Regulation of invertase in *Aspergillus nidulans*: effect of different carbon sources

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Aspergillus nidulans produces an extracellular β -D-fructofuranoside fructohydrolase (invertase) when grown on a medium containing the β -fructofuranosides sucrose or raffinose, indicating that synthesis is subject to induction by the substrate. On a growth medium containing sucrose, production was maximal at 15 h in cultures incubated at 28 °C. After this time the level of detectable invertase in the cultures declined. A proportion of the enzyme was secreted during the linear growth phase of the fungus. Various sugars were investigated for induction of invertase, but only the two β -fructofuranosides induced high production levels; with the other sugars, the enzyme was produced only at a low constitutive level. Mycelium grown under repressive conditions (1% glucose), rapidly produced invertase when transferred to sucrose-containing medium. After 80 min the invertase level in these cultures was 26-fold higher than the constitutive level. The repressive effect of other sugars, e.g. glucose and xylose, on invertase production was also demonstrated in this experimental system.

Introduction

Sucrose is utilized by almost all micro-organisms via its conversion to glucose and fructose catalysed by invertase enzymes. Two types of invertase, β -D-fructofuranosidase fructohydrolase (EC 3.2.1.26) and α -D-glucoside glucohydrolase (EC 3.2.1.20), can be distinguished according to whether the fructose or glucose end, respectively, of the molecule is cleaved (Neuberg & Mandl, 1950).

The formation of invertase (β -D-fructofuranosidase fructohydrolase) by *Saccharomyces cerevisiae* and *Neurospora crassa* is primarily controlled by catabolic repression, particularly by hexoses. The effects of various levels and types of sugar have been examined in detail (Metzenberg, 1962; Sargent & Woodward, 1969). Two sizes of active enzyme are present in *Neurospora* (Eilers *et al.*, 1964; Metzenberg, 1964) and there is a preferential release of the smaller form during growth (Trevithick & Metzenberg, 1966). Invertase biosynthesis in yeast is not inducible by its substrates but is subject to repression by the glucose concentration in the culture medium (Hackel & Khan, 1978; Sarokin & Carlson, 1986). Two forms of the yeast enzyme are produced: a heavily glycosylated

form secreted into the periplasmic space and an apparently non-glycosylated form retained within the cell (Neumann & Lampen, 1967; Gascon & Lampen, 1968; Gascon *et al.*, 1968; Lampen, 1968). The glycosylated form has been used as a probe for the process of glycoprotein biosynthesis and secretion (Novick *et al.*, 1981; Trimble *et al.*, 1983; Esmon *et al.*, 1987).

Several factors affect invertase production in different micro-organisms and among these, glucose has been found to play an important role as repressor of synthesis. Substantial information on invertase regulation, structure and gene control exists for *S. cerevisiae* (Gascon *et al.*, 1968; Carlson & Botstein, 1982; Perlman *et al.*, 1982) and *N. crassa* (Metzenberg, 1962; Sargent & Woodward, 1969; Meachum *et al.*, 1971; Casanova *et al.*, 1987). However, few data exist concerning the enzyme produced by *Aspergillus* spp. (Andres & Peberdy, 1974; Olutiola & Cole, 1980).

Invertase content in *A. nidulans* is dependent on growth conditions and especially on carbon source. The enzyme is produced only in response to its substrates (sucrose and raffinose) and is repressed by its end products (glucose and fructose). Thus, invertase provides a good model system for investigating the production of an enzyme under dual control by induction and catabolic repression.

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Methods

Organism. *Aspergillus nidulans* (Eidam) strains 2.1, 2.124, 2.108 (University of Nottingham collection) and *Aspergillus niger* (B60) obtained from Professor Dr C. P. Kubicek (Technical University of Vienna, Austria) were used. Strains used routinely were kept at 40 °C on malt extract/yeast extract/glucose agar (MYG) containing (g l⁻¹): malt extract, 5; yeast extract, 2.5; glucose, 10; agar, 20; they were subcultured regularly every 3 to 4 months.

Culture conditions. *Aspergillus* minimal medium (AMM) (Pontecorvo *et al.*, 1953) was inoculated with 10⁷ conidia ml⁻¹ in 250 ml conical flasks containing 25 ml of medium supplemented with the appropriate carbon source (1%, w/v) and incubated as shaken cultures at 180 r.p.m. at 28 °C for up to 24 h. Glucose, fructose and xylose were autoclaved separately and all other sugars used were sterilized by filtration with a 0.2 µm membrane.

