

STUDY OF SOLVENT ACCESSIBILITY OF NEUROTOXIN FROM *Mesobuthus Tamulus*

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ABSTRACT

Mesobuthus tamulus is an Indian red scorpion, which is the most lethal species of the Buthidae family in India. We study the hydrophobicity of amino acid side-chains in peptides are fundamental in understanding protein folding, stability, function, protein-protein interactions and to design synthetic peptide vaccine. Several hydrophobicity scales have been developed for various uses. In this study, we have predicted the binding affinity of neurotoxin having 64 amino acids, which shows antigenic Peptide. These antigenic fragments of the neurotoxin can be use in rational vaccine design and to increase the understanding of roles of the immune system in neurotoxin studies.

Keywords: Neurotoxin, Peptide, Antigen, Surface activity

I. INSTRUCTION

Neurotoxin from *Mesobuthus tamulus* is active peptides with different biological properties that have ability to target specifically neural components and have therapeutic potentials. Highly acidic neurotoxin from scorpion Buthus tamulus acts on nerve cells by interacting with membrane proteins [1,2]. Generally amino acid residues are located in the surface of a protein that serve as active sites and interact with ligands. Solvent accessibility used to identify active site of functionally important residues in membrane proteins and also indicates hydrophobic and hydrophilic characteristics of amino acids and scales are developed for predicting potential antigenic sites of proteins that are rich in charged and polar residues. Solvent-accessible surface areas and backbone angles are continuously varying because proteins can move freely in a three-dimensional space [3-7].

II. MATERIAL AND METHODS

2.1. Antigenic epitope Prediction

Antigenic epitopes are determined by exploitation Bepipred Epitope Prediction. Predictions are on the basis of supported plots that ensure the prevalence of amino acid residues in experimentation notable segmental epitopes [30,31]. The peptide vaccines concept is based on identification and chemical synthesis of B-cell and T-cell epitopes which are immunodominant and can induce specific immune responses.

Deeper knowledge of antigens of neurotoxin from *Mesobuthus tamulus*, mechanisms of immune response and the development of effective and safe adjuvants give hope that the effective peptide vaccines will be developed in the future [32,33].

2.2. Solvent accessible regions and surface accessibility

For the study of solvent accessible regions in proteins different measurement was performed that are useful for the prediction of antigenic activity, surface region of peptides. *Emani et al.*, 1985 [8] predicts the highest probability 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*[9] predict backbone or chain flexibility on the basis of the known temperature B factors of the α -carbons. 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.

2.3. Study of Hydrophobicity of neurotoxin from *Mesobuthus tamulus*

Hydrophobicity scales are developed for the prediction of the protein folding patterns, polar and nonpolar residue with a protein sequence such analysis has the goal of predicting membrane spanning segments hydrophobic or regions that are likely exposed on the surface of proteins (Hydrophilic domain) and therefore potentially antigenic. Hydrophobicity determines where the amino acid will be located in the final structure of the protein. We used hydrophobicity plot of Sweet et al., Kyte & Doolittle, Abraham & Leo, Bull & Breese scale, Guy, Miyazawa et al., Roseman MA, Chothia, Eisenberg et al., Chothia, Eisenberg et al., Manavalan et al., Black, Fauchere et al., Janin, Rao MJK, Tanford, Rose et al., Wolfenden et al., Wilson & et al.

III. RESULTS

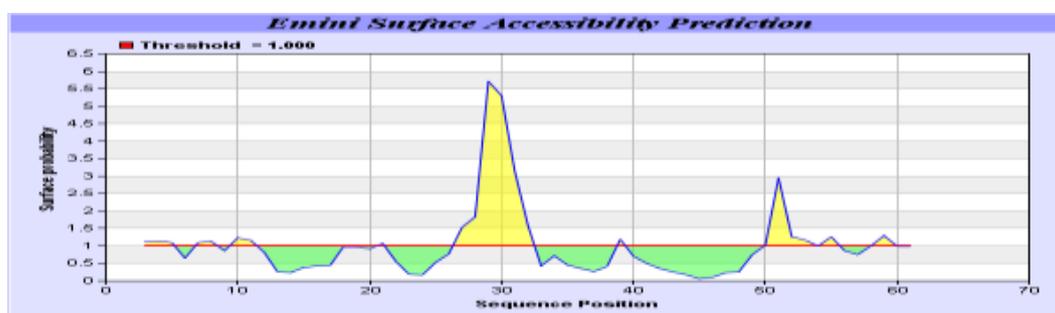
Protein Sequence

Neurotoxin [*Mesobuthus tamulus*]

GEDGYIADGDNCTYICTFNKYCHALCTDKKKGDSGACDWWVPYGVVCWCEDLPTVPPIRGSGKCR

Theoretical pI: 4.47, Mw (average mass): 7040.87Mw (monoisotopic mass): 7036.04

3.1. Emini surface accessibility prediction



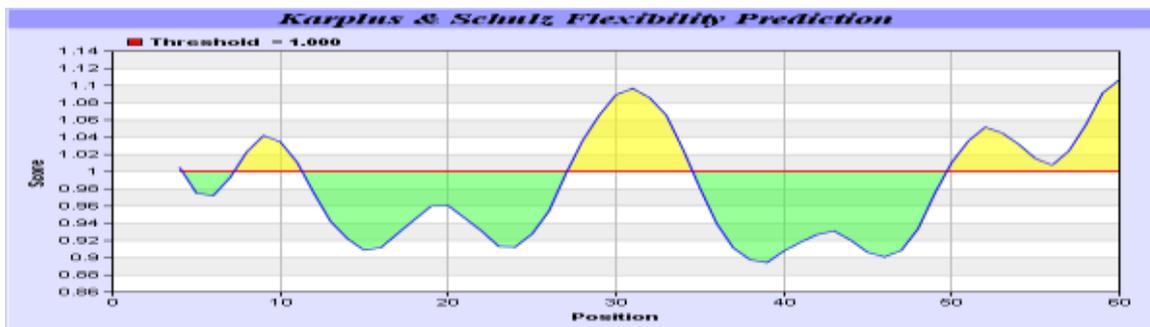
Average: 1.000 Minimum: 0.124 Maximum: 7.808 Threshold: 1.000

Fig-1 Emini surface accessibility prediction revealed an epitope with 6 amino acid residues maximum (7.808) in the sequence positions 27- TDKKGD -32 of neurotoxin from *Mesobuthus tamulus*.

