Toxins of Animal and Plant Origin

Volume 2 Edited by A. de Vries and E. Kochva
Tel-Aviv University, Israel

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GORDON AND BREACH SCIENCE PUBLISHERS
New York London Paris

Gordon and Breach, Science Publishers, Inc.
440 Park Avenue South
New York, N.Y. 10016

Editorial Office for the United Kingdom

Gordon and Breach, Science Publishers Ltd.
42 William IV Street
London WC2

Editorial Office for France

Gordon & Breach
7–9 rue Emile Dubois
Paris 14e

Proceedings of the Second International Symposium on Animal and Plant Toxins, held at Tel-Aviv University, Tel-Aviv, Israel, February 22–28, 1970.

Library of Congress catalog card number 71-130967. ISBN 0 677 12440 6. Printed in England.

PREFACE

The field of research of toxins from various sources, mainly animal, has been active during the last two decades and has resulted in several international meetings, and in a series of original and review publications. There has been rapid progress in this field, not only in its clinical and pharmacological aspects, but also on the cellular and molecular level. The knowledge accumulating is so extensive and intensive that it is imperative from time to time to review together the activities of the numerous laboratories occupied with toxin research throughout the world. Such a survey of the field was made possible by the Second International Symposium on Animal and Plant Toxins held at Tel-Aviv University in February 1970, the proceedings of which are presented here. The participants of the Symposium have covered in their papers not only the results of accomplished studies, but also preliminary findings obtained in their laboratories.

We have included in these volumes both extensive reviews and original technical reports, arranged according to disciplines rather than taxonomy. We have restricted editing to the bare minimum necessary for better comprehension and organisation of the material.

In these tasks we were aided by several colleagues from Tel-Aviv University who read some of the manuscripts, especially Drs. A. Ar, A. Bdolah, S. Ben-Efraim and B. Moaz. Mrs. Ruth Manneberg, the secretary of the Department of Zoology, Tel-Aviv University, took charge of the arranging of the manuscripts and the preparation of the book for the printer and Miss Ziva Yehezkel compiled the subject index. We thank them all for their efforts, and Gordon and Breach of London and their local representative, Miss Miriam Balaban for dealing so patiently and efficiently with the extensive texts and numerous illustrations.

