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Enzymes of hepatopancreas and thromp of *Rapana venosa* (Valenciennes 1846)

Rapana venosa'nın (Valenciennes 1846) hepatopancreas ve tromp'unun enzimleri

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Abstract

In this study were investigated the extraction and activities of the enzymes from the hepatopancreas and thromp of *Rapana venosa*. The examined enzymes were amylase, lipase and protease. Higher activity was found in hepatopancreas than in thromp. Amylase, lipase and protease activities of thromp were determined first time. Lipase activity was also assayed first in hepatopancreas of *R.venosa*. Moreover the activities of amylase and lipase found were higher than those given hereto.

Keywords: *Rapana venosa,* hepatopancreas, thromp, amylase, lipase, protease.

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Introduction

Rapana venosa (Valenciennes 1846) (formerly *R.thomasiana* thomasiana) (Mollusca, Gastropoda, Prosobranchia, Muricidae) is of Japan Sea origin. It is believed that the gastropod was introduced into the Black Sea by a ship carrying its eggs attached to its hull in 1946. In 1950 it depleted the Guda osysters bank in the Caucasus and began to feed on the mussels living near the southern shores of the Crimea and near the Bulgarian coasts (Drapkin, 1953; Ivanoui, 1946 a, b). It was first detected in Turkish coasts in 1960 (Fisher-Piettee, 1960). The distribution of *R.venosa* in the Black Sea coasts of Turkey was investigated by Bilecik (1975, 1990). In 1970's it penetrated into the Sea of Marmara (Drapkin, 1953). Its anatomy was studied by Lupu (1977).

The contents of *R.venosa* were identified as raparin, a heparinoid, (Güven *et.al.*, 1991; Genç *et al.*, 1996), insulin (Akıncı *et al.*, 1998), lipids (Rosoiu and Serban, 1981) and fatty acids in whole animal (Chirstie *et al.*, 1988) and fatty acids and sterols in various organs (Güven *et al.*, 1988), hemocyanin (Boteva *et al.*, 1991; Idakieva *et al.*, 1995; Stoeva *et al.*, 1995), bioglycan (Mikheiskaya *et al.*, 1986).

R.venosa enzymes were also studied as amylase (Rosoiu, 1977), Protease (Mirza, 1976; Rosoiu 1990) and cholinesterase (Grigor'eva, 1990).

Other compounds studied were: Pepsin inhibitor (Rosoiu, *et al.*, 1989), FMFR amid (Yanagowa *et al.*,1988; Kobayashi and Sakata, 1992) and dye in hypobranchial gland (Shimoyama and Noda, 1992).

The radionuclide contamination of *R.thomasiana* was investigated after Tchernobyl accident (Bulut *et al.*, 1990).

Enzymes, the subject in this work are essential constituents of living organisms. The metabolism in living cells involves thousands of chemical reactions, mostly occurred in the presence of enzymes (Ruyssen and Lauwers, 1978). The most important enzymes in digestive tract are amylase, lipase and protease which are similar in mamalian and marine organisms (Anon., 1974). In the molluscs, liver and saliva are the main digestive juices. Amylase, emulsin, glycogenase, lactase, lipase, maltase, pepsin and urease are found within the contents of their stomachs and intestine. In gastropods carbohydrases occur in the digestive juice, salivary glands and digestive diverticula (Oven, 1966). Intestine in prosobranche contains protease and amylase (Franc, 1968). Enzymes in vertebrates were found in pancreas, pyloric caecum, intestinal fluid and bile (in anchovy). Pancreas in fish, is present as a discrete organ or as diffused tissue surrounding the pyloric caecum (Sargent, 1976). Amylase, β-galactosidase is isolated (Alemany and Rosell-Perez, 1973; Berkkan, 1991; Kuşçu and Berkkan, 1992; Özsoy and Berkkan, 1997) and (Dumitri *et al.*, 1978) from Mytilus sp.

