| Cercetări marine | I.R.C.M. | Nr.14 | 239-247 | 1981 |
|---|---|---|---|---|

# ACTIVITY OF ∝-AMYLASE IN SOME SPECIES OF MOLLUSCS AND FISH

*Natalia Roşoiu*[1] *and Mihai Şerban*[2].

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

ABSTRACT:

**The work is presenting some new data concerning the activity of -amylase in 3 species of molluscs (Rapana thomasiana, Mytilus galloprovincialis and Mya arenaria) and 3 species of fish (Squalus acanthias, Sprattus sprattus sprattus and Engraulis encrasicholus ponticus) in the Romanian coastal waters of the Black Sea, in a cephalopod (Illex illecebrosus) and 5 species of fish (Etrumeus sadina, Trachurus trachurus trachurus, Scomber japonicus colias, Merluccius sp. and Balistes capriscus) captured in different areas of the World Ocean.**

Amylases, ∝-1,4-glucane-4-glucanohydrolase (EC 3.2.1.1.) and ∝-1,4-glucane-4-glucohydrolase (EC 3.2.1.3) - enzymes which catalyse hydrolysis of the ∝ 1-4 glycosidic bounds of polysaccharides - have made the object of multiple research works carried out with marine organisms all over the world during the recent period. In this respect, the bivalve molluscs have been the most frequently studied class. Consequently, the study of the amylases has been approached - during the last years -with several mollusc species characterized by mass growth along the Romanian coast of the Black Sea, namely: Mytilus galloprovincialis Lmk., Mya arena-

ria (L) and Rapana thomasiana Grosse, the results obtained having been published in a series of previous papers (7, 8, 11, 12, 15, 16 and 17 - 20).

As a continuation to these research works, the present paper is bringing some new data concerning the activity of $\alpha$-amylase in 3 mollusc and 3 fish species along the Romanian coast of the Black Sea and in a cephalopod and 5 fish species from different areas of the World Ocean.

MATERIAL AND METHODS

The work has been done on organs and tissues of the molluscs Rapana thomasiana, Mytilus galloprovincialis, Mya arenaria and Illex illecebrosus, the fishes Etrumeus sadina, Trachurus trachurus trachurus, Scomber japonicus colias, Merluccius sp. and Balistes capriscus and on viscera of the fishes Squalus acanthias, Sprattus sprattus sprattus and Engraulis encrassicholus ponticus. The last 3 fishes, collected from the Black Sea, were processed as freshlycaught; the other fishes, as well as the cephalopod Illex illecebrosus, were in frozen state, as they had been caught in the coastal waters of West Africa and Guinea and were processed 4 - 6 months after.

Protein extracts were obtained by grinding the organs and tissues from at least 10 individuals of each species in a Potter homogenizer; the extraction ratio was 1 g tissue per 10ml 0,4% $CaCl_2$. The extracts were kept for an hour at +4°C, then were centrifuged for 20 minutes at 3,000 r.p.m.

Dosage of enzymatic activity was carried out by the METAIS and BIETH method (13), adapted to the optimum parameters of the amylasic activity for each species separately. Thus, for Rapana thomasiana, determinations were done at pH 6.2 and 37°C (16); for Mytilus galloprovincialis, at pH 6.0 and 35°C (15); for Mya arenaria, at pH 6.9 and 33°C (20) and for the other species, at pH 6.9 and 37°C. Specific amylasic activity was expressed in mU/mg protein/minute.

Concentration of proteins extracted was determined by the method of LOWRY and collab. (10) and was expressed in mg/ml.

Partial purification of $\alpha$-amylase was achieved by fractional precipitations with saline solutions and organic solvents (5, 17).

In our assays for stabilization of partally purified $\alpha$- amylase, we used different ingredients - aminoacids, proteins with small molecular mass, glycogen, starch and glycerine - which were added in percentage of 1 g to 100 g of protein with amylasic activity. Enzyme samples were preserved at cold (+4°C) and determinations were carried out every week for four months.

## RESULTS AND DISCUSSION

In animal organisms, $\alpha$- and $\gamma$-amylases are found prevalently, these enzymes being clearly different as regards both the optimum parameters of action and the final reaction products. We have shown in the previous papers (16 - 20) that $\alpha$-amylase is present in *Mytilus galloprovincialis*, *Mya arenaria* and *Rapana thomasiana*; the organ that concentrates amylase in the molluscs is the hepatopancreas and in the fish, the liver, the amylasic activity being a species characteristic. More recent data, obtained on an increased number of species (molluscs and fishes), give support to these statements which agree with other authors' results (1, 3, 4, 6, 9, 14 and 21).

