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Escherichia coli Mutants Impaired in Maltodextrin Transport

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Wild-type *Escherichia coli* K-12 was found to grow equally well on maltose and on maltodextrins containing up to seven glucose residues. Three classes of mutants unable to grow on maltodextrins, but still able to grow on maltose, were investigated in detail. The first class, already known, was composed of phage λ-resistant mutants, which lack the outer membrane protein coded by gene *lamB*. These mutants grow on maltose and maltotriose but not at all on maltotetraose and longer maltodextrins which cannot cross the outer membrane. A second class of mutants were affected in *malE*, the structural gene of the periplasmic maltose binding protein. The maltose binding proteins isolated from the new mutants were altered in their substrate binding properties, but not in a way that could account for the mutant phenotypes. Rather, the results of growth experiments and transport studies suggest that these *malE* mutants are impaired in their ability to transport maltodextrins across the outer membrane. This implies that the maltose binding protein (in wild-type strains) cooperates with the λ receptor in permeation through the outer membrane. The last class of mutants described in this paper were affected in *malG*, or perhaps in an as yet undetected gene close to *malG*. They were defective in the transfer of maltodextrins from the periplasmic space to the cytoplasm but only slightly affected in the transport of maltose.

At least five proteins are specifically involved in the transport of maltose and maltodextrins across the envelope of *Escherichia coli* (14a, 20a). The genes coding for these proteins are clustered in two adjacent operons, *malE-malF-malG* and *malK-lamB*, which together compose the *malB* region of the genetic map (Fig. 1) (14a, 20a). The *lamB* protein is located in the outer membrane and was initially recognized as the receptor for phage λ (15). The *malE* protein is periplasmic and has an affinity for maltose and maltodextrins (10, 19). The *malF* protein is located in the cytoplasmic membrane (21; H. A. Shuman, T. J. Silhavy, and J. R. Beckwith, manuscript in preparation), whereas the *malG* and *malK* proteins have not yet been identified. All *malE*, *-F*, *-G*, or *-K* mutants described so far were selected as maltose negative (Mal^-) and later found to be also unable to grow on longer maltodextrins (Dex^-). All *lamB* mutants, on the other hand, have been selected for resistance to phage λ (9, 18) or, more recently, to phage TP1 (26) or K10 (M. Roa, J. Bacteriol., in press). Some of the *lamB* mutants, the nonsense mutants in particular, were later found to be impaired in the transport of maltose and maltodextrins (24, 25). The phenotype of these *lamB* nonsense mutants with respect to substrate transport is, however, clearly different from that of mutant *malE*, *-F*, *-G*, or *-K* since, under the usual growth conditions, they are Dex^- but Mal^+; their defect in maltose transport can only be observed at very low substrate concentrations (24). More precisely, it was found that the apparent affinity of the transport system for maltose was 100 to 500 times lower in a *lamB* nonsense mutant than in a wild-type strain, whereas the maximum rate of transport observed at saturating maltose concentrations, such as those normally present in growth media, was the same in mutant and wild type (25). In view of this result, and considering the location of the *lamB* protein in the cell, it was proposed that this protein facilitates the diffusion of maltose and maltodextrins across the outer membrane. However, the outer membrane of *E. coli* contains about 10^5 protein molecules (the porins) which constitute diffusion channels for hydrophilic substances of a molecular weight lower than 600 (12). Accordingly, two questions are posed about the function of the *lamB* protein. First, why does this protein facilitate the transport of maltose, a compound which is small enough (molecular weight, 342) to go through the porins? Second, is the role of the *lamB* protein in the transport of maltodextrins only to provide larger channels for these substrates (2, 3), which are too bulky to go through the porins?

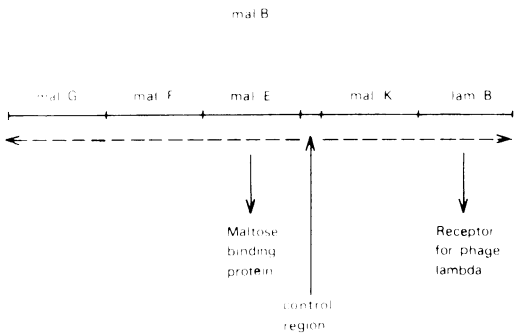

FIG. 1. *malB region of the E. coli chromosome. The direction of transcription of the two operons is indicated by the broken lines.*

In trying to answer these questions and, more generally, to understand the mechanism whereby the *lamB* protein facilitates the diffusion of maltose and maltodextrins across the outer membrane, it seemed essential to find out whether the *lamB* protein acts alone in this process or, on the contrary, cooperates with one or several other envelope components. If such other components exist, mutations in the gene(s) coding for their synthesis ought to lead to the same phenotype as do *lamB* mutations, i.e., Dex^- Mal^+. The result of a systematic search for mutants bearing this phenotype is presented in this paper, and one class of mutants, which are affected in the *malE* gene, is described in detail. Their characterization indicates that the *malE* protein cooperates with the *lamB* protein in bringing maltose and maltodextrins into the periplasmic space.

MATERIALS AND METHODS

Bacterial and bacteriophage strains. The bacterial strains used are listed in Table 1. Phage λV (a virulent mutant of phage λ) and its host range mutants λVho and λVh4h*16 are described in reference 9.

Media and standard techniques. Most growth media (6) and techniques for transduction (11), conjugation (14a), complementation, and mutagenesis with ethylmethane sulfonate or *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (18) were already described.

The dextrin media were prepared as follows: 100 g of amylose was suspended in 500 ml of 1 N sulfuric acid at 100°C for 20 min. The suspension was then rapidly cooled by adding 500 ml of ice-cold water, and the hydrolysate was neutralized by adding solid barium hydroxide. The resulting precipitate and the remaining insoluble amylose were removed by centrifugation at 5,000 rpm for 10 min. The supernatant was concentrated 10-fold by evaporation in a rotary evaporator and then dialyzed for about 10 h at 4°C against 3 liters of distilled water. After a second concentration by evaporation, the solution was sterilized and used diluted in liquid and solid media. The amount to be used in media was determined empirically so as to give good growth of a $lamB^+$ strain and very poor growth of a *lamB* nonsense mutant. The concentration corresponds to about 5×10^{-3} mol of glucose residues per liter of medium. The average degree of polymerization of the crude dextrin mixture was about 12 glucose residues per dextrin, as determined from the content of glucose residues (20) and reducing groups (23).

