Identification and Structural Characterization of a New Three-Finger Toxin Hemachatoxin from Hemachatus haemachatus Venom

Vallerinteadive Mavelli Girish1,9, Sundramurthy Kumar1,9, Lissa Joseph1, Chacko Jobichen1, R. Manjunatha Kini1,2*, J. Sivaraman1,9*

1 Department of Biological Sciences, Faculty of Science, National University of Singapore, Singapore, Singapore, 2 Department of Biochemistry, Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia, United States of America

Abstract

Snake venoms are rich sources of biologically active proteins and polypeptides. Three-finger toxins are non-enzymatic proteins present in elapid (cobras, kraits, mambas and sea snakes) and colubrid venoms. These proteins contain four conserved disulfide bonds in the core to maintain the three-finger folds. Although all three-finger toxins have similar fold, their biological activities are different. A new three-finger toxin (hemachatoxin) was isolated from Hemachatus haemachatus (Ringhals cobra) venom. Its amino acid sequence was elucidated, and crystal structure was determined at 2.43 Å resolution. The overall fold is similar to other three-finger toxins. The structure and sequence analysis revealed that the fold is maintained by four highly conserved disulfide bonds. It exhibited highest similarity to particularly P-type cardiotoxins that are known to associate and perturb the membrane surface with their lipid binding sites. Also, the increased B value of hemachotoxin loop II suggests that loop II is flexible and may remain flexible until its interaction with membrane phospholipids. Based on the analysis, we predict hemachatoxin to be cardiotoxic/cytotoxic and our future experiments will be directed to characterize the activity of hemachatoxin.

Introduction

Snake venoms are rich sources of biologically active proteins and polypeptides [1]. Apart from its crucial role in paralyzing and digesting prey, snake venom is also an excellent source for novel toxins. Understanding the mechanisms of action of unique toxins, helps in the discovery of novel receptors and in the development of lead therapeutic molecules [2,3]. Snake venom toxins can be broadly categorized as enzymatic and non-enzymatic proteins. They are also classified into various toxin superfamilies. Each superfamily contains structurally similar toxins that exhibit varied pharmacological activities. Some of the well characterized superfamilies of snake venom proteins include three-finger toxins (3FTxs), C-type lectin like proteins (CLPs), phospholipase A2s (PLA2s), serine proteases and metalloproteases [4–6]. 3FTxs, non-enzymatic snake venom proteins, are the most abundant toxins found in elapid (cobras, kraits, mambas and sea snakes) and colubrid venoms [4,7]. Besides they have been reported from viperid venom [8,9]. 3FTxs are composed of 60–74 amino acid residues and 4–5 disulfide bridges. Structurally, all 3FTxs have a stable fold with three β-stranded loops extending from a central core containing all four conserved disulphide bridges, resembling the three fingers of a hand, and hence their common name [10,11]. The conserved cysteine residues, along with invariant residues, such as Tyr25 and Phe27, contribute to proper folding [12]. Some 3FTxs have an additional fifth disulfide in loop I and II as in the case of non-conventional toxins and long-chain neurotoxins, respectively [11,13]. In general, 3FTxs exist as monomers. However, a few of them exist as homo- or heterodimers in which the subunits are held together by either non-covalent interactions or by covalent (disulfide) linkages. For example, κ-bungarotoxin [14] and haditoxin [15] exist as non-covalent homodimers where the individual subunits are structurally related to long-chain and short-chain neurotoxins, respectively. The individual subunits are arranged in anti-parallel orientation and are held together mostly by hydrogen bonds between main-chain and side-chain atoms [15–17]. On the other hand, covalently linked 3FTxs include the homodimeric α-cobratoxin (α-CT) [18] and the heterodimeric irditoxin [19]. The structural analysis of the homodimeric α-CT [18] reveals the presence of a β-strand swap as well as two disulfide linkages between loop I of the individual subunits, thereby stabilizing the entire dimeric structure [20]. In irditoxin, the individual subunits are covalently linked through a single disulfide bond between loop I of irditoxin B and loop II of irditoxin A [19]. 3FTxs also exhibit minor structural variations in the length and conformation of the loops, and...
presence of longer C-terminal or N-terminal extensions (for details, see [4]). Despite overall similar fold, 3FTxs recognize a broad range of distinct molecular targets resulting in diverse biological activities [21,22]. Based on their biological properties, 3FTxs can be classified as postsynaptic neurotoxins targeting the nicotinic [23] and muscarinic [24] acetylcholine receptors, cardiotoxins/cytotoxins targeting phospholipid membranes [25], fasciculins targeting acetylcholinesterase (AChE) [26], calciseptins and FS2 toxins targeting L-type calcium channels [27,28], anticoagulants like naniproin, exactin and siamextin [R. M. Kini and colleagues, unpublished data] targeting various coagulation complexes, β-blockers like β-cardiotoxin targeting β1- and β2-adrenergic receptors [29], dendroaspin targeting αvβ3 (glycoprotein IIB-IIIa) [30], cardiotoxin A5 targeting αβ3 integrins [31] and antagonists of α1A [32] and α2A [33]adrenergic receptors. The ability of 3FTxs to recognize various molecular targets signifies the need for understanding structure-function relationships of these toxins. The three-finger fold is also observed in various other proteins like xenoxins from X. laevis [34] and HEP21 from hen egg white [35], as well as mammalian Ly-6 alloantigens [36], urokinase-plasminogen activator receptor [37] and complement regulatory protein CD59 [38]. 3FTxs in snake venoms are thought to be evolved from non-toxic ancestral proteins through gene duplication and accelerated evolution [39,40].