In this way, analysing the data registred in Table 1, one can find that $\alpha$-amylase is present in the digestive organs and glands in all the species studied: salivary glands, stomach, intestine, liver, pancreas, hepatopancreas (in less developed organisms) and viscera. Amylasic activity was discerned also in nondigestive organs such as: mantle and foot of *Rapana*, mantle and branchiae of *Mytilus*, muscles of *Illex* and gonads of *Balistes*. The presence of amylase in those organs indicates a complementary role of the enzyme in the rapid mobilization of the sugar reserves in certain physiological states of the organisms.

In earlier studies (11, 12) on the $\alpha$-amylasic activity in the hepatopancreas, mantle, branchiae and hemolymph of the mussel we found that it is in direct proportion to the glycogen content and closely connected with the internal biological rhythm

Table 1

Activity of ∝-amylase in some species of molluscs and fish. Distribution in organs and tissues

| No. | Species (organ, tissue) | Amylasic activity mU/mg protein/min Partially purified enzymatic preparation* | Raw protein extract |
|---|---|---|---|
| | MOLLUSCA | | |
| | GASTROPODA | | |
| 1. | Rapana | | |
| | total body | | 70.44 |
| | mantle | | 4.48 |
| | foot | | 22.18 |
| | stomach | | 13.50 |
| | hepatopancreas | | 126.28 |
| | salivary glands | | 12.50 |
| | BIVALVIA | | |
| 2. | Mytilus | | |
| | total body | 7,050 | 438.00 |
| | total body | 5,833 | 430.00 |
| | mantle | | 112.00 |
| | hepatopancreas | | 1,102.00 |
| | branchiae | | 182.55 |
| 3. | Mya | | |
| | total body | 1,241 | 105.00 |
| | total body | | 485.00 |
| | hepatopancreas | | 587.00 |
| | CEPHALOPODA | | |
| 4. | Illex** | | |
| | muscles | | 37.00 |
| | viscera | | 90.00 |
| | liver | | 116.00 |
| | PISCES | | |
| 5. | Squalus | | |
| | viscera | 2,169 | 55.80 |
| 6. | Sprattus | | |
| | viscera | 3,179 | 254.90 |
| 7. | Engraulis | | |
| | viscera | 3,611 | 300.00 |
| 8. | Etrumeus** | | |
| | viscera | | 75:00 |
| | intestine | | 113.00 |
| | liver | | 120.00 |
| 9. | Trachurus** | | |
| | viscera | | 60.00 |
| | intestine | | 54:00 |
| | liver | | 131.00 |
| 10. | Scomber** | | |
| | viscera | | 61.00 |
| | intestine | | 103:00 |
| | liver | | 108.00 |
| 11. | Merluccius** | | |
| | viscera | | 31.00 |
| | intestine | | 57.00 |
| | liver | | 60.00 |
| 12. | Balistes*** | | |
| | viscera | | 151.70 |
| | intestine | | 146.00 |
| | stomach | | 166:90 |
| | liver | | 212.20 |
| | pancreas | | 104.00 |
| | gonads | | 93.30 |

OBSERVATIONS

* Purification of ∝-amylase was carried out by fractional precipitation with organic solvents-process described in the licence no. 75,140/1980.

** The specimens were captured in the West African waters of the Atlantic Ocean during September-November,1978, and the assays were performed in March,1979, after having congealed the biological material for 4-6 months.

*** The specimens were collected near the coast of Guinea during the summer of 1978 and the assays were performed in April 1979.

of the mollusc during the osmotic control process. The final products of glycogen amylolytic degradation, maltose and glucose, on one side take part in the process of glycogen synthesis, and on the other side, stimulate forming of lactic acid via glycolyse. Our data pointed out an increased amylolytic degradation of glycogen, in hypo- and hypersalinity stress physiological states of the mussel.

The amylasic activity in Mytilus galloprovincialis and Mya arenaria is comparatively higher than the amylasic activity in Rapana thomasiana, Illex illecebrosus and the fishes analysed. On this point, our data are but partially in concordance with those obtained by other authors (2).

