Proteinase-Inhibitors in Albumin Glands of *Achatina fulica*

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Three different polyvalent proteinase-inhibitors have been detected in the albumin gland of the snail, *Achatina fulica* and identified by means of fibrin-agar immunelectrophoresis. The inhibitory activity of these substances against several proteinases from different sources was investigated.

Introduction

In previous communications, we have shown that the albumin glands of snails (*Helix, Cepaea, Ampullaria*) contain proteinase-inhibitors apart from erythro-agglutinins, anti-bacterial agglutinins and glycosubstances [1–4]. The albumin glands of the African giant snail, *Achatina fulica* contain a heterogeneous mixture of glycosubstances with a broad lectin receptor spectrum; but no detectable human erythro-agglutinins [5].

We report here the new finding that proteinase-inhibitors also exist in the albumin glands of this land snail.

Results and Discussion

The fibrin-agar electrophoretic patterns of the semi-purified extract tested with trypsin as proteolytic enzyme is presented in Fig. 1 b. The presence of an inhibitory protein is revealed by the undigested field. The fibrin-agar immunelectrophoresis with human serum and rabbit anti-human immunserum employed as reference is shown in Fig. 1 a.

Within the undigested field Fig. 1 b can be localized at least 3 proteinase inhibitors from comparative analysis with the pattern Fig. 1 a obtained for the reference material. We designate according to their source (and by analogy to the nomenclature used for the description of the *Helix pomatia* inhibitors [2] AF_1, AF_2, AF_3, from the cathode to the anode and of the plates). Their electrophoretic mobility lies compared with the serum proteins, for the AF_1 in the alpha, for the AF_2 in the beta, and for the AF_3 in the alpha-2 field (Table I).

Frequently three to four inhibitors have been revealed in the albumin gland extract of other snail species by the same technique (Table II).

Another experimental evidence for the presence and also topographical distribution of these inhibitors has been provided by an histochemical study approach with fluorescein labelled proteinases [7].

The FITC-pancreatic proteinases gave bright fluorescence at the reaction sites with the proteinase-inhibitors. The intensity of fluorescence indicat-

Table I. Inhibition of proteinases by different inhibitor active proteins from the albumin gland of *Achatina fulica* as revealed by fibrin-agar electrophoresis

Inhibitor active protein	AF_1	AF_2	AF_3
Electrophoretic mobility	γ_1	β_1	α_2
α-chymotrypsin	+	+	Ø
Trypsin	+	+	Ø
Proteinase K	+	+	+
Pronase P	+	+	+
Thermolysin	Ø	Ø	Ø
Kallikrein	Ø	Ø	Ø
Subtilisin Novo	+	+	Ø

+, Strong inhibition; (+), weak inhibition; Ø, no inhibition.

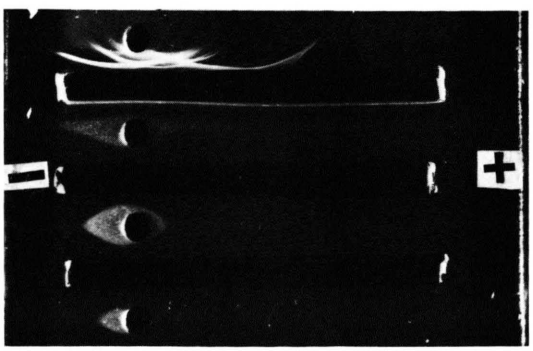

Fig. 1. Fibrin-agar immunelectrophoresis of inhibitor preparation from albumin glands of *Achatina fulica. Top upper hole:* human serum (10 µl); *top upper trough;* rabbit anti-human immunserum (100 µl); *other holes: Achatina fulica* inhibitor preparation (0.1% w/v); *trough:* trypsin (0.001% w/v).

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Table II. Comparative characteristics of the proteinase-inhibitors in snails (*Helix*, *Ampullaria* and *Achatina fulica*) and their inhibition pattern changes with proteinases from different sources.

	Helix pomatia [1]				*Helix aspersa* [4]			*Ampullaris australis* [3]				*Achatina fulica* *		
Inhibitor	1	2	3	4	1	2	3	1	2	3	4	1	2	3
Mobility	γ_2	γ_1	β_1	α_2	γ_1	β_1	α_2	γ_2	γ_1	β_1	α_2	γ_1	β_1	α_2
α-chymotrypsin	Ø	+	+	+	(+)	+	+	+	+	(+)	Ø	+	+	Ø
Trypsin	Ø	+	+	Ø	Ø	+	+	+	+	(+)	Ø	+	+	Ø
Pronase	Ø	+	+	+	(+)	+	Ø	+	+	+	Ø	+	+	Ø
Proteinase K	+	+	+	+	+	+	Ø	Ø	+	+	+	+	+	+
Subtilisin	+	+	+	+	+	+	Ø	Ø	+	+	+	+	+	Ø
Thermolysin	Ø	+	+	+	+	+	Ø	Ø	Ø	+	Ø	Ø	Ø	Ø
Kallikrein	Ø	+	+	Ø	Ø	+	Ø	Ø	Ø	+	Ø	Ø	Ø	Ø

+, Strong inhibition; (+), weak inhibition;
Ø, no inhibition;
*, this study

ed that these inhibitors were localized predominantly as globular structures within the lobules of the gland tissue [7].

