INTERNATIONAL JOURNAL OF APPLIED BIOLOGY AND PHARMACEUTICAL TECHNOLOGY

www.ijabpt.com ISSN : 0976-4550

Volume-6, Issue-3, July-Sept-2015 *Coden IJABFP-CAS-USA*

Received: 8th June-2015 *Revised: 22nd July -2015* *Accepted: 22nd July-2015*

Research article

PREDICTION OF ANTIGENIC AND BINDING SITES OF NEUROTOXIN 23 OF SCORPION (*LYCHASMUCRONACTUS SP.*)

Bharati K. Thosare* and Arun G. Ingale

Department of Biotechnology, School of Life Sciences, North Maharashtra University, Jalgaon, Maharashtra, India

Corresponding author: Tel: (0257)2257423 Email:bharati.thosare@gmail.com, agibiotech@gmail.com

ABSTRACT: Identification of antigenic and binding site of protein is highly desirable for the design of vaccines and immunodiagnostics. The present exercise deals with a prediction of antigenic as well as binding sites of neurotoxin 23 of *Lychasmucronactus.* This species of scorpion having diverse molecules of toxic peptide, the peptide neurotoxin 23 is 96 amino acids long of which 23 to 96 specifically code for neurotoxin. The total of 27 such different ligand binding residue were identified by ConSurf and Raptor X server. The web tool Ellipro which implements Modeller and Jmol viewer, predicted and visualized the linear and discontinuous antibody epitopes ofneurotoxin 23 protein sequence.Thus the information discussed here provides a clue for understanding antigenic site and molecular function of neurotoxin 23.

Key **words:** Epitope, Binding residue, Functional site, Ligands, Neurotoxin.

INTRODUCTION

Neurotoxins are well known for their profound physiological effect and enormous potential in bio-medical application. Neurotoxins originated from animal sources are most powerful with maximum mortality rate throughout the world. There are wide group of animals such as snake, scorpion species, spiders which produces potent neurotoxin to kill and immobilize their prey or to protect themselves from the predators. The total number of scorpion stings throughout the world has been estimated as 1000-2000 deaths each year (Karalliedde, 1995). The scorpion venom enhances the excitability of nerve and muscle cells while other has effect on neurons and neuro-transmitter release. Acetylcholine, noradrenaline and serotonin are the main components of scorpion venom. The scorpion bites are also responsible for hypertension by releasing catecholamines, toxic myocarditis, arrhythmias, heart failure and pulmonary edema (Karalliedde, 1995).

Neurotoxin 23 producing species *L ychasmucronatus* is widely distributed in Southeast Asia and Southern China. The neurotoxin 23 is expressed by venom gland, having 96 AA in toxin protein of which 1-22 are signal peptide and 23-96 i.e. 74 AA code specifically for neurotoxin 23 (Zhao et al. 2010).It is a wild toxin protein with very less mutation but yet antigenically and functionally unknown for its molecular function, ligand binding site as well as evoking of immune response due to neurotoxin 23. Functional site detection is important for targeting specific site in structure based drug designing. It causes a better prediction or development of therapeutic agent if we know specific antigen binding site of protein. So in order to translate the genomic information into biological-knowledge and to determine the molecular function neurotoxin 23 at molecular level, binding site as well as to understand the complex antigen antibody binding process, we have predicted the antigen (epitope) binding site of toxin. Furthermore, the emerged information of antigenic and functional site study could be beneficial for various aspects of toxins viz. drug discovery, drug development, experimental toxicology, exploring molecular mechanisms of action and personal health prediction (Bianchi et al. 2013).

MATERIALS AND METHODS

Retrieval of protein sequence:

The protein sequence of the neurotoxin 23 of *Lychasmucronactus* was retrieved from UniProt sequence database. Here we have predicted secondary as well as 3D structure of antigenic epitope binding site and functional site in protein sequence neurotoxin 23. Having taxonomical information: family: Buthidae Genus: Lychas Species: *Lychas Mucronactus.* The length of query protein is 96 amino acid residues. It was ascertained that the information of antigenic and information of functional site of neurotoxin 23 is not available in literature. Hence, the present exercise was undertaken.