Results

Isolation and Purification of Hemachatoxin

The H. haemachatus crude venom was fractionated on a gel filtration (Superdex 30) column. Peak 3 (Figure 1A) from gel filtration chromatography contained proteins that mostly belong to 3FTx family. Hemachatoxin (black arrow) was purified from peak 3 on a C18 reverse-phase column (Figure 1B) and further purified to homogeneity using a shallow gradient on the same column (Figure 1C). The homogeneity and mass of hemachatoxin was determined to be 6835.68 ± 0.94 Da.

Figure 1. Purification of hemachatoxin from the venom of H. haemachatus. (A) Size-exclusion chromatogram of the crude venom. The proteins were eluted using 50 mM Tris-HCl, pH 7.4 and monitored at 280 nm. The fractions of peak 3 (black horizontal bar) were pooled and sub-fractionated on RP-HPLC. (B) RP-HPLC chromatogram of peak 3 using a linear gradient of 28–50% solvent B. The elution was monitored at 215 nm. The black arrow indicates the elution of hemachatoxin. (C) The re-purification of hemachatoxin on a shallow gradient of 35–45% solvent B. The elution was monitored at 215 nm. (D) The ESI-MS profile of hemachatoxin showing the three peaks of mass/charge (m/z) ratio ranging from +4 to +6 charges. The mass of hemachatoxin was determined to be 6835.68 ± 0.94 Da.

doi:10.1371/journal.pone.0048112.g001
determined by electrospray ionization mass spectrometry (ESI-MS). ESI-MS showed 3 peaks of mass/charge (m/z) ratio ranging from +4 to +6 charges (Figure 1D). The mass was calculated to be 6835.68 ± 0.94 Da.

Sequence Determination and Analysis

We determined the complete amino acid sequence of hemachatoxin by automated Edman degradation. The first 45 amino acid residues were determined by sequencing the native protein while the remaining sequence was determined from overlapping fragments of chemically-cleaved S-pyridylethylated hemachatoxin (Figure S1A,S1B, S2) (Table S1). The calculated mass of 6836.4 Da from the hemachatoxin sequence agrees well with the experimentally determined molecular mass (6835.68 ± 0.94 Da). The crystal structure (see below) with well defined electron density for the entire hemachatoxin molecule was used to confirm the experimentally determined sequence of the protein as described earlier [41]. The BLAST search [42] showed that hemachatoxin is closely related (70% identity) to cardiotoxins/cytotoxins, a subgroup of 3FTxs (Figure 2A). Hemachatoxin exhibited highest identity to cytotoxin 1 (97%) [43], cytotoxin 2 (89%) and cytotoxin 3 (84%) [44], purified from Naja haemachatus venom. Hemachatoxin differs from cytotoxin 1 [43] in two amino acid positions (Leu27Met28 is replaced by Met27Leu28). This difference was confirmed by ESI-MS (CNBr cleavage site and mass of peptides, Table S1), Edman degradation (Figure S3A, S3B and S3C) and electron density map (see below).

Thus hemachatoxin belongs to the 3FTx family based on sequence similarity and the position of cysteine residues (Figure 2).

