

ENZYME ACTIVITIES OF RIGHT MASSIVE GLAND SALIVARY OF *RAPANA VENOSA*
(VALENCIENNES 1846)

RAPANA VENOSA (VALENCIENNES 1846)'NİN SAĞ MASİF İFRAZ BENZİNİN ENZİM
AKTİVİTELERİ

SERAP AKINCI¹, KASIM CEMAL GÜVEN¹, MÜNİRE HACİBEKİROĞLU²,
MUTLU KÜÇÜK³, ERDOĞAN OKUŞ¹

¹Institute of Marine Sciences and Management, Müşküle sok. 11, 34470 Vefa İstanbul

²Fikret Biyal Research Center, Cerrahpaşa Faculty of Medicine, İstanbul

³DETAM Experimental Medical Research Institute, University of İstanbul
Turkey

In this study the enzymes were investigated from the right massive gland salivary of Rapana venosa. The enzymes determined were amylase, lipase and protease. Lipase and protease activities were proved first in right massive gland salivary of R. venosa.

Bu çalışmada Rapana venosa'nın sağ masif ifraz bezinin enzimleri üzerinde çalışılmıştır. Bu enzimler, amilaz lipaz ve proteazdır. R. venosa'ın sağ masif ifraz bezinin içerdiği lipaz ve proteaz üzerindeki tayin literatürde ilktir.

Keywords: *Rapana venosa*; Right massive gland salivary; Amylase; Lipase; Protease

Anahtar kelimeler: *Rapana venosa*; Sağ masif ifraz bezi; Amilaz; Lipaz; Proteaz

Introduction

Shellfish *Rapana venosa* (Valencien- nes 1846) (formerly *R. thomasi* Grosse) (Mollusca, Gastropoda, Prosobranchia, Muricidae) was of Japan Sea origin and introduced into the Black Sea by a ship carrying the eggs attached to its hull in 1946. In 1950 it depleted the Guda oysters bank in the Caucasus and began to feed on the mussels living near the southern shores of the Crimea and near the Bulgarian coasts (1,2,3). It was first detected in Turkish coasts in 1960 (4) and the scatter of *R. venosa* in the Black Sea coasts of Turkey was investigated by Bilecik(5,6). In 1970's it penetrated into the Sea of Marmara(1).

The anatomy of *R.venosa* was studied by Lupu(7). There are two massive gland salivary as left and right, which are connected to digestive channel. They secrete enzymes such as amylase(8).

Protease activity was found in the stomach and hepatopancreas of *R. venosa* (9). Lipase activity of hepatopancreas and tromp was first demonstrated by Akinci et

al.(11a). Amylase and protease activities were also investigated in the study(11b).

Lipase is an important enzyme for digestion and usually covers a large series of enzymes hydrolizing various esters. Detection of lipase activity in marine organisms was also interesting. A 1,3-specific triacyl glycerol lipase to that occurring in mammalian pancreas was demonstrated. On the other hand various types of esterases were detected in marine organisms.

Enzymes of fish and shellfish, in the elasmobranch, pepsin is present when the stomach of the intestines are acidic but no tripsin. Gastric diastase occurs when the stomach is either neutral or alkaline. It is also said that lipase does not occur when the stomach is acidic. In the pancreas, lipase, diastase and tripsin were found. In the mollusc, liver juices and saliva are the main digestive juice. Various enzymes were found among the contents of their stomach and intestines. In vertebrates proteolytic ability of pepsin was found in

the stomach. The liver and pancreas contain tripsin and amylase. Lipase activity has also been detected in the wall of pancreas(12).

The contents of *R.venosa* were identified as raparin, a heparinoid, (13,14), insulin (11a), lipids (15) and fatty acids in whole animal (16) fatty acids and sterols in various organs(17) and enzymes in hepatopancreas(11b).

Enzyme activities were expressed in U/mg or μ U/mg. This unit was defined with reference to the estimation method used. The determination methods were titrimetric or spectrophotometric. The activities of the enzymes were investigated in this work according to amylase by iodometric(18), enzymatic dye-test(19), lipase by titrimetric (20), spectrophotometric (21-23), protease by spectrophotometric using Folin-Ciocalteau reagent(24), and Van Urk reagent (25) methods.

