

# The Maltodextrin System of *Escherichia coli*: Metabolism and Transport

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The maltose/maltodextrin regulon of *Escherichia coli* consists of 10 genes which encode a binding protein-dependent ABC transporter and four enzymes acting on maltodextrins. All *mal* genes are controlled by MalT, a transcriptional activator that is exclusively activated by maltotriose. By the action of amyloamylase, we prepared uniformly labeled [<sup>14</sup>C]maltodextrins from maltose up to maltoheptaose with identical specific radioactivities with respect to their glucosyl residues, which made it possible to quantitatively follow the rate of transport for each maltodextrin. Isogenic *malQ* mutants lacking maltodextrin phosphorylase (MalP) or maltodextrin glucosidase (MalZ) or both were constructed. The resulting *in vivo* pattern of maltodextrin metabolism was determined by analyzing accumulated [<sup>14</sup>C]maltodextrins. MalP<sup>-</sup> MalZ<sup>+</sup> strains degraded all dextrins to maltose, whereas MalP<sup>+</sup> MalZ<sup>-</sup> strains degraded them to maltotriose. The labeled dextrins were used to measure the rate of transport in the absence of cytoplasmic metabolism. Irrespective of the length of the dextrin, the rates of transport at a submicromolar concentration were similar for the maltodextrins when the rate was calculated per glucosyl residue, suggesting a novel mode for substrate translocation. Strains lacking MalQ and maltose transacetylase were tested for their ability to accumulate maltose. At 1.8 nM external maltose, the ratio of internal to external maltose concentration under equilibrium conditions reached 10<sup>6</sup> to 1 but declined at higher external maltose concentrations. The maximal internal level of maltose at increasing external maltose concentrations was around 100 mM. A strain lacking *malQ*, *malP*, and *malZ* as well as glycogen synthesis and in which maltodextrins are not chemically altered could be induced by external maltose as well as by all other maltodextrins, demonstrating the role of transport per se for induction.

The *Escherichia coli* maltodextrin system has become a paradigm for the understanding of a complex sugar-utilizing system in bacteria (5, 55). The regulon is controlled by MalT, the central activator of the system, and consists of 10 coordinately regulated genes that are geared for the utilization of maltose and maltodextrins. Five genes encode a high-affinity and binding protein-dependent ABC transporter comprising LamB, a specific outer membrane diffusion porin, the λ receptor or maltoporin (27, 51); a high-affinity maltose/maltodextrin binding protein in the periplasm, MalE, the major recognition site of the system (32); two tightly membrane-bound permease subunits, MalF and MalG (13, 28); and MalK, the ATP-hydrolyzing subunit of the transporter (located at the cytoplasmic side of the transporter) providing the necessary energy for active transport (1, 40, 58). Structural information on three components of the transporter is available: the structures of the λ receptor (53), MalE (57, 59), and MalK (11) have been solved. The functional interaction between the subunits (41) has been studied, and models of active transport based on the biochemical characteristics of the transport components have been proposed previously (12, 14). The specificity of the intact transporter is limited by the specificity of the periplasmic maltose binding protein (MBP [= MalE]). MBP binds with high-affinity maltose (60) and maltodextrins composed of linearly α(1-4)-glucosidically linked glucose polymers, including amylose and starch (24), as well as α(1-4)-glucosidically linked cyclodextrins (60). There are two modes of ligand binding by MBP. High affinity requires a free reducing end of the malto-

dextrin molecule, while binding to extended polysaccharide chains is of lower affinity (29). Maltodextrins whose reducing end is blocked by residues larger than a methyl group (21), even though bound well by the binding protein, cannot be transported, indicating a polarity in the mechanism in which linear dextrins are channeled into the transporter (25, 52, 62). While maltose transport has been studied to some extent (21, 23, 38, 60), the transport of maltodextrins is less well understood. Ferenci (22) has synthesized labeled maltodextrin by the transfer of unlabeled cyclic maltodextrins as the dextrinyl donor and [<sup>14</sup>C]maltose as the acceptor, resulting in linear, labeled dextrins of different chain lengths. With these dextrins in transport assays, it was established that there is a size limit for the maltose transporter that occurs at around 8 to 10 glucosyl residues. However, from the technique of synthesis, the specific radioactivity of the different dextrins remained undefined, not allowing a quantitative transport analysis between the different linear maltodextrins (22). From studies using unlabeled maltodextrins of different chain lengths as a carbon source, it has been concluded that maltodextrins of up to seven glucosyl units exhibit about the same growth rate. Longer dextrins resulted in an increasingly reduced growth rate, again indicating that these dextrins were no longer transported (63). Traditionally, binding protein-dependent transport systems are regarded as unidirectional, where the transport step is coupled to the hydrolysis of ATP (4). In the maltose system, the exit of accumulated maltose from mutant cells unable to metabolize it can be observed. This exit, unlike the entry by the transporter, is blocked by uncouplers of oxidative phosphorylation. Thus, the exit appears to be independent of the entry reaction and catalyzed by a different system (23).

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The metabolism of maltodextrins once inside the cell is achieved by three enzymes whose encoding genes are controlled by MalT. MalQ is an amyloamylase (39, 47, 65, 66), an obligatory glucanotransferase producing from any linear maltodextrin a mixture of maltodextrins plus glucose (44). Mutants lacking amyloamylase can no longer grow on maltose but can grow on maltodextrins with a chain length of four or more glucosyl residues (55). This is due to the second enzyme, maltodextrin phosphorylase encoded by *malP*, which removes by phosphorolysis the nonreducing glucosyl residue to yield  $\alpha$ -glucose-1-phosphate (glucose-1-P) (56, 64). Maltodextrin phosphorylase recognizes maltodextrins at a minimum of four glucosyl residues. Mutants lacking this enzyme still grow on maltose but then accumulate large amounts of linear dextrans due to the action of amyloamylase. Under these conditions, the cells become large and stain blue in the presence of iodine. Thus, metabolism of maltose and maltodextrins in a wild-type strain leads to the production of glucose and glucose-1-P, which enter glycolysis after the transformation to glucose-6-P by glucokinase and phosphoglucosyltransferase, respectively.

