Biochemistry & Physiology: Open Access

Gomase and Sherkhane, Biochem Physiol 2014, 3:3
DOI: 10.4172/2168-9652.1000136

Research Article Open Access

Prediction of Antigenic Peptide and MHC Binder from ITX-3 *Tegenaria agrestis*: Current Approach for Synthetic Vaccine Development

Sherkhane AS and Gomase VS*

The Global Open University, Nagaland, India

***Corresponding author:** Gomase VS, The Global Open University, Dimapur - 797 112, Nagaland, India, Tel: 91-9987770696; E-mail: gomase.viren@gmail.com

Rec date: May 23, 2014, **Acc date:** Jun 05, 2014, **Pub date:** Jun 12, 2014

Abstract

Venom of *Tegenaria agrestis* species causes necrosis in humans. ITX-3 is a toxin with 68 amino acids. Antigenic peptides of *Tegenaria agrestis* toxic protein are most suitable for synthetic peptide vaccine development because with single epitope, the immune response can be generated in large population. In this research, we used PSSM and SVM algorithms for the prediction of MHC class I & II binding peptide, antigenicity, Solvent accessibility, polar and nonpolar residue to analyze the regions that are likely exposed on the surface of proteins which are potentially antigenic that allows potential drug targets to identify active sites as well as to design synthetic peptide vaccine.

Keywords: ITX-3; *Tegenaria agrestis*; Antigenic peptides; MHC-Binders; TapPred; PSSM; SVM; Nonamers

Introduction

Tegenaria agrestis is a member of the genus of Tegenaria known scientifically known as aggressive house spider [1,2]. Venom of *Tegenaria agrestis* species causes necrosis, loss of limbs, fatal to healthy humans and necrotic skin lesions [3,4]. ITX-3 toxins act directly on central nervous system neurons and paralyze insects [5]. These toxins have great potential for synthetic peptide vaccine. Antigenic peptides from *Tegenaria agrestis* are most suitable for the development of synthetic peptide vaccine because a single toxin subunit can generate sufficient immune response. Major histocompatibility complex (MHC) molecules are cell surface proteins that binds to the peptides derived from host or antigenic proteins, and present them at the cell surface for recognition by T-cells. T cell recognition is a fundamental mechanism of the adaptive immune system by which the host identifies and responds to foreign antigens [6,7]. There are two types of MHC molecule and are extremely polymorphic. MHC class I molecules present peptides from proteins synthesized within the cell, whereas, MHC class II molecule present peptides derived from endocytosed extracellular proteins. Identification of MHC-binding peptides and T-cell epitopes helps improve our understanding of specificity of immune responses [8-11].

Methodology

Database searching

There are many different types of databases available; the antigenic protein sequence of *Tegenaria agrestis* was retrieved from GenBank, UniProtKB/Swiss-prot [12-14].

Prediction of antigenicity

Prediction of antigenicity program predicts those segments from neurotoxin protein that are likely to be antigenic by eliciting an antibody response. In this research work antigenic epitopes of ITX-3 *Tegenaria agrestis* are determined by using the Hopp and Woods, Welling, Parker, Bepipred, Kolaskar and Tongaonkar antigenicity methods [15-19].

Prediction of MHC Binding Peptide

MHC peptide binding of ITX-3 *Tegenaria agrestis* is predicted using neural networks trained on C terminals of known epitopes. Rankpep predicts peptide binders to MHC-I ligands whose C-terminal end is likely to be the result of proteosomal cleavage using Position Specific Scoring Matrices (PSSMs). Support Vector Machine (SVM) based method for prediction of promiscuous MHC class II binding peptides from protein sequence; SVM has been trained on the binary input of single amino acid sequence [20-24].

Prediction of Antigenic Peptides by Cascade SVM based TAPPred method

In the present study, we predict cascade SVM based several TAP binders which was based on the sequence and the features of amino acids [25]. We found the MHCI binding regions (Table 3), the binding affinity of ITX-3 *Tegenaria agrestis*.

Solvent Accessible Regions

We also predict solvent accessible regions of proteins having highest probability that a given protein region lies on the surface of a protein Surface Accessibility, backbone or chain flexibility by Emani et al. [26] and Karplus and Schulz [27]. By using different scale, the hydrophobic and hydrophilic characteristics of amino acids that are rich in charged and polar residues were predicted [28-37].

Results and Interpretations

ITX-3 *Tegenaria agrestis* contain a long residue with 68 amino acids.

MKLQLMICLVLLPCFFCEPDEICRARMTNKEFTYKSNVCNGCG DQVAACEAECFRNDVYTACHEAQKG

Prediction of antigenic peptides

In this study, we found the antigenic determinants by finding the area of greatest local hydrophilicity. The Hopp-Woods scale Hydrophilicity Prediction Result Data found high pick in position 21-23,27-28 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). 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 and Prediction Result Data found high in position 62-64 (Figure 2). We also study Hydrophobicity plot of HPLC/Parker Hydrophilicity Prediction Result Data found 39-CNGCGDQ-45 (5.314), 61-TDDCNPH-67 (5.400), 60-STDDCNP-66 6.029 (maximum) (Figure 3), BepiPred predicts the location of linear B-cell epitopes Result found that, 32-FT-33, 39-CNGCGDQVA-47, 64-EAQKG-68 (Figure 4), Kolaskar and Tongaonkar [19] antigenicity methods (Figure 5) Predicted peptides result found i.e. 4-QLMICLVLLPCFFCEPDEICRA-25, 35-KSNVCNGCGDQVAACEAE-52 and the predicted antigenic fragments can bind to MHC molecule is the first bottlenecks in vaccine design.

