ENZYME KINETCS DATA OF A-AMYLASE EXTRACTED AND PURIFIED FROM MARINE MOLLUSCS *MYTILUS GALLOPROVINCIALIS* LMK. AND *MYA ARENARIA* L.

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Abstract

The amylolytic activity in *Mytilus galloprovincialis* and *Mya arenaria*, from Romanian Bleak Sea Coast is similar to that in the benthonophage fishes and the higher mammals – rabbit, cow, dog – and it is several times greater than in the crustaceans and the predatory fishes. The purified a-amylase of *Mytilus galloprovincialis* Lmk. and *Mya arenaria* L. has an optimum activity at pH 6.0 at, 35°C, and, at pH 6.9 - 7.0 at 32°C. $CaCl_2$, NaCl and $MgCl_2$ in concentrations between 1-10 mM activate the α-amylase and protect it against thermal inactivation. The Ca^{2+} an Cl^- are essential for the enzymatic activity of marine mollusca α-amylase.

Key words: α-amylase, marine molluscs, enzyme kinetic.

Amylase, 1,4-α-glucan-glucanohydrolase (3.2.1.1.) enzyme which catalyses hydrolysis of the α - 1.4 glycosidic bounds of polysaccharides, have been the object of many research projects carried out with marine organisms all over the world during the recent period. This paper represents original data on a-amylase investigates in *Mytilus galloprovincialis* Lmk. and *Mya arenaria* L.Similar data obtained on unpurified protein extracts from hepatopancreas and whole body, as well as on the purified a-amylase from hepatopancreas and whole body, indicate the presence of a single enzyme with amylolytic activity in both bivalves. On the other hand, the more intense enzymatic activities, associates with the hepatopancreas, show that this is the organ which concentrates the amylase. Amylase activity was detected also in non digestive organs, such as mantle and branchie, as well as in hemolymph. 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 (Table 1).

Table 1. Activity of a-amylase in Mytilus galloprovincialis and Mya arenaria. Distribution in organs and tissues.

Species (organ,tissue)	Amylase activity (mU/mg protein/minute) partially purified enzymatic preparation	crude protein extract
MOLLUSCA BIVALVIA		
1. *Mytilus galloprovincialis*		
total body	7,050	438.00
mantle		112.00
hepatopancreas		1,102.00
gills		182.55
2. *Mya arenaria*		
total body	1,241	105.00
hepatopancreas		587.00

We found that the α-amylase activity in hepatopancreas, mantle, gills and hemolymph of the mussel is in direct proportion to the glycogen content and closely connected with the internal biological rhythm of the mollusca during the osmotic control process (Figure 1).

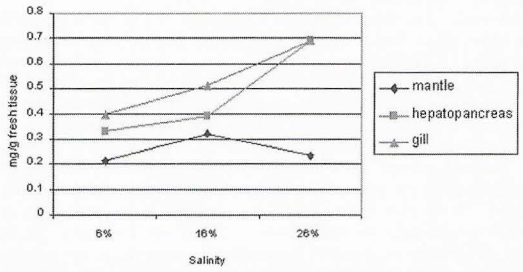

Fig. 1. Variation of glycogen concentration in *Mytilus galloprovincialis* in hepatopancreas, mantle, gills and hemolymph in conditions of variable salinities.

Our data show an increased amylolytic degradation of glycogen, under hypo- and hypersalinity states of the mussel (Figure 2). Glucose, the final product of glycogen amylolytic degradation, is involved in the process of glycogen synthesis, and on the other hand, stimulates forming of lactic acid via glycolysis. Our data show an increased amylolytic degradation of glycogen, in both hypo- and hypersalinity states of the mussel (1).

The amylase activity in *Mytilus galloprovincialis* (1,102 mU/mg/minute) is comparatively higher than the amylase activity in *Mya arenaria* (587 mU/mg /minute).

The purified α-amylase of the hepatopancreas and whole body of Mytilus galloprovincialis Lmk., has an optimum activity at pH 6.0 and 35°C, and the purified α-amylase of the hepatopancreas and whole body of *Mya arenaria* L. has an optimum activity at pH 6.9 - 7.0 and 32°C (2).

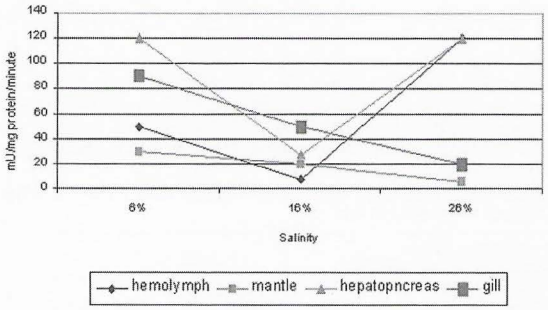

Fig 2. Amylase activity in *Mytilus galloprovincialis* in hepatopancreas, mantle, gills and hemolymph in conditions of variable salinities (determined by Metais-Bieth's method using starch as substrate).

In our assays for stabilization of the isolated and purified α-amylase of *Mytilus galloprovincialis* and *Mya arenaria*, we used different ingredients, such as: amino acids, small molecular mass proteins, glycogen, starch and glycerol. Stability of the purified α-amylase was enhanced by aspartic acid, glycine and a mixture of serine, tyrosine and tryptophan while valine, cysteine and bovine serum albumin activated the amylase (Table 2). The purified α-amylase it self shows stability, over time. After four months refrigerating (+4°C), 100% of its enzymatic activity was still found, in the absence of any ingredient. $CaCl_2$, NaCl and $MgCl_2$ in concentrations between 1-10 mM activate the a-amylase and protect it against thermal inactivation (3).

Table 2 - Time - stability of purified a-amylase of Mya arenaria

Ingredient added to enzymatic preparation	Specific amylase activity (mU/mg proteine/minute at pH 6.8 and 370C)				
	Initial	After 2 weeks	After 4 weeks	After 2 months	After 4 months
CONTROL Vaccuum-dried enzymatic preparation	4,350	4,127	4,305	4,926	4,504
aspartic acid	5,345	6,734	6,695	5,127	5,005
valine	29,600	8,343	9,290	5,732	5,700
glutamic acid	3,067	3,117	3,238	2,872	2,990
glycine	4,548	7,987	5,055	5,819	5,201
cysteine	20,500	9,283	9,427	8,235	7,300
amino acid mixture - serine - tyrosine - tryptophan	4,667	7,824	7,158	6,629	6,310
bovine serum albumine	28,182	10,345	7,647	7,125	7,480
glycogen	6,549	6,504	7,297	4,886	4,897
starch	9,680	8,196	6,126	4,307	4,245
glycerine	8,020	2,931	2,257	2,344	2,463

The Ca^{2+} an Cl^- are essential for the enzymatic activity of mollusca α-amylase. In conclusions, the amylase activity in *Mytilus galloprovincialis* and *Mya arenaria*, from Romanian Bleak Sea Coast is similar to that in the benthonophage fishes and the higher mammals - rabbit, cow, dog - and it is several times greater than in the crustaceans and the predatory fishes.

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