The third enzyme, maltodextrin glucosidase encoded by *malZ* (52), is not essential for maltose or maltodextrin utilization. Its activity can be described as an enzyme that removes glucose residues from the reducing end of maltodextrins more than two glucose units long (61). Maltose itself is not a substrate of the enzyme. The enzyme also hydrolyzes  $\gamma$ -cyclodextrin very effectively (45), even though it is unclear how this sugar should arise inside the cell. *E. coli* does not transport  $\gamma$ -cyclodextrin. Mutants lacking *malZ* have no maltose-negative phenotype, but they cannot grow on maltose in the absence of phosphoglucosyltransferase that transforms glucose-1-P reversibly into glucose-6-P. Mutants lacking this enzyme but containing MalZ grow normally on maltose (45). The last MalT-dependent enzyme is a periplasmic amylase, MalS (54). This enzyme cleaves maltodextrins more than two glucose units long as well as cyclodextrins (26). The preferred dextrin released from long dextrans is maltohexaose (27). The role of this enzyme is unclear. Mutants lacking it show only a slight disadvantage in growth on long maltodextrins.

The regulation of the maltose system is specifically controlled by MalT acting as an inducer-dependent *mal* gene activator (50). Even though in vivo, the maltose system is induced by all maltodextrins in the medium serving as a carbon source, in vitro, MalT is activated only to stimulate *malP* transcription by maltotriose (49). Thus, it is assumed that the exclusive inducer maltotriose is formed internally by the metabolism of maltose and maltodextrins.

A particular regulatory feature of the maltose system is its constitutivity, as observed in *malQ* mutants. This is due to endogenously produced maltotriose that is derived from the degradation of glycogen. Obviously, degradation of glycogen also occurs in the wild type, but in this case, the maltodextrins formed from glycogen are effectively funneled into glucose and glucose-1-P, thus keeping the inducer concentration low (15). The maltose system is subjected to catabolite repression as well as inducer exclusion. Thus, the expression of *malT* as well as some of the MalT-dependent genes (i.e., the ones encoding transport proteins) is controlled by the cyclic AMP/catabolite gene activator protein system (10), which in turn is controlled by the PtsG-mediated transport of glucose (46). EIIA<sup>Glc</sup>-me-

TABLE 1. Strains and plasmids used in this study

Strain or plasmid	Known genotype	Source or reference
<i>E. coli</i> K-12 strains		
CB47	MC4100 <i>malQ maa</i>	8
HS3166	MC4100 <i>malQ am20</i>	H. Shuman
JH1	HS3166 <i>malZ::spec</i>	This study
MC4100	<i>araD139 deoC1 flbB5301 ptsF25 rbsR relA1 rpsL150 Δ(argF-lac)U169</i>	9
MD11	MC4100 <i>malT::Tn10</i>	Michael Dahl
ME469	MC4100 $\Phi P(malP-lacZ)$	Michael Ehrmann
RD22	JH1 <i>malS::Tn10</i>	This study
RD33	JH1 <i>malP::Tn10 glgA-lacZ</i>	This study
ST103	MC4100 $\Phi P(malP-lacZ)$	This study
Plasmids		
pBAD-YabM	Harbors YabM under the pBAD promoter	36
pCHAP113	Harbors <i>malQ</i> under the <i>lac</i> promoter control	47

diated catabolite repression on *malT* is also exerted by growth on glycerol, most likely affecting the EIIA<sup>Glc</sup>-P-dependent stimulation of adenylate cyclase by glycerol-3-P (19, 20). The target of inducer exclusion is MalK, the ATP-hydrolyzing subunit of the maltose/maltodextrin ABC transporter (33). In this process, unphosphorylated EIIA<sup>Glc</sup> of the glucose-specific phosphotransferase system (PTS) is thought to interact with the regulatory domain of MalK to curb transport activity (2, 11). The regulatory domain of MalK also interacts with MalT, inhibiting its activity as a transcriptional activator (31). There are several indications that it is the state of transport activity which controls the interaction of MalK with MalT and thus the activity of the latter (2, 6).

Not the activity but the expression of *malT* is controlled by Mlc, a transcriptional regulator acting as a repressor (16). The activity of Mlc as repressor is controlled by the PtsG-mediated transport of glucose. Instead of acting as an inducer for Mlc, this repressor is inactivated by sequestration to unphosphorylated (and actively transporting) membrane-bound PtsG (34).

Here, we report the synthesis of <sup>14</sup>C-labeled maltodextrins up to maltoheptaose and their use in metabolic studies as well as in quantitative transport analysis.

## MATERIALS AND METHODS

**Bacterial strains and growth conditions.** We used *E. coli* K-12 strains derived from strain MC4100. The isogenic derivatives of these strains were constructed by P1<sub>vir</sub> transduction (37) using selectable antibiotic resistance insertions in the relevant genes. These strains are listed in Table 1.

**Synthesis of radiolabeled maltodextrins.** The synthesis was done according to the method of Pajatsch et al. (43) with alterations. Strain ST103 lacking MalQ, MalP, and MalZ was transformed with pCHAP113 harboring *malQ* under *lac* promoter control (47). The strain was grown in Luria broth and ampicillin (100  $\mu$ g/ml) in the absence of isopropyl- $\beta$ -D-thiogalactopyranoside (IPTG) (the strain did not contain the Lac repressor). Outgrown cells were harvested and resuspended in 50 mM Tris-HCl (pH 7.5)–10 mM MgSO<sub>4</sub>. Cellular extracts were obtained by passing the suspension through a French pressure cell at 16,000 lb/in<sup>2</sup>. The high-speed supernatant of the extract was dialyzed three times against 2 liters of the same buffer and adjusted to a protein concentration of 4.5 mg/ml. Analytical experiments for the synthesis of <sup>14</sup>C-labeled maltodextrins were done as described in the legend to Fig. 1. To preparatively synthesize the dextrans, 1.25  $\mu$ Ci [<sup>14</sup>C]maltose (600 mCi/mmol, repurified; Hartmann) in 1,250  $\mu$ l water con-

taining 3% ethanol was buffered to 10 mM Tris-HCl, pH 7.5, and 120  $\mu$ l of the dialyzed extract described above was added. In preliminary experiments, it was established that ethanol up to a concentration of 10% did not interfere with the reaction. After 10 min at room temperature, the entire mixture was heated to the temperature of boiling water, the precipitate was removed by centrifugation, and the supernatant was applied as a single streak onto one sheet of Whatman 3MM chromatography paper and developed with butanol-ethanol-water at a ratio of 5:3:2 (vol/vol/vol) for 4 days by descending chromatography. Under these conditions, glucose as the sugar migrating fastest had left the paper and maltose was positioned close to the end of the 47-cm long paper. The paper was dried and exposed to X-ray film for 3 h. Strips containing the different maltodextrins up to maltoheptaose were cut out and eluted with water by descending chromatography. The eluates were collected in Eppendorf tubes, with the first 5 drops (about 250  $\mu$ l) containing all the radioactivity. The different solutions were separately chromatographed once again under the same conditions. Based on the reaction mechanism of amyloamylase (44), all [ $^{14}$ C]maltodextrins synthesized contained the same specific radioactivity with respect to their glucosyl residues. The purified samples were kept frozen.