Predicted peptides:

No.	Start Position	End Position	Peptide	Peptide Length
1	27	32	TDKKGD	6

3.2. Karplus-Schulz flexibility prediction



Average: 0.999 Minimum: 0.916 Maximum: 1.084 Threshold: 1.000

Fig-2 Karplus-Schulz prediction of chain flexibility in neurotoxin from *Mesobuthus tamulus* (threshold setting = 1.000) Karplus-Schulz analysis revealed a predicted epitope which shows high score in amino acid 28-DKKGDS-33, 58-RGS-60 (1.084 (maximum))

3.3. Bepired Epitope Prediction

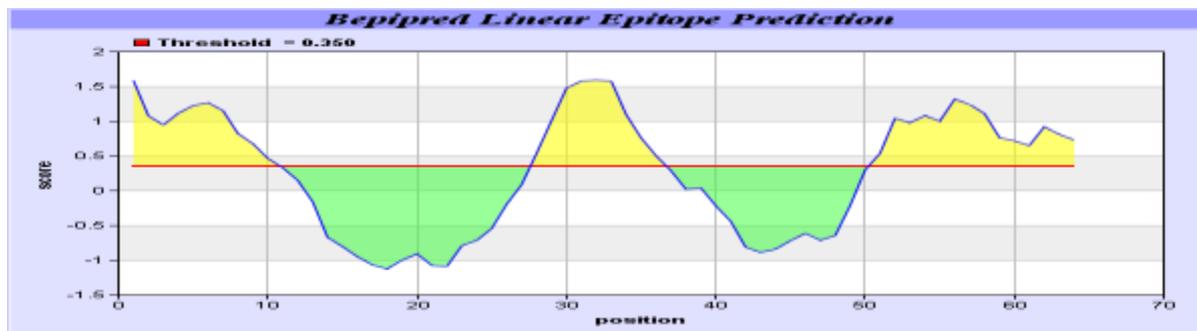


Fig-3 Bepired Epitope Prediction in neurotoxin from *Mesobuthus tamulus* (threshold setting = 0.350) Bepired Epitope Prediction analysis revealed a predicted epitope with 19 amino acid residues in the sequence positions 1- GEDGYIADGD -10, 28-DKKGDSGAC -36.

Table-3 Predicted peptides of neurotoxin from *Mesobuthus tamulus*.

No.	Start Position	End Position	Peptide	Peptide Length
1	1	10	GEDGYIADGD	10
2	28	36	DKKGDSGAC	9

4. Surface activity: Hydrophobicity and Hydrophilicity of neurotoxin from *Mesobuthus tamulus*

Hydrophobic are nonpolar regions located inside of the membrane (buried in the protein core), usually membrane-spanning proteins (hydrophobic) whereas hydrophilic groups are polar, which are located on the

surface of proteins (hydrophilic domains) on the outside of the protein, interacting with the water by hydrogen bonding in the cytosol. Secondary structure of protein depends on the hydrophobic properties of the amino acid residue side chains that are stabilized by non-covalent interactions i.e., hydrophobic, Vander Waals, electrostatic and hydrogen bonding interactions. Interaction between the non-polar amino-acid residues and the aqueous environment provides a strong hydrophobic force for protein folding [10] forming a hydrophobic core in the protein interior. The Calculation of hydrophobicity identifies the location of potential protein features which is antigenic, exposed or the buried residues. The amino acids making up the epitope are usually charged and hydrophilic in nature so their accurate determination of hydrophobicity/ hydrophilicity of amino acid side-chains in peptides are fundamental in understanding protein folding, stability, function, protein-protein interactions and to design synthetic peptide vaccine. Several hydrophobicity scales have been developed for various uses. Sweet *et al.* (1983), Kyte & Doolittle (1982), Abraham & Leo (19987), Bull and Breese (1974), Guy (1985), Miyazawa, *et al.* (1985), Roseman (1988), Chothia (1976), Eisenberg *et al.* (1984), Manavalan, *et al.* (1978), Black (1991), Fauchere, *et al.* (1983), Janin (1979), Rao and Argos (1986), Tanford (1962), Rose *et al.* (1985), Wolfenden *et al.* (1981), Wilson *et al.* (1981) [11-29]. The maximum region of hydrophilicity is to be considered as an antigenic having hydrophobic characteristics. Because the N- and C- terminal region of proteins are usually solvent accessible and antigenic which form antibodies.

Hydrophobicity plot of Sweet *et al.*

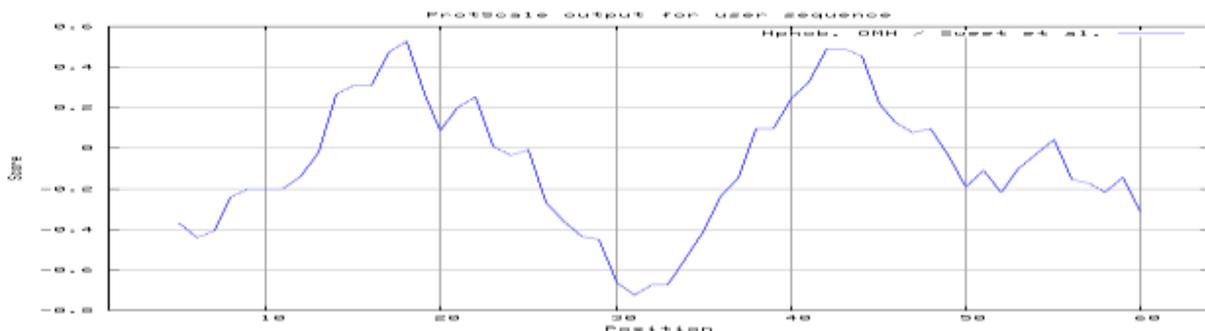


Fig-1Hydrophobicity plot of Sweet *et al.* (1983) of neurotoxin from *Mesobuthus tamulus* shows at position 15- Score: 0.309, Position: 16- Score: 0.309, Position: 17- Score: 0.476, Position: 18 Score: 0.526 (max).

