Acta Pharmaceutica Turcica
XLII (2,3) (80-84) 2000

β-GALACTOSIDASE ACTIVITY IN THE MANTLE TISSUE OF THE MUSSEL MYTILUS GALLOPROVINCIALIS LAM.

MYTILUS GALLOPROVINCIALIS LAM. MANTO DOKUSU β-GALAKTOZİDAZ AKTİVİTESİ

NİHAN SOMAR, NURTEN ÖZSOY, HAKAN BERKKAN

Department of Biochemistry, Faculty of Pharmacy, University of Istanbul, 34452, Istanbul, Turkey

In the present study β-galactosidase was purified 19 fold from the mantle tissue of *Mytilus galloprovincialis* Lam. by extraction, ammonium sulphate precipitation and hydroxylapatite column chromatography and some of its kinetic properties were examined.

Experiments on β-galactosidase showed that optimum temperature and pH of the enzyme were 1.85 and 49°C, respectively. The Km value for p-nitrophenyl-β-D-galactopyranoside was 1.59 x 10^{-3} M.

Galactosyltransferase activity of β-galactosidase was determined by identification of some oligosaccharides on thin-layer chromatograms as a consenquence of the action of the enzyme on lactose.

The results of the present study lead to suggest that β-galactosidase is responsible for hydrolysis of β-galactoside linkages in the mantle tissue of mollusc as well as for synthesis of the aforesaid bonds.

Türkiye sahillerinde bulunan midye türü olan *Mytilus galloprovincialis* Lam. manto dokusu β-galaktozidazı ekstraksiyon, amonyum sülfat ile çöktürme ve hidroksilapatit kolon kromatografisi yöntemleri kullanılarak 19 kez saflaştırıldı ve bazı kinetik özellikleri incelendi.

β-Galaktozidaz aktivitesi gösteren fraksiyon ile yapılan deneyler sonunda, enzimin optimal pH'sının 1.85, optimal temperatürünün ise 49° C olduğu belirlendi. p-Nitrofenil-β-D-galaktopiranozide karşı Km değerinin 1.59 x 10^{-3} M olduğu saptandı.

Midye manto dokusu β-galaktozidazının galaktoziltransferaz aktivitesi, enzimin laktoz üzerine etkisi sonucu meydana gelen bazı oligosakkaridlerin ince tabaka kromatografisi ile belirlenmesi sonucu saptandı.

Yukarıda özetlenen bulgulardan β-galaktozidazın midye manto dokusundaki β-galaktozid bağı içeren bileşiklerin hidrolizi yanında, adı geçen bağların sentezini de sağladığı ileri sürüldü.

Keywords: *Mytilus galloprovincialis Lam.; mussel; mantle tissue; β-galactosidase*

Anahtar kelimeler: *Mytilus galloprovincialis Lam.; midye;mantodokusu; β- galaktozidaz*

Introduction

β-Galactosidase(β-D-galactoside galactohydrolase, EC 3.2.1.23) catalyses the hydrolysis of β-D-galactosidic linkages present in simple and complex oligo- and polysaccharides. This enzyme is of interest because of its many potential biotechnologic applications. β-Galacto-sidase is commercially used in the dairy industry for the hydrolysis of lactose to its monosaccharides to improve digestibility of dairy products by lactose-intolerant individuals (1,5). β-Galactosidase plays an important role in the structural studies of carbohydrates. For this purpose a more active and stable β-galactosidase preparation is required. Many research have been done on the purification of this enzyme from different sources (2,3,4,6,7,10,-11,14), including mussels. An investigation on the whole tissue extract of *Mytilus galloprovincialis* revealed the presence of four different β-galactosidase isoenzymes (14), in a study on another mussel species *Mytilus edulis* (11), the mantle tissue of

which discarded, β-galactosidase have been found only in the hepatopancreas. Four β-galactosidase isoenzymes have been obtained from the hepatopancreas of *Mytilus gallopro-vincialis* (4).

The present study aims to isolate the mantle tissue β-galactosidase of *Mytilus galloprovincialis* and to elucidate the biochemical functions of the enzyme.

Materials and Methods

Chemicals: Hydroxylapatite was prepared in our laboratory according to the modified method of Tiselius et al. (12). For enzyme assay 4-nitrophenyl-β-D-galactopyranoside (Fluka 73670) was used as substrate. Bovine serum albumin (Serva 11920) and 4-nitrophenol (Fluka 73560) were employed as standards for protein determination and enzyme activity tests, respectively.

Protein determination: Protein contents of the samples were determined by means of Zamenhof Microbiuret method (15). Optical densities obtained by the application of method to the samples were substituted in the regression equation of bovine serum albumin and protein amounts of the samples were calculated.

