Role of maltase in the utilization of sucrose by *Candida albicans*

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Two isoenzymes of maltase (EC 3.2.1.20) were purified to homogeneity from *Candida albicans*. Isoenzymes I and II were found to have apparent molecular masses of 63 and 66 kDa on SDS/PAGE with isoelectric points of 5.0 and 4.6 respectively. Both isoenzymes resembled each other in similar N-terminal sequence, specificity for the $\alpha(1\rightarrow4)$ glycosidic linkage and immune cross-reactivity on Western blots using a maltase II antigen-purified rabbit antibody. Maltase was induced by growth on sucrose whereas β -fructofuranosidase activity could not be detected under similar conditions. Maltase I and II were shown to be unglycosylated enzymes by neutral sugar assay, and more than 90% of α -glucosidase activity was recoverable from spheroplasts. These data, in combination with other results from this

laboratory [Geber, Williamson, Rex, Sweeney and Bennett (1992) *J. Bacteriol.* **174**, 6992–6996] showing lack of a plausible leader sequence in genomic or mRNA transcripts, suggest an intracellular localization of the enzyme. To establish further the mechanism of sucrose assimilation by maltase, the existence of a sucrose-inducible H^+ /sucrose *syn*-transporter was demonstrated by (1) the kinetics of sucrose-induced [^{14}C]sucrose uptake, (2) recovery of intact [^{14}C]sucrose from ground cells by t.l.c. and (3) transport of 0.83 mol of H^+ /mol of [^{14}C]sucrose. In total, the above is consistent with a mechanism whereby sucrose is transported into *C. albicans* to be hydrolysed by an intracellular maltase.

INTRODUCTION

Candida albicans is a pathogenic diploid yeast responsible for infections of the skin, mucous membranes and deep organs in both previously healthy and immunocompromised persons. Assimilation of carbohydrates such as sucrose may play a role during pathogenesis. For example, an inability to assimilate sucrose has been shown to correlate with avirulence in *C. albicans* var. *stellatoidea* (Kwon-Chung et al., 1990) which otherwise shares over 90% homology with *C. albicans* (Kwon-Chung et al., 1989). In addition, spontaneous mutation of *C. albicans* var. *stellatoidea* to a sucrose-utilizing prototroph was shown to result in karyotropic shifts accompanied by increased virulence in mice (Wickes et al., 1991).

Maltase is an exo- α -glucosidase (EC 3.2.1.20) which hydrolyses the α -glucopyranosyl bond of various substrates including disaccharides, oligosaccharides and certain aryl and alkyl α -glucopyranosides. In *Saccharomyces cerevisiae*, maltase is an intracellular enzyme responsible for hydrolysis of maltose (DeKroon and Koningsberger, 1970), whereas the extracellular invertase (EC 3.2.1.26), a β -fructofuranosidase, is primarily responsible for hydrolysis of sucrose (DeLa Fuente and Sols, 1962). *C. albicans* assimilates sucrose, but its inability to assimilate raffinose (Meyer et al., 1984) makes possession of an invertase less likely. Thus sucrose as well as maltose utilization may be dependent on the same maltase activity. Indeed, *C. albicans* strains that lack the ability to assimilate sucrose lack α -glucosidase activity (Wickes et al., 1991). However, the mechanism of sucrose assimilation by *C. albicans* has remained unclear. For example, genetic studies that reported the cloning of a putative regulatory gene, CASUC 1, used hydrolysis of *p*-nitrophenyl α -D-glucoside (PNPG) in intact cells as a marker of transformation. However, the target gene of CASUC 1 could not be identified, since a whole cell assay may not discriminate

between transport and hydrolytic activities (Kelly and Kwon-Chung, 1992). Thus identification and characterization of enzymes and transporters with a role in sucrose utilization is an essential step in genetic studies of disaccharide metabolism in *C. albicans*.

In the present study we have undertaken the purification and characterization of two species of maltase from *C. albicans*, as well as studies of sucrose transport in order to help to define the role of maltase in sucrose metabolism.

