JOURNAL OF BACTERIOLOGY, Feb. 1980, p. 431-435
0021-9193/80/02-0431/05$02.00/0

Vol. 141, No. 2

Periplasmic Maltose-Binding Protein Confers Specificity on the Outer Membrane Maltose Pore of *Escherichia coli*

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ompB mutants of *Escherichia coli* K-12 are markedly deficient in porin in their outer membrane. This results in a decreased rate of uptake for many substrates: the maltose pore (λ receptor) can in some circumstances, in the absence of the periplasmic maltose-binding protein, compensate for the consequent defects in permeability to lactose, mannitol, glycylglycyl-L-valine, and tri-L-ornithine. It is postulated that the maltose-binding protein associates with the maltose pore and confers on it the specificity for maltose, and that the absence of the maltose-binding protein leaves the pore open and results in enhanced transmembrane diffusion of molecules other than maltose. This paper presents evidence to support this hypothesis.

The outer membrane of gram-negative bacteria serves as a permeability barrier for molecules above a certain size, but allows rapid diffusion of small hydrophilic molecules up to 600 daltons (7). This diffusion of low-molecular-weight hydrophilic molecules is due to the presence of pores formed by proteins (termed porins) in the membrane. Several studies have shown that, in *ompB* mutants (23) whose outer membranes lack detectable proteins 1a and 1b, periplasmic enzymes are cryptic for certain substrates (3, 4), and that these mutants are less efficient in the uptake of many metabolites (2, 30).

The in vitro studies of Nakae (19, 20), using membrane vesicles made from phospholipid and lipopolysaccharide, showed that the addition of protein 1a of *E. coli* B made the otherwise impermeable vesicles permeable to molecules up to 600 daltons. This and other experiments show that proteins 1a and 1b constitute the major porins of *E. coli*. In addition to these nonspecific pores, there exist pathways which facilitate the diffusion of specific classes of substrates across the outer membrane. Examples of such systems are the λ receptor for maltose and maltotriose transport (28), the *tsx* protein (bacteriophage T6 receptor) for nucleoside transport (13), the *tonA* protein for ferrichrome transport (33), the *fepA* (*cbr*) protein for ferrienterochelin transport (12, 21, 32), and the *btuB* protein involved in vitamin B_{12} transport (9). The λ receptor protein has been studied in some detail and has been purified (22, 24, 26). The role of the λ receptor in maltose transport has only been recently demonstrated (14, 27), although the *lamB* gene, which encodes it, has long been known to be the part of a *mal* operon (29). The λ receptor facilitates the uptake of maltose at low concentrations, but has no effect at higher maltose concentrations, when diffusion across the outer membrane barrier is presumably not rate limiting. The λ receptor is, however, essential for uptake of maltotriose (27).

There is no measurable affinity of maltose for the receptor either as a purified protein or when present in the outer membrane (14, 27). We are thus faced with the dilemma of explaining the specificity of this pore when the protein concerned has no binding affinity for the molecule that is preferentially able to diffuse through the pore.

In this paper we test the hypothesis that the specificity of the λ receptor as a pore is due to association with the periplasmic maltose-binding protein (coded for by the *malE* gene [16]). We postulate that the association of the maltose-binding protein with the outer membrane λ receptor impedes the diffusion of most solutes, but the associated binding protein is available for maltose binding. This hypothesis is tested by looking at the ability of the λ receptor to substitute for the major porins using *ompB* mutation to reduce the level of the major porins, *malT* mutation to make λ receptor synthesis constitutive, and *malE* mutation to remove the binding protein.

MATERIALS AND METHODS

Bacterial strains. The *E. coli* K-12 strains used for strain construction are listed in Table 1. Strains constructed for this work are described below under "Genetic methods." Colicinogenic strains have been described previously (6, 11).

