Evolutionary Distance and Conserved Domain Analysis of Divergent Phylogenetic Lineages from Genus Naja

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Introduction

Naja Naja is one of the poisonous snakes in the genus Naja of Elapids family and commonly called Indian cobras and are mostly found in Asia and Africa. They are highly venomous species having a cocktail of chemicals that have different effects may be speed the heart rate, blood pressure and interfere with the normal function of the nervous system and potently block α7 homo-oligmeric neuronal AChRs causing paralysis. The aim of the present study is to identify the origin of neurotoxin, prediction of structure and function of neurotoxin by multiple sequences analysis and observing the conserved pattern of amino acid residues and to construct the phylogenetic tree for organizing evolutionary history of N. Naja of genus Naja from Elapidae family.

Abstract

Naja Naja is one of the poisonous snakes in the genus Naja of Elapids family; commonly called Indian cobras and are mostly found in Asia and Africa. They are highly venomous species having a cocktail of chemicals that have different effects may be speed the heart rate, blood pressure and interfere with the normal function of the nervous system and potently block α7 homo-oligmeric neuronal AChRs causing paralysis. The aim of the present study is to identify the origin of neurotoxin, prediction of structure and function of neurotoxin by multiple sequences analysis and observing the conserved pattern of amino acid residues and to construct the phylogenetic tree for organizing evolutionary history of N. Naja of genus Naja from Elapidae family.

Materials and Methods

Sources and sequence information of genus Naja neurotoxins

Thirteen species has taken from genus Naja of Elapidae family, in which targeted neurotoxins protein data were used to observe molecular resemble of related protein by phylogenic analysis (Table 1) [16,17].

| Accession | Description | Identity % | E Value | Total Score |
|-----------|-------------|------------|---------|-------------|
| P60814.1  | Naja naja   | 100%       | 5e-55   | 176         |
| Q8YGI4.1  | Naja naja   | 98%        | 1e-52   | 170         |
| O42255.1  | Naja sputatrix | 97%     | 5e-52   | 168         |
| P82935.2  | Naja kaouthia | 88%    | 2e-40   | 139         |
| Q9YG2.1   | Naja naja   | 72%        | 4e-35   | 125         |
| P25679.2  | Naja kaouthia | 85%    | 7e-31   | 114         |
| O93422.1  | Naja naja   | 72%        | 1e-27   | 106         |
| P01399.1  | Naja annulifera | 67%    | 2e-24   | 97.8        |
| P85520.1  | Naja oxiana | 69%        | 6e-24   | 96.3        |
| P25680.1  | Naja naja   | 68%        | 5e-23   | 94.0        |
| P01401.1  | Naja haje haje | 62%    | 3e-21   | 89.4        |
| P01400.1  | Naja melanoleuca | 62%  | 2e-20   | 87.0        |
| Q9W717.1  | Naja naja   | 54%        | 8e-20   | 86.3        |

Table 1: Sequences producing significant alignments.

Multiple sequence alignment of genus Naja neurotoxins

Multiple sequence alignment [MSA] is conducted by COBALT, which aligns thirteen neurotoxin protein sequences of similar Naja genus using a combination of distance matrix and approximate parsimony methods. Numerical setting method is used to study the relative entropy threshold, in bits, that must be met for an alignment column to be displayed in red. A larger number indicates higher degree of conservation. The relative entropy is computed as: Σi fi log2 (fi / pi), where i is residue type, fi is residue frequency observed in the multiple alignment column, and pi is the background residue frequency. Identity setting used for only columns with one residue type will be colored in red [18].

Construction of a phylogenetic tree for neurotoxin from genus Naja of Elapidae family

Phylogenetic analyses were performed by fast minimum evolution algorithm and Neighbor Joining algorithms to allow the reconstruction phylogenetic tree of the molecular evolutionary history of various aligned sequences that are useful to align highly evolved gene families clearing evolutionary relationships such as multiple actin proteins [19,20]. Trees were obtained by the methods fast minimum evolution algorithm and Neighbor Joining algorithms. Evolutionary distance is studied by Grishin (protein) model [21,22] and distance between two sequences modeled as expected fraction of amino acid substitutions per site given the fraction of mismatched amino acids in the aligned region and can be computed for fraction of mismatched amino acids larger than 0.75 [23-26].