Preparation of maltodextrins of defined lengths. Amylopectin-free amylose (10 g; Sigma grade III) was hydrolyzed by suspension in 100 ml of 1 N sulfuric acid at 100°C for 20 min. The hydrolysate was cooled in ice and neutralized by adding solid barium hydroxide. The resulting precipitate was removed by centrifugation, and the supernatant was collected and freeze-dried. The dried dextrins were dissolved in 10 ml of distilled water. Undissolved material (including some large-molecular-weight dextrins) was removed by centrifugation and discarded. Separation of dextrins according to molecular weight was achieved by chromatography on a column (85 by 4 cm) of Bio-Gel P2 (200–400 mesh), with water as elution solvent. Fractions of 5 ml were collected at a flow rate of 60 ml/h, and each fraction was tested for the presence of dextrins by thin-layer chromatography on Kieselgel 60 (Merck) plates, using pyridine-ethyl acetate-water (8:4:3, by volume) as the solvent system. Sugars were detected by charring in the presence of 20% (wt/vol) H_2SO_4. Column fractions containing similarly migrating dextrins were combined and concentrated by freeze-drying, and their purity was rechecked by thin-layer chromatography as shown in Fig. 2. The resulting dextrin fractions were not all pure but were resolved into fractions with narrow molecular weight distributions. Up to maltodecaose, impurities consist only of the next higher or lower dextrin homolog, but longer fractions were less well resolved. The average degree of polymerization of each combined dextrin fraction was confirmed by the techniques described in the preceding section.

Growth rates on maltodextrins. Wild-type and mutant bacteria were taken from exponentially growing cultures in minimal medium containing 0.8% maltose plus amino acid supplements. The bacteria were collected by centrifugation, washed twice with minimal medium without carbon source, and suspended at an optical density of 0.1 at 578 nm in 2.5 ml of minimal medium containing the required amino acids at 40 μg/ml and the specified dextrins. Growth was followed by measurements of optical density at 578 nm.

Isolation of Dex^- Mal^+ mutants. After mutagenesis with ethyl methane sulfonate or *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, independent cultures were grown to saturation in maltose minimal medium to lower the proportion of Mal^- Dex^- mutants. The cultures were then diluted in dextrin minimal medium and grown for two generations to an optical density of 0.4 at 578 nm. Penicillin was then added to each culture at a concentration of 2,000 IU/ml (11). After 75 min of incubation, the cells were centrifuged, washed, and resuspended in maltose minimal medium. After overnight growth, the cultures were plated on dextrin plates. After 2 days of incubation at 37°C the tiny colonies were picked and purified. Only 10 to 20% proved to be really Dex Mal^+. Three of the mutants described were isolated after ethyl methane sulfonate mutagenesis without penicillin treatment (*malE88*,

TABLE 1. *Bacterial strains*

Strain	Genotype	Origin
HfrG6	Hfr *his*	Hofnung et al. (9)
pop 1021	Hfr *metA trpE*	Raibaud et al. (14a)
pop 1080	Hfr *metA trpE lamB102*	Hofnung et al. (9)
pop 1092	Hfr *metA trpE lamB114*	Hofnung et al. (9)
pop 1759	Hfr *his malE17*	Kellerman and Szmelcman (10)
pop 271	F⁻ *thi thr leu tonA rpsL ΔmalB204*	Raibaud et al. (14a)
pop 282	F⁻ *thi thr leu tonA rpsL ΔmalB242*	Raibaud et al. (14a)
pop 284	F⁻ *thi thr leu tonA rpsL ΔmalB213*	Raibaud et al. (14a)
pop 287	F⁻ *thi thr leu tonA rpsL ΔmalB237*	Raibaud et al. (14a)
pop 716	F⁻ *thi thr leu tonA rpsL ΔmalB3*	Raibaud et al. (14a)
pop 720	F⁻ *thi thr leu tonA rpsL ΔmalB7*	Raibaud et al. (14a)
pop 722	F⁻ *thi thr leu tonA rpsL ΔmalB9*	Raibaud et al. (14a)
pop 724	F⁻ *thi thr leu tonA rpsL ΔmalB11*	Raibaud et al. (14a)
pop 726	F⁻ *thi thr leu tonA rpsL ΔmalB13*	Raibaud et al. (14a)
pop 728	F⁻ *thi thr leu tonA rpsL ΔmalB15*	Raibaud et al. (14a)
pop 729	F⁻ *thi thr leu tonA rpsL ΔmalB16*	Raibaud et al. (14a)
pop 733	F⁻ *thi thr leu tonA rpsL ΔmalB103*	Raibaud et al. (14a)
pop 737	F⁻ *thi thr leu tonA rpsL ΔmalB107*	Raibaud et al. (14a)
pop 740	F⁻ *thi thr leu tonA rpsL ΔmalB112*	Raibaud et al. (14a)
pop 468[a]	F′ *argH⁺ malB⁺/argH thy rpsL recA ΔmalB101*	Hofnung (8)
pop 469[b]	F′ *argH⁺ malE⁺ malF⁺ malG⁺/ argH thy rpsL recA ΔmalB101*	Hofnung (8)
pop 794[a]	F′ *argH⁺ malE⁺ malK⁺ lamB⁺ malF7/argH thy rpsL recA ΔmalB101*	Hofnung (8)
pop 796[a]	F′ *argH⁺ malE10 malK⁺ lamB⁺ malF⁺/argH thy rpsL recA ΔmalB101*	Hofnung (8)
pop 1151	Hfr *his malE88*	EMS[c]-induced mutant of HfrG6
pop 1152	Hfr *his malE89*	EMS-induced mutant of HfrG6
pop 1153	Hfr *his malE254*	EMS-induced mutant of HfrG6
pop 1154	Hfr *metA trpE malE110*	NTG[d]-induced mutant of pop 1021
pop 1155[e]	Hfr *his malB102*	NTG-induced mutant of HfrG6
pop 1156[e]	Hfr *metA trpE malB455*	NTG-induced mutant of pop 1021

[a] The episome in this strain is KLF12.
[b] The episome in this strain is KLF10.
[c] EMS, Ethyl methane sulfonate.
[d] NTG, *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine.
[e] The *malB* mutations in these strains are probably in *malG* (see text).

malE89, *malE254*) and the others were isolated after *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine mutagenesis and penicillin treatment.

Blue test. The blue test was performed as described by Schwartz (17).

Maltose transport. The transport assays were essentially performed as described previously (24).

Purification of mutant maltose binding proteins. Maltose binding protein from the four *malE* mutants was purified by a slight modification of the affinity chromatographic method described for wild-type protein (5).

Bacteria were grown in minimal medium with 0.2% glycerol plus 0.2% maltose as carbon sources plus appropriate amino acid supplements.

Osmotic shock fluids were prepared and chromatographed on cross-linked amylose as previously described (5). In contrast to wild-type binding protein, the mutant proteins could not be eluted with 10 mM maltose from amylose columns and required much higher concentrations of maltose for elution (0.5 M). The presence of maltose binding protein in the eluted fractions was determined by cross-reactivity against antibodies raised against wild-type binding protein (5). The purity of the preparations was tested by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, using 10% gels in the buffer system of Weber et al. (27).

Binding of substrates to the *malE* protein. The affinity of the *malE* protein for maltose was determined in shock fluids by equilibrium dialysis as described previously (19). The affinity of purified *malE* protein for maltose and maltodextrins was determined by using the fluorescence quenching assay (25) with a Perkin-Elmer MPF3 fluorescence spectrophotometer.

