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Maltose-Binding Protein Does Not Modulate the Activity of Maltoporin as a General Porin in *Escherichia coli*

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Maltoporin (λ receptor) is part of the maltose transport system in *Escherichia coli* and is necessary for the facilitated diffusion of maltose and maltodextrins across the outer membrane. Maltoporin also allows the diffusion of nonmaltodextrin substrates, albeit with less efficiency. The preference of maltoporin for maltodextrins in vivo is thought to be the result of an interaction of maltoporin with the maltose-binding protein, the *malE* gene product. In a recent report Heuzenroeder and Reeves (J. Bacteriol. 144:431–435, 1980) suggested that this interaction establishes a gating mechanism which inhibits the diffusion of nonmaltodextrin substrates, such as lactose. To reinvestigate this important conclusion, we constructed *ompR malT*^{c} strains carrying either the *malE*$^+$ gene, the nonpolar *malE444* deletion, or the *malE254* allele, which specifies an interaction-deficient maltose-binding protein. Lactose uptake was measured at different concentrations below the K_m of this transport system and under conditions where transport was limited by the diffusion through maltoporin. We found no difference in the kinetics of lactose uptake irrespective of the *malE* allele. We conclude that the maltose-binding protein does not modulate the activity of maltoporin as a general outer membrane porin.

Passage of substrates across the outer membrane of *Escherichia coli* is accomplished by general porins OmpC and OmpF, which do not seem to belong to a particular transport system (3, 20), and by specific porins (e.g., the maltoporin that is part of the maltose transport system) (11, 21, 35). Maltoporin specifically increases the diffusion rate for maltose and maltodextrins (maltoporin is the λ receptor) and also allows diffusion of nonmaltodextrin substrates (3), albeit with less efficiency. In vivo the rate of diffusion of maltose present at an external concentration of 1 μM through maltoporin is about 100 times higher than the rate of *ompC*- and *ompF*-mediated diffusion (33, 34). Maltodextrins cannot pass at all through these nonspecific porins (36). Whether maltoporin alone is sufficient for this remarkable specificity is not clear at this time. In vitro experiments with liposomes containing the *E. coli* B general porin or maltoporin have shown that these two channels exhibit the same permeability toward glucose, whereas permeability toward maltose is about 50 times lower through the *E. coli* B porin or the OmpF or OmpC porins of *E. coli* K-12 than through maltoporin (18, 24, 25). The specificity of maltoporin for maltose in vitro in the absence of maltose-binding protein (MBP) is a function of chemical selectivity and, to a smaller extent, of solute size.

However, there is evidence that the MBP might also be involved in determining the specificity of the maltoporin channel. Workers have isolated MBP mutants (e.g., *malE254*) which were not able to grow on maltodextrins, although MBP was only slightly impaired in dextrin binding in vitro (K_d for maltohexaose of mutant *malE254* MBP, 25 μM [36]). Growth on maltose at millimolar concentrations was not impaired (Mal$^+$ Dex$^-$ phenotype). This phenotype, which is similar to that of maltoporin-negative strains, was taken as evidence that the MBP of Dex$^-$ mutants has lost its ability to interact with maltoporin (36). This interaction was postulated to be essential for uptake of dextrins and of maltose at low concentrations. Indeed, binding of solubilized maltoporin to affinity columns could be demonstrated by using immobilized wild-type MBP, whereas no binding was seen if MBP from Dex$^-$ strains was used (2, 4).

Independent support for a MBP-maltoporin interaction has come from the work of Heuzenroeder and Reeves (15), who measured the activity of maltoporin as a general porin in the presence of wild-type or mutant MBP in *malT*^{c} *ompR* strains devoid of general porins OmpC and OmpF. These workers concluded that wild-type MBP inhibited the general porin activity of maltoporin. Their conclusion has gained considerable attention as an example for binding protein-mediated control of porin activity. We reexamined this conclusion and studied the effect of the presence or absence of MBP on the maltoporin-mediated permeation of lactose through the outer membrane. We used two different *malE* alleles (the nonpolar deletion *malE444* and *malE254*, which specifies an interaction-deficient MBP) in an *ompR* background and measured lactose transport in strains with normal and fourfold-reduced maltoporin contents in which permeation of lactose through maltoporin is rate limiting for the overall uptake process at low lactose concentrations. We show that MBP has no influence on the permeation of nonmaltodextrin sugars through maltoporin.

MATERIALS AND METHODS

Reagents. The urea used for polyacrylamide gels was recrystallized twice in ethanol. Tetracycline and mitomycin C were obtained from Sigma Chemical Co., St. Louis, Mo.; acrylamide, bisacrylamide, tetramethylenediamine, and *ortho*-nitrophenylgalactoside were obtained from Serva, Heidelberg, Federal Republic of Germany, and isopropyl-β-D-thiogalactoside (IPTG) was obtained from Calbiochem-Behring, La Jolla, Calif. The Ouchterlony double-diffusion immunoprecipitation plates used were from Hyland Laboratories, Inc., Costa Mesa, Calif.

