peptides (Brusic et al., 1998; Bhasin and Raghava, 2005). The average accuracy of SVM based method for 42 alleles is ~80%. This method will be useful in cellular immunology, vaccine design, immunodiagnostics, immunotherapeutics and molecular understanding of autoimmune susceptibility. 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 predict those MHC ligands from which C-terminal end is likely to be the result of proteosomal cleavage. The identification of peptides that can stimulate cytotoxic T Lymphocytes (CTLs) is one of the major challenges in subunit vaccines design. The existing epitope predic-

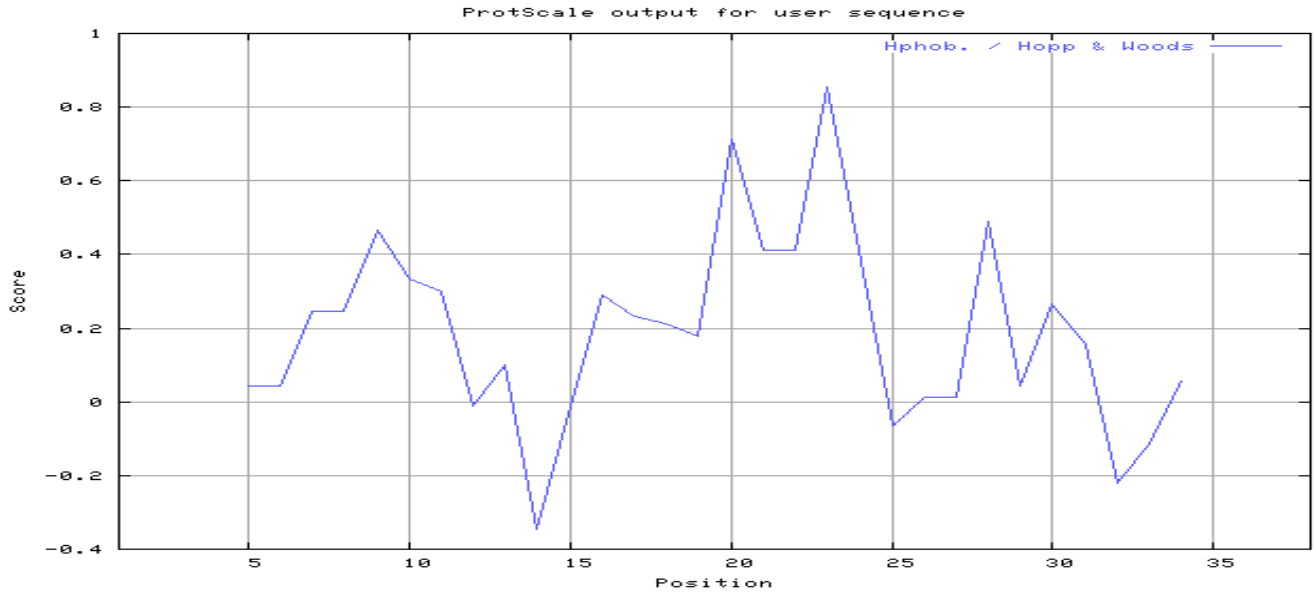

Figure 1. Hydrophobicity plot of neurotoxin alpha-KTx 3.8 by Hopp TP, Woods KR (1981).

tion methods are based on identification of MHC binding peptides. It is not necessary that all MHC binders can act as T cell epitopes. There is a need to develop a highly accurate prediction method for CTL epitopes instead of MHC binders. The use of artificial neural network and support vector machine on the recent and high quality CTL epitopes and non-epitopes data is explored as a means to meet these challenges (Gomase and Kale, 2008a,b).

RESULTS

The neurotoxin protein sequence is 38 residues long as:

GVPINVKCRGSPQCIQPCRDAGMRFGKCMNGKCHCT PQ

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 (Figures 3-5).

Secondary alignment

The Robson and Garnier method predicted the secondary structure of the neurotoxic protein. Each residue is assigned values for alpha helix, beta sheet, turns and coils using a window of 7 residues (Figure 6). 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.

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 neurotoxin protein is hydrophobic in nature and contains segments of low complexity and high-predicted flexibility (Figures 7-27).

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 MHC 1 and MHC 2 binding regions (Tables 1, 2). MHC molecules are cell surface glycel-

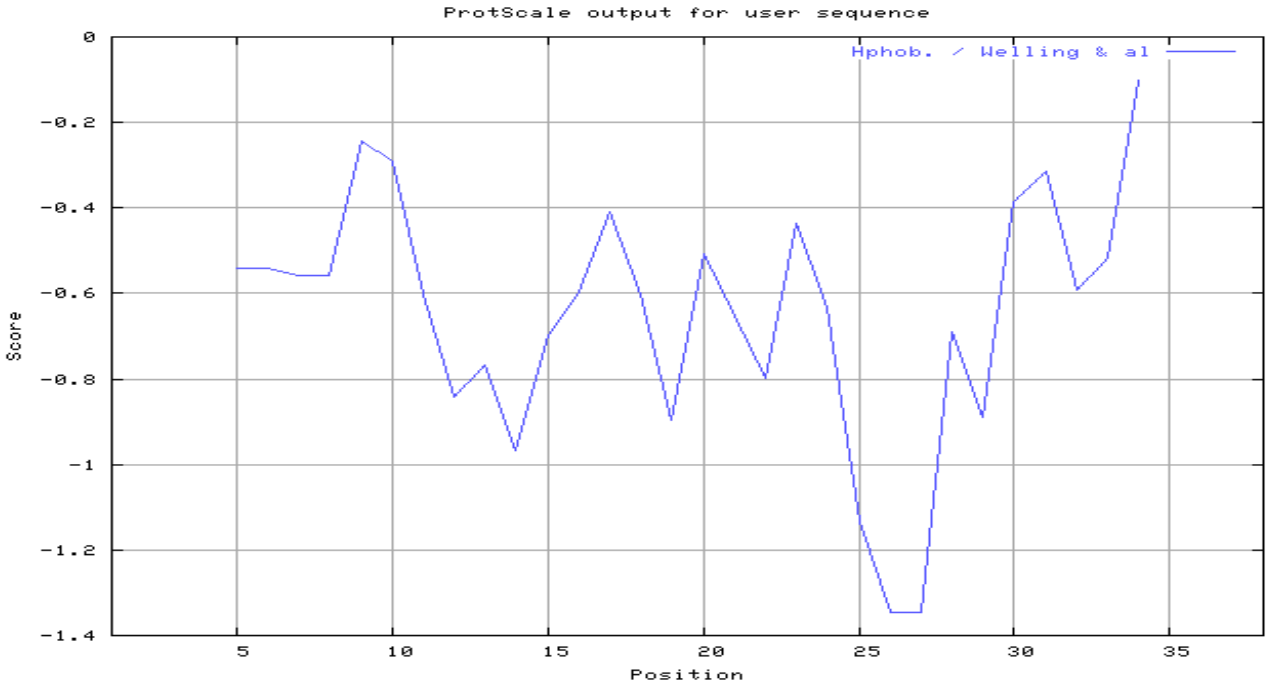

Figure 2. Hydrophobicity plot of neurotoxin alpha-KTx 3.8 by Welling GW, Weijer WJ, van der Zee R, Welling-Wester S (1985).

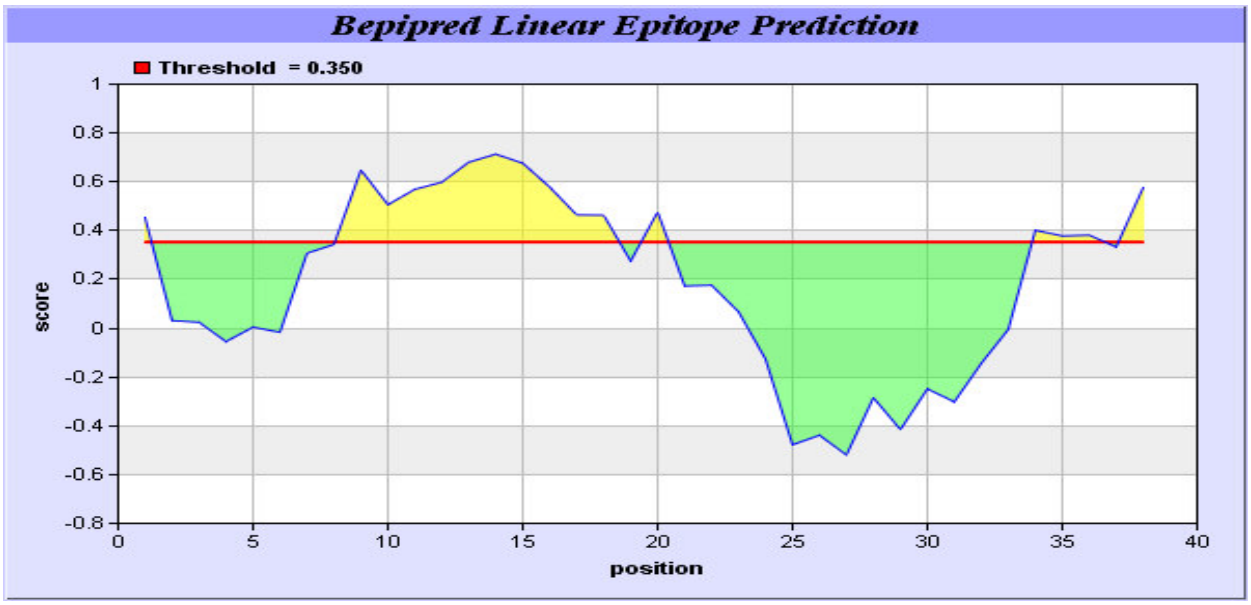

Figure 3. B-cell epitopes are the sites of molecules that are recognized by antibodies of the immune system for the neurotoxin protein.

proteins, which take active part in host immune reactions and involvement of MHC class 1 and MHC 2 in response to almost all antigens. In this assay we predicted the binding affinity of neurotoxin protein having 38 amino acids, which shows different nonamers (Tables 1 and 2). For development of MHC binder prediction method, an elegant machine learning technique supports 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 neurotoxin pro-

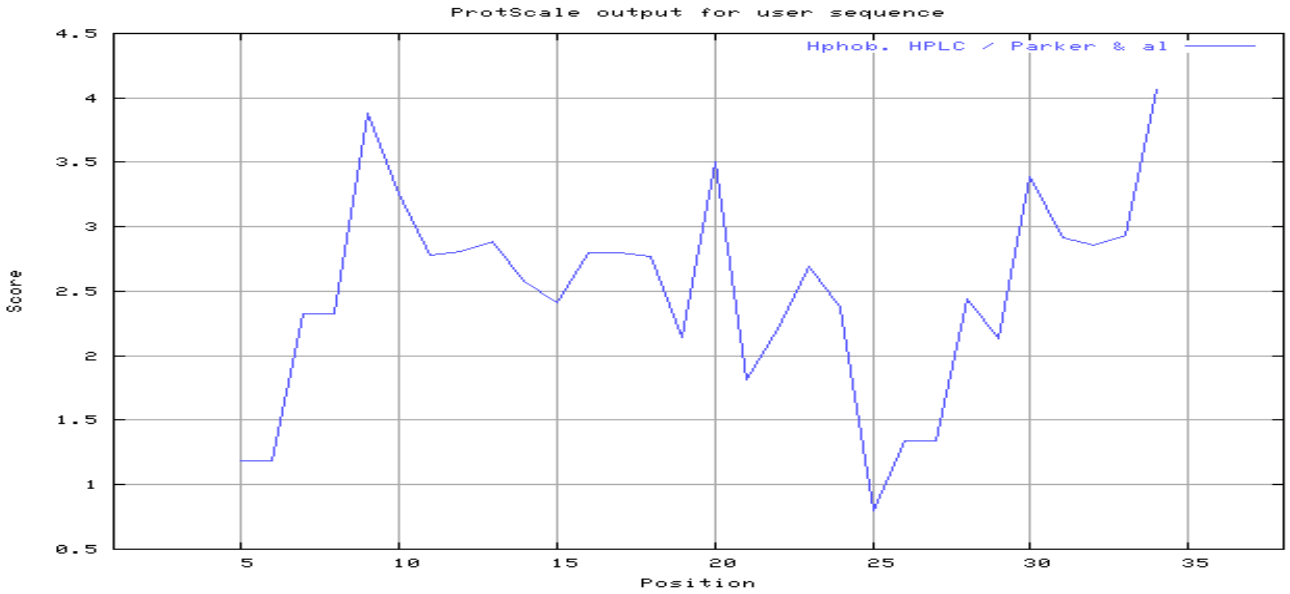

Figure 4. Hydrophobicity plot of neurotoxin alpha-KTx 3.8 by HPLC Parker JMR, Guo D, Hodges RS (1986).

