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ANTIGENIC DETERMINANTS OF NEUROTOXIN FROM MESOBUTHUS TAMULUS: NEW APPROACH FOR SYNTHETIC PEPTIDE VACCINES

Smruti S. Changbhale[1], Sonu Mishra[2], Virendra Gomase[3]

[1]*The Global Open University, Dimapur, Nagaland, (India)*

[2,3]*Department of Biotechnology, Mewar University, Chittorgarh, Rajasthan, (India)*

ABSTRACT

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. These disadvantages led to the development of subunit vaccines, including synthetic peptides as antigen, which consist of a specific part of the whole antigen which has been demonstrated to stimulate an immune response by eliciting antibodies that neutralize the biological activity of proteins.*

Keywords: neurotoxin, peptide, Antigenicity

I. INTRODUCTION

Neurotoxin from *Mesobuthus tamulus*are characterized to envision the antigenicity and solvent accessible regions that allows potential drug targets to identify active sites against a versions reactions. Prediction of antigenicity predicts those segments within neurotoxin that are antigenic by eliciting an antibody response[11-20]. Antigenic peptides should be located in solvent accessible regions and contain each hydrophobic and hydrophilic residue which believed that majority surface exposed regions of a protein are potential antigenic. Prediction of peptides those are in the N- and C-terminal region of the protein, because the N- and C- terminal regions of proteins are usually solvent accessible and unstructured hence Antibodies against those regions are also likely to recognize the native protein that can help to design of synthetic peptide vaccine and immuno-diagnostic reagents [20-32].

II. MATERIAL AND METHODS

Antigenic epitopes are determined by exploitation the Hopp and Woods, Welling, Parker, Kolaskar, Tongaonkar antigenicity method and Bepipred Epitope Prediction [**1-5**]. Predictions are on the basis of supported plots that ensure the prevalence of amino acid residues in experimentation notable segmental epitopes [6-10].

III. RESULTS

3.1. Protein Sequence

Neurotoxin [*Mesobuthus tamulus*]

GEDGYIADGDNCTYICTFNNYCHALCTDKKGDSGACDWWVPYGVVCWCEDLPTPV PIRGSGKCR

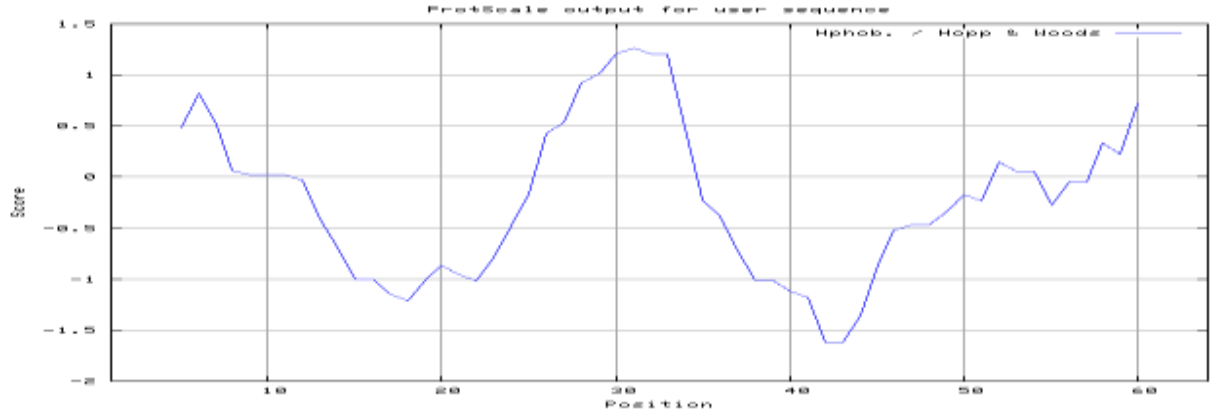
Theoretical pI: 4.47

Mw (average mass): 7040.87

Mw (monoisotopic mass): 7036.04

3.2. Hopp and Woods antigenicity methods

Hopp and woods method predicts antigenic determinants by searching protein sequences of neurotoxin from *Mesobuthus tamulus*to find the area of greatest local hydrophilicity and the hydrophilic regions in the protein are located on the surface and are potentially antigenic. The point of highest local average hydrophilicity is located in or adjacent to an antigenic determinant. In this scale the amino acid value is starting from -3 (most hydrophobic) to 3 (most hydrophilic).

