Presentation of β-galactosidase activity secreted by plant cells

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Summary

Extracellular plant β-galactosidase from opium poppy and celandine was detected on agar plates by the presence of dyed zones from 1-naphthyl-β-D-galactopyranoside used as a synthetic substrate. Evaluating the azo-dye zones assessed the degree β-galactosidase activity. No coloration of the agar medium was observed on non-inoculated parts, in medium inoculated with heat – inactivated cells (10 min. at 100°C) or in medium without substrate with all calli tested. On the agar plates with substrate and sterile opium poppy, celandine or gherkin seedlings (2–6 days old) changes in coloration were observed showing the release of β-galactosidase from the roots during germination.

Key words: β-galactosidase, enzyme secretion, callus culture, seedlings

The enzyme β-D-galactoside-galactohydrolase (EC 3.2.1.23), known as β-galactosidase (lactase), which hydrolyses lactose to glucose and galactose, has evoked considerable interest due to its application in the food industry, nutrition and medicine [1, 2]. Cow milk contains about 4.8% of lactose. This sugar is poorly soluble in water and insufficiently sweet. It may also have a mild laxative effect when consumed in large quantities. Many adults and some infants have also a low tolerance for it [3]. Hence the production of low-lactose milk may be of great economic value [5]. The use of cheese whey is largely limited by lactose due to its low sweetness, poor solubility and problem of lactose intolerance [6, 7]. The

enzymatic hydrolysis of lactose by β-galactosidase to glucose and galactose constitutes a potential route for decreasing its tolerance for human beings [8]. Several microbial sources of β-galactosidase have been used for this purpose [2, 4] but new sources of this enzyme are still of great value.

The determinations of β-galactosidase activity of cells play an important role in many fields of basic and applied research. The quality of human nutrition's is beside others; strongly depend on the quantity, quality, structure and physicomechanical properties of compounds in food [9]. The sugars' biotransformation plays an important role in some biotechnological processes.

The application of a simple and rapid screening method to detect β-galactosidase activity is of great importance both for scientific and production purposes.

Although β-galactosidase is generally present also in plants, this source has not been used previously [10, 11]. Therefore, the aim of the present work is the presentation of the intra- and extracellular activity of β-galactosidase in plant, and elaboration of a simple and rapid method for the determination of extracellular β-galactosidase.

MATERIALS AND METHODS

Plant materials

Long-term callus cultures and cell suspensions were derived from *Chelidonium majus* L. or *Papaver somniferum* L. *cv. Amarín* resp. seedlings and were cultivated as previously described [12, 14].

Seedlings of poppy, celandine and gherkin grew for 2–6 days under sterile conditions [13, 14].

Determination of fresh and dry mass

Fresh and dry masses of cell suspensions were determined gravimetrically. In order to determine the dry mass, samples were dried to the constant mass at 100°C.

Identification of extracellular enzyme activity

1-Naphthyl-β-D-galactopyranoside was used for the identification of extracellular β-galactosidase. β-Galactosidase hydrolysed the substrate, releasing 1-naphthol with hexazonium p-rosaniline, the corresponding azo-dye was formed. Extracellular β-galactosidase was identified by method of [15, 16].

1-Naphthyl-β-D-galactopyranoside (10 mg) was dissolved in 0.5 ml of dimethylformamide and 10 ml of Mc Ilvaine buffer containing Fast Blue BB (10 mg) pH 5.1 or 10 ml of buffered hexazonium p-rosaniline solution were added (9.4 ml Mc Ilvaine buffer pH 5.0 adjusted with 0.1 N NaOH). 10 ml of 2% agar in Mc Ilvaine buffer

(pH 5.0) were added to above mentioned mixture and autoclaved as usual [13]. Then agar plates were inoculated with cells from growing callus cultures or 2–6 days old seedlings of opium poppy, celandine or gherkin resp. and were cultivated for 20–100 min. Hexazonium p-rosaniline was prepared according to [15].

Solution A: 400 mg of p-rosaniline was dissolved in 8 ml of distilled water and 2 ml of conc. HCl was added. Solution B: 4% sodium nitrite. Solution A and B were mixed in equal volume.

Determination of intracellular and extracellular activity of β-galactosidase

Enzyme preparation

The cell suspension culture was used to determine the intracellular β-galactosidase activity. The cells (10 g) were filtrated off and washed with 3 l of distilled water. Soluble proteins were extracted by grinding the cells in a precooled (4°C) mortar using a ratio 1:1 (g/ml) of cells and Mc Ilvaine buffer pH 4.8 at 4°C. The homogenate was squeezed through two layers of a nylon cloth and centrifuged at 15.000 x g for 15 min at 4°C, the supernatant was used for the determination of intracellular enzyme activity [17].

For the determination of extracellular enzyme activity the cultivation medium was centrifuged at 15.000 x g for 15 min at 4°C (microscopically controlled for the cells absence). After centrifugal filtration through CENTRIFLO CF 25 (Amicon Co) diaphragms at 3.500 xg for 15 min. at 4°C, the filtrate was used for the determination of extracellular enzyme activity [17].

Enzyme assay

The enzyme assay was performed by the modified method [18] of using o-nitrophenyl-β-D-galactopyranoside (PNG) as the substrate. The reaction mixture contained a suitable amount of enzyme preparation (0.1 – 0.5 ml), 3 mM substrate in 2 ml of Mc Ilvaine buffer (pH 4.8). The control contained boiled (10 min., 100°C) enzyme preparate. After incubation for 15 min. at 30°C the reaction was terminated by addition of 2 ml 1 M Na_2CO_3 and liberated o-nitrophenol was measured at 410 nm against control. The enzyme activity is expressed in katals. Protein content was determined by the method of Doumas et al. [19] using bovine serum albumin as a standard.