Enzyme activity were expressed in U/mg or μU/mg. This unit was defined with reference to the estimation method used. The determination methods were titrimetric or spectrophotometric. The activities of the enzymes investigated in this work were according to amylase by iodometric (Anon, 1968), enzymatic dye-test (Anon., Boehringer), lipase by titrimetric (Willstatter *et.al.*. 1923), by spectrophotometric (Duncombe, 1963; Güven *et al.*, 1960, Güven and Aran, 1981), proteas by spectrophotometric using Folin-Ciocalteau reagent (Anson, 1938), van Urk reagent (Güven and Hallı, 1984) methods.

In this work was examined amylase, lipase and protease activities in hepatopancreas and thromp of *R.venosa.*

Material

Rapana venosa was obtained from Mimtur, Rumeli Feneri, Istanbul collected from the Black Sea near the Bosphorus at 20-40 m deep. It was stored at -30°C. After dissection, the parts of hepatopancreas and thromp were separated and re-stored at -30°C.

The subtances used:
Ammonium sulfate (Merck)
Accacia gum (Konig-Wiegan)
Sodium taurocholate (Kali-Chemie). 4 g in 50 ml water.
Tris-chloride buffer (60.6 mg tris, 234 mg sodium chloride in 100 ml water).
Thymolphtalein (Merck). Solution 1% in alcohol.
Calcium chloride anhidre (Merck)
0.I.N. KOH
Egg white, fresh.
Olive oil, neutralized
Hemoglobin (Sigma)
Thyrosine (Merck)
Trichloro acetic acid (Merk). Solution 5%.
Folin-Ciocalteau reagent
Pancrease-α-Amylase EPS BM/Hitachi 717 (Boehringer Mannheim)

Methods

1. Extraction techiques

1.1. Hepatopancreas

1.1.1. Hepatopancreas of 10 *R.venosa* were cut, homogenized in 500 ml alcohol, cooled at –3°C, after maceration, centrifuged. The residue was re-macerated in 150 ml saline by mixing occasionally, re-centrifuged. The upper phase was taken (H_1).

1.1.2. 350 g Hepatopancreas was mixed with 300 ml saline and 2.5 ml chloroform (to preserve) for 30 min in a blender then centrifuged: the supernatant

was taken and shaken with 50 ml dichlormethane to remove lipids (H_2). The H_1 and H_2 extracts were lyophilized.

1.2. Thromp

1.2.1. Thromp of 10 *R.venosa* were cut and shaken mechanically with 150 ml saline for 30 min. After filtration, acetone was added, filtered, the residue was taken and dialized in Wisking tubing (36/32, θ4.5 cm), centrifuged and lyophilized (T_1).

1.2.2. 55 g Pieced thromp was mixed with 50 ml saline and 2.5 ml chloroform for 30 min in a mixer, filtered, shacked with petroleum ether to remove lipids, centrifuged. The residue was re-extracted with saline as indicated above. The extracts were combined and lyophilized (T_2).

1.2.3. 90 g Pieced thromp was macerated with 200 ml saline for 3 h in a refrigerator. After centrifugation, upper phase was taken and lyophilized (T_3).

2. Activity assays

2.1. Amylase activity (Anon., Boehringer)

Amylase activity was determined by using 4.6-ethylidene G_7PNP (G_1-α, d-maltohaptoside) as substrate.

Reaction scheme:

$$5\text{-Ethyliden-}G_7PN+5H_2O \xrightarrow{\text{Pancreas α-amylase}} 2\text{ ethyliden-}G_5+2G_2PNP$$

$$+2\text{ ethyliden- }G_4+2G_3PNP+\text{ethyliden-}G_3+G_4PNP$$

$$2G_2PN+2G_3PNP+10H_2O \xrightarrow{\alpha\text{ glucosidase}} 4PNP+10G$$

PNP=p-nitrophenol, G=glycose

2.2. Lipase (Willstätter et al., 1923)

2.5 g Neutralized olive oil was mixed in a mortar with 1.25 g acacia gum and 1.25 ml water then diluted with water to 15 ml. The enzyme extract obtained from hepatopancreas and thromp was dissolved in 1 ml water and added 8 ml tris-chloride buffer 2 ml sodium taurocholate solution; 10 mg $CaCl_2$, 15 mg egg white. The mixture was stirred for 3 min and incubated at 37°C for 57 min in water bath, then cooled and added a mixture of 125 ml alcohol and 10 ml ether. 10 drops of thymolphtalein solution and titrated with 0.1 N KOH solution.

