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Predicted structure model of Bungarotoxin from *Bungarus fasciatus* snake

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Abstract:
Snake venoms are cocktails comprising combinations of different proteins, peptides, enzymes and toxins. Snake toxins have diverse characteristics having different molecular configuration, structure and mode of action. Many toxins derived from snake venom have distinct pharmacological activities. Venom from *Bungarus fasciatus* (commonly known as banded krait) is a species of elapid snake found on the South East Asia and Indian sub-continent, mainly contains neurotoxins. Beta bungartotoxin is the major fraction of *Bungarus* venom and particularly act pre-synaptically by obstructing neurotransmitter release. This toxin in other snake species functionally forms a heterodimer containing two different subunits (A and B). Dimerization of these two chains is a pre-requisite for the proper functionality of this protein. However, *B. fasciatus* bungartotoxin contains only B chain and their structural orientation in yet to be resolved. Therefore, it is of interest to describe the predicted structure model of the toxin for functional insights. In this work we analyzed the neurotoxic nature, their alignments, secondary and three dimensional structures, functions, active sites and stability with the help of different bioinformatical tools. A comprehensive analysis of the predicted model provides approaching to the functional interpretation of its molecular action.

Keywords: Snake venom, Bungarotoxin, 3D structure, Bioinformatics.

Background:
Snake venoms are assortment of different biologically active proteins and polypeptides which are produced and stored in highly specialized venom glands. Venom toxins are of biological interest because of their diverse and selective pharmacological and physiological effects through their interaction with different molecular targets **[1].** However, these protein cocktails, often neurotoxins, conferring potency against a spectrum of targets and prey (reviewed in **[2, 3]).** For instance, *Bungarus multicinctus* venom contains both α-bungarotoxin, which blocks acetylcholine receptors on the postsynaptic membrane of neuromuscular junctions, and β-bungarotoxin, a kind of beta- neurotoxin from the phospholipase A_2 family, which inhibits neurotransmitter release from presynaptic membranes **[4].** So far, as many as 16 isoforms of the β-BuTx have been reported from *Bungarus* species **[5]** and 8 of them, namely b1–b5 **[6]** and SPI–SPIII **[7]** have been studied in detail. Structurally, they are covalently linked heterodimers; chain A is homologous to Group I PLA_2 enzyme, while chain B is structurally similar to Kunitz-type serine protease inhibitors and dendrotoxins. However, dimerization is a pre-requisite for the proper functionality of complete bungarotoxin. Beta2-bungarotoxin A chain consists of 120 amino acid residues in a single chain. A chain has 13 cysteine residues of which 12 form six disulphide bridges. An extra cysteine residue at the 15th position forms an inter-chain disulphide forms the disulphide bridge with chain B at 55 th position **[8]** and also determines the X-ray crystal structure

