Identification of sucrase activity in cell suspension and culture medium of melon

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ABSTRACT: The activity of (soluble acid) sucrase was detected in a culture medium of the cell suspension culture of watermelon (*Citrullus vulgaris* L.). A simple and rapid procedure for the identification and determination of extracellular sucrase from a culture medium of watermelon cell suspension cultures is described. Sucrose was used as a substrate for the determination of extracellular and intracellular activities of the enzyme. Intracellular activity was estimated from the cell suspension. The results show a 91.5–92.0% intracellular and 8.0–8.5% extracellular distribution of sucrase activity. The described method enables to carry out a rapid, simple and specific detection of extracellular sucrase in plants.

Keywords: intracellular sucrase; extracellular sucrase; watermelon

Sources of numerous natural compounds are limited. Synthetic preparation of these compounds complements their insufficiency, which is limited by natural sources. Solution of this problem is aided by biotechnologies. Biotransformation and production of high-value fine and special chemicals are known from recent time. The knowledge of totipotency and mastering of 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.

Plant glycosidases that catalyse the hydrolysis of aryl and alkyl glycosides are involved in a wide variety of biological processes. In the last decades, several methods for determining the activities of a variety of glycosidases have been developed. Various naturally occurring or synthetic substrates may be used for these purposes (Kim et al. 2002; Hashizume et al. 2003; Machová 1994).

Sucrase (β-D-fructofuranosidase EC 3.2.11.26) invertase, called also saccharase, catalyses hydrolysis of sucrose to glucose and fructose at concentrations lower than 10% (wt/vol.) and has a transfructosylating activity at sucrose concentrations higher than 10% (wt/vol.) (Rubio et al. 2002). The enzyme is an important industrial product with applications in the production of noncrystallisable invert sugars and soft-centred chocolates (Wiseman 1979). Invertase has been widely studied especially in yeasts and fungi (Costalioli et al. 1997; Romero-Gómez et al. 2000).

The development of a new method for identification and determination of biocatalysts is closely connected with progress of biotechnological processes. Although generally invertase is also present in plants, this source was not used previously.

The aim of this paper is to report on our investigations concerning the hydrolysis of sucrose by intra- and extracellular acid soluble sucrase from melon cells. Sucrose was used for the study of intracellular and extracellular sucrase activity.

MATERIALS AND METHODS

Tissue cultures

Long-term tissue cultures and cell suspension were derived from seedlings of watermelon *Citrul-*

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lus vulgaris cv. Samara as previously described by Tilemann et al. (2003).

Identification and determination of intra- and extracellular sucrase

Using sucrose as a substrate the intra- and extracellular activity of soluble acid sucrase was identified. Eight days old cell suspension cultures were used to determine the intracellular enzyme activity. The cells (5 g) were filtered and washed twice with 1,000 ml of distilled water. Soluble proteins were extracted by grinding the cells in a pre-cooled mortar using a ratio 1:2 (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 g at 4°C. For determinations of extracellular enzyme activity the cultivation medium (without cells) was used after centrifugation (15,000 g, 10 min at 4°C).

Enzyme assay

Enzyme activity was determined by a modified method of Rubio et al. (2002) using sucrose as a substrate. The reaction mixture contained a suitable amount of enzyme (0.2–0.4 ml) and 0.4 mM sucrose in Mc Ilvaine buffer pH 4.5 in a final volume of 2 ml. Enzyme activity was determined at 30°C for 30 to 60 min. The control contained temperature-inactivated enzyme (100°C, 10 min). The enzyme activity was expressed in katals. Proteins were determined by the method of Bradford (1976) using bovine serum albumin as a standard protein. The glucose content released by the enzyme was determined by the method of Trinder (1969).

RESULTS AND DISCUSSION

Synthetic chromogenic substrates are very suitable for biochemical and histochemical studies of hydrolytic enzymes. In the last decades, several methods for the determination of hydrolytic enzymes have been developed. Various naturally occurring chromogenic substrates may be used for these purposes (Kim et al. 2002; Mičieta et al. 2002). p-nitrophenyl-β-D-fructofuranoside and naphthyl- β-D-fructofuranoside were not synthesised and only a natural substrate – sucrose was used for the study of sucrase.

