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Catabolite inactivation of the yeast maltose transporter is due to proteolysis

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The maltose transport capacity of fermenting *Saccharomyces cerevisiae* rapidly decreases when protein synthesis is impaired. Using polyclonal antibodies against a recombinant maltose transporter-protein we measured the cellular content of the transporter along this inactivation process. Loss of transport capacity was paralleled by a decrease of cross-reacting material which suggests degradation of the transporter. We also show that in ammonium-starved cells the half-life of the maltose transporter is 1.3 h during catabolism of glucose and > 15 h during catabolism of ethanol.

Catabolite inactivation; Maltose transport; Transport inactivation; Transport turnover; *Saccharomyces cerevisiae*

1. INTRODUCTION

Most proteins in *Saccharomyces cerevisiae* are stable for long periods of time under different metabolic conditions [1–4]. Sugar transporters behave differently in this respect, in that a rapid and irreversible inactivation is observed upon arrest of protein synthesis [4–7]. This inactivation, that mainly affects the V_{max}, follows first-order kinetics and is an energy-dependent process stimulated by fermentable substrates [8–10]. The characteristics of the inactivation as well as preliminary results obtained with antibodies against an epitope attached to the maltose transporter [11] suggest that this inactivation might be due to proteolysis. This research attempts to check this possibility. For this purpose we have prepared polyclonal antibodies against a recombinant maltose transporter-protein and have measured the cellular content of this transporter along the inactivation process.

Two distinct maltose transporters have been identified in *Saccharomyces* that are expressed in maltose fermenting strains: one encoded by the Gene 1 of the *MAL* loci (*MAL11* through *MAL41* and *MAL61*) [12,13] and another one encoded by the *AGT1* gene [11]. These two transporters show great sequence similarity and are induced by maltose and repressed by glucose [11]. Both are proton symporters and are similarly inactivated upon protein synthesis impairment [11]. Actually, their only known difference concerns its specificity: while the MAL transporter only transports maltose and turanose, the AGT1 transporter transports isomaltose, α-methylglucoside, and maltotriose as well as maltose and turanose [11]. In this work two different strains have been used: a strain that only express the *MAL1* locus and that, therefore, allowed us to investigate the behaviour of a unique MAL transporter, and another strain that might express more than one *MAL* locus. The results obtained with both strains strongly indicate that the maltose transport inactivation is due to proteolysis.

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2. EXPERIMENTAL

The following strains of *S. cerevisiae* were used: MAL1-1[c]-D (*MAT* a *leu1 trp1 MAL13-1[c] [mal22 mal23]::TRP1*) [14], ATCC 42407 (*MAT* a *suc[-] MAL GAL*), and MAL1-1[c]-D-pRM1-1 (strain MAL1-1[c]-D transformed with plasmid pRM1-1). Plasmid pRM1-1 is a multicopy plasmid that carries the *MAL1* locus [14]. Cells were grown at 30°C in liquid medium containing 1% yeast extract/2% peptone/3 ppm antimycin A and 2% maltose or glucose as indicated. Cell growth was monitored by optical absorbance measurement at 640 nm. Inactivation of the maltose transport was achieved by suspending exponentially growing cells in an ammonium-free medium as described in [9] in the presence of 2% glucose or ethanol as indicated in each case.

Maltose transport was measured as in [15] and fermentation as in [16]. A fusion-gene was constructed containing 658 bp *Eco*RI-*Hin*cII fragment of the maltose transporter gene and the lacZ' gene as present in plasmid pEX-3 [17]. The resulting plasmid, pEXMPO3, was used to transform *E. coli* strain POP2136 (Boehringer-Mannheim Biochimica catalogue, 90/91). Isolation of inclusion bodies was performed essentially as described in [18]. To purify the fusion-protein, the inclusion bodies were successively treated with 1 ml of 5 M and 8 M urea in 0.1 M Tris-HCl, pH 8.5. Both batches were combined and dialyzed in the cold against 1 litre of 1.4 M NaCl/27 mM KCl/15 mM KH_2PO_4/81 mM Na_2HPO_4, pH 7.4 (PBS). The precipitated fusion-protein was recovered by centrifugation and suspended in 2 ml of PBS. A total of 10.6 mg protein was obtained with a estimated purity of about 80%. Antibodies were raised in rabbits by 3 injections of approx. 250 μg of the fusion-protein.