Kyte & Doolittle hydrophobicity plot

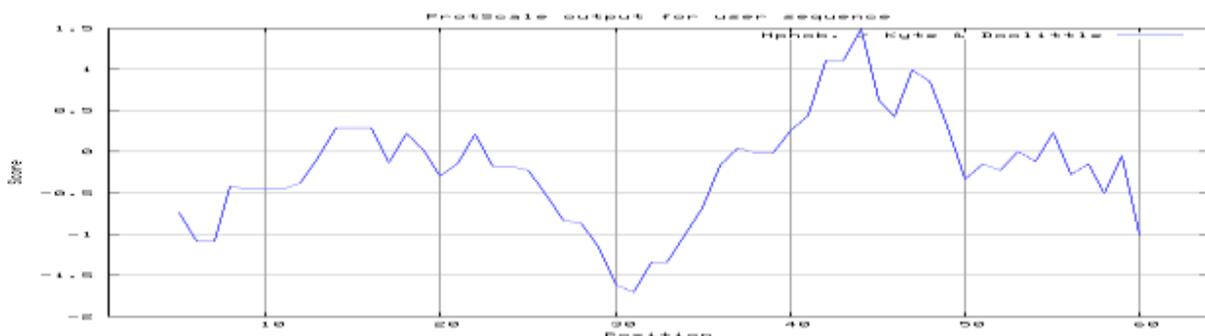


Fig-2Kyte & Doolittle hydrophobicity plot of neurotoxin from *Mesobuthus tamulus* shows at position MAX: 1.614. Regions with value above (0) are hydrophobic in nature. A window size of 5-7 generally works well for

predicting putative surface-exposed regions as well as transmembrane regions. Position: 42- Score: 1.111, Position: 43- Score: 1.111, Position: 44 Score: 1.489 (max).

Abraham & Leo hydrophobicity plot

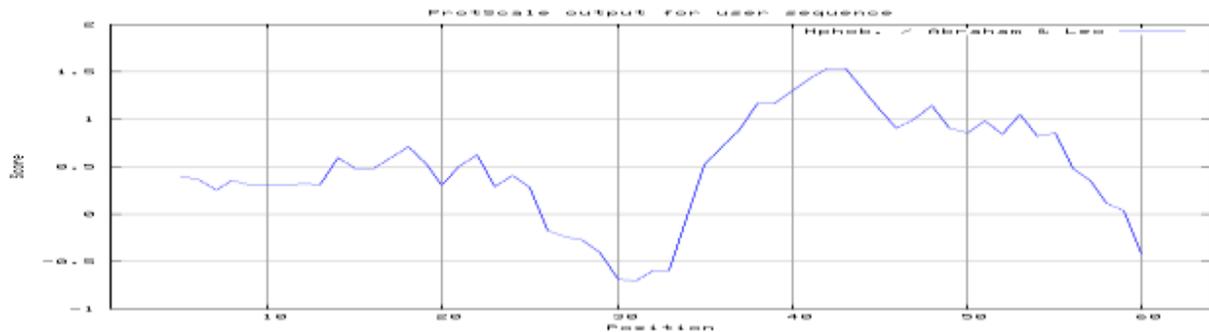


Fig-3Abraham & Leo hydrophobicity plot of neurotoxin from *Mesobuthus tamulus* shows Position: 40 - Score: 1.308,Position: 41- Score: 1.436,Position: 42- Score: 1.534 (max), Position: 43- Score: 1.534 (max), Position: 44- Score: 1.314.

Bull & Breese scale use surface tension to measure hydrophobicity

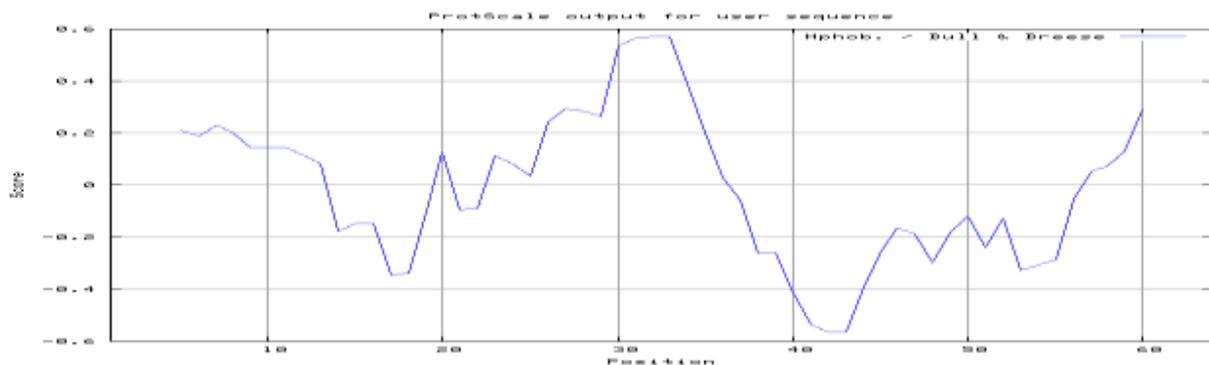


Fig-4Bull & Breese use surface tension to measure hydrophobicity and also uses negative values to describe the hydrophobicity of neurotoxin from *Mesobuthus tamulus*.Position: 30 Score: 0.537, Position: 31- Score: 0.564, Position: 32- Score: 0.572 (max), Position: 33 -Score: 0.572 (max),Position: 34 Score: 0.388.

Hydrophobicity scale of Guy

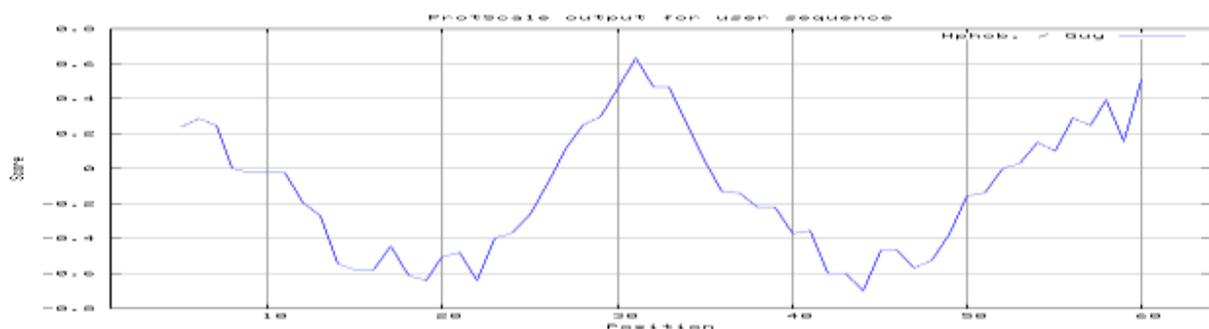


Fig-5Hphob. /Guy. (1985) of neurotoxin from *Mesobuthus tamulus* that shows Position: 30 Score: 0.466, Position: 31- Score: 0.634 (max), Position: 32 Score: 0.469,Position: 33 Score: 0.469.