**Analysis of the metabolic fate of the different  $^{14}$ C-labeled maltodextrins in different strains.** The strains mentioned in the legend to Fig. 2 were grown overnight in NZA medium (10 g NZ-amine A [Sheffield Products Inc.]-5 g yeast extract-7.5 g NaCl in 1 liter distilled water). They were washed once with minimal medium A (MMA) (37) and resuspended in MMA to an optical density at 578 nm ( $OD_{578}$ ) of 0.5. A total of 0.015 to 0.02  $\mu$ Ci of the different  $^{14}$ C-labeled maltodextrins was added, the suspensions were centrifuged after 10 min of incubation, and the pellets were treated with 5  $\mu$ l 12% trichloroacetic acid (TCA), kept on ice for 10 min, and centrifuged. The TCA supernatant was spotted on thin-layer chromatography (TLC) plates, developed in *n*-butanol-ethanol-water at a ratio of 5:3:2 (vol/vol/vol), and autoradiographed for 1 week.

**Transport assays of [ $^{14}$ C]maltose as a measure of *mal* gene expression.** Strains were grown in MMA plus 0.4% Casamino Acids (CAA) or MMA plus 0.4% glycerol as the sole source of carbon. Alterations are indicated in the tables. After being washed in MMA three times, the cells were resuspended in 3 ml MMA to an  $OD_{578}$  of 0.1 and the assay was initiated by the addition of 70 nM [ $^{14}$ C]maltose (600 mCi/mmol; Amersham) or labeled maltodextrins of identical specific radioactivities (with respect to the glucosyl moieties). Five 0.5-ml samples were removed within a time interval of 2 min. The initial rate of uptake at time zero was extrapolated mathematically. When higher substrate concentrations were used, the same amount of radioactive substrate was mixed with unlabeled maltodextrin prior to its addition to achieve the desired final concentration. To determine the equilibrium accumulation of [ $^{14}$ C]maltose in strain CB47, the latter was grown in MMA plus glycerol and resuspended in 5 ml MMA to an  $OD_{578}$  of 0.02. Next, 29 nM [ $^{14}$ C]maltose (final concentration, 600 mCi/mmol; Amersham) was added. Fifteen minutes after the addition, the entire volume was filtered through nitrocellulose filters (pore size, 0.45  $\mu$ m; Schleicher und Schüll) and the filter was assayed without washing for radioactivity in a scintillation counter. The filtrate was collected, and 0.1 ml was also counted to determine the [ $^{14}$ C]maltose remaining in the medium. Care was taken to ensure that net uptake had ceased after 15 min. When higher maltose concentrations were used, [ $^{14}$ C]maltose was mixed with unlabeled maltose of the desired concentration prior to addition to the medium. The same amount of radioactivity was used in all experiments.

**Exit of accumulated [ $^{14}$ C]maltose from strain CB47 in the presence and absence of the plasmid-encoded sugar exit transporter.** Both strain CB47 (lacking MalQ as well as maltose transacetylase, the *maa* gene product) and strain CB47 transformed with pBAD-YabM were grown overnight in MMA plus glycerol. Cells were washed three times with MMA and resuspended in 6 ml MMA to an  $OD_{578}$  of 0.2. [ $^{14}$ C]maltose was added at an initial concentration of 137 nM. The concentration of [ $^{14}$ C]maltose accumulated internally was determined to be 0.7 mM for both strains. After 15 min, 1 mM unlabeled maltose was added. Samples (0.5 ml) were removed at the time intervals shown in Fig. 3, filtered through nitrocellulose filters (pore size, 0.45  $\mu$ m; Schleicher und Schüll), and counted in a scintillation counter.

## RESULTS

**Synthesis of [ $^{14}$ C]labeled maltodextrins of equal specific radioactivities.** A crude extract of a strain lacking the *mal* encoded enzymes but harboring pCHAP113 encoding amyloamylase (47) was used as the source for the enzymatic conversion of [ $^{14}$ C]maltose into [ $^{14}$ C]glucose and [ $^{14}$ C]maltodextrins.

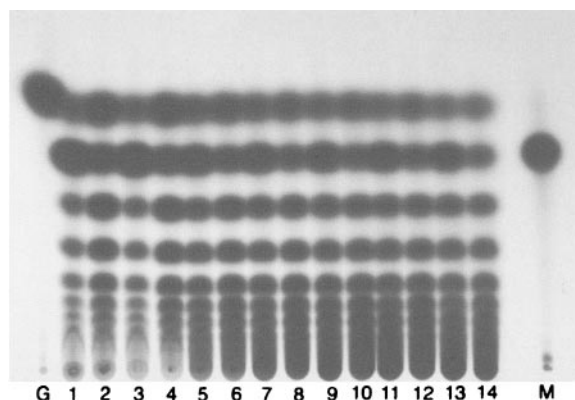


FIG. 1. TLC analysis of amyloamylase-mediated synthesis of  $^{14}$ C-labeled maltodextrins. The incubation mixture contained in 15  $\mu$ l of 10 mM Tris-HCl, pH 7.5, crude dialyzed extract (final concentration, 1.5 mg protein/ml) of a strain containing overexpressed amyloamylase, 20  $\mu$ M [ $^{14}$ C]maltose, and the following unlabeled sugars at a 3 mM final concentration: in lanes 1 and 2, no addition; in lanes 3 and 4, maltose; in lanes 5 and 6, maltotriose; in lanes 7 and 8, maltotetraose; in lanes 9 and 10, maltopentaose; in lanes 11 and 12, maltohexaose; and in lanes 13 and 14, maltoheptaose. Lane M, flanking right, standard [ $^{14}$ C]maltose; lane G, flanking left, standard [ $^{14}$ C]glucose. Six-microliter samples were spotted on a TLC plate at 1 min after addition of the enzyme (odd-numbered lanes) and after 15 min (even-numbered lanes). Autoradiography was for 24 h.