Solvent accessible regions

We also predict solvent accessible regions in proteins; different measurement was performed for the prediction of antigenic activity, surface region of peptides. Emani et al. [26], (Figure 6) predicts the highest probability i.e. found Maximum in 26-RMTNKEFTYK-35, that a given protein region lies on the surface of a protein and are used to identify antigenic determinants on the surface of proteins. Karplus and Schulz [27] (Figure 7) High score is found i.e. found 1.042 maximum in 26-RMTNKEF-32. Predict backbone or chain flexibility on the basis of the known temperature B factors of the a-carbons. The hydrophobicity and hydrophilic characteristics of amino acids is determined by using different scales that are rich in charged and polar residues i.e. Sweet et al. [28] hydrophobicity prediction Result Data found high in position 8-9, 12-14, Kyte and Doolittle [29] result high in position 8-9, Abraham and Leo [30] result high in position 8-10, 12-13, Bull and Breese [31] result high in position 41-43,63-65, Miyazawa [32] result high in position 8-9, Roseman [33] result high in position 8-10,12-14, Wolfenden [34] result high in position 8-10,11-12, Wilson et al. [35] 8-12,13-15, Cowan [36] 6-7,8-10, Chothia [37] 6-9,10-14 (Figure 7).

Prediction of MHC Binding Peptide

We found binding of peptides to a number of different alleles using Position Specific Scoring Matrix. ITX-3 *Tegenaria agrestis* sequence is 68 residues long, having 60 nonamers. MHC molecules are cell surface proteins, which actively participate in host immune reactions and involvement of MHC-I and MHC-II in response to almost all antigens. We have predicted MHC-I peptide binders of ITX-3 *Tegenaria agrestis* was tested with on a set of 4 different alleles i.e. H2-Db (mouse) 8mer, H2-Db (mouse) 9mer, H2-Db (mouse) 10mer, H2-Db (mouse) 11mer (Table 1) and MHC-II peptide binders for I_Ab, I_Ad, I_Ag7 alleles highlighted in red represent predicted binders (Table 2). Here RANKPEP report PSSM-specific binding threshold and is obtained by scoring all the antigenic peptide sequences included in the alignment from which a profile is derived, and is defined as the score value that includes 85% of the peptides within the set. Peptides whose score is above the binding threshold will appear highlighted in red and peptides produced by the cleavage prediction model are highlighted in violet. We also use a cascade SVM based TAPPred method which found 17 High affinity TAP Transporter peptide regions (Table 3) which represents predicted TAP binders residues which occur at N and C termini from ITX-3 *Tegenaria agrestis* [38-43].

MHC-I Allele	POS.	N	SEQUENCE	C	MW (Da)	SCORE	% OPT.
8mer_H2_Db	27	RAR	MTNKEFTY	KSN	1015.14	9.688	0.1846
8mer_H2_Db	9	MIC	LVLLPCFF	CEP	933.23	7.091	0.1351
8mer_H2_Db	39	SNV	CNGCGDQV	AAC	776.83	3.069	0.0585
8mer_H2_Db	4	MKL	QLMICLVL	LPC	914.23	1.244	0.0237
9mer_H2_Db	38	KSN	VCNGCGDQV	AAC	875.96	16.026	0.3182
9mer_H2_Db	1		MKLQLMICL	VLL	1074.46	8.026	0.1594
9mer_H2_Db	4	MKL	QLMICLVLL	PCF	1027.39	7.799	0.1548
9mer_H2_Db	27	RAR	MTNKEFTYK	SNV	1143.31	4.598	0.0913
9mer_H2_Db	14	LLP	CFFCEPDEI	CRA	1084.25	3.759	0.0746
9mer_H2_Db	26	CRA	RMTNKEFTY	KSN	1171.33	2.737	0.0543
9mer_H2_Db	8	LMI	CLVLLPCFF	CEP	1036.37	2.51	0.0498
9mer_H2_Db	46	GDQ	VAACEAECF	RND	924.07	2.491	0.0495
10mer_H2_Db	26	CRA	RMTNKEFTYK	SNV	1299.5	4.964	0.0843
10mer_H2_Db	29	RMT	NKEFTYKSNV	CNG	1211.33	1.44	0.0245
10mer_H2_Db	13	VLL	PCFFCEPDEI	CRA	1181.37	0.32	0.0054
11mer_H2_Db	49	VAA	CEAECFRNDVY	TAC	1330.47	11.083	0.1394
11mer_H2_Db	36	TYK	SNVCNGCGDQV	AAC	1077.14	7.455	0.0938
11mer_H2_Db	44	GCG	DQVAACEAECF	RND	1167.29	3.148	0.0396
11mer_H2_Db	25	ICR	ARMTNKEFTYK	SNV	1370.58	1.845	0.0232