Assay of β-galactosidase activity: β-Galactosidase activity was determined by incubating 100 µl of enzyme solution with 100 µl of 20 mM p-nitrophenyl-β-D-galactopyranoside and 100 µl of Mc Ilvaine citrate-phosphate buffer (pH 5.8) at 37°C for 30 minutes. The reaction was stopped by adding 3 ml of 0.2 M Na_2CO_3 solution. Optical density of the colour developed by p-nitrophenol liberation was measured at 420 nm. The activity was expressed as µmol of p-nitrofenol liberated per minute.

Assay of galactosyltransferase activity: Equal volumes of sample and lactose solution (10%) were incubated at 37°C for 2.5, 5 and 24 hours. Hydrolysates were detected by thin-layer chromatography on silicagel coated plastic sheets. The solvent system was n-butanol/methanol/-0.03M boric acid (5:3:1) (8). After drying the chromatoplate, spots were revealed with a mixture of 0.5 ml of concentrated $AgNO_3$ solution and 100 ml of acetone, followed by treatment with 2 % (w/v) NaOH in metanol (13) and kept at 110°C for 2 minutes.

Enzyme purification: The partial purification of β-galactosidase from mantle tissue of *Mytilus galloprovincialialis* Lam. involved 3 steps:

1. Isolation: 160 g mantle tissue of freshly collected mussels were homogenized in 300 ml of 0.9 % NaCl solution by means of a homogenizer, then 300 ml more saline were added and the homogenate was stirred by means of magnetic stirrer for 4 hours and left overnight in the refrigerator. The homogenate was centrifuged at 20 000 rpm (45 700 g) for 30 minutes in a refrigerated centrifuge (Cryofuge 20-3 Hereaus-Christ) and the supernatant (crude extract) was collected.

2. Ammonium sulphate fractionation: The crude extract was precipitated by ammonium sulphate at 25% saturation and left overnight in the refrigerator. The precipitate, separated by means of centrifugation at 20 000 rpm for 30 minutes was discarded and the supernatant brought up to 50 % saturation with ammonium sulphate and left overnight at 4°C. The enzyme active precipitate thus formed (named 25 - 50% ammonium sulphate fraction) was separated by centrifugation at 20 000 rpm for 30 minutes, dissolved in distilled water and dialysed against distilled water.

3. Hydroxylapatite column chromatography: The dialysed β-galactosidase active 25-50% ammonium sulphate fraction was applied to hydroxylapatite column (bed dimensions: 2cm x 11cm) 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 and M). The fractions showing β-galactosidase activity were collected separately and dialysed against distilled water. All of these operations were carried out at 4^0 C.

Kinetic properties of β-galactosidase:

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

2. Effect of temperature: The effect of temperature on the activity of the enzyme was studied between 5°C and 60°C under standard assay conditions.

3. Substrate specificity: The effect of substrate concentration on the velocity of the enzyme reaction was investigated by using varying concentrations (10 mM – 0.625 mM) of p-nitrophenyl-β-D-galactopyranoside as substrate. Km and Vmax-values were calculated by means of the equation of Lineweaver - Burk plot (9).

Results and Discussion

Elution of β-galactosidase active 25 - 50% ammonium sulphate fraction through hydroxylapatite column with stepwise molarity gradient of phosphate buffer (pH 6.8) resulted in appearance of the enzyme at 50 mM buffer concentration (Fig.1).

Table 1. Purification of β-galactosidase of *Mytilus galloprovincialis* Lam. from 160 g of mantle tissue.

Purification step	Volume (ml)	Protein (mg)	Total activity* (U)	Specific activity** (U/mg)	Recovery (%)	Purification fold
1. Crude extract	270	975	2889	2.96	100	1
2. 25 - 50 % ammonium sulphate fraction	45	75.9	774	10.2	26.8	3.4
3. Hydroxylapatite column chromatography 50 mM eluate	29	4.75	264	55.5	9.1	18.7

