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Categorizing Molecular Features of Venom Toxins using Bioinformatics tools

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ABSTRACT

Poisonous organisms are represented in many taxa, including kingdom Animalia. During evolution, animals have developed special organs for production and injection of venoms. Animal venoms are complex mixtures, compositions of which depend on species producing venom. The most known and studied poisonous terrestrial animals are snakes, scorpions and snails. Venomous animals produce a myriad of important pharmacological components. The individual components, or venoms (toxins), are used in ion channel and receptor studies, drug discovery, and formulation of insecticides. Knowing the key clinical applications of venom, the present investigation was carried out to understand the molecular basis of venom toxins of animals like snake (L-amino acid oxidases), cone snail (Contulakin-G) and scorpion (Chlorotoxin) by retrieving the protein sequence information, deducing various physicochemical properties, predicting secondary structural elements, homology modelling and depicting the potent antigenic regions using various bioinformatics tools and soft-wares. Because of their remarkable molecular diversity, venoms are key, albeit challenging, resource for pharmacological discovery that contribute to the development of drugs that act as anti-tumor agents, heart stimulants and therapies for neurological diseases. Venom-informatics is a systematic bioinformatics approach in which classified, consolidated and cleaned venom data are stored into repositories and integrated with advanced bioinformatics tools for the analysis of structure and function of toxins. Venom-informatics complements experimental studies and helps reduce the number of essential experiments.

Keywords : Venom, Toxins, Venom-Informatics, Therapeutics, Snake, Cone Snail, Scorpion

I. INTRODUCTION

Venomous organisms are widespread throughout the globe and represented in many biological taxa (Yuri N Utkin,2015). Venom is a complex mixture of proteins and peptides, and presents several medical and pharmaceutical applications. Over the years, venoms and fractions there, have been displayed several biological activities/applications, including antibacterial, anti-protozoarian, antiviral (human immunodeficiency virus), analgesic, treatment of cancer and for many such diseases (Carolina Alves Nicolau *et* al. 2018). It is important to note that some of those aforementioned activities can be related not only to medium to high abundance specific venom toxins but also to low abundance components and, eventually, to their synergistic effects.

Venomous animals permanently or periodically contain toxins in their organisms, which are toxic to other species. Even small doses of such compounds in the body of another animal cause painful disorders and sometimes death. The pathophysiological complications related to a single sting of such animals are noteworthy to recognize venomous animal envenomation as a universal health problem. However, the information about peptides and proteins from toxins is not standardized in these resources, especially the naming of toxins and pharmacological activities, and mining for venom peptides is difficult. By molecular cloning and classical biochemistry, several proteins and peptides (related to toxins) are characterized. The revelation of many other novel components and their potential activities in different fields of biological and medicinal sciences revitalized the interests in the field of animal venomics and venom-informatics (Yuri N Utkin,2015).

Venom have been of great interest to researchers due to their significant roles in pharmacology (Ortiz *et* al., 2015). In our current investigation we have studied three different toxins from three different venomous organisms namely L- amino acid oxidases (snake), Contulakin-G (cone snail), Chlorotoxin (scorpion). Snake venoms are sources of molecules with proven and potential therapeutic applications. However, it is still challenging to associate signaling pathways identified through functional genomics to the pathophysiology of snakebite (assessed through well-established biochemical and biological assays, screening for hemorrhagic, hypotensive, edematogenic, neurotoxic, and myotoxic activities) (Paola G. Ojeda *et* al. 2017). The geographic cone is the most toxic of the known species, and several human deaths have resulted from envenomation. Severe cases of cone snail stings involve muscle paralysis, blurred/double vision, and respiratory paralysis, leading to death. The animals produce a potent venom to paralyse their prey. The venom contains a complex mixture of substances that includes neurotoxins, which are chemicals that block the conduction of nerve impulses (Kapil S *et* al. 2022). Scorpion's venom is primarily considered as the cocktail of chemicals which is injected into the prey/predators to disrupt their biological systems. Chlorotoxin possesses targeting properties towards cancer cells including glioma, melanoma, small cell lung carcinoma, neuroblastoma and medulloblastoma (Lucie Dardevet *et* al. 2017).