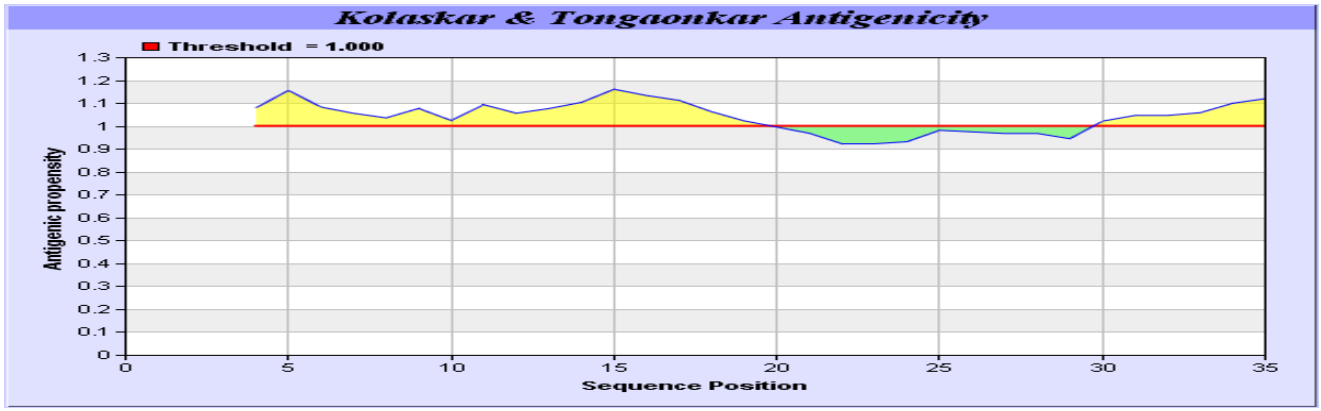

Figure 5. Kolaskar and Tongaonkar antigenicity are the sites of molecules that are recognized by antibodies of the immune system for the neurotoxin protein.

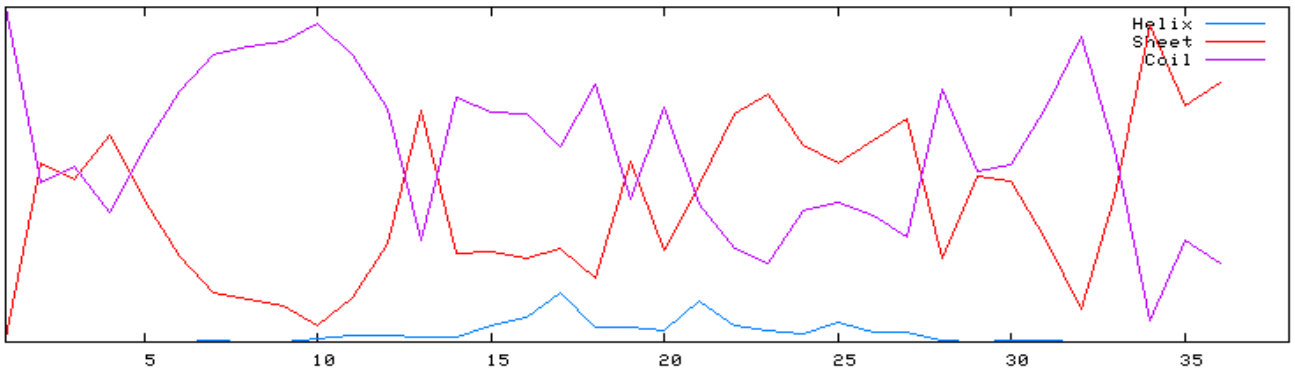

Figure 6. Secondary structure plot of the neurotoxin protein.

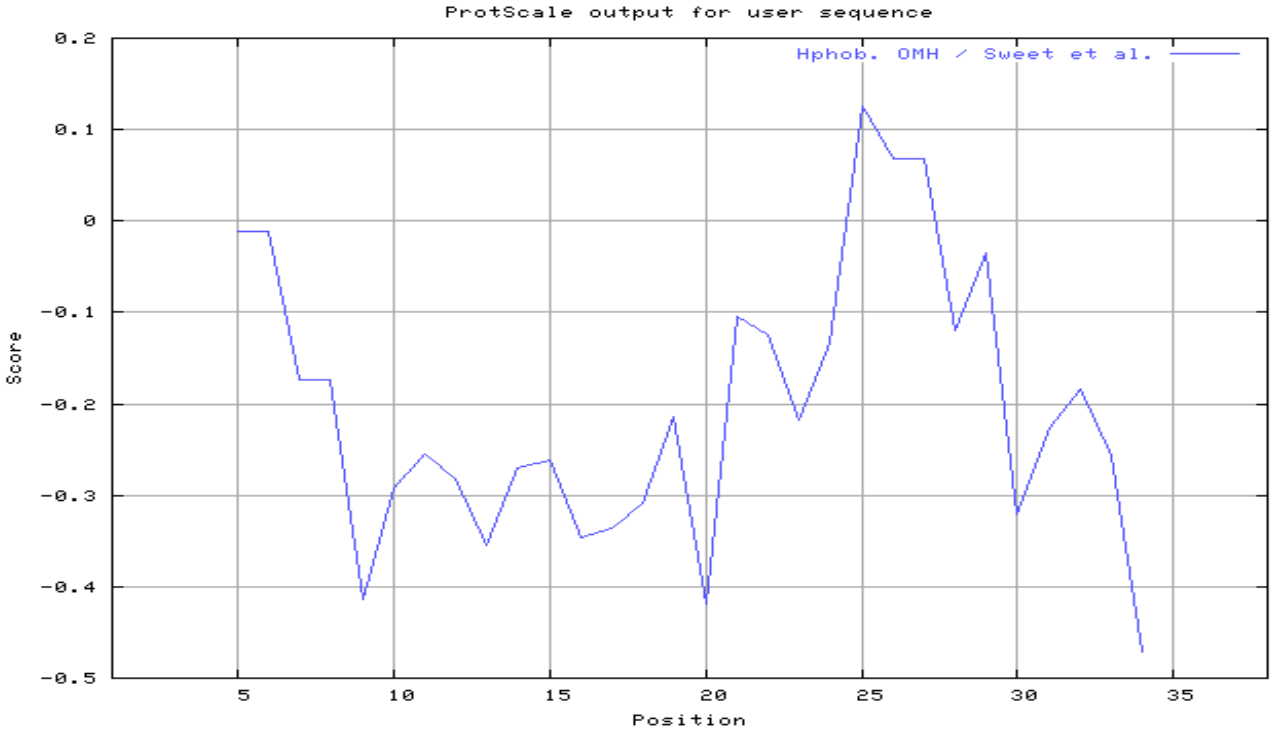

Figure 7. Hydrophobicity plot of neurotoxin alpha-KTx 3.8 by OMH Sweet RM, Eisenberg D (1983).

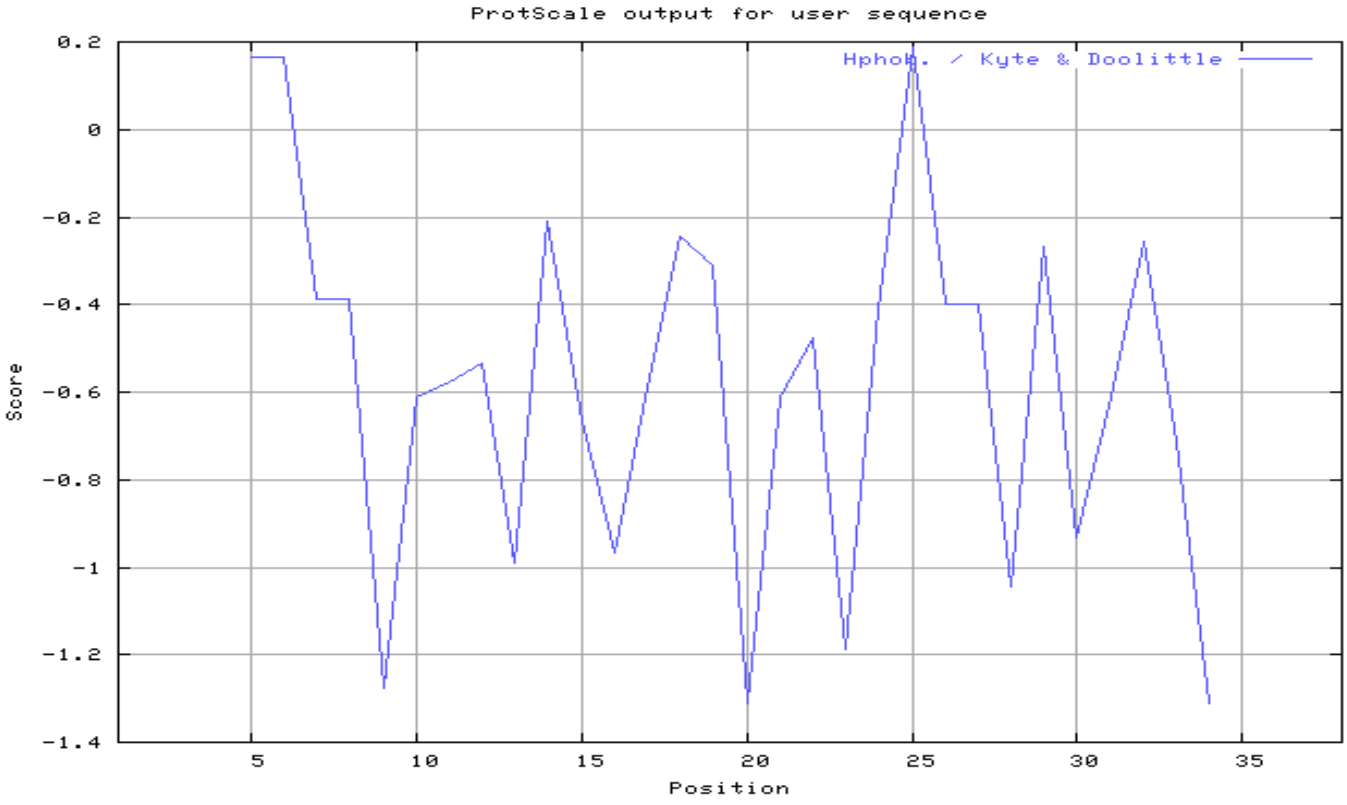

Figure 8. Hydrophobicity plot of neurotoxin alpha-KTx 3.8 by Kyte J, Doolittle RF, (1982).