To test enzyme production at various growth phases, whole cultures were harvested at frequent time intervals for determination of dry weight, enzyme activity and protein. For induction experiments, 18-h-old mycelium was produced in 25 ml of AMM supplemented with 1% (w/v) glucose and transferred, after washing, to the same medium with 1% (w/v) sucrose for a further 2 h.

Preparation of enzyme fractions. Three enzyme fractions were derived from the cultures, namely soluble and insoluble mycelial fractions and a culture filtrate preparation. Mycelium was harvested by centrifugation at 4000 g for 10 min, washed twice in ice-cold 25 mM-Tris/phosphate buffer pH 6.7, frozen at -70 °C and freeze-dried overnight. Freeze-dried mycelium was weighed and then ground to a fine powder with liquid nitrogen, resuspended at a concentration of 100 mg ml⁻¹ in lysis buffer [25 mM-Tris/phosphate pH 6.7, 10% (v/v) glycerol, 0.1 mM-dithiothreitol, 1 mM-EDTA and 2 mM-phenylmethylsulphonyl fluoride, prepared as a 40 mM stock solution in 95% (v/v) ethanol] (Carlson *et al.*, 1981) and centrifuged in an Eppendorf microcentrifuge at 13000 g for 30 min at 4 °C. The supernatant obtained after centrifugation was used as the soluble fraction. The pellet was washed twice by centrifugation in lysis buffer, resuspended in the same buffer and used as insoluble fraction (wall fraction).

Culture filtrates were dialysed overnight against 10 mM-acetate buffer pH 5.0 at 4 °C, to remove residual glucose before determination of enzyme activity.

Analytical determinations. Invertase was assayed by the following method: 80 µl of appropriately diluted enzyme preparation was mixed with 160 µl 0.2 M-acetate buffer pH 5.0 and 80 µl of a 0.5 M-sucrose or raffinose solution (final concentration 125 mM). After incubation for 30 min at 30 °C the reaction was stopped by addition of Sumner's 3,5-dinitrosalicylic acid reagent (1 ml) (Miller, 1959); the tubes were boiled for 10 min for colour development. Reducing sugars were estimated using glucose (0.1–0.5 mg ml⁻¹) as a reference standard. In this and all enzyme assays two control reactions were set up, one in which the enzyme preparation was replaced by buffer solution and the second in which the substrate solution was similarly replaced. The absorbance at 550 nm of the control mixtures was subtracted from the assay mixture absorbance to correct for endogenous reducing components. The suspended material in the insoluble mycelial fraction did not interfere with the spectrophotometric assays. One unit of invertase was defined as the amount of enzyme liberating 1 µmol of reducing sugar (as glucose) in 1 min at 30 °C.

Malate dehydrogenase (EC 1.1.1.37) in the soluble mycelial and culture filtrate fractions was measured spectrophotometrically (Galbraith & Smith, 1969). The reaction mixture contained 50 µl of appropriately diluted enzyme preparation, 0.25 µM-NADH, 260 µM-phosphate buffer pH 7.4 and 30 µM-oxaloacetate in a total volume of

3 ml. One unit was defined as the amount of enzyme which converted 1 µmol of oxaloacetate in 1 min at 25 °C.

Maltase (α -glucosidase; EC 3.2.1.20) activity was determined by its hydrolysis of the substrate *p*-nitrophenyl α -D-glucopyranoside (PNPG) into *p*-nitrophenol, which is detected by its absorbance at 410 nm (Kratky *et al.*, 1975). A total of 0.5 ml of assay mixture (1 mg PNPG ml⁻¹ in 0.05 M-phosphate buffer pH 7.0) was placed in a test tube in a 37 °C water bath. After temperature equilibration, 50 µl of enzyme solution was added to start the reaction. After 30 min the reaction was stopped with 1 ml 0.2 M-NaHCO₃. The absorbance was read at 410 nm against a distilled water blank. One unit of maltase was defined as the amount of enzyme capable of liberating 1 µmol of *p*-nitrophenol min⁻¹ at 37 °C.

Glucose was measured by the procedure of Raabo & Terkildsen (1960), using the Sigma diagnostic kit.

Protein was determined by the method of Bradford (1976) using the Bio-Rad Protein Assay Reagent and bovine albumin as standard.