Prior to the synthesis, the extract was carefully and extensively dialyzed to remove any unlabeled maltodextrins from the extract. Even though it has been reported that amyloamylase does not utilize maltose as substrate but utilizes it only as acceptor in the dextrinyl transfer reaction (44), we found that incubation of [ $^{14}$ C]maltose (20  $\mu$ M) with the extensively dialyzed extract led to the formation of  $^{14}$ C-labeled maltodextrins of various lengths as well as [ $^{14}$ C]glucose. After the equilibrium was reached in little more than 1 min, the composition of the dextrin mixture did not change, even after long incubation periods (15 min). The samples were analyzed by TLC and autoradiographed (Fig. 1). To quantitatively separate the different  $^{14}$ C-labeled maltodextrins (Fig. 1, lane 2), we used chromatography paper (Whatman 3MM). After autoradiography, the respective paper strips were eluted with water. All  $^{14}$ C-labeled maltodextrins from maltose to maltoheptaose were chromatographed twice by this method and appeared essentially homogenous by the TLC criterion (Fig. 2).

Quantitative analysis of the amyloamylase-catalyzed reaction with [ $^{14}$ C]maltose at a 20  $\mu$ M concentration in the absence of any unlabeled maltodextrin (Fig. 1, lane 2) revealed a curious, nearly twofold stepwise decrease in molar yields with increasing chain lengths of the dextrin formed (Table 2). The presence of 3 mM unlabeled dextrans from maltose to maltoheptaose in the reaction mixture altered the equilibrium composition of the  $^{14}$ C-labeled dextrans formed with increasing chain lengths of the unlabeled dextrans (Fig. 1). Thus, in the presence of unlabeled maltoheptaose, glucose qualitatively appeared to be the least prominent spot of the equilibrium mixture. This method is ideally suited to synthesizing radioactively labeled maltodextrins up to maltoheptaose with specific radioactivities up to the original activity of the starting [ $^{14}$ C]maltose. The limit in heptaose is given by

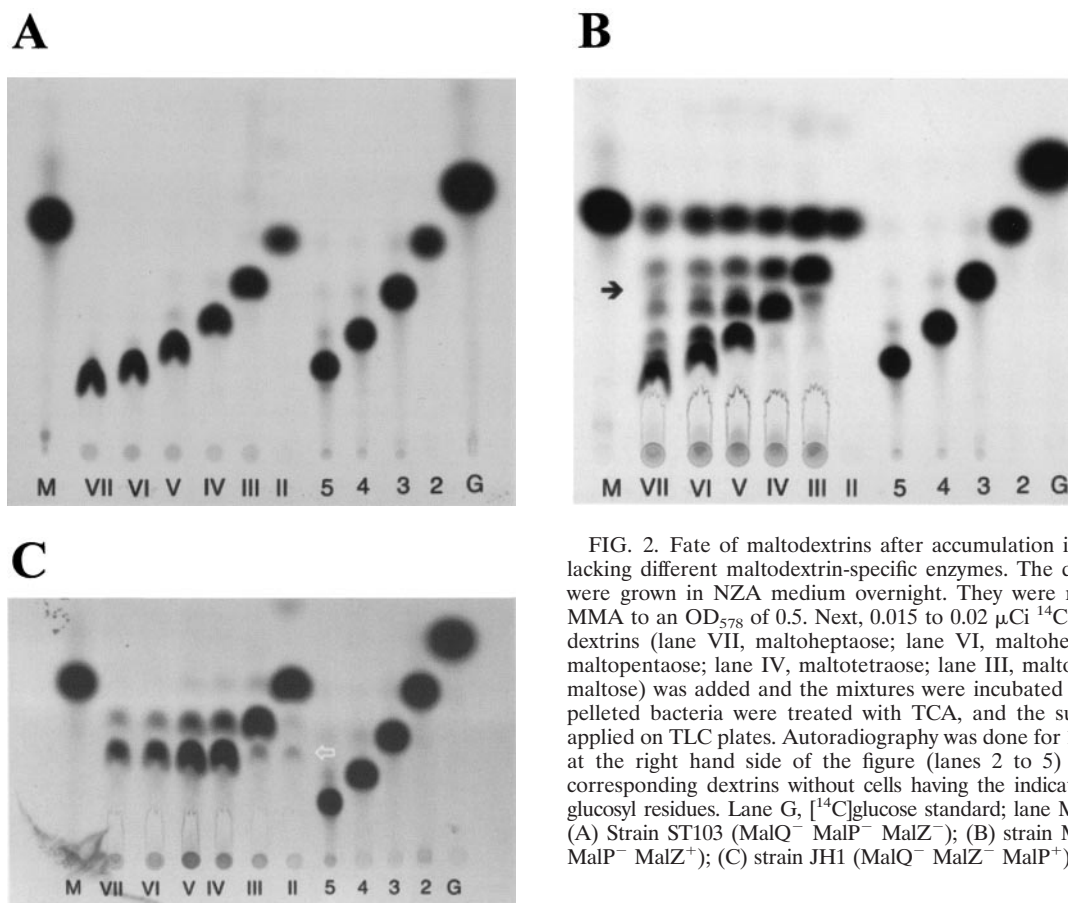


FIG. 2. Fate of maltodextrins after accumulation in *malQ* strains lacking different maltodextrin-specific enzymes. The different strains were grown in NZA medium overnight. They were resuspended in MMA to an OD<sub>578</sub> of 0.5. Next, 0.015 to 0.02 μCi <sup>14</sup>C-labeled maltodextrins (lane VII, maltoheptaose; lane VI, maltohexaose; lane V, maltopentaose; lane IV, maltotetraose; lane III, maltotriose; lane II, maltose) was added and the mixtures were incubated for 5 min. The pelleted bacteria were treated with TCA, and the supernatant was applied on TLC plates. Autoradiography was done for 1 week. The set at the right hand side of the figure (lanes 2 to 5) represents the corresponding dextrans without cells having the indicated numbers of glucosyl residues. Lane G, [<sup>14</sup>C]glucose standard; lane M, [<sup>14</sup>C]maltose. (A) Strain ST103 (MalQ<sup>-</sup> MalP<sup>-</sup> MalZ<sup>-</sup>); (B) strain ME469 (MalQ<sup>-</sup> MalP<sup>-</sup> MalZ<sup>+</sup>); (C) strain JH1 (MalQ<sup>-</sup> MalZ<sup>-</sup> MalP<sup>+</sup>).

the limited separation power on chromatography paper under the given conditions of paper quality and solvent used.