II. METHODOLOGY

1. Retrieval of sequences

Protein sequence data of L-amino acid oxidases (snake), contulakin-G (cone snail) and chlorotoxin (scorpion) have been retrieved by UniprotKB database and saved in FASTA format with their respective UniprotKB IDs.

2. Analysis of physico-chemical properties

Physico-chemical properties of L-amino acid oxidases (snake), contulakin-G peptide (cone snail) and chlorotoxin (scorpion) were analysed by using Protparam tool available at Expasy server which included isoelectrical point, theoretical pI, extension

coefficient, half-life, instability index, Aliphatic index, Grand average, hydropathicity, etc.

3. Secondary structure prediction

The secondary structures of L-amino acid oxidases (snake), contulakin-G (cone snail) and chlorotoxin (scorpion) were predicted by SOPMA, which gives the information of Alpha helix, beta sheets, extended strands and random coils.

4. Tertiary structure prediction

The tertiary structures of L-amino acid oxidases (snake), contulakin-G (cone snail) and chlorotoxin (scorpion) were obtained by using SWISS-MODEL tool and PDBsum by selecting the template with maximum homology and with optimized parameters. The obtained structures were stored in pdb format for visualization.

5. Visualization of tertiary structures

The predicted tertiary structures of L-amino acid oxidases (snake), contulakin-G (cone snail) and chlorotoxin (scorpion) were visualized by using structure visualization tool Rasmol. Visualization was done using different models and formats to understand structural features of respected proteins.

6. Antigenicity Prediction

The antigenicity of L-amino acid oxidases (snake), contulakin-G (cone snail) and chlorotoxin (scorpion) were obtained by using Antigenicity prediction tool. Kolaskar and Tongaonkar predict the segment within a protein sequence that are likely to be antigenic by eliciting an antibody response.

7. Prediction of Helical wheel projection

The properties of alpha helices in proteins were obtained by a helical wheel projection tool, which is a type of plot or visual representation. The amino acids sequence that makes up a helical region of the protein's secondary structure are plotted in a rotating manner where the angle of rotation between consecutive amino acids is 100°, so that the final representation looks down the helical axis.

III. RESULTS AND DISCUSSION

1. Retrieval of sequences

The sequences of L-amino acid oxidases (snake contulakin-G (cone snail) and chlorotoxin (scorpion) were retrieved from Uniprot KB. The sequences obtained were stored in FASTA format with their respective UniprotKB IDs. The UniprotKB IDs, sequence length and protein names are shown in Table 1 below.

Organism	Snake	Cone snail	Scorpion
Protein	L-amino acid oxidases	Contulakin-G	Chlorotoxin
UniprotKB ID	Q6TGQ9	Q9XYR5	P45639
Amino acid length	497	76	36

Table 1. Retrieval of protein sequences

2. Analysis of Physico-chemical properties

The analysis of physicochemical properties of L-amino acid oxidases (snake), contulakin-G (cone snail) and chlorotoxin (scorpion) was done by using Protparam tool and respective results were shown in Table 2. The theoretical pI depicts that chlorotoxin (scorpion) is basic in nature, while L-amino acid oxidases (snake) and contulakin-G (cone snail) both are acidic. Further, the instability index indicates that contulakin-G (cone snail) is unstable but L-amino acid oxidases (snake) and chlorotoxin (scorpion) are stable.

Physico-chemical analysis	L-amino acid oxidases (Snake)	Contulakin-G (Cone snail)	Chloro-toxin (Scorpion)
Molecular weight	56288.67	8260.61	4004.75

Theoretical pI	5.78	6.08	8.50
Instability index	38.82 (stable)	45.51 (unstable)	37.911 (stable)
Aliphatic index	78.09	97.50	13.61
GRAVY	-0.450	0.013	-0.547

Table 2. Physicochemical analysis

3. Secondary structure prediction

The secondary structures of L-amino acid oxidases (snake), contulakin-G (cone snail) and chlorotoxin (scorpion) were predicted by SOPMA tool. The secondary structure elements alpha helix, extended strand and random coils were calculated shown in Table 3. According to Table 3 the percentage of alpha helix was found to be more in chlorotoxin (scorpion) and least in L-amino acid oxidases (snake). Further, the percentage of extended strand was found to be more in L-amino acid oxidases (snake) and least in chlorotoxin (scorpion).

