Indian Journal of Biotechnology
Vol 8, July 2009, pp 259-265

The saga of cytotoxin evolution–Switching of destructive role to a constructive role

Subhamay Panda[1*], Santamay Panda[2] and Jayanta Sinha[3]

[1*]Department of Biological Sciences, Gupta College of Technological Sciences, Asansol 713 301, India
[2]Department of Physics, B C College, Asansol 713 304, India
[3]Department of Zoology, B B College, Asansol 713 303, India

Received 30 May 2008; revised 27 November 2008; accepted 20 February 2009

Snake venom contains the toxin proteins, cytotoxins. Cytotoxins exert their effect upon the target cells by interacting with membrane lipids and proteins. Ultimate objective of a cytotoxin is to destroy the target cells. These cytotoxins contain cysteine residues responsible for disulphide linkage between them. Similar variety of peptides enriched with cysteine is also found in many other organisms. But interestingly, in those cases they never have a cell destructive function, in turn, they act to be cell-friendly. In this work, we analysed the cytotoxins and related peptides in terms of amino acid percentage profile, multiple sequence alignment, codon usage, isoelectric point determination, protein secondary structure prediction and phylogenetic tree construction through different softwares. Among all the interesting results, lysine profile was very much informative. High amounts of lysine are conserved in all the cytotoxins whereas in other related peptides it is in less numbers. Phylogenetic tree showed a stepwise dynamic evolution of these interesting molecules. This paper, therefore, showed that there is a great possibility to turn harmful natural peptides into a beneficial engineered molecule for the betterment of lives of mankind.

Keywords: Cytotoxin, cysteine, lysine, molecular evolution, phylogenetic analysis, snake

Introduction

Evolution is a dynamic process as evidenced from a journey through different biological molecules. With the completion of the Human Genome Project there is a clear indication that a higher vertebrate genome is not a junkyard but it significantly carries the footsteps, which are necessary for this journey. Different primitive molecules evolved in the vertebrate genome out of a necessity. In this paper, we searched the answer why the cytotoxins found in snakes, evolved and altered in human and some other animals for a novel function.

It is believed that the snakes started their journey on earth 100-120 million years ago during the lower cretaceous period[1]. With the most interesting body anatomy, snakes also possess a fascinating weapon i.e., its venom. Secreted in the venom gland, venom is a colourless to dark amber fluid with a viscosity range of 1.5-2.5[2]. Snake use this venom in defence as well as in assault[3]. Snake venom toxins comprise of large number of homologous proteins, cytotoxins (cardiotoxins). In many cases, they represent more than fifty per cent of the whole venom[4]. The name cytotoxin is given because they exert toxic effect upon all types of excitable and non-excitable cells[5]. These proteins are also cardiotoxic[6]. Cytotoxins are comprised of a single polypeptide chain of 60-62 amino acid residues[7]. Molecular weight of snake venom cytotoxins ranges from 6.5-7.0 kDa, made up mainly of β-sheet proteins cross-linked by four disulfide bridges[7]. Highly basic cytotoxins show a wide range of biological functions, for example, lysis of erythrocytes, contraction of cardiac muscles, and selective killing of certain types of tumour cells[8]. Cytotoxins exert their action upon target cells by interaction with membrane lipids of the cell[9,10]. Cytotoxicity of cytotoxins not only occurs by interaction with membrane lipids but also due to their indirect inhibition of Na^+, K^+, ATPase and this gives more toxic characteristics to the peptides[11].

Cytotoxins belong to a broad superfamily of three finger toxins[12]. The family members of this multigene family contain 60-74 amino acid residues highly enriched with disulfide bonds, 4 disulfide bonds being conserved in all the peptides[13]. These proteins acquire the same kind of protein folding having characteristic three loops extending from a central core, which looks like three fingers of a hand and hence the name three finger toxin[14,15].

*Author for correspondence:
Tel: 91-3412-213120; Fax: 91-3412-213120
E-mail: subhamay.panda@gmail.com;
subhamay_panda@rediffmail.com

Snake cytotoxins are critically found in snakes but other related peptides are also being found in many other organisms, such as peptides used in complement system (CD59), lymphocytes (Ly-6) and also in brain (Lynx 1), etc[16,17]. In this study, we closely analysed and compared the cytotoxins with other related peptides in different aspects such as amino acid profile, secondary structure, isoelectric point, codon usage and phylogenetic tree analysis. We found that a dynamic molecular evolution is happening most probably from a common ancestor molecule in different organisms encompassing different functions of cytotoxins and other related proteins. The destructive function of cytotoxins has evolved into different molecules having much cell-friendly functions.