Structural Analysis

The structure of hemachatoxin was determined by the molecular replacement method using Naja nigricollis toxin coordinates (PDB code 1TGX) as a search model. There were two hemachatoxin molecules in an asymmetric unit with each molecule consisting of residues from Leu1 to Asn61 (Figure 3A). Both monomers are well defined in the electron density map (Figure 3B). The model was refined to a final R value of 0.23 (Rfree = 0.28) (Table 1). The stereochemical parameters of the model were analyzed by PROCHECK [45] and all residues are in the allowed regions of the Ramachandran plot. Each monomer of the asymmetric unit consists of 6 anti-parallel β-strands (β2Qβ1Qβ4Qβ3Qβ5Qβ6) that form two β-sheets (Figure 3A). The first β-sheet consists of two anti-parallel β-strands, β1 (Lys2-Lys6) and β2 (Phe10-Thr14), while the second contains four anti-parallel strands, β3 (Leu21-Thr26), β4 (Ile35-Thr40), β5 (Ala42-Ser47) and β6 (Lys51-Asn56). The fold of hemachatoxin is maintained by four disulfide bonds, and these cysteines are strictly conserved among the 3FTxs. The three fingers of hemachatoxin consist of the secondary structures β1Vβ2, β3Vβ4 and β5Vβ6 (Figure 3A). The electrostatic surface representation shows that loops I and II are predominantly charged residues, whereas loop III is highly hydrophobic in nature (Figure 3C). The sequence alignment revealed the conserved
residues of hemachatoxin as well as its identity to cardiotoxins/cytotoxins (Figure 4A). Also, hemachatoxin shared the common three-finger fold and molecular shape when compared to its structural homologues (Figure 4B) [46].

Discussion

The three-dimensional structures of snake venom 3FTxs, particularly that of neurotoxins [15,20,47,48] and cardiotoxins/cytotoxins [49–52] have been extensively studied. Here we report the structural characterization of a new 3FTx, hemachatoxin from the venom of H. haemachatus. The structural analyses indicate that hemachatoxin belongs to cardiotxin/cytotoxin subgroup of 3FTx family. It exhibited 97% sequence identity to cytotoxin 1 [43], whose crystal structure has not been determined. ESI-MS, Edman degradation and crystal structure data indicates that hemachatoxin differs from cytotoxin 1 in two amino acid positions (Leu27Met28 is replaced by Met27Leu28) and hence are isoforms. Multiple isoforms of 3FTxs are known to be present in single snake venom [53,54].

As mentioned in the introduction section, 3FTxs, including hemachatoxin, share overall structural similarity (Figure 4B), but they differ from each other in their biological activities. Subtle variations in the size and conformation of β-sheet loops dictate the biological specificities in 3FTxs. For example, the well characterized long-chain (e.g. α-cobratoxin, α-bungarotoxin) and short-chain (e.g. erabutoxin a, toxin-α) neurotoxins that differ in loop size and length of C-terminal extension, exhibit distinct specificity for nAChR subtypes. Short-chain neurotoxins has a longer loop I (12–13 amino acid residues [aa] vs. 9–12 aa in long-chain neurotoxins), a shorter loop II (15–16 aa vs. 19–20 aa in long-chain neurotoxins) and C-terminal tail (2 aa vs. 7–24 aa in long-chain neurotoxins) when compared to long-chain neurotoxins. This longer loop I of short-chain neurotoxins contains key functional residues that are important for recognizing the nicotinic acetylcholine receptor [55,56], while shorter loop I of long-chain neurotoxins lacks these functional residues. The long C-terminal tail appears to substitute for the loop I functional residues and contribute to the receptor binding [57,58]. The deletion of this C-terminal tail reduces the binding affinity [59,60]. Similarly, the difference in the conformations of the three loops appears to dictate the biological specificities of these neurotoxins. Both short-chain and long-chain neurotoxins exhibit equi-potency towards muscle αβγδ nAChR [56,60] but only long-chain neurotoxins, not short-chain neurotoxins, bind to neuronal α7 nAChR with high affinity [61,62]. Detailed structure-function studies indicate that the presence of the fifth disulfide bond in loop II enables long-chain neurotoxins to recognize α7 nAChR. The short helical segment formed by the fifth disulfide is thought to be crucial for the target receptor recognition [62,63]. Thus, size and conformation of the loops indeed affects the interaction of neurotoxins with their receptor. Similarly, structures of loop I in fasciculin [64], and loop III in FS2 [65] and dendroaspin [66] have distinct conformations. Hence, subtle conformational differences in the loops of 3FTxs may help in identifying putative functions.