SSE	L-amino acid oxidases (Snake)	Contulakin-G (Cone snail)	Chloro-toxin (Scorpion)
Alpha helix	36.84%	48.68%	84.62%
Extended strand	7.89%	6.58%	0%
Random coil	50%	42.11%	9.23%

Table 3. Secondary structure prediction

4. Homology Modelling

The 3D structures of L-amino acid oxidases (snake), contulakin-G (cone snail) and chlorotoxin (scorpion) were predicted by using automated mode of SWISS-MODEL server and PDBsum.

5. Visualization of tertiary structures

The predicted tertiary structures of L-amino acid oxidases (snake), contulakin-G (cone snail) and chlorotoxin (scorpion) were visualized by using structure visualization tool RasMol. The generated structures are shown in Figure 1-5.

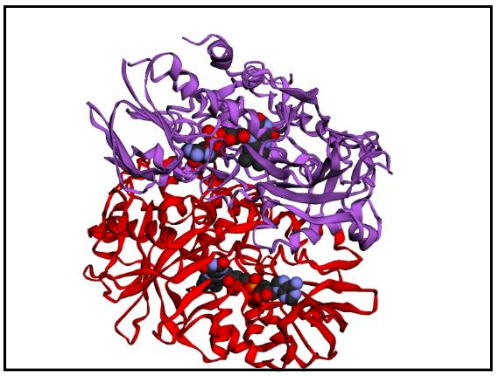


Fig 1. L-amino acid oxidases (snake)

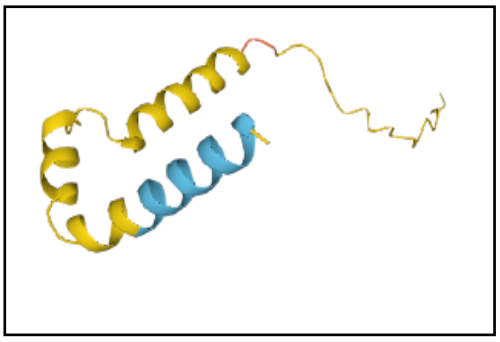


Fig 2. Contulakin-G (cone snail)

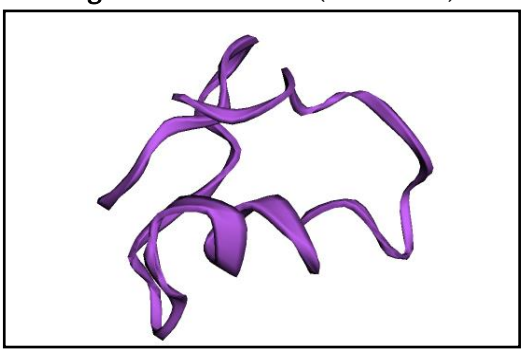
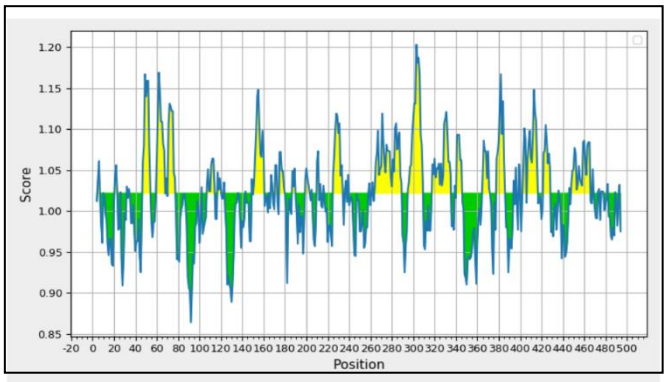


Fig 3. Chlorotoxin (scorpion)

6. Antigenicity Prediction

Kolaskar and Tongaonkar's method predicts antigenic epitopes of given sequence, based on physicochemical properties of amino acid residues that frequently occur in experimentally determined antigenic epitopes. In-silico studies reveals that L-amino acid oxidase with 14 antigenic peptides is found to be more

antigenic while contulakin-G with 2 and chlorotoxin with 1 antigenic peptide being lower antigenic nature.

