The MalT-dependent and malZ-encoded Maltodextrin Glucosidase of Escherichia coli Can Be Converted into a Dextrinyltransferase by a Single Mutation*

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malZ is a member of the mal regulon. It is controlled by MalT, the transcriptional activator of the maltose system. MalZ has been purified and identified as an enzyme hydrolyzing maltotriose and longer maltodextrins to glucose and maltose. MalZ is dispensable for growth on maltose or maltodextrins. Mutants lacking amylosylase (encoded by malQ), the major maltose utilizing enzyme, cannot grow on maltose, maltotriose, or maltotetraose, despite the fact that they contain an effective transport system and MalZ. From such a malQ mutant a pseudorevertant was isolated that was able to grow on maltose. The suppressor mutation was mapped in malZ. The mutant gene was cloned. It contained a Trp to Cys exchange at position 292 of the deduced protein sequence. Surprisingly, the purified mutant enzyme was still unable to hydrolyze maltose as was the wild type enzyme, while both were able to release glucose from maltodextrins. However, the mutant enzyme had gained the ability to transfer dextrinyl moieties to glucose, maltose, and other maltodextrins. Thus, it had gained an activity associated with amylosylase. It was the MalZ292-associated transferase reaction that allowed the utilization of maltose. In addition, we discovered that mutant and wild type enzymes alike were highly active as γ-cyclodextrinases.

The Escherichia coli maltose system (1, 2) contains two enzymes that are necessary for the utilization of maltose and maltodextrins. Maltotriose, after having been taken up by the high affinity and binding protein-dependent ABC (ATP binding cassette) transport system (3) is recognized by amylosylase (encoded by malQ) (4), the reducing end glucose is released and the amylosyl residue is transferred to another maltotriose molecule thus forming maltopentaose (5, 6). The repetition of this cycle leads to the formation of long maltodextrins and free glucose which, after phosphorylation by glucokinase enters glycolysis. Maltopentaose and longer maltodextrins are recognized by maltodextrin phosphorylase (encoded by malP) (7, 8) which, by phosphorylisis, releases the nonreducing end glucose as glucose 1-phosphate. Thus, the final products of maltodextrin metabolism by these two enzymes are glucose and glucose 1-phosphate.

The degradation of maltose, the smallest member of maltodextrins, also requires amylosylase, and malQ mutants are Mal−. However, amylosylase does not recognize maltose as glucosyl donor, only as maltodextrinyl acceptor (6). Therefore, in order to metabolize maltose, amylosylase requires a maltodextrin primer with the minimal size of maltotriose. Within the cell, the required maltodextrin primer can originate from the degradation of glycogen or from the action of an as yet uncharacterized maltose/maltotriose phosphorylase with glucose and glucose 1-phosphate as starting material (9).

There are two more enzymes, members of the mal regulon, that are not essential for the metabolism of maltose and small maltodextrins. One is a periplasmic α-amylase encoded by malS (10, 11). This enzyme hydrolyzes larger dextrans in the periplasm preferentially to maltohexaose (12) which can then be transported by the maltose/maltodextrin transport system. The other is a cytoplasmic enzyme encoded by malZ (13, 14) whose function in maltodextrin metabolism is unclear. The enzyme degrades linear maltodextrins up to a chain length of 7 glucose units but not maltose. It sequentially deaves glucose from the reducing end of the maltodextrin chain. Even though it can hydrolyze maltotriose to glucose and maltose, malQ mutants cannot grow on maltotriose or maltotetraose even when malZ is overexpressed (14).

The endogenous formation of maltotriose required for maltose utilization is also an important aspect of the regulation of the mal system. All mal genes are under the control of MalT, the positive activator of the system (15). In vitro MalT-dependent mal gene expression requires the presence of maltotriose (16). Cells growing in the presence of maltose or any other maltodextrin will result in the elevated expression of the mal system even though only maltotriose is the effective internal inducer. We have argued that the increased formation of endogenous maltotriose during exogenous induction by maltodextrins is driven by glucose and glucose 1-phosphate, the degradation products of maltodextrin metabolism. This reaction is most likely catalyzed by maltose/maltotriose phosphorylase, the same enzyme that is required for the maltodextrin primer synthesis mentioned above (9).

