Cercetări marine	I.R.C.M.	Nr.14	223-237	1981

DETERMINATION OF SOME NATURAL INHIBITORS OF PEPSIN, TRYPSIN AND CHYMOTRYPSIN IN SEVERAL INVERTEBRATE AND FISH SPECIES ALONG THE ROMANIAN COAST OF THE BLACK SEA

Natalia Rôşoiu[1], *Mihai Şerban*[2] *and Maria Panait*[1]

1) Romanian Marine Research Institute - Constanţa
2) Faculty of Veterinary Medicine - Bucharest

ABSTRACT:

The paper is presenting preliminary data on some natural inhibitors of pepsin, trypsin and chymotrypsin in the protein extracts from Actinia aequina, Rapana thomasiana, Squalus acanthias, Odontogadus merlangus euxinus and Engraulis encrassicholus ponticus.

Within the frame of an extensive research program for turning the biological resources of the Black Sea to the best account, we have had in view, in this paper, to determine and investigate the natural inhibitors of pepsin, trypsin and chymotrypsin in several invertebrate and fish species.

A great number of references to proteolytic enzym inhibitors separated from different animal or vegetal sources are cited in the literature. Investigations concerning determination and isolation of these bioactive principles also in invertebrates were initiated during the latest years. Consequently, trypsin and chymotrypsin inhibitors were isolated and purified from Taenia pisiformis (6) and a chromoproteide - ovorubine - in the eggs of the amphibian snail Pomacea canaliculata australis (1, 3 and 7)

with anti-tryptic and anti-chymotryptic properties, resembling the ovomucoid (4).

We have not found any data referring to such may of research with marine organisms in the available literature.

In this work, we are presenting some preliminary data on the occurence activity of certain natural inhibitors of protease provided by several invertebrate and fish species along the Romanian coast of the Black Sea.

MATERIAL AND METHODS

The work was carried out with five species of organisms which live in the Romanian coastal waters of the Black Sea: a coelenterate - Actinia aequina, a gastropod - Rapana thomasiana and three fishes - Squalus acanthias, Odontogadus merlangus euxinus and Engraulis encrassicholus ponticus. Assays were done on organs, tissues and total body.

The entire organisms and the organs and tissues sampled from the above mentioned species were property washed with running water, then with distilled water. After drying by tamponing between filter papers, the biological material was thoroughly weighed and macerated with a Potter homogenizer; the extraction ratio was 1 g tissue for 10 ml distilled water. The resulted homogenate was preserved for an hour at +4°C for completion of protein extraction and thereafter was centrifuged at 4,000 x g for 15 minutes; the supernatant which resulted in this way was considered as total protein extract.

The protein concentration, the activity of pepsin, trypsin and chymotrypsin, as well as the activity of inhibition of pepsin, trypsin and chymotrypsin, were determined in the total protein extract (diluted as the case standed).

The activity of the protease inhibitors was measured as percent of the standard enzym activity lost in the presence of the inhibitors; therefore the same methods were used for determining both the activity of pepsin, trypsin and chymotrypsin, and their respective inhibitors. The inhibitor solution (in distilled water) and pepsin, trypsin and chymotrypsin solutions (also in distilled water) were mixed together and let react by incubation

at 37°C in a thermostat for an hour; then the residual activity of the enzym was determined.

As the activity of the inhibitors is expressed in percent of the lost enzymatic activity,it is necessary that standard preparations of the respective enzyms should be used at each determination.

In order to annihilate any source of error,three series of determinations were carried out as follows:

- 1.o ml of total protein extract + 1.o ml of distilled water were incubated for an hour in a thermostat at 37°C, after which the activity of the respective enzym in the total protein extract was determined;

- 1.o ml of standard enzym solution+1.o ml of distilled water were incubated for an hour in a thermostat at 37°C, after which the standard enzym activity was determined;

- 1.o ml of standard enzym solution + 1.o ml of total protein extract (the inhibitor solution) were incubated for an hour in a thermostat at 37°C, after which the standard enzym residual activity was determined.

By calculation, percentage of standard enzym inhibition by the protein extract from the investigated organisms was found.

Determination of protein concentration in the extracts from the investigated species was carried out by the method of LOWRY and collab. (5).

