CROTALUS DURISSUS TERRIFICUS VENOM: CROTOXIN AND INTER-CRO

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Crotoxin from *Crotalus durissus terrificus* represents almost 50% of the dry weight of the venom and is the main toxic component of the latter.[6] Crotoxin was separated into two fractions by two different methods, using carboxymethyl-cellulose and DEAE-cellulose chromatography:[15,9] an acid, nontoxic fraction denoted Crotapotin, and a basic, low toxicity fraction with enzymatic activity denoted *Phospholipase* A_2. Crotapotin (acid) potentiates the toxicity of phospholipase A (basic) *in vivo* but inhibits its hemolytic activity *in vitro*.

Both laboratories showed that Crotoxin is a complex formed by the strong association of crotapotin with phospholipase A_2. The recombination of these fractions restores the toxicity of crotoxin. Phospholipase A_2 has a molecular weight of 15,000 daltons, with a P.I. of 8.6.[15,10] It consists of a single polypeptide chain of 133 residues.[8] The acid component crotapotin has a P.I. of 3.4 and a molecular weight of 9,000 daltons according to Horst[10], and a P.I. of 3.7 according to Breithaupt,[5] who, however, determined the same molecular weight. Breithaupt[5] reduced crotapotin withβ-mercaptoethanol followed by carboxyamide methylation and separated three peptide chains linked by disulfide bridges:

Chain A with 40 amino acid residues, MW 4,300
Chain B with 34 amino acid residues, MW 3,700
Chain C with 14 amino acid residues, MW 1,600

The N-terminal of the peptide chains only supplies serine for chain A, indicating that the N-terminals of chains B and C are blocked.[5] Crotoxin at high doses acts on the post-synaptic membranes, stabilizing the acetylcholine receptors.[3,17]. Crotapotin only potentiates the toxicity of crotalic phospholipase A, a fact that could be interpreted as a pharmacokinetic phenomenon. This acid fraction can protect phospholipase A against inactivation by binding to low affinity sites, thus preserving it for high-affinity receptors.

There is no evidence of *in vivo* formation of the crotoxin complex. Jeng[11] and Bon[2] proposed a model which they called "chaperon" effect for crotapotin,

which is considered to be a phospholipase A "transporter" to the specific site of the appropriate receptor.

Using a totally different process, Laure[13] obtained crotapotin from crotoxin in order to determine its primary structure by the following methodology: crotoxin was isolated by filtration on Sephadex G-75 with 0.05 M ammonium formiate, pH 3.5, dialyzed and lyophilized. Crotapotin and phospholipase A_2 were isolated on SP-Sephadex C-25 with 0.8 M to 3.0 M guanidine chloride, pH 4.2. After dialysis and lyophilization, crotapotin was filtered on superfine Sephadex G-50 with 0.1 M NH_4HCO_3, pH 8.2.

Crotapotin was tested for purity by amino acid analysis, polyacrylamide gel electrophoresis, N-terminal on polyamide plates by dansylation and then reduced with dithiothreitol and carboxymethylated with iodoacetic acid by standard methods, followed by desalting on Sephadex G-10 and lyophilization. The lyophilized product was submitted to filtration on Sephadex G-50 S.F. with 0.1 M NH_4HCO_3, pH 8.2, and three peaks corresponding to chains A, B and C were obtained.

Chain C was isolated and sequenced by Edman-dansyl and dabitization, in collaboration with my graduate student Gabilan. Chain B was isolated and cleaved with BrCN and peptides I and II were isolated by filtration on Sephadex G-50 S.F. Sequencing was done by my post-graduate student Conti using the same process as for chain C. Chain A was submitted to different enzymatic treatments, the peptides were isolated and purified and sequenced by the Edman-Dansyl method. Using sequencing via Edman-Dansyl and dabitization, the primary structures of chains A, B and C of crotapotin were determined and are presented below:

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                    10                  20          30          38
Chain A:  SYG(C)YCGAGGGG(W)PQDASDRCCFZHBCCYAKLTGCBPT

                   10                  20          30     34
Chain B:  pERDTKZBZVCDPCGCEIMGCDTAAAICFEDSEDG

                   10     14
Chain C:  pEFSPEBCZGESZPC
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Papers presented by Aird[12,1] at the 8th World Congress on Animal, Plant and Microbial Toxins and published in Biochemistry have presented the three crotapotin chains, chains C and A being complete and chain B incomplete.

Inter-Cro

The same methodology[14] used for crotoxin separation, was used for the Inter-Cro separation except that the dimension of the column used was three times that of the previous system for the same venom load. A fraction between crotoxin and crotamine, with the same characteristic as crotoxin was thus obtained. When this fraction was submitted to the same procedure as used to obtain crotapotin, via SP-Sephadex, it unfolded into the two fractions mentioned earlier (crotapotin and phospholipase A_2). Studies currently underway will confirm whether this is an iso-crotoxin. Different isoforms of crotoxin have been mentioned by Faure and Bon[6,7,4].

In our Laboratory we observed that Inter-Cro is much more insoluble than crotoxin, and has when injected in mice intraperitonially, a delayed toxic effect i.e. 20-30 minutes compared to 3-5 min. with crotoxin.

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