

β-GLUCOSIDASE ACTIVITIES IN THE MANTLE TISSUE OF THE MUSSEL *MYTILUS GALLOPROVINCIALIS* L.

MYTILUS GALLOPROVINCIALIS L. MANTO DOKUSU β-GLUKOZİDAZ AKTİVİTESİ

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Two different β-glucosidase isoenzymes were separated from the mantle tissue of *Mytilus galloprovincialis* L., a mollusc species existing in the coast of Turkey, by extraction, ammonium sulphate precipitation and hydroxylapatite column chromatography and some of their properties were examined.

It has been determined that the optimum temperatures for the two isoenzymes are 47°C and 49°C pH optima lies between 5.4-6.4 and Km values with 4-nitrophenyl-β-D-glucopyranoside were found 0.64 mM and 0.14 mM respectively.

Türkiye sahillerinde bulunan midye türü olan *Mytilus galloprovincialis* L.'in manto dokusundan ekstraksiyon, amonyum sülfat ile çöktürme ve hidroksilapatit kolon kromatografisi yöntemleri kullanılarak iki farklı β-glukozidaz izoenzimi elde edildi ve bazı kinetik özellikleri incelendi.

Optimum sıcaklıkları 47°C ve 49°C olarak belirlenen izoenzimlerin 5.4-6.4 arasındaki pH'larda en yüksek aktiviteyi gösterdikleri ve 4-nitrofenil-β-D-glukopiranozide karşı Km değerlerinin sırasıyla 0.64 mM ve 0.14 M olduğu bulundu.

Keywords : *Mussel; Mytilus galloprovincialis* L.; Mantle tissue; β-glucosidase

Anahtar kelimeler: *Midye; Mytilus galloprovincialis* L.; Manto dokusu; β-glukozidaz

Introduction

Although β-glucosidases from plants and yeasts have been extensively studied, the mollusc β-glucosidases are relatively unexplored. It has been reported previously that oysters contain several glycosidases, including α and β-glucosidases, galactosidases and mannosidases, with a fairly wide range of action on oligosaccharides (1). The occurrence and distribution of several enzymatic hydrolytic activities of glycosidic linkages have been studied in mussels. In *Mytilus edulis* two different β-glucosidase activities have been found (2). Another study on the hepatopancreas of *Mytilus edulis* indicated the presence of α - D - galactosidase, β - D - galactosidase, α - D - mannosidase, β - D - glucosidase, α - L - fucosidase and N - acetyl - β - D - glucosaminidase(3). Two β - glucosidase isoenzymes have been obtained from the hepatopancreas of *Mytilus galloprovincialis* L., a mollusc species existing in the coast of Turkey (4).

In the present study β-glucosidases of the mantle tissue of this mollusc species were investigated.

Materials and Methods

Chemicals

Hydroxylapatite was prepared in our laboratory according to the modified method of Tiselius et al. (5).

For enzyme assay 4-nitrophenyl-β-D-glucopyranoside (Fluka 73673) was used as substrate. Bovine serum albumin (Serva 11920) and 4-nitrophenol (Fluka 73560) were employed as standarts for protein determination and enzyme activity tests respectively.

Protein determination

Protein contents of the samples were determined by means of Zamenhof Microbiuret method (6). Optical densities obtained by the application of the method to the samples were substituted in the regression equation of bovine serum albumin and protein amounts of the samples were calculated.

Enzyme assay

β - glucosidase activity was determined by incubation of 100 µl of the enzyme solution with 100 µl of 20 mM 4-nitrophenyl-β-D-glucopyranoside and 100 µl of Mc Ilvaine buffer (pH 5.8) for 30 minutes at 37°C. The reaction was stopped by adding 3 ml of 0.2 M Na₂CO₃ solution. The extinction of the solution was measured at 420 nm by Shimadzu UV-1208 spectrophotometer. One unit of enzyme activity was defined as the amount of enzyme which would liberate 1 µmol of 4-nitrophenol per minute under the assay conditions. The specific activity was expressed as enzyme units per mg of protein.

Enzyme purification

The partial purification of β-glucosidase isoenzymes from mantle tissue of *Mytilus galloprovincialis* L. involved 3 steps:

1. *Isolation.* The mussels were obtained on the day of experiment. The mantle tissue of wet weight 98 g

removed from 50 mussels was homogenized in 150 ml of % 0.9 NaCl solution then 150 ml more saline was added and the homogenate was stirred by means of magnetic stirrer for 30 minutes and left overnight in the refrigerator. The homogenate was centrifuged at 20 000 rpm (45 700 g) for 30 min. in a refrigerated centrifuge (Cryofuge 20-3 Hereaus-Christ) and the supernatant (crude extract) was collected.