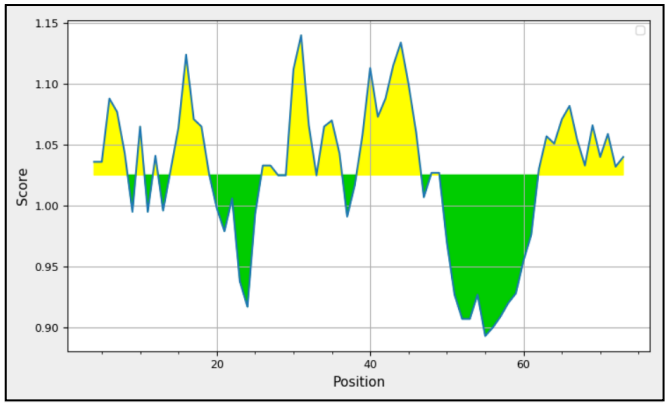


Average: 1.022 Minimum: 0.864 Maximum: 1.203

Predicted peptides:

No.	Start	End	Peptide	Length
1	47	54	KRVVIVGA	8
2	60	67	SAAYVLAN	8
3	71	77	QVTVLEA	7
4	107	114	KHRIVREY	8
5	151	160	GVLDYPVKPS	10
6	225	234	GYYVSFIESL	10
7	264	289	IQEKVHLNARVIKIQQDVKEVTVTYQ	26
8	295	309	TLSVTADYVIVCTTS	15
9	318	335	EPPLPPKKAHALRSVHYR	18
10	365	371	SRFIYYP	7
11	378	385	GVGVIIAY	8
12	407	416	INDLSLIHQL	10
13	422	428	QAICRPS	7
14	448	465	PYQFQHFSEALTAPVDRI	18

Fig 4. Antigenicity of L-amino acid oxidases (snake)

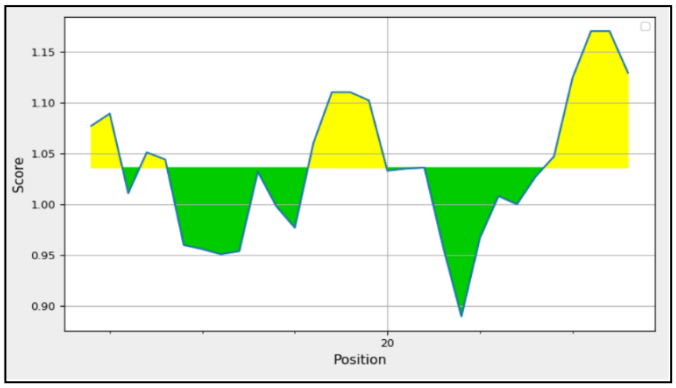


Average: 1.025 Minimum: 0.893 Maximum: 1.140

Predicted peptides:

No.	Start	End	Peptide	Length
1	14	19	WIAAPL	6
2	39	46	PQLILGSL	8

Fig 5. Antigenicity of Contulakin-G (cone snail)



Average: 1.036 Minimum: 0.890 Maximum: 1.170

Fig 6. Antigenicity of Chlorotoxin (scorpion)

7. Prediction of helical wheel projection

Helical wheels are a standard way to predict protein sequence segments with either helical or non-helical potential. These diagrams are projections of a protein's amino acid sequence onto a plane perpendicular to the axis of the helix. Comparing helical wheel representations for helical segments and non-helical segments made it clear that residues with hydrophobic side chains occur significantly more often in helical regions. One the other hand, the non-helical regions have more polar side chains.