The protease inhibitors in *Achatina fulica* gland extract also exhibited different reactions of proteinase inhibition with proteinases from different sources (Table II). The inhibition pattern changes with the proteinases were not too different from those observed for other snail species (Table II). However, Thermolysin and Kallikrein were not inhibited by any of the inhibitors from *Achatina fulica*, but these enzymes were affected by the isoinhibitors from *Helix species* [1, 4]. The significance of this difference is unknown. Remarkably, these inhibitors reacted strongly with the bacterial proteinases: Proteinase K, Pronase and Subtilisin. This phenomenon has been observed with inhibitors from other snail species (Table II). This finding supports the postulated protective role of these substances with respect to the snail eggs [1].

In conclusion, the results of this investigation has provided the first biochemical evidence for the presence of proteinase-inhibitors in the albumin glands of this snail species. Furthermore, the polyvalent nature of these inhibitors as found previously in some snail species [3] has also been demonstrated by the fibrin-agar electrophoresis technique.

Experimental

Enzyme preparation

Fresh albumin gland tissue of the snail *Achatina fulica* was homogenized in saline (1:2 w/v) for 5–7 min. The homogenate was filtered by suction through a plug of cheese-cloth. The filtrate was subjected to salt (ammonium sulphate) fractionation procedure as described for the isolation of proteinase-inhibitors in snails [6]. The supernatant at 63% $(NH_4)_2SO_4$ saturation was dialyzed thoroughly against cold water at 4 °C and lyophilized. The final product constituted the semi-purified material which was examined in this investigation.

Chemicals

Trypsin (E.C. 3.4.21.1) cryst. lyophil., Thermolysin (E.C. 3.4.24.4), Pronase P (Streptomyces griseus), Trypsin-inhibitor from soyabean, Agarose, were all purchased from Serva, Heidelberg, FRG, Proteinase K was bought from E. Merck, Darmstadt, FRG. Kallikrein (E.C. 3.4.4.21) was obtained from Bayer Biochemical laboratories, Wuppertal, FRG and Subtilisin Novo cryst. was from Novo, Mainz, FRG.

Fibrin-agar electrophoresis and fibrin-agar immunelectrophoresis

These were performed according to the procedure as described by Sprenger [4]. Thus, 9 vol. parts of agarose (Serva, Heidelberg) 0.8% solution in barbital buffer, 0.05 M pH 8.2, 2 vols of 0.4% bovine fibrinogen (Behring-Werke, Marburg) in barbital buffer were heated in a water-bath for 30 min at 47 °C until a fine floculate formed and then immediately distributed on the plates.

Electrophoretic separation was performed at room temperature using the LKB Flat Bed electrophoresis equipment at 150 V for 2 h. A fibrin-agar

immunelectrophoresis with trypsin as the proteinase, human serum as the antigen mixture and rabbit anti-human immunserum serves as the reference for each run. The diffusion time was 12 to 18 h for the immunsera and 5 to 16 h for the proteinases at room temperature.

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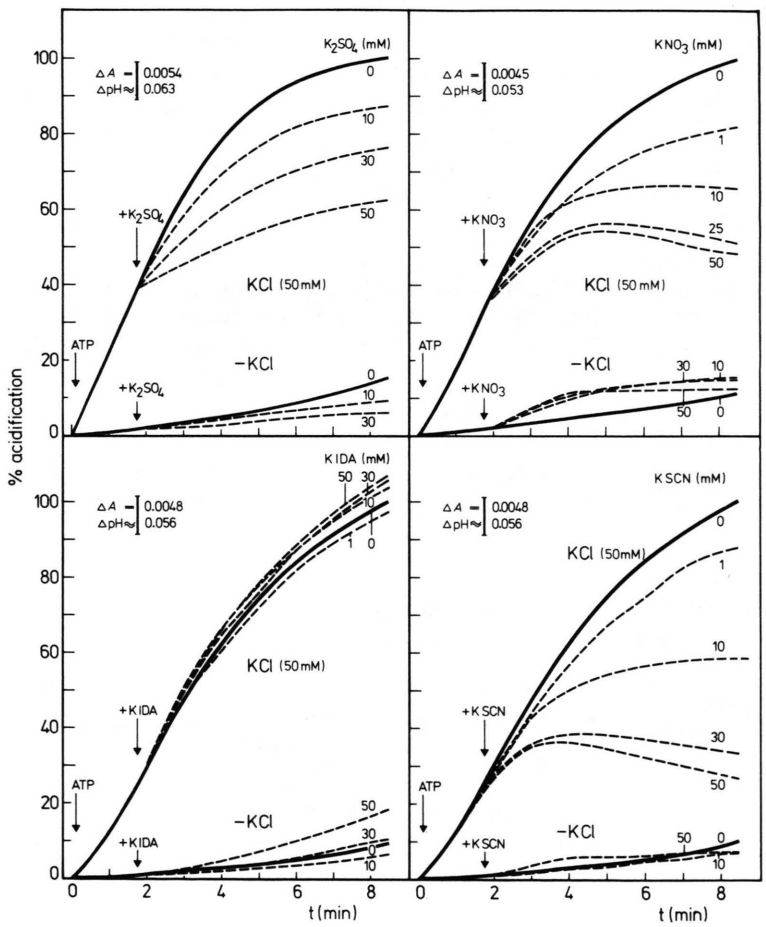
Erratum

A. Hager and M. Helmle, Properties of an ATP-Fueled, Cl^--Dependent Proton Pump Localized in Membranes of Microsomal Vesicles from Maize Coleoptiles, Z. Naturforsch. **36 c,** 997 – 1008 (1981).



Page 1003:
Fig. 9 is corrected in the following way:

Page 999:
The sentence on page 999, line 15 – 18 should be read:
The obtained difference spectra indicates a considerable ATP-dependent acidification within the vesicles corresponding to a ΔpH-value of 1 after 22 min.

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