Pepsin activity was determined by the ANSON method (2), using hemoglobin at pH 2.o as a substrate; trypsin and chymotrypsin activities were also determined by the ANSON method (2), using adulterated hemoglobin at pH 7.5 and 8.o, respectively. The activity of these proteases was expressed in nmoles tyrosine/mg protein/minute at 37°C.

Pepsin, trypsin and chymotrypsin inhibition activities were determined by the same method,except that standard solutions of pepsin, trypsin and chymotrypsin were put to the work after having been incubated with the inhibitor solution.

Pepsin and trypsin provided by the Merck firm and chymotrypsin provided by the BDH were used as standard enzyms.

Table 1

Evidencing the activity of pepsin inhibition in Rapana thomasiana. Distribution in organs and tissues

Organ or tissue	Protein conc. mg/ml	Enzym/ Inhibitor mg/mg	Dosage of activity in:	nmoles tyr/ mg prot/min, 37°C	Inhibition %
Branchiae	3.43	4/0.69 = 5.80	Protein extract	0	88.5
			Pepsin std.sol.	9,189	
			Pepsin std.sol. incub.with extract	1.058	
Salivary glands	12.22	4/2.44 = 1.64	Protein extract	0	100
			Pepsin std.sol.	9,189	
			Pepsin std.sol. incub.with extract	0	
Vitelline gland	8.89	4/1.78 = 2.25	Protein extract	0	100
			Pepsin std.sol.	9,189	
			Pepsin std.sol. incub.with extract	0	
Stomach	2.70	4/0.54 = 7.41	Protein extract	3,950	100
			Pepsin std.sol.	9,189	
			Pepsin std.sol. incub.with extract	0	
Hepato-pancreas	10.11	4/2.02 = 1.98	Protein extract	0	89.9
			Pepsin std.sol.	9,189	
			Pepsin std.sol. incub.with extract	929	
Kidney	6.07	4/1.21 = 3.31	Protein extract	2,635	100
			Pepsin std.sol.	9,189	
			Pepsin std.sol. incub.with extract	1.334	
Testis	1.29	4/0.26 = 15.38	Protein extract	6,625	100
			Pepsin std.sol.	9,189	
			Pepsin std.sol incub.with extract	663	
Mantle	2.09	4/0.42 = 9.52	Protein extract	0	88.5
			Pepsin std.sol.	9,189	
			Pepsin std.sol. incub.with extract	1.058	
Foot	3.58	4/0.72 = 5.56	Protein extract	0	79.8
			Pepsin std.sol.	9,189	
			Pepsin std.sol. incub.with extract	1,858	
Total	4.74	4/0.95 = 4.21	Protein extract	0	81.3
			Pepsin std.sol.	9,189	
			Pepsin std.sol. incub.with extract	1,720	

RESULTS AND DISCUSSION

I. Peptic activity and pepsin inhibition activity in protein extracts from Rapana thomasiana, Squalus acanthias, Odontogadus merlangus euxinus and Engraulis encrassicholus ponticus

Both proteolytic activity at pH 2.o and standard pepsin inhibition activity were discerned in the total protein extracts from all the species investigated.

While studying the distribution in organs and tissues of the peptic and pepsin inhibition activities of the protein extracts from Rapana thomasiana, the following were found out (Table 1):

- pH 2.o proteolytic activity is null in branchiae, salivary glands, vitelline gland, hepatopancreas, mantle, foot and total body, while in stomach, kidney and testis it takes values of 3,95o; 2,635 and respectively, 6,625 nmoles tyrosine/mg protein/minute, at 37°C.

- Pepsin inhibition activity is very intense in all the organs studied. Thus, values renging from 79.8% (foot) to 100% (salivary glands, vitelline gland, stomach, kidney and testis) are recorded, as standard pepsin inhibition percent; it is 81.3% in total body.

From the analysis of the same distribution in the protein extracts from Squalus acanthias, the following resulted (Table 2):

- The greatest pH 2.o proteolytic activity appeared in stomach (1,383 nmoles tyr./mg prot./min.) and the smallest, in eggs (178 nmoles tyr./mg prot./min.) - at 37°C.

- Standard pepsin inhibition percent varies from 30%, in liver, to 92.5% in visceral liquid.