Construction of strains. The bacterial strains used were constructed by standard genetic techniques (19) and are listed in Table 1. The mutations in the *malB* region used in this work are shown in Fig. 1. The *malE254* mutation confers

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TABLE 1. Bacterial strains and phages

Strain or phage	Known markers	Construction	Source and/or reference(s)
E. coli			
pop1153	HfrG6 *his malE254*		36
HS3018	F⁻ *araD139* Δ(*argF-lac*)*U169 relA1 rpsL150 thiA fibB5301 deoC1 ptsF25 malT*ᶜ*-1* Δ*malE444*		30
LA5612	HfrG6 *malT*ᶜ*-1*		10
JB42	LA5612 *lamB102 zjb-729*::Tn*10*	P1 of MM407 (*lamB102 zjb-729*::Tn*10*)	M. D. Manson
JB55-29	HS3018 *zjb-729*::Tn*10*	P1 of MM129 (Δ*malE444 zjb-729*::Tn*10*)	9
JB55-42	HS3018 *zja-742*::Tn*10*	P1 of MM310 (*zja-742*::Tn*10*); select Tcʳ, screen Mal⁻	9
JB56-29	pop1153 *zjb-729*::Tn*10*	P1 of MM129; select Tcʳ, screen Mal⁺	This study
JB56-42	pop1153 *zja-742*::Tn*10*	P1 of JB55-42; select Tcʳ, screen Mal⁺	This study
JB100	LA5612 *ompR*::Tn*10*	P1 of TK871 (*ompR*::Tn*10*)	5
JB103	JB100 Tcˢ *malE*⁺	Selection for Tcˢ	Bochner (6)
JB104	JB103 *zjb-729*::Tn*10*	P1 of JB55-29; select Tcʳ, screen Mal⁺	This study
JB105	JB103 Δ*malE444 zjb-729*::Tn*10*	P1 of JB55-29; select Tcʳ, screen Mal⁻	This study
JB106	JB103 *malE254 zjb-729*::Tn*10*	P1 of JB56-29 into JB105; select Tcʳ, screen Mal⁺	This study
JB107	JB103 *lamB102 zjb-729*::Tn*10*	P1 of MM407 (*lamB102 zjb-729*::Tn*10*)	M. D. Manson
JB108 through JB110	JB104 through JB106 *zjb-729*::Tn*10* (Tcˢ) *zah-735*::Tn*10* Δ(*argF-lac*)*U169*	P1 of SH205 (*zah-735*::Tn*10* Δ*lacU169*) into Tcˢ isolates of JB104 through JB106 (still contain P10 of *zjb-729*::Tn*10* [9])	6, 29; this study
JB114	JB103 *zja-742*::Tn*10*	P1 of JB55-42; select Tcʳ, screen Mal⁺	This study
JB115	JB103 Δ*malE444 zja-742*::Tn*10*	P1 of JB55-42; select Tcʳ, screen Mal⁻	This study
JB116	JB103 *malE254 zja-742*::Tn*10*	P1 of JB56-42 into JB103; select Tcʳ, screen Dex⁻ as maltose chemotaxis deficient	This study
JB118 through JB120	JB114 through JB116 *zja-742*::Tn*10* (Tcˢ) *zah-735*::Tn*10* Δ(*argF-lac*)*U169*	P1 of SH205 (*zah-735*::Tn*10* Δ*lacU169*) into Tcˢ isolates of JB114 through JB116	6, 29; this study
JB154	JB108 *zjb-729*::Tn*10* (Tcˢ) *zah-735*::Tn*10* Δ(*argF-lac*)*U169 malE*⁺ λ288	Lysogeny of JB108 with a single plaque lysate of λ288; select Lac⁺	This study
JB125	JB154 Δ*malE444* Δ288	Lysogeny of JB109 with a single plaque lysate of λ288; select Lac⁺	This study
JB126	JB154 *malE254* Δ288	Lysogeny of JB110 with a single plaque lysate of λ288; select Lac⁺	This study
JB134	JB118 *zja-742*::Tn*10* (Tcˢ) *zah-735*::Tn*10* Δ(*argF-lac*)*U169 malE*⁺ λ288	Lysogeny of JB118 with a single plaque lysate of λ288; select Lac⁺	This study
JB135	JB134 Δ*malE444* λ288	Lysogeny of JB119 with a single plaque lysate of λ288; select Lac⁺	This study
JB136	JB134 *malE254* λ288	Lysogeny of JB120 with a single plaque lysate of λ288; select Lac⁺	This study
JB127	JB134 *malT*::Tn*10 ompR*⁺	P1 of TST3 (*malT*::Tn*10*) into Tcˢ derivative of JB134	T. J. Silhavy
JB288	HS3018 λ288	Lysogeny of HS3018 λ61-4; select Lac⁺, screen Mal⁻	This study
Phages			
λ61-4	λpΦ(*lamB-lacZ*)Hyb61-4 *malE*⁺		32
λ288	λpΦ(*lamB-lacZ*)Hyb61-4 *malB* p_R⁺ *malE*⁻	Mitomycin induction of JB288	This study

a Dex⁻ phenotype due to an impaired interaction of this gene product with the maltoporin (36). Δ*malE444* is a nonpolar deletion within the *malE* gene which removes 765 base pairs of the gene (10a) but does not affect the other *malB* genes (7, 8, 30). The *malT*ᶜ*-1* allele localized at min 74 on the standard linkage map (1) and carried by strain LA5612 allowed constitutive synthesis of the genes of the *malB* regulon (10).