Hydrophobicity plot of Miyazawa et al.

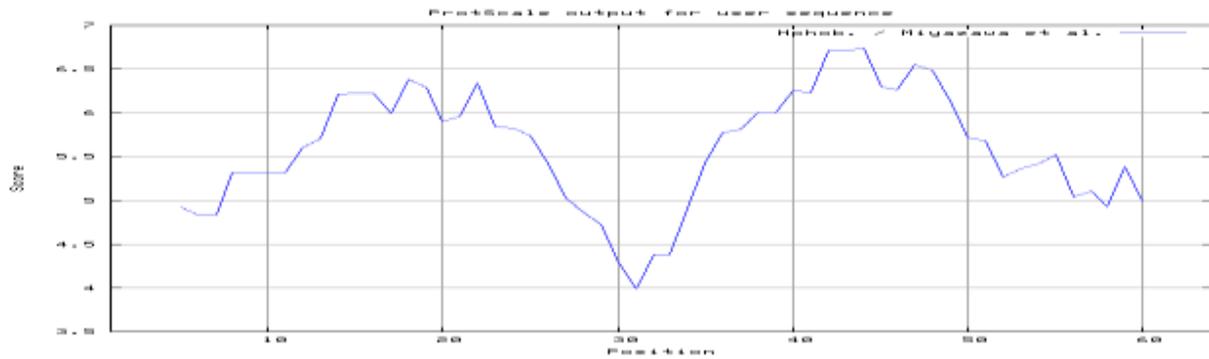


Fig-6Hydrophobicity plot of Miyazawa *et al.* (1985) of neurotoxin from *Mesobuthus tamulus* which shows high score at Position: 40 Score: 6.260,Position: 41 Score: 6.227,Position: 42 Score: 6.709,Position: 43 Score: 6.709,Position: 44 Score: 6.739 (max),Position: 45 Score: 6.297,Position: 46 Score: 6.266,Position: 47 Score: 6.552,Position: 48 Score: 6.484,Position: 49 Score: 6.136.

Hydrophobicity plot of Roseman MA

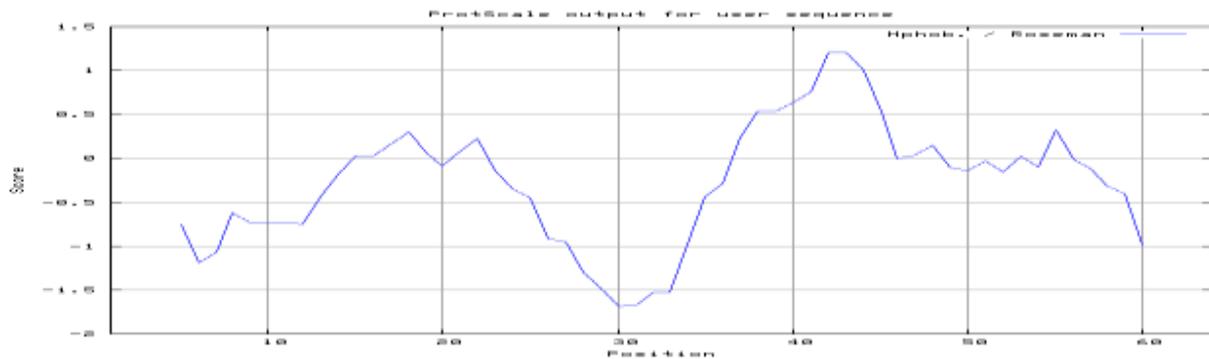


Fig-7Hydrophobicity plot of Roseman MA (1988) of neurotoxin from *Mesobuthus tamulus* which shows high score at Position: 38 Score: 0.539, Position: 39 Score: 0.539, Position: 40 Score: 0.640, Position: 41 Score: 0.757, Position: 42 Score: 1.208 (max), Position: 43 Score: 1.208 (max), Position: 44 Score: 0.999, Position: 45 Score: 0.531.

Hydrophobicity plot of Chothia

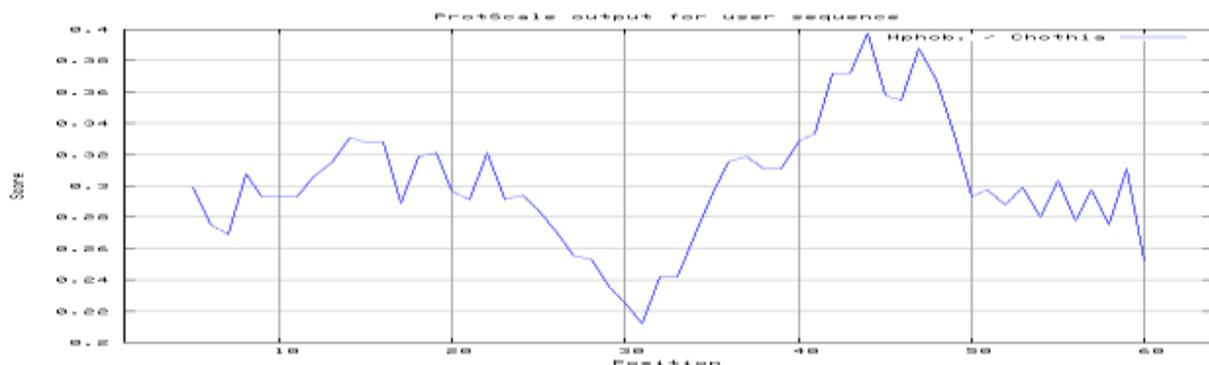


Fig-8Hphob. /Chothia. (1976) of neurotoxin from *Mesobuthus tamulus* which shows high score at Position: 42 Score: 0.372, Position: 43 Score: 0.372, Position: 44 Score: 0.398 (max),Position: 45 Score: 0.358,Position: 46 Score: 0.354,Position: 47 Score: 0.388 Position: 48 Score: 0.368