Hemachatoxin shows highest similarity to P-type cardiotoxins [67] (Figure 2A). Similar to these P-type cardiotoxins, hemachatoxin has the preserved Pro31 and cytolytic site. The three-dimensional structure is similar to P-type cardiotoxins (Figure 4B) (RMSD values, 0.8 to 2.1 Å for 58 to 60 Cα atoms; Z score values, 12.2 to 9.8). Besides, hemachatoxin shows considerable structural identity with S-type cardiotoxins (RMSD 1.1 to 2.8 Å for 58 to 59 Cα atoms; Z score values, 10.5 to 6.3) (data not shown). However, the similarity with other groups of 3FTxs, such as neurotoxins, muscarinic toxins, fasciculin, FS2 or dendroaspin, is relatively low (Figure 2B, Table 2). The P-type cardiotoxins bind to phospholipids and perturb the membrane surface with their lipid binding sites (6–13, 24–37 and 46–50 amino acid positions in the tip of loop I, II and III, respectively) [67–69]. These hydrophobic residues flanked by cationic residues form cytolytic region in
Table 1. Crystallographic data and refinement statistics.

| Data collection* |  |
|------------------|---|
| Unit Cell (Å)    | a = 49.7, b = 50.1, c = 57.8 |
| Resolution range (Å) | 50.2-4.3 (2.47-2.43) |
| Wavelength (Å)   | 1.5418 |
| Observed reflections | 28936 |
| Unique reflections | 5614 |
| Completeness (%) | 96.2 (84.5) |
| Redundancy       | 3.9 (3.7) |
| *R_{sym}         | 0.05 (0.17) |
| </SigI           | 20.6 (11.7) |

Refinement

Resolution range (Å) (I > 2σ(I)) 30-2.43

Ramachandran statistics

Most favored regions (%) 98.31
Allowed regions (%) 1.69
Disallowed regions (%) 0

Statistics from the current model.

R_{work} = \sum_{i\neq j} F_{o,i} - F_{c,i} \sum_{i\neq j} |F_{o,i}| / \sum_{i\neq j} |F_{c,i}|

Theoretical R-factor and goodness of fit:

R_{work} = 0.23

Water molecules (938 atoms) 40.30
Water molecules (62 atoms) 37.1
Wilson B value 36.54

For further details on the crystallography of hemachatoxin, see Table 1. Cardiotoxins [70,71]. We compared the B values of the cardiotoxin loops with those of hemachatoxin. All three loops in P-type cardiotoxins showed a high B value (an increase of 5-8 Å²) compared with the rest of the molecule. A similar increase in B values (up to 8 Å² increase) was observed in hemachatoxin compared with the rest of the molecule. A similar increase in B values was also observed in hemachatoxin.

Future experiments will be directed to characterize the activity of hemachatoxin. Additional experiments are required to fully characterize the cardiotoxins and may remain flexible until its interaction with membrane contacts. Nonetheless this analysis suggests that loop II is flexible and may remain flexible until its interaction with membrane phospholipids. These structural analyses also suggest that hemachatoxin might be having cardiotoxic/cytotoxic activity and our future experiments will be directed to characterize the activity of hemachatoxin.

Conclusion

In summary we report the isolation, purification and structural characterization of a new 3FTx, hemachatoxin from H. haemachatus venom. The structural and sequence analysis reveals hemachatoxin to be a P-type cardiotoxin. Close comparison of the loops of hemachatoxin with other 3FTxs suggests that hemachatoxin has structural features similar to the well characterized cardiotoxins. The structural analysis combined with literature predicts hemachatoxin to have cardiotoxic/cytotoxic properties. Additional experiments are required to fully characterize the activity of hemachatoxin.

Materials and Methods

Protein Purification

Lyophilized H. haemachatus crude venom was purchased from South African Venom Suppliers (Louis Trichardt, South Africa). Size-fractionation of the crude venom (100 mg in 1 ml of distilled water) was carried out on a Superdex 30 gel-filtration column (1.6×60 cm) pre-equilibriated with 50 mM Tris-HCl buffer (pH 7.4). The proteins were eluted with the same buffer using an AKTA purifier system (GE Healthcare, Uppsala, Sweden). Peak 3 from the gel-filtration chromatography was sub-fractionated by reverse-phase high-performance liquid chromatography (RP-HPLC) on a Jupiter C18 column (10×250 mm) equilibrated with solvent A (0.1% TFA). The bound proteins were eluted using a linear gradient of 28-50% solvent B (80% acetonitrile in 0.1% TFA). The mass of each fraction were analyzed on a LCQ Fleet™ Ion Trap LC/MS system (Thermo Scientific, San Jose, USA). Xcalibur™ 2.1 and ProMass deconvolution 2.8 software were used, respectively, to analyze and deconvolute the raw mass data. The peak corresponding to hemachatoxin was pooled and rechromatographed using a shallow gradient of 35–45% solvent B on the same column. The mass and homogeneity of purified hemachatoxin was analyzed as described above.