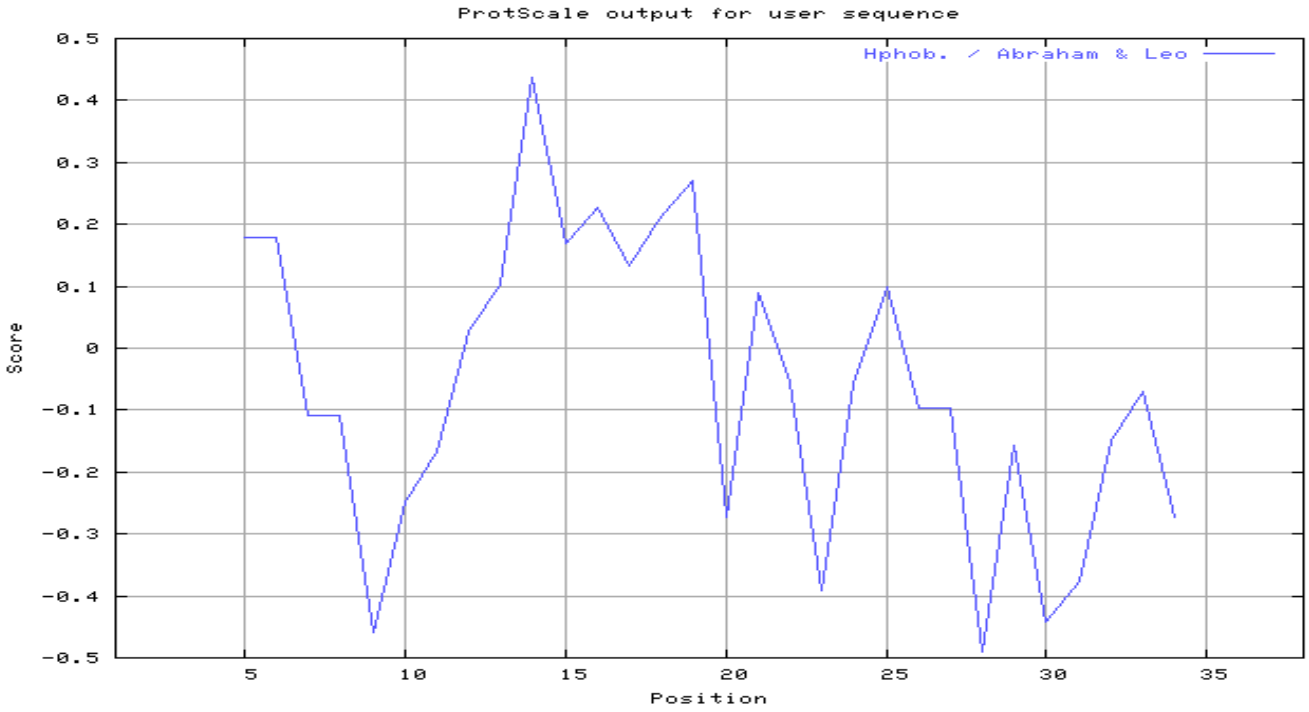

Figure 9. Hydrophobicity plot of neurotoxin alpha-KTx 3.8 by Abraham and Leo.

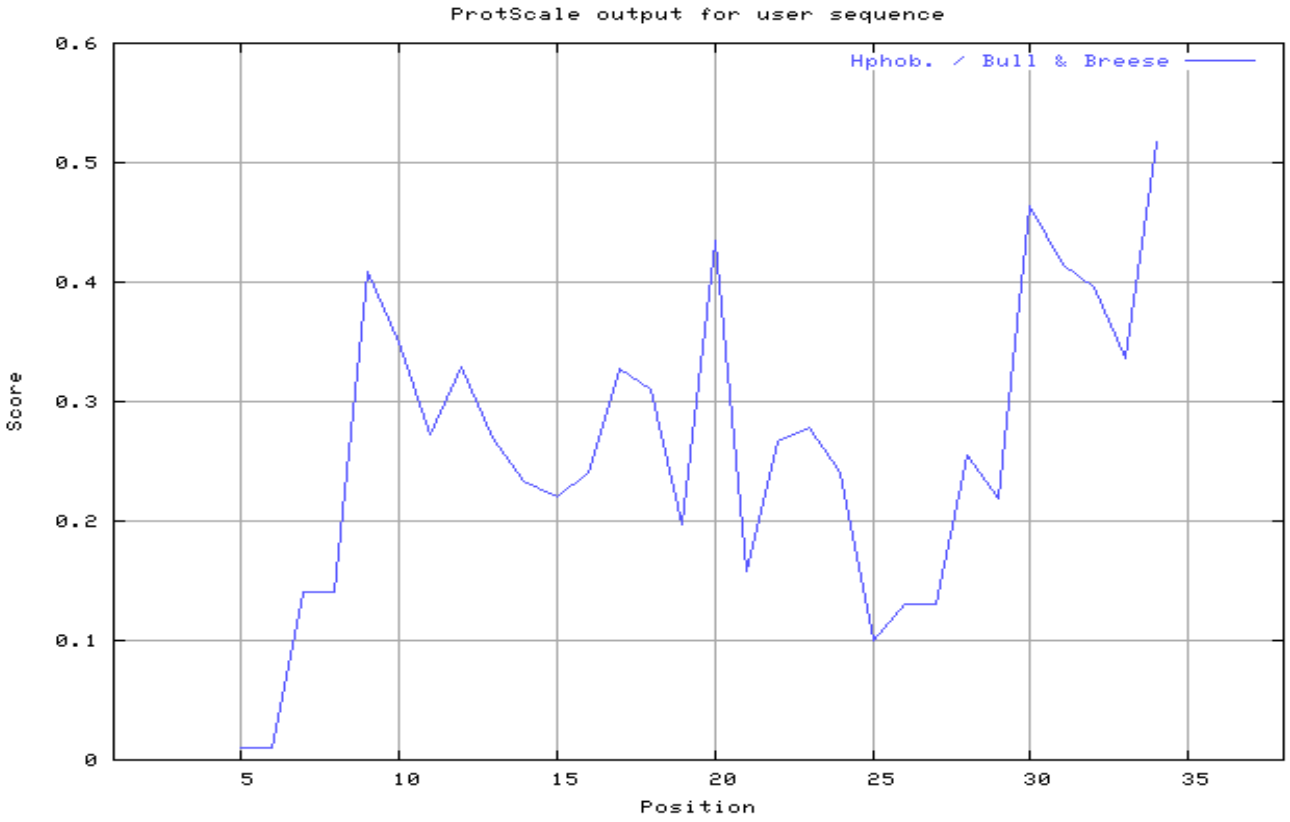

Figure 10. Hydrophobicity plot of neurotoxin alpha-KTx 3.8 by Bull HB, Breese K (1974)

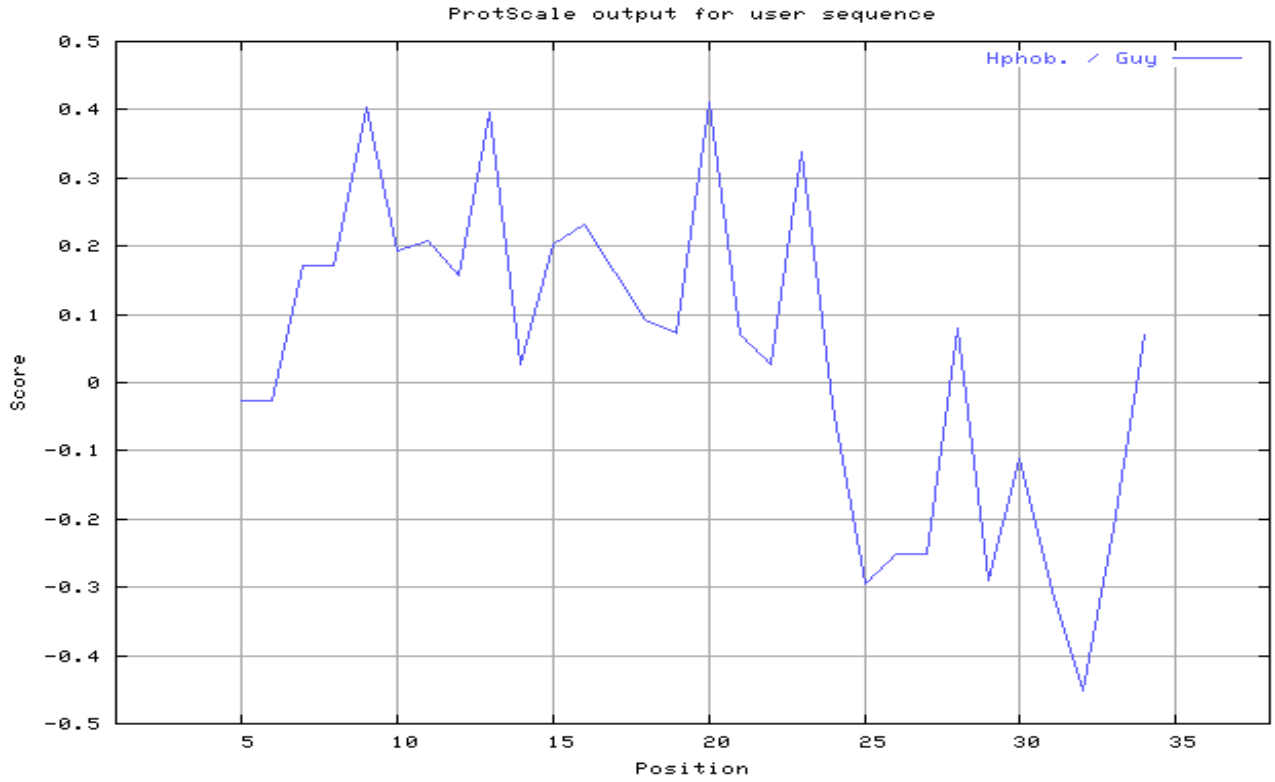

Figure 11. Hydrophobicity plot of neurotoxin alpha-KTx 3.8 by Guy H R (1985).

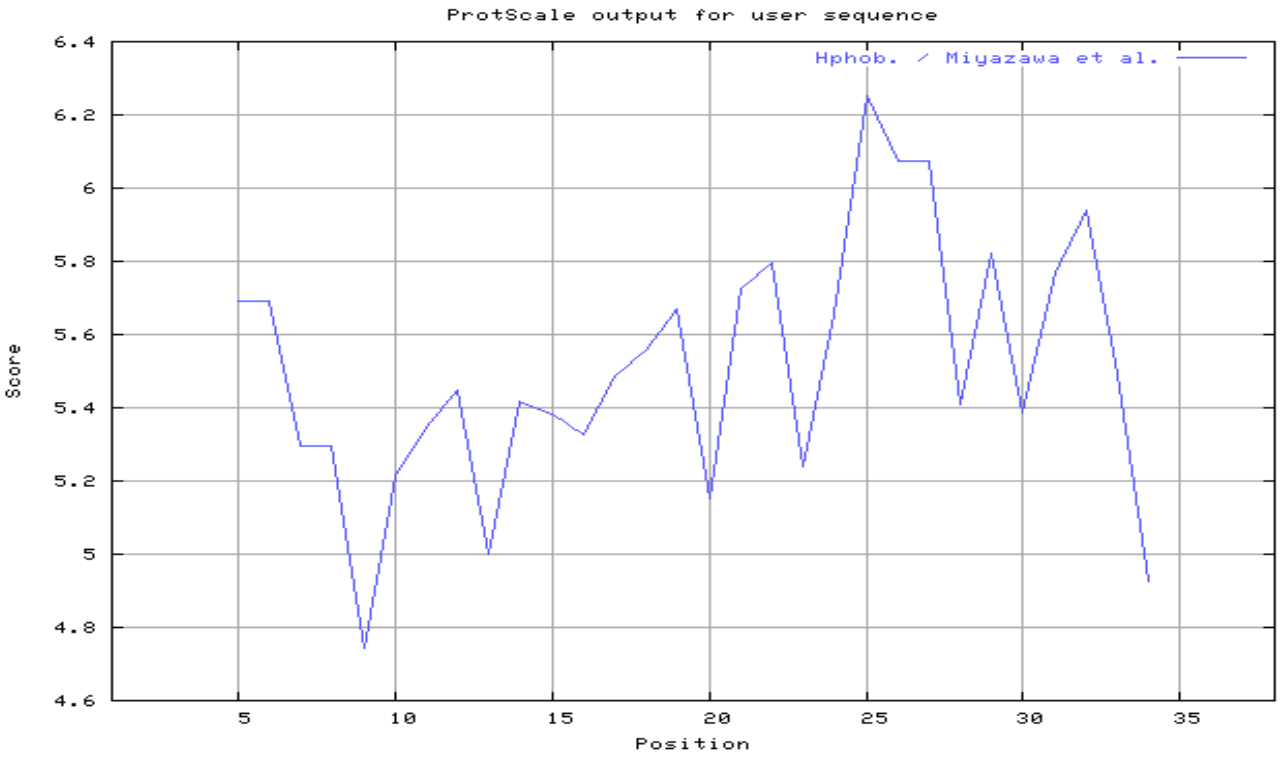

Figure 12. Hydrophobicity plot of neurotoxin alpha-KTx 3.8 by Miyazawa S, Jernigen RL (1985).