An *ompR*::Tn*10* insertion localized at min 74 (5) was introduced by P1 transduction into strain LA5612 and selection for tetracycline resistance (Tcʳ) on double-strength yeast extract–tryptone agar (19). Transductants were tested for constitutive *malB* expression by measuring maltose transport after growth in minimal medium A (MMA) (19) containing either 0.4% glycerol or 0.4% maltose. Of 10 transductants, 2 were identified as *ompR*::Tn*10 malT*ᶜ recombinants (one was strain JB100). A Tcˢ isolate was selected (strain JB103) by the method of Bochner et al. (6) to allow subsequent introduction of the Δ*malE444* and *malE254* mutations by cotransduction with the *zja-742*::Tn*10* insertion or with the *zjb-729*::Tn*10* insertion.

zja-742::Tn*10* is inserted between *metA* and *malB* (9), whereas *zjb-729*::Tn*10* is localized within the *malB* region between *malK* and *lamB* (Fig. 1). *zjb-729*::Tn*10* allows Tn*10*-dependent constitutive expression of *lamB* at a level that is 25% of the wild-type level (9). This low level of expression of *lamB* was an advantage for this study, since lactose permeation through the outer membrane is the rate-limiting step up to fairly high lactose concentrations (0.2 mM) for the overall uptake process in strains carrying *zjb-729*::Tn*10* (see below).

Tcʳ *malE*⁺, Δ*malE444*, and *malE254* transductants carrying the *zjb-729*::Tn*10* insertion (strains JB104, JB105, and JB106, respectively) or carrying the *zja-742*::Tn*10* insertion (strains JB114, JB115, and JB116, respectively) were se-

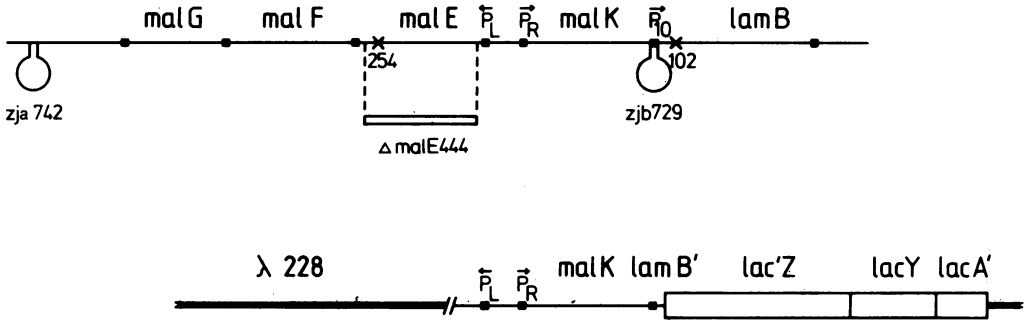

FIG. 1. Genetic organization of the *malB* region of *E. coli* and of the fusion phage λ288 *lamB-lacZ*. The *malB* region consists of two divergent operons transcribed from promoters p_L and p_R (16, 27, 31). The *malE* gene codes for MBP, *lamB* codes for maltoporin, and *malF,G,K* code for cytoplasmic membrane components of the maltose transport system (14, 30). Transposon *zjb-729*::Tn*10* is located between *malK* and *lamB* and allows *malT*-independent transcription of *lamB* at 25% of the level in *malB*$^+$ cells from a Tn*10* promoter (P10) (9). Transposon *zja-742*::Tn*10* is located outside the *malB* region and shows 60% cotransduction with *malE*. The localization of the nonpolar deletion Δ*malE444* (30), the point mutation *malE254* (Mal$^+$ Dex$^-$) (36), and the early *lamB102* amber mutation (17) is also shown. Phage λ288 is a Δ*malE* derivative of phage λ61-4 carrying a functional *malB* p_R promoter, the protein fusion Φ(*lamB-lacZ*)Hyb61-4, and a functional *lacY* gene. The fusion joints of fusion 61-4 are within the *lamB* signal sequence, thus specifying a cytoplasmically localized *lacZ* gene product (32).

lected on double-strength yeast extract–tryptone agar containing tetracycline. Screening was either on minimal agar containing 0.4% maltose and the appropriate amino acid supplements for the ability or inability (Mal$^-$ phenotype) of the cells to grow on maltose or on minimal swarm plates [0.3% agar, 10 mM potassium phosphate buffer (pH 7.0), 1 mM $(NH_4)SO_4$, 1 mM Mg_2SO_4, 40 μg of histidine per ml, and 10^{-4} M maltose] for the inability of cells to form chemotactic rings on this medium. Strains carrying *malE254* (Dex$^-$ phenotype) are similar to *lamB* mutants and are not able to respond to maltose gradients established by growth of the cells on these plates (M. D. Manson, personal communication).

Tc^s isolates of strains carrying *zjb-729*::Tn*10* which still showed constitutive low levels of expression of *lamB* from the Tn*10* promoter (9) and Tc^s isolates of strains carrying *zja-742*::Tn*10* were selected by the method of Bochner et al. (6) to allow subsequent introduction of the Δ*lacU169* deletion by cotransduction with the closely linked Tn*10* insertion *zah-735*::Tn*10* (29).