Prediction of antigenicity and functional residues:

ConSurfe server (http://consurf.tau.ac.il/) was used to determine conserved and functional residues (Fabian et al. 2003). The default parameters were selected to obtained homologous sequences and functional residues of neurotoxin 23. By using empirical bayesian or maximum likelihood paradigm the ConSurf calculates and evaluates the position specific conservation score. Antigenic epitope is determined using Ellipro which implemented modified version of Thornton's method. Ellipro asked for input of sequence, according to sequence ellipro suggested structural template for homology modeling (Julia et al. 2008). The neurotoxin originated from new world scorpion *Centruroides sculpturatus* PD Bid 1VNA was selected as template of 0.00091 E values. Once template has been selected and submitted to server the Ellipro program predicted continuous as well as discontinuous epitope sequence of neurotoxin 23that are likely to be antigenic by eliciting an antibody response.

RESULT AND DISCUSSION

Conserved site

Identification of functionally important regions in proteins was done by ConSurf. It is an automated web-based tool which detects the evolutionary conserved amino acids and functional site by surface mapping. According to ConSurf 1, 30, 46, 54, 57, 71, 76 are functional residues highly conserved and exposed to environment and 28, 30, 38, 40, 48, 53, 61, 63, 68, 70, 71 are structurally conserved residue shown in figure 1

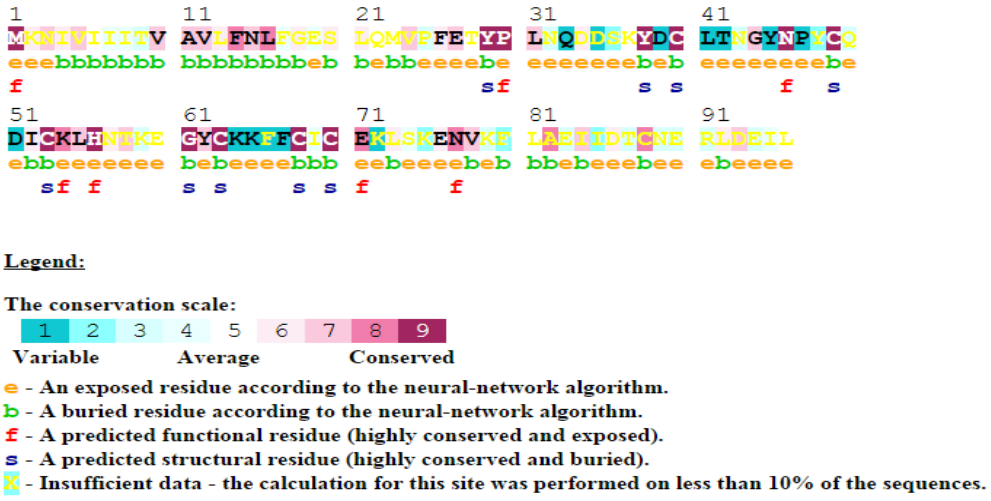

Figure 1: Variable, average and conserved residue of neurotoxin 23 mapped by ConSurf.

Antigenic site

Antigen antibody having a remarkable feature, they having high affinity and strict specificity. It is known that antibody recognize the unique conformation and spatial locations on the surface of antigen. Computational prediction of sequence of amino acid in protein is often crucial in determining the role of protein in biological processes. According to information of sequence the conserved and antigenic site of toxin was predicted. Ellipro implemented the MODELLAR program and Jmol viewer which allows prediction and visualization of antibody epitopes in a given protein sequence or structure (Eswar et. al 2008). Elipro gave 0.732 AUC value, when most significant prediction was consider for query protein.For neurotoxin 23 the Elliproserver predicted 7 linear epitope site and 4 discontinuous epitope sites of which first 3 predicted sites are best predicted sites.