{We performed the amyломaltase-catalyzed reaction with commercial [<sup>14</sup>C]maltose from two sources, Amersham and Hartmann. Using Hartmann [<sup>14</sup>C]maltose, we observed that with some samples, all maltodextrins formed appeared on chromatography paper (but not on TLC plates) as doublets, allowing their separation. The analysis of this phenomenon revealed that all of the commercial [<sup>14</sup>C]maltose preparations tested contained various amounts (2 to 40%) of a disaccharide that is very similar to [<sup>14</sup>C]maltose (possibly isomaltose, α-1-6

glucosyl glucose) and that was stable and not interconvertible with maltose; it could be separated from maltose by using extended paper chromatography but was not a substrate of the *E. coli* maltose transporter and could not act as a dextrinyl donor in the amyломaltase reaction. However, it acts as an acceptor in the amyломaltase reaction with maltose as the substrate and gives rise to a series of maltodextrins whose reducing end is most likely composed of the unknown disaccharide. The identity of this disaccharide was not further studied, but care was taken to use purified maltose in amyломaltase-catalyzed maltodextrin formation to ensure the identity of the radioactive dextrans with the series of linear α(1-4)maltooligosaccharides.}

**Fate of maltodextrins after their accumulation in strains lacking different maltodextrin-metabolizing enzymes.** To avoid fast degradation of the maltodextrins to glucose, all strains carried a mutation in *malQ*, abolishing amyломaltase (MalQ) activity. The strains carried various additional mutations, i.e., in *malS* (periplasmic amylase), *malP* (maltodextrin phosphorylase), *malZ* (maltodextrin glucosidase), *amyA* (cytoplasmic amylase), and *glgA* (glycogen synthase). Being MalQ<sup>-</sup>, they exhibited partially constitutive maltodextrin transport activity not requiring induction (15). The cells were exposed to the different purified <sup>14</sup>C-labeled maltodextrins from maltose to maltoheptaose, incubated for 5 min, centrifuged, washed free from the external medium, and precipitated with TCA. The TCA supernatant was analyzed by TLC and autoradiographed (Fig. 2). The absence of MalQ, MalP, and MalZ was sufficient

TABLE 2. Quantitative analysis of [<sup>14</sup>C]maltodextrins formed from [<sup>14</sup>C]maltose by MalQ<sup>a</sup>

Maltodextrin	Relative molar activity (cpm) (10 <sup>-3</sup> )
Glucose.....	202
Maltose.....	102
Maltotriose.....	48.6
Maltotetraose.....	19.8
Maltopentaose.....	7.69
Maltohexaose.....	3.39
Maltoheptaose.....	1.84

<sup>a</sup> Equilibrium composition of [<sup>14</sup>C]maltodextrins after incubation of [<sup>14</sup>C]maltose (20 μM) with amyломaltase (Fig. 1, lane 2). The material was chromatographed on Whatman 3MM filter paper, the chromatogram was autoradiographed, and the spots were cut out and counted in a scintillation counter.

to allow the accumulation of all maltodextrins in a chemically unaltered form (Fig. 2A). In the absence of MalP but in the presence of MalZ, the end product of all maltodextrins was maltose, a sugar that does not induce the maltose system (Fig. 2B). Figure 2B also shows that the products left from maltoheptaose and maltohexaose are less prominent than those from maltose and maltotriose. Since the initial radioactivity was the same in all cases, this imbalance was due to the metabolism of glucose-1-P, glycolysis, and loss as CO<sub>2</sub> cleaved off from the different dextrans. In the absence of MalZ but in the presence of MalP, the larger maltodextrins were all quickly degraded to maltotetraose. Maltotetraose at the given time of incubation was then partially transformed into maltotriose, whereas maltotriose itself was not further hydrolyzed (Fig. 2C). Here again, due to the formation of glucose by MalZ, followed by phosphorylation, glycolysis, and loss as CO<sub>2</sub>, the total product formation was smaller for larger dextrans than for smaller dextrans. The comparison between Fig. 2B and C shows that the action of MalP on the different dextrans (larger than maltotetraose) was much faster than that of MalZ. The ability of serving as a substrate for MalP decreased strongly with dextrans shorter than maltopentaose but still allowed maltotetraose to be a substrate for the formation of the inducer maltotriose. As is shown in the accompanying publication (17), the action of MalP is crucial to form glycogen-derived maltotriose, the inducer of the system. In contrast, the action of MalZ is to degrade maltotriose to the noninducer maltose. We noticed the formation of small amounts of a labeled compound that runs slightly ahead of maltotetraose (Fig. 2B and C, arrow). It is formed even when maltose is used as a substrate. Situated in its chromatographic behavior on TLC plates between maltotriose and maltotetraose, this compound behaved chromatographically similarly to  $\alpha$ -cyclodextrin (six glucosyl residues in a ring) (43). It is unclear how this compound could be synthesized from maltodextrins via MalZ or MalP. MalZ is known to hydrolyze  $\gamma$ -cyclodextrin (and  $\beta$ -cyclodextrins weakly) to maltose and glucose, but not  $\alpha$ -cyclodextrin (45). The presence or absence of glycogen did not affect its synthesis. We did not observe it in strains lacking all the *mal* enzymes (Fig. 2A). The compound was not further studied.

Occasionally, when maltose was accumulated (for instance, as seen in Fig. 2C, lane II), a small spot of radioactivity was seen migrating faster than glucose. This spot has previously been identified as acetyl maltose and was due to the action of maltose transacetylase. This enzyme does not use maltodextrins larger than maltose as a substrate.

The presence or absence of *amyA* encoding a cytoplasmic amylase (48) had no effect on any of the above patterns (data not shown). Also, the presence or absence of *glgA* (encoding glycogen synthase) or *malS* (encoding periplasmic amylase) had no effect on the maltodextrin pattern seen in a *malQ malZ malP*<sup>+</sup> or *malQ malZ*<sup>+</sup> *malP* background (not shown).