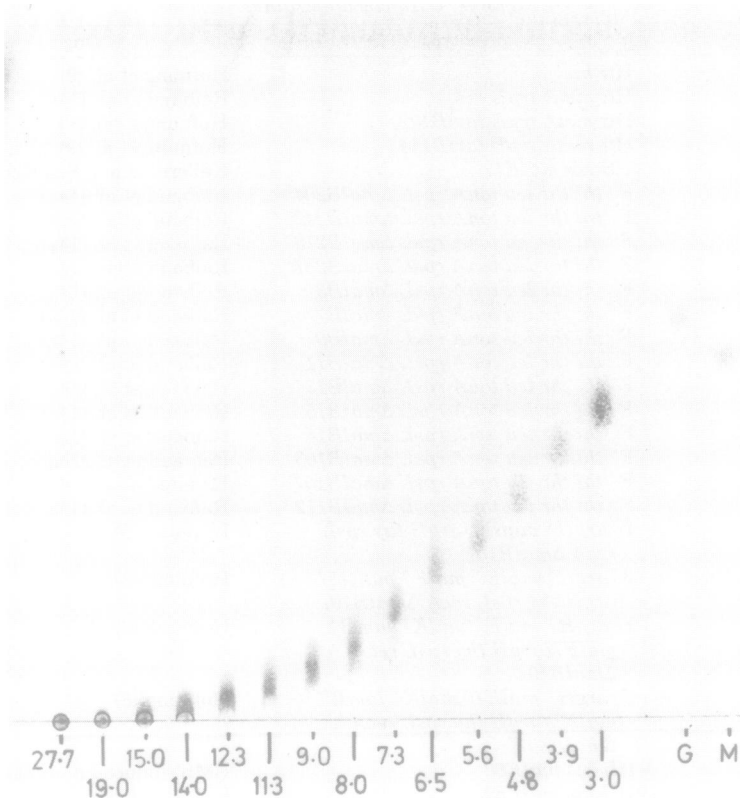

FIG. 2. *Purity of the isolated maltodextrin fractions. Gel filtration of an amylose hydrolysate was used to resolve maltodextrins into fractions of narrow molecular weight distribution, as described in the text. Between 10 and 20 μg of total sugar of each fraction was chromatographed on a thin-layer plate (Kieselgel 60), using pyridine-ethyl acetate-water (8:4:3, vol/vol/vol) as solvent. Spots were visualized by spraying the dried developed plate with 20% (vol/vol) sulfuric acid and charring at 120°C for 10 min. The number below each fraction indicates the average degree of polymerization of each dextrin fraction determined by measuring the total glucose content (20) and number of reducing groups (23) in each fraction. G and M refer to 5-μg glucose and 5-μg maltose standards, respectively.*

RESULTS

Isolation of Dex$^-$ Mal$^+$ mutants. To obtain strains which might be affected in a component cooperating with the *lamB* protein in the transport of maltodextrins, we proceeded to select mutants which, like *lamB* nonsense mutants, bear a Dex$^-$ Mal$^+$ phenotype. Three classes were found.

The first class was composed of strains resistant to λ as well as to its host range mutants λVho and λVh4h*16. This pattern of phage resistance is characteristic of *lamB* mutants completely devoid of *lamB* protein (9).

The second class consisted of mutants impaired in the degradation of maltodextrins inside the cell. Such mutants, affected in *malP*, *pgm*, or *pgl*, could be easily recognized because, due to the action of the amylomaltase, they accumulate long maltodextrins (amylose) when grown on maltose, and their colonies turn blue in the presence of iodine (1).

The last class, not previously described, was composed of Dex$^-$ Mal$^+$ mutants that were sensitive to λ and did not stain blue with iodine.

In one given selection, among 32 Dex$^-$ Mal$^+$ mutants, 25 belonged to the first class, 4 to the second, and 3 to the last. Six mutants of the last class, obtained as a result of this and another selection, are described in this paper.

Genetic mapping of the mutations. When stocks of phage P1 grown on strains carrying the Dex$^-$ Mal$^+$ mutations were used to transduce the *metA* strain pop 1021, about 10% of the Met$^+$ transductants were Dex$^-$, as would be expected if the six Dex$^-$ mutations belonged to the *malB* region (16). Complementation tests performed with F′*malB* episomes carrying mutations in the

various genes of the *malB* region demonstrated that four of the six mutations were in *malE*. The results were inconclusive for the other two mutations. The *malE* mutations were mapped by Hfr × F^- crosses, using F^- strains which carry deletions in the *malB* region. The two other mutations were also mapped by the same technique and found to be located either in *malG* or farther to the left in an as yet unidentified gene of the *malB* region (Fig. 3).

Growth of the wild-type and Dex$^-$ Mal$^+$ strains on maltodextrins of various lengths. The dextrins used in the genetic studies (22; 14a; this paper) were a mixture of molecules of various lengths. They were obtained by performing a limited acid hydrolysis of amylose followed by dialysis to eliminate the smallest hydrolysis products (see Materials and Methods). The average chain length of these maltodextrins, as determined from the reducing power, was about 12 glucose units. To test the detailed growth properties of the mutants, maltodextrins were purified by gel filtration into fractions of narrow molecular weight distribution (Fig. 2). These defined maltodextrins, at a concentration of 5×10^{-4} M, were used to grow the wild-type strain or the Dex$^-$ Mal$^+$ mutants (Fig. 4). The growth rate of the wild-type strain was essentially the same for maltodextrins containing between two and seven glucose residues but then dropped abruptly for longer dextrins. As expected (4, 24, 25), *lamB* mutants grew like the wild-type strain on maltose, but their growth rate was much reduced on maltotriose and negligible on larger oligomers. The new *malE* mutants fall into two classes. The *malE254* and *malE110* strains behaved similarly to *lamB* mutants, growing at wild-type rates on maltose but with sharply reduced growth rates between maltose and maltotetraose. With the other two *malE* mutants, the sharp drop in growth rate occurred between maltotetraose and maltohexaose, but still earlier than found with wild-type strains. These latter mutants also grew better on maltotriose than on maltose. The growth patterns of the two presumptive *malG* mutants were very similar to those of the *lamB* mutants.

Effect of increased extracellular dextrin concentration on growth of a *lamB* and a Dex$^-$ Mal$^+$ *malE* mutant. The growth defect of *lamB* nonsense mutants on maltose and maltotriose could be completely overcome by increasing the concentration of these substrates in the medium (24; Fig. 5). However, the growth rate of the same mutants on maltotetraose does not increase significantly when the concentration of this substrate is increased from 5×10^{-4} to 3.1×10^{-3} M (Fig. 5). These results are best explained by assuming that, in the absence of *lamB* protein, maltose (molecular weight, 342) and maltotriose (molecular weight, 504) can diffuse through the porins, whereas maltotetraose (molecular weight, 666) is too bulky to do so. The transport of maltotetraose and higher maltodextrins, therefore, stringently depends on the presence of a functional *lamB* protein in the outer membrane.