Sequencing

Hemachatoxin (1.2 mg) was dissolved in 500 µl of denaturation buffer (130 mM Tris-HCl pH 8.5, 1 mM EDTA, 6 M guanidine HCl). After the addition of the reducing agent β-mercaptoethanol (1.23 µl; 25×molar excess of disulfide bonds), the reaction mixture was incubated under a nitrogen stream for 3 h at room temperature. Subsequently, the alkyllating reagent 4-vinylpyridine (3.7 µl; 3×molar excess of β-mercaptoethanol) was added and incubated under a nitrogen stream for another 2 h at room temperature. The S-pyridylethylated protein was immediately separated from the reaction mixture by RP-HPLC on a Jupiter C18 column (4.6×250 mm) using a linear gradient of 20-60% solvent B and the mass was determined by ES-TOF-MS as discussed above. For cyanogen bromide (CNBr) cleavage, the S-pyridylethylated protein (0.82 mg) was dissolved in 410 µl of 70% TFA to which CNBr (67.7 µl; 70% TFA) was added in order to yield a final protein concentration of 1 μg/µl. CNBr was used at a molar ratio to methionine residue of 200:1. The reaction tube was incubated in complete darkness for 24 h at room temperature. After 24 h, 8.2 ml of Milli-Q water (10×v/v) was added into the reaction tube and, subsequently, the reaction tube was lyophilized overnight [72]. The lyophilized sample was resolubilized in 3 ml of 0.1% TFA for separation by reverse-phase chromatography on a Jupiter C18 column (4.6×250 mm) using a linear gradient of 10-50% solvent B. The masses of the peptide fragments were determined by ES-TOF-MS (data not shown). The terminal sequence of native hemachatoxin and peptides generated by CNBr cleavage (identified by mass spectrometry data) were determined by automated Edman degradation using a PerkinElmer Life Sciences Model 494 pulsed liquid-phase sequencer (Piscataway, Foster City, USA) with an on-line Model 789A phenylthiohydantoin-derivative analyzer. The complete amino acid sequence of hemachatoxin was determined by automated Edman degradation using a PerkinElmer Life Sciences Model 494 pulsed liquid-phase sequencer.
Hemachatoxin from Ringhals Cobra Venom
acid sequence of hemachatoxin was determined by overlapping sequences.

Crystallization and Structure Determination

Crystallization screens were performed with the hanging drop vapor diffusion method using Hampton Research and Jena Bioscience screens. The protein was at a concentration of 35 mg/ml, and 1:1 crystallization drops were set up with the reservoir solution. The diffraction quality crystals of hemachatoxin were obtained from a reservoir solution containing 150 mM ammonium acetate, 100 mM sodium acetate (pH 4.6) and 25% polyethylene glycol. Crystals were grown up to 10 days and were cryo-protected with 20% (w/v) glycerol supplemented (the mother liquor concentration was maintained by exchanging with water with glycerol) with the crystallization condition. Hemachatoxin molecules located in the asymmetric unit. The resultant electron density map was of good quality. Several cycles of model building/refitting using the program Coot [75], and alternated with refinement using the program Phenix [76], lead to the convergence of R-values (Table 1). Non-crystallographic symmetry (NCS) restraints were used throughout the refinement process.

Accession Numbers

The protein sequence data reported in this paper will appear in the UniProt Knowledgebase under the accession number B3EWH9. The three dimensional coordinates and structure factors of hemachatoxin were deposited in the RCSB (www.pdb.org) database with the access code 3VTS.

Supporting Information

Figure S1 Reduction and pyridylethylation of hemachatoxin. (A) The S-pyridylethylated hemachatoxin (black arrow) was purified on a linear gradient of 20–60% solvent B. (B) The ESI-MS profile of S-pyridylethylated hemachatoxin showing the four peaks of mass/charge (m/z) ratio ranging from +4 to +7 charges. The mass was determined to be 7685.12 ± 1.14 Da. (TIF)

Figure S2 Separation of peptides derived from cyanogen bromide cleavage of the S-pyridylethylated hemachatoxin on RP-HPLC. A linear gradient of 10–50% solvent B was used. The peptides A and B were sequenced by Edman degradation method. (TIF)
Figure S3  Chromatographic profiles of PTH-amino acid (phenylthiohydantoin-amino acid) residues 27 and 28 of the Edman degradation cycles 29 and 30. (A) Elution profile of standard PTH-amino acid residues. (B) Cycle 29 of Edman degradation showing the 27th residue, PTH-L, PTH-T and PTH-M, denote the cyclohexane, 28th and 29th cycle, respectively. (C) Cycle 30 of Edman degradation showing the 28th residue, PTH-M. PTH-L denote the cyclohexane from 29th cycle.