System and Methods

Amino Acid Sequence Searching

The amino acid sequences of proteins were collected from Swiss-Prot/TrEMBL (http://www.expasy. org/ sprot) database. The detailed information regarding sequences i.e. accession number in the database, molecule name and function. For better understanding a sequence ID code was given to each molecule (Table 1).

Amino Acid Percentage Profile

Amino acid percentage analysis was carried out using the program MEGA version 3.1[18].

Multiple Sequence Alignment

Multiple sequence alignment is done with ClustalW software.

Codon Usage

Codon usage pattern of two important amino acids, namely lysine and cysteine was studied and predicted by Emboss program[19].

Isoelectric Point Determination and Prediction of Secondary Structures

Isoelectric point determination of each peptide chain was done using an algorithm and program described by Dr Luca Toldo (http://www.embl-

Table 1—Swiss-prot Acc. no. and sequence ID of cytotoxins and related proteins and their corresponding functions

No	Sequence ID	Swiss-prot Acc. no.	Organism	Functions
1.	CX1.a	P01468	*Naja pallida*	Shows cytolytic activity
2.	CX1.b	P01467	*N. mossambica*	Shows cytolytic activity
3.	CX1.c	P01455	*N. haje annulifera*	Shows cytolytic activity
4.	CX6	P80245	*N. pallida*	Shows cytolytic activity
5.	CX3	P24777	*Hemachatus haemachatus*	This protein lyses RBC and has cardiotoxic & hypotensive activities
6.	CX2	P24776	*H. haemachatus*	This protein lyses RBC and has cardiotoxic & hypotensive activities
7.	PJ10	Q71TU4	*Plethodon jordani*	Pheromon & receptor activation
8.	XEN1	Q09022	*Xenopus laevis*	Lacks α-neurotoxic activity, channel protein activation
9.	CD59	P58019	*Mus musculus*	Potent inhibitor of the complement membrane attack complex (MAC) action
10.	LMP1	Q9UAD1	*Eptatretus stoutii*	Acts upon complement system
11.	HEP21	Q8AV77	*Gallus gallus*	Related to Ly-6 protein
12.	GML	Q99445	*Homo sapiens*	May play a role in the apoptotic pathway or cell-cycle regulation induced by p53 after DNA damage
13.	SLR.M	Q9Z0K7	*M. musculus*	T cell activation & cell to cell adhesion
14.	SLRH	P55000	*H. sapiens*	Has an antitumour activity
15.	SLR2	Q86SR0	*H. sapiens*	T cell differentiation/activation
16.	LX.M	Q9WVC2	*M. musculus*	Seems to modulate nicotinic acetylcholine receptors
17.	LX.Mc	P61050	*Macaca mulatta*	Seems to modulate nicotinic acetylcholine receptors
18.	LX.H	Q9BZG9	*H. sapiens*	Seems to modulate nicotinic acetylcholine receptors

CX1.a: Cytotoxin 1; CX1.b: Cytotoxin 1; CX1.c: Cytotoxin 1; CX6: Cytotoxin 6; CX3: Cytotoxin3;
CX2: Cytotoxin 2; PJ10: Pheromone Pj-10 isoform F [Precursor]; XEN1: Xenoxin 1[Precursor]; CD59: CD59B glycoprotein [Precursor]; LMP1: Leukocyte membrane protein 1; HEP21: Hep21 protein [Precursor]; GML: Glycosyl-phosphatidylinositol-anchored molecule-like protein[Precursor]; SLR.M: Secreted Ly-6/uPAR-related protein 1 [Precursor]; SLRH: Secreted Ly-6/uPAR-related protein 1 [Precursor]; SLR2: Secreted Ly6/uPAR related protein 2;LX.M: Ly-6/neurotoxin-like protein 1 [Precursor]; LX.Mc: Ly-6/neurotoxin-like protein 1 [Precursor]; LX.H: Ly-6/neurotoxin-like protein 1 [Precursor].

heidelberg.de/cgi/pi-wrapper.pl)[20]. Chofas program was used for secondary structure prediction of protein in terms of helix, turns and sheet (http://bioinfo.hku.hk/FASTA/chofas.htm)[21].