The Dex$^-$ Mal$^+$ *malE254* mutant behaved very similarly to the *lamB* mutant (Fig. 5). Its growth defect on maltotriose, but not on maltotetraose, could be overcome by increasing the extracellular concentration of these substrates. The same behavior was found also for *malE89* and *malE110* (not shown).

Maltose transport in the mutants. The rate of maltose transport was determined for the various mutants at different maltose concentrations (Fig. 6). The four *malE* mutants behaved very similarly to nonsense *lamB* mutants: they were strongly affected in the transport of maltose when this sugar was present at a low concentration (10^{-5} M and below) and essentially unaffected at a high concentration (higher than 10^{-4} M). The two presumptive *malG* mutants were slightly affected in maltose transport but to a similar degree at all concentrations. These determinations were performed in strains that metabolize maltose and therefore may not represent true measures of the transport activities. However, some of the *malE* mutations (*malE88* and *malE254*) were transduced into a *malQ* strain (devoid of amylomaltase). The results were essentially identical to those shown in Fig. 6. Also, in studies not shown, the transport properties of *malE89* in strain pop 1152 were found to resemble *malE58* in pop 1151, *malE110* in pop 1154 behaved like *malE254* in pop 1153, and *malB102* in pop 1155 behaved like *malB455* in pop 1156.

Characterization of the *malE* protein in the Dex$^-$ Mal$^+$ mutants. A simple explanation accounting for the phenotype of the Dex$^-$ Mal$^+$ *malE* mutants would be that their *malE* protein had lost its affinity for dextrins while it retained affinity for maltose. To investigate this possibility, we proceeded to look for the *malE* protein in the mutants. A cold osmotic shock was performed on the six strains, according to the procedure of Neu and Heppel (13). The resulting shock fluids were subjected to electrophoresis on polyacrylamide gels in the presence of sodium dodecyl sulfate. A protein migrating like maltose binding protein was present in all cases and in about the same amount as found in wild-type shock fluids (data not shown). Equilibrium dialysis experiments were then performed, using

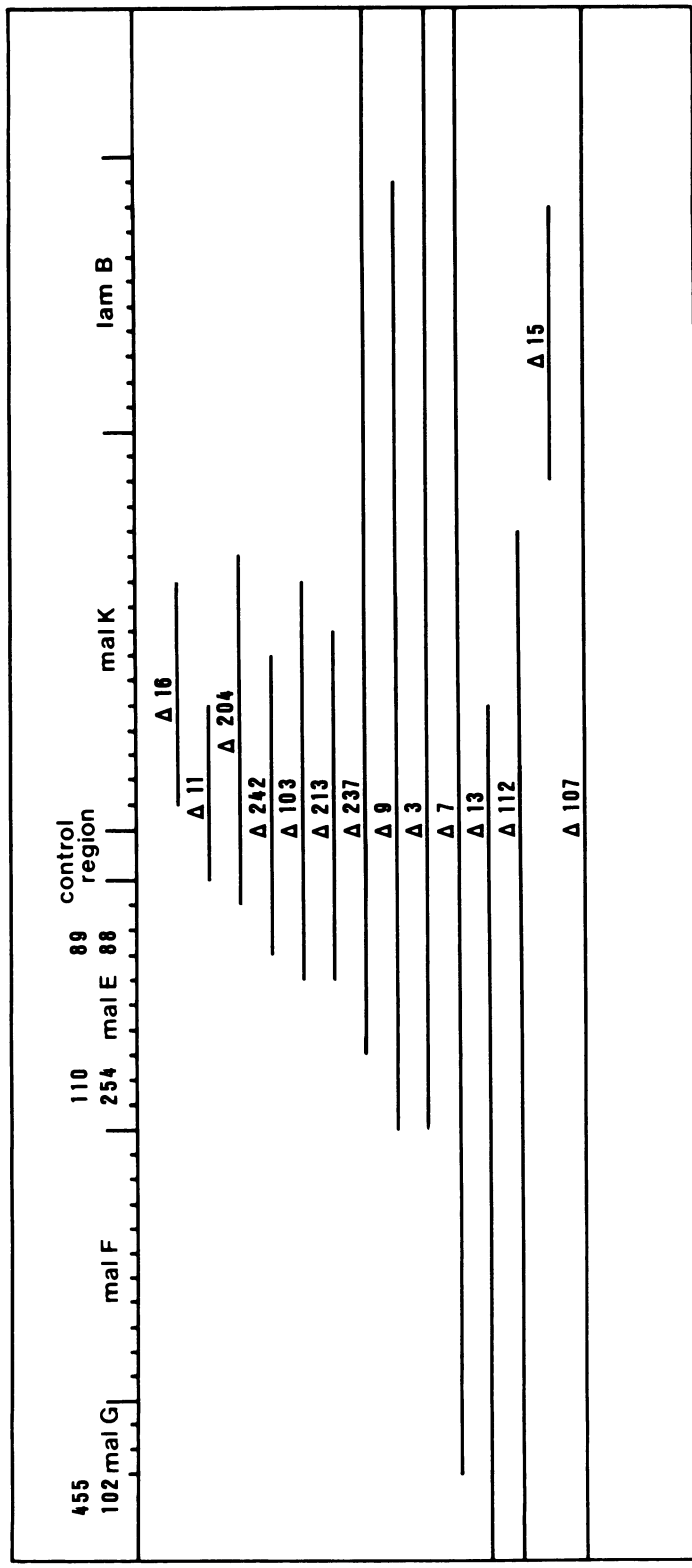

FIG. 3. *Genetic location of the Dex⁻ Mal⁺ mutations. The divisions within the genes correspond to the endpoints of all the deletions that have been used to construct this map (20a). The deletions used in the present work are shown by heavy bars below the map. The position of the Dex⁻ Mal⁺ mutations are shown by numbers above the map. The left end point of the malG gene is not defined on the figure to indicate the fact that mutations 102 and 455 may be inside or outside this gene.*