of beta2 bungarotoxin which showed different conformation of B chain from native bovine pancreatic trypsin inhibitors and accounts for its lack of protease inhibitor activity. Structurally, the a helices and the Ca^{2+} binding loop in chain A are similar to other PLA_2 molecules. However, there is a conformational change in the region where it interacts with chain B (residue 13–16 and 74–76), as well as in the substrate binding loop (residue 63–65). Similarly, the C-terminal region of chain B shows conformational variation due to the interaction with chain A, and this conformational change accounts for the lack of protease inhibitor activity. Moreover, ionic interactions between Glu16 of chain A and Lys 48 of chain B and a hydrogen bond between Arg 75 of chain A and carbonyl oxygen atom of Leu58 of chain B in the interface play important roles with interchain disulphide bridge and contribute to the overall actability of this covalent complex. The B-chain consists of 61 amino acid residues which is homologous to mammalian pancreatic and snake venom protease inhibitors (Kunitz type) and also the facilitatory dendrotoxins from mamba (*Dendroaspis*) venom **[9, 10].** The B-chain, however, does not have any intrinsic protease inhibitor activity. The B-chain is thought to act as an affinity probe to guide the toxin to its target (probably a potassium channel) on nerve terminals and the different B-chains may account for the differences in lethality between the isoforms **[11]**. β-BuTx binds to the presynaptic site at the neuromuscular junction and disrupts the release of acetylcholine. The effect of β-BuTx on the nerve terminal and summarized the series of event during neurotoxicity as: (1) initial weak reduction in the spontaneous acetylcholine release, (2) subsequent enhancement of the release of acetylcholine and (3) a final decrease in the release of acetylcholine leading to complete failure of neurotransmission **[12].** Subsequently, several studies were done to understand the effect of β-BuTx in the nerve terminals. As mentioned earlier, chain B is similar to dendrotoxin from *Dendroaspis* venoms, which blocks voltage sensitive K^{2+} channels. Hence, in organ bath assay, when muscle preparation is pretreated with dendrotoxin, the neuromuscular block by β-BuTx slows down. This indicates that both beta bungarotoxin and dendrotoxin target the same receptor/acceptor and that chain B exhibits the blocking activity by binding to the voltage gated K^{2+} channel **[13].** Chemical modification studies show that the inhibition of PLA_2 activity of chain A restrains the neurotoxic effect, suggesting the involvement of enzymatic activity in the neurotoxicity **[14].** Chain B binds to the K^+ channels in the target presynaptic site owing to its high affinity interaction and in turn assists chain A to bind to the presynaptic site. This novel functional characteristic of beta-bungarotoxin to act on the pre-synaptic site is of particular interest in pharmacology for therapeutic development. *B. fasciatus* is an elapid snake species indigenous to Bangladesh and south-east Asian locations also contains bungarotoxins into its venom gland. While searching for beta-bungarotoxin protein sequences in NCBI and UnitProtKB/SwissProt Database, reports of only two isoform of B chain from *B. fasciatus* are found. So far, there is no report of existence of A chain in bungarotoxin from *B. fasciatus* although significant works has been carried out on this species. Therefore, we predicted the three dimensional structure of the B chain using popular web-based servers and validated the output structure for elucidation of *B. fasciatus*, beta-bungarotoxin structure.

Methodology:

The complete protein sequences of two beta bungarotoxin BF B1 (B2KTG2) and BF B2 (B2KTG3) chain of *Bungarus fasciatus* were retrieved from the biological database, National Centre for Biotechnology Information (NCBI), cited at http://www.ncbi.nlm.nih.gov.

Prediction and Identification of Neurotoxin

Neurotoxic nature of the protein sequences of beta bungarotoxin from *Bungarus fasciatus* were confirmed through online server (www.imtech.res.in/raghava/ntxpred/). NTXpred predicts neurotoxins and classify them based on their function and origin. The server uses a number for predicting neurotoxins using residue composition based on feed-forwarded neural network (FNN), recurrent neural network (RNN) and support vector machine (SVM).

Sequence Alignment

In 3D structure, template may be a predefined layout to give an idea about the unknown structure of the query molecule. The NCBI BLAST **[15]** (Basic Local Alignment Search Tool) was used to identify the template for modeling the three dimensional structure of beta bungarotoxin B chain. Protein protein BLAST (blastp) was performed using PSI-BLAST **[16]** search at NCBI server. The sequence of the target molecule in FASTA format was submitted for blastp against Protein Data Bank database which yields the suitable template and finally the alignment was performed with the target protein sequences with Protein Data Bank (PDB code: 1bun) template using CLUSTALW **[17].**

Signal Peptide Detection

SignalP 4.1 server **[18]** was used for the detection of signal peptide sequence in the amino acid sequences. SignalP 4.1 server predicts the presence and location of signal peptide cleavage sites in amino acid sequences from different organisms. The method incorporates a prediction of cleavage sites and a signal peptide/non-signal peptide prediction based on a combination of several artificial neural networks.

Secondary Structure Prediction

The PSIPRED (http://bioinf.cs.ucl.ac.uk/psipred//? Program =psipred) protein structure prediction server was used for the detection of secondary structure and fold recognition. PSIPRED, **[19]** is considered as a highly accurate secondary structure prediction method.