Results and Interpretation

Evolutionary distance

This study, thirteen neurotoxin protein from genus Naja is

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RecName: Full=Neurotoxin-like protein NTL2; Flags: Precursor [Naja atra]

RecName: Full=Probable weak neurotoxin NNAM2; AltName: Full=TA-N9; Flags: Precursor [Naja atra]

RecName: Full=Probable weak neurotoxin NNAM3; Flags: Precursor [Naja atra]

RecName: Full=Weak neurotoxin 5; Short=Wtx-5; Flags: Precursor [Naja spatulata]

RecName: Full=Weak tryptophan-containing neurotoxin; Short=WTX; Flags: Precursor [Naja kaouthia]

RecName: Full=Weak toxin CM-8a [Naja kaouthia]

RecName: Full=Probable weak neurotoxin NNAMI; Flags: Precursor [Naja atra]

RecName: Full=Long neurotoxin homolog; AltName: Full=Kappa-cobrotoxin; Flags: Precursor [Naja atra]

RecName: Full=Oxiana weak toxin [Naja oxiana]

RecName: Full=Weak toxin CM-11 [Naja haje haje]

RecName: Full=Weak toxin HC11 [Naja enanilfera]

RecName: Full=Weak toxin CM-13b [Naja annulifera]

RecName: Full=Weak toxin CM-10 [Naja nivea]

RecName: Full=Neurotoxin-like protein NTL2; Flags: Precursor [Naja atra]

RecName: Full=Probable weak neurotoxin NNAM2; AltName: Full=TA-N9; Flags: Precursor [Naja atra]

RecName: Full=Probable weak neurotoxin NNAM3; Flags: Precursor [Naja atra]

RecName: Full=Weak neurotoxin 5; Short=Wtx-5; Flags: Precursor [Naja spatulata]

RecName: Full=Weak tryptophan-containing neurotoxin; Short=WTX; Flags: Precursor [Naja kaouthia]

RecName: Full=Weak toxin CM-9a [Naja kaouthia]

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RecName: Full=Weak toxin CM-10 [Naja nivea]
summarized to study the evolutionary distance. The identification of the origin of neurotoxin protein from genus *Naja*, multiple sequences analysis, observing the conserved amino acid residues and reconstruct the phylogenetic tree specify the evolutionary history, relationship of *N. Naja* a with different species (Table 1). Rectangle tree shows rectangular shaped rooted tree, where root is places in the longest edge. Fast minimum evolution algorithm produce un-rooted tree such as ones shown as radial or force in the tabs below. The rooted trees are created by placing a root in the middle of the longest edge (Figures 1-4). Slanted tree shows similar to rectangle, but with triangular tree shape. Neighbour Joining algorithms produce un-rooted tree such as ones shown as radial or force in the tabs below. The rooted trees are created by placing a root in the middle of the longest edge.

**MSA**

Multiple sequence alignment analysis shows columns with no gaps are colored in blue or red. The red color indicates highly conserved regions and blue indicates less conserved ones. The Conservation analysis can be used to select a threshold for determining which columns are colored in red (Figure 5). Multiple sequence alignment identify conserved motifs and to predict functional role in the variable sites as well as conserved sites show the sequence divergence profile of these neurotoxin proteins, which demonstrate the sequence enrichment strategy of these sequences for adaptation to different physiological systems. Here we observed that from all sequences of neurotoxin proteins that Cys(c), Thr (T), Asn (N) (Hydrophilic amino acid) Phe(F), Gly(G), Ala(A), Pro(P) (hydrophobic amino acid), Lys (K), Arg(R), Positive charged, Asp(D), Negative charged which is conserved in all peptides having a common ancestor. That all of these peptides share eight highly conserved cysteines which were involved in the formation of β-strands are almost conserved. Cysteine (C) is conserved in all sequences at 8 sites. Multiple sequence alignment is carried out by COBALT of *Naja* genus.

**Conserved domain analysis**

Molecular study of *N. Naja* shows conserved domains and having one of snake toxin superfamily with user query added Superfamily (Figures 6 and 7). Snake toxin domains are present in short and long
RecName: Full=Weak toxin S4C11 [Naja mel... 
RecName: Full=Weak toxin CM-13b [Naja an... 
RecName: Full=Oxiana Weak toxin [Naja ox... 
RecName: Full=Weak toxin CM-10 [Naja niv... 
RecName: Full=Long Neurotoxin homolog. A... 
RecName: Full=Probable weak neurotoxin N... 
RecName: Full=Weak toxin CM-9a [Naja kan... 
RecName: Full=Weak tryptophan-containing... 
RecName: Full=Weak neurotoxin 5; Short =W... 
RecName: Full=Neurotoxin-like protein NT... 
RecName: Full=Probable weak neurotoxin N... 

**Figure 4:** Force tree - Grishin (protein) model- showing Phylogeny of genus *Naja* with the help of rendering tree. Force tree is similar to radial, where nodes are pushed away from one another for better presentation.

**Figure 5:** Multiple sequence alignment by COBALT of genus *Naja*. Here columns with no gaps are colored in blue or red. The red color Cys(c),Thr(T),Asn(N) hydrophilic polar, Phe(F),Gly(G),Ala(A),Pro(P) hydrophobic nonpolar,Lys(K),Arg(R) Positive charged, Asp(D) Negative charge indicates highly conserved columns and blue indicates less conserved ones. The Conservation Setting can be used to select a threshold for determining, which columns are colored in red.
future, different neurotoxin may be converted in laboratories through protein engineering to design synthetic peptide vaccine that have a much positive role.

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