- However, an effect of standard enzym activation (152.8%) was found in the protein extracts from the shark eggs.

Analysis of the data in Table 3 shows an appreciable pH 2.o proteolytic activity in total body and viscera of Odontogadus merlangus euxinus (1,391 and respectively, 1,949 nmoles tyr./mg prot./min. at 37°C), while in Engraulis encrassicholus ponticus, pH 2.o proteolytic activity is null both in total body and viscera.

Table 2

Evidencing the activity of pepsin inhibition in Squalus acanthias. Distribution in organs and tissues

Organ or tissue	Protein conc. mg/ml	Enzym/ Inhibitor mg/mg	Dosage of activity in:	nmoles tyr/ mg prot/min 37oC	Inhibit. %	Activation %
Eggs	29.87	2/29.87 = o.o7	Protein extract	178.o		
			Pepsin std.sol.	9,547.5	-	152.8
			Pepsin std.sol. incub.with extract	4,586.o		
Liver	14.2o	2/14.2o = o.14	Protein extract	78o,o		
			Pepsin std.sol.	9,547.5	3o.o	-
			Pepsin std.sol. incub.with extract	7,54o,o		
Pancreas	21.5o	2/21.5o = o.o9	Protein extract	525.4		
			Pepsin std.sol.	9,547.5	39.7	-
			Pepsin std.sol. incub.with extract	6,282.o		
Stomach	9.94	2/9.94 = o.2o	Protein extract	1,382.5		
			Pepsin std.sol.	9,547.5	83.7	-
			Pepsin std.sol. incub.with extract	2,943.o		
Visceral liquid	54.3o	2/54.3o = o.o4	Protein extract	196:2		
			Pepsin std.sol.	9,547.5	92.5	-
			Pepsin std.sol. incub.with extract	911,o		

Table 3

Evidencing the activity of pepsin inhibition in Odontogadus merlangus euxinus and Engraulis encrasicholus ponticus

Species	Protein conc. mg/ml	Enzym/ Inhib. mg/mg	Dosage of activity in:	nmoles tyr/ mg prot/min 37oC	Inhibit. %	Activation %
Odontogadus (total body)	1o.o5	2/2.o1 = 1.oo	Protein extract	1,391		
			Pepsin std.sol.	6,383	-	134
			Pepsin std.sol. incub.with extract	8,83o		
Odontogadus (viscera)	8.21	2/1.64 = 1.22	Protein extract	1,949		
			Pepsin std.sol.	6,383	18	-
			Pepsin std.sol. incub.with extract	5,648		
Engraulis (total body)	13.17	2/2.63 = o.76	Protein extract	0		
			Pepsin std.sol.	6,383	2o	-
			Pepsin std.sol. incub.with extract	5,114		
Engraulis (viscera)	1o.82	2/2.16 = o.93	Protein extract	0		
			Pepsin std.sol.	6,383	1oo	-
			Pepsin std.sol. incub.with extract	0		

Standard papsin inhibition percent takes low values in viscera of Odontogadus merlangus euxinus (18%) and in total body of Engraulis encrassicholus ponticus (20%) and a maximum value - 100% inhibition- in viscera of Engraulis (full of roe and milt).

134% standard pepsin activation was recorded in total body of Odontogadus merlangus euxinus.

II. Tryptic activity and trypsin inhibition activity in protein extracts from Actinia aequina, Rapana thomasiana, Squalus acanthias, Odontogadus merlangus euxinus and Engraulis encrassicholus ponticus

A lower activity of trypsin inhibition was discerned in protein extracts from organs and tissues,as well as from total body of Actinia aequina, Rapana thomasiana, Squalus acanthias, Odontogadus merlangus euxinus and Engraulis encrassicholus ponticus, this being even null in some cases. A slight tryptic activity is present in other organs and tissues from the species above, except the small fishes, in which considerable enzymatic activities were found.

In the settled experimental conditions, no tryptic activity was discerned in the coelenterate Actinia aequina. The protein extract obtained from its total body gives 37.7% trypsin inhibition activity, at the ratio Enzym/Inhibitor of 2.47 (Table 4).