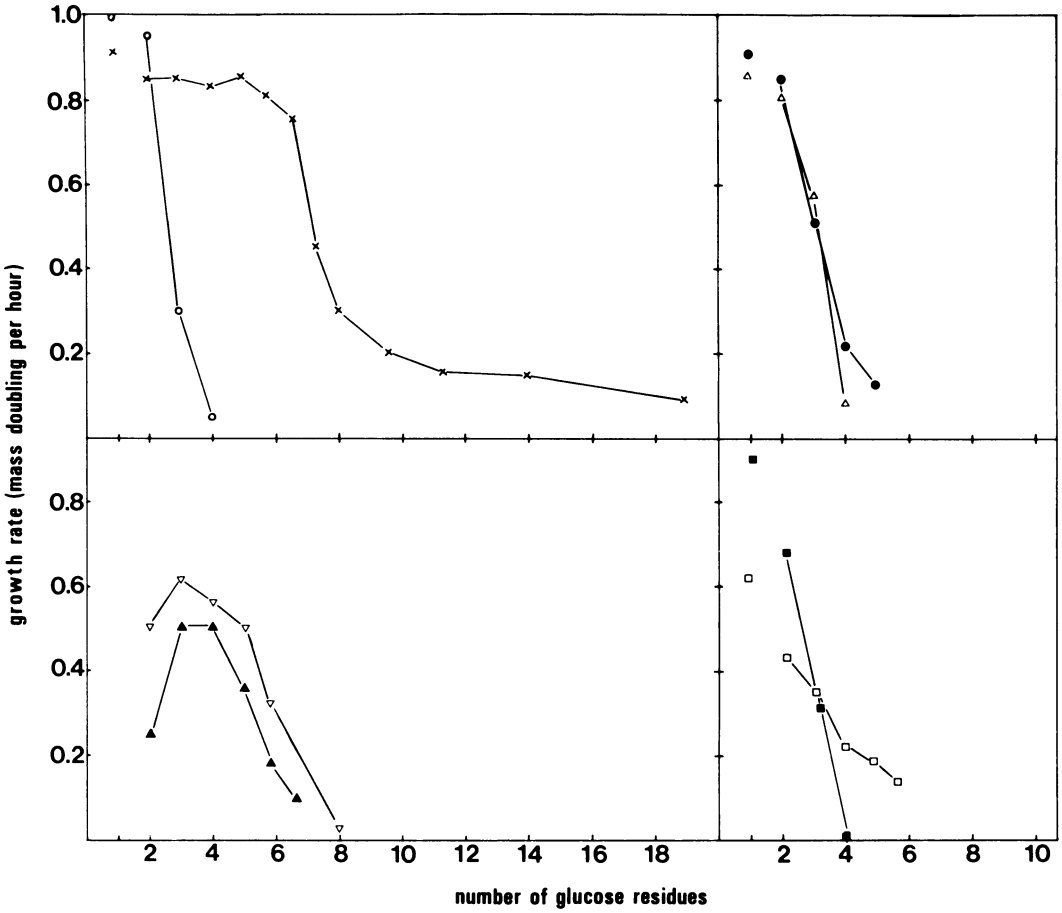

FIG. 4. *Growth rate of the wild-type strain and Dex⁻ Mal⁺ mutants on maltodextrins of various lengths. All substrates were used at a concentration of 5 × 10^{-4} M. The size of the maltodextrins is expressed on the abscissa as the average number of glucose residues in the tested maltodextrin fraction. The value of the growth rate on glucose (n = 1) is shown as a reference for all strains except pop 1151 and pop 1152 (not done). Symbols: Wild-type strain HfrG6 (×); lamB102 mutant pop 1080 (○); malE110 mutant pop 1154 (●); malE254 mutant pop 1153 (△); malE88 mutant pop 1151 (▲); malE89 mutant pop 1152 (▽); malB102 mutant pop 1155 (□); malB455 mutant pop 1156 (■).*

[^{14}C]maltose (10^{-5} M) as ligand. No binding activity could be detected in the concentrated shock fluids containing about 4 mg of protein per ml from the four *malE* mutants, whereas normal activity was found in the two other mutants (presumptive *malG* mutants). Since the *malE* mutants grow on maltose, the lack of detectable binding activity in the shock fluids could have been due either to an inactivation of the proteins during extraction or to the fact that the affinity of the proteins for maltose is too low (K_d higher than 10^{-4} M) for the activity to be found by equilibrium dialysis. Evidence that the proteins had not lost substrate binding activity first came from the observation that they were retained on a cross-linked amylose column like wild-type *malE* protein (5). The binding proteins from the four *malE* mutants could be eluted from the column by adding maltose, but a much higher concentration (0.5 M) of this sugar was required than in the case of wild-type *malE* protein (10^{-3} M). These results not only indicated that these proteins were probably more affected in their affinity for maltose than for amylose, but also provided a method for purifying the mutant proteins (Fig. 7). The affinity of these pure proteins for maltose and various maltodextrins could then be determined by measuring the maltose- or maltodextrin-dependent fluorescence quenching resulting from the binding of these substrates (25). The results (Table 2) are in agreement with the observed behavior

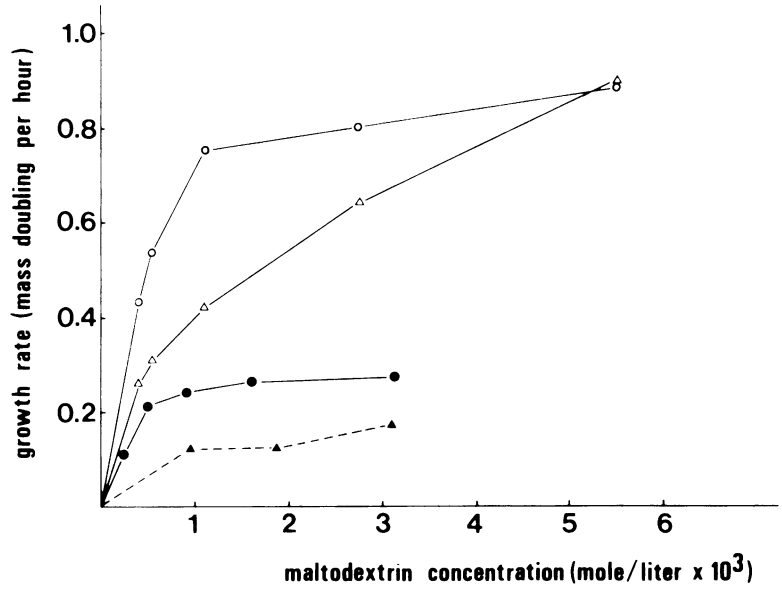

FIG. 5. *Dependence of growth rate on maltodextrin concentration. The experimental conditions were as in the legend to Fig. 4, except for the concentration of substrates. lamB102 mutant pop 1080 grown on: maltotriose* (△); *maltotetraose* (▲). *malE254 mutant pop 1153 grown on: maltotriose* (○); *maltotetraose* (●).

of the protein on the cross-linked amylose column. The substrate binding properties of the mutant *malE* proteins are modified, but the modification is the opposite of that which would have been predicted from the growth properties of the mutants. Indeed, the mutant proteins have the lowest affinity for maltose and maltotriose, which are the best growth substrates, and the highest affinity for dextrins larger than tetraose, which are hardly utilized by the mutant cells. An attempt at resolving this paradox is presented below.