Table 1: Promiscuous MHC ligands, having C-terminal ends are proteosomal cleavage sites of ITX-3 *Tegenaria agrestis*

MHC-II Allele	POS.	N	SEQUENCE	C	MW (Da)	SCORE	% OPT.
MHC-II I_Ab	16	PCF	FCEPDEICR	ARM	1093.26	17.265	0.4845

MHC-II I_Ab	57	FRN	DVYTACHEA	QKG	990.06	14.247	0.3998
MHC-II I_Ad	42	CNG	CGDQVAACE	AEC	876.96	18.515	0.3484
MHC-II I_Ad	60	DVY	TACHEAQKG		926.01	12.673	0.2385
MHC-II I_Ad	23	DEI	CRARMTNKE	FTY	1090.28	8.553	0.1609
MHC-II I_Ag7	49	VAA	CEAECFRND	VYT	1068.16	11.53	0.2821

Table 2: Prediction of MHCII ligands all rows highlighted in red represent predicted binders.

Peptide Rank	Start Position	Sequence	Score	Predicted Affinity
1	54	FRNDVYTAC	8.382	High
2	15	FFCEPDEIC	8.379	High
3	17	CEPDEICRA	8.26	High
4	20	DEICRARMT	8.194	High
5	37	NVCNGCGDQ	7.924	High
6	28	TNKEFTYKS	7.699	High
7	41	GCGDQVAAC	7.682	High
8	48	ACEAECFRN	7.629	High
9	31	EFTYKSNVC	7.588	High
10	6	MICLVLLPC	7.375	High
11	14	CFFCEPDEI	7.364	High
12	21	EICRARMTN	6.852	High
13	57	DVYTACHEA	6.735	High
14	4	QLMICLVLL	6.453	High
15	34	YKSNVCNGC	6.394	High
16	55	RNDVYTACH	6.252	High
17	27	MTNKEFTYK	6.093	High

Table 3: cascade SVM based High affinity TAP Binders of ITX-3 *Tegenaria agrestis*

Discussion

In this study, we found the antigenic determinants by finding the area of greatest local hydrophilicity. Hopp and Woods hydrophobicity scale is used to identify of potentially antigenic sites in proteins. Hydrophilicity Prediction result data found high in sequence position at 21-23, 27-28 in a protein this scale is basically a hydrophilic index where a polar residues have been assigned negative values. The Window size of 5-7 is good for finding hydrophilic regions, greater than 0 values are consider as hydrophilic which is consider as antigenic.

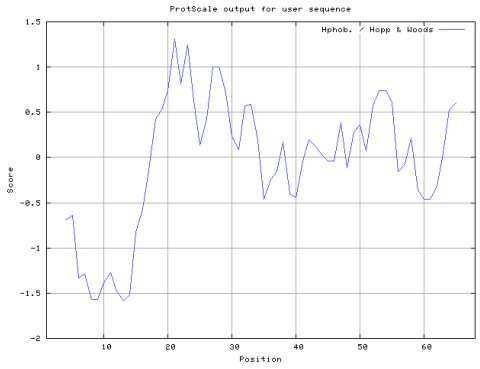

Figure 1: Hydrophobicity plot of Hopp and Woods [15] of ITX-3 *Tegenaria agrestis*

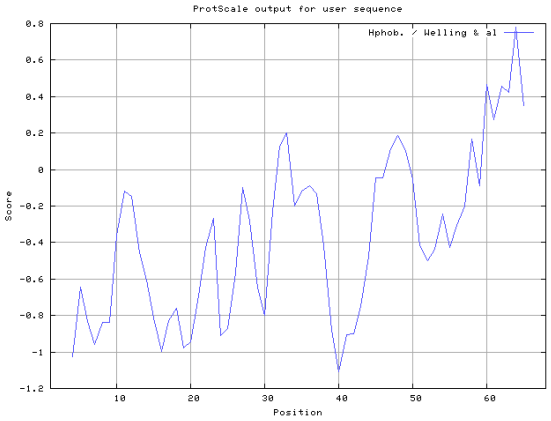

Figure 2: Hydrophobicity plot of Welling et al. [16] of alpha-neurotoxins of ITX-3 *Tegenaria agrestis*

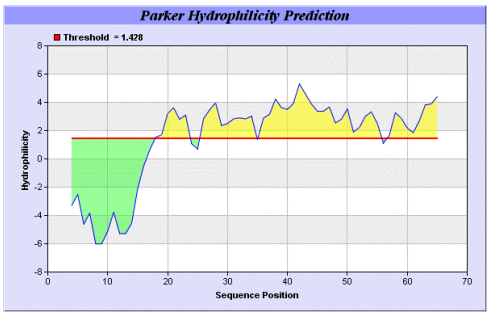

Figure 3: Hydrophobicity plot of HPLC/Parker et al. [17] of alpha-neurotoxins of ITX-3 *Tegenaria agrestis*

Welling used information on the relative occurrence of amino acids in antigenic regions to make a scale which is useful for prediction of antigenic regions and the predicted result data found high in sequence position 62-64. 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.

