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MAL11 and *MAL61* Encode the Inducible High-Affinity Maltose Transporter of *Saccharomyces cerevisiae*

QI CHENG AND CORINNE A. MICHELS*

Department of Biology, Queens College and the Graduate School of CUNY, Flushing, New York 11367

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We have investigated the transport of maltose in a genetically defined maltose-fermenting strain of *Saccharomyces cerevisiae* carrying the *MAL1* locus. Two kinetically different systems were identified: a high-affinity transporter with a K_m of 4 mM and a low-affinity transporter with a K_m of 70 to 80 mM. The high-affinity maltose transporter is maltose inducible and is encoded by the *MAL11* (and/or *MAL61*) gene of the *MAL1* (and/or *MAL6*) locus. The low-affinity maltose transporter is expressed constitutively and is not related to *MAL11* and/or *MAL61*. Both maltose transporters are subject to glucose-induced inactivation.

Maltose fermentation in *Saccharomyces* yeast strains requires the presence of one or more of five unlinked *MAL* loci, *MAL1*, *MAL2*, *MAL3*, *MAL4*, and *MAL6*, which exhibit extensive sequence and functional homology to each other (1, 5, 7, 12, 17, 18). Each locus is a complex locus consisting of three genes essential for maltose fermentation (5, 7, 17, 18). GENE 1 has been suggested to encode maltose permease based on genetic analysis of mutations in *MAL61*, the GENE 1 located at the *MAL6* locus (4). This suggestion is strongly supported by sequence analysis of the *MAL61* gene, which indicates that the product of *MAL61* is an integral membrane protein and belongs to a family of sugar transporters including the *GLUT1* human glucose transporter (8). GENE 2 encodes maltase and GENE 3 encodes the *MAL* activator, a positive *trans*-acting factor regulating the transcription of GENE 1 and GENE 2 (3, 10).

Early biochemical studies in baker's *Saccharomyces* strains revealed one maltose transporter with a K_m of 4 mM (13). Later studies on genetically undefined strains of *Saccharomyces cerevisiae* indicated the apparent presence of at least two forms of the maltose transporter: a low-affinity transporter with a K_m of 70 mM, and a high-affinity transporter with a K_m of 4 mM (2). Given the polygenic nature of the *MAL* loci, we wished to explore the possibility that these two kinetically distinct transporters could be the products of different *MAL* loci or that at least one of them could be the product of GENE 1. Therefore, the kinetics of maltose uptake was studied in genetically well-characterized strains carrying only a single copy of either the *MAL11* or the *MAL61* gene, GENE 1 of the *MAL1* and *MAL6* loci, respectively.

The method used to measure the uptake of radiolabeled maltose was as described in Kruckeberg and Bisson (16) with slight modifications (22). Yeast strains were grown on YP medium (1% yeast extract, 2% peptone; Difco Laboratories) supplemented with the appropriate sugar as indicated. A 0.1 M tartaric acid-Tris (pH 4.2) buffer was used to resuspend the cells for assaying maltose uptake. We determined the initial velocity of maltose uptake over a range of substrate concentrations from 0.2 to 200 mM and plotted the data as velocity (V) versus velocity/substrate concentrations (V/S), using the Eadie-Hofstee transformation (11, 15).

The kinetics of maltose uptake were studied in a maltose-fermenting *S. cerevisiae* strain, 600-1B (*MAT***a** *SUC1 MAL13 MAL12 MAL11 ura3-52 leu2-3,112*). This strain contains only the *MAL1* locus and no others and thus contains a single copy of the *MAL11* gene and no other *MAL11* gene homologs (5). Figure 1A shows the Eadie-Hofstee analysis of maltose uptake by strain 600-1B grown under inducing growth conditions (in the presence of 2% maltose). Uptake is clearly biphasic, implying the presence of at least two forms of maltose transporter in this strain. One is a high-affinity transporter with a K_m of 4 mM, and the other is a low-affinity transporter with a K_m of 70 to 80 mM. Following growth of strain 600-1B under noninducing growth conditions (in the absence of maltose but with 2% galactose as the sugar source), the high-affinity transport activity decreases to an undetectable level and the low-affinity transport activity remains unaffected (Fig. 1B), indicating that the high-affinity transporter is maltose inducible but that the low-affinity transporter is constitutively expressed. The high specific activity of radiolabeled maltose needed to detect this low-affinity system tends to give high background levels and variable results. Nevertheless, this apparent low-affinity transport activity is reproducibly present.