* μmol 4-nitrophenol/min. ** μmol 4-nitrophenol/min./mg protein

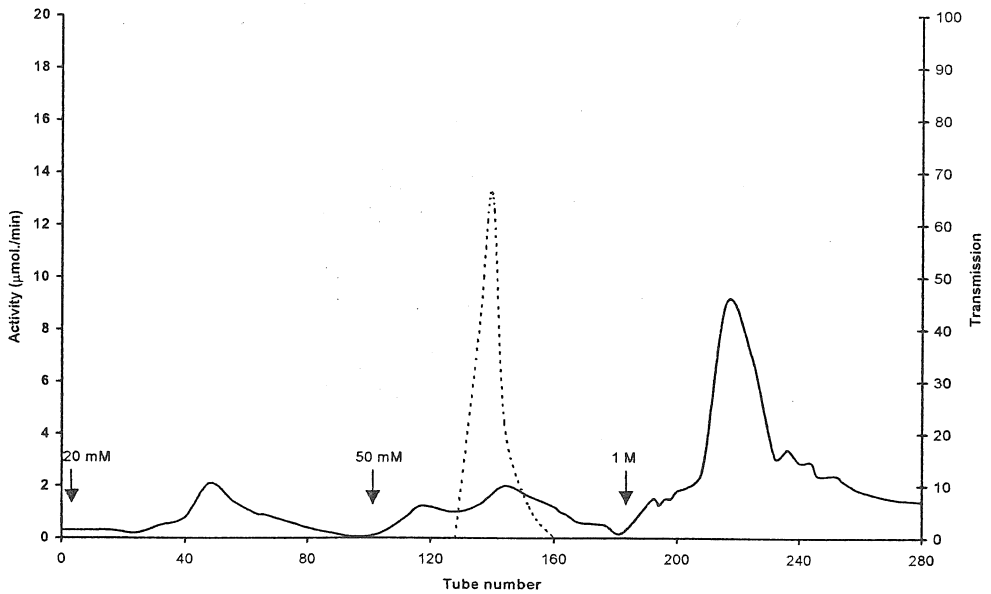

Fig. 1. Elution profile of the 25 - 50% ammonium sulphate fraction of *Mytilus galloprovincialis* Lam. mantle tissue crude extract. Column: 110x20 mm, sample volume: 45 ml, flow rate: 30 ml/hour, eluant: 20 mM, 50 mM and M potassium phosphate buffers pH 6.8.
Transmission of proteins at 280 nm (—), activity of β-galactosidase (····)

The enzyme was purified approximately 19 fold over the crude homogenate and was obtained in 9 % yield. The results of partial purification of β-galactosidase from the mantle tissue of *Mytilus galloprovincialis* Lam. are summarized on Table 1. No multiple forms appeared during purification of β-galactosidase. The existence of multimolecular forms of β-galactosidase has been reported in hepatopancreas and whole tissue extract of *Mytilus galloprovincialis* Lam. (4,14). It

was observed that β-galactosidase activity of the mantle tissue eluted with 50 mM buffer was actually the activity of one of the isoenzymes, found in whole tissue homogenate which existed in 50 mM eluate.

Optimum pH of the enzyme was 1.85 (Fig. 2). *Mytilus galloprovincialis* β-galactosidases exhibited their pH optimum activity at acidic reagions with maximum values at 2.1; 4; 1.5 and 2 for β-galactosidase isoenzymes, isolated from the whole tissue's homogenate (14) and 2.7; 3.9; 2.0 and 2.1 for β-galactosidase isolated from the hepatopancreas (4). Optimum pH value for β-galactosidase determined in the present study is within the mentioned range.

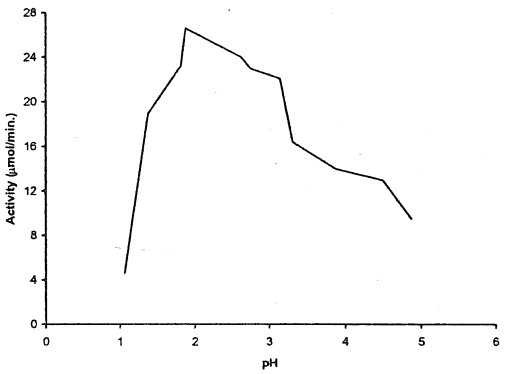

Fig. 2. Effect of pH on *Mytilus galloprovincialis* Lam. mantle tissue β-galactosidase activity

β-Galactosidase showed maximum activity at 49° C (Fig. 3). This value is higher then that reported for β-galactosidase from *Mytilus edulis* (35°C) (11). High optimum temperature is expec-ted from Mytilus galloprovincialis as found in the present study since it is a mollusc variety lives in the Mediterranean sea.

Km of β-galactosidase was found 1.59 x 10^{-3}M for p-nitrophenyl-β-D-galactopyranoside. Variation of β-galactosidase activity in connection with concentration

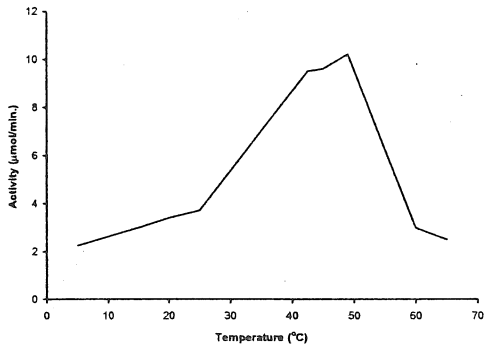

Fig. 3. Effect of temperature on *Mytilus galloprovincialis* Lam. mantle tissue β-galactosidase activity