Lac$^-$ derivatives were lysogenized with phage λ288 (*att*$^-$) carrying the *malB* promoter p_R and the protein fusion Φ(*lamB-lacZ*)Hyb61-4. This fusion carries a wild-type *lacY* gene (13, 32) but no functional *malE* gene (Fig. 1).

The *malE*-deficient phage λ288 was isolated as a derivative of phage λ61-4 by screening independent Lac$^+$ lysogens of strain HS3018 [Δ*malE444* Δ(*argF-lac*)*U169*] for the ability to grow on MMA plates containing maltose (19); 2 of the 500 lysogens tested were Mal$^-$. The periplasmic proteins of one Mal$^-$ lysogen (strain JB288) were examined by using polyclonal anti-MBP antibodies in the Ouchterlony double-diffusion immunoprecipitation test, and they were found to be free of any cross-reacting material. Thus, phage λ288 which is integrated in strain JB288 lacks most of the *malE* gene. A single plaque lysate was then used for further lysogenization steps. Lac$^+$ lysogens carrying phage λ288 were selected on MacConkey agar plates (19) containing lactose and purified on the same plates containing in addition 10^8 PFU of λ *cI h80* for counterselection of nonlysogenic cells.

We selected Lac$^+$ lysogens which contained the *malE*$^+$, Δ*malE444*, or *malE254* gene either in strains carrying the *zjb-729*::Tn*10* insertion with a fourfold-reduced maltoporin content (strains JB154, JB125, and JB126) or in strains carrying the *zja-742*::Tn*10* insertion with wild-type levels of maltoporin (strains JB134, JB135, and JB136). All of these strains carried a wild-type *lamB* gene in addition to the *lamB-lacZ* fusion. The *lamB* gene is constitutively expressed either with normal efficiency from the *malB* p_R promoter due to the presence of the *malT*^{c}-*1* allele or with fourfold-reduced efficiency from the Tn*10* promoter in the *zjb-729*::Tn*10* insertion (9) (Fig. 1 and 2). The *lamB-lacZ* and *lacY* genes were constitutively expressed from the *malB* promoter in these strains (Table 2). The V_{max} of lactose transport measured at a lactose concentration of 600 μM was very similar in the λ288 lysogens described above to the V_{max} of a wild-type strain fully induced with IPTG (see below).

Growth of the cells and transport assays. Cultures were pregrown in liquid double-strength yeast extract–tryptone medium at 37°C, diluted 20-fold in MMA containing 0.4% glycerol and 0.2% Casamino Acids, and grown at 37°C to an optical density at 578 nm of 1. Since all of the strains contained the *malT*^{c}-*1* allele, the maltose transport system (and, in strains lysogenic for the fusion phage λ288, the lactose permease) was expressed constitutively. In strains devoid of λ288 and carrying a wild-type *lac* operon (strains JB42 and JB107), lactose permease was induced by adding 10^{-3} M IPTG. After they were washed three times in MMA, 2×10^8 cells were suspended in 1 ml of MMA at room temperature. The initial rates of transport were determined at two different maltose concentrations (6.75×10^{-6} and 2.3×10^{-4} M) and at seven different lactose concentrations (between 2×10^{-5} and 6×10^{-4} M). Samples (150 μl) were withdrawn at time intervals between 10 and 80 s, filtered onto membranes (pore diameter, 0.45 μm; Millipore Corp., Bedford, Mass.), and washed three times with 5 ml of MMA. The membranes were dried, and radioactivity was determined by liquid scintillation counting. Rates of uptake were expressed as picomoles of maltose or lactose taken up per minute by 150 μl of cells suspended to an optical density at 578 nm of 0.5 (3×10^7 cells). Mean values of the transport rates from at least two and three independent experiments are given for the strains with normal and reduced maltoporin contents, respectively. For the latter strains, the standard deviation (σ) of the experimental data was calculated as follows: $\sigma = [\Sigma x^2 - (\Sigma x)^2/\eta]/\eta - 1$, where x is transport rate and η is number of experiments. The standard deviations were found to be less than 30% (mostly around 15%) of the mean value.