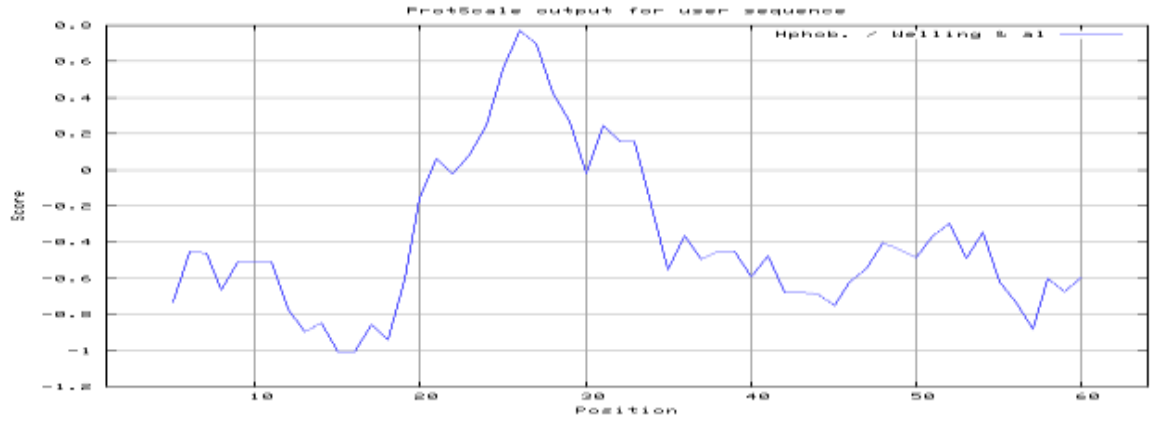


Min: -0.857, Max: 1.243 score at (position 35-36, 61-63) window-7

Fig-1 Hopp and Woods antigenicity plot of neurotoxin from *Mesobuthus tamulus*. In this scale the amino acid value is starting from -3 are consider (most hydrophobic) to 3 (most hydrophilic). The values greater than 0 are consider to be hydrophilic that are exposed on the surface of the folded protein.

3.3. Welling antigenicity methods

Welling antigenicity method is based on the percentage of each amino acid present in known antigenic regions (neurotoxin from *Mesobuthus tamulus*) compared to the percentage of the amino acids in the average composition of a protein. Previous strategies are based on the assumption that antigenic regions are primarily hydrophilic at the surface of the protein. This method is better than the Hopp-Woods scale of hydrophobicity which is also used to identify antigenic regions.

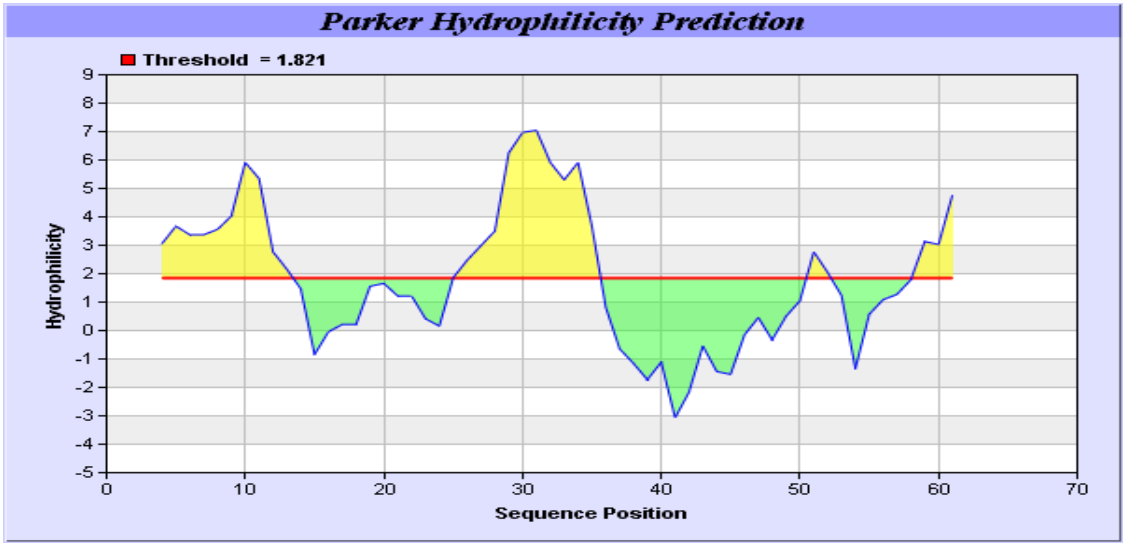


Min: -1.075, Max: 0.231 score at (position 23-24, 38-40) window-11

Fig-2 Welling antigenicity plot of neurotoxin from *Mesobuthus tamulus*. The Max: 0.231 score which was shown at the (Position: 25- Score: 0.557, Position: 26 Score: 0.771 (max), Position: 27- Score: 0.700, Position: 28 Score: 0.426).