Hydrophobicity plot of Eisenberg *et al.*

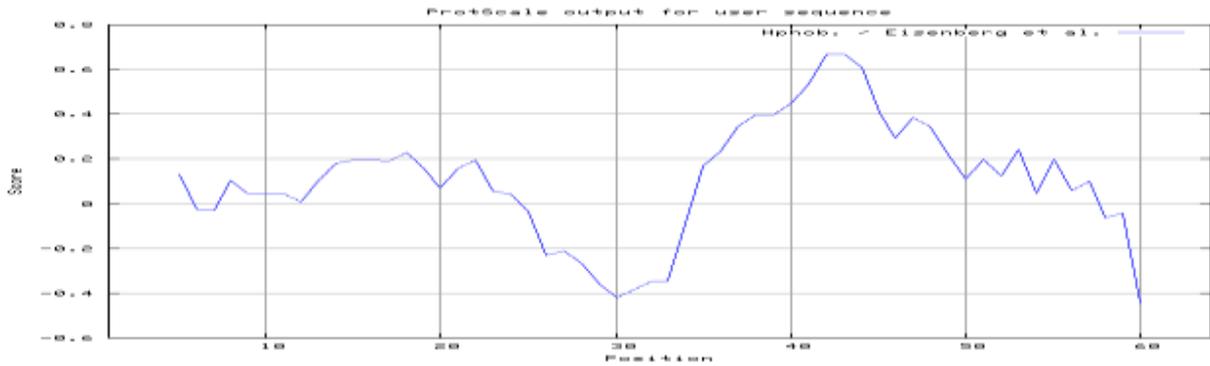


Fig-9Hydrophobicity plot of Eisenberg *et al.* (1984) of neurotoxin from *Mesobuthus tamulus* which shows high score at Position: 40 Score: 0.448,Position: 41 Score: 0.536,Position: 42 Score: 0.668 (max),Position: 43 Score: 0.668 (max),Position: 44 Score: 0.610, Position: 45 Score: 0.408

Hydrophobicity plot of Manavalan *et al.*

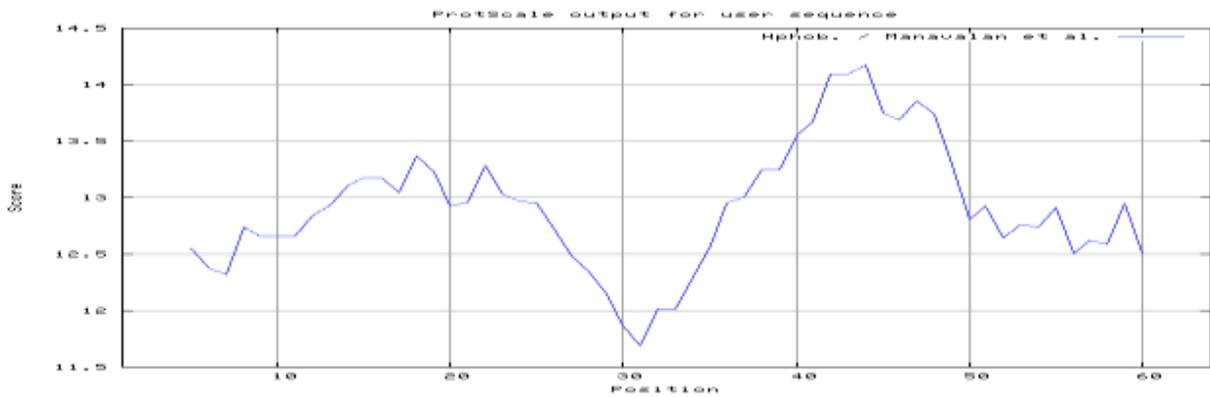


Fig-10Hydrophobicity plot of Manavalan *et al.* (1978) of neurotoxin from *Mesobuthus tamulus* which shows high score at Position: 41 Score: 13.673,Position: 42 Score: 14.093,Position: 43 Score: 14.093,Position: 44 Score: 14.171 (max),Position: 45 Score: 13.747,Position: 46 Score: 13.689,Position: 47 Score: 13.853,Position: 48 Score: 13.736.

Hydrophobicity plot of Black

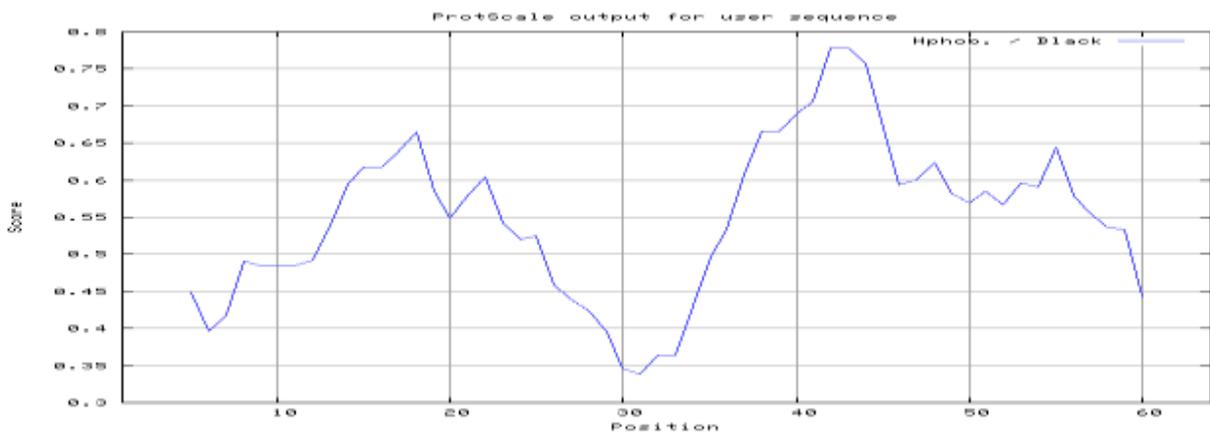


Fig-11 Hphob. /Black. (1991) of neurotoxin from *Mesobuthus tamulus* which shows high score at Position: 38 Score: 0.666, Position: 39 Score: 0.666, Position: 40 Score: 0.690, Position: 41 Score: 0.706, Position: 42 Score: 0.778 (max), Position: 43 Score: 0.778 Position: 44 Score: 0.756, Position: 45 Score: 0.669

Hydrophobicity plot of Fauchereet al.