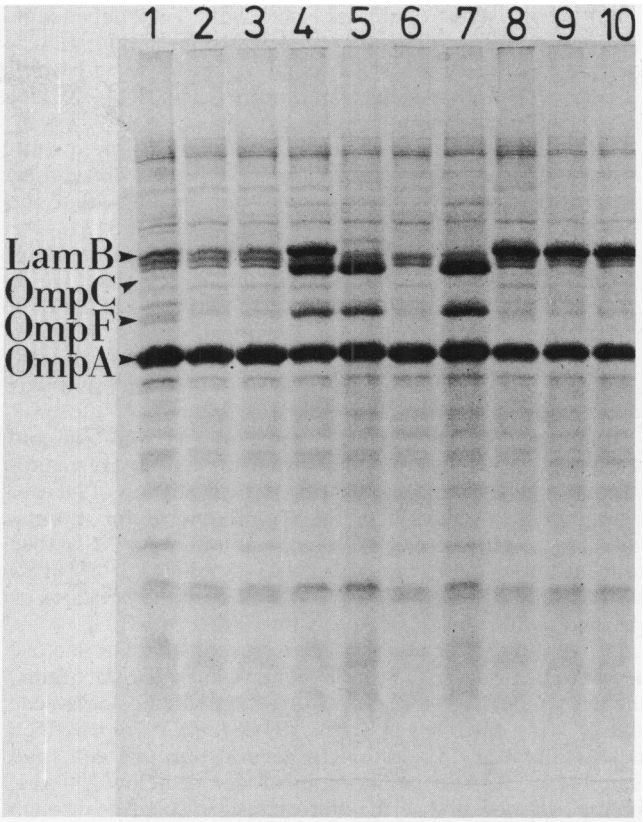

FIG. 2. Maltoporin contents of cell envelopes from *malE*$^+$ and *malE* strains with reduced and normal *lamB* expression, as estimated by urea-polyacrylamide gel electrophoresis. Cell envelope proteins from 2 × 10^8 cells were resolved. The positions of major outer membrane proteins OmpA, OmpC, OmpF, and LamB (maltoporin) are indicated. Lane 1, strain JB154 (*ompR malT*^{c}*-1 zjb-729*::Tn*10* λ288); lane 2, strain JB125 (*ompR malT*^{c}*-1* Δ*malE444 zjb-729*::Tn*10* λ288); lane 3, strain JB126 (*ompR malT*^{c}*-1 malE254 zjb-729*::Tn*10* λ288); lane 4, strain LA5612 (*malT*^{c}*-1*); lane 5, strain JB42 (*malT*^{c}*-1 lamB102 zjb-729*::Tn*10*); lane 6, strain JB107 (*ompR malT*^{c}*-1 lamB102 zjb-729*::Tn*10*); lane 7, strain JB127 (*malT*::Tn*10 ompR*$^+$ λ288); lane 8, strain JB134 (*ompR malT*^{c}*-1 zja-742*::Tn*10*288); lane 9, strain JB135 (*ompR malT*^{c}*-1* Δ*malE444 zja-742*::Tn*10* λ288); lane 10, strain JB136 (*ompR malT*^{c}*-1 malE254 zja-742*::Tn*10* λ288).

Ouchterlony double-diffusion immunoprecipitation tests. Stationary cultures (2 ml; grown on double-strength yeast-tryptone agar) of λ288 lysogenic strains carrying either a wild-type *malE* gene (strain JB154), the *malE254* mutation (strain JB125), or the *malE444* deletion (strains JB126 and JB288) were centrifuged and then suspended in 50 μl of 10 mM Tris (pH 7.2). After 10 μl of a toluene-chloroform mixture (1:1, vol/vol) was added, the cells were shaken for 30 min at 37°C and then pelleted. Samples of the cell-free extracts were placed in the wells of Ouchterlony plates. By using antibodies raised against purified MBP (7), precipitation bands were clearly seen after 20 h at 37°C.

Preparation of cell envelopes and polyacrylamide gel electrophoresis. Cultures grown logarithmically in MMA containing 0.4% glycerol (optical density at 578 nm, 1) were disrupted by passing them three times through a French pressure cell (10,000 lb/in^2). The crude extract was centrifuged for 1 h at 150,000 × *g* at 4°C. The pellet containing inner and outer membranes was rinsed with MMA at room temperature, suspended in 200 μl of sample buffer (10 mM Tris-hydrochloride [pH 7.3], 1 mM dithiothreitol, 1% sodium dodecyl sulfate), solubilized by heating to 100°C for 5 min, and cleared by centrifugation for 2 min at 13,000 × *g* in an Eppendorf centrifuge. Samples (50 μl) of the supernatant were electrophoresed on 9% polyacrylamide slab gels containing 8 M urea by the method of Pugsley and Schnaitman (26). Estimation of the maltoporin content of whole cell envelopes has the advantage of simplicity and avoids the loss of maltoporin observed with all other procedures which isolate outer membrane proteins by extraction or by membrane separation (12).

Determination of maltoporin expression in strains containing the *lamB-lacZ* protein fusion. To determine maltoporin expression, we measured the expression of *lamB-lacZ* protein fusion 61-4 (32). Cells were grown and broken as described above. The β-galactosidase activity of the crude extracts was determined as described by Miller (19), except that Z-buffer was replaced by MMA containing 0.05 M β-mercaptoethanol. β-Galactosidase specific activities are expressed as micromoles of *ortho*-nitrophenylgalactoside hydrolyzed per minute per milligram of protein at room temperature.