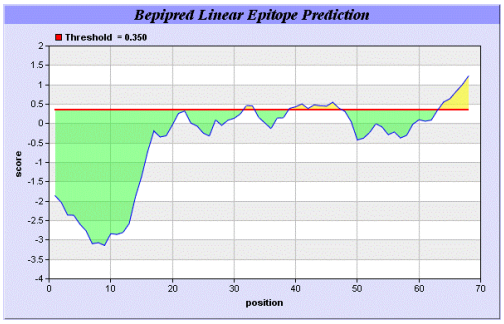

Figure 4: Bepipred Linear Epitope Prediction plot showing antibody recognized B-cell epitopes of alpha-neurotoxins of ITX-3 *Tegenaria agrestis*

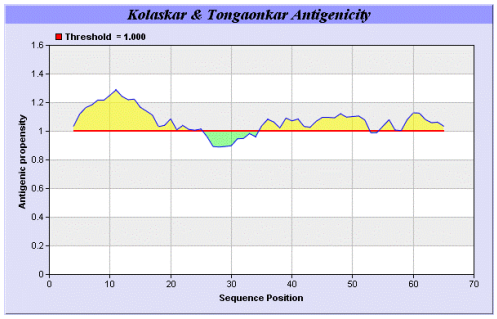

Figure 5: Kolaskar and Tongaonkar [19] antigenicity plot for the alpha-neurotoxins of ITX-3 *Tegenaria agrestis*

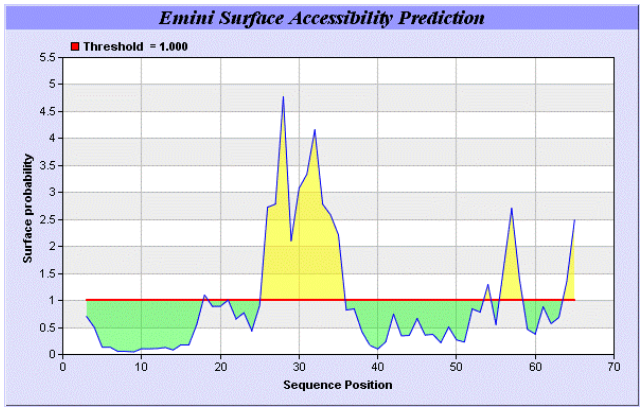

Figure 6: Emini [26] Surface Accessibility Prediction plot of alpha-neurotoxins of ITX-3 *Tegenaria agrestis*

We also study Hydrophobicity plot of HPLC / Parker Hydrophilicity Prediction Result Data found 39-CNGCGDQ-45 (5.314), 61-TDDCNPH-67 (5.400), 60-STDDCNP-66 6.029 (maximum). BepiPred predicts the location of linear B-cell epitopes Result found that 32-FT-33, 39-CNGCGDQVA-47, and 64-EAQKG-68. There are 3 antigenic determinant sequences is found by Kolaskar and Tongaonkar [19] antigenicity scales the results show highest pick at position 4-QLMICLVLLPCFFCEPDEICRA-25, 35-KSNVCNGCGDQVAACEAE-52 (Figure 1-5).

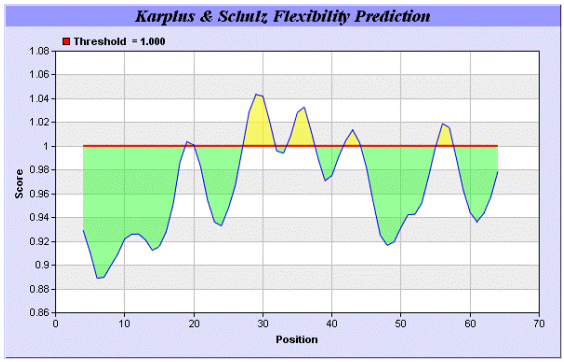

Figure 7: Karplus and Schulz [27] Flexibility Prediction of alpha-neurotoxins of ITX-3 *Tegenaria agrestis*

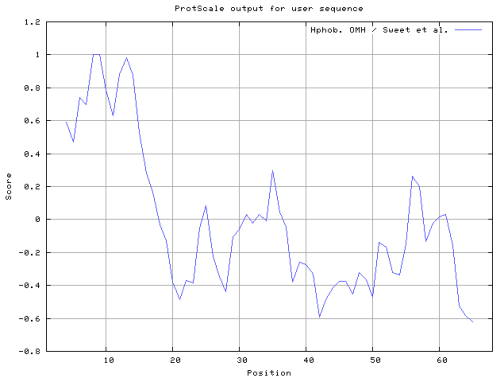

Figure 8: Hydrophobicity plot of Sweet and Eisenberg [28] of alpha-neurotoxins of ITX-3 *Tegenaria agrestis*