In an attempt to identify the postulated maltose/maltotriose phosphorylase, we reasoned that the action of this enzyme should be reversible, and it should therefore be able to split maltose to glucose and glucose 1-phosphate. Thus, malQ mutants which are Mal− should be able to grow again on maltose after a mutational event resulting in the overproduction of the postulated maltose/maltotriose phosphorylase. Among the phenotypic revertants in such a selection, we found one mutation that did not map in malQ. In this paper, we report the analysis of this second site revertant. We found that the mutation had occurred in malZ. We cloned and sequenced the mutant malZ (malZ292). We purified the encoded enzyme and characterized its enzymatic activity. We present the explanation for the ability of the mutant enzyme to support growth on maltose by its acquisition to transfer dextrinyl residues to maltose, followed...
TABLE I  
Bacterial strains and plasmids  

| Strains         | Known genotype                          | Reference |
|-----------------|-----------------------------------------|-----------|
| MC4100         | F-/araD139 Δ(argF-lac)I169 flbB5301 decC1 relA1 rbsR rpsL 150 pst2 F- thi-1 |           |
| HfrG6          | malT’ his                                |           |
| POP4063        | HfrG6 malP::Tn10                         | Institut Pasteur |
| POP4064        | HfrG6 malQ::Tn10                         | Institut Pasteur |
| BW10320        | MC4100 phoB519::Tn5-312 (Tet’ cbr510)    | B. Wanner |
| DW18           | MC4100 Φ(glac::lacZ) (λpLM615)           |           |
| NK9054 (W1485) | NK9051 pgem::Tn10                       |           |
| RH97           | MC4100 glgC200::Tn10                     |           |
| RH98           | MC4100 glgA::Tn10                        |           |
| ME469          | MC4100 malS malT’::10 Φ(malP::lacZ::Y’-X’) malQ::Tn10 (Tet’ cbr510) |           |
| HS3166         | MC4100 malQ’::10                         |           |
| SFC1           | HS3166 malZ92                           | This study |
| RP90           | HS3166 phoB519::Tn5-312 (Tet’ cbr510)    | This study |
| RP91           | SFC1 pgm::Tn10                           | This study |
| RP92           | SFC1 Φ(glac::lacZ) (λpLM615)            |            |
| RP93           | RP92 pgm::Tn10                           | This study |
| RP94           | RP92 glgC200::Tn10                       | This study |
| RP95           | SFC1 glgC200::Tn10 malQ’::10             | This study |
| RP96           | MC4100 malQ::Tn10 malZ::spec             |           |
| RP97           | SFC1 malP::Tn10                          |           |
| RP98           | SFC1 malQ::Tn10                          |           |
| RP99           | ME469 pgem::Tn10                         | This study |
| RP100          | MC4100 pgem::Tn10 malZ::spec             |           |
| RP101          | MC4100 pgem::Tn10                        |           |
| Plasmids       |                                         |           |
| pACYC177       | Ap’, Kn’                                |           |
| pRP100         | pACYC177 Kn’ phoB 3 malZ292              |           |
| pCHAP 113      | Kn’ malQ’                               |           |
| pDH832         | Ap’ malF’                                |           |
| pRP112         | pDH832 malF’ Ap’ malZ292                 |           |
| pBR322         | Ap’, Tet’                               |           |
| pST4           | pBR322 Ap’, malZ’                        |           |
| pRP117         | pBR322 Ap’, malZ292                      |           |
| pCYTEXP1       | Ap’                                      |           |
| pRP118         | Ap’ malZ292                             |           |
| pRP119         | Ap’ malZ’                                |           |

Phenotype: DNA Methods—Chromosomal DNA was isolated as described by Silhavy et al. (18). Plasmid DNA was prepared by the alkaline-SDS method according to Birnboim and Doly (19). Other standard recombinant DNA techniques are from Sambrook et al. (20).

Construction of pRP100 and Derivatives—Chromosomal DNA of S. C. CamHi and PstI. The 10–14 kb fraction was eluted from an agarose gel and ligated into pACYC177 that had been digested with the same enzymes. Strain RP90 was transformed with this ligation mixture. Colonies were selected for kanamycin resistance and screened for the ability to derepress alkaline phosphatase on G + L medium (21) with 0.1 mM P, and 0.2% glucose as carbon source and 40 μg/ml 5-bromo-4-chloro-3-indolyl phosphate (X-P) as indicator for alkaline phosphatase. The 672 bp of which were able to derepress alkaline phosphatase were also able to grow on MMA-plates containing 0.2% maltose. One of these plasmids, pRP100, contained a chromosomal insert of about 12 kb and was used for further studies. pRP102 was obtained by deleting two MluI fragments (together 6 kb) from pRP100.

