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Crystalline Phospholipase A associated with a Cobra Venom Toxin

COBRA venom has been used as a source of phospholipase A activity¹⁻³, and Braganca and Sambray showed that there are multiple forms of phospholipase A in cobra venom⁴. In the course of a study of the toxic components of the venom of the Indian cobra, *Naja naja*, we discovered that a very potent central nervous system toxin (corticotoxin I) is associated with a high phospholipase A activity. This component was isolated by ammonium sulphate fractionation and chromatography on carboxymethyl 'Sephadex G-25' and finally crystallized. A photograph of the crystalline product is shown in Fig. 1. The specific phospholipase activity of the crystals, assayed in ether suspension by a modification of the technique described by Long and Penny⁵, was 150 μ /mg. (A phospholipase unit is the amount of activity which results in the liberation of 1 μ mole of fatty acid per min at 20° C.) There was no detectable myoneural toxic activity, as determined by the procedure described by Vick *et al.*⁶, using anaesthetized dogs. The crystals can

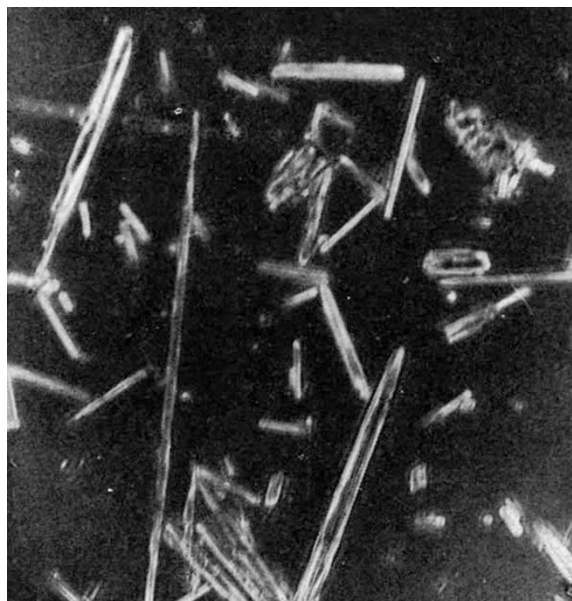


Fig. 1. Crystals of phospholipase A from Indian cobra venom ($\times c. 100$). Crystallization was accomplished by preparing a salt-free solution of a chromatographically purified fraction at a concentration of about 60 mg/ml. The pH was adjusted to 5.2 at 2° C and the crystals formed overnight.

be separated into two major components either by electrophoresis on cellulose acetate strips or by chromatography on DEAE cellulose. The specific phospholipase activity of both components was similar in the conditions of our assay. The amino-acid composition of the acid hydrolysates of the two components was nearly identical, as shown in Table 1. Amide content has not been determined so far, and there may be a difference in the number of free carboxyl groups. Work is now in progress to compare the substrate specificities, kinetic parameters and physicochemical properties of the two components.

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Table 1. AMINO-ACID COMPOSITION OF THE TWO PHOSPHOLIPASE COMPONENTS

Amino-acid	Peak 1	Peak 2
Aspartic acid	41.5	40.0
Glutamic acid	15.2	15.0
Threonine	8.2	7.9
Serine	9.7	9.4
Proline	7.5	6.3
Glycine	18.6	17.2
Alanine	22.5	21.5
Valine	8.1	8.0
Half-cystine	19.0	21.9
Methionine	0.0	0.0
Isoleucine	7.7	7.9
Leucine	10.0	10.0
Tyrosine	13.9	13.7
Phenylalanine	8.0	8.0
Ammonia	32.0	24.0
Lysine	10.1	10.0
Histidine	2.3	2.2
Arginine	10.0	11.6
Tryptophan	7.2	5.7

Determination by amino-acid analysis of acid hydrolysates. The values are averages of 5 runs, calculated with leucine set arbitrarily at 10 so as to adjust the molecular weight to approximately 24,000 to conform with preliminary estimates based on sedimentation rate and the calculated molar extinction coefficient. Tryptophan was estimated spectrophotometrically by the method of Edelhoch⁷. Variations in cystine and ammonia were great, so the values cannot be used as a basis for proving a difference between the two peaks.

Evidence for Order in the Structure of α -Elastin

THE structure of elastin, the insoluble constituent protein of elastic fibres occurring in the intercellular spaces of connective tissues, has been investigated and different models have been proposed. While a fibril structure is supported by electron microscope results¹⁻³, the rubber-like properties of the water swollen elastin have been interpreted as evidence for a cross-linked network of randomly coiled chains⁴⁻⁶. According to Partridge^{7,8}, elastin should be composed of globular protein molecules arranged in a tetrahedral packing, but Ramachandran^{9,10}