3.4. Parker Hydrophilicity Prediction

Parker scale predicts antigenicity by identifying regions of greatest native hydrophilicity of neurotoxin from *Mesobuthus tamulus*. It was derived from the Hopp-Woods scale however, these uses the HPLC retention times of model peptides to determine hydrophilicity. Parker hydrophilicity scale is sequence-based method that has been shown recently to perform prediction of linear epitopes of neurotoxin from *Mesobuthus tamulus*.



Average: 1.821 Minimum: -3.071 Maximum: 7.043

Fig-3 Parker antigenicity plot of neurotoxin from *Mesobuthus tamulus*hydrophilic scale based(threshold setting = 1.678). Parker antigenicity scale predicted 28 length peptides this scale predicted maximum score at position 26-KKGDSG-31, 9-ODN-11 under the threshold value 1.821.

Table 1-Parker Hydrophilicity Prediction Result Data Average: *1.821* ***Minimum:*** *-3.071* ***Maximum:*** *7.043*

Position	Residue	Peptide start position	Peptide end position	Peptide	Score
4	**G**	1	7	GED**G**YIA	3.057
5	**Y**	2	8	EDG**Y**IAD	3.671
6	**I**	3	9	DGY**I**ADG	3.371
7	**A**	4	10	GYI**A**DGD	3.371
8	**D**	5	11	YIA**D**GDN	3.557
9	**0**	6	12	IAD**G**DNC	4.029
10	**D**	7	13	ADG**D**NCT	5.914
11	**N**	8	14	DGD**N**CTY	5.343
12	**C**	9	15	GDN**C**TYI	2.771
13	**T**	10	16	DNC**T**YIC	2.157
14	**Y**	11	17	NCT**Y**ICT	1.471
15	**I**	12	18	CTY**I**CTF	-0.843
16	**C**	13	19	TYI**C**TFN	-0.043
17	**T**	14	20	YIC**T**FNN	0.214
18	**F**	15	21	ICT**F**NNY	0.214
19	**N**	16	22	CTF**N**NYC	1.557
20	**N**	17	23	TFN**N**YCH	1.657
21	**Y**	18	24	FNN**Y**CHA	1.214
22	**C**	19	25	NNY**C**HAL	1.214
23	**H**	20	26	NYC**H**ALC	0.414
24	**A**	21	27	YCH**A**LCT	0.157
25	**L**	22	28	CHA**L**CTD	1.857
26	**C**	23	29	HAL**C**TDK	2.471
27	**T**	24	30	ALC**T**DKK	2.986
28	**D**	25	31	LCT**D**KKG	3.500
29	**K**	26	32	CTD**K**KGD	6.243

30	**K**	27	33	TDK**K**GDS	6.971
31	**G**	28	34	DKK**G**DSG	7.043 (maximum)
32	**D**	29	35	KKG**D**SGA	5.914
33	**S**	30	36	KGD**S**GAC	5.300
34	**G**	31	37	GDS**G**ACD	5.914
35	**A**	32	38	DSG**A**CDW	3.671
36	**C**	33	39	SGA**C**DWW	0.814
37	**D**	34	40	GAC**D**WWV	-0.643
38	**W**	35	41	ACD**W**WVP	-1.157
39	**W**	36	42	CDW**W**VPY	-1.729
40	**V**	37	43	DWW**V**PYG	-1.114
41	**P**	38	44	WWV**P**YGV	-3.071 (minimum)
42	**Y**	39	45	WVP**Y**GVV	-2.171
43	**G**	40	46	VPY**G**VVC	-0.543
44	**V**	41	47	PYG**V**VCW	-1.443
45	**V**	42	48	YGV**V**CWC	-1.543

3.5. Kolaskar&Tongaonkar Antigenicity

Kolaskar&Tongaonkar Antigenicity is a semi-empirical method for the prediction of antigenic regions including information of surface accessibility and flexibility. The method was able to predict antigenic determinants with an accuracy of 75%.