Homology modeling

The X-crystal structural coordinates of beta bungarotoxin of *Bungarus multicinctus* were available at 2.45 Å resolution (PDB: 1BUN) and were used as template structure to generate the three- dimensional model of bungarotoxin. The Swiss-Pdb Viewer 4.0 homology modeling software was used to generate the 3-D structure of beta bungarotoxin B.

Validation of the modeled structure

In order to assess the reliability of the modeled structure of bungarotoxin B1 and B2 chain, we calculated the root mean square deviation (RMSD) by superimposing it on the template structure using a three dimensional structural superposition (3d-SS) tool **[20].** The backbone conformation of the modeled structure was calculated by analyzing the phi (Φ) and psi (ψ)

torsion angles using PROCHECK, as determined by Ramachandran plot statistics. Finally, the quality of the consistency between the template and the modeled structure was evaluated using ProSA **[21],** during which the energy criteria for the modeled structure were compared with the potential mean force obtained from a large set of known protein structures.

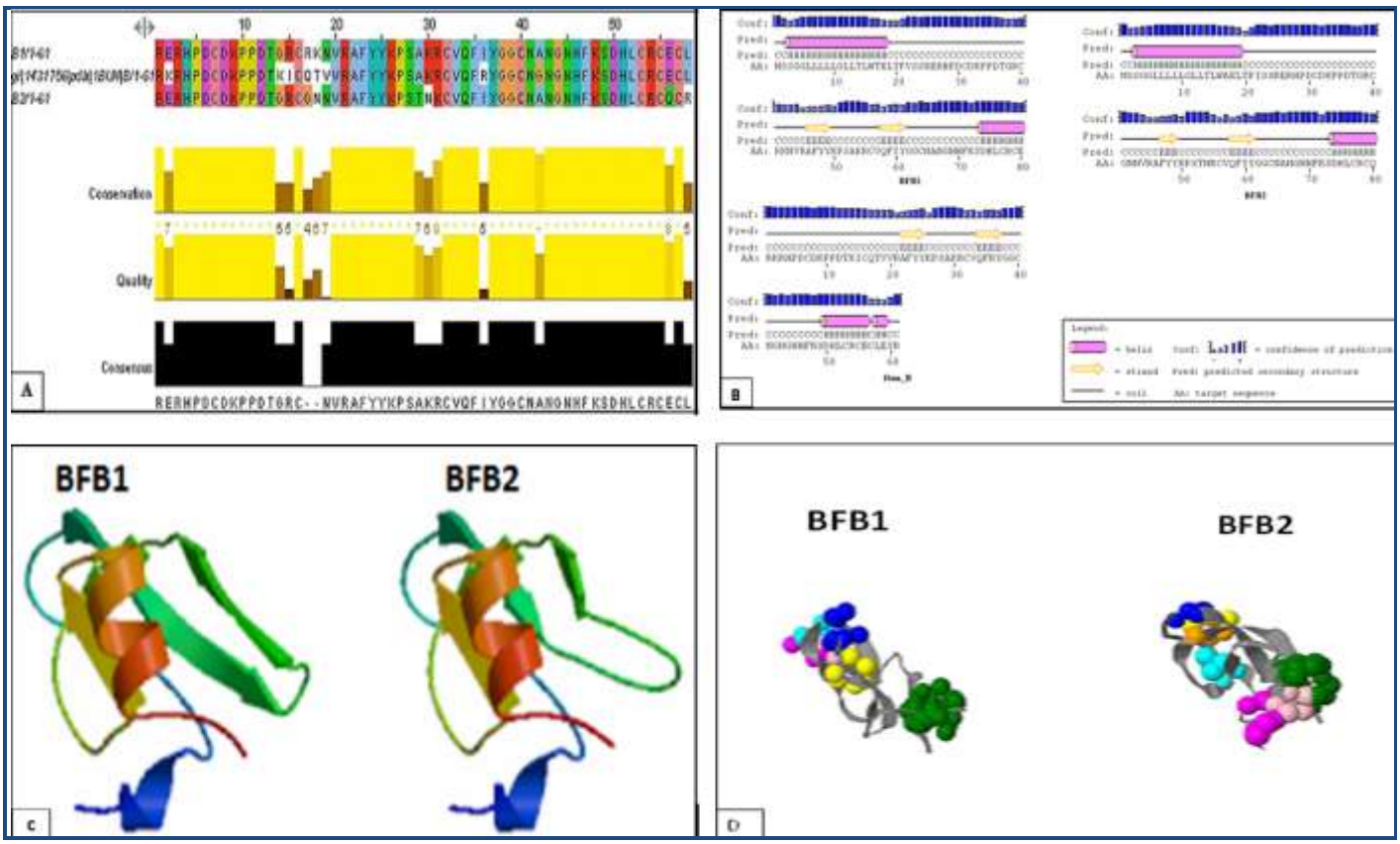

Figure 1: Sequence, structure and active site analysis of beta bungarotoxin B chain in Bungarus fasciatus: **A)** Multiple sequence alignment of BF B1 and BF B2 with template 1BUN_B used for structure modeling; **B)** Secondary structure prediction from PSIPRED server; **C)** Three dimensional structures of BF B1 and BF B2 predicted from Swiss-Modeller; **D)** CASTP prediction of the active site residues on the modeled structures; Active site residues are shown in colored position.

Function annotations of the protein

To functionally annotate the B chain, 'Profunc' was used and to find the conserved domains in protein to identify its family, it was searched against close orthologous family members. NCBI Conserved Domain Database (NCBI CDD) **[22]** was used to find the conserved domains or ancient domains in the protein sequence.