2. *Ammonium sulphate fractionation*: The crude extract was first precipitated by ammonium sulphate at 40% saturation and left overnight in the refrigerator. The precipitate, separated by means of centrifugation at 20 000 rpm for 30 min. was discarded and the supernatant brought up to 60% saturation with ammonium sulphate and left again overnight at 4°C. The enzyme active precipitate thus formed (40-60% cut) was separated by centrifugation at 20 000 rpm for 30 min., dissolved in distilled water and dialysed against distilled water.

3. *Hydroxylapatite column chromatography*: The dialysed β -glucosidase active 40-60% cut was applied to hydroxylapatite column (1.8x14 cm) equilibrated with 5 mM potassium phosphate buffer (pH 6.8). The elution was performed by washing the column with the same buffer of increasing concentrations (20 mM, 50 mM, 100 mM, 200 mM). Fractions were collected in 2.5 ml volumes and each one analysed for β -glucosidase activity. All the active fractions were pooled and dialysed against distilled water.

Kinetic properties of β -glucosidase isoenzymes:

1. *Effect of pH* : The relationship between the pH variation and the activity of the isoenzymes was investigated at pH range of 1.2-9.2 by using Sorensen's glycine 1 pH 1.2-3.6, Mc Ilvaine pH 2.6-7.0 and Sorensen's phosphate pH 7.0-9.2 buffers.

2. *Effect of temperature* : The effect of temperature on the rate of hydrolysis of the isoenzymes was studied

between 5°C and 60°C under standart assay conditions.

3. *Substrate specificity* : The effect of substrate concentration on the velocity of the enzyme reaction was investigated by using varying concentrations (0.15 mM-2.5 mM) of 4-nitrophenyl- β -D- glucopyranoside as substrate. Michaelis constants (K_m) and maximum velocity (V_{max}) values were calculated by means of the equation of Lineweaver-Burk plot (7).

Results

Elution of β -glucosidase active 40-60% ammonium sulphate cut through hydroxylapatite column with stepwise molarity gradient of phosphate buffer (pH 6.8) resulted in two enzyme active peaks. These isoenzymes collected at 20 mM and 50 mM phosphate buffer eluates (pH 6.8) were named F_1 and F_2 respectively (Fig.1).

The results of partial purification of β -glucosidase isoenzymes from the mantle tissue of *Mytilus galloprovincialis* L. are summarized on Table 1.

The properties of two β -glucosidase isoenzymes studied in the present investigation are given on Table 2.

Variation of β -glucosidase activity in connection with concentration change of 4-nitrophenyl- β -D-glucopyranoside is shown in Fig.2

The effect of temperature and pH on β -glucosidase activity are shown in Fig.3 and Fig4. respectively.

Table 1. Purification of β -glucosidase isoenzymes of *Mytilus galloprovincialis* L. from 98 g of wet mantle tissue.

Purification step	Volume (ml)	Total protein (mg)	Total activity (U)	Specific activity (U/mg)
1. Crude extract	340	2829.05	1959	0.69
2. 40-60% ammonium sulphate cut	20	427.8	346.36	0.81
3. Hydroxylapatite column chromatography				
F_1	18	9.59	18.3	1.9
F_2	20	6.09	32.8	5.4

Table 2. Some properties of β -glucosidase isoenzymes from the mantle tissue of *Mytilus galloprovincialis* L.

Fractions	Optimum pH	Optimum temperature(°C)	K_m (M)	V_{max} (U)*
F ₁	5.4-6.4	47	6.4×10^{-4}	1.6×10^{-3}
F ₂	5.4-6.4	49	1.4×10^{-4}	1.4×10^{-3}