Willstätter unit is equivalent to the hydrolysis of 2.5 g olive oil in ratio 24% i.e., the number of ml 0.1 N KOH solution x 6.25= Willstätter unit.

2.3.Proteas (Anson, 1938)

5 g Hemoglobin solution (2 g hemoglobin was dissolved in 35 ml water and added 38 urea and 8 ml 2N NaOH) was heated at 35.5°C for 5 min, added enzyme extract of *R.venosa*, dissolved in water. The mixture was heated at 35.5°C for 30 min, 10 ml trichloroacetic acid solution added and stored for 10 min at room temperature and filtered. 5 ml filtrate was taken and added 10 ml 0.5NaOH and 3 ml Folin-Ciocalteau reagent (diluted 2:1 in water). 5 min later the absorbance was read at 752 nm in a spectrophotometer (Shimadzu UV-1601) against the blank which had been prepared in the same manner omitting the enzyme. The absorbance was read in a spectrophotometer and tyrosine amount found by multipliying with 3.6 (dissolution factor) and proteolytic activity of enzyme extracts was calculated from the liberated tyrosine from hemoglobin.

Standard curve of tyrosine

Tyrosine stock solution: 60.4 mg tyrosine was dissolved in 0.1 N HCl and the volume was adjusted to 100 ml with 0.1 N HCl. 0.5, 1, 2, 3, 4 ml stock solution of tyrosine was put in a volumetric flask and the volume was adjusted to 5 ml and the standard curve was plotted for the concentrations of 0.0367-0.2896 µg/ml.

Result and Discussion

1. Amylase activity
1.1. Hepatopancreas

Amylase activity of hepatopancreas extracts are shown in Table 1. H_2 extract showed the highest activity.

1.2. Thromp

Amylase activity of thromp extracts are shown in Table 1. T_3 extract showed the highest activity.

Table 1. Amylase activity of hepatopancreas and thromp extracts.

H- Hepatopancreas T-Thromp

Extract	µ U/mg
H_1	320
H_2	1400
T_1	160
T_2	300
T_3	480

2. Lipase activity

Lipase activity of hepatopancreas extract was as follows:

H_2 44.2μM/mg

Lipase activity was not found in thromp extract

3. Proteolytic activity

Proteolytic activity of hepatopancreas extract was found as 6.67 μ U/mg.

Proteas activity was not found in thromp extract.

The comparison of our results with the earlier findings were:

Rosoiu (1976) found amylase activity as 48-120 μ U/mg/min., we found 1400 μ U/mg, Mirza (1976) found proteolytic activity as 50-10 n mol tyrosine/min/mg in stomach, whereas Rosoiu (1990) 3950 n mol tyrosine/min/mg but no activity in hepatopancreas, contrary to these earlier findings we found a proteolytic activity as 6.7 μU/mg.

Our result on amylase activity in hepatopancreas was found 10 times as high than by Rosoiu (1976) finding.

Only amylase, activity was found in thromp.

In conclusion amylase, lipase in hepatopancreas and amylase activitiy in thromp were determined first time in *R.venosa.* Additionally protease activity was also demonstrated in hepatopancreas.

Özet

Bu çalışmada *R.venosa*'nın hepatopankreas ve trompunun enzimleri üzerinde aktivite tayinleri yapılmıştır. Hepatopankreas'da amilase, lipase ve proteas tayinleri ile trompta amilase tayini bu çalışmada ilk defa yapılmıştır. Amilase aktivitesi literatürdeki kayıtlara nazaran daha yüksek bulunmuştur. Lipase aktivitesi ise hepatopankreasda tespit edilmiş, trompta ise tespit edilmemiştir. Hepatopankreasda, protease aktivitesi literatürdeki kayıtlara nazaran daha yüksek bulunmuştur. Hepatopankreasda beklenen bu enzimatik aktivitelerin yanında, trompta amilas aktivitesinin bulunması sindirimin başlangıcının tespiti bakımından *R.venaso* için önem taşımaktadır.

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