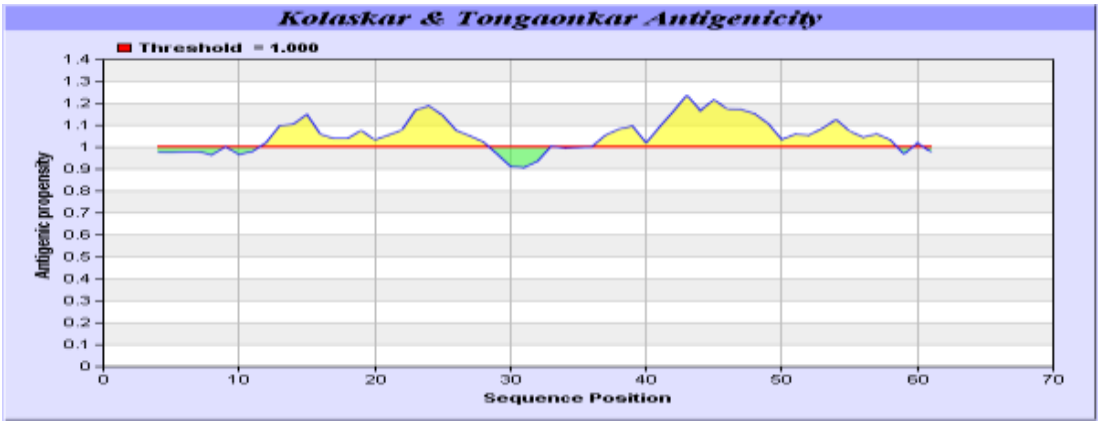


Fig-4 Kolaskar and Tongaonkar antigenicity prediction plot of neurotoxin from *Mesobuthus tamulus* predicts those segments among a protein sequence that are to be antigenic by eliciting an antibody response (threshold setting = 1.000).This scale predicted a 40 length peptide in the positions 12-CTYICTFNNYCHALCTD –28, 36-CDWWVPYGVVCWCEDLPTPVPIR -58

Table -2*Predicted peptidesof* neurotoxin from *Mesobuthus tamulus.*

No.	Start Position	End Position	Peptide	Peptide Length
1	12	28	CTYICTFNNYCHALCTD	17
2	36	58	CDWWVPYGVVCWCEDLPTPVPIR	23

Table -3*Kolaskar&Tongaonkar Antigenicity Result Data* **Average:** 1.056 **Minimum:** 0.907 **Maximum:** 1.237

Position	Residue	Peptide start position	Peptide end position	Peptide	Score
4	**G**	1	7	GED**G**YIA	0.977
5	**Y**	2	8	EDG**Y**IAD	0.976
6	**I**	3	9	DGY**I**ADG	0.980
7	**A**	4	10	GYI**A**DGD	0.980
8	**D**	5	11	YIA**D**GDN	0.966
9	**G**	6	12	IAD**G**DNC	1.001
10	**D**	7	13	ADG**D**NCT	0.967
11	**N**	8	14	DGD**N**CTY	0.981
12	**C**	9	15	GDN**C**TYI	1.021
13	**T**	10	16	DNC**T**YIC	1.098
14	**Y**	11	17	NCT**Y**ICT	1.104
15	**I**	12	18	CTY**I**CTF	1.149
16	**C**	13	19	TYI**C**TFN	1.059
17	**T**	14	20	YIC**T**FNN	1.040
18	**F**	15	21	ICT**F**NNY	1.040
19	**N**	16	22	CTF**N**NYC	1.077
20	**N**	17	23	TFN**N**YCH	1.033
21	**Y**	18	24	FNN**Y**CHA	1.055
22	**C**	19	25	NNY**C**HAL	1.078
23	**H**	20	26	NYC**H**ALC	1.169