In Illex and the fishes which were caught in different areas of the World Ocean, a remarkable stability of the enzyme is observed. An appreciable amylasic activity was discerned in these organisms after 4-6 months from the capture, although they had been preserved in a frozen state (Table 1).

By fractional precipitation with organic solvents (17), enzymatic preparations with $\alpha$-amylasic activity were obtained (Squalus acanthias), having a purification degree up to 40 x. $\alpha$-amylase stabilization research was carried out on these purified enzymatic preparations.

The enzyme inactivation rate depends on a number of factors, such as: temperature, pH of the medium, protein concentration, ionic strength of the medium and existence of protecting substances in the reaction medium. In our assays for stabilization of the partially purified $\alpha$-amylase, we used different ingredients, such as: aminoacids, small molecular mass proteins, glycogen, starch and glycerine. In this respect, the results obtained on the partially purified enzymatic preparation from Mya arenaria is presented hereinafter.

It may be observed, from the data in Table 2, that the partially purified amylase shows good stability in time, after having been subjected to vaccuum drying; after having been kept in a refrigerator for four months at $+4^{\circ}C$, the amylasic activity was still found in proportion of 103%. Aspartic acid, glycocol and

a mixture of serine, tyrosine and tryptophan proved to have been the best stabilizers. At the same time, it was found that valine, cysteine and bovine serum albumin have the property of activating the amylase (activity 5 times stronger as compared to the control);however,the amylasic activity decreases in the course of time - after four months' refrigerating, only 35% of the initial activity of the enzyme is still found, however this is almost twice stronger as compared to the control.

Table 2

Time-stability of partially purified $\alpha$-amylase from Mya arenaria

| Ingredient added to enzymatic preparation | Specific amylasic activity mU/mg protein/min at 37oC | | | | |
|---|---|---|---|---|---|
| | Initial | After 2 weeks | After 4 weeks | After 2 months | After 4 months |
| CONTROL - Vaccuum-dried enzymatic preparation | 4,35o | 4,127 | 4,3o5 | 4,926 | 4,5o4 |
| Aspartic acid | 5,345 | 6,734 | 6,695 | 5,127 | 5,oo5 |
| Valine | 29,6oo | 8,343 | 9,29o | 5,732 | 5,7oo |
| Glutamic acid | 3,o67 | 3,117 | 3,238 | 2,872 | 2,99o |
| Glycocol | 4,548 | 7,987 | 5,o55 | 5,819 | 5,2o1 |
| Cysteine | 2o,5oo | 9,283 | 9,427 | 8,235 | 7,3oo |
| Bovine serum albumine | 28,182 | 1o,345 | 7,647 | 7,125 | 7,48o |
| Aminoacid mixture: | 4,667 | 7,824 | 7,158 | 6,629 | 6,31o |
| - Serine | | | | | |
| - Tyrosine | | | | | |
| - Tryptophan | | | | | |
| Glycogen | 6,549 | 6,5o4 | 7,297 | 4,886 | 4,897 |
| Starch | 9,68o | 8,196 | 6,126 | 4,3o7 | 4,245 |
| Glycerine | 8,o2o | 2,931 | 2,257 | 2,344 | 2,463 |

NOTE: The enzymatic reaction was carried out at pH 6.8 and 37°C.

CONCLUSIONS

- An important $\alpha$-type amylasic activity has been observed in all the investigated mollusc and fish species.

- Having studied its distribution in organs and tissues, it was found that $\alpha$-amylase prevails in the digestive organs and glands. In certain physiological conditions of the organisms,

this enzyme is also present in some non-digestive organs, having a complementary role in the rapid mobilization of the glucidic reserves.

- The organ which concentrates amylase in the molluscs is the hepatopancreas, while in the fish it is the liver.

- The $\alpha$-amylasic activity has specific characteristics; Mytilus galloprovincialis and Mya arenaria are the species in which the strongest $\alpha$-amylasic activity has been observed.

- In Illex illecebrosus, Etrumeus sadina, Trachurus trachurus trachurus, Scomber japonicus colias, Merluccius sp. and Balistes capriscus an appreciable amylasic activity was found even after 4 to 6 months from their capture, the organisms having been kept frozen for that entire duration.

- Having assayed the time-stability of the partially purified $\alpha$-amylase from Mya arenaria, aspartic acid, glycocol and a mixture of serine, tyrosine and tryptophan were found to be its most efficient stabilizers, while valine, cysteine and bovine serum albumin have the property of activating the amylase.