Extracts and crude and purified plasma membrane preparations were obtained as in [19]. When indicated the extracts were obtained in the presence of the following proteinases inhibitors: 5 mM EDTA,

Table I

Transport and fermentation rate by the used strains

Strain	Transport (mmol · g protein^{-1} · h^{-1})	Fermentation (mmol · g protein^{-1} · h^{-1})		
	Maltose	Maltose	Isomaltose	α-Methylglucoside
ATCC 42407	2.6	10.1	< 0.5	< 0.5
MAL1-1^c-D-pRM1-1	2.7	15.0	< 0.5	< 0.5

Cells were grown with 2% maltose, harvested during logarithmic growth, washed with water and suspended in fresh medium containing 2% of the indicated sugars in the presence of 3 ppm antimicyn A. Transport and fermentation were measured as indicated in section 2.

1 mM PMSF, 1 mM benzamidine, 1 mM *o*-phenanthroline, 1 mM iodoacetate, 10 μg leupeptin, 20 μg chymostatin, and 3 μg aprotinin. Proteins were separated by electrophoresis in 10% SDS-polyacrylamide and transferred to nitrocellulose BA-85 by semidry blotting using 25 mM Tris, 0.19 M glycine and 20% methanol as buffer [20]. Immunodetection was achieved using a 1/1000 dilution of the immune sera and alkaline phosphatase [21]. The cellular content of the maltose transporter was measured by immunoblotting increasing amounts (5–20 μg protein) of crude extracts obtained at different intervals after the start of the inactivation. In all cases representation of the intensity of the transporter bands plotted versus the total protein was a straight line (see below) whose slope was taken as proportional to the amount of transporter. Intensity of the bands was quantified by scanning densitometry with a SilverScan connected to a Macintosh IICi using the NIH image 1.42 software. Peak area was quantified in pixels after equalizing the acquired image.

Protein was determined after precipitation with trichloroacetic acid using the method of Lowry et al. [22].

3. RESULTS AND DISCUSSION

3.1. *Suitability of the used strain to estimate the cellular content of a unique maltose transporter*

The polyclonal antibodies raised against the maltose transporter fusion-protein (see section 2) will cross-react with the five maltose transporters (product of the Gene 1 of the five *MAL* loci) and, probably, with the isomaltose (AGT1) transporter with which the former show a great sequence similarity [11]. Therefore, to measure the cellular content of a unique maltose (MAL) transporter, a strain that only expresses one *MAL* locus and that does not express AGT1 is required. To meet this requirement we used a mutant (MAL1-1^c-D) defective in all maltose transporters [14]. As expected, this mutant was unable to grow on maltose as well as to transport and to ferment this sugar (results not shown). This mutant was transformed with a multicopy plasmid that carries the *MAL1* locus [14]. The resulting strain (MAL1-1^c-D-pRM1-1) grew, transported, and fermented maltose at a similar rate as a wild type strain (ATCC 42407) (Table I). Both the transformed strain, that only expresses one MAL transporter, as well as the wild type strain, that might express more than one MAL transporter, were unable to ferment two specific substrates of the isomaltose transporter, isomaltose and α-methylglucoside (Table I) [11]. These results indicate that the AGT1 gene is not expressed in these strains.

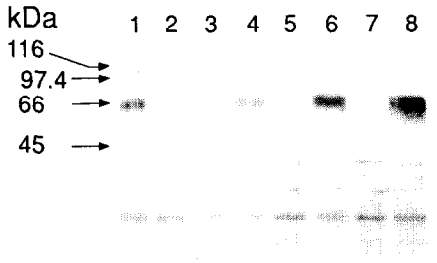

Fig. 1. Immunodetection of the maltose transporter in crude extracts and membrane preparations. Strains ATCC 42407 (lanes 1,2) and MAL1-1^c-D-pRM1-1 (lanes 3–8) were grown on glucose (lanes 2,3,5,7) or maltose (lanes 1,4,6,8). Aliquots containing 12 mg protein of crude extracts (lanes 1–4), crude membrane preparations (lanes 5,6), or plasma membrane preparations (lanes 7,8) were analyzed using the immune sera.