A. de VRIES and E. KOCHVA

Tel-Aviv

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IV PHARMACOLOGY

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Volume 3

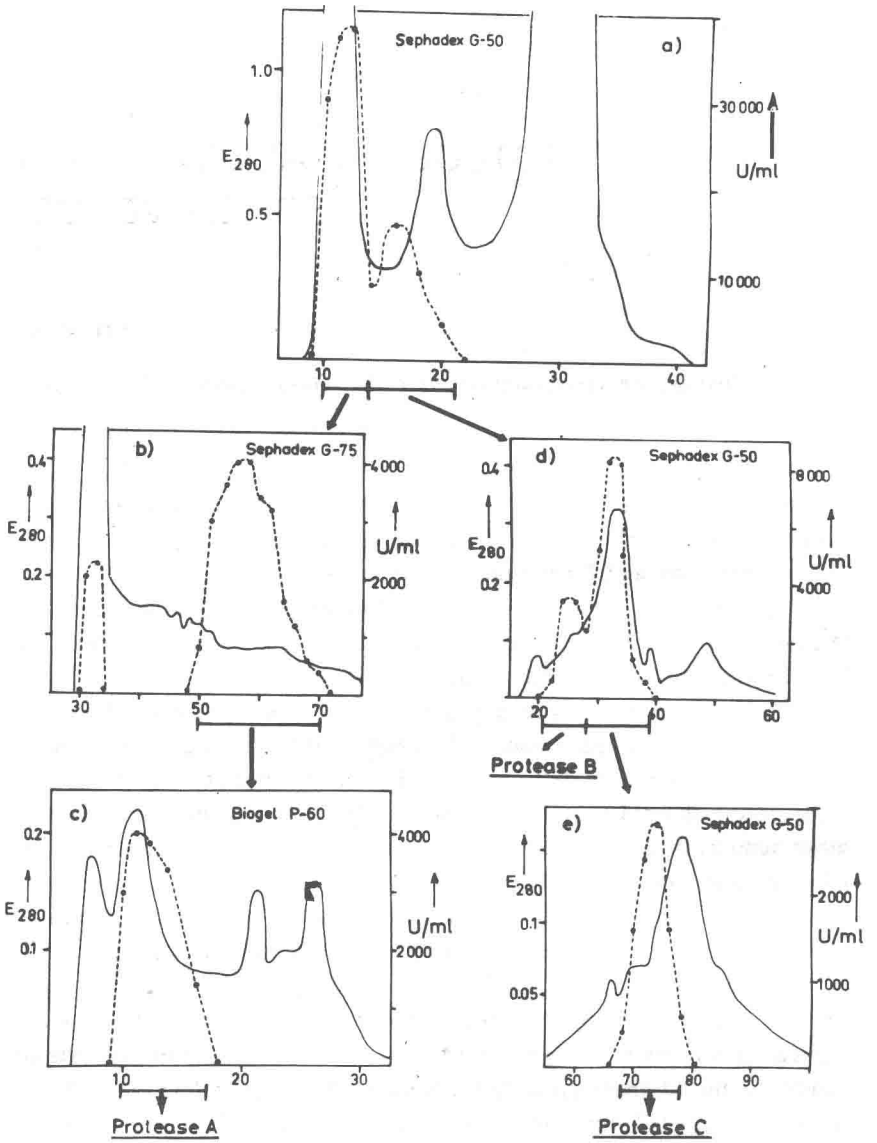

FIGURE 1. Chromatographic separation of protease activity of *Pamphobeteus roseus* venom

a) Gel filtration of 52 mg crude venom on a Sephadex G-50 column (0.9 × 90 cm), eluted with 0.06 M phosphate buffer pH 7.0 (2 ml fractions); proteolytic activity (broken line) tested on casein hydrolysis was expressed in units (U) per ml. b) Chromatography of the first proteolytic active fraction on Sephadex G-75 (1.5 × 130 cm column, 2 ml fractions) and c) on Biogel P-60 (100–200 mesh, 1.1 × 58 cm column, 2 ml fractions, 0.06 M phosphate buffer pH 7.0). d) and e) Rechromatography of the second proteolytic active fraction on Sephadex G-50 (d) 1.5 × 70 cm column and e) 1.5 × 150 cm column, 2 ml fractions)