Therefore only sucrose was used to study the activity of intracellular and extracellular (acid soluble) sucrase. The highest specific activity of the studied enzyme was recorded on day 8 of cultivation (Table 2) in undifferentiated cells. Homogenised cell suspension cultures and culture medium alone after 8-day cultivation were used to assay the activity of intracellular and extracellular soluble acid sucrase (Hashizume et al. 2003). The distribution of intracellular and extracellular enzyme activity is shown in Tables 1–3. The data indicate a 91.5–92.0% intracellular and 8.0–8.5% extracellular distribution of the enzyme activity tested. The intracellular specific enzyme activity is 1.72–1.84 times higher.

The production of extracellular glycosidases as well as proteolytic enzymes (Balasubramaniam et al. 2005; Luan et al. 2005; Mertová et al. 2002) that are released from plant cells might be of some importance for biotechnological applications in the food and pharmaceutical research and industry (Shibano et al. 2004; Willits et al. 2004; Asano et al. 2000; El Ashry et al. 2000a,b,c; Watson et al.

Table 1. Sucrase activity in 6 days old cell culture and culture medium of watermelon

Fraction	Volume (ml)	Protein (mg/g of fresh weight)	Activity (nkat/g of fresh weight)	Specific activity (nkat/mg of protein)
Intracellular activity (homogenate of isolated cells)	5.0	2.75	553	201.1
Extracellular activity (culture medium without cells)*	4.3	0.44	48	109.1

For Table 1–3: *corresponding to the amount of isolated cells

Table 2. Sucrase activity in 8 days old cell culture and culture medium of watermelon

Fraction	Volume (ml)	Protein (mg/g of fresh weight)	Activity (nkat/g of fresh weight)	Specific activity (nkat/mg of protein)
Intracellular activity (homogenate of isolated cells)	5.0	2.80	568	202.9
Extracellular activity (culture medium without cells)*	4.3	0.45	53	117.8

Table 3. Sucrase activity in 10 days old cell culture and culture medium of watermelon

Fraction	Volume (ml)	Protein (mg/g of fresh weight)	Activity (nkat/g of fresh weight)	Specific activity (nkat/mg of protein)
Intracellular activity (homogenate of isolated cells)	5.0	2.84	562	197.9
Extracellular activity (culture medium without cells)*	4.3	0.45	51	113.3

2001). These enzymes are generally present in plants. They have not been used in biotechnological processes until now (Balasubramaniam et al. 2005; Neubert et al. 2004).

It is a very well-known fact that glycosidase inhibitors such as many mono- and bicyclic polyhydroxylated pyrrolides, piperidines and azepines (referred to as iminosugars or azasugars) have the potential as antiviral, anticancer and antidiabetic agents. Some of these inhibitors have already been put on the market for treatment of diabetes. The mechanisms of these effects have been studied extensively. Some of them are naturally occurring and owing to the pronounced biological activity of this class of compounds various synthetic routes have been designed for the syntheses of many of them (El Ashry et al. 2000a,b,c; Watson et al. 2001; Matsuura et al. 2004).

Extracellular sucrase, 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 (Luan et al. 2005; Mičieta et al. 2002; Watson et al. 2001; Matsuura et al 2004; Mučaji et al. 2001). Sucrase could be used for biotransformation of sucrose to glucose and fructose (invert sugars) enabling the successive production of fructose containing a preparation suitable for the food industry and diabetics (Schlee, Kleber 1991; Mansfeld et al. 1992; Asano 2003). These enzymes as well as galactosidases and proteases (Mičieta et al. 2002; Mertová et al. 2002) are generally present in plants. They have not been used in biological processes until now (Balasubramaniam et al. 2005; Luan et al. 2005; Bilka et al. 2002).

Due to its simplicity and reproducibility the method presented here could be useful for the detection of sucrase in plants and their enzymatic improvement.

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Identifikácia sacharázy v kultivačnom médiu melóna

ABSTRAKT: Sacharáza (kyslá solubilná) sa detegovala v kultivačnom médiu suspenzných kultúr dyne červenej (*Citrullus vulgaris* L.). Vypracovala sa jednoduchá a rýchla metóda dôkazu a stanovenia extracelulárnej sacharázy v kultivačnom médiu suspenzných kultúr dyne červenej. Pri stanovení aktivity intracelulárnej a extracelulárnej sacharázy sa ako substrát použila sacharóza. Intracelulárna enzýmová aktivita sa stanovila v bunkách suspenznej kultúry. Výsledky ukazujú, že 91,5–92 % sacharázy je v bunkách a 8,0–8,5 % v kultivačnom médiu. Predložená metóda umožňuje jednoduchý, rýchly a špecifický dôkaz enzýmu – extracelulárnej sacharázy v rastlinách.

Kľúčové slová: intracelulárna sacharáza; extracelulárna sacharáza; dyňa červená

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