Electrophoresis procedures. Samples were prepared as described above and diluted (1:1) with twice-concentrated electrophoresis sample buffer [125 mM-Tris buffer pH 6.8; 4% (w/v) SDS; 20% (v/v) glycerol and 0.05% (w/v) bromophenol blue as a tracking dye], and loaded onto a 7.5% (w/v) acrylamide slab gel (acrylamide:bisacrylamide ratio, 30:0.8) made up in 37 mM-Tris buffer pH 8.8, plus 0.1% (w/v) SDS. The running buffer system used was 25 mM-Tris, 192 mM-glycine containing 0.1% (w/v) SDS. Electrophoresis was run at a constant current at 4 °C until the tracking dye front was 0.5 cm from the bottom edge of the gel.

Invertase activity was detected *in situ* by a modification of the staining procedure of Gabriel & Wang (1969).

Immediately after electrophoresis the gels were incubated for 1 h in 2.5% (v/v) Triton X-100 with gentle shaking at room temperature. The gels were then washed with distilled water and incubated at 30 °C for 30 min in 0.1 M-sucrose in 0.1 M-acetate buffer pH 5.0. On completion of the enzymic reaction, the gels were thoroughly rinsed with distilled water, transferred to 0.1 M-iodoacetamide, kept in this solution for 5 min at room temperature, and then rinsed well with distilled water. The gels were next immersed in a freshly prepared solution of 0.2% (w/v) 2,3,5-triphenyltetrazolium chloride monohydrate in 1 M-NaOH and heated in a boiling water bath for 1–2 min until the red formazan bands appeared. Immediately after the heating step, the gels were rinsed with distilled water, fixed for 30 min in 7.5% (v/v) acetic acid and stored at 4 °C.

Results

The kinetics of invertase production are illustrated in Fig. 1. Synthesis of the enzyme was strongly influenced by the sugar used as the main carbon source. Of the sugars tested (fructose, glucose, raffinose and sucrose), only the sucrose-grown and the raffinose-grown cultures possessed invertase activity, indicating that the enzyme is substrate inducible. Under the conditions described, invertase production was greatest with young cultures (15 h) and fell progressively with increasing time (Fig. 1*a, b*). Total enzyme activity increased at a rate proportional to the increase in total protein within the culture (Fig. 2*a*). Secretion of total protein also increased with the age of the culture (Fig. 2*b*). At least half of the total enzyme in the system was secreted to the culture

Fig. 1. Growth kinetics (a) and invertase secretion (b) by *A. nidulans* (2.124) on different carbon sources (each 1%, w/v): fructose (▲), glucose (○), raffinose (●) and sucrose (△). Values are the means of triplicate flasks for each time point. Standard deviations were always less than 10% of the mean.

medium during growth. Secretion of invertase by mycelium grown on sucrose, expressed as a percentage of total enzyme, increased with the age of the culture (Fig. 3). However, enzyme synthesis declined with age, as did the concentration of free glucose in the medium (Fig. 4). Malate dehydrogenase, an essential intracellular enzyme, was not found in the culture filtrates, indicating that the invertase was truly secreted.

The mycelial soluble fraction and the secreted enzymes had the same optimum pH (5.2) and temperature (60 °C) values for the reaction, indicating that they are the same protein (M. H. Vainstein, L. M. Boddy & J. F. Peberdy, unpublished results). The enzyme hydrolysed sucrose and raffinose, showing it to be a β -fructofuranosidase. No maltase activity was found in the system under the conditions tested.

Experiments were carried out to verify the regulatory effect of sucrose and glucose on invertase synthesis and secretion. Mycelium grown overnight on glucose/salts medium was harvested by centrifugation, washed twice in the salts medium, transferred to a fresh medium with sucrose or glucose as a carbon source, and incubated for a further 80 min. As shown in Table 1, glucose did not

Fig. 2. Effect of carbon source (1%, w/v) on the total protein content (a) and protein secretion (b) in *A. nidulans* strain 2.124: fructose (▲), glucose (○), raffinose (●) and sucrose (△). Values are the means of triplicate flasks for each time point. Standard deviations were always less than 10% of the mean.

Fig. 3. Percentage of invertase present in different fractions of *A. nidulans* strain 2.124 during growth on 1% (w/v) sucrose medium. ▨, Secreted enzyme; □, mycelial soluble fraction; ▩, mycelial insoluble fraction (see Methods).

