# AMINO-ACID COMPOSITION AND TERMINAL AMINO ACIDS OF THE TOXINS OF THE VENOM OF THE CENTRAL ASIAN COBRA

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From the venom of the central Asian cobra we have isolated two neurotoxins the homogeneity of which has been shown by electrophoresis, disc electrophoresis, and rechromatography [1, 2]. The present paper gives the results of a determination of the amino-acid composition and terminal amino acids of these toxins.

The amino-acid compositions of the two toxins of the venom of N. oxina Eich. were compared with those of the toxins of the venoms of N. nigricollis and N. n. atra (Table 1). In toxin (I) four alanine residues were found, which are not present in the composition of the other toxins. The molecules of none of the toxins contain free sulfhydryl groups, and they each contain four cystine residues, i.e., in all cases the definite configuration of the molecule is fixed by four disulfide bridges. All the toxins contain from five to eight asparagine residues and six or seven glutamic acid residues (some of them, probably, in the amide form [3, 4]), and they each contain one tryptophan residue. From a calculation of the molar extinction, it is not excluded that the number of tryptophan residues is two. The total number of amino-acid residues in both the toxins that we have studied is 62.

Isoleucine and leucine, respectively, have been found at the N-ends of the molecules of the toxins (I) and (II). When the toxins were treated with carboxypeptidase, regardless of the time of incubation, we found only asparagine. Calculations of the amount of asparagine split off after incubation of the toxins with carboxypeptidase for 4 and 12 h showed that it rises to two moles per mole of toxin. With a further increase in the time of incubation of the toxins with the enzyme no other amino acids were detected; appar-

TABLE 1. Amino-acid Composition of some Toxins of the Venoms of the Family Elapidae (moles/mole)

| Amino acid | N. oxiana Eich. | | N. nigricollis [3] | N. n. atra [4] |
|---|---|---|---|---|
| | toxin I | toxin II | | |
| Lysine | 6 | 6 | 6 | 3 |
| Histidine | 1 | 2 | 2 | 2 |
| Arginine | 2 | 4 | 3 | 6 |
| Tryptophan | 1—2 | 1—2 | 1 | 1 |
| Aspartic acid | 5 | 8 | 7 | 8 |
| Threonine | 7 | 6 | 8 | 8 |
| Serine | 3 | 4 | 2 | 4 |
| Glutamic acid | 6 | 7 | 6 | 7 |
| Proline | 6 | 4 | 5 | 2 |
| Glycine | 4 | 5 | 5 | 7 |
| Alanine | 4 | — | — | — |
| Cystine (1/2) | 8 | 8 | 8 | 8 |
| Valine | 1 | 2 | 2 | 1 |
| Isoleucine | 4 | 2 | 3 | 2 |
| Leucine | 2 | 2 | 2 | 1 |
| Tyrosine | 2 | 1 | 1 | 2 |
| Total | 62 | 62 | 61 | 62 |

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ently, the next amino acid from the C-end is cystine which, after the clevage of the peptide bond, remains connected to the residues of the molecule by a disulfide bridge. This permits the conclusions that from their C-ends the peptide chains of the molecules of both toxins have the sequence Cys-Asn-Asn-COOH. The leucine at the N-end and the sequence Cys-Asn-Asn-COOH at the C-end were also found in an analysis of the structures of the toxins of the venoms of N. nigricollis and N. n. atra.

We have determined the role of the individual amino acids in the lethal action of the toxins of the venom of the Central Asian cobra. Toxin (I), which contains one mole less of histidine, arginine, and glycine than the smallest amount in the other three toxins investigated and two moles less of aspartic acid, acts on mice considerably more feebly (its $LD_{50}$ is 0.56 mg/kg body weight, and that of the toxin (II) is 0.13 mg/kg). It may be assumed that some of the amino acids listed are extremely important for the manifestation of a high efficiency by the toxins. After treatment with phenyl isothiocyanate, both toxins lost their activity, regardless of whether the N-terminal amino acid was split off or not. The degree of lethal effect of the modified toxins can be judged from the figures given below.

| Material Investigated | Dose, mg/kg | Effect* |
|---|---|---|
| Toxin I | 0.85 | 6/0† |
| Toxin II | 0.17 | 6/0 |
| PTC-toxin I | 8.5 | 0/6 |
| PTC-toxin II | 1.7 | 0/6 |
| RC-toxin I | 8.5 | 0/6 |
| RC-toxin II | 1.7 | 0/6 |
| Toxin I with a split-off C-end (Cys-Asn-Asn) | 0.85 | 6/0 |
| Toxin II with a split-off C-end (Cys-Asn-Asn) | 0.17 | 6/0 |

* PTC-toxin – phenylthiocarbamoylated toxin; RC-toxin – reduced carboxymethylated toxin.

† The denominator represents the number of animals that survived and the numerator the number that died.

It is likely that in the realization of the lethal effect of the toxins the ε-amino group of lysine has a decisive significance [5]. When the two asparagine residues were split off from the C-end there was no change in the efficiency of either of the toxins of the venom of the Central Asiatic cobra. It has not yet been possible to establish the role of the amino-acid residues following the carboxy end of the peptide chain, since this required the preliminary reduction of the disulfide bonds, which was accompanied by a loss of biological effect. Thus, after the reduction of the SS bonds with β-mercaptoethanol and alkylation of the liberated sulfhydryl groups with monoiodoacetic acid, the administration of the toxins in doses corresponding to 10 times their $LD_{50}$ [2] did not lead to a lethal outcome (see Table 1).

## EXPERIMENTAL

The toxins were obtained from the venom of the Central Asian cobra N. oxiana E. by the method described previously [2]. The molecular weights of the toxins were approximately the same (6000 and 6500). The amino-acid compositions of the toxins were determined on a Hitachi KLA 3B amino-acid analyzer. The toxins (2.5 mg each) were hydrolyzed with double-distilled 6 N hydrochloric acid (1.0-1.5 ml) in tubes sealed under vacuum. Hydrolysis was performed at 105°C for 24, 48, and 72 h. The amount of terminal amino acids of the toxins from the N-end was determined by the fluorodinitrobenzene and phenyl isothiocyanate PITC methods [6, 7] and those from the C-end by the carboxypeptidase method [8]. The amounts of tryptophan in the molecules of the toxins were determined spectrophotometrically [9] and the amounts of free sulfhydryl groups by amperometric titration [10]. The reduction and alkylation of the sulfhydryl groups was performed as described by Krestfield et al. [11]. The toxicities were determined by the intraperitoneal administration of the toxins to white mice weighing 18-20 g followed by the statistical treatment of the results as described by Belen'kii [12].

## CONCLUSIONS

1. The amino-acid compositions of two neurotoxins from the venom of the Central Asian cobra have been established. The N-terminal amino-acid residue of toxin (I) is isoleucine and that of toxin (II) leucine. There is the same sequence from the C-end of both toxins – Cys-Asn-Asn-COOH.

2. The role of the individual functional groups (ε-amino groups and disulfide bridges) of the amino acids in the lethal action of the toxins has been investigated and discussed.

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