MATERIALS AND METHODS

The D-glucose, D-fructose, D-sorbitol detection kit was from Boehringer-Mannheim, Mannheim, Germany. Phenyl-Sepharose, DEAE-Sepharose, PBE 94 and polybuffer 74 were obtained from Pharmacia-LKB, Uppsala, Sweden. Biogel HTP hydroxyapatite was from Bio-Rad Laboratories, Watford, Herts., U.K. *S. cerevisiae* α -glucosidase was purchased from U.S. Biochemicals, Cleveland, OH, U.S.A. Invertase, glucose oxidase type V-S and peroxidase type XII were from Sigma, St. Louis, MO, U.S.A. Filters (0.45 μ m) were obtained from Millipore Corp., Bedford, MA, U.S.A. Synchropak RP-4 h.p.l.c. column was obtained from Synchrom, Lafayette, IN, U.S.A. All other reagents were of the highest purity available. Rosewell Park Memorial Institute (RPMI) 1640 media was from Biofluids, Rockville, MD, U.S.A. Silica gel t.l.c. plates (GL) were from Analtech, Newark, DE, U.S.A.

Enzyme assays

α -Glucosidase activity was measured with PNPG by a method similar to that of Ramirez et al. (1989). Enzyme solutions (10 μ l) were incubated in 1 ml of 10 mM sodium phosphate, pH 7.5, with 0.2 mM PNPG at 25 °C for 10 min and the absorbance at 400 nm was recorded. One unit was defined as 1 absorbance unit

in 10 min of assay. Glucose was determined with a glucose oxidase/peroxidase-coupled continuous assay similar to that of Somogyi and Nelson (1952). Similarly, after isoelectrofocusing of samples on a pH 3–10 gel, glucose was detected by overlaying the gel strips with 10 ml of a solution of 186 units/ml glucose oxidase, 20 units/ml peroxidase and 0.2 mg/ml 3,3-diaminobenzidine in 50 mM sodium phosphate, pH 7.5, at 30 °C with a 10 mM concentration of the indicated disaccharide.

β -Fructofuranosidase (invertase) activity was measured by determination of fructose production from raffinose using a D-glucose, D-fructose, D-sorbitol detection kit according to the manufacturer's directions. One unit of activity was defined as 1 μ mol of fructose produced/min of assay.

Enzyme purification

For production of *C. albicans* α -glucosidase, the pathogenic laboratory specimen A.T.C.C. 32354 (B311) was grown overnight in a 50-litre glass fermenter in 1 % yeast extract/2 % peptone/2 % sucrose (YEPS). In a typical preparation, 100 g of cells was harvested and washed twice in ice-cold distilled water. Cells were then resuspended in 0.5 vol. of 0.01 M sodium phosphate, pH 7.5, containing 1 mM phenylmethanesulphonyl fluoride (PMSF) and broken by mechanical shaking in a Braun homogenizer with an equal volume of acid-washed glass beads (0.45 mm diameter) at 4 °C. Unless otherwise noted, all further procedures were carried out at 4 °C. The extract was spun at 5000 g for 10 min whereupon an equal volume of a satd. $(\text{NH}_4)_2\text{SO}_4$ solution was added to the supernatant. After being allowed to stand for 15 min at 4 °C, precipitated material was removed by centrifugation at 10000 g for 10 min. The supernatant, which contained more than 98 % of the enzyme activity, was then applied to a phenyl-Sepharose column (5.0 cm $\times$ 13 cm) equilibrated in 50 %-satd. $(\text{NH}_4)_2\text{SO}_4$, 0.01 M sodium phosphate, pH 7.5, and washed with 1 litre of a cold solution of 0.01 M sodium phosphate, pH 7.5, to which had been added solid $(\text{NH}_4)_2\text{SO}_4$ sufficient to raise the conductivity to 47 mmho at 25 °C. The enzyme was then eluted with a 500 ml gradient formed from equal volumes of the previous buffer and 0.01 M sodium phosphate, pH 7.5, without $(\text{NH}_4)_2\text{SO}_4$. Fractions containing enzyme activity were then pooled and dialysed for 16 h against 4 litres of 0.01 M sodium phosphate, pH 7.5. This material was then applied to a DEAE-Sephacel column (2.5 cm $\times$ 18 cm) previously equilibrated in the same buffer. The column was washed with a solution containing 0.08 M NaCl and 0.01 M sodium phosphate and then eluted with 500 ml of a linear gradient of 0.08–0.3 M NaCl in the same buffer. Fractions with enzyme activity were pooled and applied to a Bio-Rad HTP column (2.5 cm $\times$ 15 cm) equilibrated with 0.02 M sodium phosphate, pH 7.5. After the column had been washed with this same buffer, the enzyme was eluted with a 500 ml gradient of 0.02–0.1 M sodium phosphate, pH 7.5.