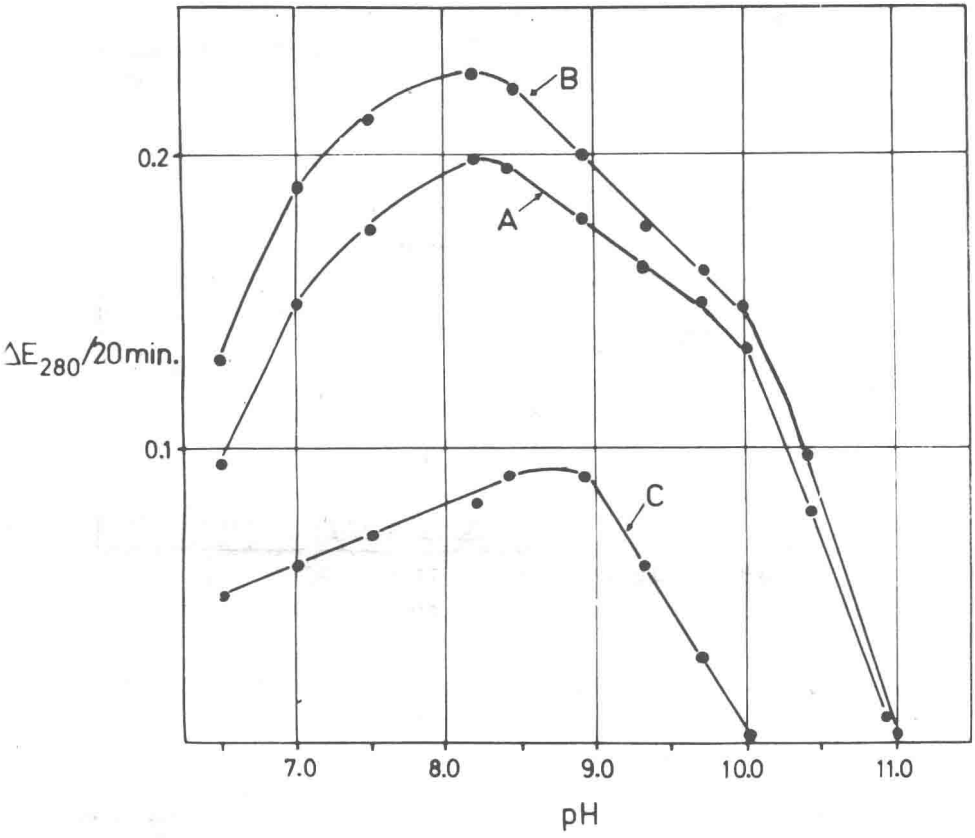

FIGURE 2. pH-optimum of the three protease fractions, tested on casein hydrolysis in the buffer system 0.04 M phosphoric acid, acetic acid and boric acid; the pH-value was titrated with 0.2 N NaOH

with 5–10 μg enzyme for 60 minutes at 37 °C, pH 7.5). Likewise the trypsin specific inhibitor N-tosyl-L-lysyl-chloromethane (5 mM) and the chymotrypsin specific inhibitor N-tosyl-L-phenylalanyl-chloromethane (5 mM) have no effect on the enzyme activity.

The molecular weight was estimated by thin-layer-gel chromatography on

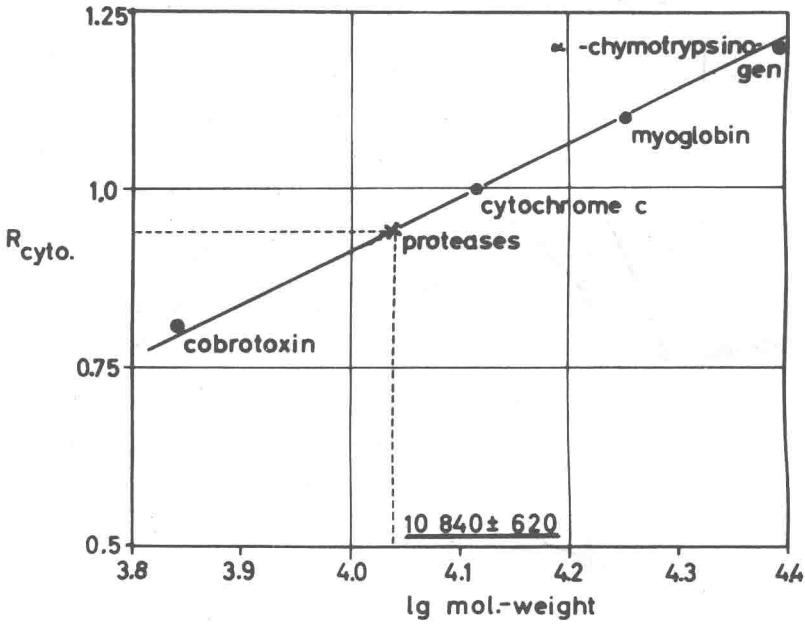

FIGURE 3. Molecular weight determination of the protease fractions by thin-layer-gel chromatography on Sephadex G-50 (superfine) using 0.05 M tris-HC1-buffer pH 8.0 + 0.5 M NaC1 as eluent

Sephadex G-50 (superfine) using cobrotoxin (mol.w. 6,950 [Yang *et al.*, 1969]), cytochrome c (mol.w. 13,000), myoglobin (mol.w. 17,800) and α-chymotrypsinogen (mol.w. 25,000) as standard compounds (Figure 3). The protease fractions showed the same diffusion velocity refered to that of cytochrome c ($R_{cyto.}$). A molecular weight of 10,840 ± 620 was calculated.