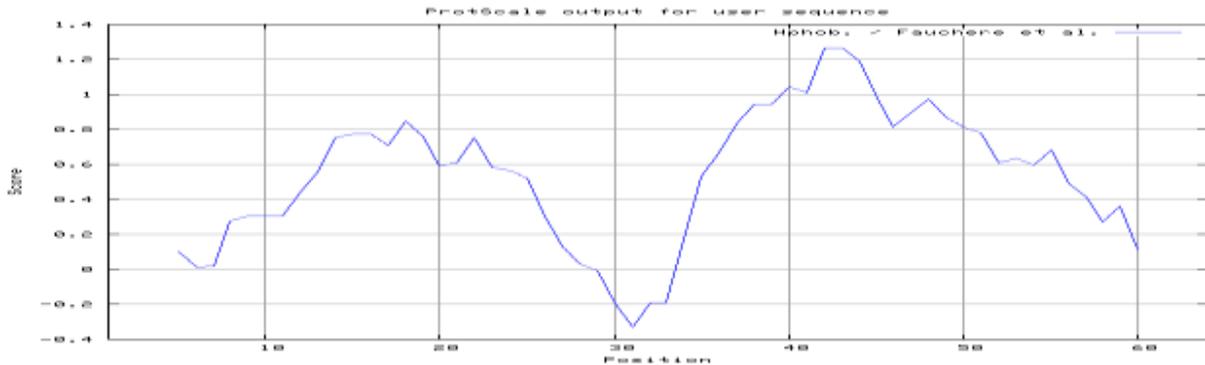


Fig-12 Hydrophobicity plot of Fauchereet al. (1983) of neurotoxin from *Mesobuthus tamulus* which shows high score at Position: 40 Score: 1.043, Position: 41 Score: 1.008, Position: 42 Score: 1.264 (max), Position: 43 Score: 1.264 (max), Position: 44 Score: 1.186 Position: 45 Score: 0.979.

Hydrophobicity plot of Janin

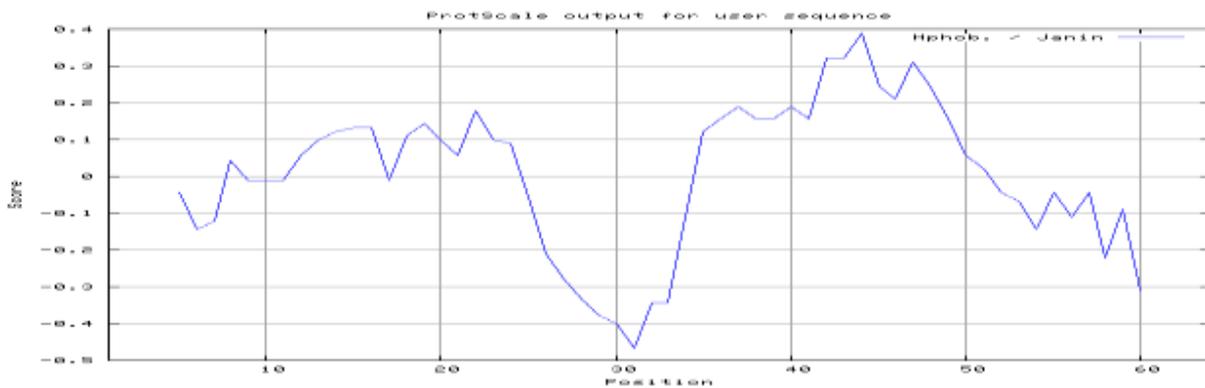


Fig-13 Hydrophobicity plot of Janin J (1979) of neurotoxin from *Mesobuthus tamulus* based on the accessible and buried amino acid residues of proteins which shows high score at Position: 42 Score: 0.322, Position: 43 Score: 0.322, Position: 44 Score: 0.389 (max), Position: 45 Score: 0.244, Position: 46 Score: 0.211, Position: 47 Score: 0.311, Position: 48 Score: 0.244.

Hydrophobicity plot of Rao MJK

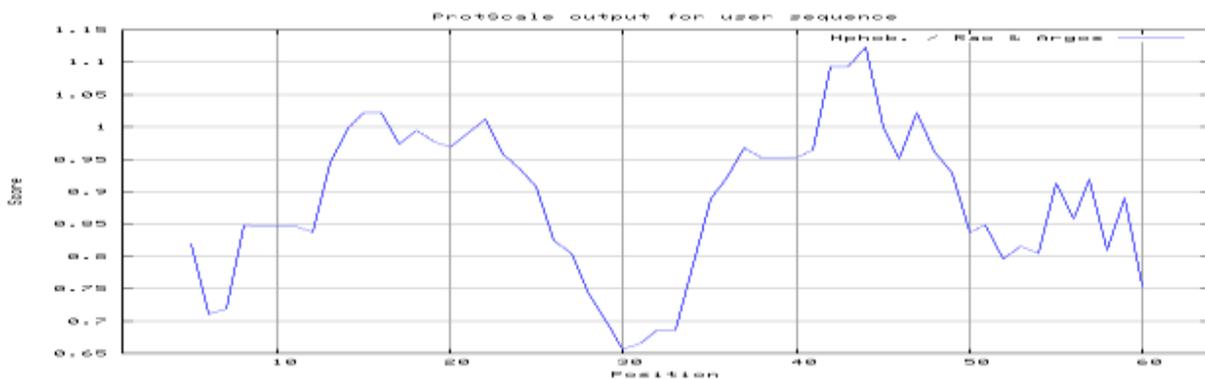


Fig-14 Hydrophobicity plot of Rao MJK (1986) of neurotoxin from *Mesobuthus tamulus* which shows high score at Position: 42 Score: 1.093, Position: 43 Score: 1.093, Position: 44 Score: 1.123 (max), Position: 45 Score: 0.999.