**The rate of transport of labeled maltodextrins declines as their size increases but appears constant when calculated per glucosyl residue.** The availability of <sup>14</sup>C-labeled linear maltodextrins of defined specific radioactivities prompted us to assay the initial rate of transport at identical substrate concentrations. We used strain ST103 lacking all maltodextrin-utilizing enzymes and thus accumulating these dextrans in their unmod-

TABLE 3. Rate of transport of maltodextrins

Maltodextrin	Initial rate of glucosyl uptake (nmol/min/10 <sup>9</sup> cells) at the following dextrin concn <sup>a</sup>	
	70 nM	10 $\mu$ M
Maltose	0.59 (0.29)	3.29 (1.64)
Maltotriose	0.76 (0.25)	5.15 (1.71)
Maltotetraose	0.86 (0.21)	7.20 (1.80)
Maltopentaose	0.76 (0.15)	7.82 (1.56)
Maltohexaose	0.76 (0.12)	7.50 (1.25)
Maltoheptaose	0.67 (0.09)	5.50 (0.78)

<sup>a</sup> Numbers in parentheses indicate nmols of the different maltodextrins taken up per min per 10<sup>9</sup> cells.

ified form (Fig. 2). The first set of experiments was done at 70 nM, well below the  $K_m$  of the transporter for these substrates, and the second set was done at 10  $\mu$ M under conditions near the maximum rate of uptake. Table 3 shows that, surprisingly, the initial rate of uptake stayed quite constant when it was calculated in glucosyl residues taken up per minute. Thus, even though the rate per mole of maltodextrin taken up per minute declined as the size increased, the rate per glucosyl residue stayed surprisingly constant. There was the possibility that the observed constant rate of uptake was not caused by the ABC transporter in the inner membrane but by the  $\lambda$  receptor, the specific diffusion pore for maltodextrins in the outer membrane. Therefore, we transformed ST103, the test strain, with a multicopy-number plasmid harboring *lamB* encoding the  $\lambda$  receptor. Transport of [<sup>14</sup>C]maltohexaose at 70 nM and 10  $\mu$ M concentrations was the same with and without additional copies of the  $\lambda$  receptor. Therefore, the constant initial rate of glucosyl residues of the tested maltodextrins was indeed a reflection of the properties of the maltodextrin ABC transporter.

**The capacity of *malQ* strains to accumulate maltose can reach 10<sup>6</sup>-fold over the external concentration and a maximal internal concentration of 100 mM.** To demonstrate the capacity of *E. coli* to accumulate maltose and to maintain the concentration gradient, uptake was tested in CB4, a *malQ* strain lacking maltose acetylase, the only remaining enzyme that is able to alter maltose (8). This enzyme slowly acetylates cytoplasmically accumulated maltose, which can then exit the cells via a non-carrier-mediated diffusion process (3). CB47 cells were exposed to various concentrations of external <sup>14</sup>C-labeled maltose and were filtered after the equilibrium of accumulation had been reached. Filter-bound radioactivity as well as radioactivity in the filtrate were measured to determine the internal and external [<sup>14</sup>C]maltose concentrations. These results are summarized in Table 4. At the low external concentration of 1.8 nM, the accumulation was 10<sup>6</sup>-fold. This ratio dropped as the external concentration was raised. It appears that the maximally attainable internal concentration is on the order of 100 mM. Since the exit of accumulated [<sup>14</sup>C]maltose could be initiated by the addition of 1 mM external unlabeled maltose (Fig. 3), it is clear that an exit system whose internal  $K_m$  must be of the same order (100 mM) acts under these conditions. Strains lacking all maltodextrin enzymes become sensitive to maltodextrins (they cease to grow), in particular, to maltotriose when present in the growth medium in an excess of 50  $\mu$ M, indicating that the accumulated sugar represents an

TABLE 4. Equilibrium accumulation of maltose in a *malQ* strain (CB47) at different external maltose concentrations<sup>a</sup>

External [ <sup>14</sup> C]maltose concn			Ratio of internal over external [ <sup>14</sup> C]maltose concn
Initial external	External at equilibrium	Internal at equilibrium	
27 nM	1.8 nM	1.89 mM	1,050,000
1 μM	95 nM	63 mM	663,000
3 μM	1.47 μM	106 mM	72,108
6 μM	4.38 μM	113 mM	25,799
10 μM	8.35 μM	115 mM	13,820

<sup>a</sup> Each test was done in 5 ml MMA without a carbon source with a cell suspension exhibiting an OD<sub>578</sub> of 0.02. Calculation of the internal maltose concentration was based on the assumption that one cell has a volume of 10<sup>-12</sup> ml and the OD<sub>578</sub> of an outgrown bacterial cell culture of 1.4 is equal to that for 10<sup>9</sup> cells per ml (37).

osmotic, and possibly also an energetic, stress. The exit of [<sup>14</sup>C]maltose was made visible by blocking the entry with unlabeled maltose. When a plasmid encoding a general sugar exit system (35) was present in strain CB47, this exit reaction was increased and the final equilibrium concentration of accumulated internal maltose was lowered by a factor of more than 2 (Fig. 3). The presence of the plasmid-encoded exit pump had no effect on the initial rate of uptake of maltose at a low external concentration, but it relieved the toxicity of maltotriose in the growth medium apparently by lowering the internal dextrin concentration. Exit of maltose must be necessary for capping an unlimited accumulation of maltose. Thus, the entry and exit of maltose are mediated via different systems and the ABC transporter is exclusively geared for the unidirectional entry reaction.

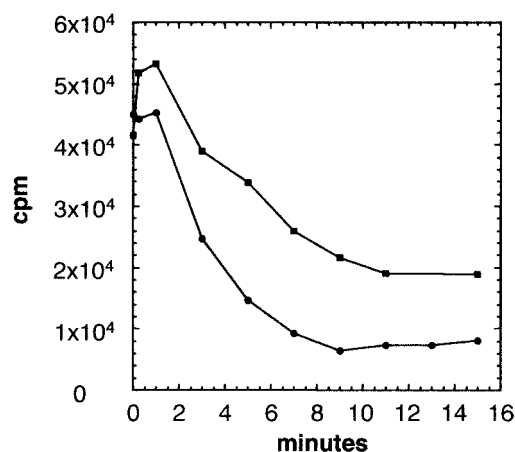


FIG. 3. Exit of [<sup>14</sup>C]maltose accumulated in CB47 by a plasmid encoding a sugar exit system. The culture was grown in MMA plus glycerol and resuspended in MMA without a carbon source to an OD<sub>578</sub> of 0.2. [<sup>14</sup>C]maltose at 0.14 μM (final concentration) was added and incubated for 15 min. The internal [<sup>14</sup>C]maltose concentration had then reached about 0.7 mM under equilibrium conditions. Unlabeled maltose (1 mM) was added at time zero to the medium, and samples were taken thereafter, filtered, and analyzed for radioactivity. Loss of radioactivity from the cell is given in cpm remaining inside the cells corresponding to a culture volume of 0.5 ml. ■, strain CB47; ●, strain CB47 harboring the plasmid-encoded uninduced levels of YabM, a sugar exit system.