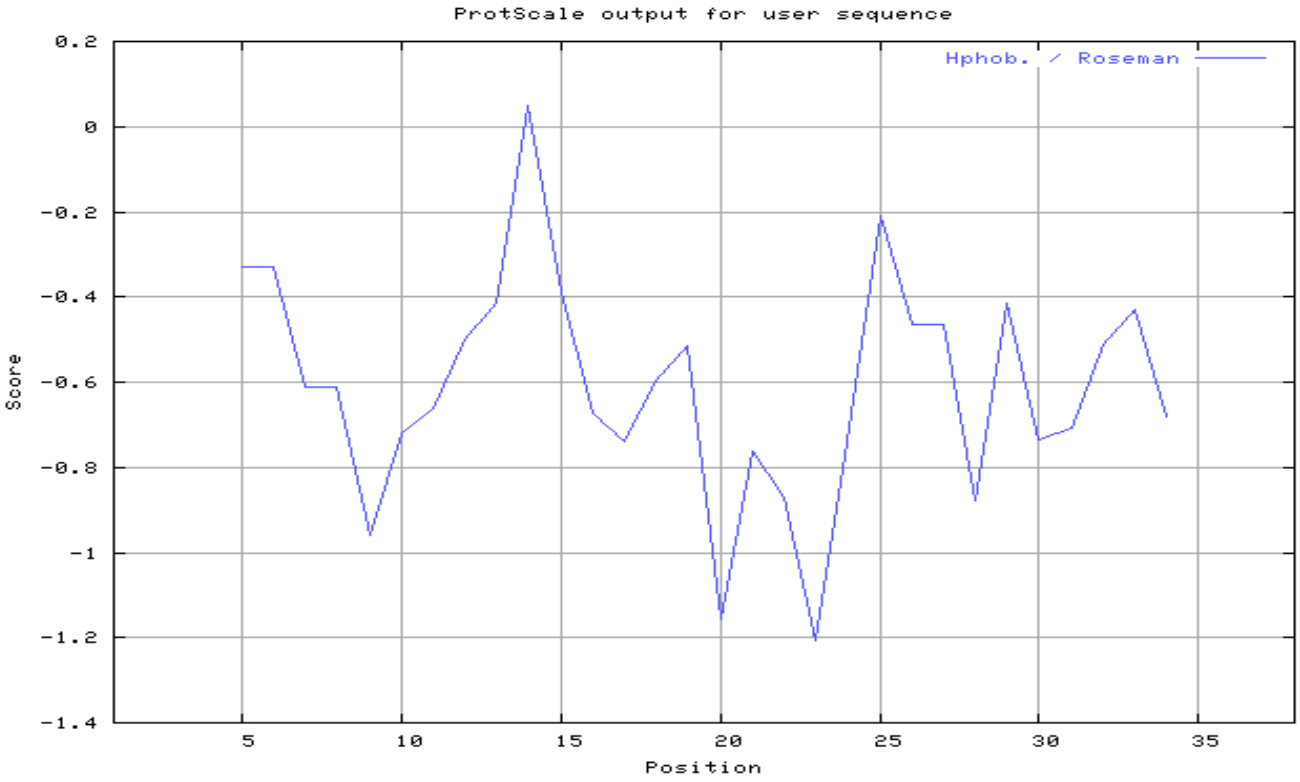

Figure 13. Hydrophobicity plot of neurotoxin alpha-KTx 3.8 by Roseman MA (1988).

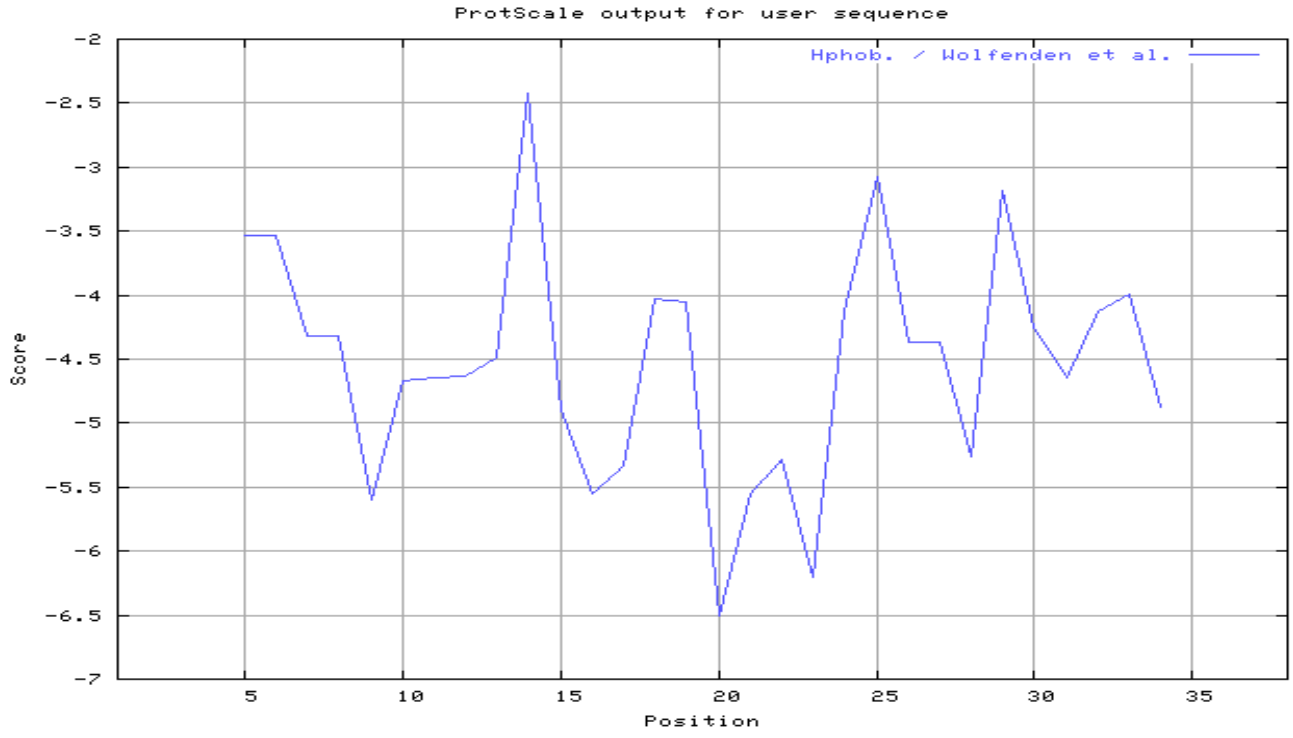

Figure 14. Hydrophobicity plot of neurotoxin alpha-KTx 3.8 by Wolfenden RV, Andersson L, Cullis PM, Southgate CCF (1981).

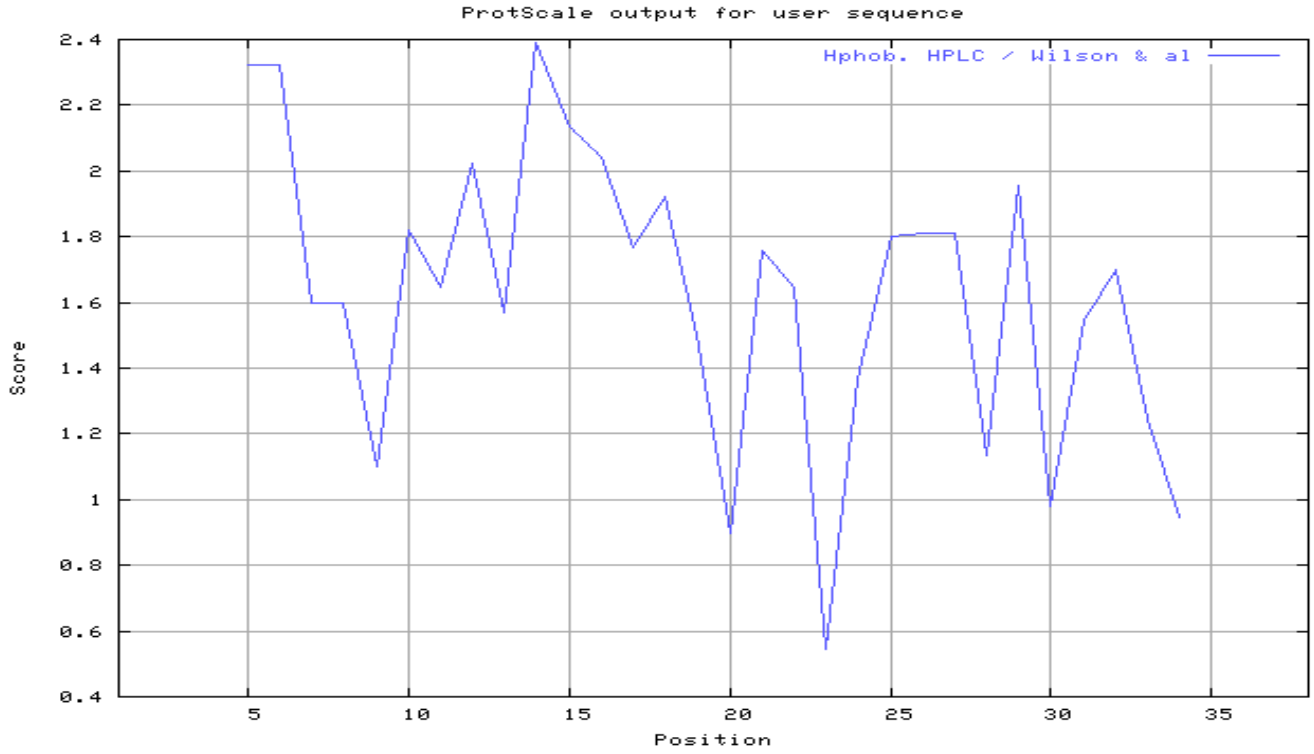

Figure 15. Hydrophobicity plot of neurotoxin alpha-KTx 3.8 on HPLC by Wilson KJ, Honegger A, Stotzel RP, Hughes GJ (1981).