Table 4

Evidencing the activity of trypsin inhibition in Actinia aequina

Protein conc. mg/ml	Enzym/ Inhibitor mg/mg	Dosage of activity in:	nmoles tyr/ mg prot/min, 37°C	Inhi- bition %
4,03	2/0.81 = 2.47	Protein extract	0	
		Trypsin std.sol.	25,386	37.7
		Trypsin std.sol. incub.with extract	15,820	

At the same time, no tryptic activity was discerned in Rapana thomasiana (Table 5).

Protein extracts obtained from kidney and testis of

Table 5

Evidencing the activity of trypsin inhibition in Rapana thomasiana
Distribution in organs and tissues

Organ or tissue	Protein conc. mg/ml	Enzym/ Inhib. mg/mg	Dosage of activity in:	nmoles tyr/mg prot/min 37°C	Inhibition %	Activation %
Branchiae	3.43	2/0.69 = 2.9o	Protein extract	0		
			Trypsin std.sol.	3,937	-	139
			Trypsin std.sol. incub.with extract	5,482		
Salivary glands	12.22	2/2.44 = o.82	Protein extract	0		
			Trypsin std.sol.	3,937	-	251
			Trypsin std.sol. incub.with extract	9,86o		
Vitelline gland	8.89	2/1.78 = 1.12	Protein extract	0		
			Trypsin std.sol.	3,937	31	-
			Trypsin std.sol. incub.with extract	2,723		
Stomach	2.7o	2/o.54 = 3.7o	Protein extract	0		
			Trypsin std.sol.	3,937	97	-
			Trypsin std.sol. incub.with extract	11o		
Hepato-pancreas	1o.11	2/2.o2 = o.99	Protein extract	0		
			Trypsin std.sol.	3,937	8	-
			Trypsin std.sol. incub.with extract	3,624		
Kidney	6.o7	2/1.21 = 1.65	Protein extract	0		
			Trypsin std.sol.	3,937	loo	-
			Trypsin std.sol. incub.with extract	0		
Testis	1.29	2/o.26 = 7.69	Protein extract	0		
			Trypsin std.sol.	3,937	loo	-
			Trypsin std.sol. incub.with extract	0		
Mantle	2.69	2/o.42 = 4.76	Protein extract	0		
			Trypsin std.sol.	3,937	38	-
			Trypsin std.sol. incub.with extract	2,447		
Foot	3.58	2/o.72 = 2.78	Protein extract	0		
			Trypsin std.sol.	3,937	-	135
			Trypsin std.sol. incub.with extract	5,335		
Total body	4.74	2/o.95 = 2.11	Protein extract	0		
			Trypsin std.sol.	3,937	9	-
			Trypsin std.sol. incub.with extract	3,569		

Rapana thomasiana inhibit by 100% the standard trypsin, while those obtained from vitelline gland, hepatopancreas, mantle and total body prove a very low inhibitory activity. The protein extract from the stomach gives 97% standard trypsin inhibition. On the contrary, a natural trypsin activating agent is present in the branchiae and salivary glands, determining an increase in the activity of the pure enzymatic preparation up to 139% and 251%, respectively (Table 5).

In Squalus acanthias, very weak tryptic activity was discerned in eggs (45.4 nmoles tyr/mg prot./min), pancreas (390 nmoles tyr/mg prot./min), stomach (833.2 nmoles tyr/mg prot./min) and viscera (191.7 nmoles tyr/mg prot./min). The tryptic activity is null in the liver (Table 6).

Table 6

Evidencing the activity of trypsin inhibition in Squalus acanthias. Distribution in organs and tissues

Organ or tissue	Protein conc. mg/ml	Enzym/ Inhibitor mg/mg	Dosage of activity in:	nmoles tyr/ mg prot/min 37°C	Inhibition %
Eggs	23.48	1/23.48 = 0.04	Protein extract	45.4	
			Trypsin std.sol.	32,340.0	60.7
			Trypsin std.sol. incub.with extract	12,766.0	
Liver	6.54	1/ 6.54 = 0.15	Protein extract	0	
			Trypsin std.sol.	32,340.0	100
			Trypsin std.sol. incub.with extract	0	
Pancreas	9.23	1/ 9.23 = 0.11	Protein extract	390.0	
			Trypsin std.sol.	32,340.0	86.1
			Trypsin std.sol. incub.with extract	4,894.0	
Stomach	9.45	1/ 9.45 = 0.11	Protein extract	833.2	
			Trypsin std.sol.	32,340.0	100
			Trypsin std.sol. incub.with extract	0	
Viscera	11.13	1/11.13 = 0.09	Protein extract	191.7	
			Trypsin std.sol.	32,340.0	37.9
			Trypsin std.sol. incub.with extract	20,272.0	