TABLE 5. Induction of the maltose transporter by maltodextrins in a wild-type strain (MC4100)

Carbon source (%)	Initial rate of maltose uptake (nmol/min/10 <sup>9</sup> cells at 70 nM maltose)
Glycerol (0.2).....	0.008
CAA (0.4).....	0.086
Maltose (0.2).....	0.161
Maltotriose (0.2).....	0.214
Maltotetraose (0.2).....	0.123
Maltopentaose (0.2).....	0.121
Maltohexaose (0.2).....	0.144
Maltoheptaose (0.2).....	0.213

**Induction of the maltose system by inducer formation and by transport.** The induction of the maltose system is controlled on one hand by the inducer maltotriose-activating MalT, the transcriptional activator of the system (49), and by MalK, the ATP-hydrolyzing ABC subunit of the transporter inactivating MalT (31). Even though highly suggestive, the effect of transport itself on induction (modulating the regulatory activity of MalK) has never been measured directly. Therefore, we measured induction by exogenous maltodextrins under two conditions. In the first set, we used strain MC4100, allowing uptake via the ABC transporter as well as metabolism of the dextrins (including internal maltotriose formation and degradation) by the three enzymes MalQ, MalP, and MalZ and final degradation by glycolysis. In the second set, we used strain RD33 lacking all three maltodextrin-metabolizing enzymes as well as glycogen. Under these conditions, maltotriose cannot be formed from the dextrins taken up nor can it be formed from the degradation of glycogen.

In the first set, strain MC4100 was grown in minimal medium with glycerol overnight and reinoculated in minimal medium in the absence of glycerol but in the presence of 0.2% of the different maltodextrins from maltose to maltoheptaose. After growth overnight, the capacity of these cells to transport maltose was taken as a measure for the induction of the maltose system (Table 5). In this comparison, maltotriose induced significantly better than the other maltodextrins even though the differences were not dramatic. The induction by the different maltodextrins seen under these conditions reflects the sum of both internal maltotriose levels as well as induction by transport. The conclusion from this set of experiments is that the internal maltotriose levels may not vary significantly in spite of different exogenous maltodextrins. The low transport activity in cultures grown on glycerol is an underestimate of the uninduced state since glycerol exerts catabolite repression on the maltose system (19). When measured after growth in minimal medium plus 0.4% CAA, a more realistic value (10-fold increased) of the uninduced state was obtained.

In the second set, by using strain RD33, which lacks MalQ, MalZ, and MalP, all internal metabolism of maltodextrins was abolished. Strain RD33 carries a *glgA-lacZ* fusion and can therefore no longer produce endogenously glycogen-derived maltotriose (18). In this strain, maltodextrins supplied from the medium are accumulated in a chemically unaltered form (as seen for a similar strain, ST103, in Fig. 2A). Thus, the formation of the inducer maltotriose does not contribute to induction. As a measure of *mal* gene expression, we again used the

TABLE 6. Induction by exogenous maltodextrins in a strain (RD33) lacking all maltodextrin enzymes

Maltodextrin added to RD33 ( <i>malQ malP malZ glgA</i> ) grown in glycerol or CAA	Transport <sup>a</sup> (nmol/min/10 <sup>9</sup> cells)
With 0.2% glycerol	
None	0.025
Maltose	0.059
Maltotriose	1.890
Maltotetraose	0.268
Maltopentaose	0.163
Maltohexaose	0.272
Maltoheptaose	0.991
With 0.4% CAA	
None	0.214
Maltose	0.539
Maltotriose	3.121
Maltotetraose	0.932
Maltopentaose	0.806
Maltohexaose	1.378
Maltoheptaose	1.949

<sup>a</sup> The effect of transport on induction and catabolite repression by glycerol is shown. *mal* gene expression was measured as initial maltose transport at a 70 nM concentration.

transport activity of maltose. The strain was grown overnight in MMA with 0.2% glycerol or with 0.4% CAA as the carbon source. As discussed above, such a strain is sensitive to maltodextrins when added in a concentration exceeding 50  $\mu$ M. At an OD<sub>578</sub> of 0.1, 10  $\mu$ M concentrations of maltodextrins (from maltose to maltoheptaose) were added for induction (Table 6). The following conclusions can be drawn. All maltodextrins are able to induce the system, maltotriose being the most effective and maltose the least effective. Assuming that most of the dextrins in the medium (initial concentration, 10  $\mu$ M) have accumulated in the outgrown culture, the maximal internal concentration is in the order of 1 mM. As judged by TLC analysis, the different maltodextrins used were homogeneous (not shown). Since they are not degraded inside the cells and since MalT *in vitro* is activated only by maltotriose (48), the induction seen under these conditions must be due to the transport of these sugars *per se*. Again, cells grown in glycerol have a much lower basal expression than cells grown in CAA. There is still the possibility that CAA may contain an inducing substance. Therefore, we compared the transport of maltose (70 nM) in RD33 cells grown on 0.2% glycerol with that in RD33 cells grown on 0.4% CAA and on 0.4% CAA plus 0.2% glycerol. The high uninduced rate of transport in CAA-grown cells (0.214 nmol per min per 10<sup>9</sup> cells) was repressed more than 10-fold in the additional presence of 0.2% glycerol during growth (0.012 nmol per min per 10<sup>9</sup> cells; the value with 0.2% glycerol alone is 0.025). This indicates that CAA does not contain an inducer but that glycerol represses *mal* gene expression.

## DISCUSSION

In this publication, we addressed two different aspects of the maltose/maltodextrin system of *E. coli*, i.e., its transport characteristics and the *in vivo* role of its enzymes. We synthesized uniformly <sup>14</sup>C-labeled maltodextrins up to seven glucosyl units

long, exhibiting defined and identical specific radioactivities with respect to their glucosyl units. The synthesis of these dextrins was accomplished by the action of amyloamylase on uniformly labeled [<sup>14</sup>C]maltose. In contrast to the proposal that amyloamylase cannot use maltose as a substrate (but as an acceptor), we found that purified [<sup>14</sup>C]maltose was easily transformed into maltodextrins and glucose. The enzyme preparation had been thoroughly dialyzed. Thus, if the enzyme indeed needs a maltodextrin larger than maltotriose, such a priming maltodextrin had to be tightly bound to it during dialysis. Or, alternatively, maltose can be recognized by amyloamylase as a substrate as well, producing increasingly better primers in the course of glucosyl transfer. In any case, we found the reaction catalyzed by amyloamylase to be a convenient and simple method to synthesize maltodextrins up to maltoheptaose of defined specific radioactivities.