24	**A**	21	27	YCH**A**LCT	1.188
25	**L**	22	28	CHA**L**CTD	1.145
26	**C**	23	29	HAL**C**TDK	1.077
27	**T**	24	30	ALC**T**DKK	1.052
28	**D**	25	31	LCT**D**KKG	1.024
29	**K**	26	32	CTD**K**KGD	0.970
30	**K**	27	33	TDK**K**GDS	0.912
31	**G**	28	34	DKK**G**DSG	0.907 (min)
32	**D**	29	35	KKG**D**SGA	0.936
33	**S**	30	36	KGD**S**GAC	1.005
34	**G**	31	37	GDS**G**ACD	0.995
35	**A**	32	38	DSG**A**CDW	0.998
36	**C**	33	39	SGA**C**DWW	1.002
37	**D**	34	40	GAC**D**WWV	1.055
38	**W**	35	41	ACD**W**WVP	1.082
39	**W**	36	42	CDW**W**VPY	1.096
40	**V**	37	43	DWW**V**PYG	1.019
41	**P**	38	44	WWV**P**YGV	1.093
42	**Y**	39	45	WVP**Y**GVV	1.163
43	**G**	40	46	VPY**G**VVC	1.237 (max)
44	**V**	41	47	PYG**V**VCW	1.167
45	**V**	42	48	YGV**V**CWC	1.217

IV. DISCUSSION

Antigenic determinants of neurotoxin from *Mesobuthus tamulus*are determined by finding the area of greatest local hydrophilicity using the Hopp-Woods method. 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. Here we found high peaks at position Position: 30 Score: 1.211, Position: 31Score: 1.267 (max), Position: 32- Score: 1.200, Position: 33 Score: 1.200by using window-7. Welling Method used to locate hydrophilic regions in a protein since, it is assumed that antigenic determinants are located on

surface which contain charged and polar residues. These methods are used to obtain a rough idea for estimation of potentially antigenic regions. However, as shown by Hopp and Woods not all antigenic regions are hydrophilic and not all hydrophilic regions are antigenic. Therefore welling developed a method based on the percentage of each amino acid present in known antigenic determinants compared with the percentage of the amino acids in the average composition of a protein. Here we found the Position: 25- Score: 0.557, Position: 26 Score: 0.771 (max), Position: 27- Score: 0.700, Position: 28 Score: 0.426by using window-11. Parker used three parameters - hydrophilicity, accessibility and flexibility to calculate the antigenic propensity using a composite plot 181. This method has improved to predict antigenic determinants as compared to Hopp and Woods' method. Parker antigenicity plot is based on (threshold setting = 1.678) and this scale predicted maximum score at position 26-KKGDSG-31,9-ODN-1111 under the threshold value 1.821.KolaskarTongaonkar antigenicity methods and predict location of antigenic determinants within neurotoxin from *Mesobuthus tamulus*that are antigenic by eliciting an antibody response. This plot predicts those segments among a protein sequence that are to be antigenic by eliciting an antibody response (threshold setting = 1.000). This scale predicted a 40 length peptide in the positions 12-CTYICTFNNYCHALCTD –28, 36-CDWWVPYGVVCWCEDLPTPVPIR -58, A typical profile show characteristic peaks and troughs, corresponding to the most hydrophobic and most hydrophilic parts of the protein respectively.Different residues which are rankings are commonly used hydrophobicity scales. While the scales differ in detail, there is a general consensus regarding the types of residue that appear at the most hydrophobic end (I, F, L, V and M) and those that appear at the most hydrophilic end (N, Q, E, D and K) (Fig-1 toFig-4). We also find the location in solvent accessible regions in protein by using the hydrophobic scale Emanisurface accessibility.

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*.Hydropathy scale is a physiochemical 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

REFERENCES

[1] Hopp TP and Woods KR. Prediction of protein antigenic determinants from amino acid sequences. Proceedings of the National Academy of Sciences USA.1980;78: 3824-3828.

[2] Welling GW, Weijer WJ, van der Zee R, Welling-Wester S. Prediction of sequential antigenic regions in proteins. FEBS Lett. 1985;188: 215–218.

[3] Parker JMR, Guo D and Hodges RS. New hydrophilicity scale derived from high-performance liquid chromatography retention data: correlation of predicted surface residues with antigenicity and X-ray derived accessible sites. Biochemistry. 1986; 25: 5425.

[4] Kolaskar AS, Tongaonkar PC. A semi-empirical method for prediction of antigenic determinants on protein antigens. FEBS Lett.1990; 276 (1-2): 172-174.

[5] Jens Erik Pontoppidan Larsen, Ole Lund and Morten Nielsen. Improved method for predicting linear B-cell epitopes. Immunome Res. 2006; 2: 2

[6] Senior K. Taking the bite out of snake venom, Lancet.1999; 353.

[7] Ahmad S, Gromiha MM, Sarai A. Real value prediction of solvent accessibility from amino acid sequence. Proteins.2003; 50:629–635.