The natural trypsin inhibitor in the shark stomach and liver presents a remarkable activity, but at Enzym/Inhibitor ratios of only 0.15 and 0.11, respectively; the standard enzym

inhibition is 100% in these circumstances. On the whole, the percent of standard trypsin inhibition by the natural inhibitor in the shark viscera, at an Enzym/Inhibitor ratio of o.o9, is only 37.9% - much lower than the inhibition percent of the pepsin by the natural inhibitor of this enzym, extracted from total body of Rapana thomasiana, specifically, 81.3%, at an Enzym/Inhibitor ratio of 4.21 (Tables 1 and 6).

An intensive tryptic activity is found in Odontogadus merlangus euxinus and Engraulis encrassicholus ponticus, specifi - cally: in total body, 1,172 and respectively, 3,241 nmoles tyr/mg prot./min at 37°C; and in viscera, 8,29o and respectively, 14,o26 nmoles tyr/mg prot./min. at 37°C; this is comparable with the enzymatic activity of Merck trypsin (Table 7).

Table 7

Evidencing the activity of trypsin inhibition in Odontogadus merlangus euxinus and Engraulis encrassicholus ponticus

Species	Protein conc. mg/ml	Enzym/ Inhibit. mg/mg		Dosage of activity in:	nmoles tyr/ mg prot/min 37°C	In- hib. %	Acti- vati- on %
Odontogadus (total body)	lo.o5	2/2.o1 = 1.oo		Protein extract	1,172		
				Trypsin std.sol.	lo,oo7		- 134.6
				Trypsin std.sol. incub.with extract	14,643		
Odontogadus (viscera)	8.21	2/1.64 = 1.22		Protein extract	8,29o		
				Trypsin std.sol.	lo,oo7	22.7	-
				Trypsin std.sol. incub.with extract	16,o23		
Engraulis (total body)	13.17	2/2.63 = o.76		Protein extract	3,241		
				Trypsin std.sol.	lo,oo7		- 141.5
				Trypsin std.sol. incub.with extract	17,4o2		
Engraulis (viscera)	lo.82	2/2.16 = o.93		Trypsin extract	14,o26		
				Trypsin std.sol.	lo,oo7	64.6	-
				Trypsin std.sol. incub.with extract	17,568		

As concerning the trypsin natural inhibitor, this is present in the viscera of the two fish species; the inhibition percents are 22.7% in Odontogadus merlangus euxinus (E/I ratio = 1.22) and 64.6% in Engraulis encrassicholus ponticus (E/I ratio = o.93). In contradistinction to Odontogadus, the viscera of Engraulis were full of roe and milt, which leades to the supposition that the trypsin inhibitor is located in these formations.

Testing the trypsin inhibition activity in total body in the two fishes, surprisingly, standard enzym activation effects of 134.6% and respectively 141.5% were found (Table 7).

It results this way, by testing the presence of the trypsin natural inhibitor in the above mentioned species, trypsin inhibition activity was made evident in some protein extracts obtained from organs and tissues or total body, but on the whole, the inhibition process proved to be much inferior to that which is induced by the pepsin natural inhibitor in the same organisms.

III. Chymotryptic activity and chymotrypsin inhibition activity in protein extracts from Actinia aequina, Rapana thomasiana, Squalus acanthias and Engraulis encrassicholus ponticus

Among all the species investigated, the highest pH 8.o proteolytic activity was perceived in Actinia aequina, specifically, 18,887 nmoles tyr/mg prot./min at 37°C as compared to 38,595 nmoles tyr/mg prot./min, 37°C, the value representing the activity of a BDH chymotrypsin preparation. The protein extract from total body inhibits standard chymotrypsin in proportion of 58.3% at an Enzym/Inhibitor ratio of 2.47 (Table 8).

