Identification and determination of extracellular saccharase in cell suspension of *Chelidonium majus* L.

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Summary

A simple and rapid procedure for identification and determination of extracellular saccharase is described, using a culture medium of *Chelidonium majus* L. (celandine) cell suspension cultures. Sucrose was used as a substrate for determination of the extracellular and intracellular activities of the studied enzyme. The culture medium (without cells) was used for identification and determination of extracellular enzyme activity. Intracellular activity was estimated using cell suspension.

Key words: Chelidonium majus L. (celandine), extracellular saccharase, intracellular saccharase activities, cell suspension cultures

The sources of numerous natural compounds are limited. Synthetic preparation of these compounds complements their insufficiency, which is limited by natural sources. Biotechnology helps to solve this problem. Biotransformation and methods of producing high-value fine and special chemicals have been invented recently. The knowledge of totipotency and mastering the plant-tissue cultivation

techniques were applied at first in agriculture, for instance in plant propagation. It was recognised later that plant cells could be used for biosynthesis and biotransformation of various substances of natural and synthetic origin [1, 2, 3].

Saccharase (β-D-fructofuranosidase, EC 3.2.1.26), called also invertase, catalyses the hydrolysis of sucrose to glucose and fructose. The studied enzyme is also used in processes leading to mixtures of glucose and fructose (invert sugars) enabling the successive production of fructose-containing preparations [4, 5].

The development of new techniques of immobilisation of biocatalysts is highly connected with the progress of biotechnological processes. Although saccharase is generally present also in plants, this source was not used previously.

The aim of this paper is to report on our investigation concerning the hydrolysis of sucrose by intra- and extracellular saccharase from celandine (*Chelidonium majus* L.) cells.

MATERIALS AND METHODS

Cell suspension cultivation

Long-term callus cultures and cell suspension were derived from seedlings of *Ch. majus* and were cultivated as described previously [6].

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 and determination of intra- and extracellular saccharase

Using sucrose as substrate, we identified and determined the intra- and extracellular activity of saccharase. Cell suspension cultures were used to determine the intracellular enzyme activity. The cells (10 g) were filtered off and washed with 1.5 l of distilled water twice.

Soluble proteins were extracted by grinding the cells in a precooled mortar using a 1:2 ratio (g/ml) of cells and Mc Ilvaine buffer pH 4.5 at 4°C. The homogenate was filtered through two layers of nylon cloth and centrifuged at 15,000 x g at 4°C.

In order to determine the extracellular enzyme activity the cultivation medium (without cells) after centrifugation (15,000 x g, 10 min at 4°C) was used.

Enzyme activity

Enzyme activity was determined by a modified method of Rubio [7] and Trinder [8], using an Accutrend GCT meter by Roche Diagnostics GmbH (Germany) and the strips for glucose determination.

The reaction mixture contained the suitable amount of enzyme preparate (supernatant or culture medium 0.1-0.3 ml) saccharose (0.04 mM) in the total volume of 2 ml of Mc Ilvaine buffer, pH 4.5, and was incubated for 60 min at 30°C. The control contained temperature-inactivated enzyme (10 min at 100°C).

The required volume (40 μl) of reaction mixture was applied on the strip for glucose determination, and concentration of the glucose was estimated using the Accutrend GCT meter. The enzyme activity was expressed in katals. Proteins were determined by the method of Bradford [9].

RESULTS AND DISCUSSION

In recent decades, several methods for determining the activity of hydrolytic enzymes have been developed. Various naturally occurring or chromogenic synthetic substrates may be used for these purposes [10, 11]. p-Nitrophenyl-β-D-fructofuranoside and naphthyl-β-D-fructofuranoside have not been synthesised and in order to study saccharase the only natural substrate, namely saccharose, was used.

Therefore only sucrose was used for the study of intracellular and extracellular saccharose activity. Homogenised cell suspension cultures and culture medium achieved after a 10-day cultivation were used for determination of intracellular and extracellular activity of saccharase, respectively. The distribution of intracellular and extracellular activity is shown in Table 1.

Table 1.

Saccharase activity in cell suspension and in cultivation medium *Chelidonium majus* L.

fraction	proteins $\left(\frac{mg}{g\ dry\ mass}\right)$	activity $\left(\frac{nkat}{g\ dry\ mass}\right)$	specific activity $\left(\frac{nkat}{mg\ protein}\right)$
intracellular activity (homogenate of isolated cells)	12.4	317	25.56
extracellular activity (cultivation medium without cells)*	7.3	46	6.30

*corresponding to the amount of isolated cells

The data indicate 87.3% of intracellular and 12.7% of extracellular distribution of the enzyme activity tested. The intracellular specific activity is 4.1 times higher. The specific activity of the extracellular α- and β-galactosidase was found two to eight times higher than that of the intracellular [11, 12].

It is very well recognised that glycosidase inhibitors, such as many mono- and bicyclic polyhydroxylated pyrrolides, piperidines and azepines (referred to as iminosugars or azasugars) may have antiviral, anticancer and antidiabetic properties. Some of these inhibitors have already been introduced to the market for diabetes treatment. The mechanisms of these effects have been studied extensively. Some of these are naturally occurring, and owing to the well-known biological activity of this class of compounds various synthetic methods have been designed to synthesise many of them [13, 14, 15, 16].

Extracellular saccharase, galactosidases, as well as proteolytic enzymes, which are released from plant cells, may be of some importance for biotechnological application in the food and pharmaceutical research and industry [11, 17]. Saccharase could be used for biotransformation of saccharose to glucose and fructose (invert sugars) enabling the successive production of fructose-containing preparation, suitable for food industry and diabetics [4, 5, 18]. These enzymes as well as galactosidases and proteases [11, 19] are generally present in plants. So far they have not been used in biotechnological processes [19, 20].

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IDENTYFIKACJA I OZNACZANIE SACHARAZY ZEWNĄTRZKOMÓRKOWEJ W KULTURZE ZAWIESIN KOMÓRKOWYCH GLISTNIKA JASKÓŁCZE ZIELE (*CHELIDONIUM MAJUS* L.)

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Streszczenie

W pracy opisano prostą i szybką metodę identyfikacji i oznaczania sacharazy zewnątrzkomórkowej z użyciem pożywki z kultur zawiesin komórkowych glistnika jaskółcze ziele (*Chelidonium majus* L.). Substratem użytym do określenia zewnątrzkomórkowej i wewnątrzkomórkowej aktywności badanego enzymu była sacharoza. Pożywkę (bez komórek) użyto do identyfikacji i oznaczenia zewnątrzkomórkowej aktywności enzymu. Aktywność wewnątrzkomórkowa oznaczona była przy użyciu zawiesin komórkowych.

Słowa kluczowe: Chelidonium majus L. (glistnik jaskółcze ziele), sacharaza zewnątrzkomórkowa, wewnątrzkomórkowa aktywność sacharazy, kultury zawiesin komórkowych