Using these labeled maltodextrins in transport assays, we found that uptake through the maltose/maltodextrin ABC transporter at a substrate concentration of 70 nM (far below their transport  $K_m$ ) as well as at 10  $\mu$ M (under  $V_{max}$  conditions) takes place with surprisingly similar rates of transport with respect to each glucosyl unit. We interpret this finding by a ratchet-type transport mechanism in which the transport of each glucosyl unit represents a repetitive cycle for the transporter. Studies of growth rates using maltodextrins of differing lengths as carbon sources have shown that growth rates from maltose up to maltoheptaose remain similar and decline only with longer dextrins (63). With the assumption that transport limits growth, this is consistent with our observation that the rate of transport declines linearly in proportion to the increased size of the substrate or that the rate of uptake per glucosyl residue remains constant independent of the length of the dextrin. Studies reporting the growth yields for different sugars showed that the yields for maltose, maltotriose, and maltohexaose grown under anaerobic conditions were 37.5, 67.6, and 155 g per mol of substrate (42). Calculated per glucosyl residue, this comes to 18.7-, 22.5-, and 25.8-g growth yields per mol glucosyl residue, respectively. The higher growth yield achieved with maltohexaose has been interpreted by the equal-ATP requirement for transporting maltose versus maltohexaose. However, in this case, the gain in yield should have been considerably higher even if only one ATP is needed for transporting one dextrin molecule. Maltohexaose contains per glucosyl residue a higher nutritional value than maltose. This is due to the conservation of energy contained in the glycosidic linkage by the phosphorylase action saving one ATP per glycosidic bond. Thus, the yield per glucosyl residue being 18.7 g in the case of maltose should be one-third ATP (or 3.3 g) more for one glucosyl residue in maltotriose (22.0 g) and two-thirds ATP (or 6.6 g) more for the glucosyl residue in maltohexaose (24.9 g). This is very close to what has been found experimentally (42), arguing against the equal-ATP requirement for the accumulation of the different dextrin molecules and for the requirement for equal transported glucosyl residues within the dextrin molecule. Thus, we propose that the energy expenditure for the transport of one molecule of maltohexaose by the ABC transporter is three times the value for one molecule of maltose.

The availability of a strain being devoid of amyloamylase as well as maltose transacetylase in which maltose cannot be

altered in the cytoplasm allowed us to reexamine (60) the relationship of internal and external maltose concentrations in the equilibrium state of accumulation where the rates of exit and entry become equal. At a 1.8 nM external concentration, a  $10^6$ -fold accumulation was reached, resulting in an internal concentration of 1.89 mM. Increasing the external concentration reduced the concentration gradient but increased the internal concentration. The latter reaches (but does not greatly exceed) 100 mM. The limit of 100 mM internal maltose is maintained by one or more exit systems with a high  $K_m$  for exit. Binding protein-dependent ABC transporters have traditionally been considered unidirectional pumps where the hydrolysis of ATP is directly coupled to the inward transport of substrate (4). This view has been challenged by a report claiming the exit of amino acids via a binding protein-dependent ABC transporter (30). In the maltose system, it has been demonstrated that [ $^{14}\text{C}$ ]maltose accumulated in a *malQ* mutant can be chased out by the addition of millimolar concentrations of external unlabeled maltose. Yet, uncouplers of oxidative phosphorylation strongly inhibit the exit of maltose but not the ABC transporter-mediated entry of maltose (23), demonstrating that entry and exit occur by two separate mechanisms. During the present study, we found that maltotriose was growth inhibitory for *malQ* strains growing on glycerol or CAA. We also found that the overexpression of *yabM* (35), a gene known to encode a sugar exit transporter, abolished the sensitivity against maltotriose in a *malQ* mutant and resulted in increased maltose exit (Fig. 3) without affecting the initial rate of entry. There is more than one sugar exit transporter (7) that could account for maltose exit. Thus, we would like to emphasize that maltose, when present at high internal concentrations, can be secreted by mediated processes exhibiting a very high  $K_m$  value. Therefore, at present, there is no convincing evidence for maltose exit through the MalEFGK<sub>2</sub> ABC transport complex.

Using mutants lacking *malP* (maltodextrin phosphorylase) or *malZ* (maltodextrin glucosidase) or both, we followed the fate of  $^{14}\text{C}$ -labeled maltodextrins after their accumulation in order to identify the role of MalP and MalZ in the metabolism of maltodextrins. To prevent the fast breakdown of the accumulated dextrins, we performed these studies in a mutant background lacking *malQ*. The conclusion of these studies (Fig. 2) was that maltodextrin phosphorylase will reduce the size of any maltodextrin to maltotetraose and, in a slow reaction, to maltotriose but not any further (17). Thus, MalP will ensure the formation of the inducer maltotriose from any longer linear maltodextrin, for instance, as those derived from glycogen break down. But MalP will not eliminate the inducer. In contrast to the inducer-producing role of MalP, MalZ, the maltodextrin glucosidase, will hydrolyze any maltodextrin, including maltotriose to maltose. Thus, MalZ will be expected to reduce glycogen-derived endogenous induction. We would like to emphasize that maltose is not recognized by either MalP or MalZ.

The availability of a mutant lacking glycogen and all maltodextrin enzymes (*malQ*, *malP*, *malZ*) preventing the degradation of the accumulated maltodextrins allowed us to test the proposal that transport itself influences induction. Following the ability to transport maltose as a measure of *mal* gene expression, we indeed found that maltotriose strongly induced

the system; it was the best of all six dextrins tested. However, all other maltodextrins, including maltose, were able to induce the system. We interpret this result by the action of MalK during transport. In this scenario, MalK engaged in transport would relieve MalT from inhibition. Exit of maltodextrins may occur through sugar exit systems, allowing continuing reentry through the ABC transporter. The different levels of induction reached by the different sugars could be explained by their unequal rates of exit. The low inducing capability of maltose in this scheme would then also be due to its removal by maltose transacetylase (8). The longer dextrins are not substrates for this enzyme.

The observation that transport of maltodextrins alone in the apparent absence of internal maltotriose can elicit induction poses an inherent problem: the activity of MalT can be controlled (inhibited) by the interaction with MalK, representing transport-controlled induction. Yet, for its activation, MalT needs to bind inducer (49). Is there an alternative inducer aside from maltotriose? In the accompanying paper (17), where we discuss the osmoregulation of endogenous induction, we come to the conclusion that, aside from the glycogen-derived inducer maltotriose that is sensitive to MalZ, there exists glycogen-independent endogenous induction that is insensitive to MalZ and therefore must be caused by an inducer distinct from maltotriose that is able to activate MalT when MalK is not inhibiting MalT. This inducer must be inefficient for induction in the presence of a nontransporting (and therefore MalT-inhibiting) MalK, but it will reveal its MalT-activating capabilities either in the absence of MalK (see accompanying publication [17]) or during transport engagement.

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