Table 8

Evidencing the activity of chymotrypsin inhibition in Actinia aequina

Protein conc. mg/ml	Enzym/ Inhibitor mg/mg	Dosage of activity in:	nmoles tyr/ mg prot/min 37°C	Inhibition %
4.o3	2/o.81 = 2.47	Protein extract	18,887	
		Chymotrypsin std.sol.	38,595	58.3
		Chymotrypsin std.sol. incub.with extract	34,971	

In Rapana thomasiana (Table 9), chymotryptic activity was discerned only in hepatopancreas (645 nmoles tyr/mg prot/min. at 37°C), kidney (895 nmoles tyr/mg prot./min. at 37°C) and total body (1,975 nmoles tyr/mg prot./min. at 37°C).

Investigating the activity of inhibition of a pure chymotrypsin preparation by protein extracts obtained from different organs and tissues, as well as from total body of Rapana thomasiana, the following were found:

Table 9

Evidencing the activity of chymotrypsin inhibition in Rapana thomasiana. Distribution in organs and tissues

Organ or tissue	Protein conc. mg/ml	Enzym/ Inhib. mg/mg	Dosage of activity in:	nmoles tyr/ mg prot/min 37°C	In- hib. %	Ac- tiv. %
Branchiae	4.97	2/o.99 = 2.o2	Protein extract	0		
			Chymotrypsin std.sol:	42,o53	6.3	-
			Chymotrypsin std.sol. incub.with extract	39,386		
Salivary glands	22.67	2/4.53 = o.44	Protein extract	0		
			Chymotrypsin std.sol.	42,o53	o.1	-
			Chymotrypsin std.sol. incub.with extract	41,998		
Vitelline gland	14.93	2/2.99 = o.67	Protein extract	0		
			Chymotrypsin std.sol.	42,o53	- 1o9.9	
			Chymotrypsin std.sol. incub.with extract	46,21o		
Stomach	6.o5	2/1.21 = 1.65	Protein extract	0		
			Chymotrypsin std.sol.	42,o53	- 1o3.1	
			Chymotrypsin std.sol. incub.with extract	43,341		
Hepato-pancreas	13.98	2/2.8o = o.71	Protein extract	645		
			Chymotrypsin std.sol.	42,o53	- 1o2	
			Chymotrypsin std.sol. incub.with extract	43,543		
Kidney	7.62	2/1.52 = 1.32	Protein extract	895		
			Chymotrypsin std.sol.	42,o53	- 1o9.5	
			Chymotrypsin std.sol. incub.with extract	46,21o		
Testis	4.47	2/o.89 = 2.25	Protein extract	0		
			Chymotrypsin std.sol.	42,o53	- 113.7	
			Chymotrypsin std.sol. incub.with extract	47,811		
Mantle	5.29	2/1.o6 = 1.87	Protein extract	0		
			Chymotrypsin std.sol:	42,o53	2.7	-
			Chymotrypsin std.sol. incub.with extract	4o,931		
Foot	6.8o	2/1.36 = 1.47	Protein extract	0		
			Chymotrypsin std.sol.	42,o53	- 1o5.1	
			Chymotrypsin std.sol. incub.with extract			
Total body	4.55	2/o.91 = 2.2o	Protein extract	1,975		
			Chymotrypsin std.sol:	42,o53	13.9	-
			Chymotrypsin std.sol. incub.with extract	38,171		

- A slight chymotrypsin inhibition activity was made evident in the branchiae (6.3%), salivary glands (o.1%), mantle (2.7%) and total body (13.9%) at Enzym/Inhibitor ratios of 2.o2, o.44, 1.87 and 2.2o, respectively.

- A natural factor which activates chymotrypsin, making the pure enzymatic preparation rise its activity to 1o2-113.7%, is present in the vitelline gland, testis, hepatopancreas, stomach, kidney and foot.