TABLE 1. *E. coli strains used for strain construction*

Strain	Genotype[a]	Source
AB1297 (CGSC 1927)	*metA28 argH1 purF1 xyl-7 supE44* Hfr PO12	P. Howard-Flanders
AB2847 (CGSC 2847)	*aroB351 tsx-354 malT354*	A. J. Pittard
pop3127	*thi rpsL araD Δlac U169 malT*^{c}	M. Schwartz
pop1753	*his thi malE11*	G. Hazelbauer
pop1754	*his thi malE12*	G. Hazelbauer
pop1755	*his thi malE13*	G. Hazelbauer
pop1756	*his thi malE14*	G. Hazelbauer
P1831	*ompB101 tsx-354 malT354*	This laboratory
P2222	*metA28 argH1 purF1 xyl-7 supE44 zij-1*::Tn*10*	This laboratory
P2335	*malT*^{c} *ompB101 metA28 lamB24 tsx-354 zij-1*::Tn*10*	This laboratory
P2342	*malT*^{c} *tsx-354 ompB101 zij-1*::Tn*10*	This laboratory
P2343	*malT*^{c} *tsx-354 zij-1*::Tn*10*	This laboratory
P2421	*malT*^{c} *malE13 lamB24 ompB101 tsx-354 zij-1*::Tn*10*	This laboratory
P2341	*malT*^{c} *malE13 ompB101 tsx-354 zij-1*::Tn*10*	This laboratory

[a] Transposon nomenclature follows that of Kleckner et al. (17).

Media. Nutrient broth (Difco) was prepared double strength plus 5 mg of sodium chloride per ml; nutrient agar was blood agar base (Difco) prepared as directed without the addition of blood. Minimal agar was prepared by the addition of 20 g of agar (Difco) per liter to liquid minimal medium (27). Glucose was added as a carbon source at a final concentration of 5 mg/ml. Glycerol was added at a final concentration of 1% (vol/vol). Growth supplements were added at a concentration of 20 μg/ml. Soft agar overlays contained 1% agar. Maltose tetrazolium agar was as described previously (1). Tetracycline was added where indicated at 16 μg/ml.

Chemicals. Glycylglycyl-L-valine was purchased from Sigma; tri-L-ornithine came from Miles-Yeda Ltd., Rehovot, Israel; tetracycline was obtained from Gist Brocade; and [^{14}C] lactose and [^{14}C]mannitol were obtained from the Radiochemical Centre, Amersham, England.

Genetic methods. A set of strains was made in a *malT*^{c} background containing combinations of *ompB101* or *ompB*$^+$, *malE* (*malE11*, *malE12*, *malE13*, *malE14*), or *malE*$^+$ and *lamB24* or *lamB*$^+$ alleles. The starting strain was AB2847; the *metA28* allele of strain AB1927 was transferred by P1 transduction, using as a donor a derivative carrying transposon Tn*10* integrated close to *metA28*. The methods of Kleckner et al. (17) were used to integrate the transposon. The AB2847 *metA*, Tn*10* derivative P2222 was used as the base strain and sequentially transduced with: (i) bacteriophage P1 grown on strain pop3127 using selection for maltose fermentation to give *malT*^{c} derivatives; (ii) phage P1 grown on strain P1831 using selection for *aroB*$^+$ followed by screening for colicin L resistance (6) to give *ompB*$^+$ and *ompB101* derivatives; or (iii) phage P1 grown on either pop1753, pop1754, pop1755, or pop1756 using selection for *metA*$^+$ followed by screening for maltose fermentation to give *malE* and *malE*$^+$ derivatives. Strains carrying the *lamB24* mutation were constructed in parallel to those above; strain P2335, a *lamB* mutant of strain AB2847, was used as the base strain to construct a parallel series of strains, using the same methods as described above. All derivatives were *aroB*$^+$ and *metA*$^+$ and retained transposon Tn*10*. A summary of the strain types produced is presented in Table 2.

Detection of maltose-binding protein. The presence or absence of the maltose-binding protein in strains thought to be carrying the *malE* mutation was verified by examination of periplasmic proteins released by osmotic shock (15). Detection of maltose-binding protein in the shock fluid was done by slab gel polyacrylamide electrophoresis and sample preparation (18). The slabs were stained with Coomassie brilliant blue (10). The maltose-binding protein was assumed to be a 40,000-dalton protein that was absent in *malE* strains and ran in the same position as purified maltose-binding protein.