3.2. *Suitability of the antibodies to estimate the cellular content of the maltose transporter*

When extracts of maltose grown cells were subjected to SDS-gel electrophoresis and probed with the immune sera raised against the MAL transporter-lacZ′ fusion protein three bands of about 67, 62, and 57 kDa were detected whose intensity increased as the sample was enriched in the plasma membrane fraction (Fig. 1). These bands were not detected with the preimmune sera and appeared only in extracts of maltose grown cells (Fig. 2). These results show that the antibodies recognize a set of plasma membrane proteins, inducible by maltose, with a molecular size close to the 68.2 kDa predicted by the sequence of the maltose transporter gene [13,23]. Therefore we concluded that these bands correspond to the maltose transporter. The observed molecular size heterogeneity probably reflects post-translational modifications, i.e glycosylation [24]. However, the possibility that the two bands of lower molecular size are proteolytic products of the transporter cannot be excluded even though these bands appeared also in samples obtained in the presence of a cocktail of proteinase inhibitors. Additional bands that appeared

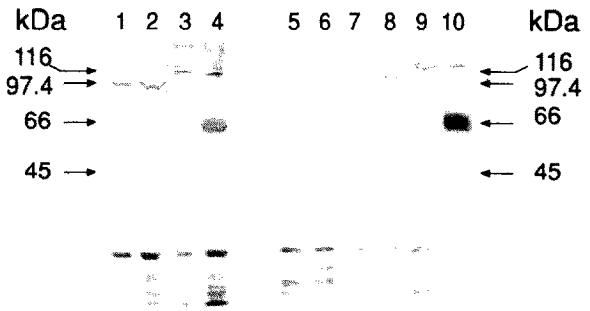

Fig. 2. Detection of the maltose transporter with the preimmune and the immune sera. Strains ATCC 42407 (lanes 1–4) and MAL1–1^c-D-pRM1–1 (lanes 5–10) were grown on glucose (lanes 1,3,5,7,9), or maltose (lane 2,4,6,8,10). Aliquots of crude extracts containing 15 μg protein were analyzed using the secondary antibody (lanes 5,6), the secondary antibody plus the preimmune sera (lanes 1,2,7,8), or the secondary antibody plus immune sera (lanes 3,4,9,10).

in the immunoblots were not related with the transporter since they were present in samples of non-induced cells and were recognized by the preimmune sera as well as by the secondary antibody (Fig. 2). Increasing amounts of crude extracts from maltose grown cells resulted in a proportional increase in the intensity of the three bands of the transporter in the immunoblot (Fig. 3). This result (see section 2) as well as those reported above show that the antibodies are suitable for the estimation of the cellular content of the maltose transporter.

3.3. *Inactivation is accompanied by a decrease in the cellular content of the transporter*

Maltose transporter is irreversibly inactivated in fermenting yeast when its synthesis is impaired [4,8]. We have measured the cellular content of this transporter during the inactivation process using the strain that only express the *MAL1* locus. To this end increasing amounts of crude extracts of non-inactivated cells (lanes 1–4, Fig. 3A) and of 4 h-inactivated cells (lanes 5–8, Fig. 3A) were analyzed. The results showed that the content of the transporter, measured by the slope of the straight line obtained plotting the intensity of the bands versus the protein content (see section 2), decreased by > 90% during 4 h under the inactivating conditions (Fig. 3B). However, during this period no substantial differences were observed in the case of a protein unrelated with the maltose transporter (Fig. 3B, internal control). A detailed study showed that the decrease of the transporter content followed first-order kinetics and run in parallel to the transport inactivation (Fig. 4). From data shown in Fig. 4 it could be calculated that the half-life of this transporter in ammonium starved cells is about 1.3 h during catabolism of glucose and > 15 h during catabolism of ethanol. Similar results were found using the strain that might express more than one *MAL* locus (results not shown).

The results presented in this work strongly indicate that the catabolite inactivation of the maltose transporter is due to proteolysis. It is well known that proteolysis is also involved in catabolite inactivation of a number of cytoplasmic proteins [25], i.e. malate dehydrogenase [26], aminopeptidase I [27], fructose-1,6-bisphosphatase [28], and phosphoenolpyruvate carboxykinase [29]. These facts suggest that proteolysis is a general mechanism of catabolite inactivation of both cytoplasmic and plasma membrane proteins. Degradation of most plasma membrane proteins occurs by endocytosis [30]. However, some of these proteins might be also degraded in the ubiquitine pathway. This is indicated by the fact that, in mammalian cells, a number of plasma membrane proteins are specifically conjugated with