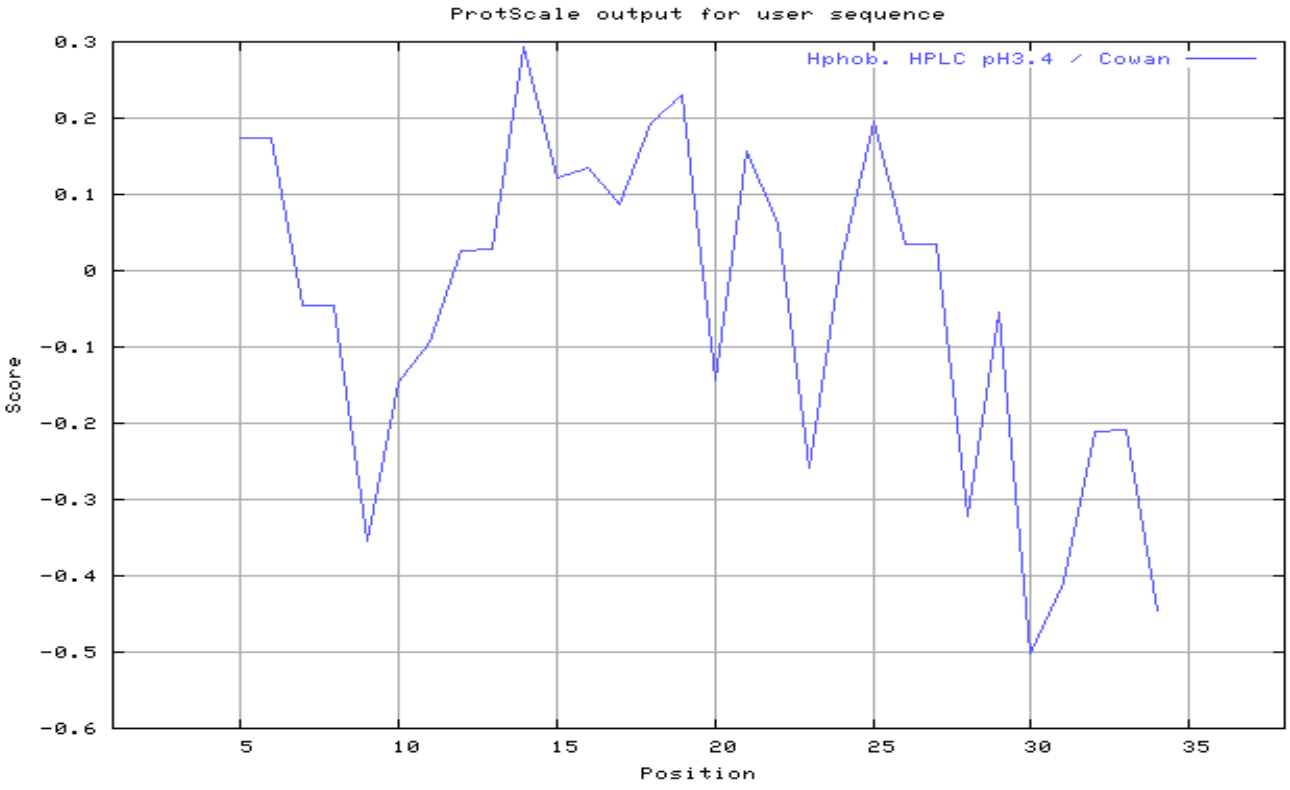

Figure 16. Hydrophobicity plot of neurotoxin alpha-KTx 3.8 by HPLC pH3.4 Cowan and Whittaker (1990)

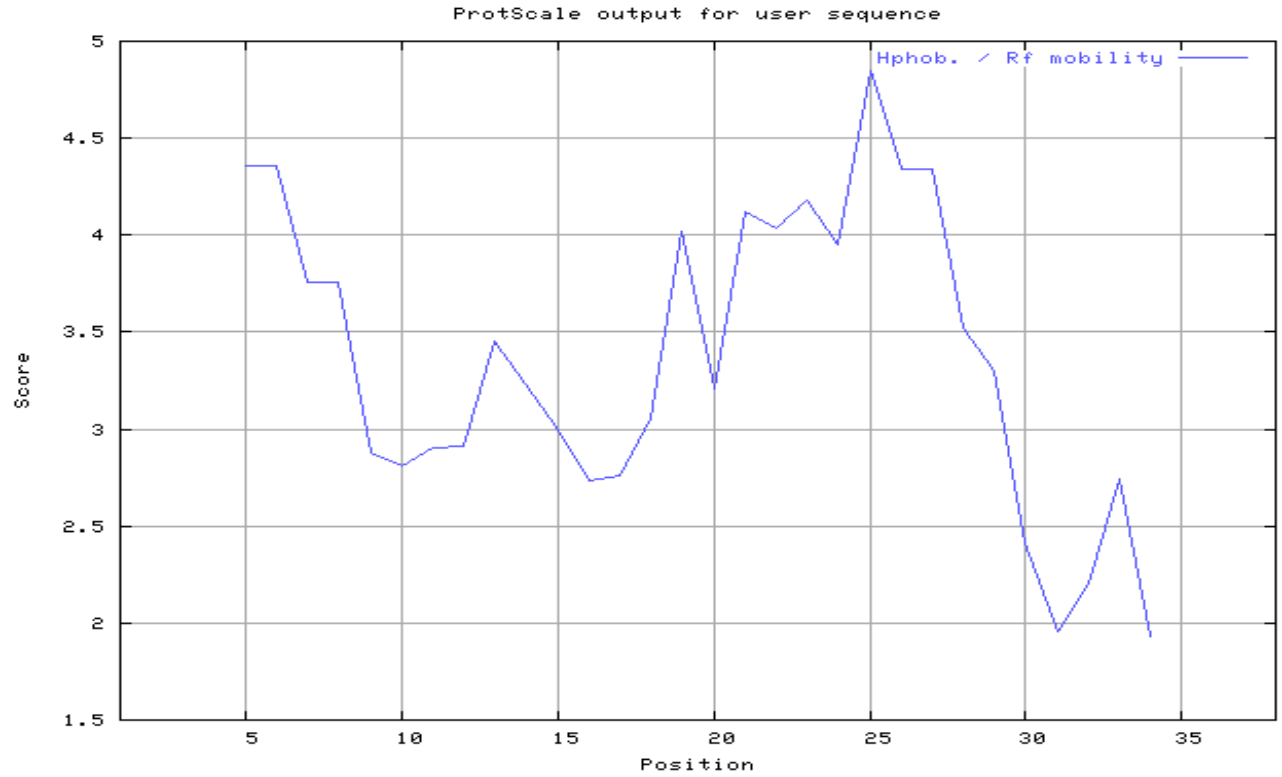

Figure 17. Hydrophobicity plot of neurotoxin alpha-KTx 3.8 by Rf mobility.

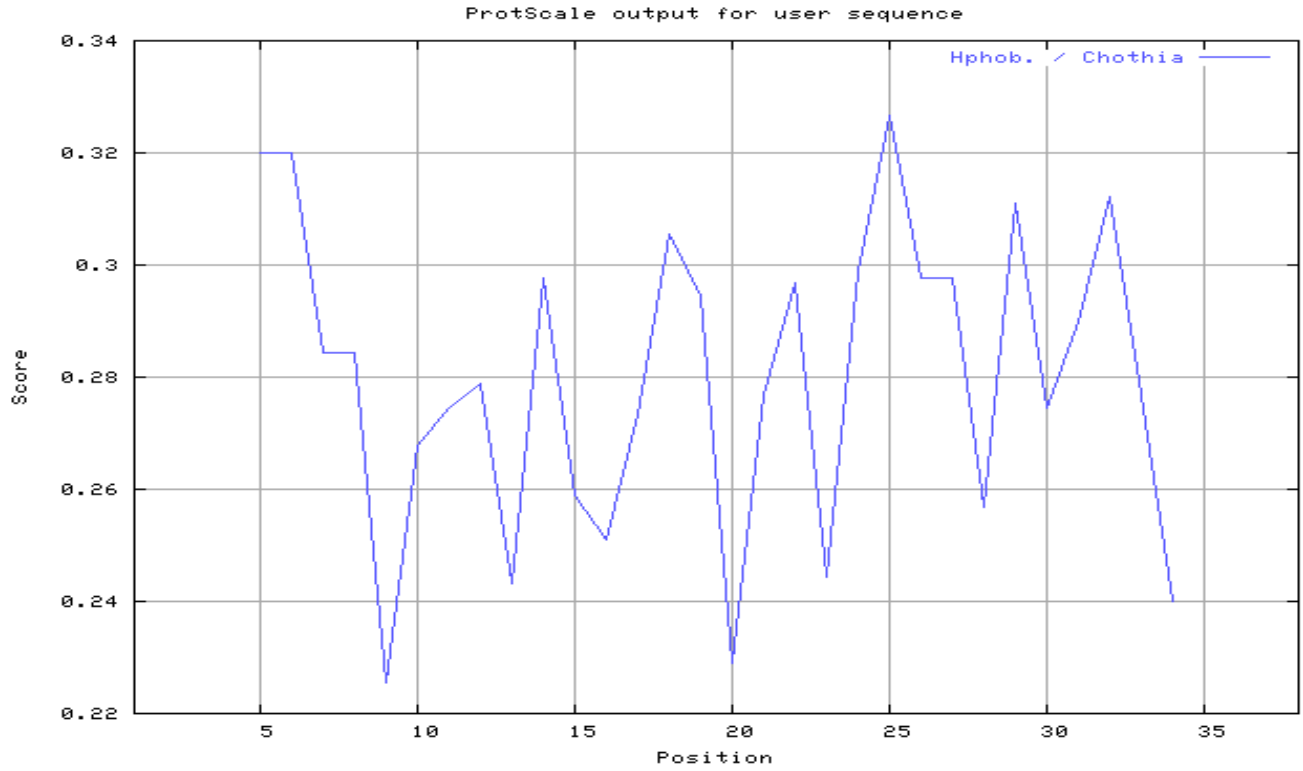

Figure 18. Hydrophobicity plot of neurotoxin alpha-KTx 3.8 by Chothia C (1976).

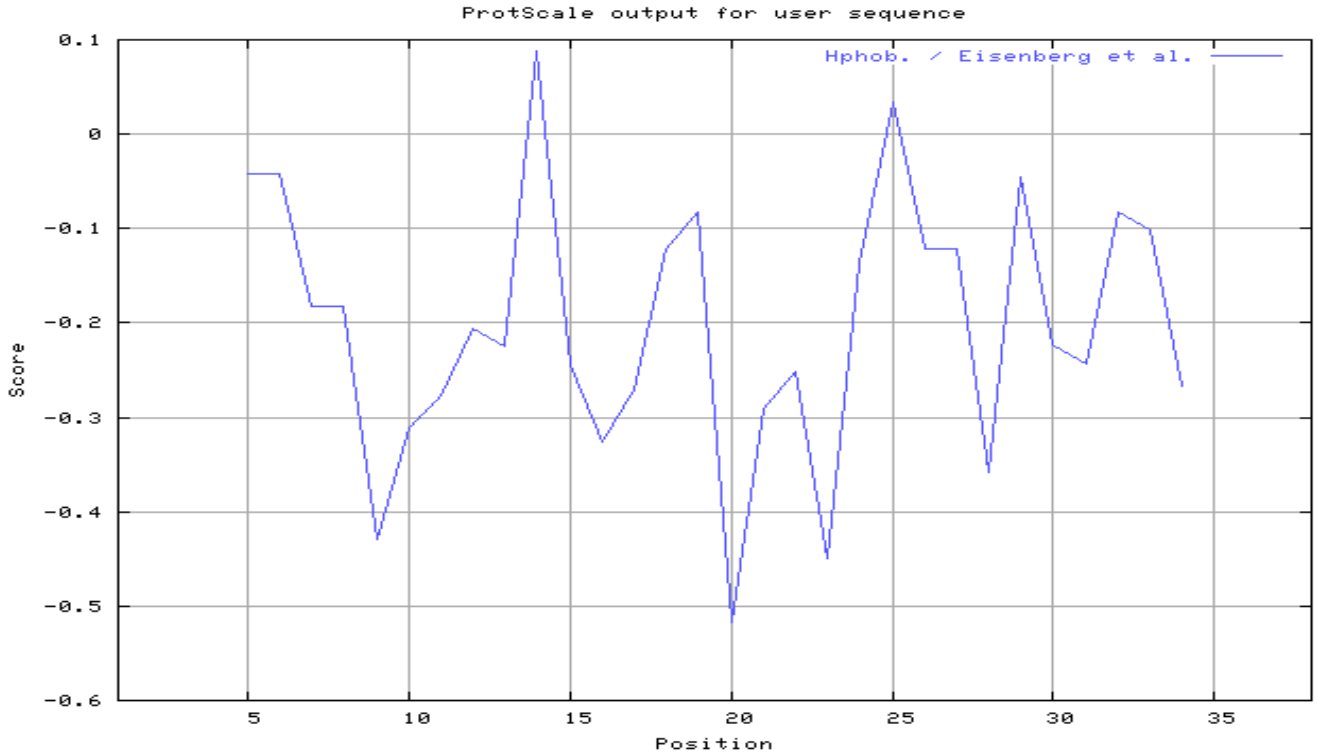

Figure 19. Hydrophobicity plot of neurotoxin alpha-KTx 3.8 by Eisenberg D, Schwarz E, Komaromy M, Wall R (1984).