Since all three proteases possess the same enzymatic properties and the same molecular weight, one could assume that it might probably be the same protease, which was split by gel filtration into three fractions as a result of aggregating to oligomeric forms or of complex formation.

Proteases of similarly low molecular weight were detected only in invertebrates (Arthropoda). Sonneborn *et al.* (1969) purified a protease with a molecular weight of 12,500 from the mid gut of the hornet larva (*Vespa orientalis*), possessing chymotryptic properties. A protease with a molecular weight of 11,000 and similar enzymatic properties like the spider venom proteases was isolated by Pfleiderer *et al.* (1967) from the crayfish *Astacus*

fluviatilis. The high proteolytic activity of the spider venom indicates its significance for the extracorporal digestion. Furthermore, low molecular weight proteases are of great interest in the elucidation of the evolution of endopeptidases.

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Paper III-21

BIOCHEMISTRY OF *HELODERMA* VENOM

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INTRODUCTION

THERE IS only one venomous lizard family among the reptiles, the Gila monsters (Helodermatidae), with two species: the North American *Heloderma suspectum* (Figure 1) and the Mexican *Heloderma horridum*. Their venom glands are located in the lower jaw and as we were able to show in earlier investigations (Mebs and Raudonat, 1966, 1967; Mebs, 1968, 1969a), they produce a venom, which possesses in addition to toxic also enzymatic properties: it contains phospholipase A, hyaluronidase, esterolytic- and kinin-releasing activities.

In the following paper the fractionation of the venom of *Heloderma suspectum* and the isolation and properties of an arginine ester hydrolyzing and of a kinin-releasing enzyme (kallikrein) from this venom are reported.

MATERIALS AND METHODS

Lyophilized venom of *Heloderma suspectum* was purchased from Miami Serpentarium, Miami, USA. The hydrolysis of N-benzoyl-L-arginine ethylester (BAEE) and of p-toluene-sulphonyl-L-arginine methylester (TAME) was assayed according to the spectrophotometric method of Schwert and Takenaka (1955), the hydrolysis of N-benzoyl-DL-arginine-p-nitroanilide (BAPA) was assayed colorimetrically according to Erlanger *et al.* (1961). Casein hydrolysis was tested according to Kunitz method (1947), one unit was defined as the amount of enzyme which caused an increase of absorbancy of 0.001 at 280 nm after 20 minutes incubation at 37° C. Kinin-releasing

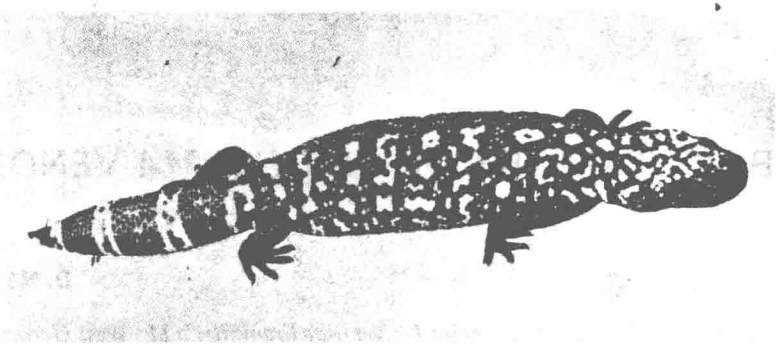

FIGURE 1. Adult specimen of the Gila monster, *Heloderma suspectum*

activity was determined on the isolated guinea pig ileum-preparation (in aerated Tyrode-solution containing 1 mg atropine and 0.1 mg Avil per liter, at 37° C) using a 3% globulin solution (bovine plasma globulins, precipitated with ammonium sulfate between 0.3 and 0.5 fold saturation) as substrate and synthetic bradykinin (Sandoz AG, Basel, Switzerland) as standard. Specific activity was expressed in μg of synthetic bradykinin equivalents released per minute per mg enzyme.