For the construction of pRP112, pRP102 was digested with ScaI, and the resulting fragments were blunt-ended with T4 polymerase. In a second step these fragments were digested with MluI. A 2.1 kb MluI/ ScaI fragment was isolated and ligated in pDHB32 that had been digested with MluI and PshAI. The resulting plasmid pRP112 contained a 2.1 kb chromosomal fragment encoding the entire mutant gene malZ292 (Fig. 1). For the construction of pRP117, pRP112 was digested with HindIII and fragment and the above 742 bp fragment was eluted from an agarose gel. Plasmid pST4 (14) was also digested with HindIII and SnB1. The above 742 bp fragment was ligated in the opened pST4 plasmid. After transformation of RP90 with the ligation mixture, all clones were able to grow on MMA plates containing 0.2% maltose. The construction of pRP118 and pRP119, the expression plasmids for malZ and malQ292, was done by polymerase chain reaction technique using two primers (ACAGGGGACATGATGTAAATGC) and (TTCCGTGTATCGGCGCC). A 1421 bp fragment of malZ was amplified in this way using pRP112 as template. The amplified fragment was digested with Ndel, and the resulting 1206 bp fragment was ligated in the heat-inducible expression vector pCYTEP1 (22). The correct orientation of the inserted insert was confirmed by restriction analysis. The 672 bp BglII/EcoRI fragment of this vector was exchanged against a 1491 bp BglII/MunI fragment from pRP112 or pRP114 completing malZ292 and malZ, respectively. pRP112 and pRP114 are equivalent with the exception of the malZ292 mutation.

DNA Sequencing—To sequence the malQ and the malZ292 genes, the plasmids pST4 and pRP112 were used. Plasmid preparation was done with the Qiagen Kit (Diagen GmbH, Hilden, Germany). Double stranded DNA was denatured (23) and sequenced by the chain termination method (24) with the Sequenase kit (U.S. Biochemical Co.) using seven primers obtained from the MWG-BIOTECH Co.

Purification of MalZ and MalZ292—Strain RP96 harboring either pRP118 or pRP119 was grown at 28°C in 2 liters of LB (17) to an optical density of 0.75 at 578 nm (A578). The culture was shifted to 42°C for 2 h, harvested by centrifugation, washed in 50 ml Heps, pH 7.0, and resuspended in 35 ml of the same buffer. The cells were disrupted by one passage through a French pressure cell at 16,000 p.s.i. DNase was added followed by incubation for 30 min. Inclusion bodies were collected by centrifugation (12,000 x g for 10 min). The pellet was solubilized in 10 ml of 50 mM Heps containing 4 mM guanidinium HCl and 200 mM dithiothreitol. The protein concentration was adjusted to 1 mg/ml (total volume 100 ml) and put into a dialysis bag with 300 ml external volume of the same buffer containing 4 mM guanidinium HCl. Renaturation was achieved by slowly (during 12 h) diluting the outside dialysate with the 5-fold volume of MMA at 4°C. Subsequently, the protein solution was dialyzed twice against 2 liters of MMA overnight. Renaturation of the precipitate was removed by centrifugation (18,000 x g for 30 min). The precipitate was supplemented with 40% saturated (NH4)2SO4, and cleared by centrifugation. The supernatant was precipitated with 60% (NH4)2SO4. The precipitate was collected by centrifugation, dissolved in 50 mM sodium phosphate buffer, pH 7.0, and dialyzed against the same buffer. The protein was further purified in 2 ml portions by fast protein liquid chromatography anion exchange column chromatography (Mono Q, Pharmacia Biotech Inc.) in 50 mM sodium phosphate buffer, pH 7.0. The column was eluted with 20 ml of a linear NaCl gradient (0–300 mM) in the same buffer.

Transport of [14C]Maltose—Cultures were grown logarithmically in MMA containing 0.4% glucose as carbon source to an A578 of 0.5. When indicated, 1 mM maltose was added 2 h previously. The cultures were harvested, washed three times with MMA, and resuspended in the same medium to an A578 of 0.5 (glycerol-grown cultures) or 0.05 by the consecutive hydrolysis of glucose from the transfer product.

EXPERIMENTAL PROCEDURES

Construction of Strains—Derivatives of E. coli K12 strains were used throughout this study. They were constructed by P1 vir transduction (17) and are listed in Table I. To isolate the malZ292 mutation, strain HS3166, a maltose constitutive malQ’::10 mutant, was grown in minimal medium A (MM) (17) containing 0.4% glucose. 2 x 10^10 cells were plated on MMA with 0.2% maltose. Colonies were tested for the reversion of the malQ’::10 mutation by transducing them with a P1 lysate prepared on POP4064 (HfrG6 malQ::Tn10). Transductants were selected for tetracycline resistance (Tet’ cbr510) and screened for a Mal’ phenotype.

