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IDENTIFICATION OF THE MALTOSE TRANSPORT PROTEIN OF *SACCHAROMYCES CEREVISIAE*

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Identification of the maltose transport protein of *Saccharomyces cerevisiae* was attempted by searching for maltose-inducible proteins in isolated plasma membranes. Membranes from maltose-grown cells contained two proteins that were absent in glucose-grown cells. The proteins differed in size, but peptide sequence analysis indicated a high degree of homology. The amino-terminal and internal sequences of the largest protein, with an apparent molecular mass of 64 kDa, were determined. These sequences were identical to predicted amino acid sequences in the MAL61 gene product. It is concluded that this protein is the inducible maltose permease of *Saccharomyces cerevisiae*.

Maltose utilization by *Saccharomyces cerevisiae* is an inducible process, requiring the presence of two maltose-specific proteins, viz. a maltose permease and an intracellular α-glucosidase [1]. Both proteins are encoded by genes that are located in a MAL locus together with a third gene coding for a positive-regulatory protein, probably involved in induction of transcription [2]. Five different MAL loci have been described (MAL1-MAL4, MAL6), some of which can be cryptic depending on the strain [3,4]. In any of these loci gene 1 is supposed to encode the maltose permease, gene 2 the maltase and gene 3 the transacting regulatory protein [2].

Sequence analysis of the MAL61 gene (identical to the MAL6T gene of *S. carlsbergensis* [5]) has revealed an open reading frame of 1842 bp, coding for a gene product consisting of 614

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amino acids [6]. The predicted protein has a strongly hydrophobic character and is most likely an integral membrane protein, having 9-12 putative membrane-spanning segments. It has a predicted sequence homology to various members of the family of sugar transporters, including yeast and bacterial sugar carriers [7-9] as well as the human GLUT glucose transporters [10,11]. The MAL61 gene can restore high-affinity maltose transport in a maltose permease-deficient strain. Therefore it is highly likely that the MAL61 gene product indeed represents the inducible maltose transporter [12].

Many genes coding for putative sugar transporters in yeast have been described. Little is known however about the proteins themselves. The aim of the work presented in this paper is to identify proteins in the plasma membrane of *S. cerevisiae* involved in maltose transport.

MATERIALS AND METHODS

Saccharomyces cerevisiae CBS 8066 was obtained from the Centraal Bureau voor Schimmelcultures (Delft, The Netherlands) and was maintained on malt agar slopes at 4 °C. The organism was grown aerobically in chemostat culture under maltose or glucose limitation as described before [13].

Plasma membranes were isolated according to the procedure described by Van Leeuwen *et al.* [13].

SDS-PAGE was performed according to Laemmli [14] using 10 % gels. Gels were prepared 1 day in advance in case amino acid sequencing was performed on material isolated from the gel after SDS-PAGE. Polypeptides were visualized by Coomassie Brilliant Blue or silver staining [15].

Amino acid sequencing of the amino-terminal part of polypeptides was carried out on material blotted onto polyvinylidene difluoride membrane, essentially as described by Matsudaira [16]. Blotting was carried out in 10 mM 3-[cyclohexylamino]-1-propanesulfonic acid, 10 % methanol (pH 11.0) for 2 h at about 1 mA/cm^2 using an LKB 2117 Multiphor II blotting apparatus [17].

In-gel proteolysis, using Endoproteinase Lys-C as the proteolytic enzyme, was performed essentially as described by Rosenfeld *et al.* [18]. Proteolysis was carried out for 20 h at 30°C with 9 mU Endoproteinase Lys-C per gel slice. After concentrating the collected extracts, peptides were separated on a Knauer 100x4 mm column, filled with 100RP18 endcapped material (Merck) at a flow rate of 0.6 ml/min using a one-hour linear gradient of 0-75 % acetonitrile and 0.1 to 0.08 % trifluoroacetic acid. The absorbance of the eluate was monitored at 220 nm and the material contained in the A_{220} peaks was collected and freeze-dried.

Amino acid sequencing was carried out with an Applied Biosystems Model 475A pulse liquid sequencer, connected on line with a Model 120A PTH-amino acid analyzer.

α-Glucosidase from yeast and Endoproteinase Lys-C were obtained from Boehringer (Mannheim).

