PURIFICATION AND PROPERTIES OF A PROTEOLYTIC ENZYME FROM COTTON SEEDS

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A new proteolytic enzyme has been isolated from the seeds of the cotton plant of variety Tashkent 1 by affinity chromatography.

An acetone-defatted powder was extracted with 0.1 M phosphate buffer, pH 5.6, for 18 h. The supernatant was diluted with water to reduce the ionic strength of the buffer to 0.01 M, and Hg^{2+} ($1 \cdot 10^{-4}$ M) and EDTA ($1 \cdot 10^{-4}$ M) were added. The presence of Hg^{2+} ions in the solution blocked the active center of the thiol proteinase and permitted it to be eliminated in the first stages of purification. Chromatography was performed on the affinity sorbent bacitracin-Silochrome. This possesses a broad specificity for various serine, carboxy, and metallo proteinases [1]. A graph of elution from the column is shown in Fig. 1.

Fraction 1, not bound to the sorbent, possessed proteolytic activity and, according to the results of electrophoresis in 7.5% polyacrylamide gel, consisted of three components. Fraction 2 was eluted by phosphate buffer, pH 5.6, containing 1 M NaCl, $1 \cdot 10^{-4}$ Hg^{2+}, and $1 \cdot 10^{-4}$ EDTA, and was then concentrated on an FMO 2 membrane filter, being freed in this way from low-molecular-weight impurities. The concentrated solution was dialyzed against distilled water for a day; the precipitate that deposited was separated off by centrifugation at 18,000 rpm, and the supernatent was chromatographed on a column of Sephadex G-75. An elution graph is shown in Fig. 2. Peak II possessed proteolytic activity.

The proteolytic enzyme obtained was homogeneous according to electrophoresis and had a molecular weight of about 32,000 Da. The new proteinase differed from the proteinase B isolated previously from cotton seeds [2] not only in its specificity but also by the absence of sugars from the molecule. The complete amino acid composition of the enzyme was determined, a high level of Glu and Asp being found: Asp_{35}, Thr_{16}, Ser_{17}, Glu_{63}, Gly_{22}, Ala_{13}, Val_{10}, Met_4, Ile_7, Leu_{15}, Tyr_8, Phe_7, His_6, Lys_{21}, Arg_{18}, Pro_{14}, and Cys_6.

The enzyme readily hydrolyzed hemoglobin and azocasein. Its maximum activity was exhibited at pH 5.8.

p-Chloromercuribenzoate and EDTA did not affect the activity of the enzyme, which showed that it did not belong to the group of thiol or metallo proteinase. Dithiothreitol activated

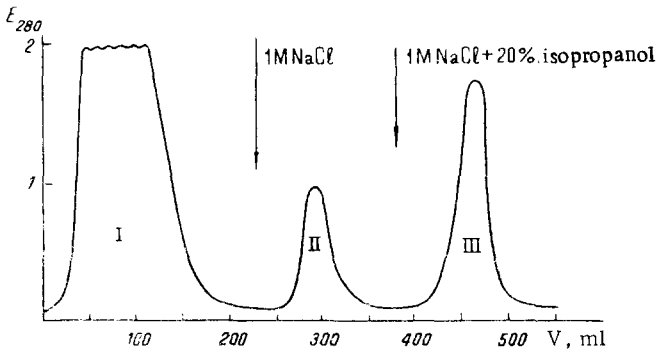

Fig. 1. Chromatography on a 15 × 5 cm column with bacitracin-Silochrome. The arrows show the beginning of elution with 1 N NaCl and with 1 M NaCl + 20% of isopropanol.

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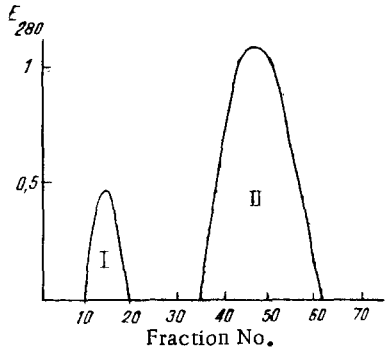

Fig. 2. Gel filtration on a 90 × 2 cm column of Sephadex G-75 in 0.01 M phosphate buffer, pH 5.7. Rate of elution 12 ml/h.

the proteinase very slightly, but this cannot be explained by the influence of the SH reagent on the active center but is rather a consequence of features of the conformational structure of the enzyme molecule. A decrease in activity was observed under the action of the soybean inhibitor. We assumed that the enzyme that we have isolated belongs to the serine group of proteinases. (The authors express their gratitude to G. N. Rudenskaya and V. M. Stepanov for providing the possibility of working with the affinity sorbent).

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ISOLATION AND IDENTIFICATION OF A COMPONENT OF THE SEX PHEROMONE OF *Orgyia gonostigma*

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In recent years, *Orgyia gonostigma* F. (*Lepidoptera:Limantriidae*) (scarce vaporer moth) has become an economically important pest in apple orchards of the intensive type. Its sex attractant is unknown, although it could be used for combatting this pest, and we have therefore begun an investigation of the sex attractant of *O. gonostigma*.

An extract of the sex attractant was prepared by steeping excised terminal segments of the abdomens of virgin females in methylene chloride. To find the components of the attractant we used an approach that we have described previously [1]. The crude extract, in an amount of 70 female-equivalents, was subjected to micropreparative gas-liquid separation in a column containing the liquid phase XE-60 into 1-minute fractions with the taking of samples into glass capillaries and the subsequent testing of the biological activity of their contents by the electroantennogram (EAG) method [2]. In this way we found one EAG-active fraction with a retention time of 8 min and retention indices of 2508 and 2200 determined on columns with polar (XE-60) and nonpolar (Apiezon L) phases. A comparison of these values with tabular values of retention indices [3], suggested to us that the EAG-active substance could be a monounsaturated ketone with 21 carbon atoms.

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