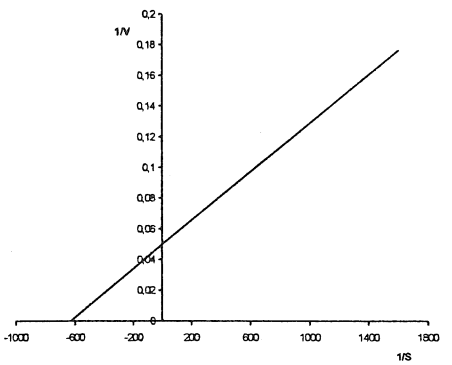

Fig. 4. Lineweaver - Burk plot of the mantle tissue β-galactosidase of *Mytilus galloprovincialis* Lam.

change of p-nitrophenyl-β-D-galactopyranoside is shown in Fig.4. β-Galactosidase investigated in this work showed higher affinity for the substrate p-nitrophenyl-β-D-galactopyranoside then the β-galactosidase isoenzymes in hepatopancreas of the same mussel (4). This higher affinity to substrate may be the result of the necessity for the mussel to carry on its biochemical functions in lower substrate concentrations.

Thin-layer chromatography revealed the presence of new oligosaccharides in addition to the hydrolysis products of lactose (Fig. 5). This results suggest that

the enzyme possibly catalyses a galactosyltransfer reaction. It has been reported previously that oysters contain several glycosydases, including α- and β-glucosidases and galactosidases, which possess a transferring action (3).

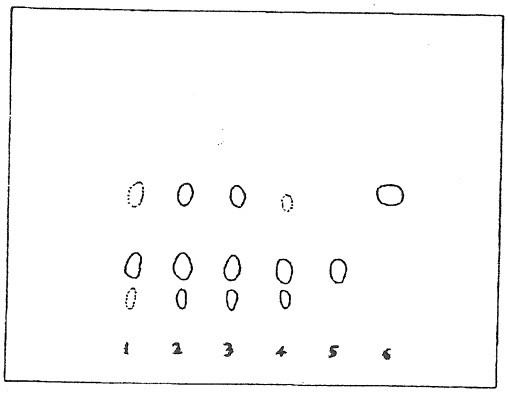

Fig. 5. Thin-layer chromatogram of hydrolysis products of lactose by the action of β-galactosidase. Hydrolysis products of lactose at 1- 0 hour; 2-2.5 hour; 3-5 hour, 4-24 hour; 5- lactose (standard); 6- glucose (standard).

In conclusion, mantle tissue of *Mytilus galloprovincialis* is responsible for hydrolysis of β-galactosidase linkages as well as for synthesis of the aforesaid bonds.

References

1. Dziezak, J.D.: Food Technology 45, 78 (1981)
2. Christacopoulos, P., Macris, B.J., Kekos, D.: Process. Biochem. International 15, 210 (1990)
3. Courtois, J.E., Petek, F., Dong, T.: Bull. Soc. Chim. Biol. 44, 11 (1962)
4. Dikmen,C.: MSc Thesis, Department of Biochemistry, Institute of Health Sciences, University of Istanbul. 1998
5. Hosinger, V.H., Kligermann, A.E.: Food Technology 45, 92 (1991).
6. Hotamisligil, S., Hale, S., Alroy, J., Fisher, I., Raghavan, S.: Comp. Biochem. Physiol. 106 (2), 373 (1993)
7. Itoh, T., Ohhashi, M., Toba, T.: Milchwisswschaft 35(10), 593 (1980)
8. Lato, M., Brunelli, B., Cinffini, G.: J. Chromatog. 36, 407 (1969)
9. Lineweaver, H., Burk, D.: J. Am. Chem. Soc. 56, 658 (1934)
10. Miller, A.L., Frost Russel, G., O'Brien, J.S.: Biochem.J. 165, 591 (1997)
11. Sanchez, M. P., Vazquez R., Ruiz, A.M.: Acta Cient. Compostel. 10, 175 (1973)
12. Tiselius, A., Hjerten, S., Levin, Ö.: Arch. Biochem. Biophys., 65, 132 (1956)
13. Trevelyan, W.E., Procter, D.P., Harrison, I.S.: Nature 444, 132 (1950)
14. Yalvaç, S., Kuşçu, I.: Acta Pharmaceutica Turcica 35 (2), 45 (1993)
15. Zamenhof, S.: In: Colowick, S.P, Kaplan, N.O.(Eds.), Methods in enzymology, 3, pp. 702, Academic Press, New York, 1957

Accepted: 23.12.1999