Fig. 4. Free glucose present in the medium during growth of *A. nidulans* strain 2.124 on different carbon sources (each 1%, w/v): fructose (▲), glucose (○), raffinose (●) and sucrose (△).

promote an increase in specific activity of invertase but sucrose did. After 80 min incubation on sucrose medium, a 26-fold increase in the total enzyme activity was noted compared with the levels on glucose medium. PAGE analysis of these samples showed that the mycelial soluble fraction and the secreted enzymes migrate at the same position in the gels (Fig. 5a). These effects on enzyme induction were verified in three different *A. nidulans* strains and *A. niger*. Data in Table 2 show a large difference in specific activity in all three *A. nidulans* strains as well as in *A. niger*. Among the *A. nidulans* strains tested, the wild-type was the best producer of invertase. However, this was still low compared with *A. niger*, but the levels of secretion were very similar (60.69 and 58.88%, respectively). *A. niger* possesses two forms of invertase instead of the one form normally observed in *A. nidulans* strains (Fig. 5b).

Once the inducibility of invertase synthesis and secretion by sucrose was known it was of interest to investigate the effect of other carbohydrates. The experimental protocol previously described was followed using the strain of *A. nidulans* best for invertase production.

As shown in Fig. 6, the lowest level of total invertase units was found in cultures with 3% (w/v) glucose. Low values for activity were also found for 0.05% (w/v) glucose but, interestingly, secretion of invertase could also be detected. Maltose, melibiose, glycerol and trehalose (all at 1%, w/v) induced invertase to the same extent. Raffinose and sucrose were the best inducers but raffinose seemed to be the best carbon source for secretion of invertase. PAGE analysis showed the same form of enzyme produced on all sugars tested (data not shown). The samples were also screened for the presence of maltase, a sucrose-hydrolysing intracellular enzyme found in the same fungi. No activity was detected under the conditions used.

Fig. 5. Electrophoretic analysis in a 7.5% SDS non-denaturing gel of invertases from *A. nidulans* (a) and *A. niger* (b). (a) Lane 1, yeast invertase; lane 2, invertase secreted into the culture filtrate; lane 3, invertase present in the mycelial soluble fraction. (b) Lane 1, mycelial soluble fraction prepared from mycelium incubated for 120 min in medium containing 1% (w/v) glucose; lanes 2 and 3, mycelial soluble fraction prepared from mycelium incubated for 45 min (2) and 120 min (3) in medium containing 1% (w/v) sucrose; lanes 4 and 5, mycelial insoluble fraction prepared from mycelium incubated for 120 min in medium containing 1% (w/v) glucose (4) or sucrose (5). The gels were stained for invertase activity as described in Methods.

The effect of sugar mixtures on invertase expression was investigated to confirm the repressive effect of a range of concentrations of glucose, fructose and xylose, in the presence of a fixed concentration of sucrose. Mycelium produced in AMM with 3% (w/v) glucose was washed as before and transferred to fresh AMM medium containing the sugar mixtures. As shown in Table 3, sucrose (1%, w/v) alone gave the highest production of invertase, the effect being significantly higher than that of various mixed sugars, according to Tukey's test at 5% probability. Glucose, 5% (w/v), had the most repressive effect in the presence of 1% (w/v) sucrose. The levels of enzyme produced in 5% fructose or xylose were similar to those obtained with 1% and 3% (w/v) glucose (Table 3).

In another set of experiments, mycelium grown overnight in 1% (w/v) sucrose medium was transferred to a fresh medium containing sucrose (1%, w/v) or glucose (1%, w/v) and incubated for a further 80 min as shaken cultures. As shown in Table 4, invertase continued to be actively synthesized and secreted in sucrose medium. However, in the presence of glucose, synthesis of invertase was severely repressed and secretion was not observed during the course of the experiment.

Discussion

The existence of external hydrolytic enzymes in micro-organisms might be attributed to the impermeability of the cell membrane to their corresponding substrates.

Fig. 6. Effect of different sugars on invertase expression in *A. nidulans* strain 2.1. ▨, Total enzyme activity in the system; ▩, mycelial soluble fraction; □, enzyme secreted into the culture medium. Abbreviations: Glc 1, 3% glucose; Man, 1% mannose; Glc 2, 0.05% glucose; Xyl, 1% xylose; Fru, 1% fructose; Gal, 1% galactose; Mal, 1% maltose; Mel, 1% melibiose; Tre, 1% trehalose; Inu, 1% inulin; Raf, 1% raffinose; Suc, 1% sucrose.