The abbreviations used are: MMA, minimal medium A; kb, kilobase(s); bp, base pair(s).
plasmid pACYC177. The phoB::Tn5 derivative of strain HS3166 (malQ<sup>am</sup>) was transformed with the ligation mixture. Colonies were selected for resistance against kanamycin, and the ability to derepress alkaline phosphatase was screened. All these clones were able to grow on maltose, pRP100, the plasmid in one of these clones, contained a chromosomal insert of about 12 kb. With the assumption that the gene in question was malZ, we cloned a 2.1-kb MluI/SacI fragment containing malZ in pDH832 yielding pRP112 (Fig. 1). This plasmid still carried the mal<sup>+</sup> second site mutation but was no longer able to complement a phoB mutation. The insert was sequenced in its entire length and verified the identity of its open reading frame as MalZ. The mutant gene contained a G to T exchange in comparison to the wild type malZ sequence resulting in the exchange of Trp to Cys at position 292 of the MalZ polypeptide chain.<ref>

Purification of Mutant and Wild Type MalZ Proteins—In order to study the enzymatic properties of the mutant MalZ enzyme free of activities that might be caused by other maltodextrin-hydrolyzing activities, the purification of the mutant and wild type MalZ was necessary. For this purpose, malZ and malZ292 were cloned into the plasmid pCYTEXP1 (22) allowing the expression of both genes under temperature-sensitive λ repressor control. In both cases, induction resulted in the formation of inclusion bodies. This material was isolated by differential centrifugation followed by solubilization in 4 M guanidinium HCl. Renaturation was achieved by slow dilution in phosphate buffer. Gel electrophoretically homogeneous protein was obtained by anion exchange chromatography in a Mono Q column. The analysis of the different purification steps by SDS-polyacrylamide gel electrophoresis is shown in Fig. 2. All tests were done with protein purified in this manner. To ensure that the observed properties were not due to the denaturation-renaturation treatment, the key experiments (transfer reaction, cyclodextrinase activity) were repeated with crude extract containing soluble native MalZ as well as MalZ292. No differences to the preparation obtained from inclusion bodies were observed.

MalZ292 Is Not Only a Hydrodase as the Wild Type MalZ but Also a Transferase—From the isolation of the malZ292 mutation as a phenotypic Mal<sup>+</sup> revertant of a malQ<sup>am</sup> mutant, it seemed likely that the MalZ mutant enzyme would have acquired the ability to hydrolyze maltose, a property that is absent in the wild type MalZ enzyme. However, maltose was not hydrolyzed by MalZ292 even after long incubation times (data not shown). Maltopentaoside was hydrolyzed (mainly to glucose and maltose) by the wild type as well as the mutant enzyme (Fig. 3A). However, the presence of trace amounts of 1<sup>4</sup>C-labeled glucose or 1<sup>4</sup>C-labeled maltose in the hydrolysis mixture revealed that the mutant MalZ enzyme was able to transfer dextrinyl residues onto the labeled glucose and maltose while the wild type enzyme was not, or only to a minor extent (Fig. 3B). Most significantly, 1<sup>4</sup>C label originally contained in maltose was released as 1<sup>4</sup>C-glucose during the experiment. In the attempt to detect the initial transfer product arising with the mutant enzyme, we tested maltose, maltotriose, maltotetraose, and maltopentaoside as glucosyl donors with 1<sup>4</sup>C-labeled glucose or 1<sup>4</sup>C-labeled maltose as acceptors (Fig. 4). As expected, maltose was not used as a glucosyl donor. When 1<sup>4</sup>C-glucose was the acceptor and unlabeled maltotriose the donor, the initial labeled product was 1<sup>4</sup>C-maltotriose, whereas, with unlabeled tetaose as donor, 1<sup>4</sup>C-tetaose was formed as initial product. This demonstrated that dextrinyl

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<sup>*</sup> Sequencing of malZ revealed some errors in the published sequence that were corrected in the GenBank<sup>TM</sup> entry under the accession number X59839.
transfer onto $[^{14}C]$glucose occurred after the enzymatic release of the reducing glucose moiety from the donor maltodextrin. When $[^{14}C]$maltose was used as acceptor, the first products to appear with maltotriose as donor were $[^{14}C]$maltotetraose as well as $[^{14}C]$maltotriose. With maltotetraose as donor, $[^{14}C]$maltopentaose as well as $[^{14}C]$maltotetraose appeared as first products. This is consistent with the mechanism of transfer onto $[^{14}C]$glucose mentioned above. Dextrinyl transfer onto $[^{14}C]$maltose at the nonreducing end would occur after the enzymatic release of the reducing end glucose moiety of the donor. This first product will thus contain two consecutive $^{14}C$-labeled glucose residues at the reducing end. This product itself is a good substrate of MalZ and will lead to the quick release of $[^{14}C]$glucose as well as a $[^{14}C]$-labeled dextrin that is smaller by one glucose moiety. With unlabeled pentaose as donor, the results are less clear since the products formed were not sufficiently separated by the TLC technique. It is noteworthy to emphasize that the MalZ292 enzyme, aside from its increased activity as a dextrinyl transferase, still shows the same activity as the wild type enzyme in its function as a maltodextrin glucosidase, i.e. in the net formation of glucose from maltodextrins.