Phylogenetic Tree Construction

Neighbour-joining analysis was carried out using the program MEGA (3.1), using Poisson-corrected distances. This helped in the construction of unrooted phylogenetic trees represented as Phylogram and Radial tree[18].

Results

Various cytotoxins and related proteins were analysed through various tools. The results of different analyses are given below.

Amino Acid Percentage Profile

Results of amino acid percentage are given in Table 2. One of the most important features of the amino acid profile was the conservation of cysteine amino acid. Lysine was detected in high amounts in cytotoxins, though in other sequences it was quite low.

Multiple Sequence Alignment

In Table 3, the variable sites as well as conserved sites show the sequence divergence profile. First position leucine (L) is conserved in all peptides except in SLR.M and SLR2. Lysine (K) is highly conserved only in CX1.a, 1.b, 1.c; CX6; CX3 and CX2. Cysteine (C) is conserved in all sequences at 7 sites. Asparagine (N) is also conserved in all sequences in a single site. This suggests that most probably cytotoxins and other related peptides have a common ancestor molecule in terms of cysteine and asparagine.

Codon Usage

Codon usage profile clearly indicates that cysteine is conserved in all the sequences (Fig. 1a). In the case of cysteine, the codon usage and amino acid percentage did not vary much as compared to lysine in different sequences. Codon usage and amino acid percentage of lysine is low in SLR and Lynx molecule of higher vertebrates (Fig. 1b). Codon usage and amino acid percentage of lysine is very high and stable in cytotoxins (CX) whereas highly variable in other molecules.

Isoelectric Point Determination and Prediction of Secondary Structures

Analysis of isoelectric point (pI) in Fig. 2a clearly indicates that cytotoxins have a very high pI (8.77-9.47) but having a narrow range whereas other related peptides are not so high in their pI (6.04-8.38).

Table 2—Amino acid percentage profile of various cytotoxins and related proteins

Sequence ID	Amino acid																			
	Ala	Cys	Asp	Glu	Phe	Gly	His	Ile	Lys	Leu	Met	Asn	Pro	Gln	Arg	Ser	Thr	Val	Trp	Tyr
CX1.a	3.33	13.3	3.33	0	1.67	3.33	0	5	15	8.33	6.67	6.67	10	1.67	3.33	3.33	5	5	1.67	3.33
CX1.b	3.33	13.3	1.67	0	1.67	3.33	0	5	15	8.33	6.67	8.33	10	1.67	3.33	3.33	5	5	1.67	3.33
CX1.c	1.67	13.3	3.33	1.67	1.67	3.33	1.67	1.67	15	6.67	3.33	5	8.33	0	1.67	6.67	6.67	13.3	1.67	3.33
CX6	6.67	13.3	3.33	0	3.33	3.33	0	3.33	13.3	8.33	3.33	6.67	8.33	1.67	3.33	3.33	3.33	10	0	5
CX3	0	13.1	4.92	1.64	1.64	3.28	1.64	3.28	16.4	9.84	4.92	4.92	8.2	0	1.64	6.56	8.2	8.2	0	1.64
CX2	1.64	13.1	3.28	1.64	1.64	3.28	1.64	3.28	19.7	9.84	3.28	6.56	8.2	0	1.64	4.92	6.56	8.2	0	1.64
PJ-10	6.17	9.88	11.1	13.6	2.47	6.17	1.23	2.47	2.47	9.88	2.47	2.47	3.7	3.7	1.23	3.7	7.41	6.17	0	3.7
XEN1	7.14	10.7	1.19	5.95	3.57	5.95	0	5.95	9.52	10.7	5.95	4.76	1.19	3.57	3.57	3.57	10.7	4.76	0	1.19
CD59B	6.98	9.3	3.1	2.33	3.88	4.65	0	3.88	5.43	15.5	1.55	6.98	1.55	5.43	3.88	10.1	4.65	6.2	1.55	3.1
LMP1	5.83	7.5	4.17	4.17	5.83	6.67	1.67	5	11.7	9.17	1.67	3.33	0.83	3.33	0.83	7.5	10.8	8.33	0	1.67
HEP21	6.54	10.3	5.61	7.48	1.87	6.54	0.93	2.8	6.54	10.3	0.93	2.8	1.87	2.8	6.54	5.61	7.48	7.48	0.93	4.67
GML	7.59	6.96	2.53	6.33	3.8	3.16	1.27	6.33	2.53	11.4	3.8	7.59	6.33	1.27	5.7	6.33	5.7	6.96	1.27	3.16
SLR.M	10.9	9.09	3.64	4.55	6.36	6.36	1.82	2.73	2.73	8.18	4.55	3.64	5.45	1.82	3.64	7.27	7.27	5.45	2.73	1.82
SLR.H	10.7	10.7	3.88	5.83	2.91	2.91	0.97	2.91	2.91	7.77	3.88	1.94	4.85	1.94	4.85	10.7	9.71	6.8	1.94	1.94
SLR2	8.25	10.3	4.12	2.06	1.03	9.28	5.15	4.12	1.03	14.4	2.06	2.06	4.12	4.12	3.09	8.25	9.28	5.15	1.03	1.03
LX.M	11.2	8.62	1.72	1.72	5.17	4.31	2.59	0	3.45	11.2	5.17	2.59	6.03	1.72	3.45	5.17	10.3	7.76	0.86	6.9
LX.Mc	10.2	8.47	3.39	0.85	2.54	3.39	1.69	0.85	2.54	14.4	5.08	3.39	7.63	1.69	2.54	6.78	10.2	7.63	0.85	5.93
LX.H	9.48	8.62	3.45	0.86	1.72	5.17	1.72	1.72	2.59	14.7	5.17	3.45	6.9	1.72	3.45	4.31	12.1	6.03	0.86	6.03