-The partially purified amylase itself shows good time-stability; after four months' refrigerating (+4°C), 10-3% of its enzymatic activity was still found, in the absence of any ingredient.

REFERENCES :


1. ALEMANY M., ROSELL-PEREZ M., 1973 - Two different amylase Activities in the Sea Mussel, Mytilus galloprovincialis L., Rev.Espan.de Fisiol., 129: 217-222.
2. ANANICHEV A.V., 1959 - Pishchevaritely'niye fermenty ryb i sezonnaya izmenchivosty' ikh aktivnosti, Biokhimia, 24,6: 1033-1040.
3. ANDREYEV A.K., 1958 - Myshechnaya amilaza ryb, Biokhimia, 23, 6: 899-904.
4. BUZINOVA N.S., 1979 - Aktivnosty' amilazy u amurov i tolstolobikov, Sovrem.vopr.skol.fiziol.ryb., Moskva: 201-206.
5. DUMITRU I.F., IORDACHESCU D., NICULESCU S., ROSOIU N., 1978 - Procedeu de izolare a unei enzime amilolitice, Patent nr.68328.

6. GOROMOSOVA S.A., SHAPIRO A.Z., 197o - Ob aktivnosti amilazy v ghepatopancrease i myshtzakh midii, Biologhiya morya, 18: 89-98.
7. IORDACHESCU D., DUMITRU I.F., 1978 - Some physicochemical properties of $\alpha$ -amylase purified from marine mollusc Mya arenaria, Rev.Roum.Biochim., 15, 4: 279-285.
8. IORDACHESCU D., NICULESCU S., DUMITRU I.F., 1975 - Amylase extraction from the hepatopancreas of Mytilus galloprovincialis Lmk., Cercetări marine, IRCM, 8: 155-161.
9. KRISTENSEN J.H., 1972 - Carbohydrases of some marine invertebrates with notes on their food and the natural occurence of carbohydrases studied, Marine Biol., 14:13o-142.
1o. LOWRY O.H., ROSENBROUGH N.J., FARR L.A., RANDALL B.I., 1951 - Protein measurement with Folin reactiv, J.Biol.Chem., 193: 265-275.
11. MAHASNEH D., ROSOIU N., 1981 - The effect of salinity on amylase activity in Mytilus galloprovincialis, Téthys, 1o, 2: 135-14o.
12. MAHASNEH D., ROSOIU N., 1981 - Variation of amylasic activity in Mytilus galloprovincialis Lmk.as a function of salinity and glycogen content of the tissues,Rapp.Comm.int. mer Médit., Cagliari, 27, 3: 45-47.
13. METAIS P., BIETH J., 1968 - Determination de l'alpha amylase par une microtechnique, Ann.Biol.Clin., 26: 133-142.
14. NEWELL R.C., PARKER J., COOK P.A., 198o - A possible role of $\alpha$-amylase isoenzymes from the style of the mussel Choromytilus meridionalis (Krauss) following thermal acclimation. J.Exp.Mar.Biol.and Ecol.,47, 1: 1-8.
15. ROSOIU N., 1976 - Dynamique de l'activité amylasique chez la moule, pendant son cycle biologique annuel. Cercetări marine, IRCM, 9: 261-266.
16. ROSOIU N., 1977 - Activité amylasique chez Rapana thomasiana Grosse, Cercetări marine, IRCM, 1o: 2o5-21o.
17. ROSOIU N., 198o - Procedeu de obţinere a unui preparat amilolitic din Mytilus galloprovincialis, Patent nr.7514o.

18. ROSOIU N., IACOVACHE C.,198o- Partial characterization of the purified ∝ -amylase from the soft clam Mya arenaria L., Rev.Roum.Biochim., 17, 4: 285-29o.
19. ROSOIU N., SERBAN M.,1981 - Activité amylasique chez quelques espèces de Mollusques des eaux roumaines de la mer Noire, Rapp.Comm.Int.mer Médit., Cagliari, 27,3: 29-3o.
2o. ROSOIU N., STOICA S.,1978 - Activité amylasique chez Mya arenaria L., Cercetări marine, IRCM, 11: 2o3-213.
21. SEIDERER L.J., NEWELL R.C., 1979 - Adjustment of the activity of ∝ -amylase extracted from the style of the black mussel Choromytilus meridionalis (Krauss) in response to termal acclimation, J.Exp.Mar.Biol.and Ecol.,39, 1 : 79-86.