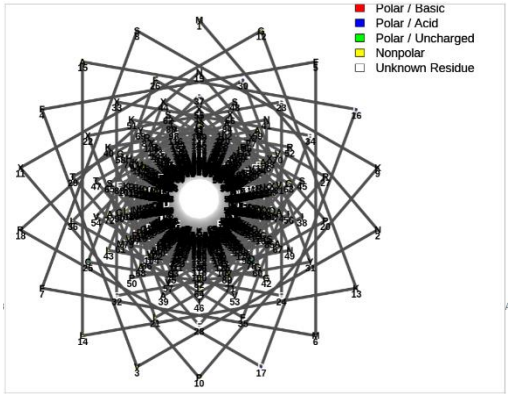


Fig 7. Helical wheel projection of L-amino acid oxidases (snake)

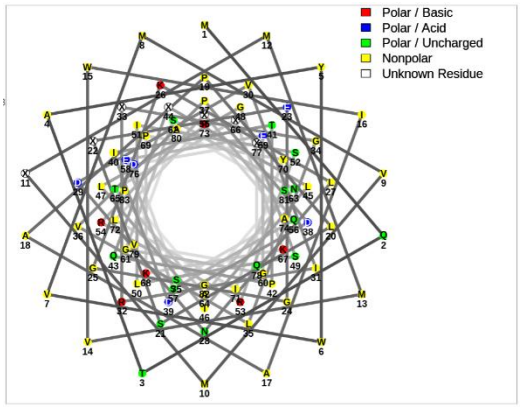


Fig 8. Helical wheel projection of contulakin- G (cone snail)

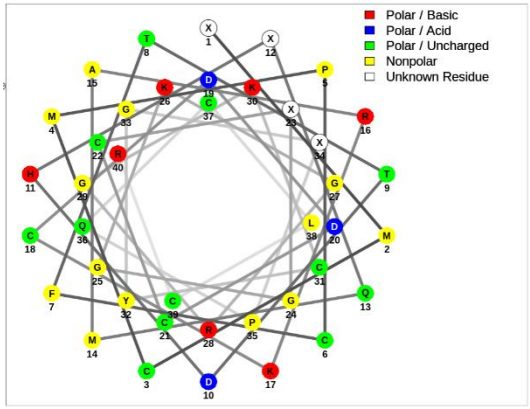


Fig 9. Helical wheel projection of chlorotoxin (scorpion)

IV. CONCLUSION

Proteins of three different venomous organisms were chosen for the investigation namely L-amino acid oxidases (snake), Contulakin-G (cone snail), Chlorotoxin (scorpion). In the present study, sequence and structural insight of L-amino acid oxidases (snake), contulakin-G (cone snail) and chlorotoxin (scorpion) emphasize their basic molecular nature with knowledge of venom toxin composition and their biological properties for designing novel scaffold and designing new peptide based drugs for the treatment of various cancers, haemostatic and other chronic disorders and as well as new tools for clinical diagnostic and assays of haemostatic parameters. Sequences of L-amino acid oxidases (snake), contulakin-G (cone snail) and chlorotoxin (scorpion) were retrieved from UniProtKB protein database, using these sequences their physicochemical properties were analysed from Protparam tool. Further, secondary structural elements were predicted from SOPMA tool and the tertiary models of respected proteins were built using SWISS-MODEL server. Additionally, antigenicity of respected proteins was predicted using kolaskar and tongaonkar's antigenicity prediction tool along with prediction of helical wheel projection.

V. REFERENCES

[1] Aboutorabi A, Naderi N, Gholamipour Pourbadiee H, Zolfagharian H, Vatanpour H. 2016. Voltage-gated sodium channels modulation by bothutous schach scorpion venom. Iranian journal of pharmaceutical sciences 12(3), 55-64.

[2] Adkins, C.L., D.N. Greenwald, D.B. Means, B. Matturro, and J. Ries. 2011. Petition to List the Eastern Diamondback Rattlesnake (Crotalus adamanteus) as Threatened under the Endangered Species Act. Petition submitted 08/11/11 to U.S. Fish and Wildlife Service (USFWS), Washington, D.C., and USFWS Region 4, Atlanta, GA.

[3] Al-Asmari AK, Islam M, Al-Zahrani AM. 2016. In vitro analysis of the anticancer properties of scorpion venom in colorectal and breast cancer cell lines. Oncology letters 11(2), 1256-1262.