Strain 100-1A (*MAT***a** *SUC1 MAL13 MAL12 mal11*Δ*::URA3 leu2-3,112*) carries a deletion/disruption of the *MAL11* gene at the *MAL1* locus (6). It does not grow on 2% (wt/vol) maltose YP medium and grows poorly on 10% (wt/vol) maltose YP medium. Eadie-Hofstee analysis of strain 100-1A grown on YP medium supplemented with both 2% maltose and 2% galactose (inducing conditions) is shown in Fig. 2A. The high-affinity transport activity is totally absent, but the low-affinity transport activity remains and appears to be unaffected. This clearly demonstrates that the high-affinity transporter alone is encoded by the *MAL11* gene.

Strain 100-1A was transformed with a CEN plasmid carrying the *MAL61* gene, plasmid pMAL61. Both the ability to grow normally on 2% (wt/vol) maltose YP medium and the ability to ferment maltose are restored in this transformant, 100-1A[pMAL61]. Transforming strain 100-1A with the vector plasmid alone does not restore these activities. Figure 2B shows an Eadie-Hofstee analysis of strain 100-1A[pMAL61] grown on YP medium supplemented with 2% maltose. The high-affinity transport activity is fully restored. When 100-1A[pMAL61] cells are grown on YP medium supplemented with both 2% maltose and 2% galactose, the same biphasic Eadie-Hofstee pattern is obtained (data not shown). These

* Corresponding author.

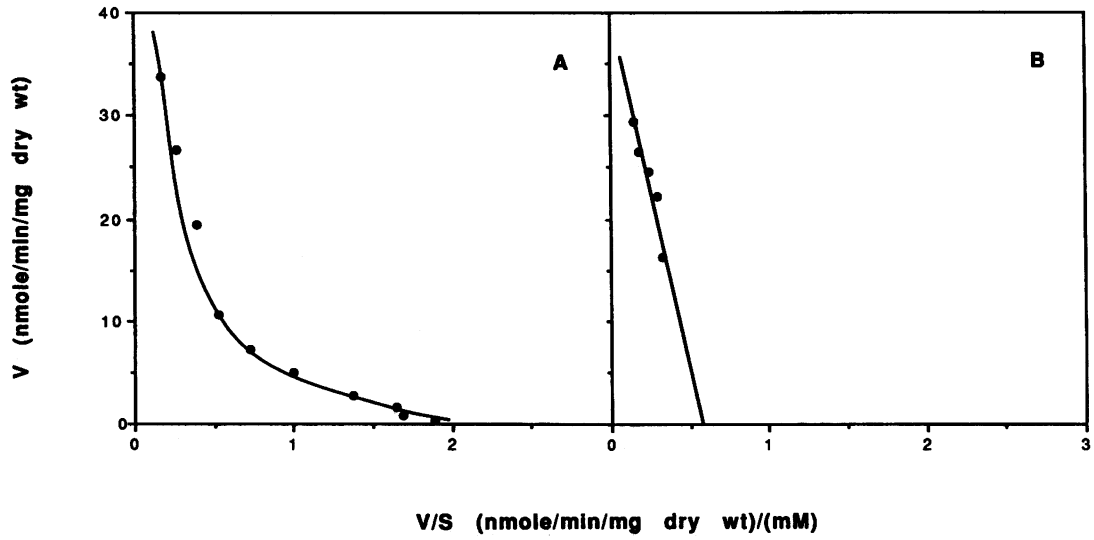

FIG. 1. Eadie-Hofstee plot of maltose uptake in the *MAL1* strain 600-1B. Strain 600-1B was grown in YP medium supplemented with (A) 2% (wt/vol) maltose and (B) 2% (wt/vol) galactose.

results clearly demonstrate that the *MAL11* and/or *MAL61* genes encode the high-affinity maltose transporter.

