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ACTIVITY OF TRYPSIN INHIBITION BY PROTEIN EXTRACTS FROM *Squalus acanthias*

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ABSTRACT:

Protein extracts from viscera of Squalus acanthias develop trypsin inhibition activity comparable to that of the natural inhibitors separated and purified from pancreas, fowl eggs and soya. Thus, a quantity of only o.lo mg of raw natural inhibitor extracted from shark viscera inhibits by 25% the enzymatic activity of 6 mg of pure trypsin (Merck).

Numerous data on the natural inhibitors of trypsin separated, purified and crystallized from different animal and vegetal organisms are cited in the literature. Thus, there are wellknown trypsin natural inhibitors which were separated from pancreas (4, 6), colostrum (7), plasmatic serum (10), fowl eggs-ovomucoid (8), soya beans (5) etc.

We have found no reference in the available literature about such kind of research on marine organisms. The first data concerning the natural inhibitors of some proteases in several invertebrate and fish species at the Romanian coast of the Black Sea were published by us in a previous paper (11).

We are presenting in this paper some new evidence on the activity of trypsin inhibition by protein extracts obtained from viscera of Squalus acanthias.

MATERIAL AND METHODS

The work was done with viscera of the Black Sea shark Squalus acanthias.

Protein extractions, determinations of trypsin activity, as well as determination of trypsin inhibition activity were carried out through the methods described in the previous paper (11). Inhibition was expressed in percents of the standard enzym lost in the presence of the inhibitor.

Trypsin provided by the Merck firm was used as standard enzym, and hemoglobin, as a substrate.

RESULTS AND DISCUSSION

Examining the distribution of trypsin inhibition activity in organs and tissues of Squalus acanthias, the following standard trypsin inhibition percents were recorded: 37.9 % in viscera; 6o.7% in eggs; 86.1% in pancreas and 100% in liver and stomach (11).

As a continuation to this research, we display in the present paper some new data on the antitryptic activity in the shark.

Incubating a constant quantity (2 mg/ml) of standard trypsin with varied quantities of inhibitor(protein concentration between o.o2 and 3.8o mg/ml) at 37°C,the antitryptic activity was determined as a function of the E/I ratio.

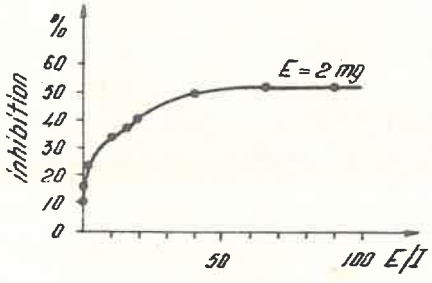

Fig.1- Antitryptic activity as a function of the ratio of Standard Enzym/Inhibitor in conditions of constant enzym concentration

It results from Figure 1 that the trypsin (pure enzymatic preparation) inhibition percent increases gradually with the increase in value of the E/I ratio, specifically, from 3.8% at E/I = o.53 to 52.2%, which corresponds with E/I = loo. This denotes that the smaller the amount of inhibitor, the higher is the percent in which the pure enzymatic preparation is inhibited.

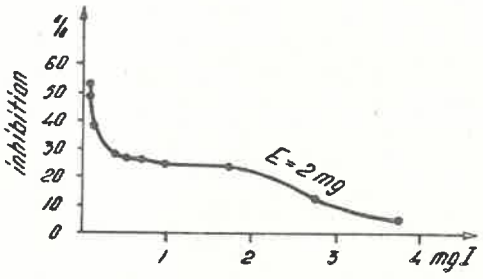

Fig.2 - Antitryptic activity as a function of the inhibitor concentration in conditions of constant enzym concentration

The experimental data plotted in Figure 2 show that the inhibition decreases from 52.2% to 3.8% along with the increase in the inhibitor concentration in the reaction medium from o.o2 mg/ml to 3.8o mg/ml. In this case it is more clearly noticed that as the inhibitor concentration in the reaction medium increases, the affinity of the enzym for the effector decreases. This may be explained by the fact that at higher concentrations of the inhibitor some intermolecular links are established between the peptidic chains of the effector, which thus becomes partially unable to interact with the enzym.

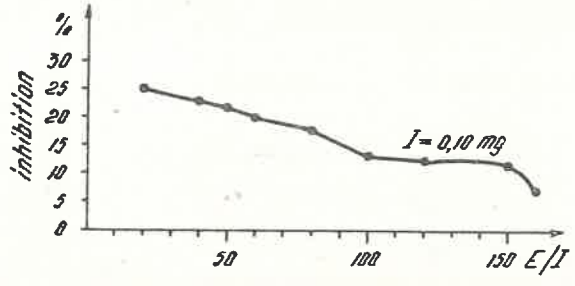

Fig.3 - Antitryptic activity as a function of the ratio of Standard Enzym/Inhibitor in conditions of constant inhibitor concentration

In the experiments where the quantity of inhibitor was kept constant (protein concentration=o.lo mg/ml) and the enzym was in variable quantity (between 2 and 16 mg/ml) a decrease in the trypsin inhibition percent was recorded, in relation to the rising value of the E/I ratio.

Thus, the antitryptic activity decreases from 25% to 7.5% for an increase of the E/I value from 2o to 16o (Fig.3). The diminution of the trypsin inhibition percent, in this case, is explained by the presence in exces of the enzym in the reaction medium against a very small quantity of inhibitor.

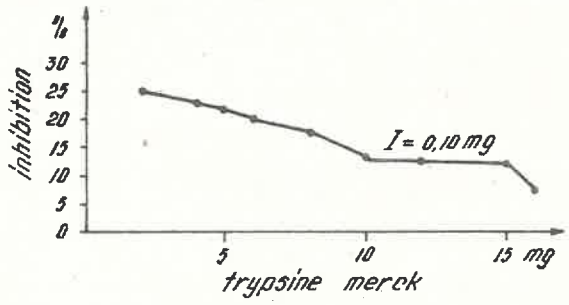

Fig.4 - Antitryptic activity as a function of the trypsin concentration in conditions of constant inhibitor concentration

Then again, the data in Figure 4 indicate that a quantity of only o.1o mg of raw natural inhibitor from shark viscera inhibits by 25% quantities between 2 and 6 mg of pure trypsin, while quantities of 8 to 16 mg of standard trypsin are inhibited by from 18% to 7.5%.

The data above show that the protein extracts obtained from shark viscera develop an activity comparable to some natural inhibitors of trypsin separated and purified from other animal and vegetal organisms (Table 1).

Table 1

Activity of trypsin inhibition by different natural inhibitors

Natural inhibitor	µg of standard trypsin inhibited by 1 µg of natural inhibitor
Trypsin inhibitor isolated from pancreas	2.5 (3)
Trypsin inhibitor isolated from soya	1.2 (3)
Ovomucoid	o.7 (1, 2)

Observation: For enzymatic determinations, the authors used hemoglobin as a substrate.

Ovorubin, a carotenoidic component extracted from the eggs of the amphibian gastropode Pomacea canaliculata australis, inhibits by considerable percents different proteinases (9).Thus,

0.02 mg of ovorubin, inhibits by 75% a quantity of 0.003 mg of trypsin, at E/I = 0.15. At an equal value of E/I, the protein extracts obtained from the shark stomach inhibits standard tryp - sin by 100%, while those from the pancreas, inhibits it by 86.1% (9, 11).

In conclusion, the data presented herein point out that the protein extracts obtained from different organs and viscera of Squalus acanthias develop a remarkable antitryptic activity.

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