[4] Brazón J, Guerrero B, D'Suze G, Sevcik C, Arocha-Piñango CL. 2014. Fibrin (ogen) olytic enzymes in scorpion (Tityus discrepans) venom. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology 168, 62-69.

[5] Carolina Alves Nicolau, Alyson Prorock, Yongde Bao, Ana Gisele da Costa Neves-Ferreira, Richard Hemmi Valente ID and Jay William Fox, Revisiting the Therapeutic Potential of Bothrops jararaca Venom: Screening for Novel Activities Using Connectivity Mapping PMID: 29415440

PMCID: PMC5848170
DOI: 10.3390/toxins10020069

[6] Ciscotto, P.; Machado de Avila, R.A.; Coelho, E.A.; Oliveira, J.; Diniz, C.G.; Farias, L.M.; de Carvalho, M.A.; Maria, W.S.; Sanchez, E.F.; Borges, A.; et al. Antigenic, microbicidal and antiparasitic properties of an L-amino acid oxidase isolated from Bothrops jararaca snake venom. Toxicon 2009, 53, 330–341. [CrossRef] [PubMed]

[7] Chippaux JP. 2012. Emerging options for the management of scorpion stings. Drug design, development and therapy 6, 165.

[8] DeBin JA, Maggio JE, Strichartz GR (February 1993). "Purification and characterization of chlorotoxin, a chloride channel ligand from the venom of the scorpion". Am. J. Physiol. 264 (2 Pt 1): C361–9.

[9] Kolaskar A, Tongaonkar PC. A semi-empirical method for prediction of antigenic determinants on protein antigens. FEBS Lett. 1990; 276: 172–174.

[10] Lourenço W. 2001. The scorpion families and their geographical distribution. Journal of Venomous Animals and Toxins 7(1), 03-23.

[11] Lucie Dardevet, Dipti Rani, Tarek Abd El Aziz, Ingrid Bazin, Jean-Marc Sabatier, Mahmoud Fadl, Elisabeth Brambilla, and Michel De Waard, Chlorotoxin: A Helpful Natural Scorpion Peptide to Diagnose Glioma and Fight Tumor Invasion 2015 Apr; 7(4): 1079–1101.Published online 2015 Mar 27. PMCID: PMC4417956 PMID: 25826056 doi: 10.3390/toxins7041079

[12] Olivera BM, Teichert RW (October 2007). "Diversity of the neurotoxic Conus peptides: a model for concerted pharmacological discovery". Molecular Interventions. 7 (5): 251–60.

[13] Ortiz E, Gurrola GB, Schwartz EF, Possani LD. (2015). Scorpion venom components as potential candidates for drug development. Toxicon 93, 125-135.

[14] Paola G Ojeda , David Ramírez Jans Alzate-Morales, Julio Caballero, Quentin Kaas, Wendy González, Computational Studies of Snake Venom Toxins PMID: 29271884 PMCID: PMC5793095
DOI: 10.3390/toxins10010008

[15] Price, Andrew H. (2009). Venomous Snakes of Texas: A Field Guide. University of Texas Press. pp. 38–39. ISBN 978-0-292-71967-5.

[16] Rao VR, Perez-Neut M, Kaja S, Gentile S. 2015. Voltage-gated ion channels in cancer cell proliferation. Cancers 7(2), 849-875.

[17] Sasha Kapil1; Stephen Hendriksen2; Jeffrey S. Cooper3 Cone Snail Toxicity In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan.

[18] Yoshiba S. [An estimation of the most dangerous species of cone shell, Conus (Gastridium) geographus Linne, 1758, venom's lethal dose in humans]. Nihon Eiseigaku Zasshi. 1984 Jun;39(2):565-72.

[19] Yuri N Utkin(2015). Animal venom studies: current benefits and future development. World J Biol Chem 6(2):28-33 ISSN1948-8454

[20] Zhang L, Shi W, Zeng XC, Ge F, Yang M, Nie Y, Guoji E. 2015. Unique diversity of the venom peptides from the scorpion Androctonus bicolor revealed by transcriptomic and proteomic analysis. Journal of proteomics 128, 231-250.

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