Resolution of the two isoenzymes for amino acid analysis, neutral sugar analysis and N-terminal sequencing was carried out by concentrating the above enzyme to 1 mg/ml in a Centriprep-10 cartridge, dialysis against distilled water, adding trifluoroacetic acid (TFA) to 0.1 % and loading it on a Synchropak RP-4 h.p.l.c. column (250 mm $\times$ 4.6 mm internal diameter) equilibrated with 41.5 % acetonitrile in water with 0.05 % TFA and eluting it with a linear gradient of 41.5–46 % acetonitrile in 0.05 % TFA over 30 min.

Purity of the final product was assessed by subjecting the indicated amount of protein to 10 % SDS/PAGE by the procedure of Laemmli (1970), as well as by isoelectric focusing on Novex slab gels, pH 10–3, according to the manufacturer's

directions. Protein was assayed using the Bio-Rad protein assay according to the manufacturer's directions.

Analysis of maltase isoenzymes

Amino acid composition and sequence analysis was performed as described by Roberts et al. (1989). Neutral sugar content of h.p.l.c.-purified lyophilized isoenzymes was performed by the method of Grollman et al. (1992).

Antibody preparation and Western blotting

Purified maltase (200 μ g) was subjected to SDS/PAGE on slab gels as described above, and the 66 kDa band was cut out and ground up in a 5 ml tissue homogenizer in 0.5 ml of 10 mM sodium phosphate, pH 7.5, mixed with complete Freund's adjuvant and injected into a New Zealand White rabbit. The procedure was repeated every 14 days, but with incomplete Freund's adjuvant. After the fourth injection the rabbit was bled to assess antibody production. The antibody was then purified by precipitation of other proteins with 7.5 % octanoic acid as described by Russ et al. (1983) and then antigen-purified by the method of Harlow and Lane (1988). Western blotting of *Candida* proteins was carried out by the method of Towbin et al. (1979).

Sucrose transport by *C. albicans*

C. albicans was grown overnight in either YEPS or 1 % yeast extract/2 % peptone/2 % glucose (YEPD) and the indicated number of cells was washed three times and incubated in 0.3 ml of 50 mM sodium phosphate, pH 6.0, in the presence of various concentrations of [14 C]sucrose (604 mCi/mmol) and incubated at either 25 °C or on ice for the times indicated in the Figure legends. After each period, 50 μ l samples were filtered on 2 cm-diameter 0.45 μ m-pore-size filters and washed rapidly with 1 ml of 100 mM HgCl₂, followed by two washes of 1 ml of 5 mM HgCl₂. The filters were then dried and counted for radioactivity by liquid-scintillation spectrometry.

In order to assess the nature of the transported radioactivity, a similar reaction to the above was carried out, except that 10⁹ cells in 1 ml were incubated with 4 μ M [14 C]sucrose and the first filter wash was performed with 1 ml of boiling 100 mM HgCl₂ to inactivate cellular enzymes. Cells then recovered from the filters were ground with an equal volume of 1 μ m-diameter glass beads for 10 min in a vortex mixer and extracted twice with 10 μ l of 10 mM sodium phosphate, pH 6.5, in distilled water. Samples were then spotted on to silica-gel t.l.c. plates impregnated with 0.1 M Na₂HPO₄ and developed twice with propan-1-ol/methanol/water (16:1:3, by vol.) as described by Prosek et al. (1991). In addition, one sample incubated with [14 C]sucrose under the same conditions and harvested at 30 s was preincubated with 1 μ g of invertase from *S. cerevisiae* before t.l.c. Standards of 5 μ l of 0.1 M solutions of either glucose, sucrose or fructose were spotted adjacent to the panel of cell extracts as well as at the origin of the cell extract corresponding to 15, 30 and 45 s respectively and developed after autoradiography with a solution of 5 % H₂SO₄ in methanol heated at 90 °C.

The stoichiometry of H⁺/sucrose cotransport was determined by the method of Brooker et al. (1985) using 2 $\times$ 10⁸ cells grown in YEPS or, as a control, YEPD-grown cells incubated anaerobically with 4 μ M [14 C]sucrose. The rise in the pH of the external media was measured over 2 min. Portions (50 μ l) were removed, filtered and assayed for radioactivity, then measured quantities of anaerobic HCl were added to calibrate observed pH changes.