Table S1  The sequence determination of hemachatoxin.

Author Contributions

Conceived and designed the experiments: JS RMK. Performed the experiments: VMG SK LJ CJ. Analyzed the data: JS RMK VMG CJ. Contributed reagents/materials/analysis tools: JS RMK. Wrote the paper: JS RMK VMG CJ.

References

1. Dufton MJ (1993) Kill and cure: the promising future for venom research. Endeavour 17: 138–140.
2. Coleghous LM, Patrick JW (1997) Pharmacology of neuronal nicotinic acetylcholine receptor subtypes. Adv Pharmacol 39: 191–220.
3. Lewis RJ, Garcia ML (2003) Therapeutic potential of venom peptides. Nat Rev Drug Discov 2: 790–802.
4. Kini RM, Doley R (2010) Structure, function and evolution of three-finger toxins: mini proteins with multiple targets. Toxicon 56: 853–867.
5. Ogawa T, Chijiiwa T, Oda-Ueda N, Ohno M (2005) Molecular diversity and accelerated evolution of C-type lectin-like proteins from snake venom. Toxicon 45: 1–14.
6. Kang TS, Georgieva D, Genov N, Murakami MT, Sinha M, et al. (2011) Neurotoxins from snake venoms. In: Harvey, A.L. (Ed.), Snake Toxins. Pergamon Press, New York, 165–222.
7. Giusti J, Bagagli P, Mazzeo M, Ottaviani F, Bonci A, et al. (2008) Naturally occurring disulfide-bound dimers of three-fingered toxins: a paradigm for biological activity diversification. J Biol Chem 283: 14571–14580.
8. Fletcher CM, Harrison RA, Lachmann PJ, Neuhaus D (1994) Structure of a tissue plasminogen activator. Comparison to other members of the Ly-6 family. J Biol Chem 269: 7937–7943.
9. Munday homologue from the venom of Ophiophagus hannah (king cobra). J Biol Chem 285: 8302–8315.
10. Holm L, Sander C (1998) Touring protein fold space with Dali/FSSP. Nucleic Acids Res 26: 2361–2370.
11. Ogawa T, Chijiwa T, Oda-Ueda N, Ohno M (2005) Molecular diversity and accelerated evolution of C-type lectin-like proteins from snake venom. Toxicon 45: 1–14.
12. Roy A, Zhou X, Chong MZ, D’hoedt D, Foo CS, et al. (2010) Structural and functional characterization of luffaculin 1, a novel type 1 ribosome-inactivating protein. BMC Struct Biol 7: 29.
13. McDowell RS, Dennis MS, Louie A, Shuster M, Mulkerin MG, et al. (1992) Mambin, a potent glycoprotein Ib-IIIa antagonist and platelet aggregation inhibitor structurally related to the short neurotoxins. Biochemistry 31: 4766–4772.
14. Karlsson E, Jolkkonen M, Mulugeta E, Onali P, Adem A (2000) Snake toxins with high selectivity for subtypes of masacrine acetylcholine receptors. Biochimie 82: 783–806.
15. Dufton MJ, Hider RC (1988) Structure and pharmacology of elapid cytotoxins. Pharmacol Ther 36: 1–40.
16. Eastman J, Wilson EJ, Cervenansky C, Rosenberry TL (1991) Calciseptine, a peptide isolated from black mamba venom, is a specific blocker of the L-type calcium channel. Proc Natl Acad Sci U S A 88: 2437–2440.
17. Yasuda O, Morimoto S, Jiang B, Karoda H, Kimura T, et al. (1994) FS2, a mamba venom toxin, is a specific blocker of the L-type calcium channels. Artery 21: 297–302.
18. Rajagopalan N, Pung YF, Zhu YZ, Wong PT, Kumar PP, et al. (2007) Beta-cardiotoxin: a new three-finger toxin from Ophiophagus hannah (king cobra) venom with beta-blocker activity. FASEB J 21: 3685–3695.
19. McDowell RS, Dennis MS, Louie A, Shuster M, Mulkerin MG, et al. (1992) Mambin, a potent glycoprotein Ib-IIIa antagonist and platelet aggregation inhibitor structurally related to the short neurotoxins. Biochemistry 31: 4766–4772.
20. Wu PL, Lee SC, Chuang CC, Mori S, Akakura N, et al. (2006) Non-cytotoxic cobratoxin X-ray structure: localization of intermolecular disulfides and folding of the three-a-helix bundle. Protein Sci 15: 1674–1684.