In this paper amylase, lipase and protease activities in right massive gland salivary of *R.venosa* were reported.

Materials and Methods

Rapana venosa was collected from Rumeli Feneri, Istanbul, the Black Sea near the Bosphorus at 20-40 m depth. It was stored at -30°C , and after dissection right massive gland salivary was separated.

Reagents

Ammonium sulfate(Merck), accacia gum (Konig-Wiegan), sodium taurochlorate (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 anhydrous (Merck), potassium hydroxide 0.1 N, egg white, fresh, olive oil, neutralized, Hemoglobin (Sigma), tyrosine (Merck), trichloro acetic acid (Merck), (5% solution in water), Folin-Ciocalteau reagent, pancrease- α -amylase EPS BM/Hitachi 717 (Boehringer, Mannheim)

1.Extraction techniques

350 g massive gland salivary was mixed with 300 ml saline and 2.5 ml chloroform (to preserve) for 30 min in a blender and then centrifuged. The supernatant was taken and shaken with 50

ml dichloromethane to remove lipids and then lyophilized.

2. Activity assays

2.1. Amylase activity (18)

The activity of amylase was determined using 4,6-ethylidene G₇PNP (G₁ - α , D - maltoheptaoside) ethylidene - G₇ PNP as substrate (Boehringer). At the end of the reaction 4PNP (p-nitrophenol) and glucose occurred was determined by enzymatic colorimetric test (sys 2, BM/Hitachi 717/911).

2.2.Lipase activity (20)

2.5 g neutralized olive oil was emulsified with 1.25 g acacia gum and 1.25 ml water then diluted with water to 15 ml. The enzyme extract was dissolved in a mixture of 1 ml water, 8 ml tris-chloride buffer, 2 ml sodium taurocholate solution. Then 10 mg CaCl₂, 15 mg fresh egg white were added. The mixture was stirred for 3 min and incubated at 37°C for 57 min in a water bath, then cooled and added a mixture of 125 ml alcohol, 10 ml ether, 10 drops of thymolphtalein solution and titrated with 0.1 N KOH solution. Calculation of unit: Willstätter unit is equivalent to the hydrolysis of 2.5 g olive oil in ratio of 24% i.e., the number of ml 0.1 N KOH solution $\times 6.25 =$ Willstätter unit.

2.3.Proteas (24)

Hemoglobin solution was used as substrat. This assay was based on the determination of liberated tyrosine from hemoglobin (2 g hemoglobin was dissolved in 35 ml water and added 38 g urea and 8 ml 2 N NaOH, mixed for 45 min). 5 ml of this solution was taken and heated at 35.5°C for 5 min, added enzyme extract of *R.venosa*. The mixture was heated at 35.5°C for 30 min, then 10 ml trichloroacetic acid solution was added and stored for 10 min at room temperature and filtered. 5 ml filtrate was taken and added 10 ml 0.5 N NaOH and 3 ml Folin-Ciocalteau reagent (diluted 2:1 in water). After 5 min the absorbance was read at 752 nm in a spectrophotometer (Shimadzu UV -1601) against the blank prepared in the same manner omitting the enzyme. The tyrosine amount was found by multiplying by 3.6 (dissolution factor) and proteolytic activity liberated of enzyme extracts was calculated on tyrosine from the hemoglobin.

Standard curve of tyrosine

Tyrosine reference solution: 60.4 mg tyrosine was dissolved in 0.1 N HCl and the volume made up to 100 ml with 0.1 N HCl. The solution contained 0.1812 mg or 1 µmol tyrosine per 3 ml. 0.5, 1, 2, 3 and 4 ml stock solution of tyrosine was transferred to a volumetric flask and the volume was adjusted to 5 ml and the standard curve plotted using Anson method as indicated above for concentrations of tyrosine as 0.0367-0.2896 µg/ml.

Results and Discussion

The equation for the standard curve of tyrosine was:

$$ABC = K^1 C + K_0$$

where $K^1 = 3.409$, $K_0 = -0.0291$

Amylase, lipase and protease activities of right massive gland salivary are shown in Table 1.