Our studies also show that both the high- and low-affinity maltose transporters are sensitive to glucose-induced inactivation. Strain 600-1B was grown to mid- to late-log phase in 2% maltose YP medium. The cells were harvested by filtration and transferred to fresh YP media containing 2% glucose or 2% glucose plus 2% maltose. After 90 min the cells were harvested and kinetic studies of maltose uptake were performed. The Eadie-Hofstee analysis results are shown in Fig. 3. Both the high- and low-affinity maltose transport activities are found to be subject to glucose-induced inactivation (Fig. 3A). At 90 min after the addition of glucose, an approximately 30% decrease in the low-affinity and 60 to 70% decrease in the high-affinity maltose transport activity is observed. The glucose-induced inactivation of maltose transport occurs even in the presence of maltose (Fig. 3B).

The kinetic studies in these genetically well-defined strains carrying a single copy of either *MAL11* or *MAL61* clearly demonstrate that *MAL11* and *MAL61* encode the maltose-inducible, high-affinity maltose transporter. A second maltose permease is present in this strain, a constitutive low-affinity transporter. This low-affinity transporter is not encoded by *MAL11* or *MAL61*. All five *MAL* loci in *S. cerevisiae* exhibit extensive sequence and functional homology (5, 7, 12, 17, 18). Thus, we suggest that GENE 1 of each *MAL* locus encodes the high-affinity maltose transporter.

The maltose transport system of *S. cerevisiae* has been described as an inducible active proton symport system (9, 14, 21, 22). The inducible high-affinity maltose transporter identified in this report as being encoded by *MAL11* and

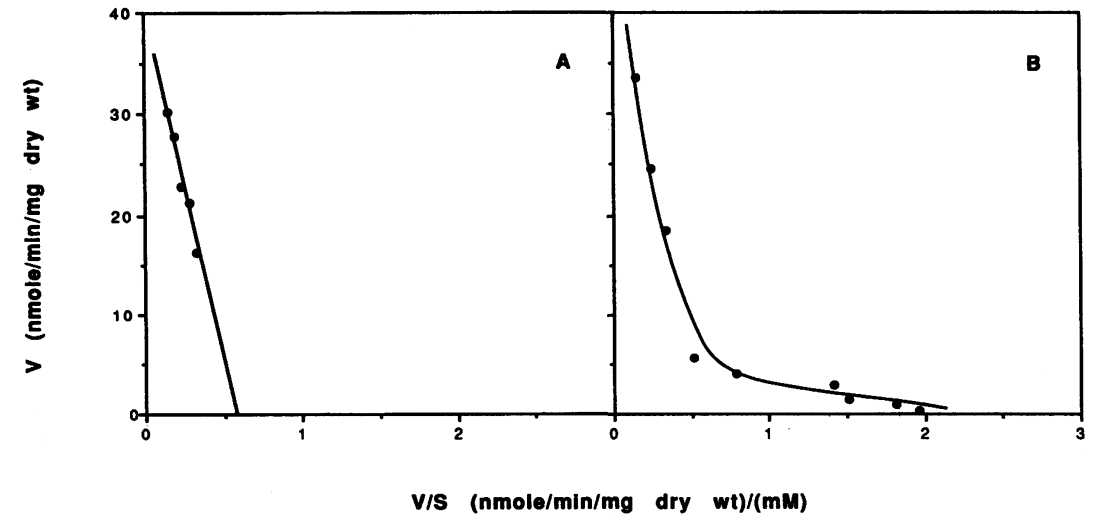

FIG. 2. Eadie-Hofstee plot of maltose uptake in the *MAL11* deletion/disruption strain 100-1A and 100-1A transformed with the plasmid-borne *MAL61* gene. (A) Strain 100-1A grown on YP medium supplemented with 2% (wt/vol) galactose plus 2% (wt/vol) maltose. (B) Strain 100-1A[pMAL61] grown on YP medium supplemented with 2% (wt/vol) maltose.