Predicted Linear Epitope(s):

No.	Chain	Start	End	Peptide	Number of residues	Score
1	_	92	96	LDEIL	5	0.76
2	_	14	29	FNLFGESLQMVPFETY	16	0.758
3	_	1	5	MKNIV	5	0.7
4	_	83	89	EIIDTCN	7	0.677
5	_	40	51	CLTNGYNPYCQD	12	0.575
6	_	54	60	KLHNTKE	7	0.56
7	_	63	67	CKKFF	5	0.515

Figure 2: Linear epitopes (amino acid residue) in peptide

Predicted Discontinuous Epitope(s):

No.	Residues	Number of residues	Score
1	_:F17, _:G18, _:E19, _:S20, _:L21, _:Q22, _:M23, _:V24, _:P25, _:F26, _:E27, _:T28, _:Y29	13	0.822
2	_:A82, _:E83, _:I84, _:I85, _:D86, _:T87, _:C88, _:N89	8	0.635
3	_:R91, _:L92, _:D93, _:E94	4	0.589
4	_:M1, _:K2, _:N3, _:I4, _:V5, _:D39, _:C40, _:L41, _:T42, _:N43, _:G44, _:Y45, _:N46, _:P47, _:Y48, _:Q50, _:D51, _:K54, _:L55, _:H56, _:N57, _:T58, _:K59, _:E60, _:C63, _:K64, _:K65, _:F66, _:F67, _:K72	30	0.585

Figure 3: Discontinuous Epitopes (amino acid residue) in peptide

The predicted epitope site and their respective AUS score for linear epitopes is shown in figure 2 and the discontinuous epiotope shown in figure 3. The 7 epitope sites are predicted for linear out of which fist 3 predicted sites are of higher confidence. The 1st epitope site contain 5 amino acid residue of o.76 score, 2nd epitope site contain 16 residues of 0.758, the 3rd contain 5 amino acids of 0.7 score, 4th have 7 amino acids of 0.677 score. The 5th epitope site contain 12 amino acids and 0.575 score, 6thpredicted epitope site contain 7 amino acid in a range with 0.56 score and the last predicted linear epitope is of 5 amino acids 0.515 score. There are 4 discontinuous epitope are predicted by Ellipro of 13, 8, 4 and 30 amino acid containing peptides with 0.822, 0.635, 0.589 and 0.585 score respectively. The predicted linear and discontinuous epitope peptides are shown in 3D structure in figure 4 and 5 respectively.

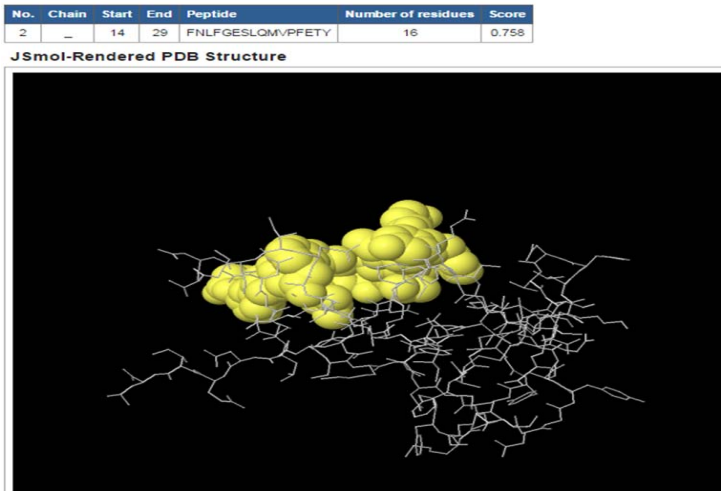

Figure 4: Predicted epitope binding site of neurotoxin 23, this is a 16 amino acid containing peptide from amino acid number14th to 29thof 0.758 AUC score.