Effect of maltotetraose on maltose transport. In wild-type strains, maltotetraose and maltose share the same periplasmic and cytoplasmic membrane components during transport. It is therefore to be expected that the presence of maltotetraose should inhibit the uptake of [^{14}C]maltose, if the two substrates compete for these shared components. Such an inhibition is indeed observed in the wild-type strain (Fig. 8A). However, if tetraose cannot reach these shared components, due to lack of entry through the outer membrane, then no tetraose inhibition of maltose transport should be observed. This was exactly what was found with the *lamB* mutant, into which maltose can enter freely through porin pores at the 9×10^{-5} M concentration used in these transport experiments (Fig. 8B). Even at a fivefold molar excess of tetraose over maltose, no significant inhibition of maltose uptake occurred in the *lamB* strain. The same competition experiment was also performed with the *malE254* mutant (Fig. 8C). This mutant was not as resistant to tetraose inhibition of maltose uptake as the *lamB* mutant but considerably more so than the wild-type strain. Half-inhibition of maltose transport needed a five- to sevenfold excess of tetraose over maltose in the *malE254* mutant, whereas in the wild-type strain more than 50% inhibition occurred at a 1:1 ratio of tetraose to maltose. This resistance to tetraose inhibition in the *malE254* strain is even more striking when one takes into account the 40-fold-higher affinity of the mutant binding protein for tetraose than for maltose (see above). This result strongly suggests that in the *malE254* mutant, as in the *lamB* strain, maltotetraose cannot reach the periplasmic space.

The effect of maltotetraose on maltose transport was also studied in the presumptive *malG* mutants. In this case maltotetraose inhibition was, if anything, more pronounced than in the wild type (Fig. 8D). This result suggests that the mutations do not affect the ability of the dextrins to reach the binding protein in the periplasm, but rather affects their transport at a later stage, probably through the cytoplasmic membrane. The enhanced inhibition is consistent with tetraose acting as a "dead-end" inhibitor in the periplasm; in the wild type, tetraose can, of course, be removed from the periplasm through further transport.

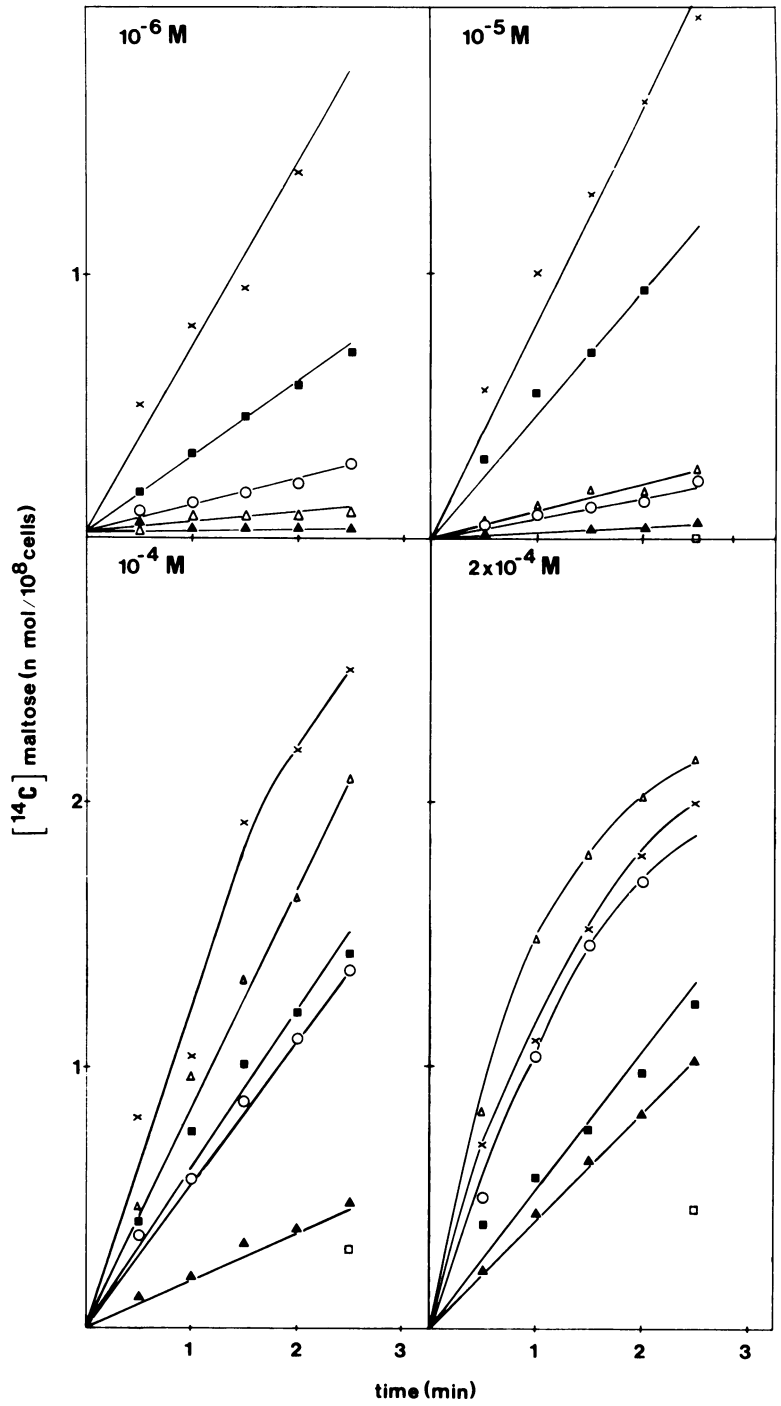

FIG. 6. *Maltose transport in the Dex⁻ Mal⁺ mutants. Maltose transport assays were performed at the concentrations shown in the four panels. The cells were grown at 37°C in maltose minimal medium and harvested in the exponential phase. (The conditions for the tests have been described [24].) The experiments were performed at 25°C. Symbols: Wild-type strain HfrG6 (×); lamB102 mutant pop 1080 (○); malE254 mutant pop 1153 (△); malE88 mutant pop 1151 (▲); malB455 mutant pop 1156 (■); malE17 mutant pop 1756 (□). This last control strain contains a nonsense mutation in malE and is therefore Dex⁻ Mal⁻.*

