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In-silico Structural and Functional Characterization of α-bungarotoxin

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Abstract: ***α- bungarotoxin (α-BTX) is a bungarotoxins, contains the venom of the elapid snake Taiwanese banded krait (Bungarus multicinctus). It is a neurotoxin protein contains 95 amino acids having 10.31 kDa with five disulphide bridges that bind competitively to the nicotinic acetylcholine receptor found at the neuromuscular junction leads to cause paralysis, respiratory failure and death in the victim. nAChRs contain two binding sites for snake venom neurotoxins. The dynamics of binding action of these sites has proved difficult, although recent studies using normal mode dynamics have aided in predicting the nature of both the binding mechanisms of snake toxins and of ACh to nAChRs. A twist-like gating motion responsible for nAChR channel opening leads to increase diameter of channel with increase in motion, although neither the twist nor the increase in channel diameter was observed when the nAChR was in complex with either one or two α-bungarotoxin molecules. The present study involves the study of physicochemical properties, secondary and tertiary structure prediction, study of nature of protein and its antigenicity by using bioinformatics tools. The detailed investigations and research on toxins of various venomous animals and their key molecular roles, can contribute as alternate mode of treatment through peptide based drugs for many diseases.***

Keywords: α-bungarotoxin, nicotinic acetylcholine receptor (nAChRs), *Bungarus multicinctus*, Bioinformatics

1. Introduction

α -bungarotoxin is one of the bungarotoxin, components of the venom of the elapid snake Taiwanese banded krait (*Bungarus multicinctus*).It is a type of α neurotoxin, a neurotoxic protein that is known to bind competitively and in a relatively irreversible manner to the nicotine acetylcholine receptor found at the neuromuscular junction causing paralysis, respiratory failure and death in the victim [1]. The multiple disulfide bonds and small amount of secondary structure seen in α-BTX is the cause of the extreme stability of this kind of neurotoxin. Since there are many entropically viable forms of the molecule, it does not denature easily, and has been shown to be resistant to boiling and strong acids [2].α-Neurotoxins antagonistically bind irreversibly to nAChRs of skeletal muscles, thereby blocking the action of ACh at the postsynaptic membrane, inhibiting ion flow and leading to paralysis. nAChRs contain two binding sites for snake venom neurotoxins [3]. Progress towards elucidation of the dynamics of binding action of these sites has proved difficult, although recent studies using normal mode dynamics [4] have aided in predicting the nature of both the binding mechanisms of snaketoxins and of ACh to nAChR. So the present study reveals the molecular characterization of alpha-bungarotoxin includes, physicochemical properties, secondary and tertiary structure prediction, study of nature of protein and its antigenicity and that can be used for further studies.

2. Materials and Methods

1) Retrieval of protein sequence information of α-bungarotoxin

For the study of α-bungarotoxin, its amino acid sequence was retrieved from the major protein sequence databases like Uniprot KB and NCBI protein database. The sequence obtained was stored in Fasta format with its accession number.

2) Analysis of Physicochemical properties

The analysis of physicochemical properties of α-bungarotoxin was done by using protein prediction tool Protparam which gives a detailed information of protein like isoelectrical point, theoretical pI, extension coefficient, half life, instability index, Aliphatic index, Grand average, hydropathicity, etc.

3) Prediction of Secondary structure of α-bungarotoxin

The secondary structure prediction of α-bungarotoxin was carried out by using online secondary structure prediction tool SOPMA, which gives the information of Alpha helix, beta sheets, extended strands and random coils.

4) Prediction of Tertiary structure of α-bungarotoxin

The tertiary structure of α-bungarotoxin was obtained by using SWISS Model tool and PDBsum by selecting the template with maximum homology and with optimized parameters. The obtained structure was stored in pdb format for visualization.

5) Visualization of tertiary structure of α-bungarotoxin

The predicted tertiary structure of α-bungarotoxin was visualized by using structure visualization tool Rasmol. Visualization was done using different models and formats to understand structural features of α-bungarotoxin.

6) Prediction of Antigenicity of α-bungarotoxin

The antigenicity of α bungarotoxin was 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 was obtained by a helical wheel projection tool, which is a type of plot or visual representation. The amino acids sequence that make 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.

3. Results and Discussion

1) Retrieval of protein sequence information of α-bungarotoxin

Figure 1 showing Protein sequence of α bungarotoxin Accession no.-p60616

>sp|P60616|3L21V_BUNMU Alpha-bungarotoxin isoform V31 OS=Bungarus multicinctus PE=1 SV=1
MKTLLLTLVVVTIVCLDLGYTIVCHTTATSPISAVTCP
PGENLCYRKMWCDVFCSSRGKVVELGCAATCPSKKP
YEEVTCCSTDKCNPHPKQRPG

2) Analysis of Physicochemical properties

Table 1: Physicochemical properties of α - bungarotoxin

Properties	Values
No. of amino acid	95
Molecular weight	10313.19
Theoretical pI	8.31
Total no. of negative charged residue (Asp+Glu)	7
Total no .of positively charged residue(Arg +Lys)	10
Atomic composition	
1)Carbon(C)	447
2)Hydrogen(H)	727
3)Nitrogen(N)	119
4)Oxygen(o)	133
5)Sulphur(s)	13
Total no .of atoms	1433
Extinction coefficient No.	10595
Half life	30hrs
Instability index	Stable (30.16)
Aliphatic index	77.89
Grand average of a hydropathicity	0.136