Active site Prediction

CastP server **[23]** was used to predict the active sites of bungarotoxin B1 and B2 chain with their respective volume and area (http://sts.bioengr.uic.edu/castp/calculation.php). The active pocket (active site) with more volume and area is always taken for further analysis. The pdb file was uploaded to get the result.

Stability of proteins

Disulfide bridges play a major role in the stabilization of the folding process for several proteins. Prediction of disulfide bridges from sequence alone is therefore useful for the study of structural and functional properties of specific proteins. The online server 'DISULFIND' **[24]** [http://disulfind.dsi.unifi.it/.] was used for predicting the disulfide bonding state of cysteines and their disulfide connectivity starting from the sequence alone.

Results & Discussion:

Sequence retrieval of Bungarotoxin

The protein sequences of bungarotoxin from *B. fasciatus* were retrieved from the NCBI database having the id as B2KTG2 and B2KTG3. The precursor sequence of beta bungarotoxin B chain is composed of 85 amino acid residues including a predicted 24-aa signal peptide and 61-aa mature peptide. Using Expasy ProtParam tool (http://web.expasy.org/protparam/) some physical parameter was obtained for the concerned sequences. The physicochemical properties like molecular weight, pI value, and percentage identity with the template protein were given **Table 1** (**see supplementary material**). To determine the nature of neurotoxin online neurotoxin prediction server NTXPRED was used. The alignment result of two bungarotoxin B chain is given in **Figure 1A**. The result showed approximately 87.059% similarity of amino acids between the two sequences. PSI blast prediction showed that both B chains of beta bungarotoxin BF B1 and BF B2 act as Ca^{2+} ion channel blocker. PSIPRED is the protein structure prediction server (http://bioinf.cs.ucl.ac.uk/psipred/), utilized for the analysis