Table 3—Conserved site as well as variable site analysis of different amino acids in various peptides

CX1.a	L K C N Q L I P P T C P K G K L C Y K M K G C I D V C P K S S L L I K Y M C C N T D K C N
CX1.b	L K C N Q L I P P T C P K G K L C Y K M K G C I D V C P K S S L L I K Y M C C N T N K C N
CX1.c	L K C H K L V P P T C P E G K L C Y K M K G C I D V C P K N S A L V K Y V C C S T D K C N
CX6	L K C N Q L I P P T C A A G K L C Y K M K G C I D V C P K S S L L V K Y V C C N T D R C N
CX3	L K C H K L V P F T C P D G K L C Y K M K G C T D T C P K S S L L V K V V C C K T D K C N
CX2	L K C H K V V P F T C P E G K L C Y K M K G C T D A C P K S S L L V N V M C C K T D K C N
PJ_10	L Q C N L D G G T E C P S D D A C V H Y Q E C T A A E D D E P E Y P M V Q C C S E D L C N
XEN1	L K C V L Q A N G E C A K E D K C L T L S T C T T M K I M S L P G E Q I T C C E G N M C N
CD59B	L K C Y C F Q F V T C S P N L S C L Y A S D C N S N Y I M D V A G I Q S K C C Q W G L C N
LMP1	L Q C Y Q E K D K N C S S G E Q C A S V T M C K K N V T I L D V T G T I K C C K K D L C N
HEP21	L Q C K C K Y K I T C E R R E R C A I I Q G C T S N C G R S R L T S R Y S C C E T D L C N
GML	L R C H C A V I N V C P Y H I R C M T I Y N C T N N C T F T N S F Y W V C C C N S M V C N
SLR.M	F R C Y C E Q P T Q C K M E D A C K T V N S C S S S C L A I G V A H P V F C C F R D L C N
SLR.H	L K C Y C K E P M R C K P E D A C M T T N S C S S S C V A I G A A H L I F C C F R D L C N
SLR2	I W C H C T G F G R C L R D S H C V T T V M C H I G C P D L G P Y V S I A C C Q T S L C N
LX.M	L E C H C A Y N G R C P A M A Y C M T T R S C V P S C F E S K H A S A T S C C Q Y Y L C N
LX.Mc	L D C H C A Y N G R C P A M V Y C M T T S S C V P S C F E S K H A S T T S C C Q Y D L C N
LX.H	L D C H C A Y N G R C P A M V Y C M T T S S C V P R C F E S K H A S T T S C C Q Y D L C N