RESULTS

In order to identify the maltose permease of *Saccharomyces cerevisiae*, plasma membranes from induced and non-induced cells were analyzed by SDS-PAGE. Figure 1A shows that the electrophoretic patterns of membrane proteins derived from maltose- or glucose grown cells were highly similar. Three differences were noted, viz. a band with an apparent molecular

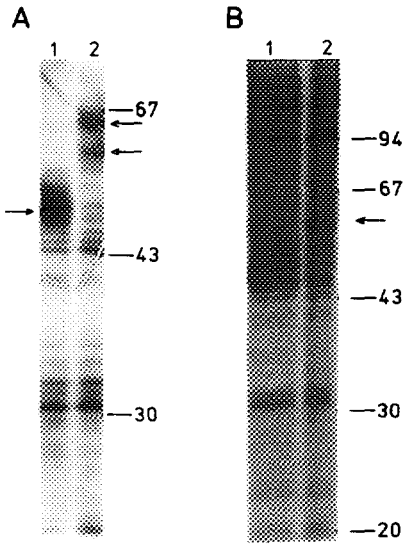

Figure 1.
SDS-PAGE analysis of plasma membranes isolated from cells grown in maltose and glucose limited chemostats. Proteins were separated on 10 % gels and visualized by silver staining (A) or Coomassie staining (B). Different membrane preparations were used for the gels shown in A and B. Lanes 1: membranes from glucose grown cells. Lanes 2: membranes from maltose-grown cells. The arrows indicate bands only present in maltose or glucose grown cells.

mass of about 48 kDa present only in plasma membranes of glucose-grown cells and 2 bands only present in plasma membranes of maltose-grown cells. The apparent molecular masses of these maltose-induced bands were found to be 64 and 59 kDa. Both bands had a diffuse appearance, suggesting the presence of a highly hydrophobic [19] or glycosylated [20] polypeptide. They did not comigrate with purified α-glucosidase from yeast, indicating that the isolated membranes did not contain significant amounts of this hydrolyzing cytoplasmic enzyme, as was concluded before [13]. The relative amounts of protein contained in the two bands varied from batch to batch. In some membrane batches the larger polypeptide was predominant whereas in others the opposite occurred (compare Figs. 1A and 1B).

To investigate the identity of both proteins, amino acid sequencing of the amino-terminus was performed. The results for the 64 kDa polypeptide, as presented in Table 1, show that its sequence corresponds to the one predicted for the amino-terminus of the MAL61 (MAL6T) gene product, i.e. MKGLSSLIN [5,6]. Amino terminal sequencing of the 59 kDa polypeptide was not successful, probably because the amino-terminus was blocked.

In order to further establish the identity of both polypeptides it was attempted to identify "internal" sequences by analysis of proteolytically-generated peptides. Therefore, in-gel proteolysis [18] was performed on both bands using a proteolytic enzyme (endoproteinase Lys-C) that cuts at the carboxyl side of most lysine residues. Separation of the peptides on a RP18

Table 1
Amino terminal sequence determination of the 64 kDa polypeptide. The indication ? means that, in four different preparations, no reliable data were obtained at the corresponding position of the polypeptide chain.

Sequencing step	amino acid
1	?
2	Lys
3	Gly
4	Leu
5	Ser
6	?
7	Leu
8	Ile
9	Asn

column resulted in chromatograms as shown in Fig. 2. About 30-40 A_{220} peaks were observed for the material originating from both bands. Moreover, both chromatograms were highly similar, suggesting that both polypeptides shared strong similarities. Amino acid sequencing was performed on a randomly chosen peak from the chromatograms, eluting at 29 min for material

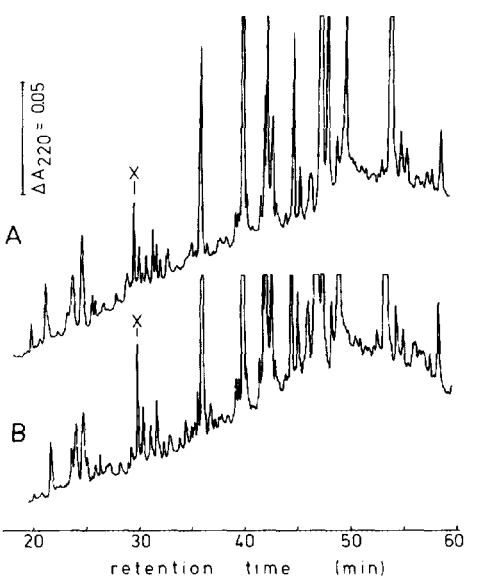

Figure 2.
HPLC analysis of the peptides generated from the 64 and 59 kDa polypeptides by endoproteinase Lys-C treatment. Chromatograms represent the A_{220} absorbing material in the eluate of a RP18 column, originating from the 64 kDa (A) or the 59 kDa (B) polypeptides. In the first 18 min of elution only some broad peaks appeared that were moreover identical in both chromatograms. This part is not shown.