Table 1. Effect of glucose and sucrose on invertase synthesis in *A. nidulans* strain 2.124

Abbreviations: CF, culture filtrate; Sol., mycelial soluble fraction; Insol., mycelial insoluble fraction (see Methods). Numbers in parentheses are standard deviations for the mean of triplicate flasks for each time point (E, exponential). Numbers in square brackets show the percentage of enzyme in the fraction.

Incubation time (min)	Invertase [units (mg dry wt mycelium) ⁻¹]							
	Sucrose (1%, w/v)				Glucose (1%, w/v)			
	Total	CF	Sol.	Insol.	Total	CF	Sol.	Insol.
20	0.005	0	0.001 [25%] (± 7E-4)	0.004 [75%] (± 1E-3)	0.005	0	0.001 [25%] (± 7E-4)	0.004 [75%] (± 1E-3)
45	0.063	0.024 [38%] (± 1.7E-3)	0.027 [43%] (± 2.1E-3)	0.012 [19%] (± 2.8E-3)	0.007	0	0.002 [28%] (± 7.1E-4)	0.005 [72%] (± 7.0E-4)
80	0.127	0.051 [40%] (± 1.5E-3)	0.052 [41%] (± 2E-3)	0.024 [19%] (± 1.3E-3)	0.007	0	0.002 [28%] (± 7E-4)	0.005 [72%] (± 7E-4)

Table 2. Induction of invertase by 1% (w/v) sucrose in different *A. nidulans* strains and *A. niger*

Values are the means of triplicate flasks for each time point, ± standard deviation (E, exponential). ND, Not determined.

	Invertase [units (mg dry wt mycelium) ⁻¹]		
	Culture filtrate	Soluble fraction	Insoluble fraction
<i>A. nidulans</i> 2.1	0.244 ± 8.1E-3	0.136 ± 2.0E-3	0.022 ± 1.5E-3
<i>A. nidulans</i> 2.108	ND	0.027 ± 1.4E-3	0.008 ± 7.0E-4
<i>A. nidulans</i> 2.124	0.051 ± 1.5E-3	0.052 ± 2.0E-3	0.024 ± 1.3E-3
<i>A. niger</i> B60	0.368 ± 3.6E-3	0.175 ± 3.0E-3	0.082 ± 2.0E-3

Some of these enzymes are formed only in the presence of their specific substrates. This situation may pertain to invertase formation in *A. nidulans*, where the enzyme appeared in high levels only in response to the presence of β -fructofuranosides (sucrose and raffinose), which are its normal substrates. In contrast, synthesis and secretion of invertase by the slime strain of *Neurospora crassa* occurred independently of the carbon source present in the culture medium, whereas catabolic repression was

normal in the wild-type strain (Casanova *et al.*, 1987). Synthesis of the larger, extracellular form of invertase in *S. cerevisiae* (Carlson & Botstein, 1982) and *Aspergillus flavus* (Olutiola & Cole, 1980) are also subject to catabolic repression.

The maximal invertase activity in *A. nidulans* was found about 15 h after inoculation into medium containing sucrose or raffinose, simultaneously with the peak of protein accumulation in the culture. In medium with

Table 3. Effect of different concentrations of glucose, xylose and fructose in the presence of 1% sucrose on invertase synthesis in *A. nidulans* strain 2.1

Values are the means of triplicate flasks for each variation $\pm$ standard deviation (E, exponential); numbers followed by different superscript letters are statistically different at 5% probability according to Tukey's test.

Sugar	Invertase activity [units (mg dry wt mycelium) ⁻¹]
Sucrose	0.2720 ^c $\pm$ 7.0E-3
Sucrose + 1% Glucose	0.2010 ^{ab} $\pm$ 1.5E-2
Sucrose + 3% Glucose	0.1780 ^{ab} $\pm$ 1.0E-2
Sucrose + 5% Glucose	0.1495 ^a $\pm$ 2.1E-2
Sucrose + 1% Fructose	0.2205 ^{bc} $\pm$ 1.7E-2
Sucrose + 3% Fructose	0.1845 ^{ab} $\pm$ 9.1E-3
Sucrose + 5% Fructose	0.2005 ^{ab} $\pm$ 1.4E-2
Sucrose + 1% Xylose	0.2145 ^{bc} $\pm$ 2.3E-2
Sucrose + 3% Xylose	0.1975 ^{ab} $\pm$ 1.3E-2
Sucrose + 5% Xylose	0.1830 ^{ab} $\pm$ 1.0E-2

sucrose, the decrease of invertase activity seems to be related to the depletion of the carbon source for mycelium growth. If low concentrations of free glucose in the medium, released in the breakdown of sucrose, caused derepression of synthesis, then higher levels of invertase activity would be expected after growth over 15 h, when the glucose levels in the culture were reaching their lowest values. The results suggested that secretion of invertase might be related to low levels of free glucose in the medium and the age of the mycelium. The percentage of the enzyme secreted increased proportionally with the age of the culture and the simultaneous decrease of both enzyme synthesis and glucose concentration.