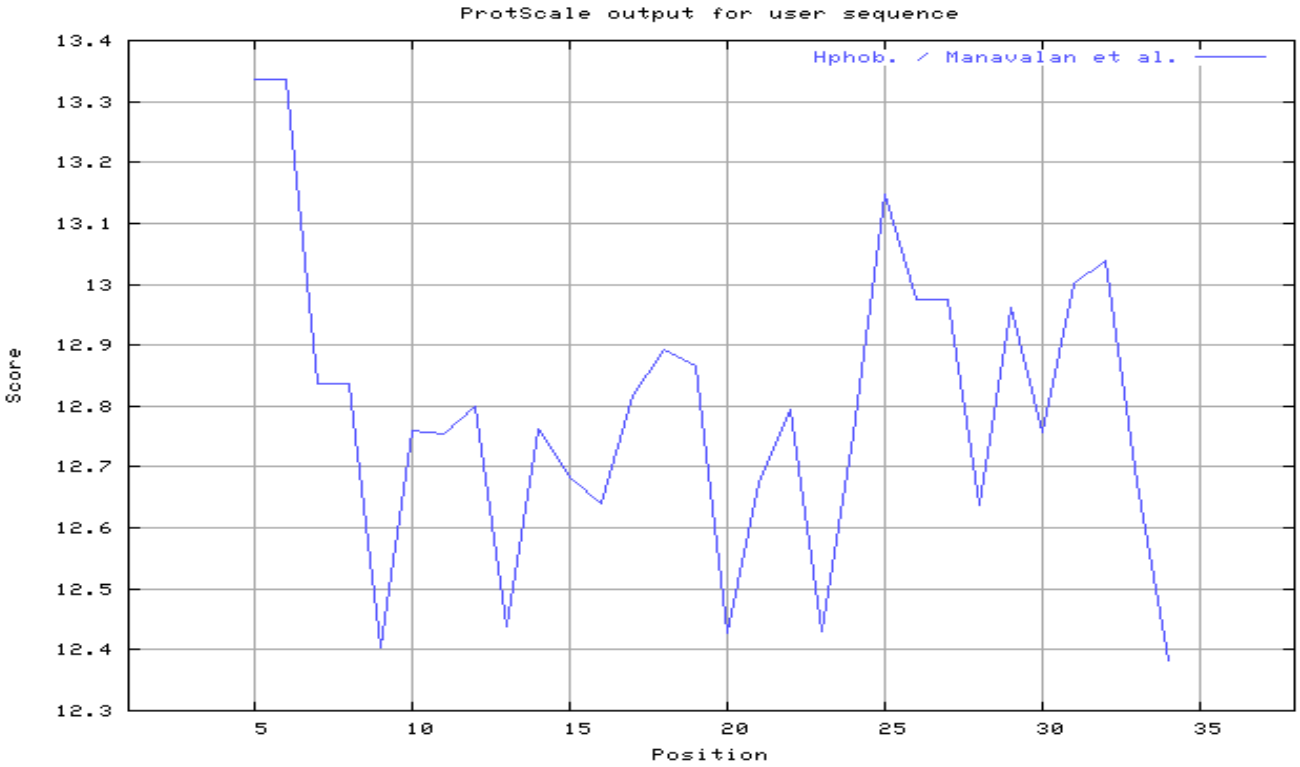

Figure 20. Hydrophobicity plot of neurotoxin alpha-KTx 3.8 by Manavalan P, Ponnuswamy PK (1978).

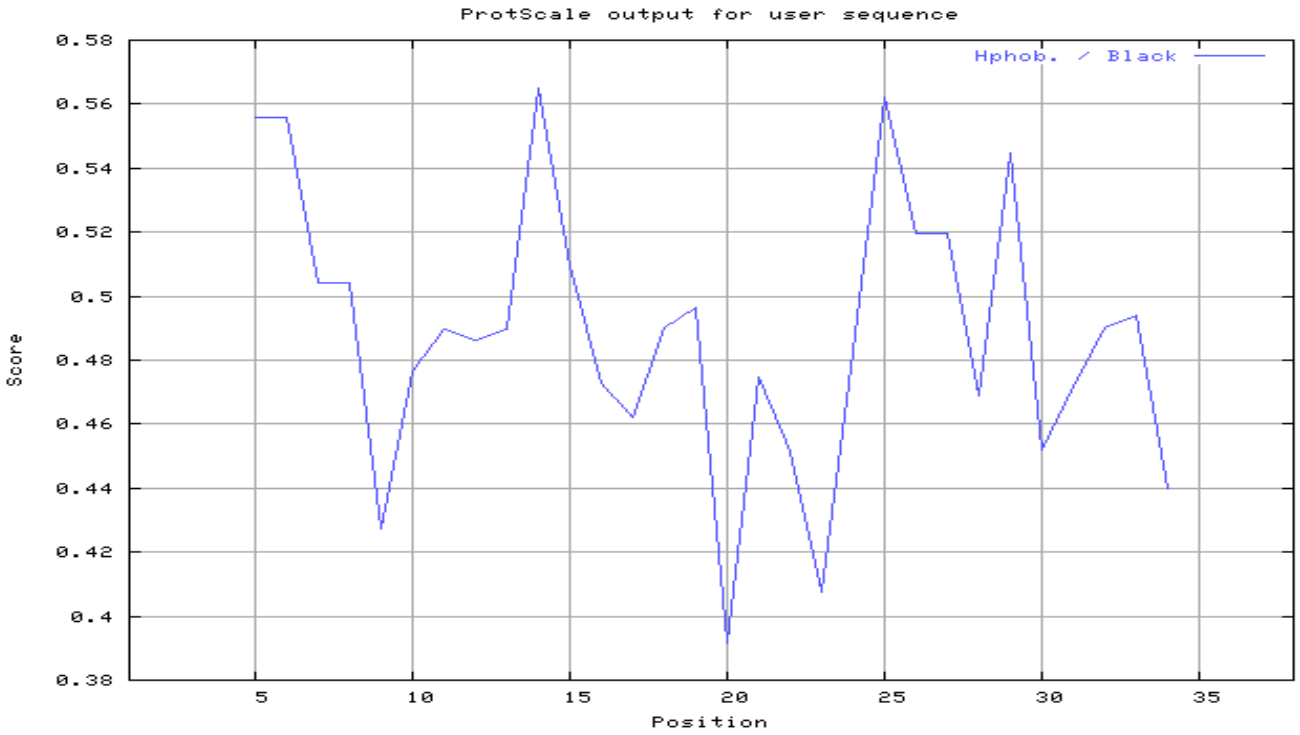

Figure 21. Hydrophobicity plot of neurotoxin alpha-KTx 3.8 by Black and Mould (1991).

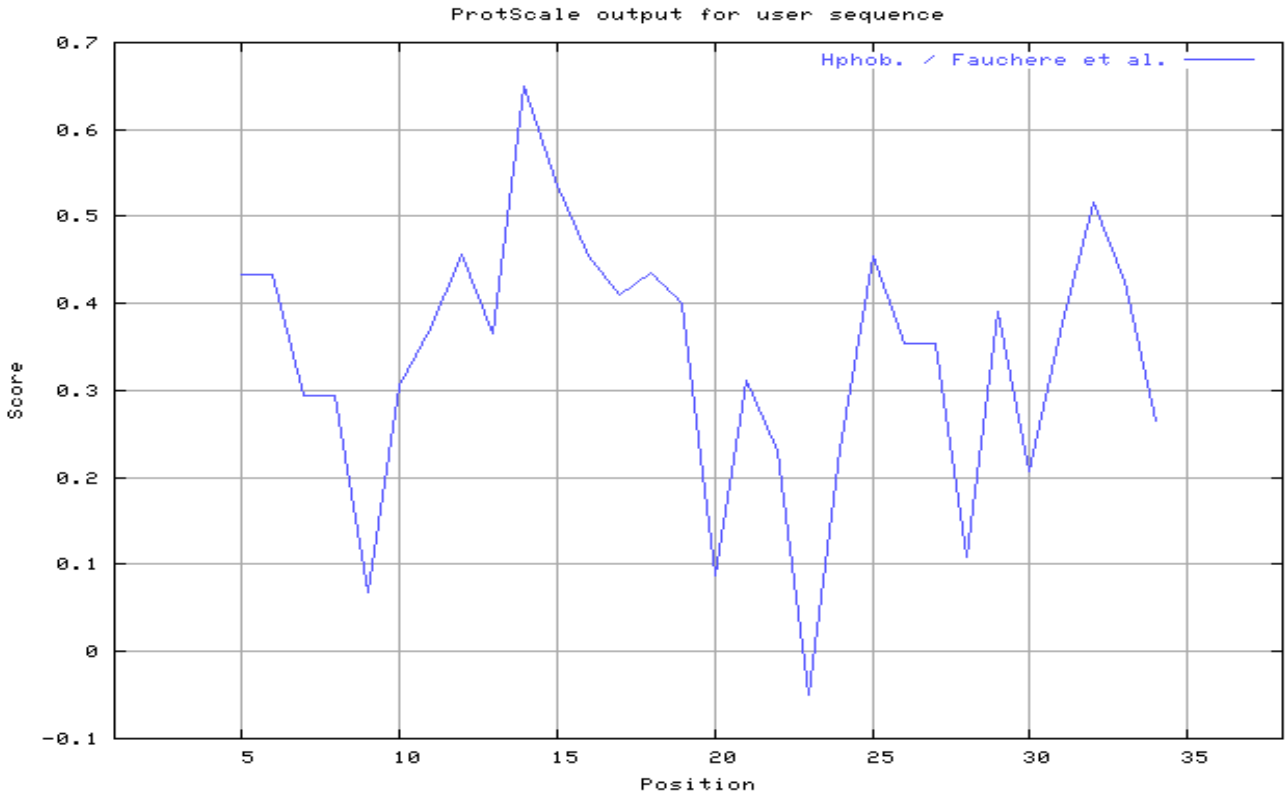

Figure 22. Hydrophobicity plot of neurotoxin alpha-KTx 3.8 by Fauchere and Pliska (1983).