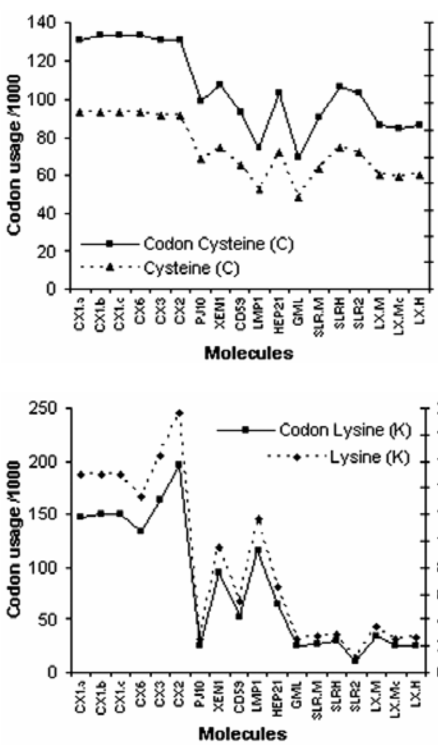

Fig. 1—**a.** Codon usage and percentage of conserved cysteine amino acid in various cytotoxins and related proteins; **b.** Codon usage and percentage of lysine amino acid in various cytotoxins and related proteins.

An interesting point to be noted in the secondary structure of cytotoxins and other related proteins is

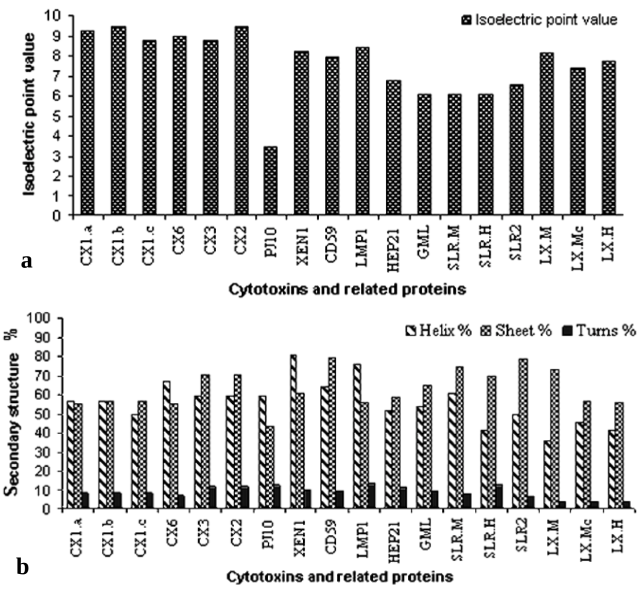

Fig. 2—**a.** Isoelectric point of cytotoxins and related proteins; **b.** Secondary structures of cytotoxins and related proteins.

that in cytotoxins the helix percentage and turn percentage is higher than Lynx-1 molecules (Fig. 2b). In cytotoxins, the helix percentage ranges from 50 to 66.7 whereas in Lynx-1 it ranges from 36.2 to 45.8. Turns also occur in high percentage i.e. 8.3-11.5 in cytotoxins than Lynx molecules (3.4%). Highest percentage of helix, sheet and turns are found in LMP1, CD59 and SLR.H molecules, respectively.

Interestingly it has been noted that two cytotoxins, namely CX3 and CX2 isolated from *Hemachatus haemachatus* showed exactly same helix sheet and turns percentage. CX1.a, 1.b, 1.c have the same turn percentage. All the Lynx molecules have same percentage of turns.

Phylogenetic Tree Construction

Phylogenetic tree of various cytotoxins and related peptides shows that these cytotoxins are a totally separate entity in terms of phylogenetic tree position (Fig. 3a & b). CX1.a, 1.b, 1.c belong to the same group (all from *Naja* sp*)* whereas CX3 and CX2 from *Hemachatus* sp. are in a separate group, but bear a common branch point. PJ10, LMP1 and HEP21 are in the same group. LMP1 and HEP21 separated from a common node. XEN1, CD59, SLR.M and SLR.H are very close to each other and rise from a common node. Whereas SLR2, GML, LX.M, LX.Mc and LX.H belong to the same group, both LX.Mc and LX.H belonging to *Macaca mulatta* and *Homo sapiens*, respectively are not very distant from each other and points towards their phylogenetic proximity.