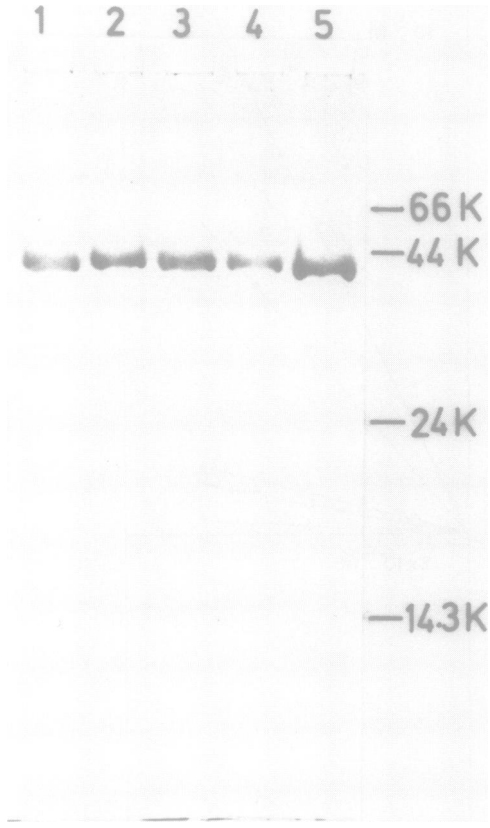

FIG. 7. *Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of wild-type and mutant maltose binding proteins. The proteins were purified by affinity chromatography using cross-linked amylose (5) and were applied to a slab gel containing 10% acrylamide and run using the buffer system of Weber et al. (27). Staining was with Coomassie brilliant blue (27). On the same gels, the positions of the molecular weight standards bovine serum albumin (66K), ovalbumin (44K), trypsinogen (24K), and lysozyme (14.3K) are also shown. Lane 1, 5 μg of wild-type protein; lane 2, 8.5 μg of malE254 protein; lane 3, 7.8 μg of malE89 protein; lane 4, 4.5 μg of malE88 protein; lane 5, 9.6 μg of malE110 protein.*

DISCUSSION

Wild-type *E. coli* grows equally well on maltose and on maltodextrins containing from three to seven glucose residues. Maltoheptaose (molecular weight, 1,150) is probably the largest compound that can be used at a rapid rate by *E. coli* as a carbon source. This paper provides a description of three classes of Dex^- Mal^+ mutants: (i) λ-resistant mutants, believed to lack the *lamB* protein, (ii) *malE* mutants, and (iii) mutants affected in *malG* or in an as yet undetected gene located to the left (see Fig. 1) of *malG*. One class of mutants that might have been expected, but has not been found, consists of *lamB* mutants with a Dex^- phenotype but still sensitive to λ. The only known Dex^- *lamB* mutants are therefore those which have no *lamB* protein in their outer membrane (14a).

The central feature of this study was the isolation of *malE* mutants that are strikingly similar to *lamB* nonsense mutants in terms of growth patterns on maltodextrins and maltose transport properties. Three properties of these mutants will be discussed in turn.

(i) These mutants are unable to grow on maltodextrins which can be utilized by wild-type strains, despite a good affinity of their binding proteins for maltotetraose, -pentaose, -hexaose, and -heptaose.

(ii) The *malE* proteins of the mutants are differentially affected in their affinity for substrates of various lengths.

(iii) The mutants transport maltose at a half-maximal rate at concentrations of this sugar significantly lower (about 10 times) than those which lead to a half-saturation of their binding protein in vitro.

The defect causing inability to grow on long dextrins cannot be simply ascribed to a substrate binding problem, as the mutant proteins retained high affinities (K_d as low a 10^{-5} M) for dextrins upon which no growth occurs. Therefore, unless the mutant binding proteins have completely different binding properties in vitro and in vivo, it seems necessary to postulate either a defect in bringing dextrins to the binding protein through the outer membrane or a faulty transmission of the dextrins from the binding protein to components of the cytoplasmic membrane. We favor the first alternative, which implies that uptake through the outer membrane is not simply diffusion through a *lamB* protein pore, but that the periplasmic binding protein and the outer membrane component must cooperate in bringing dextrins into the periplasm. Evidence in favor of this idea comes from the clear difference between growth patterns on maltotriose, which can just about pass through the porin pores with a molecular weight cut-off of about 600 for sugars, and maltotetraose, which requires a *lamB* protein-dependent mechanism. The *malE254* strain and a *lamB* nonsense mutant are both unable to grow on maltotetraose even when this substrate is present at a concentration of 3×10^{-3} M. The resistance of maltose transport to inhibition by maltotetraose in the *malE254* strain and the *lamB* nonsense mutant provides strong additional support for the existence of a decreased access of the large dextrins to the periplasm in both strains. The peculiar growth pattern of two of the *malE* strains, which

TABLE 2. *Affinity of the mutant binding proteins for maltose and maltodextrins of various degrees of polymerization*[a]

Binding protein from:	K_d (mol/liter) for:				
	Maltose ($n = 2$)	Maltotriose ($n = 3$)	Tetraose, pentaose ($n = 4, 55$)	Hexaose, heptaose ($n = 6, 4$)	Decaose ($n = 10$)
malE$^+$	1.9×10^{-6}	8×10^{-7}	1.6×10^{-6}	2.8×10^{-6}	1.1×10^{-6}
malE254	1.7×10^{-3}	3.3×10^{-4}	3.9×10^{-5}	2.5×10^{-5}	1.6×10^{-5}
malE110	1.1×10^{-3}	4.5×10^{-4}	7.4×10^{-5}	2.3×10^{-5}	3.3×10^{-5}
malE88	5×10^{-3}	9×10^{-4}	1.6×10^{-5}	1.1×10^{-5}	1.1×10^{-5}
malE89	1.1×10^{-2}	7×10^{-4}	4×10^{-5}	2.7×10^{-5}	9×10^{-6}

[a] Maltose binding protein was purified, as described in the text, from strains carrying the *malE* alleles shown. The binding of various maltodextrin fractions containing the average number of glucose residues indicated by the value of n on the first line was determined using fluorescence spectroscopy (25). Excitation of protein fluorescence was at a wavelength of 280 nm, and emission was measured at 350 nm. Measurement of the substrate concentration-dependent quenching of fluorescence at this wavelength was used to calculate the given K_d's (5, 25).

grow better on maltotriose than either on maltose or on long dextrins (Fig. 4), can be explained by a superimposition of two effects. One would be the effect of these mutations just described on the transport of long dextrins across the outer membrane, hence, the better growth on maltotriose than on long dextrins. The other would result from the exceptionally low affinity of their binding protein for maltose ($K_d = 10^{-2}$ M), much lower than for maltotriose, hence, the better growth on maltotriose than on maltose.

The four mutant *malE* proteins are affected in their substrate binding properties in a differential manner for substrates of various lengths. If the Dex$^-$ Mal$^+$ phenotype of the mutants results, as suggested above, from the inability of their *malE* proteins to cooperate with the *lamB* protein, there does not seem to be any reason a priori why their *malE* proteins should also be affected in substrate binding. It could very well be, however, that the substrate binding site on this protein is in close proximity to, or even partially overlapping with, a site involved in an interaction with the *lamB* protein. The *malE* protein seems to bear a multiplicity of presumably interacting functional sites, which may be very difficult to inactivate individually without affecting the others. This protein, indeed, not only must bind dextrins of various lengths and perhaps interact with the *lamB* protein (see above), but also must interact with at least two components of the cytoplasmic membrane, one involved in the further transport of the substrate into the cell and the other in the detection of maltose as a chemoattractant (7).