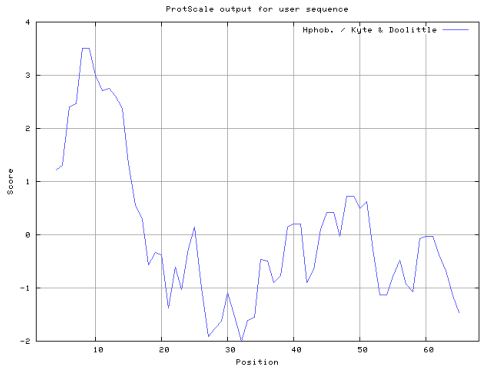

Figure 9: Kyte and Doolittle [29] hydrophobicity plot of alpha-neurotoxins of ITX-3 *Tegenaria agrestis*

Result of determined antigenic sites on proteins has revealed that the hydrophobic residues if they occur on the surface of a protein are more likely to be a part of antigenic sites. This method can predict

antigenic determinants with about 75% accuracy and also gives the information of surface accessibility and flexibility. Further this region form beta sheet which show high antigenic response than helical region of this peptide and shows highly antigenicity.

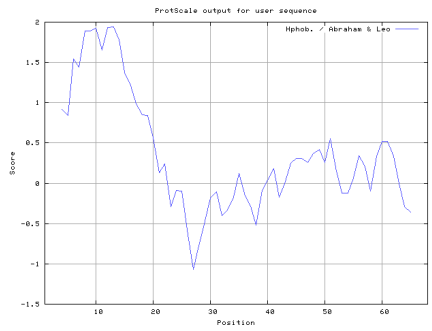

Figure 10: Abraham and Leo [30] hydrophobicity plot of alpha-neurotoxins of ITX-3 *Tegenaria agrestis*

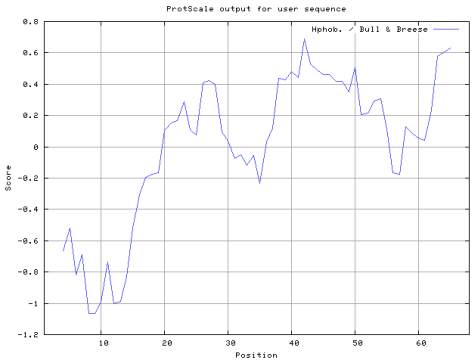

Figure 11: Bull and Breese [31] use surface tension to measure hydrophobicity and also uses negative values to describe the hydrophobicity of alpha-neurotoxins of ITX-3 *Tegenaria agrestis*

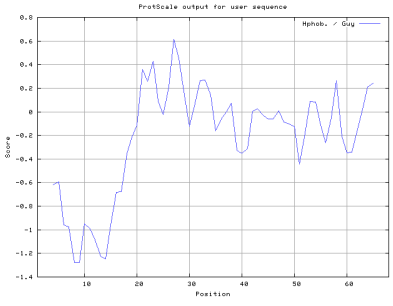

Figure 12: Hydrophobicity plot of alpha-neurotoxins of ITX-3 *Tegenaria agrestis*

We predict Solvent accessibility by using Emani et al., the result found the highest probability i.e. found 26-RMTNKEFTYK-35, that a given protein region lies on the surface of a protein and are used to identify antigenic determinants on the surface of proteins [26]. This algorithm also used to identify the antigenic determinants on the surface of proteins and Karplus and Schulz [27] predict backbone or chain flexibility on the basis of the known temperature B factors of the a-carbons here we found the result with High score is i.e. found 1.042 maximum in 26-RMTNKEF-32 (Figures 6 and 7). We predict Solvent accessibility of alpha-neurotoxins of ITX-3 *Tegenaria agrestis* for delineating hydrophobic and hydrophilic characteristics of amino acids. Solvent accessibility used to identify active site of functionally important residues in membrane proteins.

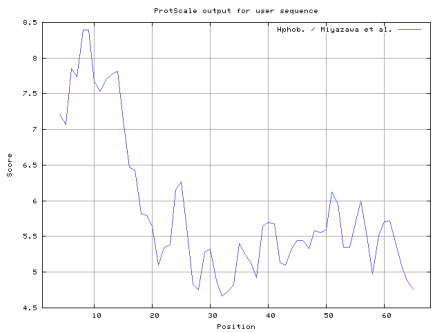

Figure 13: Hydrophobicity plot of Miyazawa and Jernigen [32] of alpha-neurotoxins of NXL1 *Naja oxiana*

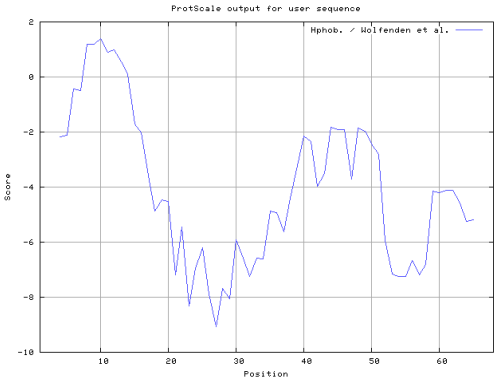

Figure 14: Hydrophobicity plot of Wolfenden et al. [34] of alpha-neurotoxins of ITX-3 *Tegenaria agrestis*

We also found the i.e. Sweet and Eisenberg [1983] hydrophobicity prediction result data found high in position 8-9, 12-14, Kyte and Doolittle [29] result high in position 8-9, Abraham and Leo [30] result high in position 8-10, 12-13, Bull and Breese [31] result high in position 41-43,63-65, Miyazawa and Jernigen [32] result high in position 8-9, Roseman [33] result high in position 8-10,12-14, Wolfenden et al. [34] result high in position 8-10,11-12, Wilson et al. [35] 8-12,13-15, Cowan and Whittaker [36] 6-7,8-10, Chothia [37] 6-9,10-14 (Figures 8-18). These scales are a hydrophilic with a polar residues assigned negative value. Because the N- and C- terminal regions of proteins are usually solvent accessible and unstructured, antibodies against those regions recognize the antigenic protein.