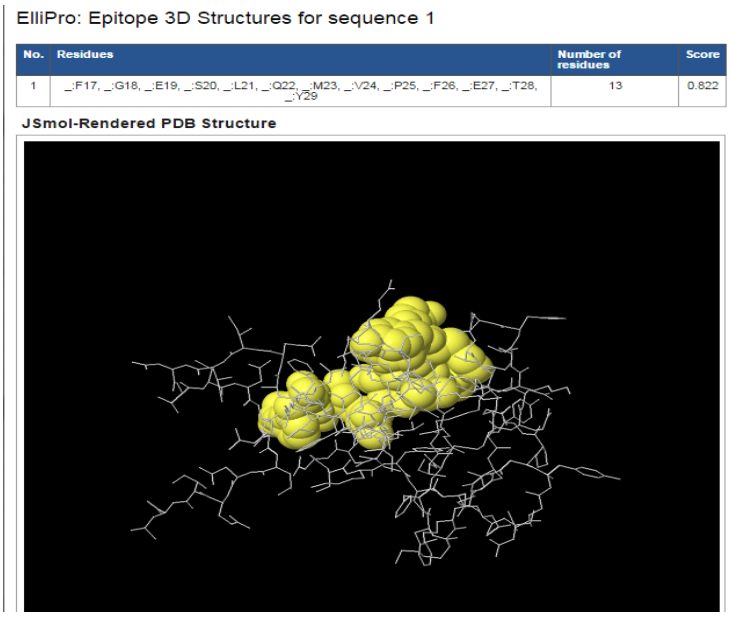

Figure 5: Predicted epitope binding site of neurotoxin 23, the figure showing 1st predicted epitope site contain 13discontinuous amino acid residues of 0.822AUC score.

DISCUSSION

The collection of data and literature of related compound is hard and very time consuming whereas in*silico* tools can give a helping hand to decrease time and hard work. By using bioinformatics tools the data emerged from antigenic site, functional site prediction will give direction for in vitro toxoid development (Tan et al. 2003). For in vitro toxoid development, information of its antigenic, binding or functional residue and information of ligands is necessary. During the development of drug/toxoid against neurotoxin 23 one should know its antigenic and binding sites. Therefore, it is important to gather antigenic and functional information of neurotoxin 23 of *Lychasmucronactus*. Reliable prediction of epitope remains challenging yet highly desirable for the design of vaccines and immunodiagnostics (Kolaskar and Tongaonkar 1990). Antigen antibody having a remarkable feature they having high affinity and strict specificity. For a protein antigen an epitope may be either a short peptide from a protein sequence, called a continuous epitope or a patch of the residues called discontinuous epitope. Continuous epiotope can be directly used for the design of vaccines and immunodiagnostics, so we having a force on prediction of antigenic site of toxin molecules. For determination of antigenic site we have used Ellipro server which is modified version of Thornton's method and together with residue clustering algorithm. The consurf was used to determine biologically functional residue which found to be conserved among the amino acid sequence. This information could be beneficial to recognize unknown amino acid function in sequence from functionally known sequences. The whole information generated from prediction of binding sites, functional residue and 3D structure of neurotoxin 23 will improve chances of development of toxoid.

REFERENCES

Bianchi V, Mangone L. (2013). web PD Binder: a server for the identification of ligand binding sites on protein structures. Nuclieic Acid Res, 41: 308-313.

Eswar N, Eramian D. (2008). Protein structure modeling with MODELLER. *Methods Mol Bio,* 426:145-159.

Glaser F, Pupko T. (2003). ConSurf: Identification of functional region in protein by surface mapping of phylogenetic information. Bioinformatics, 19: 163-164.

Julia Ponomeranko, huynh-Hoa Bui. (2008) Ellipro: a new structure based tool for the prediction of antibody epiotope. BMC Bioinformatics, 9: 514.

Karalliedde L. (1995). Review article animal toxins. British journal of Anasthesia, 74: 319- 327.

Kolaskar AS, Tongaonkar PC. (1990). A semi-empirical method for prediction of antigenic determinants on protein antigens. FEBS Lett, 76:172–174.

Tan T J, Khan A M. (2003). Bioinformatics for venom and toxin science. Briefing in bioinformatics, 4(1): 53-62.

Zhao R, Ma Y. (2010). Comparative venom gland transcriptome analysis of the scorpion *Lychasmucronatus* reveals intraspecific toxic gene diversity and new venomous components. *PMC Genomics*, 11: 452.

ISSN : 0976-4550

INTERNATIONAL JOURNAL OF APPLIED BIOLOGY AND PHARMACEUTICAL TECHNOLOGY

Email : ijabpt@gmail.com

Website: www.ijabpt.com