The differential effect of the *malE* mutations on the affinity of the binding proteins for dextrins of various lengths is rather striking. In all cases the affinity of the mutant proteins for long maltodextrins is two or three orders of magnitude greater than for maltose. This finding may indicate that the binding sites for the various substrates are not strictly identical, even though they certainly overlap.

It is commonly accepted that, in transport systems that involve a periplasmic binding protein, the apparent K_m for transport in vivo equals the K_d of the binding protein for the same substrate as measured in vitro (14, 25). This is indeed the case for maltose in the wild-type strain, but not in the *malE* mutants (Table 2). In these mutants the K_d of the binding protein is about 10-fold higher than the K_m for transport. This observation led us to reconsider the relationship to be theoretically expected between these two values. Without going into an extensive discussion of this point, we tend to conclude that there is, in fact, no compelling reason for these two values to be identical. It could very well be, for instance, that the components involved in the transfer of substrate from the binding protein to the cytoplasm are saturated when only a fraction of the maltose binding protein sites are saturated. If this were true, the apparent K_m of the transport system would be lower than the K_d of the binding protein, as found in the mutants. The quasi identity of these two values in the wild-type strain might be coincidental, due, for instance, to the fact that at low concentrations of substrate (10^{-6} M) the association of maltose to maltose binding protein could be limited by the rate of diffusion into the periplasmic space rather than by the binding reaction itself. Another possibility to explain the difference between transport K_m's and binding protein K_d's in the *malE* mutants would be that the binding protein has a different affinity for maltose in vivo, because of its interaction with some other envelope components. This possibility, which was already referred to in an earlier

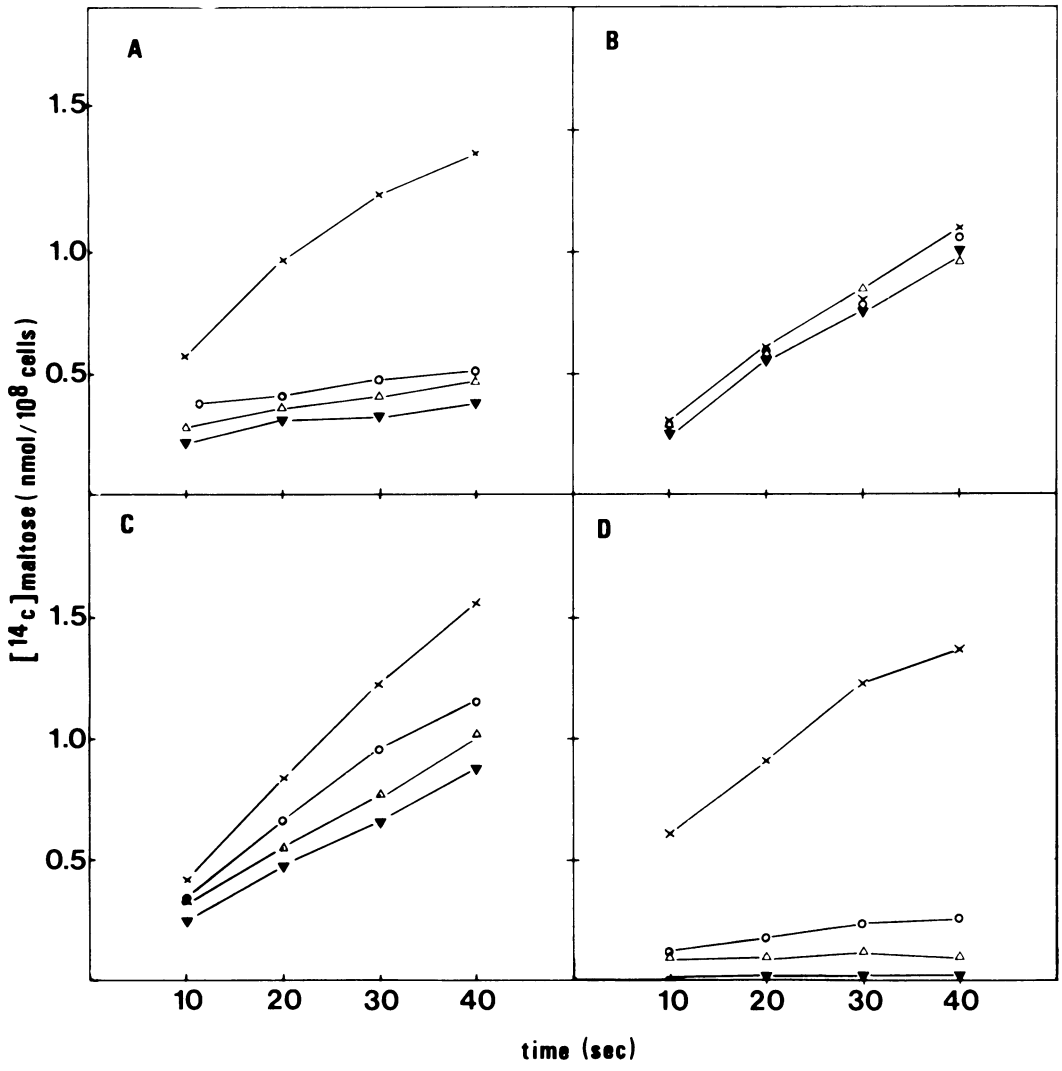

FIG. 8. *Inhibition of [^{14}C]maltose uptake by maltose uptake by maltotetraose. Exponentially growing cells on maltose minimal medium were harvested, washed twice, and suspended in minimal medium to a density of 3 × 10^8 cells per ml. A 200-μl suspension was mixed at zero time with a 20-μl mixture of [^{14}C]maltose with or without maltotetraose (i.e., no preincubation with inhibitor). A 50-μl suspension was filtered at various times, and the radioactivity in the sample was determined. The strains used were: (A) wild-type strain pop 1021; (B) lamB102 mutant pop 1080; (C) malE254 mutant pop 1153; (D) malB455 mutant pop 1155. Symbols: Maltose alone, 90 μM* (×); *maltose-tetraose, 1:1 (i.e., 90 μM:90 μM)* (○); *maltose-tetraose, 1:2 (i.e., 90 μM:180 μM)* (△); *maltose-tetraose, 1:5 (i.e., 90 μM:450 μM)* (▼).

part of this discussion, is very difficult to exclude at present.

Two Dex$^-$ Mal$^+$ mutations have been obtained which seem to map in *malG*, although the possibility that they map in yet another gene, located to the left of *malG* (see Fig. 3), has not been excluded. The existence of another such gene could have gone undetected until now if its inactivation does not lead to a Mal$^-$ phenotype. In strains bearing these mutations maltotetraose strongly inhibits maltose transport. Therefore, these mutations do not seem to affect the transfer of maltodextrins across the outer membrane but rather their transfer from the periplasmic space to the cytoplasm. Since they lead to a Dex$^-$ Mal$^+$ phenotype, it must be

concluded that the transport system can differentiate at this step between maltose and longer maltodextrins, if only on the basis of size.

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