RESULTS

Induction of α -glucosidase activity

C. albicans strain A.T.C.C. 32354 was grown for 16 h in the defined medium RPMI with either 2% maltose, 2% sucrose, 2% xylose, 2% succinic acid or 2% glucose and fractured by vortexing with an equal volume of 0.1 mm-diameter glass beads in the presence of 1 mM PMSF. The specific activity of the extract, as measured by PNPg, was induced to highest levels by maltose (66 units/mg of protein) or sucrose (64 units/mg of protein) and was repressed by glucose (0.4 unit/mg of protein); all values are the average of two measurements. This induction with maltose and repression by glucose is similar to that reported for *S. cerevisiae* (Bradley and Creevy, 1966). Similarly, growth in xylose and succinic acid led to a low activity of α -glucosidase in cell extracts (0.2 unit/mg of protein and 0.7 unit/mg of protein respectively). However, β -fructofuranosidase activity was not detected in response to growth in sucrose, in contrast with control experiments using *S. cerevisiae* A.T.C.C. 26108 (0.19 unit/mg of protein; level of detection < 0.002 unit/mg) as was reported previously (DeMot and Verachtert, 1987).

Purification of maltase from *C. albicans*

Maltase from *C. albicans* was purified with a 16% yield using a combination of $(\text{NH}_4)_2\text{SO}_4$ precipitation and chromatography on phenyl-Sepharose, DEAE-Sepharcel and hydroxyapatite as

Table 1 Purification of *C. albicans* maltase

Step	Total activity (units)	Total protein (mg)	Specific activity (units/mg)	Yield (%)
Extract	36221	1688	21	100
$(\text{NH}_4)_2\text{SO}_4$	37572	803	47	104
Phenyl-Sepharose	24378	42	583	67
DEAE-Sepharcel	13410	5	2483	37
Hydroxyapatite	5849	3	1828	16

Figure 1 SDS/PAGE (a) and isoelectric focusing (b–d) of maltase from *C. albicans*

(a) 20 μ g of maltase stained with Coomassie Blue; (b) 10 μ g of maltase on a gradient pH 3–7 gel stained with Coomassie Blue; (c) 10 μ g of maltase on a gradient pH 3–7 gel stained with an enzymic assay for glucose using maltose as substrate; (d) 10 μ g of maltase on a gradient pH 3–7 gel stained with an enzymic assay for glucose using sucrose as substrate.

shown in Table 1. Isolation of the enzyme required breakage of washed cells as no activity could be detected in the growth media. Similarly suggesting an intracellular localization, cell wall digestion of RPMI-grown *C. albicans* by the method of Goshorn et al. (1992) yielded no detectable enzyme, whereas addition of 0.5% Triton to rupture spheroplasts yielded 59 units/mg of *Candida* protein, similar to the 64 units/mg of protein obtained by glass-bead breakage described above. Figure 1(a) shows an electrophoretogram of the enzyme purified to homogeneity. As shown in Figure 1(a), the enzyme from *Candida* is composed of two bands with molecular masses equivalent to 63 and 66 kDa on SDS/PAGE. This is similar to the value of 60 kDa reported by Khan and Eaton (1967) for *S. cerevisiae*. Purified maltase from *Candida* migrated as two distinct bands on isoelectrofocusing gels with pI equal to 5.0 (maltase I) and 4.6 (maltase II) (Figure 1b). SDS/PAGE of excised bands identified maltase I as the 63 kDa species and maltase II as the 66 kDa species (results not shown).