* μmol 4-nitrophenol/min

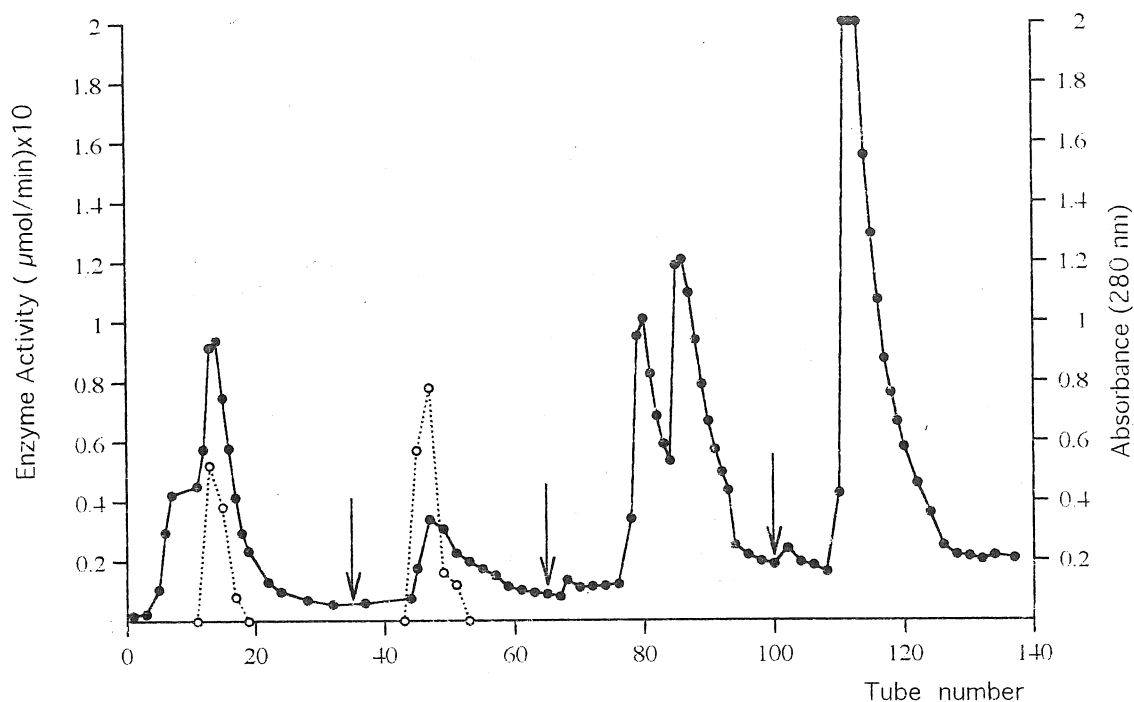


Fig. 1. Elution of β -glucosidase isoenzymes from the mantle tissue of *Mytilus galloprovincialis* L. through hydroxylapatite column. Adsorbent dimension: 1.8/14 cm, flow rate: 50 ml/hr. Arrows indicate stepwise application of 20 mM, 50mM, 100mM, 200mM phosphate buffers pH 6.8. Absorbance of proteins at 280 nm (—), activity of β -glucosidase isoenzymes (----)

Discussion

The results indicate that β -glucosidase exists in two forms in the mantle tissue of *Mytilus galloprovincialis* L.. The presence of α -glucosidases (8) and β -galactosidase (9) have been also demonstrated in the same tissue of *Mytilus galloprovincialis* L., which suggests that the mantle tissue is a good source for

glycosidases.

Optimum pH values for β -glucosidases found in another mollusc species, *Mytilus edulis*, are in acidic range, one of them has been reported to exist at pH 4.2 (2) and the other at pH 6 (3). However, β -glucosidases of *Mytilus galloprovincialis* L. were found