21. Kolbe HV, Huber A, Cordier P, Rasmussen UB, Bouchon B, et al. (1993) Xenoxins, a family of peptides from dorsal gland secretion of Xenopus laevis a toad related to snake venom cytotoxins and neurotoxins. J Biol Chem 268: 16458–16464.
22. Sato Y, Takeuchi M, Kato K, Osawa K, Suzuki Y, et al. (2009) Increased expression of the murine Ly-6 family of molecules. Immunol Cell Biol 73: 277–283.
24. Karlsson E, Jolkkonen M, Mulugeta E, Onali P, Adem A (2000) Snake toxins with high selectivity for subtypes of masacrine acetylcholine receptors. Biochimie 82: 783–806.
25. Dufton MJ, Hider RC (1988) Structure and pharmacology of elapid cytotoxins. Pharmacol Ther 36: 1–40.
26. Eastman J, Wilson EJ, Cervenansky C, Rosenberry TL (1991) Calciseptine, a peptide isolated from black mamba venom, is a specific blocker of the L-type calcium channel. Proc Natl Acad Sci U S A 88: 2437–2440.
27. Yasuda O, Morimoto S, Jiang B, Karoda H, Kimura T, et al. (1994) FS2, a mamba venom toxin, is a specific blocker of the L-type calcium channels. Artery 21: 297–302.
28. Rajagopalan N, Pung YF, Zhu YZ, Wong PT, Kumar PP, et al. (2007) Beta-cardiotoxin: a new three-finger toxin from Ophiophagus hannah (king cobra) venom with beta-blocker activity. FASEB J 21: 3685–3695.
29. McDowell RS, Dennis MS, Louie A, Shuster M, Mulkerin MG, et al. (1992) Mambin, a potent glycoprotein Ib-IIIa antagonist and platelet aggregation inhibitor structurally related to the short neurotoxins. Biochemistry 31: 4766–4772.
30. Wu PL, Lee SC, Chuang CC, Mori S, Akakura N, et al. (2006) Non-cytotoxic cobratoxin X-ray structure: localization of intermolecular disulfides and folding of the three-a-helix bundle. Protein Sci 15: 1674–1684.
31. Kolbe HV, Huber A, Cordier P, Rasmussen UB, Bouchon B, et al. (1993) Xenoxins, a family of peptides from dorsal gland secretion of Xenopus laevis a toad related to snake venom cytotoxins and neurotoxins. J Biol Chem 268: 16458–16464.
32. Sato Y, Takeuchi M, Kato K, Osawa K, Suzuki Y, et al. (2009) Increased expression of the murine Ly-6 family of molecules. Immunol Cell Biol 73: 277–283.
33. Ploug M, Ellis V (1994) Structure-function relationships in the receptor for urokinase-type plasminogen activator. Comparison to other members of the Ly-6 family and snake venom alpha-toxins. FEBS Lett 349: 163–168.
34. Fletcher CM, Harrison RA, Lachmann PJ, Neuhoff D (1994) Structure of a soluble, glycosylated form of the human complement regulatory protein CD59. Structure 2: 185–199.
35. Kordis D, Gubensek F (2000) Adaptive evolution of animal toxin multigene families. Gene 261: 43–52.
36. Zupunski V, Kordis D, Gubensek F (2000) Adaptive evolution of animal toxin multigene families. Gene 261: 43–52.
51. Forouhar F, Huang WN, Liu JH, Chien KY, Wu WG, et al. (2003) Structural
50. Wang CH, Liu JH, Lee SC, Hsiao CD, Wu WG (2006) Glycosphingolipid-
48. Love RA, Stroud RM (1986) The crystal structure of alpha-bungarotoxin at
47. Corfield PW, Lee TJ, Low BW (1989) The crystal structure of erabutoxin A at
46. Sutcliffe MJ, Jose JA, Hyde EI, Lo X, Williams JA (1994) Three-dimensional
45. Chien KY, Chiang CM, Hsu YC, Yuen AA, Rule GS, et al. (1994) Two distinct
type of cardiotoxin as revealed by X-ray crystallographic analysis of their
interaction with zwitterionic phospholipid dispersions. J Biol Chem 269: 14473–
14483.
44. Dubovki PV, Dementieva DV, Bocharov EV, Utkin YN, Arseniev AS (2001)
Different interactions between MT7 toxin and the human muscarinic M1
receptor. Proc Natl Acad Sci U S A 98: 9209–9215.