Substrate specificity of maltase

Hydrolysis of substrates by a mixture of the purified *Candida* α -glucosidase isoenzymes was measured in a glucose oxidase/peroxidase-coupled assay in 10 mM sodium phosphate, pH 7.5. At a concentration of 10 mM, maltose, sucrose and α -D-methyl glucopyranoside were found to be substrates for the *Candida* enzyme hydrolysing 1.3, 1.8 and 1.6 μ mol/min per mg of protein for each respective substrate. However, no glucose could be detected after incubation of 20 μ g of purified *Candida* α -glucosidase with 10 mM methyl β -D-glucopyranoside, cellobiose, isomaltose, α,α -trehalose, nigerose or α -cyclomaltose for 30 min (limit of detection 0.1 nmol). The lack of hydrolysis of methyl β -D-glycoside or cellobiose shows the enzyme's specificity for the α -glycosidic linkage. The specificity for the $\alpha(1\rightarrow4)$ linkage was supported by a lack of hydrolysis of the $\alpha(1\rightarrow6)$ glycosidic linkage of isomaltose, the $\alpha(1\rightarrow1)$ glycosidic linkage of trehalose and the $\alpha(1\rightarrow3)$ glycosidic linkage of nigerose. In addition, the lack of hydrolysis of the $\alpha(1\rightarrow4)$ linkage of α -cyclohexamaltose is most consistent with the enzyme being an exoglucosidase only. In order to determine qualitatively if each of the two isoenzymes had different substrate specificities, *in situ* production of glucose from either maltose or sucrose was measured by electrofocused proteins using a glucose oxidase/peroxidase-coupled overlay assay. As shown in Figures 1(c) and 1(d), each isoenzyme hydrolyses both sucrose and maltose. Contaminating β -fructofuranosidase activity was assessed by assaying the mixture of the two isoenzymes for production of fructose using raffinose as substrate as described in the Materials and methods section; no fructose was detectable (limit of sensitivity 0.1 nmol) on incubating 20 μ g of the unresolved isoenzymes for 20 min at pH 7.5. The ability of the enzyme to hydrolyse glycosidic linkages of larger polymers was shown by glucose production of 0.0014 nmol/min per unit after incubation of the enzyme with 1 mg/ml bovine liver glycogen that had been exhaustively dialysed against distilled water and lyophilized to remove smaller potential substrates. This hydrolysis of glycogen is similar to that reported for rabbit liver α -glucosidase (Onodera et al., 1989). Thus, in summary, the behaviour of both the isoenzymes from *Candida* resembles a maltase with some glycogenolytic activity.

Separation and analysis of α -glucosidase isoenzymes by reversed-phase h.p.l.c.

As shown in Figure 2, the two isoenzymes were resolved using reversed-phase h.p.l.c. in about 50% yield. Unfortunately, the

Figure 2 Separation of maltase isoenzymes by reversed-phase h.p.l.c. and analysis by SDS/PAGE and Western blot

Elution profiles of maltase I and II are shown. Inset: (a) Western blot of whole-cell extract of sucrose-grown *C. albicans* boiled in 1% SDS using antigen-purified anti-(maltase II) antibody; (b) Coomassie-stained SDS/polyacrylamide gel of maltase I (lane 1) and maltase II (lane 2); (c) Western blot of 10 µg of maltase I (lane 1) and maltase II (lane 2).

harsh conditions of the separation results in inactivation of the enzyme. However, Coomassie-stained gels of the separated proteins (Figure 2b) show peak I (maltase I) to have a molecular mass of 63 kDa and peak II (maltase II) to have a molecular mass of 66 kDa, corresponding to each of the molecular masses of the two bands in the unresolved mixture. In addition, N-terminal sequencing of the two isoenzymes (I: XIEYTWK-DATYQIWPA; II: XIEYTWKDATYQIWPA) shows that the sequences are similar to each other, within the error expected in gas-phase sequencing (Niece et al., 1991), and possess a 61% identity with an 18-amino acid N-terminal fragment of the MAL 6 structural gene from *Saccharomyces* (Hong and Marmur, 1986) beginning eight amino acid residues from the *Saccharomyces* N-terminus. In addition, the similarity between the two isoenzymes is shown by immune cross-reactivity of rabbit antibody which had been antigen-purified using purified *C. albicans* peak II (Figure 2c). The sensitivity of the antibody is shown by detection of a small amount of contaminating peak II in lane 1 of Figure 2(c) which was undetectable in Coomassie staining in Figure 2(b). In order to characterize maltase isoenzymes in the intact organism, Western blots were performed using whole cell extract made from YEPS-grown *Candida* which were first killed and had their proteolytic enzymes inactivated by rapid plunging into a boiling-water bath in the presence of 1% SDS. Figure 2(a) shows two immunoreactive bands at 66 and 63 kDa, corresponding to the molecular masses of the copurified mixture (Figure 1a) and the h.p.l.c.-resolved isoenzymes (Figure 2b), making it unlikely that the presence of two isoenzymes is an artifact of purification. No immunoreactive bands were seen when *C. albicans* was grown in YEPD-grown cells (results not shown); this correlated with the lack of maltase activity from cells grown in RPMI with glucose.

