TRYPSIN INHIBITOR FROM LIVER OF THE SQUID Berryteuthis magister

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Protein inhibitors of proteinases and, in particular, trypsin inhibitors (TIs) are widely used in clinical medicine and scientific investigation [1]. The majority of inhibitors obtained from plant raw material exert a toxic action on the organism and therefore cannot be used as drugs. TIs from animal tissues are less common and less accessible for isolation. In the present paper we show the presence of a TI in the liver of a squid – a waste material from the processing of marine products – and describe the preparation of a partially purified material.

The amount of the TI in solutions was monitored from the fall in the activity of trypsin in the hydrolysis of casein [2]. The activity of the TI was expressed in the amount (mg) of trypsin inhibited by 1 ml or 1 mg of protein in the solution under investigation. Allowance was made for the fact that the amount of trypsin in a commercial preparation (Spofa) determined by titration with soybean trypsin (Reanal) (1 ml of commercial soybean inhibitor suppresses the activity of 1 mg of trypsin with an activity of 10,000 units for BAEE) amounts to 50% of the total weight.

The TI was isolated in the following way: 150 g of liver after preliminary mechanical comminution was defatted by washing with 500 ml of butanol–hexane (1:1) three times. The water-soluble proteins were extracted with 500 ml of 0.01 M HCl solution for 10 h, and were then subjected to heat coagulation (80°C, 10 min) to precipitate ballast proteins. The subsequent purification of the TI was carried out with the aid of affinity chromatography on polyamide sorbent with trypsin covalently bound through glutaric dialdehyde. This operation was performed in a column (0.8 × 9 cm) containing 1 g of sorbent equilibrated in 0.05 M phosphate buffer, pH 8.0. The same buffer solution was used for sorption and washing. The TI was eluted with 20 ml of a 0.1 M solution of CH_3COOH in 15% isopropanol (Fig. 1). As a result, the TI was obtained with a degree of purification of 296 as compared with the initial extract and with a yield of 22% on the amount deposited in terms of activity. With respect to the specific activity index, the preparation obtained was little inferior to a known commercial trypsin inhibitor [1.0 unit/mg for soybean TI (Reanal), and 1.0 unit/mg for chick ovamucoid TI (Sigma)].

Thus, the liver of industrial species of squid, especially B. magister can serve as a raw material for obtaining a TI. It is possible to obtain about 10 units of the preparation from 1 kg of liver.

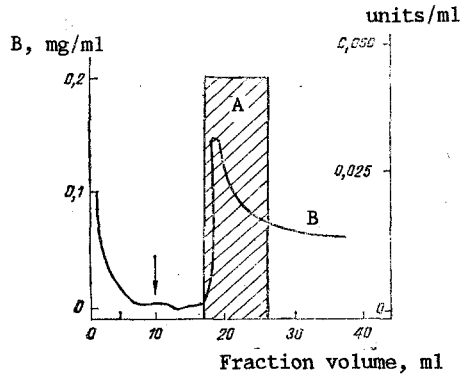

Fig. 1. Profile of the elution of the squid liver trypsin inhibitor from the biospecific sorbent polyamide-tripsin: A) TI activity; B) protein. The arrow indicates the beginning of elution.

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LITERATURE CITED

1. K. Ya. Veremeenko, Proteolytic Enzymes and Their Inhibitors in Medical Practice [in Russian], Zdorov'ya, Kiev (1961), p. 36.
2. M. L. Anson, J. Gen. Physiol., 22, 79 (1938).

PURIFICATION OF ISOENZYME I OF PHOSPHOLIPASE C FROM Clostridium perfringens

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Phospholipases, including phospholipase C (PL-C, EC 3.1.4.3), are widely used in investigations of lipid metabolism and its disturbances and of the mechanisms of heterogeneous catalysis and in the analysis and chemcial synthesis of lipids, and are irreplaceable in the study of the structure and functions of biological membrane. The directions mentioned impose high demands on the purity of the preparations used. However, the majority of known methods of isolating and purifying PL-C from various sources cannot be regarded as satisfactory because of their low yield and the multistage nature of the processes.

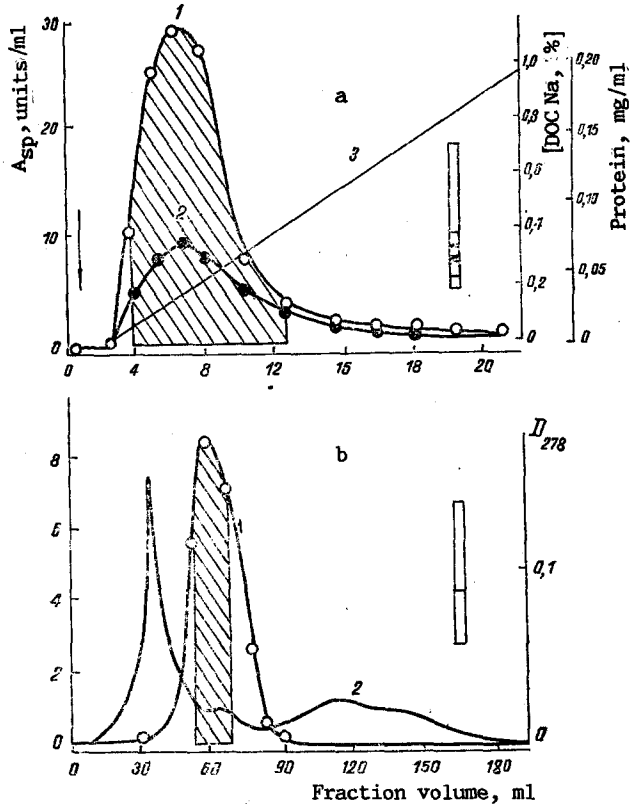

Fig. 1. Purification of isoenzyme I of phospholipase C from Cl. perfringens. a. Affinity chromatography on polikefamid: 1) activity; 2) protein; 3) sodium deoxycholate (arrow – beginning of elution). b) Gel filtration of AcA-54. The hatched region shows the fractions that were combined.

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