RESULTS AND DISCUSSION

Venom fractionation by column chromatography

400 mg of crude *Heloderma* venom, dissolved in 3 ml 0.05 M sodium acetate-buffer pH 7.0 was first filtrated on a Sephadex G-75 column (Figure 2). Two BAEE-hydrolyzing fractions were obtained, of which the first one contained the kinin-releasing activity, but not the second one which was more active in arginine ester hydrolysis and still had toxic properties. For the purification of the kinin-releasing enzyme, the kallikrein fraction I desalted on a Sephadex G-25 column was applied onto a DEAE-cellulose column and eluted with a linear gradient of increasing buffer concentration (0.005 M to 0.5 M acetate buffer pH 7.0). Under these conditions (Figure 3) most of the adsorbed protein remained irreversibly bound to the cellulose and the BAEE-hydrolyzing and kinin-releasing fraction was obtained free of phospholipase A activity. The rechroma-

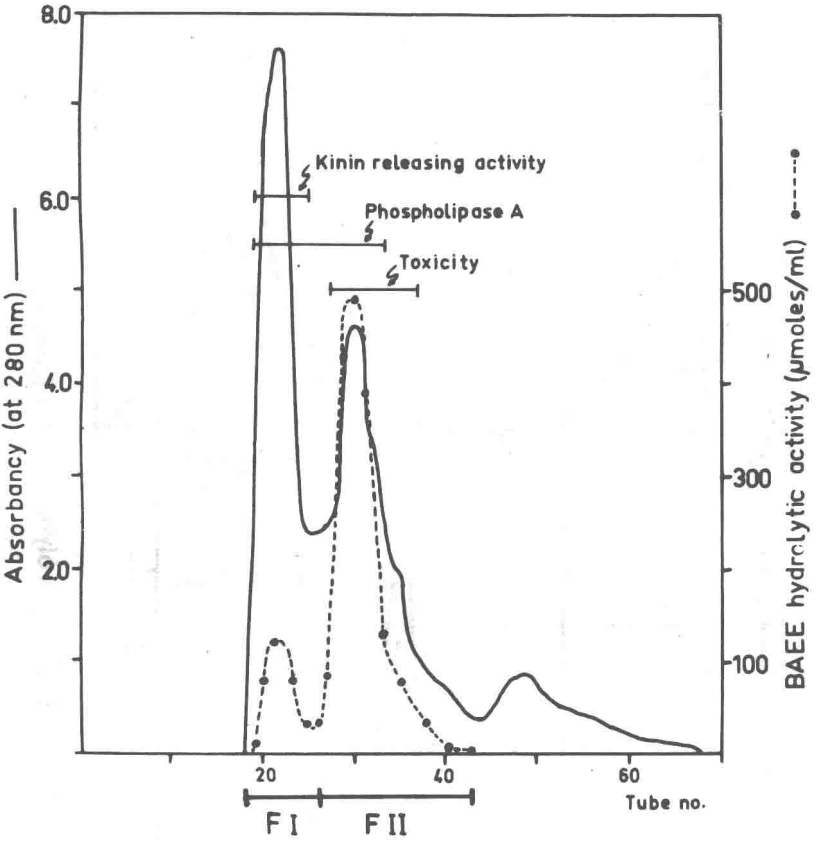

FIGURE 2. Gel filtration of 400 mg crude venom of *Heloderma suspectum* on Sephadex G-75 (1.5 X 140 cm column), eluted with 0.05 M acetate buffer pH 7.0. The fractions (5 ml) containing kallikrein (F I, kinin-releasing and arginine ester hydrolytic) and arginine esterase activity (F II) were lyophilized and desalted on a Sephadex G-25 column (2.2 X 80 cm)

tography of the desalted fraction on DEAE-cellulose showed a symmetrical peak. The enzyme was obtained with an 11 fold increase in its kinin-releasing activity; its esterolytic activity was about 20% of that of the crude venom.