MalZ is a $\gamma$-Cyclodextrinase—In our previous publication on the properties of MalZ, we reported that the enzyme only hydrolyzed linear maltodextrins up to seven glucose residues in chain length. The two cyclodextrins tested, $\alpha$- and $\beta$-cyclodextrin with a length of six and seven glucosyl residues, were not hydrolyzed (14). In testing the substrate specificity of the mutant MalZ enzyme, we found that it readily hydrolyzed $\gamma$-cyclodextrin (containing eight glucosyl residues) and to a very minor extent $\beta$-cyclodextrin but not $\alpha$-cyclodextrin. The same was true for the wild type MalZ enzyme (Fig. 5). Following the time-dependent appearance of the products, we first observed the formation of linear maltooctaose, followed by linear maltoheptaose. The same products and not the starting material ($\gamma$-cyclodextrin) became labeled from the trace amounts of $[^{14}C]$glucose present as acceptor. Only after some time, glucose, maltose, and small amounts of maltotriose became labeled (Fig. 6). Apparently linear maltoctaose and maltoheptaose are rather poor substrates. Only after the removal of glucose or maltose moieties from the reducing end of these dextrins does the subsequent hydrolysis proceed rapidly. Again, the transfer reaction occurred in significant amounts only when the mutant MalZ292 enzyme was used but not with the wild type MalZ.

![Fig. 1. Cloning of malZ292. a, region of the E. coli chromosome around minute 9 of the genetic map that is cotransducible with the malQ\textsuperscript{am} suppressor mutation. b, plasmid pRP100 that had been selected for its ability to complement a phoB::Tn5 mutation and which carried the malZ292 mutation. c and d, subcloning of malZ292 for minimal size. The letters above the lines show the restriction sites: B, BamHI; M, MluI; P, PstI, Ps, PsAlI; S, SaeI.](http://www.jbc.org/)

![Fig. 2. SDS-polyacrylamide gel electrophoresis of the different fractions in the purification of MalZ and MalZ292. Lane 1, molecular mass standards; lanes 2–5, purification of MalZ292; lanes 6–9, purification of wild type MalZ. Lanes 2 and 6, crude extracts of cells that have undergone heat induction; lanes 3 and 7, guanidinium HCl-insoluble pellet remaining after solubilization and renaturation of inclusion bodies; lanes 4 and 8, renatured protein after solubilization of inclusion bodies; lanes 5 and 9, homogeneous protein after Mono Q ion exchange chromatography. Except for the pure protein, 10 µg of total protein was applied on each lane. The gels consisted of 10% polyacrylamide and were stained with Coomassie Blue.](http://www.jbc.org/)
We conclude that the hydrolysis of \(\gamma\)-cyclodextrin by the mutant as well as the wild type enzyme occurs by opening the ring followed by the consecutive release of glucose or maltose from the reducing end of the linear dextrin finally yielding glucose and maltose.

Determination of Glucose after MalZ-dependent Hydrolysis of Maltodextrins—The qualitative appearance on TLC plates of about equimolar amounts of glucose and maltose after the hydrolysis of \(\gamma\)-cyclodextrin, obviously incompatible with a consecutive release of glucose residues after opening the circular dextrin, prompted us to reanalyze the amount of glucose released from different dextrins after end point hydrolysis with the MalZ enzyme. These data are shown in Table II. A consecutive hydrolysis of glucosyl residues from the reducing end of the maltodextrins would result in the liberation of 1, 2, 3, and so forth glucose from maltotriose, maltotetraose, maltopentaose, and so on. Whereas maltotriose and small dextrins followed this pattern, from increasingly longer dextrins too little glucose was released. With heptaose as substrate instead of 5 eq of glucose, only three were released. From \(\gamma\)-cyclodextrin instead of six glucose molecules, only four were released. Thus, it was likely that MalZ not only liberates glucose but also maltose or maltotriose from longer maltodextrins.

When the mutant MalZ enzyme was used in this assay, the amount of glucose released was significantly higher with all maltodextrins tested. We interpret this as the consequence of the maltodextrinyl transfer reaction associated with the mutant enzyme. This would result in the transfer of dextrinyl moieties onto maltose and subsequent release of glucose. This reaction is essentially the reason why the malZ292 mutation in a malQ background gives rise to maltose utilization.

Hydrolysis of Maltotriose and Maltotetraose \(^{14}\)C-labeled at the Reducing End Glucose Residue—In our previous publication on the mechanism of the MalZ enzyme, we had concluded that the enzyme is a glucosidase releasing glucose consecutively by opening the ring followed by the consecutive release of glucose or maltose from the reducing end of the linear dextrin finally yielding glucose and maltose.