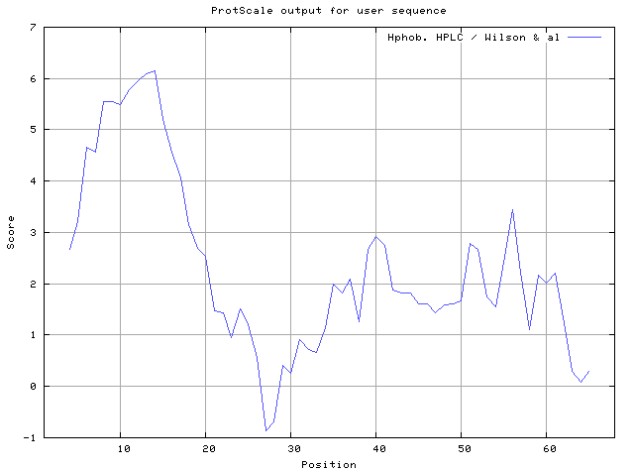

Figure 15: Hydrophobicity/HPLC plot of Wilson et al. [35] of alpha-neurotoxins of ITX-3 *Tegenaria agrestis*

Solvent-accessible surface areas and backbone angles are continuously varying because proteins can move freely in a three-dimensional space. The mobility of protein segments which are located on the surface of a protein due to an entropic energy potential and which seem to correlate well with known antigenic determinants.

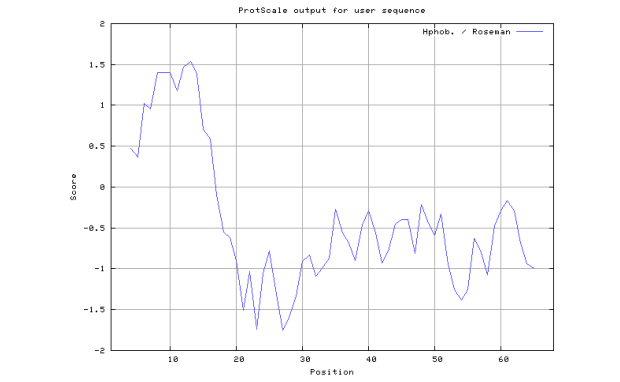

Figure 16: Hydrophobicity plot of Roseman MA [33] of alpha-neurotoxins of ITX-3 *Tegenaria agrestis*

In this study, we found predicted MHC-I peptide binders of toxin protein for 4 different alleles i.e. H2-Db (mouse) 8mer, H2-Db (mouse) 9mer, H2-Db (mouse) 10mer, H2-Db (mouse) 11mer (Table 1) and MHC-II peptide binders for I_Ab, I_Ad, I_Ag7 alleles highlighted in red represent predicted binders (Table 2). We also use a cascade SVM based TAPPred method which found 17 High affinity TAP Transporter peptide regions which represents predicted TAP binders residues which occur at N and C termini from ITX-3 *Tegenaria agrestis*. TAP is an important transporter that transports antigenic peptides from cytosol to ER. TAP binds and translocate selective antigenic peptides for binding to specific MHC molecules. The efficiency of TAP-mediated translocation of antigenic peptides is directly proportional to its TAP binding affinity. Thus, by understanding the nature of peptides, that bind to TAP with high affinity, is important steps in endogenous antigen processing. The correlation coefficient of 0.88 was obtained by using jackknife validation test.

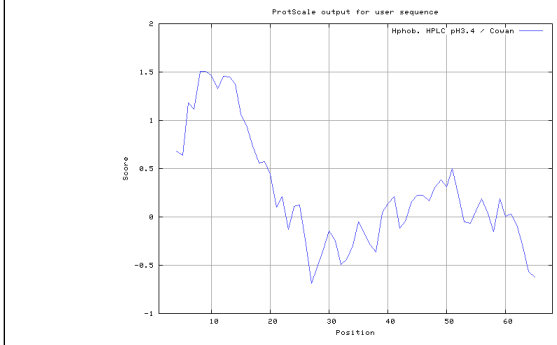

Figure 17: Hydrophobicity/HPLC pH 3.4/ plot of Cowan and Whittaker [36] of alpha-neurotoxins of ITX-3 *Tegenaria agrestis*