of the secondary structure of our concerned amino acid sequences. B chain of beta-2 bungarotoxin from *B. multicintus* was also used for the comparison of the B chain from *B. fasciatus*. Results obtained from the PSIPRED server are shown in **Figure 1B**. BLAST P result for the template selection of bungarotoxin BF B1 chain and BF B2 against PDB database showed 87% and 78% sequence similarity with beta-2 bungarotoxin from *B. multicinctus* (PDB code: 1BUN B). E value for the selected template for BF B1 and BF B2 chain against 1BUN were respectively 1e-32 and 1e-28. The three dimensional structure of beta bungarotoxin BF B1 and BF B2 chain of *B. fasciatus* given in **Figure 1C** and was evaluated with scores of target protein and template. In this study, we observed significantly similarity of score and dope score (minimum energy was considered), target and template protein. Though cysteine residues found in both the sequences at position 7,16,32,40,53,55,57; CYS_REC and DISULFIND server predicted presence of disulfide bond between 7-57, 16-40, 32-53 residues. Since cysteine residue at 55 positions has no bonding partner with A chain (A chain was not found in *B. fasciatus* in NCBI database and BLAST search) as in *B. multicinctus*, we predict that the free cysteine at position 55 of BF B1 will form a disulfide bond with cysteine 55 of BF B2. Active site of the 3D structure predicted by Swiss-Model is shown in Figures, respectively, and the amino acids involved in pocket formation in both the models are displayed in **Figure 1D**.The possible binding sites of B chain was searched based on the structural comparison of template and the model build with CASTP server. Since, BF B1 and BF B2 and the 1BUN_B are well conserved in both sequence and structure; their biological function should be identical. The predicted results are interesting and **Figure 1D** shows the amino acid position are predicted to conserved with the active site.