Analysis of neutral sugars in 42 µg of h.p.l.c.-purified maltase I yielded 0.01, 0.21 and 0.17 mol of galactose, glucose and mannose respectively per mol of enzyme. Similarly, 62 µg of maltase II yielded 0.11 mol of glucose/mol but no other neutral sugars were detected. Since no sugars were found to be present at a concentration greater than 1 mol of sugar/mol of protein, it

Table 2 Amino acid analysis of maltase I and II (mol %)

Amino acid	Isoenzyme I	Isoenzyme II
Cysteic acid	0.7	0.4
Asp + Asn	13.6	15.0
Thr	3.2	1.1
Ser	4.9	1.1
Glu + Gln	11.5	17.2
Gly	9.2	18.8
Ala	7.0	14.8
Val	3.0	—
Met	2.6	—
Iso	6.1	0.7
Leu	6.5	9.3
Tyr	5.0	—
Phe	5.7	—
His	3.4	1.2
Lys	9.5	8.0
Arg	4.8	—

Figure 3 Uptake of [^{14}C]sucrose by *C. albicans*

Yeast cells were grown in either sucrose- (■, △) or glucose- (□) containing media; 3×10^8 cells were incubated in the presence of 3 µM [^{14}C]sucrose and incubated at 25 °C (■, □) or on ice (△). Portions (0.05 ml) were filtered at the indicated times and counted for radioactivity as described in the Materials and methods section.

is doubtful that either of the two isoenzymes are significantly glycosylated. Amino acid analysis of the separated isoenzymes yielded different quantities of some amino acids (Table 2). Thus the differences in migration patterns on SDS and isoelectric focusing of these unglycosylated enzymes are most likely due to small differences in the amino acid backbone in an area different from the region of the N-terminus.

[^{14}C]Sucrose transport by *C. albicans*

The absence of invertase as well as any extracellular or cell-wall-lytic-enzyme-releasable α -glucosidase from *C. albicans* cells grown in sucrose suggests that sucrose hydrolysis may occur intracellularly. Such intracellular hydrolysis of sucrose by an enzyme such as maltase presumes a transport mechanism for sucrose in *C. albicans*. As shown in Figure 3, *C. albicans* grown in the presence of sucrose, but not glucose, showed a time-dependent accumulation of [^{14}C]sucrose at 25 °C, but not at 0 °C. The lack of significant uptake by glucose-grown cells makes it unlikely that the observed sucrose uptake in sucrose-grown cells is due to periplasmic trapping of radioactivity. In addition, the rate of initial uptake over 30 s was found to be dependent on

Figure 4 T.l.c. of [^{14}C]sucrose uptake by *C. albicans*

(a) [^{14}C]sucrose ($4\ \mu\text{M}$) was incubated with 1×10^9 cells of *C. albicans* at 5 s, 15 s, 30 s, 60 s, 2 min, 4 min and 8 min (lanes 1–7 respectively). Samples were filtered, ground with glass beads and chromatographed as described in the Materials and methods section. (b) Standards run under identical conditions: lane 1, sucrose; lane 2, glucose; lane 3, fructose.

the concentration of sucrose, and double-reciprocal plots exhibited saturable kinetics with a K_m of $7.1\ \mu\text{M}$, consistent with a facilitated transport mechanism.

In order to verify that accumulated radioactivity was not from an extracellularly or periplasmically hydrolysed product, t.l.c. was performed on lysates of cells incubated with [^{14}C]sucrose for various times and treated with boiling HgCl_2 solutions to inactivate glycosidases and synthetases. Figure 4 shows time-dependent accumulation of a radioactive material that comigrates with sucrose and is different from the presumed hydrolysis products, glucose or fructose. Liquid scintillography of silica scraped from the plates indicates that, at 30 s, approx. 80% of the total radioactivity was still in the sucrose-comigrating band, indicating an early accumulation of sucrose before degradation. However, prolonged incubation times (greater than 1 min) resulted in the disappearance of the sucrose band and the appearance of other products with low mobility on t.l.c. Similar t.l.c. of glucose-grown cells subjected to the same incubation with [^{14}C]sucrose showed no defined spots on autoradiography, as predicted by the small quantities of total radioactivity detected in Figure 3. To identify further the material comigrating with sucrose on t.l.c., incubation of a sample of cell lysate (at the 30 s point) with invertase converted the sucrose-comigrating product into two radioactive products comigrating with glucose and fructose standards (results not shown).