RESULTS AND DISCUSSION

Maltose transport in *ompR malT*^{c} strains carrying either the *malE444* deletion, the *malE254* mutation, or a wild-type *malE* gene. The purpose of this work was to study the effect of MBP on the activity of maltoporin as a general porin in *ompR* strains devoid of the OmpF and OmpC porins. First, it was necessary to demonstrate the presence and the effect of the different *malE* alleles. Therefore, we measured initial rates of maltose transport in *ompR* strains JB134 (*malE*$^+$), JB135 (Δ*malE444*), and JB136 (*malE254*), which had wild-type levels of maltoporin, and in isogenic *ompR* strains JB154 (*malE*$^+$ *zjb-729*::Tn*10*), JB125 (Δ*malE444 zjb-729*::Tn*10*), and JB126 (*malE254 zjb-729*::Tn*10*), which had fourfold-reduced maltoporin contents, as well as in control strains JB42 (*ompR*$^+$ *lamB*), JB107 (*ompR lamB*), and JB127 (*ompR*$^+$ *malT*::Tn*10*). Transport was measured at two different maltose concentrations (2.3 × 10^{-4} and 6.75 × 10^{-6} M) (Table 2). Strains JB134 and JB154 and wild-type control strain LA5612 showed high initial rates of maltose uptake at both high and low maltose concentrations. Because of the loss of MBP, strains JB135 and JB125 showed very low transport rates at both maltose concentrations. The same

TABLE 2. Maltose transport in wild-type and *malE* mutant strains

Strain (relevant genotype)	Maltose transport (pmol/min per 3 × 10^7 cells) at a maltose concn of:	
	6.75 × 10^{-6} M	2.3 × 10^{-4} M
LA5612 (*malT*^{c}*-1*)	172	226
JB42 (*malT*^{c}*-1 lamB102*)	5.2	169
JB107 (*malT*^{c}*-1 ompR lamB*)	0.7	27
JB127 (*malT*::Tn*10 ompR*)	0.2	16
JB134 (*malT*^{c}*-1 ompR*)	260	294
JB135 (*malT*^{c}*-1 ompR* Δ*malE444*)	0.5	20
JB136 (*malT*^{c}*-1 ompR malE254*)	15	215
JB154 (*malT*^{c}*-1 ompR*)	260	291
JB125 (*malT*^{c}*-1 ompR* Δ*malE444*)	0.4	20
JB126 (*malT*^{c}*-1 ompR malE254*)	14	235

TABLE 3. Expression of *lamB-lacZ* protein fusion 61-4 in wild-type and *malE* strains lysogenic for phage λ288

Strain	Genotype	β-galactosidase activity (μmol of ONPGal hydrolyzed per min per mg of protein)
JB134	*ompR malT*^{c}*-1 malE*$^+$ λ288	3.1
JB135	*ompR malT*^{c}*-1* Δ*malE444* λ288	3.4
JB136	*ompR malT*^{c}*-1 malE254* λ288	3.1
JB127	*ompR malT*::Tn*10* λ288	0.05

[a] ONPGal, *ortho*-Nitrophenylgalactoside.

low rates were observed in *malT*::Tn*10* control strain JB127 and in *ompR lamB* double mutant strain JB107, which is deficient in all of the major porins of the outer membrane. Strains JB136 and JB126 showed high uptake rates similar to the uptake rate of strain JB134, but only at the high maltose concentration. This phenotype, characteristic of Dex$^-$ strains (36), is very similar to that of strain JB42 (*lamB*) (33, 34). The fourfold reduction in maltoporin content in strains JB154, JB125, and JB126 had no effect on the rate of maltose uptake at these concentrations tested. Permeation of maltose through maltoporin in *malE*$^+$ strains with fourfold-reduced maltoporin contents has been found to be limiting for the overall uptake process only at maltose concentrations below 10^{-6} M (9). These results showed that permeation of maltose into Dex$^-$ strains did not rely on the presence of the general porins. At high substrate concentrations (2.3×10^{-4} M), maltoporin functions as an open pore for maltose, supposedly in the absence of an MBP-maltoporin interaction.

We also tested the possibility that phage λ288 still carried most of the *malE* gene and specified an incomplete MBP molecule which could bind to maltoporin in *malE444* deletion strains. In Oucherlony tests in which antibodies raised against purified MBP (7) were used, precipitation bands were clearly observed with periplasmic extracts of strains JB154 (*malE*$^+$ λ288) and JB126 (*malE254* λ288), whereas extracts of strains JB288, JB125, and JB135 (Δ*malE444* λ288) did not show any precipitation (data not shown). This indicates that phage λ288 lacks most of the *malE* gene.

Maltoporin expression in constitutive wild-type and *malE* mutants. To use the different *malE* alleles for detecting a differential effect of the MBP-maltoporin interaction on lactose transport, it was necessary to test whether the amounts of constitutively expressed maltoporin were the same in wild-type and *malE* strains. Therefore, *lamB* expression was measured in wild-type and *malE* strains both by determining the β-galactosidase activity of a *lamB-lacZ* protein fusion carried by phage λ288 and by estimating the maltoporin content of isolated cell envelopes after polyacrylamide gel electrophoresis.

Figure 2 shows the urea-polyacrylamide gel electrophoresis separation of the cell envelope proteins from strains which had reduced and normal maltoporin contents and carried either wild-type or mutant *malE* alleles. The cell envelope of wild-type strain LA5612 (Fig. 2, lane 4) showed the four major outer membrane proteins, OmpA, OmpF, OmpC, and LamB. Proteins OmpC and OmpF were easily identified, since they were missing in all *ompR* strains (Fig. 2, lanes 1 through 3, 6, and 8 through 10). The maltoporin band (LamB) was missing in *lamB* strains (strains JB42 and JB107 [lanes 5 and 6]) and in *malT*::Tn*10* *ompR*$^+$ strain JB127 (lane 7). We observed no difference in the amount of maltoporin in wild-type and *malE* mutant strains. This held true for both series of strains, those with Tn*10*-dependent, fourfold-reduced expression of *lamB* (strains JB154, JB125, and JB126) (Fig. 2, lanes 1 through 3) and those with *malB* p_R-dependent normal *lamB* expression (strains JB134, JB135, and JB136) (lanes 8 through 10).