Table 1. Enzyme activities of right massive gland salivary (U/g).

Amylase	Lipase	Tripsin
1000	19	7

The comparison of our results with the earlier findings were: Rosoiu (8) found amylase activity in one ml protein extract as 12.56 µ U/mg/min but no activity was found in gland salivary(10), whereas we found a proteolytic activity as 7 U/g.

Amylase, lipase and tripsin activities were higher in hepatopancreas (11b) than right massive gland salivary.

Lipase and protease in right massive gland salivary was determined first time in *Rapana venosa*.

References

1. Drapkin, E.I.: Priroda, 9, 92 (1953) ref: Zaitsev, Y and Mamaev, V: Biological Diversity in the Black Sea, Black Sea Environmental Series Vol.3 pp 62 United

- Nations Publications, New York, Küre Basım, Istanbul (1977)
2. Ivanov, A.I.: Zh.Biol.No:9D5; Leningrad R. 164 (1965); Biol. Abst. 83056 (1966)
3. Ivanov, A.I.: Dokl. Akad. Naul, SSSR. Ser. Biol. Abstr. 46 10004640 (1965)
4. Fisher-Piettee, E.: Hidrobiologie Ser. B. 5,1-2.51 (1960)
5. Bilecik, N.: Rapp. Comm. Int. Mer. Medit. 23, 169 (1975)
6. Bilecik, N.: Deniz Salyangozu "Rapana venosa" (V.) nin Türkiye'nin Karadeniz Sahillerindeki Dağılışı ve Karadeniz Balıkçılığındaki Etkisi. Tarım, Orman ve Köyfleri Bakanlık, Su Ürünleri Araştırma Enstitüsü, Bodrum, Seri B, Yayın No.1, 1990
7. Lupu, D.: Trav Mus. Hist. nat. "Gr. Antipa", 18, 57 (1977)
8. Rosoiu, S.N.: Cercetari Marine 10, 205 (1977)
9. Mirza, M.: Ibid. 255 (1976)
10. Rosoiu, S.N.: Rapp. Comm. Int. Mer Medit 32, 147 (1990)
- 11a. Akıncı, S., Güven, K.C., Küçük, M., Hacıbekiroğlu, M., Koyuncuoğlu, M., Okufl, E.: Pharmazie 53, 9 (1998)
- 11b. Akıncı, S., Hacıbekiroğlu, M., Küçük, M., Okufl, E., Güven, K.C.: Turkish f. Mar. Sci. 4, 29 (1998)
12. Roussel, F.S., Yonge, C.M.: Advances in Marine Biology, Vol. 12. p. 39, 188 Academic Press, London 1974
13. Güven, K.C., Özsoy, Y., Öztürk, B., Topaloğlu, B., Ulutin, O.N.: Pharmazie 46, 547 (1991)
14. Genç, L., Özsoy, Y. Güler, E., Ulutin, O.N.: Turkish J.Mar. Sci. 2, 3 (1996)
15. Rosoiu, N., Serban, M.: Rapp. Comm. Int. Mer Medit 27, 3 (1981)
16. Christie, W.W., Brenchany, Y.E., Stefanov, K.: Chem. Phys. Lipids 46, 127 (1988)
17. Güven, K.C., Yazıcı, Z., Akıncı, S., Okufl, E.: (In press)
18. Anon.: J. Mond. Pharm. 3, 337 (1968)
19. Anon.: Enzymatischer Farb-test method, Boehringer, Mannheim
20. Willstätter, R., Waldschmidt-Leitz, E., Memmen, F.: Z. Physiol. Chem. 125, 93 (1923)

21. Duncombe, W.G.: *Biochem. J.* 88, 7 (1963)
22. Güven, K.C., Bergişadi, N., Güler, E.:
Fette Seifen Anstrich-mittel 81, 152 (1976)
23. Güven, K.C., Aran, B.: *Sci. Pharm.* 49, 14
(1981)
24. Anson, M.L.: *J.Gen.Physiol.* 22, 79 (1938)
25. Güven, K.C., Hallı, U.: *Acta Pharm. Turcica*
26, 57 (1984)

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