RESULTS AND DISCUSSION

The synthetic substrates o-nitrophenyl-β-D-galactopyranoside and 1-naphthyl-β-D-galactopyranoside used in this study provide to be suitable substrates in studying the activity of β-galactosidase.

Both culture media (agar plates with and without substrate 1-naphthyl-β-D-galactopyranoside and hexazonium p-rosaniline or Fast Blue BB resp.) were inoculated with the cells from growing callus cultures and then cultivated for 0.5 – 2 h. The activity of extracellular β-galactosidase was detected by the presence of dyed reddish-brown or reddish-violet zones beneath and around the areas of growth on the agar plates. Extracellular β-galactosidase was also to be present when reddish-brown or reddish-violet zone staining occurred after 20 – 60 min in zones around the root tip of 2 – 6-days-old opium poppy, celandine or gherkin seedlings resp. on the agar plates.

No coloration of the agar medium after inoculation with heat inactivated calli or seedlings (10 min., 100°C) were observed.

For enzyme activity determination the medium concentrated by ultrafiltration centrifugation (microscopically controlled for the absence of cells) was used. Homogenates of cells of suspension cultures and culture medium alone after 14 days cultivation (without cells) were used for assaying the activity of intracellular and extracellular β-galactosidase. In both cases o-nitrophenyl-β-D-galactopyranoside was used as the substrate.

The distribution of the intra- and extracellular activity resp. of enzyme studies is presented in Tables 1 and 2. The data given point to 31.9 – 35.8% intracellular and 64.2 – 68.1% extracellular distribution of β-galactosidase activity. The extracellular specific activity was 7.23 – 7.97 times higher. Interestingly, the activities of studied extracellular enzymes are much higher than invertase [36].

Table 1.

β-Galactosidase activity in *Papaver somniferum* L. cells and culture medium after 14 days of cultivation.

fraction	Volume (ml)	protein (mg/g dry mass)	activity (nkat/g dry mass)	specific activity (pkat/mg protein)
homogenate of isolated cells	10	14.6	2.85	195.2
culture medium without cells*	5	6.9	5.12	742.1

*corresponding to the amount of isolated cells

Table 2.

β-Galactosidase activity in Chelidonium majus L. cells and culture medium after 14 days of cultivation.

fraction	Volume (ml)	protein (mg/g dry mass)	activity (nkat/g dry mass)	specific activity (pkat/mg protein)
homogenate of isolated cells	10	12.1	2.31	190.9
culture medium without cells *	5	7.3	4.92	673.9

*corresponding to the amount of isolated cells

β-Galactosidase and some other hydrolytic enzymes in plants play an important role in the cell wall metabolism. They remove and/or loosen cell structure, and β-galactosidase appears involved in the removal of β-galactose from cell wall components [20]. It has been suggested that this enzyme is involved in fruit ripening and

seed and pollen germination [21-23]. Microbial β-galactosidase hydrolyses of lactose and also catalyses the synthesis of branched oligosaccharides analogues [2].

The production of low-lactose milk may be of great economic value. Another related industrial problem concerns whey utilization. The enzymatic hydrolysis of lactose by lactase to glucose and galactose constitutes a potential route for decreasing its intolerance for human beings. Several microbial sources of β-galactosidase have been used for this purpose, but new sources of this enzyme are still of great value [2, 5]

It is now well recognized that galactosidase inhibitors such as many mono- and bicyclic polyhydroxylated pyrrolides, piperides and azepines (referred to iminosugars or azasugars) have the potential as antiviral, anticancer and antidiabetic agents. Some of these inhibitors have already been put on the market for diabetes treatment. The mechanism of these effects has been studied extensively. Some of these compounds are naturally occurring and owing to the pronounced biological activity of this class of compounds. Various synthetic routes have been designed for synthesis of many of them [24-26].

β-Galactosidase as well as other hydrolytic enzymes [27-29] of plant origin can be useful in the study of structure of some natural compounds [30-32].

The production of β-galactosidase and other hydrolytic enzymes [33, 34] by plant cells coupled with their simple detection, might be of some importance for application in the food and pharmaceutical research [35].

Although β-galactosidase as well as other hydrolytic enzymes: α-galactosidase, invertase [36-38] are generally present in the plants, they have not been used previously in biotechnological techniques. Our results indicate some possibilities for biotechnological application of β-galactosidase of plant origin.

Due to the simplicity and reproducibility of the described procedure this method could be successfully used for detection of plant producers of extracellular β-galactosidase.

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PREZENTACJA AKTYWNOŚCI β-GALAKTOZYDAZY WYDZIELANEJ PRZEZ KOMÓRKI ROŚLINNE

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Streszczenie

Autorzy badali aktywność b-galaktozydazy wydzielanej przez komórki roślinne maku polnego i glistnika jaskółcze ziele na agarze metodą oceny zabarwionych stref z 1-naftylo-b-D-galaktopiranozydem użytym jako syntetyczny substrat. Zmiany zabarwienia wskazują na zanikanie b-galaktozydazy z korzeni podczas procesu kiełkowania.

Słowa kluczowe: β-galaktozydaza, wydzielanie enzymów