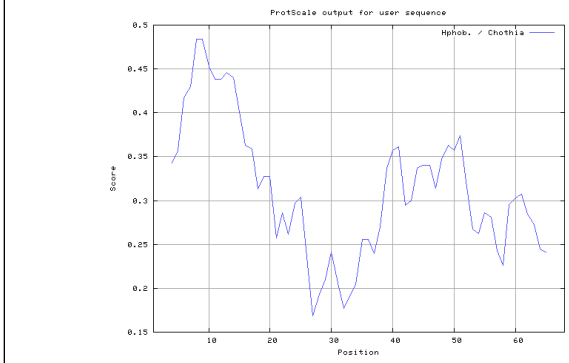

Figure 18: Hydrophobicity plot of Chothia [37] of alpha-neurotoxins of ITX-3 *Tegenaria agrestis*

In this test, we found the MHCI and MHCII binding regions. T cell immune responses are derived by antigenic epitopes hence their identification is important for design synthetic peptide vaccine. T cell epitopes are recognized by MHCI molecules producing a strong defensive immune response against alpha-neurotoxins of ITX-3 *Tegenaria agrestis*. Therefore, the prediction of peptide binding to MHCI molecules by appropriate processing of antigen peptides occurs by their binding to the relevant MHC molecules. Because, the C-terminus of MHCI-restricted epitopes results from cleavage by the proteasome and thus, proteasome specificity is important for determining T-cell epitopes. Consequently, RANKPEP also focus on the prediction of conserved epitopes. C-terminus of MHCI-restricted peptides is generated by the proteasome, and thus RANKPEP also determines whether the C-terminus of the predicted MHCI-peptide binders is the result of proteasomal cleavage. Moreover, these sequences are highlighted in purple in the output results. Proteasomal cleavage predictions are carried out using three optional models obtained applying statistical language models to a set of known epitopes restricted by human MHCI molecules as indicated here.

Conclusion

From the above result and discussion it is concluded that the ability of RANKPEP to predict MHC binding peptides, and potential T-cell epitopes. Antigenic peptide that bind to MHC molecule are antigenic that means hydrophilic in nature. This means the increase in affinity of MHC binding peptides may result in enhancement of immunogenicity of ITX-3 *Tegenaria agrestis* hence helpful in the designing of synthetic peptide vaccine. This approach can help reduce the time and cost of experimentation for determining functional properties of ITX-3 *Tegenaria agrestis*. Overall, the results are encouraging; both the sites of action and physiological functions can be predicted with very high accuracies helping minimize the number of validation experiments.

