SCORPION NEUROTOXINS: A FAMILY OF HOMOLOGOUS PROTEINS

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1. Introduction

Eleven neurotoxins have been purified from the venoms of three scorpion sub-species, *Androctonus australis Hector, Buthus occitanus tunetanus* and *Leiurus quinquestriatus quinquestriatus* [1]. These proteins all consist of a single polypeptide chain of 57 to 66 amino acid residues cross-linked by four disulfide bridges. The complete amino acid sequence of toxins I and I′ of *A. australis Hector* has been recently determined [2, 3]. In this communication we report the sequence of the first 22 to 26 amino acid residues from the *N*-terminal end of six additional neurotoxins. It is shown that scorpion neurotoxins form a new set of homologous proteins. Furthermore, this set can be divided in three subgroups when additional amino acid sequence homologies and specific toxities are taken into account.

2. Materials and methods

The pure scorpion neurotoxins were obtained according to Miranda et al. [1]. Reduction by 2-mercaptoethanol and quantitative conversion of the cysteine residues thus formed into *S*-methylcysteine residues were performed as previously described [4]. *N*-Terminal amino acid sequence determinations were carried out on 0.25 to 0.70 μmole of the reduced and *S*-methylated proteins by the sequenator procedure of Edman and Begg [5].

3. Results and discussion

Fig. 1. shows the *N*-terminal sequence, 22 to 26 amino acid residues in length, of the eight neurotoxins studied. These proteins were obtained either from the same sub-species or from scorpions of different genera. Several homologous sequences are obvious. If it is assumed that in toxins AI′, AI and AIII a deletion of two amino acid residues has occurred following Pro-19 one can align the four first half-cystine residues in all neurotoxins. As far as the three-dimensional structure is concerned, this gap may be compensated for by the peculiar steric properties of the sequence Pro-Pro at positions 18 and 19. It is thus not unlikely that the pairing of half-cystine residues is the same in all eight neurotoxins. Invariant residues other than half-cystine residues occupy positions 3, 5, 6 and 11. In fig. 1 the dotted line surrounds amino acid sequences common to the eight molecules if positions 2, 4, 7, 13, and 14, where a single conservative amino acid substitution has occurred, are included. These changes (Lys→Arg, Gly or Val→Ala, Val→Thr, Tyr→Phe) will not alter the charge of this part of the molecule.

Since scorpion neurotoxins show the same pharmacological activity [6–10] and since their overall amino acid composition, in spite of some similarities, are rather different [1], it is probable that the *N*-terminal sequence which shows a large degree of homology might play an important role in the biological function.

It is possible to divide the neurotoxins into 3 groups according to similarities both in structure and specific

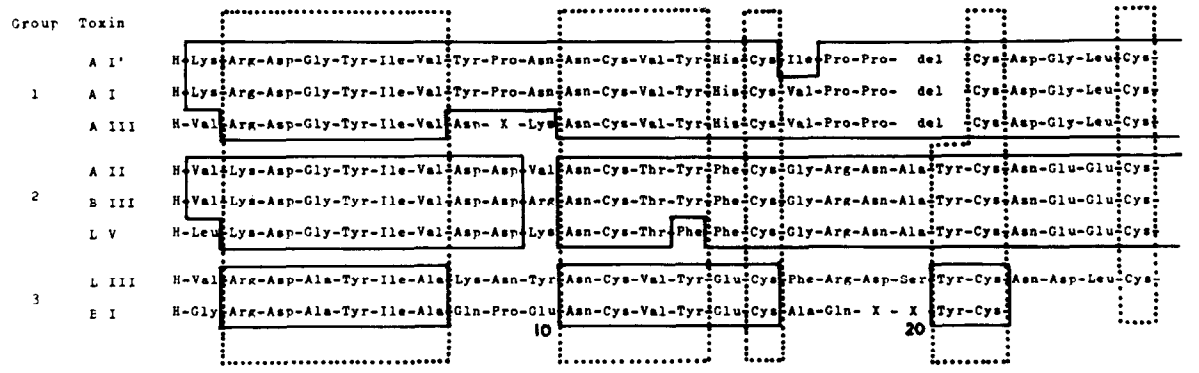

Fig. 1. *N*-Terminal amino acid sequence of scorpion neurotoxins. AI, AI′, AII and AIII, toxins of *Androctonus australis Hector*; BI and BIII, toxins of *Buthus occitanus tunetanus*; LIII and LV, toxins of *Leiurus quinquestriatus quinquestriatus*. del, deletion; X, not determined. The horizontal full-lined contours surround sequences common to toxins of each group. The vertical dot-lined contours correspond to quasi-invariant residues of the eight toxins.

toxicity (table 1). Group 1 would then comprise toxins AI, AI′ and AIII, group 2 toxins AII, BII and LV and group 3 toxins BI and LIII. Groups 1 and 2, which mainly differ by a deletion of two amino acid residues following Pro-19 consist of very toxic proteins. Toxins of group 3 are 3- to 10-times less active than toxins of groups 1 and 2, and show a lesser degree of homology with the other two groups.

If it is assumed that the *N*-terminal sequence plays a major role in the biological activity of scorpion toxins, the nature of the amino acid residue in position 10 might be of importance. In group 1 toxins AI and AI′ have an identical specific toxicity whereas toxin AIII is less active. This might be related to the change of an asparagine residue in AI and AI′ by a lysine residue in AIII. In group 2 an arginine residue in BIII and a lysine residue in LV in position 10 are replaced by a valine residue in AII which is 2- to 2.5-times more potent than BIII and LV, respectively. Therefore it appears that in groups 1 and 2 the replacement of a neutral residue in position 10 by a basic residue might be associated with a decrease of the specific toxicity.

Some amino acid residues are found in the same position in two groups but not in the third. However, the matching groups are not always the same. Thus arginine, valine and leucine occur in positions 2, 13 and 25, respectively, in groups 1 and 3 and not in group 2. Again, glycine and valine are found in positions 4 and 7, respectively in groups 1 and 2, but not in group 3. Finally, tyrosine and asparagine in positions 21 and 23 are common only to groups 2 and 3. These similarities indicate that the three groups represent evolutionary lines diverging from a common ancestry. It is an interesting possibility that a toxin much closer in structure to the ancestral may be produced by a species now living.

Table 1
Specific toxicity of scorpion toxins [1].

Toxin	LD_{50}/mg
AI′	2,985
AI	2,985
AIII	2,210
AII	5,539
BIII	2,443
LV	2,000
LIII	788
BI	548

For nomenclature of toxins see fig. 1. LD_{50}, lethal dose 50% for the 20 g mouse.

Snake venom neurotoxins form another family of homologous basic proteins of small molecular weight [11–15]. However these proteins differ from scorpion neurotoxins by their different pharmacological activity and primary structure. In contrast to scorpion toxins the specific toxicities of known snake toxins are very similar. This suggests that the comparison of primary structures of scorpion toxins could be more profitable in the study of structure-activity relationships.

Acknowledgements

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