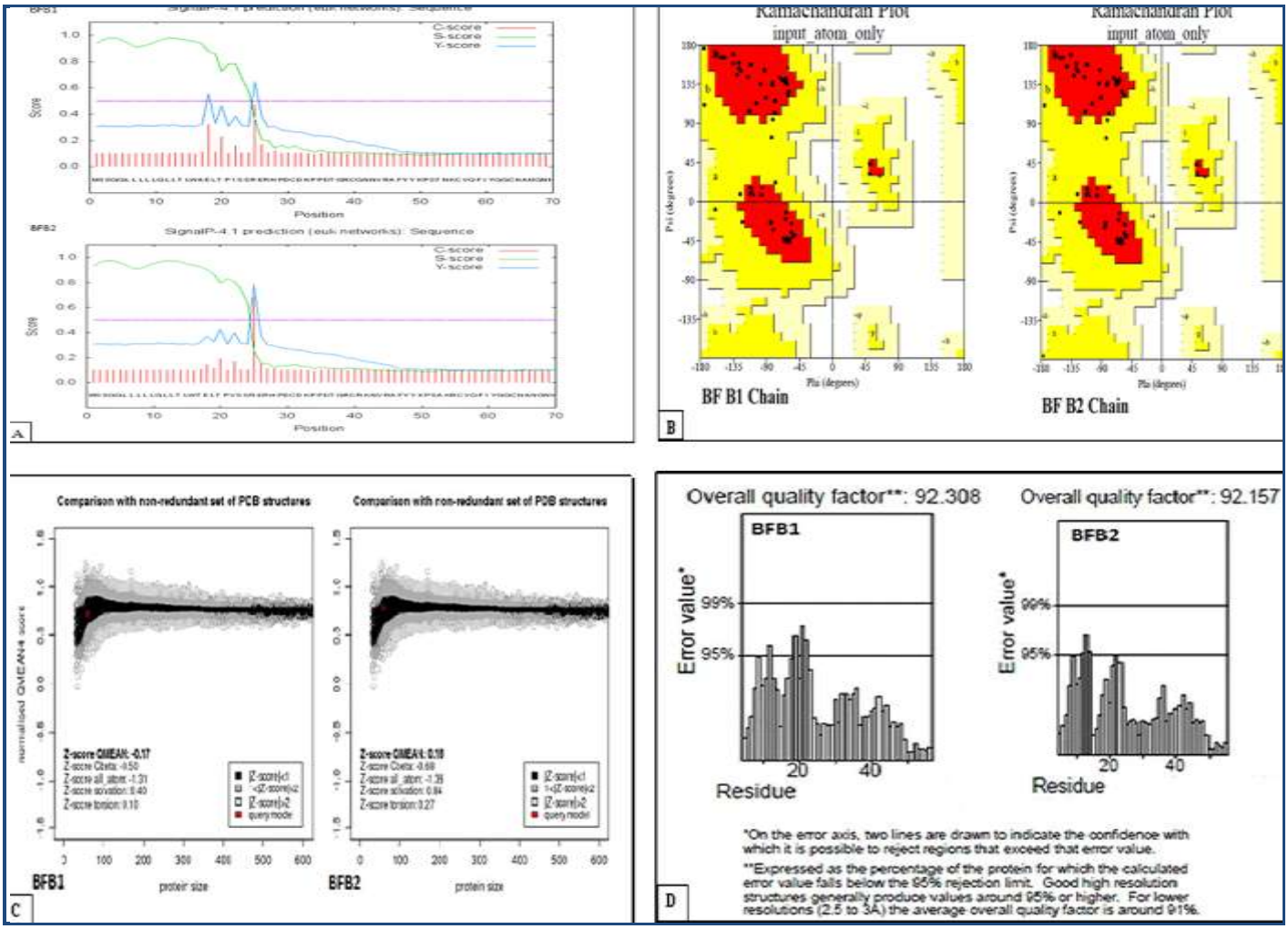

Figure 2: Determination of Signal peptide and different computational analysis of beta bungarotoxin B chain: **A)** Prediction of signal pepetide from signalP 4.1; **B)** Ramachandran plot showing predicted model reliability; **C)** Z-scorre QMEAN showing the validation of the models; **D)** ERRAT value showing overall model quality.

SignalP 4.1 server was used to recognize the presence and location of signal peptide cleavage sites in amino acid sequences **Figure 2A**. In this analysis, Ramachandran plot for bungarotoxins of *B. fasciatus* fulfilled the test ~ 84% in the most favored region. There was no amino acid residue present in the disallowed region of the Ramachandran plot. The overall G – factor of neurotoxins was 0.09 to 0.13 for the three 3-D structures. The result deduced that 3-D structure of neurotoxin was favored for a good satisfactory model. Ramachandran plot for neurotoxin of *B. fasciatus* was shown in **Figure 2B**.Verify 3D analysis revealed that 100% of the residues had an average 3D-1D score of <0.2, predicting that

the model is perfectly fitted with its sequence. The amino acid environment was evaluated using ERRAT plots, which assess the distribution of different types of atoms with respect to one another in the protein model and is used for making decision about its reliability **Figure 2C & 2D**. ERRAT showed an overall quality factor of 92.308 (BF B1) and 92.157 (BF B2), a result expected for crystallographic models with resolution >2.5A.

Conclusion:

β-BuTx is an interesting pre-synaptic neurotoxin found in different *Bungarus* snake sp. and as yet it has been studied extensively in *B. multicinctus*. *B. fasciatus* is a deadly elapid which is found in Bangladesh, as well Indian subcontinent. The venom of this species is predominantly neurotoxic which corroborates with the presence of bungarotoxin in it. However, in *B. fasciatus* so far there are reports of existence of protein that are homologous to the B chain. But in order to obtain proper functionality two chain of bungerotoxin (chain A and Chain B) needs to be dimerized. Therefore, three dimensional structural model of complete β-BuTx from *B. fasciatus* was created using only B chain. B chain of β-BuTx from *B. fasciatus* contains 13 cyctein residues which are predicted to form 6 disulfide bonds among them; furthermore from the predicted structure it is evident that the odd cystein takes part in forming inter-chain disulfide linkage. Thus, in absence of chain A in *B. fasciatuas* two B chain froms homodimer instead of the heterodimer of conventional β-BuTx and retains its neurotoxic activity.

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Supplementary Material:

Table 1: Physicochemical Properties of Bungarotoxin Protein of *Bungarus fasciatus*

No	Designation of Beta-bungarotoxin (β-BuTx)	MW (Da)	*pI*	Identity with PDB Template	Nature predicted through NTXPRED
1	BF B1 chain	7152.1	8.95	87%	Neurotoxin
2	BF B2 chain	7037.7	8.55	78%	Neurotoxin