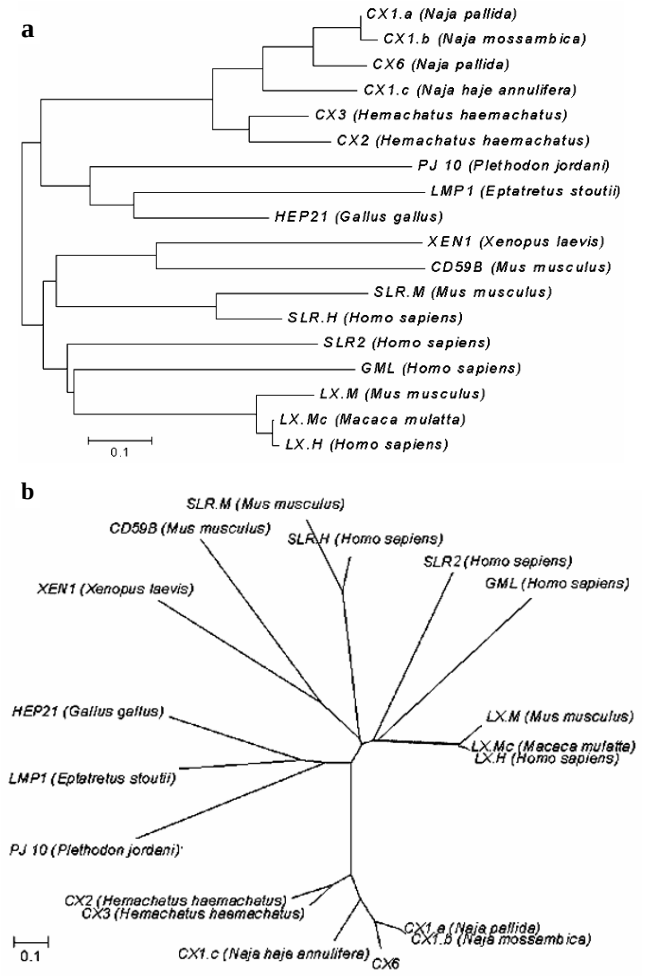

Fig. 3—**a.** Phylogram of various cytotoxins and related proteins; **b.** Radial tree of various cytotoxins and related proteins. Bar represents distance scale.

Discussion

In this paper, we have shown that cytotoxins and related peptides are similar in their cysteine profiles but significantly different in terms of other amino acid composition. The amino acid percentage profile clearly indicates that there is a conservation strategy for cysteine, because this amino acid is responsible for disulfide bridging which is actually essential for the maintenance of protein folding pattern. But strikingly there is an excessive variation in lysine profile. In cytotoxins, lysine ranges from 15% to 19.7%. But in other related peptides it is quite low. We know that lysine has the ability to form ionic bonds with other charged species in the cell[22]. The position of this amino acid on the outer side of the molecule (Fig. 4) is important for tight attachment of this molecule to various kinds of component of the cell. Irreversible depolarisation of the cell membranes is due to the attachment of cytotoxins to the target cell[23]. Lethal potency of cytotoxin (cardiotoxin) is governed by an invariant lysine residue at position 44

Fig. 4—Structure of cytotoxin 6(CX6) and lysine position in the protein. Dashed line area includes lysine residues (PDB ID 1UG4). PDB File downloaded from Protein Data Bank file format and visualized by Raswin software.

in these peptides[24]. Cytotoxins first bind to lipid phase of axonal membrane and subsequently inhibit the sodium-potassium activated adenosine triphosphatase[9]. Lysine in the scorpion neurotoxin is responsible for toxicity[25]. The basic residues of cytotoxins (cardiotoxins) are mainly the lysines, which interact specifically with the negative polar head groups of phospholipids of the membrane[26]. Another study revealed that lysines are a critical factor for the cytolytic activity of cytotoxins (cardiotoxins), initiated through the binding of the hydrophobic clusters (formed by lysines) on the cytotoxins to the hydrophobic moiety of membrane phospholipids[27]. The cytotoxins bind and exert their effects on cells by destroying the cell membrane may be in a non-specific detergent like manner[28]. Thus this study reveals that higher percentage of lysine in the cytotoxins may be related to toxicity of these molecules. In contrast, decrement of lysine percentage presumably may have a role in toxicity reduction.

Cysteine amino acids are found to be conserved at seven sites of all the peptide sequences studied, including the cytotoxins. Presence of cysteine residues points towards their involvement in disulfide bond formation, which gives the characteristic secondary structure to these molecules.

The observations on codon usage pattern for the two important amino acids, lysine and cysteine, revealed that cysteine being the conserved amino acid sequence shows least deviation for the cytotoxin and lynx molecules, whereas the lysine molecules are fully conserved in the molecules of cytotoxins and points towards the structural and functional similarity of these molecules.