3) Secondary structure prediction of α-bungarotoxin

Table 2: Secondary structure information of α-bungarotoxin

Secondary structureelement	Residues	Percentage
Alpha helix (Hh)	14	14.74%
310helix (Gg)	0	0.00%
Pi helix (Ii)	0	0.00%
Beta bridge (Bb)	0	0.00%
Extended strand (Ee)	31	32.63%
Beta Turn (Tt)	8	8.42%
Bend region (Ss)	0	0.00%
Random coil (Cc)	42	44.21 %
Ambiguous states	0	0.00%

4) Prediction of Tertiary structure of α-bungarotoxin

Table 3: Template details for α-bungarotoxin

Name	Title	Identity	Oligostate
1)2qc1.1.A	Alpha -bungarotoxin	100	Hetero-dimer
2)4uy2.2B	Alpha-bungarotoxin isoform V31	100	Hetero-dimer
3)1hc9.1.A	Alpha-bungarotoxin isoform V31	100	Hetero-dimer
4)4uy2.1B	Alpha-bungarotoxin isoform V31	100	Hetero-dimer
5)1ik8.1.A	Long neurotoxin 1	98.65	Monomer
6)1abt.1.A	Alpha-bungarotoxin	98.65	Hetero-dimer

5) Visualization of tertiary structure of α-bungarotoxin

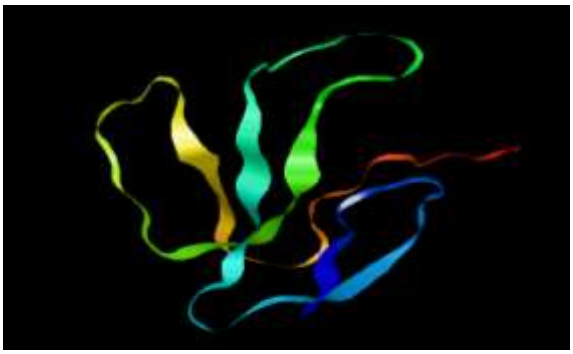

Figure 2: Tertiary structure of α-bungarotoxin

6) Prediction of Antigenicity of α-bungarotoxin

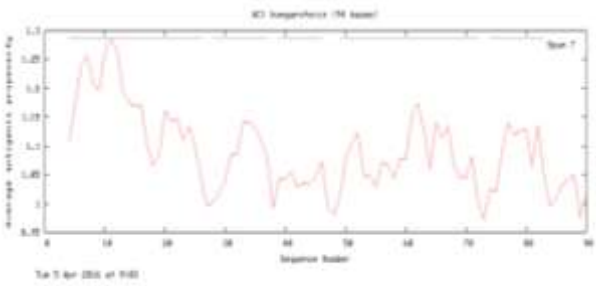

Figure 3: Antigenicity of α- bungarotoxin

n	Start Position	Sequence	End Position
1	4	LLLTLVVVTIVCLDLGYTIVCHT	26
2	28	ATSPISAVTC	37
3	39	PGENLCYR	46
4	49	WCDAFCSSRGKVVELGCAATCPSK	72
5	74	PYEEVTCCST	83

Contact Pedro Reche
Last Update: 5 April 2016

7. Prediction of Helical wheel projection

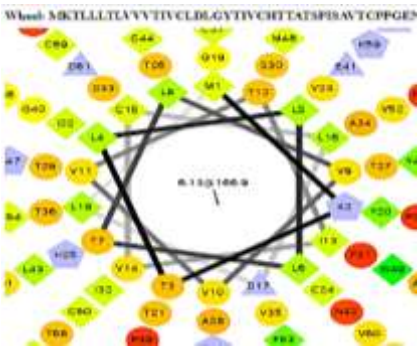

Figure 4: Helical wheel projection of α-bungarotoxin

4. Discussion

The various scientific communities were studied the structure of the alpha bungarotoxin .While some have studied the effects of the toxins and some other were studied on the mechanism .α-bungarotoxin binding to Acetylcholine receptor membranes studied by low angle "X-Ray Diffraction". So we aimed to study the physiochemical properties, prediction of secondary and tertiary structure, antigenisity, helical wheel projection by using bioinformatics tools to get the complete information of protein. The physicochemical analysis was carried out by Protparam, gives the information of the toxins on the basis of their physical and chemical properties such as molecular weight of about10.31319kDa, theoretical pI was 8.31, half-life was about 30hours etc. The secondary structure was predicted by using the SOPMA method which gave us the information about the secondary structure elements i.e. helixs, turns and coil while the 3Dstructure was predicted by using the SWISS Model which build the model by searching the template, model was further visualized by using Rasmol. The antigenicity study used to check the antigenic properties of proteins which elect the antibody response against antigen. The helical wheel projections were used to study the helical nature of protein.

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Author Profile

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