The trisaccharide raffinose supported cultures with the highest invertase activity. In this respect, *A. nidulans* had a similar behaviour to *Neurospora* (Metzenberg, 1962) and this may be related to the absence of free glucose among the enzymic cleavage products of raffinose. The ability of *A. nidulans* to synthesize very low levels of invertase in fructose medium indicates that fructose may suffice as a carbon source for the initial synthesis of the enzyme (Gupta & Bhatia, 1982). However, subsequent synthesis depends on the presence of sucrose or raffinose in the medium.

Transfer and induction experiments showed the production of a constitutive level of invertase in a wide range of sugars, with 3% glucose giving the lowest activities and 1% raffinose and sucrose the highest. A low concentration of glucose (0.05%) did not cause significant derepression or induction of invertase. In this respect invertase synthesis in *A. nidulans* differs from that in *S. cerevisiae*, where production of the secreted enzyme is highly regulated by the concentration of glucose present in the medium. Cells grown in medium containing high levels of glucose do not produce and secrete invertase, but a shift to medium containing low glucose concentrations causes derepression (Hackel, 1975; Hackel & Khan, 1978; Carlson, 1984).

Using mixtures of sugars with a range of levels of the repressor, the repressive effect of glucose on invertase synthesis was less. Even at glucose concentrations as high as 5% the enzyme continued to be synthesized and secreted as long as the inducer was present. This evidently suggests that a correlation between the molecular structure of the inducer and the catalytic centre on the enzyme is inherently involved in the mechanisms of induction (Jacob & Monod, 1961). The signal leading to induction of synthesis of invertase could

Table 4. Effect of glucose and sucrose on invertase synthesis in *A. nidulans* strain 2.124

Abbreviations: CF, culture filtrate; Sol., mycelial soluble fraction (see Methods). Numbers in parentheses are standard deviations for the mean of triplicate flasks for each time point (E, exponential).

Incubation time (min)	Invertase [units (mg dry wt mycelium) ⁻¹]					
	Sucrose (1%, w/v)			Glucose (1%, w/v)		
	Total	CF	Sol.	Total	CF	Sol.
0	0.117 ($\pm$ 2E-2)	0	0.117 ($\pm$ 2E-2)	0.117 ($\pm$ 2E-2)	0	0.117 ($\pm$ 2E-2)
20	0.178 ($\pm$ 2E-3)	0.142 ($\pm$ 3E-3)	0.035 ($\pm$ 2E-3)	0.072 ($\pm$ 7E-3)	0	0.072 ($\pm$ 7E-3)
45	0.355 ($\pm$ 2E-3)	0.255 ($\pm$ 7E-3)	0.100 ($\pm$ 9E-3)	0.078 ($\pm$ 5E-3)	0	0.078 ($\pm$ 5E-3)
80	0.461 ($\pm$ 1E-2)	0.351 ($\pm$ 1E-2)	0.110 ($\pm$ 4E-3)	0.081 ($\pm$ 4E-3)	0	0.081 ($\pm$ 4E-3)

result from the binding of the substrate to the cell membrane, or from the appearance in the cytoplasm of hydrolysis products of the substrate, resulting from the presence of constitutive levels of invertase on the cell surface.

The absence of a smaller internal form of invertase in the mycelium of *A. nidulans* and the presence of two forms in *A. niger*, together with the fact that all three enzymes are glycoproteins (Vainstein, 1990), shows that the currently accepted model for the synthesis and subcellular location of invertase in *S. cerevisiae* may not be extrapolated to these filamentous fungi. In *S. cerevisiae* a single structural gene codes for two different polypeptides, one of which is glycosylated and then secreted and the other remains free of carbohydrate in the cytosol (Perlman & Halvorson, 1981; Rodriguez *et al.*, 1981; Carlson & Botstein, 1982; Perlman *et al.*, 1982).

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