Isoelectric profile proves that the cytotoxins, which are highly toxic, are basic in nature. Such aspect is seen in cytotoxins such as CX1.a, 1.b, 1.c, CX3 and CX2. But it evolves to cell-friendly molecules such as SLR2, GML, LX.M, LX.Mc and LX.H, which turns to be less basic or sometimes acidic. It may happen that reduction of basicity of a protein is important to make it act less toxic and thus have a more cell-friendly role.

On the other hand, Lynx-1 of humans, macaca and mouse proved to be a novel modulator of nicotinic receptor i*n vitro*[29]. In cytotoxins, the lysine amount is about 15% but in Lynx-1 it is suddenly decreased to merely 2.5%. An intermediate molecule, Xenoxin-1, also supports this view. It is less toxic than the cytotoxins and have a property to activate dihydropyridine sensitive Ca^{+} channels in mammalian epithelial cells[30] and this molecule has an intermediate lysine percentage of about 9.52. Earlier studies showed that cytotoxins have specific cellular receptors in the cell[31-33]. Lynx-1 and Xenoxin-1 also act as receptor specific modulators.

Phylogram of various cytotoxins and related peptides supports that all the cytotoxins belong to the same group. High lysine amount most probably plays an important role in governing the specified pattern. This property is reflected through their similar structure and amino acid quality putting them in the same group in the phylogram. Less basicity and more of cell-friendly function makes other related peptides distant from cytotoxins.

The results obtained in the present study hint towards a dynamic molecular level evolution from cytotoxin to other related peptides (Lynx-1, Xenoxin-1, etc.). The destructive role of a toxic molecule is switched into a much cell-friendly role.

This evolution pattern suggests that medicinal importance of many peptides may rise by virtue of active protein engineering. In future, different natural toxin molecules may be converted in laboratories through protein engineering to molecules that have a much positive role.

References

1 Harris J B, Phospholipases in snake venoms and their effects on nerve and muscle, in *Snake toxins*, edited by A L Harvey (Pergamon Press, New York) 1991, 91-129.

2 Devi A, The protein and non-protein constituents of snake venoms, in *Venomous animals and their venoms*, edited by W Bucherl, E Buckley and V Deulofeu (Academic Press, NewYork) 1968, 119-165.

3 Hider R C, Karlsson E & Namiranian S, Separation and purification of toxins from snake venoms, in *Snake toxins*, edited by A L Harvey (Pergamon Press, New York) 1991, 1-34.

4 Louw A I, Snake venom toxins. The complete amino acid sequence of cytotoxin VII4 from the venom of *Naja mossambica mossambica*, *Biochem Biophys Res Commun,* 58 (1974) 1022-1029.

5 Harvey A L, Cardiotoxins from cobra venoms: Possible mechanisms of action, *J Toxicol Toxin Rev*, 4 (1985) 41-69.

6 Sarkar N K, Action mechanism of cobra venom, cardiotoxin and allied substances on muscle contraction, *Proc Soc Exp Biol Med*, 78 (1951) 469-471.

7 Kumar T K S, Jayaraman G, Lee C S, Arunkumar A I , Sivaraman T *et al*, Snake venom cardiotoxins: Structure, dynamics, function and folding, *J Biomol Struct Dyn*, 15 (1997) 431-463.

8 Dufton M J & Hider R C, The structure and pharmacology of elapid cytotoxins, in *Snake toxins,* edited by A L Harvey (Pergamon Press, New York) 1991, 259-302.

9 Vincent J P, Schweitz H, Chicheportiche R, Fosset M, Balerna M *et al*, Molecular mechanism of cardiotoxin action on axonal membranes, *Biochemistry*, 15 (1976) 3171-3175.

10 Bougis P, Rochat H, Pieroni G & Verger R, Penetration of phospholipid monolayers by cardiotoxins, *Biochemistry*, 20 (1981) 4915-4920.

11 Chiou S H, Hung C C, Huang H C, Chen S T , Wang K T *et al*, Cardiotoxins and cobratoxins isolated from Taiwan cobra, *Biochem Biophys Res Commun,* 206 (1995) 22-32.

12 Kini R M, Molecular folds with multiple missions: Functional sites in three finger toxins, *Clin Exp Pharmacol Physiol*, 29 (2002) 815-822.

13 Endo T & Tamiya N, Current view on the structure-function relationship of postsynaptic neurotoxins from snake venoms, *Pharmacol & Ther*, 34 (1987) 405-512.