The level of *lamB* expression was measured more quantitatively from the β-galactosidase activities of *lamB-lacZ* protein fusions in these strains. Table 3 shows that the presence of the wild-type *malE* gene (strain JB134), the *malE444* deletion (strain JB135), and the *malE254* mutation (strain JB136) had no influence on *lamB* expression. *lamB-lacZ* expression was reduced to 1.5% in a *malT*::Tn*10* derivative (strain JB127). In recombinant strains, in which *lamB-lacZ* expression was brought under control of the P10 promotor of the *zjb-729*::Tn*10* transposon, expression was reduced to 25% (9).

Maltoporin-mediated lactose permeation in wild-type and *malE* mutant strains. We determined the activity of maltoporin as a general porin mediating the permeation of lactose in *ompR* strains devoid of the OmpC and OmpF porins. Initial rates of uptake were measured at different [^{14}C]lactose concentrations below the K_m of this system (0.3 mM [28]) between 2×10^{-5} and 6×10^{-4} M lactose in the presence or absence of 10^{-4} M unlabeled maltose (Fig. 3).

It is evident that maltoporin is responsible for lactose permeation through the outer membrane in *ompR* strains, such as strain JB134 (*ompR*). Only negligible rates of lactose uptake were observed in strain JB107 (*ompR lamB*) which lacks maltoporin in addition to general porins OmpC and OmpF (Fig. 3). At concentrations of lactose below 200 μM, maltoporin-mediated permeation of this disaccharide through the outer membrane was the rate-limiting step for the overall uptake process in strains with fourfold-reduced contents of maltoporin. This can be concluded from the finding that strains JB154, JB125, and JB126 showed up to threefold-reduced rates of uptake of lactose at concentrations below

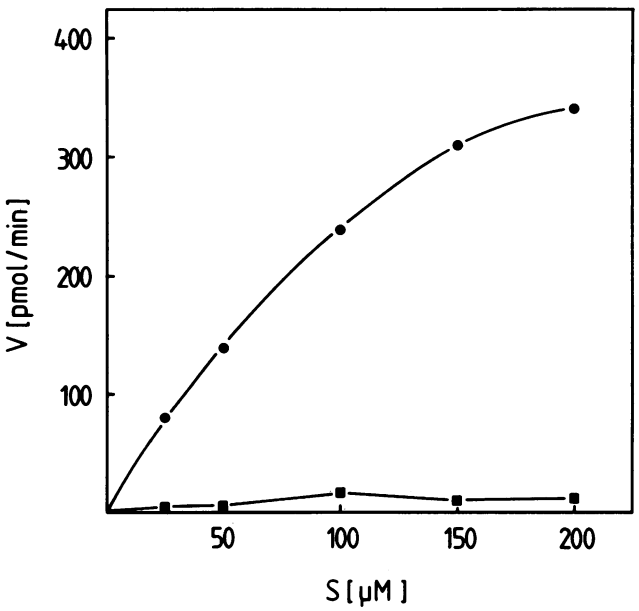

FIG. 3. Lactose transport in *ompR* strains is dependent on maltoporin. Values for transport of lactose in strains JB134 (*ompR malT*^{c}*-1* Δ*lacU169* λ288) (●) and JB107 (*ompR malT*^{c}*-1 lamB102*) (■) grown in the presence of 10^{-3} M IPTG are expressed as picomoles of lactose taken up per minute by 3×10^7 cells.