Table 1o

Evidencing the activity of chymotrypsin inhibition in Squalus acanthias. Distribution in organs and tissues

Organ or tissue	Protein conc. mg/ml	Enzym/ Inhibit. mg/mg	Dosage of activity in:	nmoles tyr/ mg prot/min 37oC	Inhibit. %
Eggs	18.6o	2/3.72 = o.54	Protein extract	0	8.6
			Chymotrypsin std.sol.	35,283	
			Chymotrypsin std.sol. incub.with extract		
Liver	7.66	2/1.53 = 1.31	Protein extract	0	14.8
			Chymotrypsin std.sol.	35,283	
			Chymotrypsin std.sol. incub.with extract	3o,o77	
Pancreas	14.39	2/2.88 = o.69	Protein extract	0	16.3
			Chymotrypsin std.sol.	35,283	
			Chymotrypsin std.sol. incub.with extract	29.544	
Stomach	7.36	2/1.47 = 1.36	Protein extract	2,55o	35.7
			Chymotrypsin std.sol.	35,283	
			Chymotrypsin std.sol. incub.with extract	25,239	

Studying the organ - distribution of the chymotryptic activity and chymotrypsin inhibition action in the protein extracts obtained from Squalus acanthias, it resulted as follows (Table 1o):

- The pH 8.o proteolytic activity is null in eggs, liver and pancreas; but a considerable chymotryptic activity was found in the stomach, specifically, 2,55o nmoles tyr./mg prot. /min., 37°C, as compared to 35,283 nmoles tyr./mg prot./min., 37°C - the activity of a pure enzymatic preparation (BDH chymotrypsin).

- The inhibition percent of standard chymotrypsine varies from 8.6% (eggs) to 35.7% (stomach) for Enzym/Inhibitor ratios having values near to one another (from o.54 to 1.36).

An intense pH 8.o proteolytic activity was made evident in the viscera full of roe and milt of Engraulis encrassicholus ponticus, specifically, 18,8o4 nmoles tyr/mg prot./min., 37°C, as well as a high inhibition percent (61%) of standard chymotrypsin (Table 11).

Table 11

Evidencing the activity of chymotrypsin inhibition in Engraulis encrassicholus ponticus

Protein conc. mg/ml	Enzym/ Inhibitor mg/mg	Dosage of activity in:	nmoles tyr/ mg prot./min 37°C	Inhibition %
8.58	2/1.72 = 1.62	Protein extract	18,8o4	
		Chymotrypsin std.sol.	38,595	61
		Chymotrypsin std.sol. incub. with extract	33,867	

Detection of higher activity of inhibition of trypsin, (Table 7) and chymotrypsin (Table 11) in the protein extracts obtained from viscera as compared to the small inhibition percents of these standard enzyms in the presence of the protein extracts from total body of Engraulis encrassicholus ponticus makes us presume that the inhibitors of both trypsin and chymotrypsin are located in the roes and milt.

CONCLUSIONS

Our research work has brought some contributions to detection of several natural inhibitors of proteolytic enzyms. Comparative data obtained show that the inhibitory activity developed had a number of specific particularities depending on the organism, and inside it, on the organ and tissue. Detection and knowledge of the degree of activity of these inhibitors may constitute experimental premises for their utilization in different applicative scopes.

REFERENCES:

1. CHEESMAN D.F., 1958 - Ovorubin, a chromoprotein from the eggs of the gastropod mollusc Pomacea canaliculata. Proc.R. Soc., 149 B: 571-587.
2. COLOWICK S.P., KAPLAN N.O., 1955 - Methods in Enzymology, II, Academic Press, New York: 3-169.
3. COMFORT A., 1947 - Lipochromes in the ovo of Pila. Nature, 160: 133-334.
4. LINEWEAVER H., MURRAY C.W., 1947 - Identification of the trypsin inhibitor of the egg white with ovomucoid, J.Biol. Chem., 171: 565-581.
5. LOWRY O.H., ROSENBROUGH L.A., FARR R.J., RANDALL R.J., 1951 - Protein in measurement with Folin reactiv. J.Biol. Chem., 193: 265-275.
6. NEMETH J., JUHASZ S., BAINTNER K., 1979 - A trypsin and chymotrypsin inhibitor from Taenia pissiformis. Int.J.Parasitol., 9, 6: 515-522.
7. NORDEN D.A., 1972 - The inhibition of trypsin and some other proteases by ovorubin, a protein from the eggs of Pomacea canaliculata. Comp.Biochem.Physiol., 42 B: 569-576.