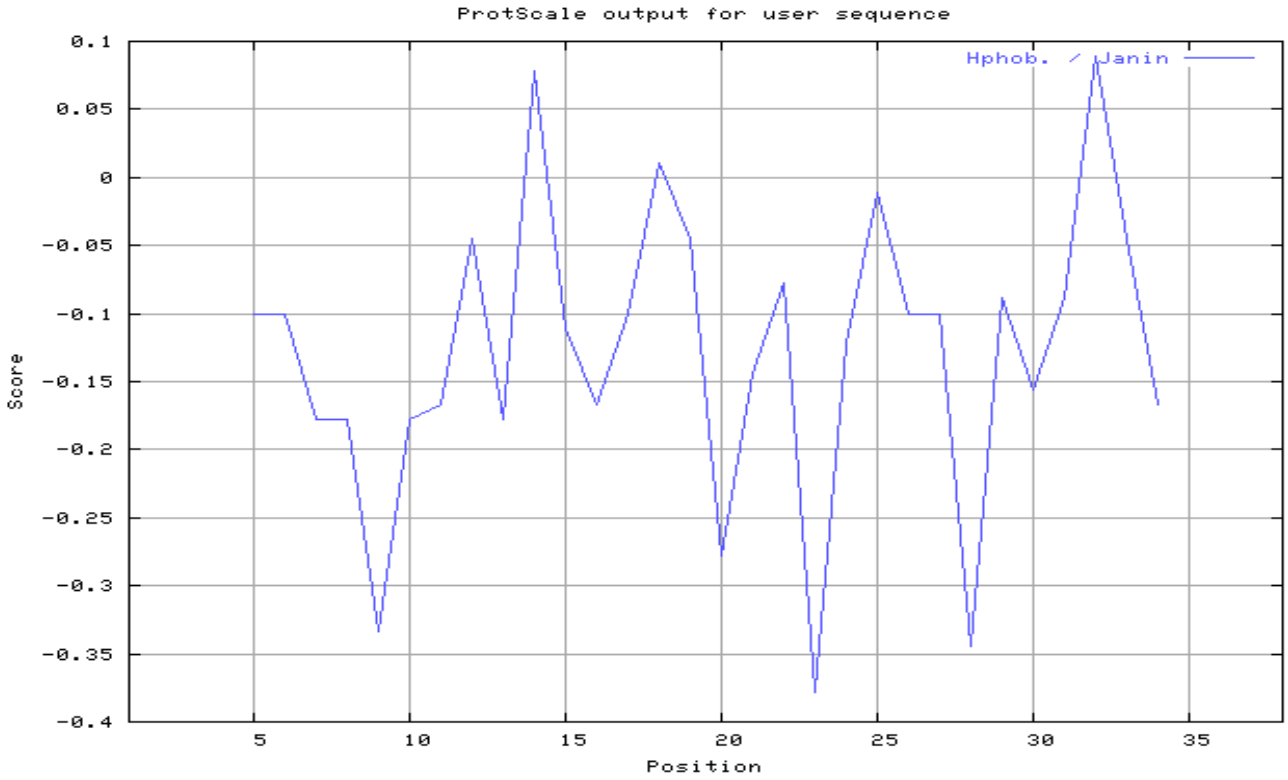

Figure 23. Hydrophobicity plot of neurotoxin alpha-KTx 3.8 by Janin (1979).

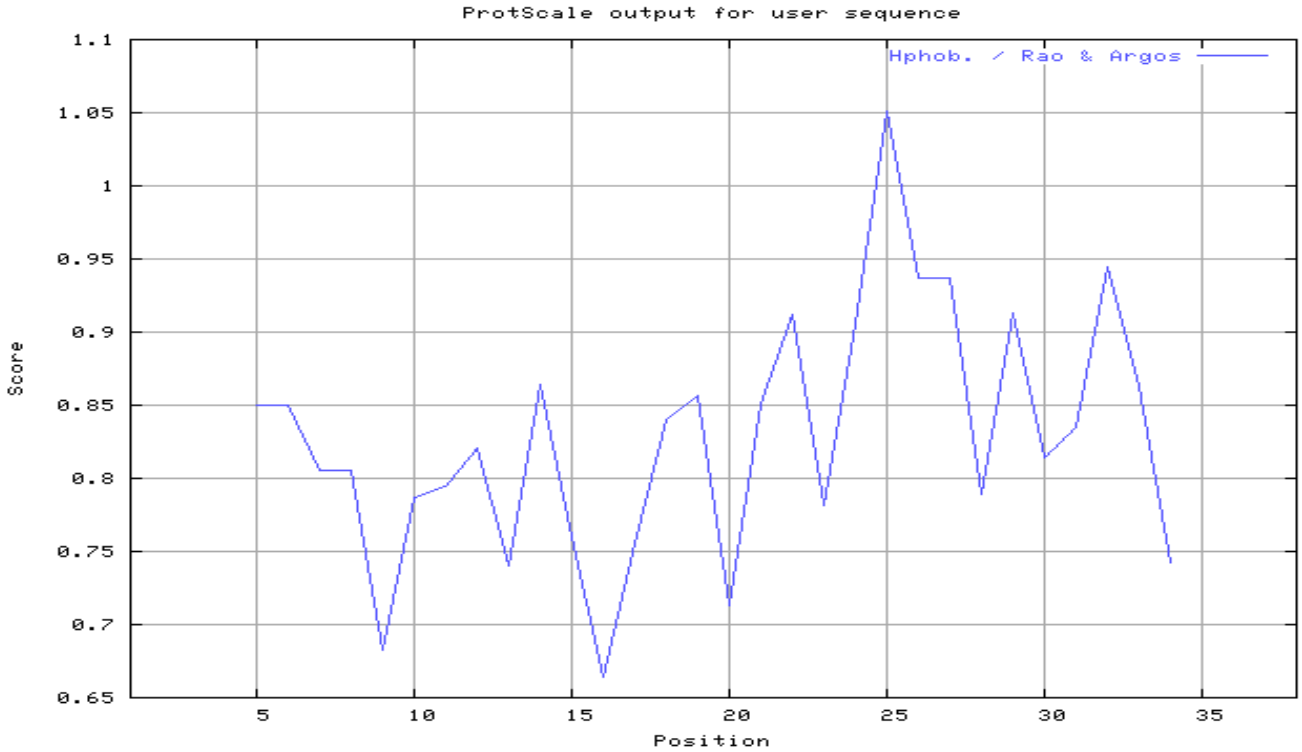

Figure 24. Hydrophobicity plot of neurotoxin alpha-KTx 3.8 by Rao and Argos. (1986)

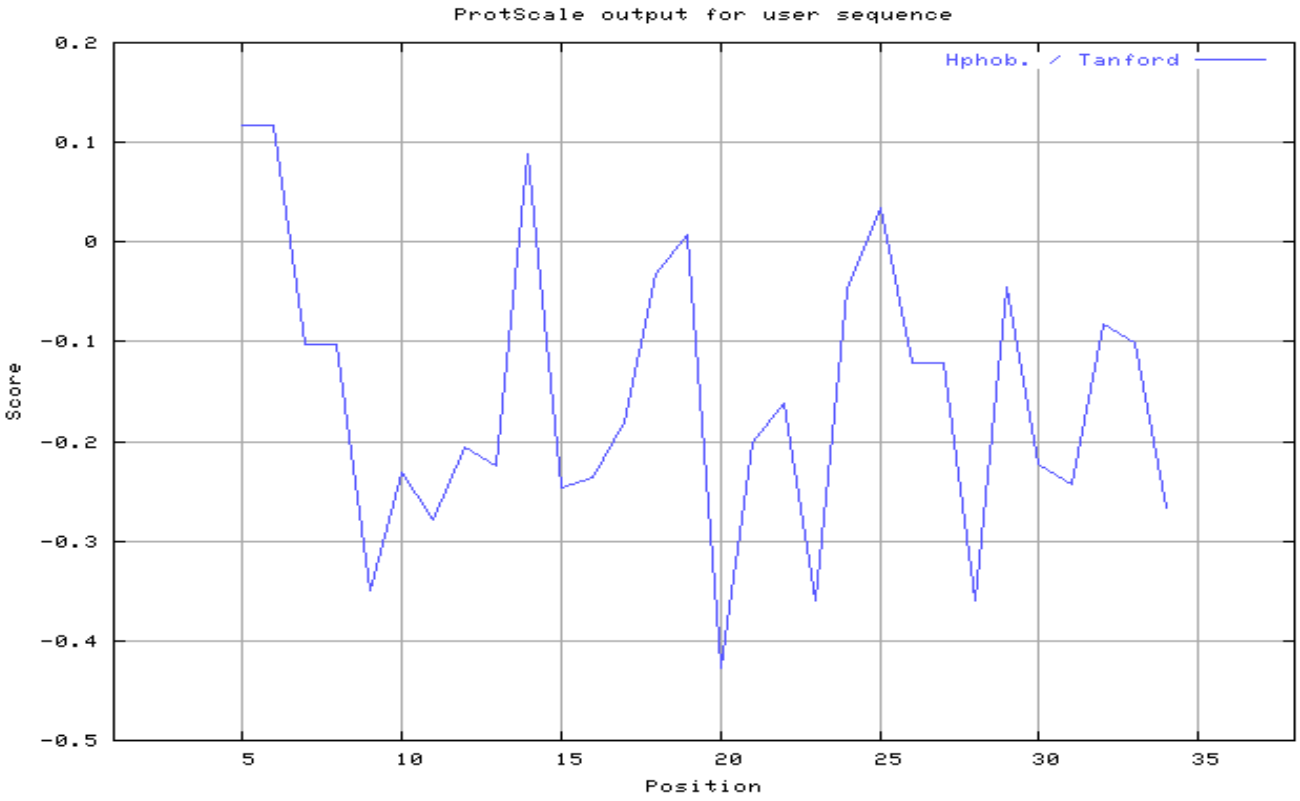

Figure 25. Hydrophobicity plot of neurotoxin alpha-KTx 3.8 by Tanford (1962).

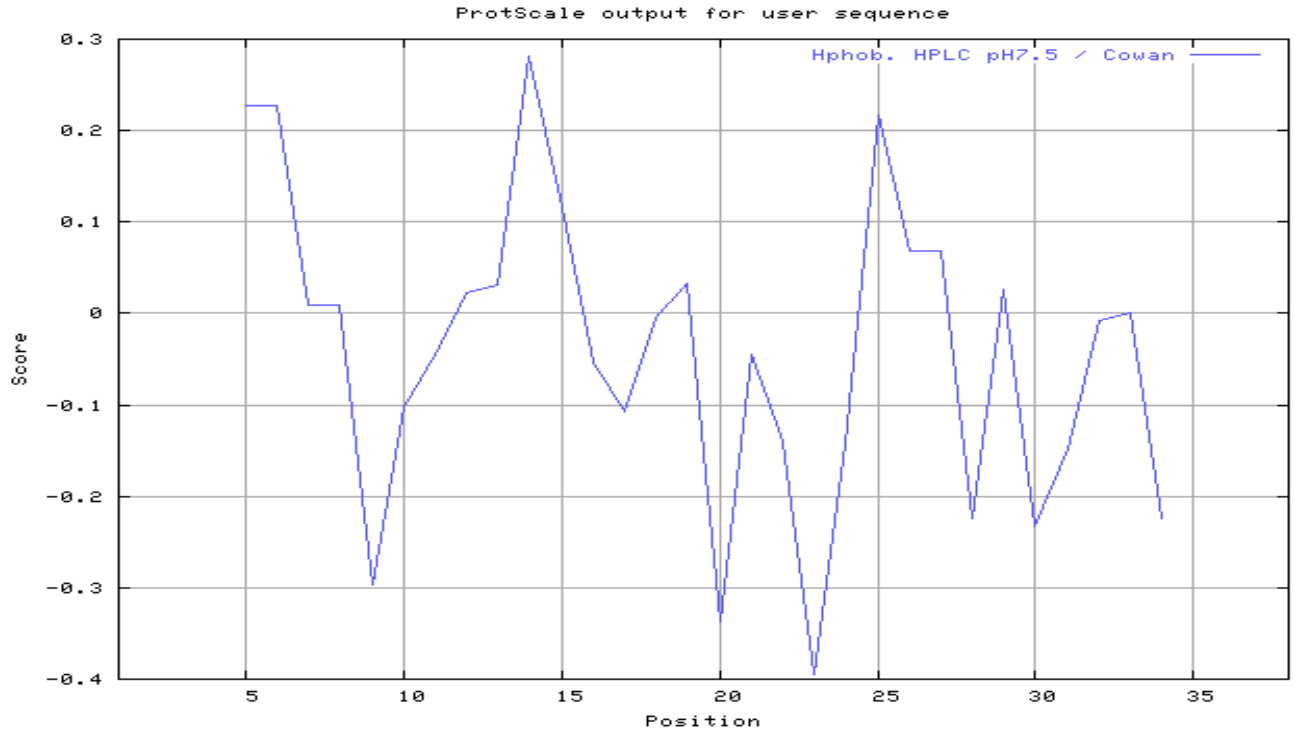

Figure 26. Hydrophobicity plot of neurotoxin alpha-KTx 3.8 by HPLC pH7.5 Cowan and Whittaker (1990)