14 Me´nez A, Functional architectures of animal toxins: A clue to drug design? *Toxicon*, 36 (1998) 1557-1572.

15 Tsetlin V, Snake venom α-neurotoxins and other 'three-finger' proteins, *Eur J Biochem*, 264 (1999) 281-286.

16 Fleming T J, Ohigin C & Malek T R, Characterization of two novel Ly-6 genes. Protein sequence and potential structural similarity to α-bungarotoxin and other neurotoxins, *J Immunol*, 150 (1993) 5379-5390.

17 Gumley T P, McKenzie I F C & Sandrin M S, Tissue expression, structure and function of the murine Ly-6 family of molecules, *Immunol Cell Biol*, 73 (1995) 277-296.

18 Kumar S, Tamura K & Nei M, MEGA3: Integrated software for molecular evolutionary genetics analysis and sequence alignment, *Briefings in Bioinformatics*, 5 (2004) 150-163.

19 Bleasby A, Rice P & Longden I, EMBOSS: The European molecular biology open software suite, *Trends Genet,* 16 (2000) 276-277.

20 Sinha J & Chatterjee P, Cysteine protease, a factor for leishmanial virulence: An *in silico* analysis, *Sajosps*, 3 (2003) 102-109.

21 Chou P Y & Fasman G D, Empirical predictions of protein conformations, *Annu Rev Biochem*, 47 (1978) 251-276.

22 Karp G, *Cell and molecular biology concepts and experiments,* 3rd edn (John Wiley and Sons, New York) 2002, 53-54.

23 Lee C Y, Chang C C, Chiu T H, Chiu P J S, Tseng T C *et al*, Pharmacological properties of cardiotoxin isolated from Formosan cobra venom, *Naunyn-Schimedberg's Archi Pharmacol*, 259 (1968) 360-374.

24 Jayaraman G, Kumar T K S, Tsai C C, Srisailam S, Chou S H *et al*, Elucidation of the solution structure of cardiotoxin analogue V from the Taiwan cobra (*Naja naja atra*) – Identification of structural features important for the lethal action of snake venom cardiotoxins, *Protein Sci*, 9 (2000) 637-646.

25 Sampieri F & Habersetzer-Rochat C, Structure-function relationships in scorpion neurotoxins: Identification of the supperreactive lysine residue in toxin I of *Androctonus australis* Hector, *Biochim Biophys Acta*, 535 (1978) 100-109.

26 Dufourcq J & Faucon J F, Specific binding of a cardiotoxin from *Naja mossambica mossambica* to charged phospholipids detected by intrinsic fluorescence, *Biochemistry,* 17 (1978) 1170-1176.

27 Gilquin B, Roumestand C, Zinn-Justin S, Ménez A & Toma F, Refined 3-dimensional solution structure of a snake cardiotoxin: Analysis of the side-chain organization suggests the existence of a possible phospholipid binding site, *Biopolymers*, 33 (1993) 1659-1675.

28 Me´nez A, Gatineau E, Roumestand C, Harvey A L, Mouawad L *et al* , Do cardiotoxins possess a functional site? Structural and chemical modification studies reveal the functional site of the cardiotoxin from *Naja nigricollis Biochimie,* 72 (1990) 575-588.

29 Miwa J M, Ibanez-Tallon I, Crabtree G W, Sanchez R, Sali A *et al*, Lynx1, an endogenous toxin-like modulator of nicotinic acetylcholine receptors in the mammalian CNS, *Neuron*, 23 (1999) 105-114.

30 Bennett H P J, James S, Macleod R J & Lambesis P, Isolation of a member of the neurotoxin/cytotoxin peptide family from *Xenopus laevis* skin which activates dihydropyridine-sensitive Ca^{2+} channels in mammalian epithelial cells, *J Biol Chem,* 273 (1998) 20046-20051.

31 Thelestam M & Mollby R, Classification of microbial, plant and animal cytolysins based on their membrane damaging effects on human fibroblasts, *Biochim Biophys Acta,* 557 (1979) 156-169.

32 Takechi M, Tanaka Y & Hayashi K, Cytolytic activity of cytotoxin isolated from Indian cobra venom against experimental tumour cells, *Biochem Int*, 11 (1985) 795-800.

33 Takechi M, Tanaka Y & Hayashi K, Binding of cardiotoxin analogue III from Formosan cobra to FL cells Le, *FEBS Lett*, 205 (1986) 143-146.