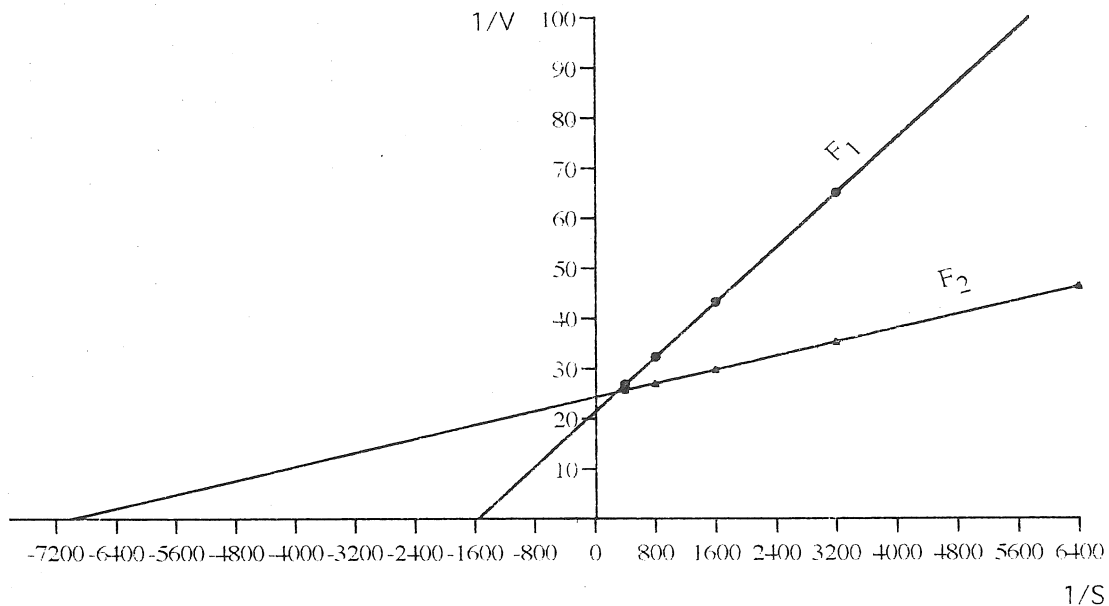


Fig.2. Lineweaver-Bruk plot of β -glucosidase isoenzymes from the mantle tissue of *Mytilus galloprovincialis* L.

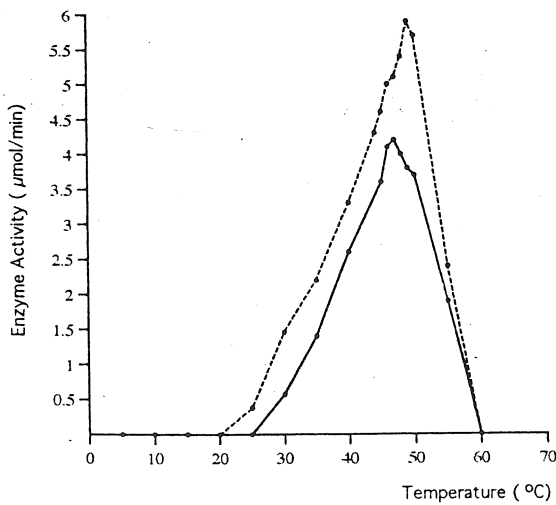


Fig.3. Effect of temperature on β -glucosidase isoenzymes F1 (—) and F2 (---) from the mantle tissue of *Mytilus galloprovincialis* L.

to have pH optima in a rather broad range from 5.4 to 6.4. Optimum temperature values for β -glucosidases (47°C and 49°C) determined in the present study are in accordance with

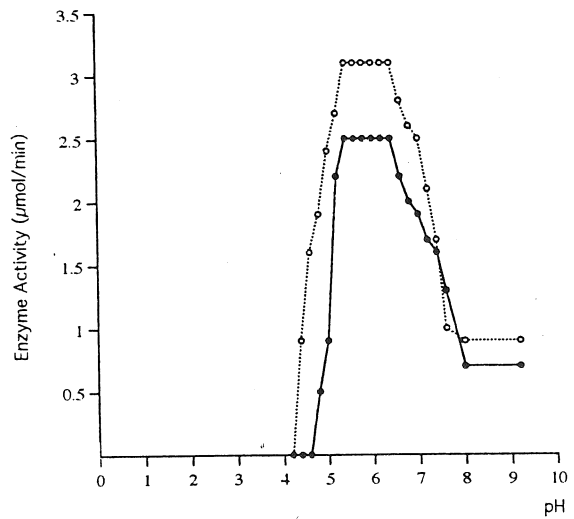


Fig.4. Effect of pH on β -glucosidase isoenzymes F1 (—) and F2 (---) from the mantle tissue of *Mytilus galloprovincialis* L. Sorensen's glycine I buffer: pH 1.2-3.6, Mc Ilvaine buffer: pH 2.2-7.2, Sorensen's phosphate buffer: pH 7.0-9.2.

values found for other glycosidases of *Mytilus galloprovincialis* L. (8,9,10). The results of the studies on kinetic properties of β -

glucosidases indicate that the two isoenzymes have the same pH optima and similar optimum temperatures but different K_m values. It was observed that β -glucosidase in F_2 shows 5 times higher affinity for the substrate 4-nitrophenyl- β -D-glucopyranoside than the isoenzyme in F_1 . The finding of different K_m values for the two isoenzymes could be the result of the different biochemical functions.

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