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**Fig. 4.** The dextrinyl transfer reaction of MalZ292. Unlabeled maltose (lanes 1 and 5), maltotriose (lanes 2 and 6), maltotetraose (lanes 3 and 7), and maltopentaose (lanes 4 and 8) at concentrations corresponding to 20 mM glucosyl residues were incubated with 10 mM \[^{14}\text{C}\]maltose (lanes 1-4) or 20 mM \[^{14}\text{C}\]glucose (lanes 5-8) in 15 l of 50 mM sodium phosphate, pH 7.0, with 1 mM of pure MalZ for 2 min. 10 l were spotted on a TLC plate and developed with butanol: ethanol: water (5:3:2). A, chemical detection by charring with sulfuric acid; B, autoradiography prior to charring.
The formation of linear maltodextrins, labeled at the reducing end glucosyl residue. The reaction mixture contained 100 mM maltotriose, 0.4 mM \([^{14}C]\)glucose (5 \(\mu\)Ci), and 0.5 mg of crude extract of a MalQ-overproducing strain in 250 \(\mu\)l of 50 mM Tris-HCl, pH 7.0. After 1 h, the mixture was chromatographed on descending paper chromatography on Whatman 3MM with butanol:ethanol:water (3:2:2, v/v/v) followed by chemical detection with charring. The figure represents a portion of the autoradiogram with the large radioactively labeled compounds at the earlier time points is not identical with \(\gamma\)-cyclodextrin, the starting material.

MalZ292-dependent in Vivo Maltose Utilization Occurs in the Absence of Amylomaltase and Maltodextrin Phosphorylase—

The properties of the MalZ292 enzyme suggested that the utilization of maltose as carbon source would, aside from the requirement of a dextrin primer, not necessitate the presence of an additional enzyme. Thus, in a net reaction, glucose would be formed from maltose by MalZ292. It was therefore necessary to demonstrate that MalZ292 would allow growth on maltose in the absence of amyloamaltase (the malQ-encoded enzyme) and maltodextrin phosphorylase (the malP-encoded enzyme).

Surprisingly, a malT\(^{+}\) malP::Tn10 mutant (strain RP97) lacking maltodextrin phosphorylase and (by the polar effect of
Maltodextrins hydrolyzed by MalZ. Samples of 0.08 µCi of maltotriose (lanes 5 and 7) and maltotetraose (lanes 6 and 8). 

The Tn10 insertion on malQ also amylomaltase and carrying malZ292 as a chromosomal mutation utilized maltose much better than strain RP98 (malT+ malQ::Tn10 malP+ with malZ292 as a chromosomal mutation. Careful analysis with the purest available maltose (Merck) revealed that strain RP98 only grew overnight to an A578 of about 0.3–0.5. After a long incubation time (between an additional 10 and 20 h), growth resumed but was due to malQ+ reversions. In contrast, strain RP97 (malP::Tn10 malQ malZ292) grew well and was fully outgrown overnight on the same purest maltose preparation (Merck). In addition, the sterile filtered spent medium of strain RP98 (malQ::Tn10 malP+ malZ292), harvested at a time when growth had stopped at A578 of 0.4, allowed full growth of strain RP97 (malP::Tn10 malQ malZ292). As tested by TLC, the spent medium still contained large amounts of maltose. This indicates that endogenously produced maltodextrin needed for the MalZ292-mediated utilization of maltose will be partially eliminated by maltodextrin phosphorylase. Apparently less pure maltose preparations contain small quantities of maltodextrins allowing the MalZ292-mediated utilization of maltose even in the presence of maltodextrin phosphorylase.

Lack of Phosphoglucomutase (Encoded by pgm) Does Not Prevent MalZ292-dependent Utilization of Maltose—We found that the introduction of a pgm mutation (lacking phosphoglucomutase) into a malQ malZ292 strain (RP91) did not prevent the utilization of maltose; under these conditions, malP+ encoding maltodextrin phosphorylase no longer prevented growth in the pgm mutant even on pure maltose. This again demonstrated firstly that in contrast to the amylomaltase/maltodextrin phosphorylase-mediated pathway the “usable glucose unit” produced from maltose in the malZ292 mutant was not glucose 1-phosphate but glucose. Secondly, it showed that the inability to utilize glucose 1-phosphate via phosphoglucomutase counteracted the maltodextrin-degrading activity of maltodextrin phosphorylase. Possibly, maltodextrin phosphorylase under conditions of excess glucose 1-phosphate even catalyzes the reverse reaction, the synthesis of maltodextrins.