For the purification of the arginine esterase, fraction II of the gel filtrated crude venom was chromatographed on DEAE-cellulose (Figure 4). By stepwise elution with increasing buffer concentrations (sodium acetate

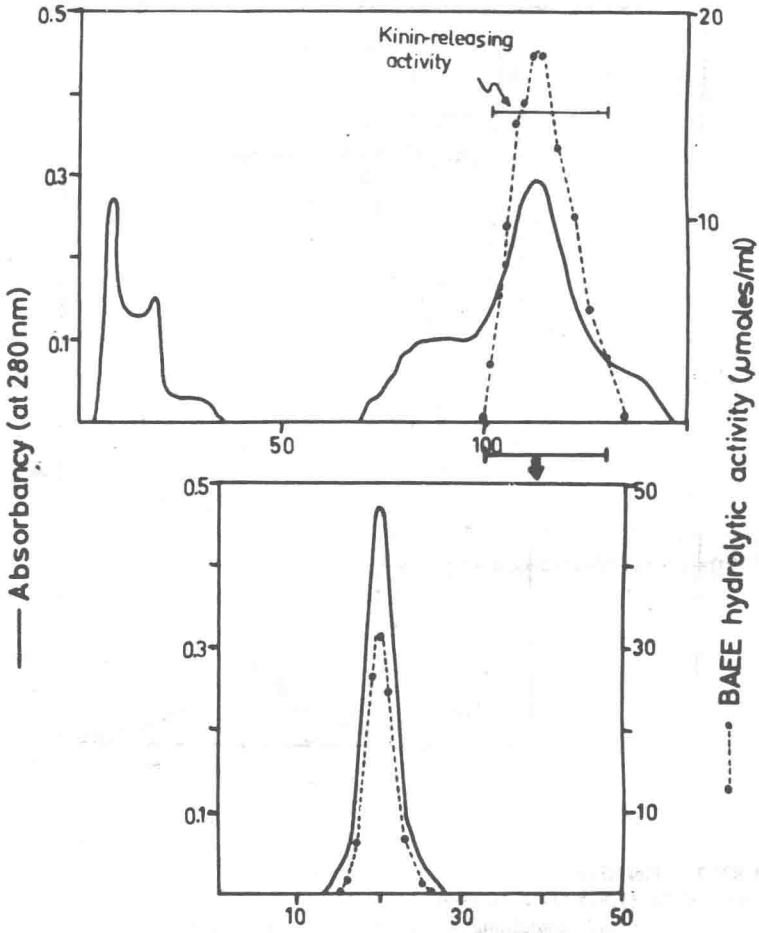

FIGURE 3. Purification of the kallikrein of *Heloderma* venom. Above: fraction I of the Sephadex G-75 column was applied onto a DEAE-cellulose-column (2.2 × 15 cm, DEAE-SN, 0.40–0.54 meq./g, from SERVA, Heidelberg, Germany) and eluted with a linear gradient of 0.005–0.5 M acetate buffer pH 7.0. The fractions (2.5 ml) containing kinin-releasing and BAEE-hydrolyzing activity were combined, lyophilized and desalted on Sephadex G-25

Below: rechromatography of the kallikrein fraction on DEAE-cellulose (1 × 12 cm column) by gradient elution with 0.1–1.0 M acetate buffer pH 7.0 (4.5 ml fractions)