43. Kini RM, Evans HJ (1989) Role of cationic residues in cytolytic activity:
modification of the effect of cibotxin from Naja nigricollis venom: its
structural similarity to coagulation factor Xa. Blood 94: 621–631.
42. Otwinowski Z, Minor W (1997) Processing of X-ray diffraction data collected
in oscillation mode. Methods in enzymology. Academic press. 307–326.
41. McCoy A (2007) Solving structures of protein complexes by molecular
replacement with Phaser. Acta Crystallogr D Biol Crystallogr 63: 32–41.
40. Larkin M, Blackshields G, Brown N, Chenna R, McGettigan P, et al. (2007)
Clustal W and Clustal X version 2.0. Bioinformatics 23: 2947–2948.
39. Gouet P, Courcelle E, Stuart D, Metoz F (1999) ESPript: analysis of multiple
sequence alignments in PostScript. Bioinformatics 15: 305–308.
38. Delano WL (2002) The PyMOL molecular graphics system.
37. Servent D, Antil-Delbeke S, Guillard C, Corringer PJ, Changeux JP, et al. (2000)
Molecular characterization of the specificity of interactions of various
neurotoxins on two distinct nicotinic acetylcholine receptors. Eur J Pharmacol 395: 197–204.
36. Sutcliffe MJ, Jose JA, Hyde EI, Lo X, Williams JA (1994) Three-dimensional
structure of the RGD-containing neurotoxin homologue deadroaspin. Nat Struct Biol 1: 802–807.
35. Kini RM, Chen KY, Wu WG, et al. (2003) Structural basis of membrane-induced cardiotoxin A3 oligomerization. J Biol Chem 278: 21899–21908.
34. Antil-Delbeke S, Gaillard C, Tamiya T, Corringer PJ, Changeux JP, et al. (2000)
Interaction of the P-type cardiotoxin with phospholipid membranes. Eur J Biochem 270: 2030–2046.
33. Liu JH, Lee SC, Hsiao CD, Wu WG (2006) Glycosphingolipid-
facilitated membrane insertion and internalization of cobra cardiotoxin. The
sulfate-cardiotoxin complex structure in a membrane-like environment
suggests a lipid-dependent cell-penetrating mechanism for membrane binding polypeptides. J Biol Chem 281: 656–667.
32. Antil-Delbeke S, Gaillard C, Tamiya T, Corringer PJ, Changeux JP, et al. (2000)
Interaction of the P-type cardiotoxin with phospholipid membranes. Eur J Biochem 270: 2030–2046.
31. Kini RM, Evans HJ (1989) Role of cationic residues in cytolytic activity:
modification of the effect of cibotxin from Naja nigricollis venom: its
structural similarity to coagulation factor Xa. Blood 94: 621–631.
30. Otwinowski Z, Minor W (1997) Processing of X-ray diffraction data collected
in oscillation mode. Methods in enzymology. Academic press. 307–326.
29. McCoy A (2007) Solving structures of protein complexes by molecular
replacement with Phaser. Acta Crystallogr D Biol Crystallogr 63: 32–41.
28. Larkin M, Blackshields G, Brown N, Chenna R, McGettigan P, et al. (2007)
Clustal W and Clustal X version 2.0. Bioinformatics 23: 2947–2948.
27. Gouet P, Courcelle E, Stuart D, Metoz F (1999) ESPript: analysis of multiple
sequence alignments in PostScript. Bioinformatics 15: 305–308.
26. Delano WL (2002) The PyMOL molecular graphics system.
25. Larkin M, Blackshields G, Brown N, Chenna R, McGettigan P, et al. (2007)
Clustal W and Clustal X version 2.0. Bioinformatics 23: 2947–2948.
24. Gouet P, Courcelle E, Stuart D, Metoz F (1999) ESPript: analysis of multiple
sequence alignments in PostScript. Bioinformatics 15: 305–308.
23. Delano WL (2002) The PyMOL molecular graphics system.
22. Larkin M, Blackshields G, Brown N, Chenna R, McGettigan P, et al. (2007)
Clustal W and Clustal X version 2.0. Bioinformatics 23: 2947–2948.
21. Gouet P, Courcelle E, Stuart D, Metoz F (1999) ESPript: analysis of multiple
sequence alignments in PostScript. Bioinformatics 15: 305–308.