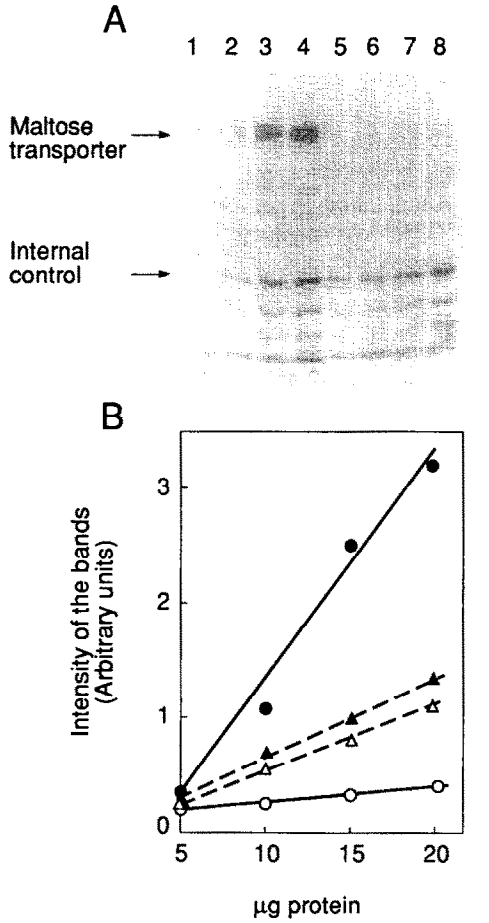

Fig. 3. Decrease in the cellular content of the maltose transporter during the inactivation process. (A) MAL1–1^c-pRM1–1 cells exponentially growing on maltose were harvested, washed and suspended in 3-times the initial volume of the ammonium-free medium in the presence of 2% glucose and 3 ppm antimycin A. Crude extracts were obtained immediately (lanes 1–4) or after 4 h incubation at 30°C (lanes 5–8). Samples containing 5 μg protein (lane 1,5), 10 μg (lanes 2,6), 15 μg (lanes 3,7), and 20 μg (lanes 4,8) were analyzed using the immune sera. (B) Intensity of the (●), (○) maltose transporter bands and of the internal control protein band (▲, △) were measured. Dark symbols correspond to the extract obtained immediately and open symbols to the extract obtained after 4 h of incubation in the inactivating conditions.

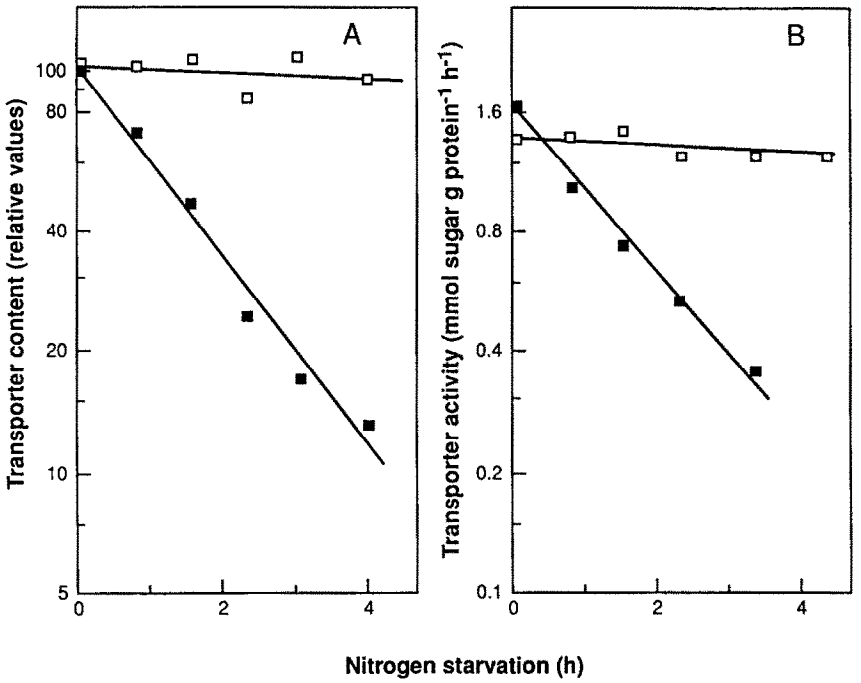

Fig. 4. Effect of the inactivating conditions on the content and activity of the maltose transporter. MAL1–1^c-pRM1–1 cells were harvested during exponential growth on maltose, washed and suspended in 3-times the initial volume of the media specified below. After incubation at 30°C for the indicated times, cells were harvested, washed and assayed for (A) maltose transporter content and (B) maltose transport activity using crude extracts. Transfer media: ammonium-free medium containing 2% glucose (■) or 2% ethanol (□).

ubiquitine [31]. It would be interesting to establish which one of these two pathways is involved in catabolite inactivation of the maltose transporter.

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