The malZ292 mutation was isolated as a second site Mal+ revertant from a malQ mutant but obviously not on pure maltose. We determined the growth rate of the malQ malZ292 mutant on maltose in comparison to a malQ malZ292 strain. While the malZ+ strain did not grow at all, we did notice that the malZ292 derivative grew on maltose at different growth rates depending on the brand of maltose that was used as a carbon source (Table III). The introduction of a pgm mutation lacking phosphoglucomutase activity, abolished the difference in the growth rates between the pure and less pure maltose and allowed reasonable growth rates and final cell densities in both cases (Table III). It was somewhat surprising that the rate of growth on maltose in a malQ pgm malZ292 strain (RP93) was dependent on the copy number of the malZ292 gene, the rate of growth becoming considerably higher when malZ292 was plasmid-encoded than when chromosomally encoded.

The MalZ292-mediated Utilization of Galactose in a pgm Mutant Requires the Presence of MalP-encoded Maltodextrin Phosphorylase—Recently we had concluded that growth on galactose in a pgm mutant (27) required the presence of all maltose utilizing enzymes, MalP, MalQ, and MalZ. Mutations in any of the genes encoding these enzymes abolished the ability to grow on galactose and to develop the galactose blue phenotype (9). It was therefore of interest whether or not the malZ292 mutation in a malQ pgm mutant would allow growth on galactose. Strain RP91 (malZ292 malQ pgm) could still grow on galactose, albeit slowly. However, RP99 (malQ malP pgm) harboring plasmid-encoded malZ292 (pPL1227) did not grow on all on galactose. Thus, the formation of maltodextrins from the galactose-derived glucose 1-phosphate (via the postulated maltose/maltotriose phosphorylase involved in inducer synthesis) and their subsequent degradation to glucose by the MalZ292 enzyme is too slow to support growth on galactose. We therefore conclude that galactose utilization in a malZ292 pgm mutant appears to require maltodextrin phosphorylase, the malP gene product, whereas maltose utilization via MalZ292 does not.

The Glycogen-derived Production of Maltodextrins Is Not Essential for the MalZ292-dependent Utilization of Maltose—The introduction of a glgA mutation that prevents glycogen formation did not alter the ability of the malQ pgm malZ292 mutant to grow on maltose (strain RP93). This demonstrates once again that aside from the glycogen-dependent pathway a second origin of maltodextrins needed as primers for the MalZ292-dependent maltose utilization must exist. Since malQ pgm or malQ pgm glgA strains are no longer constitutive for the expression of the maltose system (see Table IV), the endogenous synthesis of maltotriose (in the absence of external maltose) must be slow. It is certainly slower than the production of maltotriose from glycogen.

The expression of the maltose system is controlled by the level of maltotriose, the endogenous inducer. Obviously, mal-
TABLE IV

| Strain | Genotype | Inducer | Rate of transport |
|--------|----------|---------|------------------|
| HS3166 | malQ<sup>am</sup> | Glycerol | 104 | 106 |
| SFC1   | malQ<sup>am</sup> malZ292 | Glycerol | 64 |
| RP94   | malQ<sup>am</sup> malZ292 glgC | Maltose | 246 | 11.7 |
| RP93   | malQ<sup>am</sup> malZ292 pgm | Glycerol | 17.2 |
| RP95   | malQ<sup>+</sup> glgC | Maltose | 96 |
| MC4100 | mal<sup>+</sup> | Glycerol | 1.87 | 105 |

Maltose transport as a measure of mal gene expression in different mutants. Transport was measured at a substrate concentration of 52 nM and is given in picomoles per min per 10<sup>9</sup> cells.

In this publication we report the mutational alteration (Trp to Cys at position 292 of the polypeptide chain) of MalZ that results in the effective utilization of maltose when present in a mutant that lacks amylomaltase, the key enzyme of maltose utilization in E. coli (1). MalZ had previously been identified as an enzyme cleaving glucose from the reducing end of maltodextrins with maltotriose as the smallest substrate. The wild type MalZ enzyme is apparently not involved in maltose utilization since mutants lacking malZ still grow normally on maltose and strains harboring the wild type malZ gene but defective in malQ cannot grow on maltose (29). However, in pgm mutants which still can grow on maltose (27), the function of the malZ-encoded enzyme becomes apparent. Growth on maltose is strongly reduced. Thus, MalZ increases the ratio of glucose over glucose 1-phosphate, the immediate products of maltose utilization.