References

1. Crawford R (2008) Hobo Spider.
2. Binford GJ (2001) An analysis of geographic and intersexual chemical variation in venoms of the spider Tegenaria agrestis (Agelenidae). Toxicon 39: 955-968.
3. Centers for Disease Control and Prevention (CDC) (1996) Necrotic arachnidism--Pacific Northwest, 1988-1996. MMWR Morb Mortal Wkly Rep 45: 433-436.
4. Vetter RS, Isbister GK (2004) Do hobo spider bites cause dermonecrotic injuries? Ann Emerg Med 44: 605-607.
5. Johnson JH, Bloomquist JR, Krapcho KJ, Kral RM Jr, Trovato R, et al. (1998) Novel insecticidal peptides from Tegenaria agrestis spider venom may have a direct effect on the insect central nervous system. Arch Insect Biochem Physiol 38: 19-31.
6. Batalia MA, Collins EJ (1997) Peptide binding by class I and class II MHC molecules. Biopolymers 43: 281-302.
7. Flower DR (2008) "Vaccines: how they work" in Bioinformatics for Vaccinology. Wiley-Blackwell, Oxford, UK. pp. 73–112.
8. Marrack P, Scott-Browne JP, Dai S, Gapin L, Kappler JW (2008) Evolutionarily conserved amino acids that control TCR-MHC interaction. Annu Rev Immunol 26: 171-203.
9. Chapman HA (1998) Endosomal proteolysis and MHC class II function. Curr Opin Immunol 10: 93-102.
10. Watts C (2004) The exogenous pathway for antigen presentation on major histocompatibility complex class II and CD1 molecules. Nat Immunol 5: 685-692.
11. Neefjes J, Jongsma ML, Paul P, Bakke O (2011) Towards a systems understanding of MHC class I and MHC class II antigen presentation. Nat Rev Immunol 11: 823-836.
12. Bairoch A, Apweiler R, Wu CH, Barker WC, Boeckmann B, et al. (2005) The Universal Protein Resource (UniProt). Nucleic Acids Res. 33: D154-159.
13. Bairoch A, Boeckmann B, Ferro S, Gasteiger E (2004) Swiss-Prot: juggling between evolution and stability. Brief Bioinform 5: 39-55.
14. Walckenaer CA (1802). Faune parisienne. Insectes. Ou histoire abrégée desinsectes de environs de Paris. Paris: Dentu. Pp. 187-250.
15. Hopp TP, Woods KR (1981) Prediction of protein antigenic determinants from amino acid sequences. Proc Natl Acad Sci U S A 78: 3824-3828.
16. Welling GW, Weijer WJ, van der Zee R, Welling-Wester S (1985) Prediction of sequential antigenic regions in proteins. FEBS Lett 188: 215-218.
17. Parker KC, Bednarek MA, Coligan JE (1994) Scheme for ranking potential HLA-A2 binding peptides based on independent binding of individual peptide side-chains. J Immunol 152: 163-175.
18. Larsen JE, Lund O, Nielsen M (2006) Improved method for predicting linear B-cell epitopes. Immunome Res 2: 2.
19. Kolaskar AS, Tongaonkar PC (1990) A semi-empirical method for prediction of antigenic determinants on protein antigens. FEBS Lett 276: 172-174.
20. Reche PA, Glutting JP, Reinherz EL (2002) Prediction of MHC class I binding peptides using profile motifs. Hum Immunol 63: 701-709.
21. Reche PA, Reinherz EL (2003) Sequence variability analysis of human class I and class II MHC molecules: functional and structural correlates of amino acid polymorphisms. J Mol Biol 331: 623-641.
22. Reche PA, Glutting JP, Zhang H, Reinherz EL (2004) Enhancement to the RANKPEP resource for the prediction of peptide binding to MHC molecules using profiles. Immunogenetics 56: 405-419.
23. Craiu A, Akopian T, Goldberg A, Rock KL (1997) Two distinct proteolytic processes in the generation of a major histocompatibility complex class I-presented peptide. Proc Natl Acad Sci U S A 94: 10850-10855.
24. Pieters J (2000) MHC class II-restricted antigen processing and presentation. Adv Immunol 75: 159-208.
25. Bhasin M, Raghava GP (2004) Analysis and prediction of affinity of TAP binding peptides using cascade SVM. Protein Sci 13: 596-607.
26. Emini EA, Hughes JV, Perlow DS, Boger J (1985) Induction of hepatitis A virus-neutralizing antibody by a virus-specific synthetic peptide. J Virol 55: 836-839.
27. Karplus PA, Schulz GE (1985) Prediction of chain flexibility in proteins: a tool for the selection of peptide antigen. Natur wissen schaften. 72: 212-213.
28. Sweet RM, Eisenberg D (1983) Correlation of sequence hydrophobicities measures similarity in three-dimensional protein structure. J Mol Biol 171: 479-488.
29. Kyte J, Doolittle RF (1982) A simple method for displaying the hydropathic character of a protein. J Mol Biol 157: 105-132.
30. Abraham DJ, Leo AJ (1987) Extension of the fragment method to calculate amino acid zwitterion and side chain partition coefficients. Proteins 2: 130-152.
31. Bull HB, Breese K (1974) Surface tension of amino acid solutions: a hydrophobicity scale of the amino acid residues. Arch Biochem Biophys 161: 665-670.
32. Miyazawa S, Jernigen RL (1985) Estimation of Effective Interresidue Contact Energies from Protein Crystal Structures: Quasi-Chemical Approximation. Macromolecules. 18: 534-552.
33. Roseman MA (1988) Hydrophilicity of polar amino acid side-chains is markedly reduced by flanking peptide bonds. J Mol Biol 200: 513-522.
34. Wolfenden R, Andersson L, Cullis PM, Southgate CC (1981) Affinities of amino acid side chains for solvent water. Biochemistry 20: 849-855.
35. Wilson KJ, Honegger A, Stötzel RP, Hughes GJ (1981) The behaviour of peptides on reverse-phase supports during high-pressure liquid chromatography. Biochem J 199: 31-41.
36. Cowan R, Whittaker RG (1990) Hydrophobicity indices for amino acid residues as determined by high-performance liquid chromatography. Pept Res 3: 75-80.
37. Chothia C (1976) The nature of the accessible and buried surfaces in proteins. J Mol Biol 105: 1-12.
38. Gomase VS (2006) Prediction of antigenic epitopes of neurotoxin Bmbktx1 from Mesobuthus martensii. Curr Drug Discov Technol 3: 225-229.
39. Sherkhane AS, Changbhale SS, Chitlange NR, Waghmare S, Gomase VS, et al. (2012) Prediction of Major Histocompatibility Complex Binding Peptides and Epitopes from Naja naja Cardiotoxin (CTX). Drug Invention Today 4: 435-438.
40. Gomase VS, Kale KV, Chikhale NJ, Changbhale SS (2007) Prediction of MHC binding peptides and epitopes from alfalfa mosaic virus. Curr Drug Discov Technol 4: 117-215.
41. Gomase VS, Chitlange NR, Sherkhane AS, Changbhale SS, Kale KV (2013) Prediction of Wuchereria Bancrofti Troponin Antigenic Peptides: Application in Synthetic Vaccine Design to Counter Lymphatic Filariasis. J Vaccines Vaccin 4: 169.

42. Gomase VS, Shyamkumar K (2009) Prediction of antigenic epitopes and MHC binders of neurotoxin alpha-KTx 3.8 from Mesobuthus tamulus sindicus. African Journal of Biotechnology 8: 6658-6676.

43. Gomase VS, Chitlange NR, Changbhale SS, Kale KV (2013) Prediction of Brugia malayi antigenic peptides: candidates for synthetic vaccine design against lymphatic filariasis. Protein Pept Lett 20: 864-887.