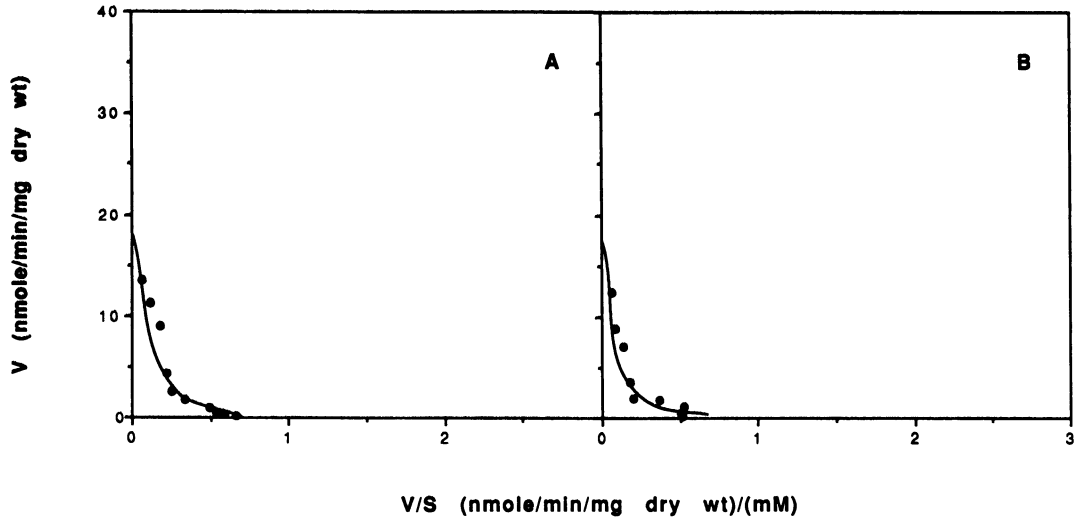

FIG. 3. Pattern of maltose uptake in *MAL1* strain 600-1B following glucose-induced inactivation. Strain 600-1B was grown to mid- to late-log phase on YP medium supplemented with 2% (wt/vol) maltose, harvested by filtration, and resuspended at time zero in YP medium supplemented with (A) 2% (wt/vol) glucose or (B) 2% (wt/vol) maltose plus 2% (wt/vol) glucose. Cells were harvested after 90 min, at which time an Eadie-Hofstee analysis of maltose transport was carried out.

MAL61 is most likely the same active maltose/proton symporter described by Serrano (22). The analysis done by Serrano (22) was carried out on cells grown under maltose-induced conditions and transport was assayed by using low 'sugar concentrations. Based on our results, almost all of the transport measured by Serrano (22) must have occurred by means of the high-affinity transporter described here. Consistent with this, we find that at pH 7.0 the maltose transport activity of induced cells is dramatically decreased (data not shown).

We have not investigated the mechanism of transport of the low-affinity transport system nor have we defined the gene(s) encoding this system. In the absence of such studies, any conclusions regarding the low-affinity transport system must be considered tentative. Nevertheless, it is possible that this low-affinity maltose transport is mediated by a common α-glucoside transport system which is less specific for maltose and capable of transporting several different α-glucosides such as melezitose, α-methyglucoside, and turanose in addition to maltose or by the combined activity of several different systems capable of transporting maltose but with reduced affinity (9, 19, 20). Only identification of the gene(s) encoding the low-affinity transporter can resolve these issues.

Our results (Fig. 3) and earlier work (2, 13) reveal that both the high- and low-affinity maltose transport systems are sensitive to glucose-induced inactivation. The mechanism of glucose-induced inactivation of maltose permease has not been determined. Work is in progress in our laboratory to fully characterize this inactivation process in genetically defined strains.

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