DISCUSSION

In this publication we report the mutational alteration (Trp to Cys at position 292 of the polypeptide chain) of MalZ that results in the effective utilization of maltose when present in a mutant that lacks amylomaltase, the key enzyme of maltose utilization in E. coli (1). MalZ had previously been identified as an enzyme cleaving glucose from the reducing end of maltodextrins with maltotriose as the smallest substrate. The wild type MalZ enzyme is apparently not involved in maltose utilization since mutants lacking malZ still grow normally on maltose and strains harboring the wild type malZ gene but defective in malQ cannot grow on maltose (29). However, in pgm mutants which still can grow on maltose (27), the function of the malZ-encoded enzyme becomes apparent. Growth on maltose is strongly reduced. Thus, MalZ increases the ratio of glucose over glucose 1-phosphate, the immediate products of maltose utilization.

The mutant enzyme MalZ292 still exhibits the same activity as the wild type MalZ enzyme. Surprisingly, maltose itself was not hydrolyzed by the mutant enzyme. The only difference that we could detect was the ability of the mutant enzyme to transfer dextrinyl residues originating from maltotriose and larger dextrans onto maltose. This indicates that it is the transfer reaction onto maltose that allows maltose utilization. The transfer reaction observed with the mutant MalZ enzyme is reminiscent of the action of amylosidase, the enzyme product of malQ. This enzyme will disproportionate any given maltodextrin longer than maltose into glucose and a series of maltodextrins in such a way that the number of glycosidic linkages will remain constant. Thus, amylosidase is exclusively a maltodextrin transferase but not a hydrolase. For every glucose released (the apparent hydrolysis reaction), it will form a new glycosidic linkage by producing a longer dextrin.

Even though the hydrolysis and the transfer reaction observed with the mutant MalZ enzyme is formally the same as in amylosidase, there is still a basic difference between the two enzymes. In the mutant MalZ, hydrolysis of glucose is not obligatorily coupled to the transfer reaction, and net hydrolysis of maltodextrins to glucose and maltose is still the predominant reaction. Nevertheless, with a continuous supply of small amounts of dextrans (maltotriose and larger), maltose can be degraded to glucose by the mutant MalZ enzyme. This does not require the presence of an additional enzyme. In contrast, when maltose is degraded to glucose by the “normal” amylosidase-mediated pathway, aside from the requirement of a maltodextrin primer, the action of maltodextrin phosphorylase, the malP product, is needed to remove the accumulation of longer dextrans (produced by amylosidase) by producing glucose 1-phosphate that enters glycolysis after phosphoglucomutase-mediated transformation into glucose 6-phosphate.

The properties of the mutant MalZ enzyme again necessitate postulating an endogenous production of maltodextrins that can act as a dextrinyl donor in the MalZ292-mediated utilization of maltose. In the malQ mutant, the major source of these dextrans including maltotriose, the inducer of the system, is clearly glycogen (9, 30). However, also the pgm or the pgm glgA derivative of the malQ malZ292 strain can still grow on maltose. These strains do not contain detectable glycogen and are not constitutive for the maltose system even though they still can be induced by maltose. Thus, it is obvious that there exists a second pathway for the synthesis of endogenous maltodextrins that do not originate from glycogen. We have previously postulated the existence of a maltose/maltotriose phosphorylase that would produce maltose from glucose and glucose 1-phosphate and additionally maltotriose from maltose and glucose 1-phosphate (9). Since phosphorylases are reversible, maltose plus phosphate would produce glucose 1-phosphate which then could give rise to the synthesis of small maltodextrins needed for the MalZ292-mediated maltose utilization in the malQ mutant background. The observation that pgm mutants in this background exhibit an increased rate of maltose
utilization is consistent with this picture. Glucose 1-phosphate produced from maltose (by the postulated maltose/maltotriose phosphorylase) would not be removed by phosphoglucomutase (forming glucose 6-phosphate followed by glycolysis). A testable prediction would therefore be that malQ pgm mutants when exposed to maltose will have an elevated level on glucose 1-phosphate even in the absence of maltodextrin phosphorylase. Clearly, the postulated maltose/maltotriose phosphorylase cannot be very active. Otherwise, this enzyme alone should give rise to growth on maltose in the absence of any other malt enzymes.

In the course of this investigation, we observed that the MalZ enzyme, wild type as well as MalZ229 mutant, was able to hydrolyze γ-cyclodextrin, but not α-cyclodextrin, and β-cyclodextrin only to a minor extent. Several cyclodextrinases have been isolated in the past and identified as a special type of maltodextrin hydrolase. They exhibit molecular weights of 66,000–72,000 and differ from α-amylases, to which they exhibit sequence homology (including their conserved motifs), by their weak activity in hydrolyzing starch. Generally, they exhibit transglycosylating activity that becomes prominent only after pullulan consistent with the claim that E. coli does not contain pullulanase (34). At present, the physiological role, if any, of the γ-cyclodextrinase activity of MalZ is unclear. E. coli is unable to transport or to grow on γ-cyclodextrin.

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