Hydrophobicity plot of Tanford

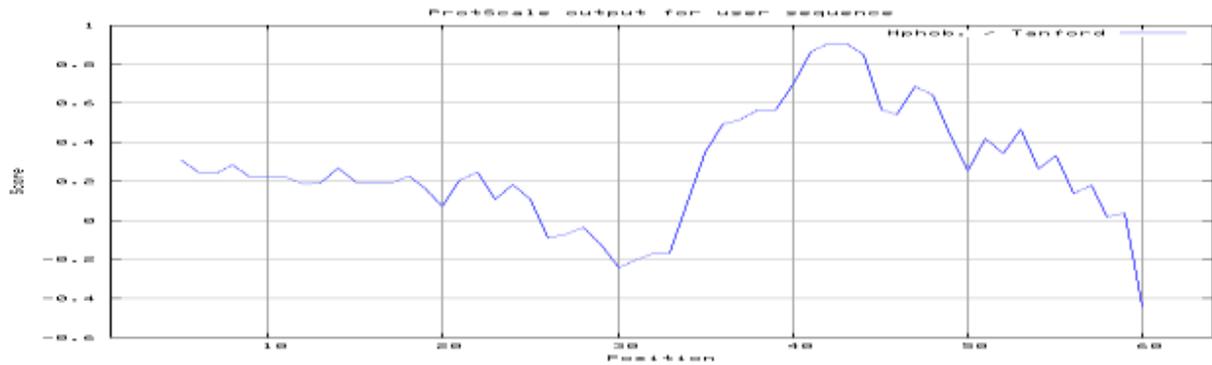


Fig-15 Hydrophobicity plot of Tanford (1962) of neurotoxin from *Mesobuthus tamulus* which shows high score at Position: 41 Score: 0.866, Position: 42 Score: 0.908, Position: 43 Score: 0.908 (max), Position: 44 Score: 0.850.

Hydrophobicity scale of Rose et al.

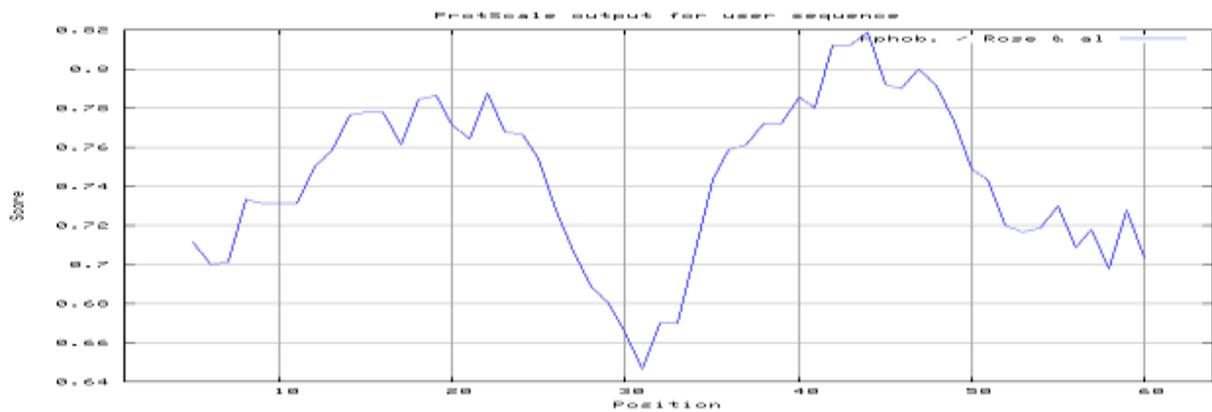


Fig-16 Hphob, Rose & al. (1985) of neurotoxin from *Mesobuthus tamulus* which shows high score at Position: 42 Score: 0.812, Position: 43 Score: 0.812, Position: 44 Score: 0.819 (max), Position: 45 Score: 0.792, Position: 46 Score: 0.790.

Hydrophobicity plot of Wolfenden et al.

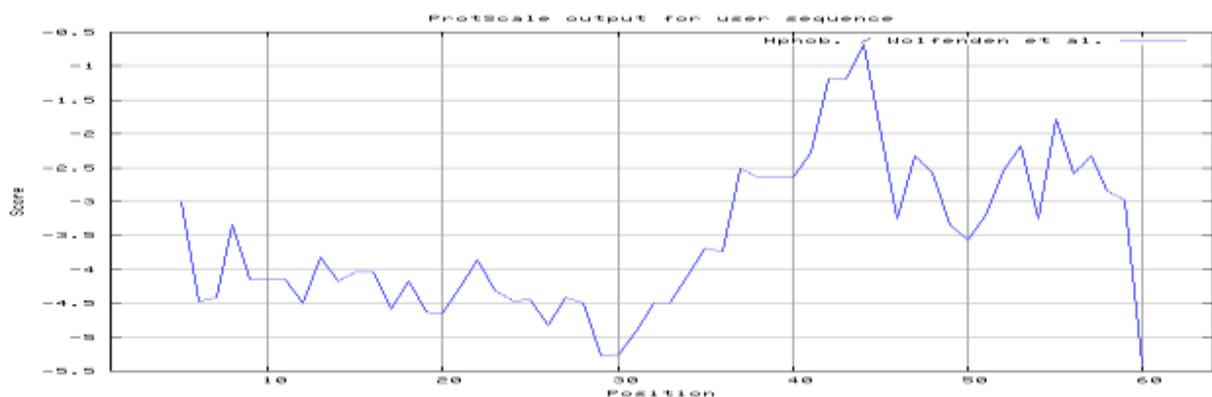


Fig-17 Hydrophobicity plot of Wolfenden *et al.* (1981) of neurotoxin from *Mesobuthus tamulus*

Hydrophobicity/HPLC plot of Wilson & *et.al*

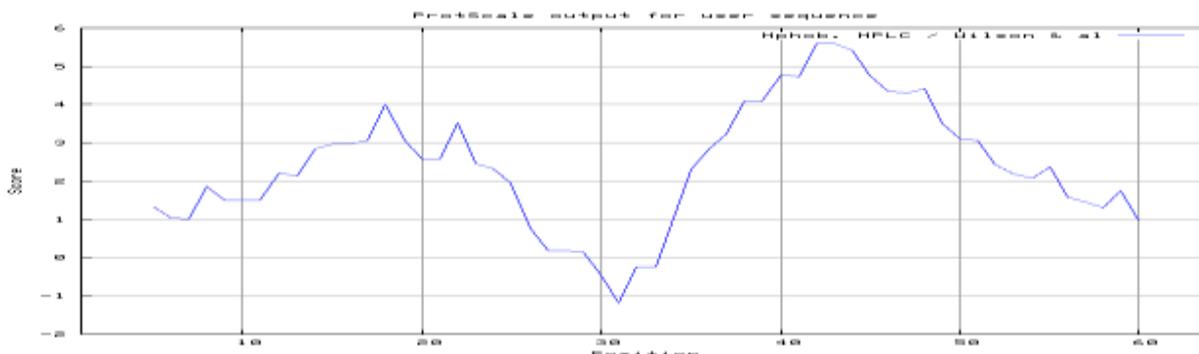


Fig-18 Hydrophobicity/HPLC plot of Wilson & *et.al* (1981) of neurotoxin from *Mesobuthus tamulus* which shows high score at Position: 40 Score: 4.778, Position: 41 Score: 4.733, Position: 42 Score: 5.589 (max), Position: 43 Score: 5.589 (max), Position: 44 Score: 5.411, Position: 45 Score: 4.756.