In order to characterize the putative sucrose transporter, a procedure similar to that of Brooker et al. (1985) was used for measurement of possible H^+ /sucrose cotransport. This procedure makes use of anaerobic conditions as well as iodoacetamide and 1-ethyl-3-(3-dimethylaminopropyl)carbodi-imide to prevent the extrusion of H^+ and also sucrose transport by energy-dependent processes. A time-dependent increase in extracellular pH of

Table 3 H^+ and sucrose uptake of sucrose- and glucose-grown *C. albicans* and calculated H^+ /sucrose stoichiometric ratios

Growth media	Uptake of sucrose (nmol/2 min)	Uptake of H^+ (nmol/2 min)	Ratio
YEPD	0.2	0.2	—
	0.2	0.3	—
	0.1	0.3	—
YEPS	92	78	0.85
	89	74	0.83
	88	70	0.80

sucrose-grown cells was observed on addition of [^{14}C]sucrose to the cell suspension; however, there was a virtually complete lack of pH change using an identical number of glucose-grown cells. Samples of each cell suspension were withdrawn after pH measurement changes and radioactivity was assessed as described previously. Table 3 shows the results of three sets of experiments contrasting high levels of H^+ and sucrose uptake in the sucrose-grown cells with low levels obtained from glucose-grown cells with an average stoichiometric ratio of 0.83 for the three experiments with sucrose-grown cells. These data again make it unlikely that the sucrose uptake observed in Figure 3 is principally due to periplasmically trapped sucrose, since passive diffusion through a cell wall should not be associated with H^+ uptake. In summary, the above data show the existence of a sucrose-inducible H^+ /sucrose cotransporter which can transport sucrose into the cell to be available for hydrolysis by an intracellular glucosidase such as maltase.

DISCUSSION

In the present study, growth of *C. albicans* in sucrose was shown to induce α -glucosidase activity rather than β -fructofuranosidase activity, as in the related yeast, *S. cerevisiae*. However, *Candida* resembles *Saccharomyces* (Khan and Eaton, 1967) in that glucose was found to repress α -glucosidase activity in *C. albicans*. In addition, the lack of significant stimulation by xylose and succinic acid suggests not just derepression by maltose and sucrose but also a direct stimulating effect similar to transcription-regulated maltase induction in *Saccharomyces* (Federoff et al., 1983).

The α -glucosidase activity in *C. albicans* was purified as two isoenzymes with molecular masses of 63 kDa (I) and 66 kDa (II) and pI values of 5.0 (I) and 4.6 (II). In comparison, the literature shows a varied array of isoelectric points for the two isoenzymes of maltase from *Saccharomyces*, ranging between 4.7 and 5.6 for the more acidic and 5.5 and 5.9 for the more basic isoenzyme (Khan and Eaton, 1967; Glemzha et al., 1981; Tabata et al., 1984). These interstrain variations probably represent heterogeneity in transcripts from multiple gene copies of the Mal locus of *Saccharomyces* (Needleman, 1991). The two isoenzymes of *C. albicans* A.T.C.C. 32354 were found to have similar substrate specificities, N-terminal sequences and shared immune cross-reactivity on Western blots. Both enzyme forms clearly behave as a maltase, as shown by their ability to cleave substrates such as sucrose and maltose. This pattern of substrate hydrolysis is similar to most preparations of isoenzymes of maltase from *Saccharomyces* (Needleman et al., 1978; Siro and Lovrgren, 1978; Krakenaite and Glemzha, 1983; Tabata et al., 1984) except for one preparation in which the two enzymes differed by their ability in turn to hydrolyse maltose or α -methyl D-glucopyranoside (Khan and Eaton, 1967). In addition, the lack of

hydrolysis of the $\alpha(1\rightarrow6)$ linkage of isomaltose distinguishes this enzyme from a glucoamylase purified from a diastotic strain of *S. cerevisiae* by Kleinman et al. (1988), and the lack of apparent secretion of this enzyme into the surrounding fluid contrasts this enzyme with the glucoamylase from *Candida tropicalis* (Sawai and Hehre, 1962), *Candida antarctica* (DeMot and Verachtert, 1987) and *Candida tsukubaensis* (DeMot et al., 1985).