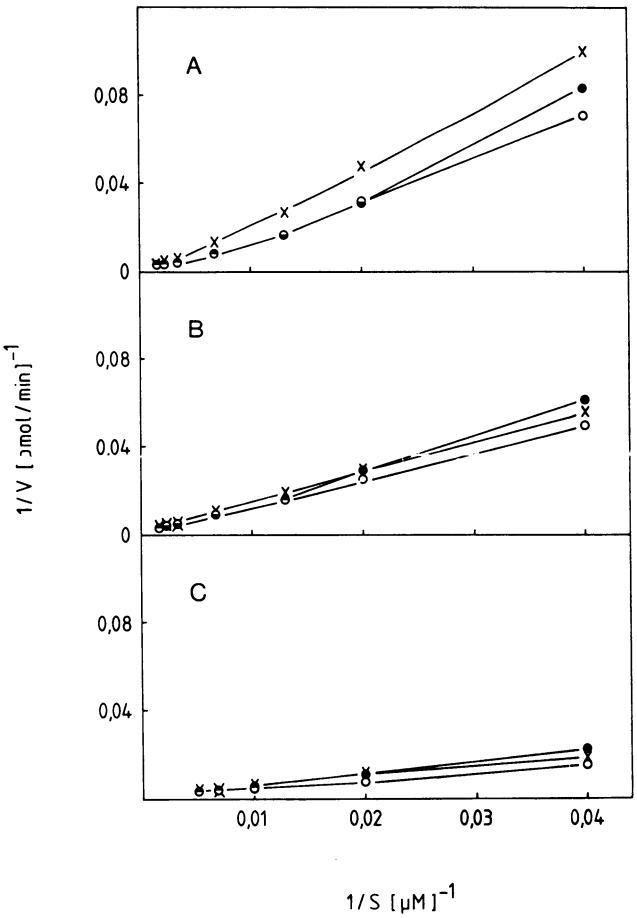

FIG. 4. Lactose transport in *ompR* strains is not affected by the presence or absence of wild-type MBP. Transport of lactose is shown as Lineweaver-Burk plots; transport rates were determined at lactose concentrations between 2×10^{-5} and 6×10^{-4} M and are expressed as picomoles of lactose taken up per minute by 3×10^7 cells. (A and B) [^{14}C]lactose transport in strains JB154, JB125, and JB126 carrying the *zjb-729*::Tn*10* insertion, resulting in fourfold-reduced maltoporin content. (A) Measured in the absence of unlabeled maltose. (B) Measured in the presence of 10^{-4} M unlabeled maltose. All of the standard deviations of the transport values (mean values of three independent experiments) were less than 30% (mostly around 15% of the mean value). (C) [^{14}C]lactose transport in strains JB134, JB135, and JB136 with wild-type levels of maltoporin, measured in the absence of unlabeled maltose (mean values of two indepenent experiments). Symbols: ○, strains carrying the *malE* wild-type allele; ●, strains carrying the *malE444* deletion; ×, strains carrying the *malE254* allele.

200 μM compared with strains JB134, JB135, and JB136 (Fig. 4A and C) and from the finding that the Lineweaver-Burk plot of the transport data of the strains with reduced maltoporin contents tended to be nonlinear (concave upward) (Fig. 4A and B). Such behavior is expected in cases where permeation of solutes through the outer membrane is rate limiting for the overall uptake process (9, 23). The V_{max} values for lactose transport observed at saturating concentrations of lactose (600 μM) were very similar in all strains which expressed the *lacY* gene product constitutively from the *malB* p_R promoter of λ288 (Fig. 1) and were close to the observed V_{max} of fully IPTG-induced wild-type strain LA5612 carrying a chromosomal *lacY* gene; the V_{max} values observed for strains LA5612, JB154, JB125, JB126, JB134, JB135, and JB136 were 526, 470, 433, 331, 400, 490, and 394 pmol/min per 3×10^7 cells, respectively.

We found no difference in the kinetics of lactose uptake within the two series of isogenic *ompR malT*^{c} strains (strains JB154, JB125, and JB126 [Fig. 4A and B] and strains JB134, JB135, and JB136 [Fig. 4C]) carrying either the *malE*$^+$ gene, the *malE444* deletion, or the *malE254* mutation. In the strains carrying *zjb-729*::Tn*10* (strains JB154, JB125, JB126), which had fourfold-reduced maltoporin contents, the number of MBP molecules per cell was estimated to be threefold higher than the number of maltoporin trimers per cell. In these strains (Fig. 4A and B) and in strains with normal amounts of maltoporin (Fig. 4C), no effect of the presence or absence of wild-type MBP on the rate of lactose uptake was measured. The inclusion of 10^{-4} M unlabeled maltose in the lactose uptake assay mixtures only slightly increased the rates of lactose uptake (Fig. 4B). This increase was more evident in strains carrying the *malE254* allele.

In summary, our data on maltose and lactose transport in *ompR malE* mutants indicate that (i) maltoporin is an open pore for maltose and lactose even in the absence of interaction with wild-type MBP, and (ii) the activity of maltoporin as a general porin is not inhibited by the presence of wild-type MBP. The latter conclusion is at variance with a previous report by Heuzenroeder and Reeves (15) and with a recent study of Neuhaus et al. (22), who claimed that they found a MBP-dependent closing of the maltoporin channel. The latter authors reported a shift in the equilibrium of open and closed maltoporin channels incorporated in black lipid films to the closed state after addition of MBP at a given polarity.

We have shown in this paper that the MBP neither interferes with nor stimulates the function of maltoporin as a general pore in vivo. Our findings do not disprove the concept of a MBP-maltoporin interaction, which may establish the specificity of the maltoporin pore for maltodextrins. This MBP-maltoporin interaction could increase the net rate of maltodextrin entry by immediate binding of maltodextrins entering the periplasm. Based on our data, we believe that the positioning of MBP in the maltoporin channel close to the external medium as suggested previously (11) is unlikely. More realistically, as suggested in a recent review (14), the maltoporin-MBP interaction has to result in a complex which leaves the maltoporin channel open.

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ADDENDUM IN PROOF

During preparation of the manuscript, we found that inclusion of maltohexaose into the [^{14}C]lactose uptake mixture strongly inhibited uptake of lactose at 1.5×10^{-4} M of strain JB135 (*malT*^{c}-*1 ompR* Δ*malE444 zjb-729*::Tn*10*) with a K_i of about 1.5×10^{-4} M. This finding indicates that inhibition of the general maltoporin activity due to binding of dextrins to the protein (11) observed in vitro by the vesicle swelling assay (M. Luckey, and H. Nikaido, Biochem. Biophys. Res. Commun. **93**:166–171, 1980) is also observed with whole cells.

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