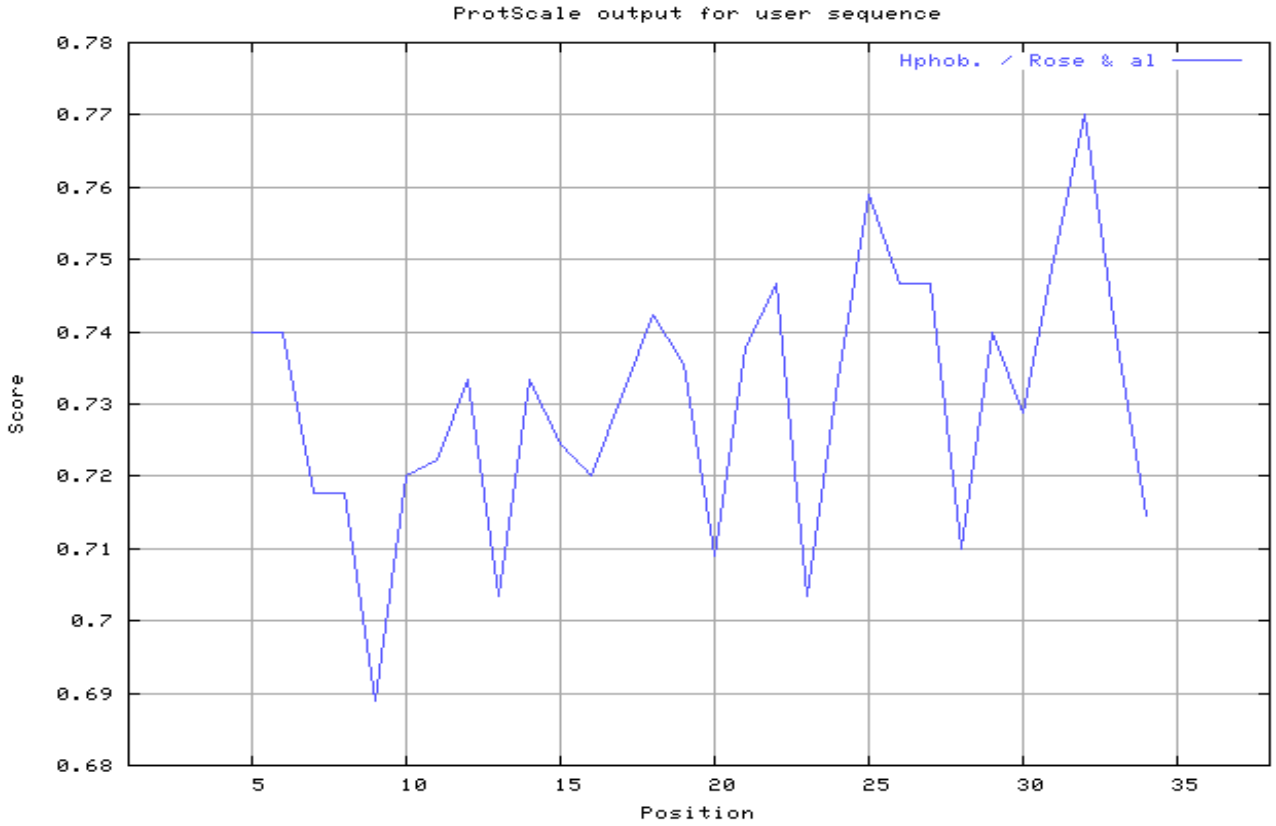

Figure 27. Hydrophobicity plot of neurotoxin alpha-KTx 3.8 by Rose GD, Geselowitz AR, Lesser GJ, Lee RH, Zehfus MH (1985).

Table 1. TAP Peptide binders of neurotoxin protein.

Peptide rank	Start position	Sequence	Score	Predicted affinity
1	17	PCRDAGMRF	5.025	Intermediate
2	7	KCRGSPQCI	4.932	Intermediate
3	19	RDAGMRFGK	4.819	Intermediate
4	21	AGMRFGKCM	4.640	Intermediate
5	1	GVPINVKCR	3.993	Intermediate
6	27	KCMNGKCHC	3.781	Intermediate
7	26	GKCMNGKCH	3.752	Intermediate
8	6	VKCRGSPQC	3.648	Intermediate
9	24	RFGKCMNGK	3.231	Intermediate
10	23	MRFGKCMNG	3.222	Intermediate

*Optimal Score for given MHC binder in mouse.

tein sequence (Alpha-KTx 3.8) having 38 amino acids, which shows 30 nonamers. Small peptide regions found as 17- PCRDAGMRF (score 5.025), 7- KCRGSPQCI (Score- 4.932), 19- RDAGMRFGK (Score- 4.819), 21- AGMRFGKCM (Score- 4.640), known as neurotoxin protein TAP transporter (Table 1). We also found the SVM based MHCII-IAb peptide regions, 26- GKCMNGKCH, 20- DAGMRFGKC, 1- GVPINVKCR, 19- RDAGMRFGK, (optimal score is 0.388); MHCII-IAd peptide regions, 20- DAGMRFGKC, 14- CIQPCRDAG, 10- GSPQCIQPC, 25- FGKCMNGKC, (optimal score is 0.386); MHCII-IAg7 peptide regions , 18- CRDAGMRFG, 17- PCRDAGMRF, 14- CIQPCRDAG, 3- PINVKCRGS, (optimal score is 1.341); and MHCII- RT1.B peptide regions, 16- QPCRDAGMR, 29- MNGKCHCTP, 8- CRGSPQCIQ, 7- KCRGSPQCI , (optimal score is -0.039)

Table 2. Peptide binders to MHCII molecules of neurotoxin protein.

Prediction method	Rank	Sequence	Residue No.	Peptide Score
ALLELE: I-Ab	1	GKCMNGKCH	26	0.388
ALLELE: I-Ab	2	DAGMRFGKC	20	0.364
ALLELE: I-Ab	3	GVPINVKCR	1	0.150
ALLELE: I-Ab	4	RDAGMRFGK	19	-0.078
ALLELE: I-Ad	1	DAGMRFGKC	20	0.386
ALLELE: I-Ad	2	CIQPCRDAG	14	0.356
ALLELE: I-Ad	3	GSPQCIQPC	10	0.320
ALLELE: I-Ad	4	FGKCMNGKC	25	0.296
ALLELE: I-Ag7	1	CRDAGMRFG	18	1.341
ALLELE: I-Ag7	2	PCRDAGMRF	17	1.197
ALLELE: I-Ag7	3	CIQPCRDAG	14	1.122
ALLELE: I-Ag7	4	PINVKCRGS	3	1.012
ALLELE: RT1.B	1	QPCRDAGMR	16	-0.039
ALLELE: RT1.B	2	MNGKCHCTP	29	-0.043
ALLELE: RT1.B	3	CRGSPQCIQ	8	-0.088
ALLELE: RT1.B	4	KCRGSPQCI	7	-0.122

*Optimal score for given MHC II peptide binder in mouse.

which represented predicted binders from neurotoxin protein (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 regions. In this assay we have also identified peptides that can stimulate Cytotoxic T Lymphocytes (CTLs). The use of artificial neural network and support vector machine on the recent and high quality CTL epitopes and non-epitopes data is explored as a means to meet these challenges. Here, machine learning techniques SVM and ANN have been used for CTL epitope prediction (Table 4). The peptides with their (ANN/SVM) scores are 1-GVPINVKCR (0.81/0.87220559), 21- AGMRFGKCM (0.96/0.38021223) and 19- RDAGMRFGK (0.89/-0.22957314).

DISCUSSION

Gomase (2007) method, B-EpiPred Server, Hopp and Woods, Welling, Parker, Kolaskar and Tongaonkar antigenicity scales were designed to predict the locations of antigenic determinants in potassium channel inhibitor Alpha-KTx 3.8 (neurotoxin). Neurotoxin protein shows beta sheets regions, which are high antigenic response than helical region of this peptide and shows highly antigenicity (Figures 1-5). We also found the Sweet hydrophobicity, Kyte and Doolittle hydrophobicity, Abraham and Leo, Bull and 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 and Argos hydrophobicity, Wolfenden hydrophobicity, Wilson HPLC hydrophobicity, Cowan HPLC pH3.4, Tanford hydrophobicity, Rf mobility hydrophobicity and Chothia hydrophobicity scales. These scales are essentially a hydrophilic index, with polar residues assigned negative values (Figures 7-27). In this assay we predicted the binding affinity of neurotoxin protein having 38 amino acids, which shows 30 nonamers. Small peptide regions found as 17- PCRDAGMRF (score 5.025), 7- KCRGSPQCI (Score- 4.932), 19-RDAGMRFGK (Score- 4.819), 21- AGMRFGKCM (Score- 4.640), known as neurotoxin protein TAP transporter. 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 1 and MHC 2 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 neurotoxin protein. Analysis shows epitopes present in the neurotoxin protein the desired immune response (Table 3). The region of maximal hydrophilicity is likely to be an antigenic site, having hydrophobic characteristics, because C- terminal regions of neurotoxin protein is solvent accessible and unstructured, antibodies against those

Table 3. Antigenic epitopes from neurotoxin protein.

No.	Start position	End position	Peptide	Peptide length
1	4	19	INVKCRGSPQCIQPCR	16

Table 4. Predicted CTL epitopes.

Peptide rank	Start position	Sequence	Score(ANN/SVM)	Prediction
1	1	GVPINVKCR	0.81/0.87220559	Epitope
2	21	AGMRFGKCM	0.96/0.38021223	Epitope
3	19	RDAGMRFGK	0.89/-0.22957314	Epitope

regions are also likely to recognize the native protein. The highest pick recorded between sequence of amino acid in the region are '4-INVKCRGSPQCIQPCR-19' (Table 3). We also found the SVM based MHCII-IAb peptide regions, 26- GKCMNGKCH, 20- DAGMRFGKC, 1- GVPINVKCR, 19- RDAGMRFGK, (optimal score is 0.388); MHCII-IAd peptide regions, 20- DAGMRFGKC, 14- CIQPCRDAG, 10- GSPQCIQPC, 25- FGKCMNGKC, (optimal score is 0.386); MHCII-IAg7 peptide regions , 18- CRDAGMRFG, 17- PCRDAGMRF, 14- CIQPCRDAG, 3- PINVKCRGS, (optimal score is 1.341); and MHCII- RT1.B peptide regions, 16- QPCRDAGMR, 29- MNGKCHCTP, 8- CRGSPQCIQ, 7- KCRGSPQCI (optimal score is -0.039) which represented predicted binders from neurotoxin protein (Table 2). The average propensity for the neurotoxin protein is found to be above 1.042 (Figure 5). All residues having above 1.0 propensity are always potentially antigenic (Table 3). The predicted segments in neurotoxin protein are '4-INVKCRGSPQCIQPCR-19'. Fragment identified through this approach tend to be high-efficiency binders, which is a larger percentage of their atoms are directly involved in binding as compared to larger molecules. Machine learning techniques SVM and ANN have been used for CTL epitope prediction; the predicted peptides with their (ANN/SVM) scores are 1- GVPINVKCR (0.81/0.87220559), 21- AGMRFGKCM (0.96/0.38021223) and 19- RDAGMRFGK (0.89/-0.22957314).

Conclusion

Neurotoxin protein sequence (alpha-KTx 3.8) of *M. tamulus* sindicus involved multiple antigenic components to direct and empower the immune system to protect the host from the neurotoxin. 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 and identification of peptides that can stimulate Cytotoxic T Lymphocytes (CTLs). This theme is implemented in designing subunit and synthetic peptide vaccines.

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