Whole-cell extracts of YEPS-grown cells boiled in 1% SDS (while still viable) to inactivate proteolytic enzymes showed the presence of both isoenzymes on Western-blot analysis. This provides evidence that both isoenzymes are present in the intact yeast, and thus one is unlikely to be only a proteolytic artifact of purification. However, boiling of the cells before lysis may not degrade proteolytic enzymes that may be a part of the intracellular processing of the enzyme. The differences in the molecular masses of the two isoenzymes may be due to differences in the amino acid backbone, as shown by differences in amino acid composition, and lack of other post-translational modifications such as glycosylation by neutral sugars. In the related yeast, *Saccharomyces*, as many as five MAL loci each coding for a maltase of different structure are responsible for multiple isoenzymes (Needleman, 1991). However, a single maltase gene in *Candida*, shown by Southern-blot analysis by Geber et al. (1992), suggests that multiple isoenzymes in *Candida* arise through either post-transcriptional processing, such as the invertase of *Saccharomyces* (Williams et al., 1985) or post-translational proteolysis.

Maltase appears to be the predominant α -glucosidase activity in *C. albicans* and is similar to the intracellular maltase of *S. cerevisiae* with respect to substrate specificity, localization inside the periplasmic membrane of spheroplasts and lack of significant glycosylation. These data, in addition to cloned sequence data from Geber et al. (1992) showing lack of an obvious leader sequence required for transmembrane transport of maltase, suggest that maltase from *C. albicans* is a predominantly intracellular enzyme. In *S. cerevisiae*, the enzyme β -fructofuranosidase (invertase) is the primary contributor toward sucrose hydrolysis. Invertase exists in an intracellular form and a heavily glycosylated periplasmic form, the latter of which is responsible for extracellular hydrolysis of sucrose with secondary transport of fructose and glucose into the cell for fermentation (DeLa Fuente and Sols, 1962). As demonstrated in this study, *Candida* does not contain significant β -fructofuranosidase activity. Thus it appears that *C. albicans* must transport sucrose into the cell if sucrose is to be hydrolysed by an intracellular enzyme such as maltase. Transport of sucrose was shown by a time-dependent, sucrose-inducible incorporation of [14 C]sucrose at 25 °C but not at 0 °C and was absent from identically treated cells that had been grown in dextrose rather than sucrose. Kinetic analysis shows this transport to be saturable (and thus facilitated) exhibiting a K_m of 7.1 μ M, similar to that of the lactose permease (1 μ M) of *Agrobacterium radiobacter* (Greenwood et al., 1990), but much lower than the sucrose permease (6 mM) described from a single strain of *Saccharomyces* (Santos et al., 1982). It appears that this uniquely adapted high-affinity transporter in *Candida* may be the result of a greater reliance of *Candida* on such a transport mechanism in comparison with *Saccharomyces*, which relies predominantly on periplasmic invertase for the assimilation of sucrose (Schekman and Novick, 1982). As shown by t.l.c., accumulation of intracellular sucrose in the presence of an active intracellular maltase predicts that the K_m of the resolved maltase isoenzymes should be much higher than that of the permease, similar to the millimolar range of most maltases of *Saccharomyces* (Glemzha et al., 1981). In addition, time-dependent formation of products less mobile than sucrose

on t.l.c. within 1 min of incubation with the sugar was observed and may represent synthesized oligosaccharides or phosphorylated sucrose similar to that shown in *Escherichia coli* (Sutrina et al., 1990). The absence of detectable hydrolytic products such as fructose or glucose may be due to the presence of phosphorylated products or to high-affinity enzymes utilizing products of α -glycosidic hydrolysis.

Further study of the presumed sucrose permease showed that it is most likely a sucrose/H⁺ *syn*-transporter as shown by similar stoichiometric ratios of H⁺ to [14 C]sucrose uptake (0.83) to those of other *syn*-transporters such as the lactose permease of *E. coli* (West and Mitchell, 1973; Booth et al., 1979; Brooker et al., 1985) or the sucrose permease of *Saccharomyces* (Santos et al., 1982). Such a nearly stoichiometric ratio of H⁺ to sucrose transport further corroborates transport of sucrose by an active process, such as by a H⁺/sucrose *syn*-transporter.

In total, these results give evidence for a model of the assimilation of sucrose whereby sucrose is transported via a sucrose-inducible permease into the intracellular compartment, where hydrolysis by an intracellular maltase takes place.

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