[8] Emini EA, Hughes JV, Perlow DS, Boger J. Induction of hepatitis a virus-neutralizing antibody by a virus-specific synthetic peptide. J Virol. 1985; 55: 836-839.

[9] Karplus PA and Schulz, GE. Prediction of chain flexibility in proteins: a tool for the selection of peptide antigen. Naturwissenschaften. 1985; 72: 212-213.

[10] Dill KA (1990). Dominant forces in protein folding. Biochemistry. 29:7133–55.

[11] Sweet RM and Eisenberg D. Correlation of sequence hydrophobicities measures similarity in three-dimensional protein structure.J. Mol. Biol.1983; 171: 479–488.

[12] Kyte J, Doolittle RF. A Simple Method for Displaying the Hydropathic Character of a Protein. J. Mol. Biol.1982; 157: 105-132.

[13] Abraham DJ, Leo AJ. Extension of the fragment method to calculate amino acid zwitterions and side chain partition coefficients. Proteins.1987; 2: 130-152.

[14] Bull HB, Breese K. Surface tension of amino acid solutions: A hydrophobicity scale of the amino acid residues. Arch. Biochem. Biophys.1974;161: 665-670.

[15] Guy HR. Amino acid side chain partition energies and distributions of residues in soluble proteins. Biophys. J.1985; 47: 61-70.

[16] Miyazawa S, Jernigen RL. Estimation of Effective Interresidue Contact Energies from Protein Crystal Structures: Quasi-Chemical Approximation. Macromolecules.1985;18: 534-552.

[17] Roseman MA. Hydrophilicity of polar amino acid side-chains is markedly reduced by flanking peptide bonds. J. Mol. Biol. 1988;200: 513-522.

[18] Chothia C. The nature of accessible and buried surfaces in proteins. J. Mol. Biol.1976;105: 1-14.

[19] Eisenberg D, Schwarz E, Komaromy M, Wall R. Analysis of membrane and surface protein sequences with the hydrophobic moment plot. J. Mol. Biol.*1984;*179: 125-142.

[20] Manavalan P, Ponnuswamy PK. Hydrophobic character of amino acid residues in globular proteins. Nature.1978;275: 673-674.

[21] Black SD, Mould DR. Development of Hydrophobicity Parameters to Analyze Proteins Which Bear Post- or Cotranslational Modifications. Anal. Biochem.1991;193: 72-82.

[22] Fauchere JL, Plisk VE. Hydrophobic parameters of amino-acid side-chains from the partitioning of N-acetyl-amino-acid amide. Eur. J. Med. Chem.1983; 18: 369-375.

[23] Janin J. Surface and inside volumes in globular proteins. Nature. 1979; 277: 491-492.

[24] Rao MJK, Argos P. A conformational preference parameter to predict helices in integral membrane proteins. Biochim. Biophys. Acta. 1986; 869: 197-214.

[25] Tanford C. Contribution of hydrophobic interactions to the stability of the globular conformation of proteins. J. Am. Chem. Soc. 1962;84:4240-4274.

[26] Welling GW, Weijer WJ, Van Der Zee R and Welling-Wester S. Prediction of sequential antigenic regions in proteins. FEBS Lett.1985; 188: 215—218.

[27] Rose GD, Geselowitz AR, Lesser GJ, Lee RH, Zehfus MH.Hydrophobicity of amino acid residues in globular proteins. Science.1985;229: 834-838.

[28] Wolfenden RV, Andersson L,Cullis PM, Southgate CCF. Affinities of amino-acid side-chains for solvent water. Biochemistry. 1985;20(4): 849-855.

[29] Wilson KJ, Honegger A, Stotzel RP, Hughes GJ. The behaviour of peptides on reverse-phase supports during high-pressure liquid chromatography. Biochem. J. 1981;199:31-41.

[30] Aguilar JC, Rodríguez EG. Vaccine adjuvants revisited. Vaccine. 2007;25(19): 3752–3762.

[31] Gomase VS, Kale KV, Chikhale NJ, Changbhale SS. Prediction of MHC binding peptides and epitopes from alfalfa mosaic virus. Curr Drug Discov Technol. 2007 Aug;4(2):117-215.

[32] Gomase VS. Prediction of antigenic epitopes of neurotoxin Bmbktx1 from Mesobuthus martensii. Curr Drug Discov Technol. 2006 Sep;3(3):225-9.