IV. RESULT INTERPRETATION & DISCUSSION

Antigenic determinants of neurotoxin from *Mesobuthus tamulus* are determined by finding the area of greatest local hydrophobicity using above methods. This method has a high success rate than other methods. The success of this method is its cautious approach to charge interactions that gives equal weight to positive and negative charged residues, whereas other methods tend to favor one or the other. The sites chosen by this method is to be highly exposed and charged regions of the protein's surface therefore, have ample opportunity to contact other proteins. This prediction revealed an epitope with 6 amino acid residues maximum (7.808) in the sequence positions 27- TDKKGD -32 of neurotoxin from *Mesobuthus tamulus*. And Karplus and Schulz chain flexibility in neurotoxin from *Mesobuthus tamulus* analysis revealed a predicted epitope with 9 amino acid residues in the sequence positions 28- DKKGDS-33, 58-RGS-60 (1.084 (maximum) (Fig-1,2,3) to predict membrane-spanning domains. We also found surface activity i.e., hydrophobic and hydrophilic regions that are likely exposed on the protein surface (Fig-1- 18) by Sweet *et al.* (1983), which shows at position 15- 18 Score: 0.526 (max). Kyte & Doolittle (1982) shows the position of MAX: 1.489. Regions with value above (0) are hydrophobic in nature. A window size of 5-7 generally works well for predicting putative surface-exposed regions as well as transmembrane regions. 42-44 Score: 1.489 (max). Abraham & Leo (19987) show the Position 40 - 42- 1.534 (max), Position: 43- 44. Bull and Breese shows 30- 32- Score: 0.572 (max), 33 - 34. , Guy (1985) shows: 30 - 31- Score: 0.634 (max), 32- 33. Miyazawa, *et al* (1985) which shows high score at Position: 40 - 44 Score: 6.739 (max), 45- 49. Roseman (1988) which shows high score at Position: 38- 43 Score: 1.208 (max), 44- 45, Eisenberg *et al.* (1984) 40- 42 Score: 0.668 (max), Position: 43- 44 , Manavalan, *et al* (1978) which shows high score at Position: 41- 44 Score: 14.171 (max), 45- 48, Black (1991) which shows high score at Position: 38- 42 Score: 0.778 (max), Position: 43-45 Score: 0.669, Fauchere, *et al* (1983) 40 Score: 1.043, Position: 41 Score: 1.008, Position: 42 Score: 1.264 (max), Position: 43 Score: 1.264 (max), Position: 44 Score: 1.186 Position: 45 Score: 0.979, Rao and Argos (1986) which shows high score at Position: 42 Score: 1.093, Position: 43 Score: 1.093, Position:

44 Score: 1.123 (max),Position: 45 Score: 0.999, Tanford (1962) shows high score at Position: 41
Score: 0.866,Position: 42 Score: 0.908,Position: 43 Score: 0.908 (max),Position: 44 Score: 0.850,
Rose *et al.* (1985) which shows high score at Position: 42 Score: 0.812,Position: 43 Score:
0.812,Position: 44 Score: 0.819 (max),Position: 45 Score: 0.792,Position: 46 Score: 0.790,
Wolfenden *et al.* (1981). ProtScale only provides a raw signal. Hydropathy scale is a physicochemical property
that quantifies the hydrophobicity of an amino acid. A window size is suggested to be 7-9 residues for predicting
surface sites. The most of used scales are hydrophobicity scales which are derived on the basis of experimental
studies on partitioning of peptides in apolar and polar solvents to predict membrane-spanning segments that are
highly hydrophobic and secondary structure conformational parameter scales. The maximum region of
hydrophilicity is to be considered as an antigenic site having hydrophobic characteristics.

V. CONCLUSION

Peptide fragments of neurotoxin from *Mesobuthus tamulus* involved multiple antigenic components to direct
and empower the immune system to protect the host. From the above result it is concluded that Antigenicity
methods predict the location of antigenic determinants neurotoxin from *Mesobuthus tamulus* that are antigenic
by eliciting an antibody response. Hence, the region spanning the sequence positions will be of greater
importance for epitope-based vaccine design. The amino acids making up the epitope are usually charged and
hydrophilic in nature. From the study of physicochemical properties it was found that, the region of maximal
hydrophilicity is likely to be antigenic site, having hydrophobic characteristics because c- terminal region of
neurotoxin from *Mesobuthus tamulus* is solvent accessible. The mobility of protein segments those are located
on the surface of a protein due to an entropic energy potential which seem to correlate well with known
antigenic determinants. These antigenic peptides can be used as their identifiers. Therefore, these antigenic
determinants are also important for synthetic peptide vaccine production.

Conflict on Interest - None

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Table-1 Emini Surface Accessibility Prediction Result Data Average: Average: 1.000 Minimum: 0.059 Maximum: 5.710

Position	Residue	Peptide start position	Peptide end position	Peptide	Score
27	T	25	30	LCTDKK	1.527
28	D	26	31	CTDKKG	1.833
29	K	27	32	TDKKGD	5.710 (max)
44	V	42	47	YGVVCW	0.173
45	V	43	48	GVVCWC	0.059 (min)

Table-2 Karplus& Schulz Flexibility Prediction Result Data Average: Average: 0.980 Minimum: 0.894 Maximum: 1.107

Position	Residue	Peptide start position	Peptide end position	Peptide	Score
38	W	35	41	ACDWWVP	0.898
39	W	36	42	CDWWVPY	0.894 (min)
40	V	37	43	DWWVPYG	0.908
59	G	56	62	PIRGSGK	1.091
60	S	57	63	IRGSGKC	1.107 (max)