Table 2
Amino acid sequence determination of isolated peptides from the 59 and 64 kDa polypeptide. Proteolysis and peptide separation by HPLC was performed as described in the Materials and Methods section. Material contained in the A_{220} peaks of Fig.2, marked with an X, was used for analysis and is indicated as 64-X and 59-X for the material derived from the 64 and 59 kDa polypeptides, respectively. n.d.: not determined.

Sequencing Step	Amino Acid	
	64-X	59-X
1	Ser + Ala	Ser + Ala
2	Thr + Ala	Thr + Ala
3	Lys + Ala	Lys + Ala
4	Val + Ala	Val + Ala
5	Asp + Glu	Asp + Glu
6	Pro + Ile	Pro + Ile
7	Phe + Asn	Phe + Asn
8	Ala + Val	Ala + Val
9	Ala + Lys	Ala + Lys
10	n.d.	Ala + Asp
11	n.d.	Pro
12	n.d.	Lys

derived from both polypeptides (indicated in Fig. 2 with an X). The results, presented in Table 2, reveal that two amino acids were measured per sequencing step, indicating that both HPLC peaks contained two peptides. Moreover, the material derived from the 59 and 64 kDa bands were exactly identical. Analysis of the predicted MAL61 gene product showed that cleavage of this protein by Endoproteinase Lys-C could generate two peptides with sequences STKVDPFAAAK and AAAAEINVKDPK, which when sequenced together would give results exactly identical to the ones given in Table 2. This strongly suggests that both polypeptides contain these sequences, that are located in the MAL61 gene product close to the carboxy terminus at residues 572-582 and 583-594.

DISCUSSION

The maltose permease, as deduced from the sequence of the MAL61 gene, is predicted to be a hydrophobic membrane protein with a molecular mass of 68 kDa [5,6]. The results presented in this paper show that plasma membranes from maltose-grown *Saccharomyces cerevisiae* contain a maltose-inducible polypeptide with an apparent molecular mass of 64 kDa, that contains an amino-terminal amino acid sequence identical to the one predicted on basis of the DNA sequence. Moreover, peptides isolated from this protein contain sequences that were also found at the carboxy-terminus of the MAL61 protein. Therefore, it is concluded that the

64 kDa polypeptide is identical to the MAL61 gene product. The fact that this protein migrates in SDS-PAGE, as a rather diffuse band, at a molecular mass smaller than that calculated for the actual protein has also been observed for other membrane spanning proteins [19-21], and is considered to be characteristic for highly hydrophobic [22] and/or glycosylated proteins.

Interestingly, this 64 kDa protein contains an amino-terminus identical to that of the predicted MAL61 gene product. Apparently, no post-translational proteolytic processing had occurred at this site, indicating that this protein does not contain a cleavable signal sequence, as observed with many proteins that are targeted towards the cell surface [23]. The absence of a cleavage site at the amino-terminus might be a common feature of transport proteins in yeast since a similar conclusion was reached for the uracil permease of *Saccharomyces cerevisiae* [21].

The data presented in Table 2 indicate that peptides generated from the second maltose-induced band, running at 59 kDa, are identical to that of the 64 kDa protein. Taking into consideration that the peptide "fingerprints" as given in Fig. 2 were highly similar for both polypeptides, it can be concluded that both proteins are very homologous. Recently, it was reported that antibodies against a Mal11-lacZ' fusion protein recognized 3 proteins in the membrane of maltose-grown *S. cerevisiae* [24] ranging from 57-67 kDa. Moreover, a double band was observed with antibodies against the putative Hxt2 glucose transport protein [25]. Thus, size heterogeneity might be a rather general phenomenon with sugar transport proteins in yeast, and could be due to posttranslational modifications [24] or the strong hydrophobicity of these proteins resulting in secondary structures even in the presence of SDS [25].

This paper is the first report on direct identification of a protein presumably involved in maltose transport in yeast. Remarkably, this protein can be detected in a wild-type yeast and appears to be one of the most abundant proteins. The high expression of the maltose permease may be due to the cultivation conditions, i.e. a maltose-limited continuous culture [26]. Obviously, the presence of high quantities of maltose transporter in *S. cerevisiae* will facilitate further work on the isolation and characterization of this sugar carrier.

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