Measurement of growth inhibition by toxic peptides. Growth inhibition was measured essentially by the method of De Felice et al. (8). Minimal glycerol plates were overlaid with 3 ml of soft minimal agar containing 200 μl of a nutrient broth-grown overnight culture. Filter paper disks (9-mm disks) were laid on the plates, and 10 μl of a 5-mg/ml solution of either glycylglycyl-L-valine or tri-L-ornithine was then added to the disk. Zone diameter was measured after overnight incubation.

Uptake of [^{14}C]lactose and [^{14}C]mannitol. Log-phase cultures (nutrient broth; 37°C; optical density at 625 nm of 1) were used. Isopropyl-β-D-thiogalactopyranoside at 10^{-3} M or 0.2% mannitol was present to induce lactose or mannitol uptake systems, respectively. The cells were then washed once in minimal medium containing 1% glycerol and suspended in the same medium containing 0.1 mg of chloramphenicol per ml. This suspension was then transferred to the uptake vessel, aerated, and kept at 37 or 25°C for 10

TABLE 2. *Relevant genotype and outer membrane proteins present in strains*

Strain group	Genotype			Protein			
	lamB	*malE*[a]	*ompB*	λ receptor	1a	1b	MBP[b]
1	+	−	*101*	+	−	−	−
2	+	−	+	+	+	+	−
3	+	+	*101*	+	−	−	+
4	+	+	+	+	+	+	+
5	*24*	−	*101*	−	−	−	−

[a] The *malE* mutation includes the four alleles *malE11*, *malE12*, *malE13*, and *malE14*.

[b] MBP, Maltose-binding protein.

min before the addition of either [^{14}C]lactose or [^{14}C]mannitol. Samples (0.1 ml) were withdrawn at various time intervals, filtered on membrane filters (0.45-μm pore diameter; Millipore Corp.), and washed with 20 ml of 0.09% (37 or 25°C) saline. The membranes were then dried, and radioactivity was counted in a Packard Tri-Carb liquid scintillation counter.

RESULTS

Inhibition by toxic peptides. The experiment using toxic peptides (8) was based on unpublished experiments using valine containing di- and tripeptides, in which *ompB* mutants showed marked resistance to these substances. It was concluded that resistance in *ompB* mutants was due to lack of porin in the outer membrane and a subsequent decrease in outer membrane permeability to these substances. A set of strains was tested against glycylglycyl-L-valine and tri-L-ornithine (Table 3). In all cases strains lacking the maltose-binding protein in an *ompB* background have enhanced inhibition zones, indicating increased uptake of the toxic peptides in comparison with strains carrying *ompB* alone. Those strains carrying the *lamB* mutation in addition to the *malE* and *ompB* mutations were more resistant to the toxic peptides than the *ompB* strains or *ompB malE* strains. It must be remembered when interpreting data based on inhibition zone diameters that the square of the radius of the zone is proportional to the logarithm of the initial concentration in the disk (5). The results demonstrate that removal of the maltose-binding protein greatly facilitates the ability of the λ receptor to compensate for an *ompB* defect in the uptake of these particular tripeptides.

Uptake of [^{14}C]lactose and [^{14}C]mannitol. Four strains were selected for uptake experiments. Selection was determined by preliminary experiments (data not shown) carried out on solid media using low-level 0.01% lactose as the sole carbon source. The uptake ability of the strains for this sugar was determined by measuring colony size after growth at 37°C for 48 h and carrying out statistical analysis to determine any significant differences. It was found, in all cases tested, that a *malE opmB* strain had a significantly larger colony size ($P < 0.001$) than *ompB* strains. On the basis of these experiments, P2421, P2341, P2342, and P2343 were chosen for liquid medium uptakes.

When radioactive sugars were supplied to *ompB* mutants at limiting concentrations, diffusion across the outer membrane became rate limiting. Our results presented in Fig. 1 and 2 confirm this observation. Furthermore, as predicted by our hypothesis, the inclusion of the *malE13* mutation substantially restores to the *ompB* strain P2342 the ability to take up both lactose and mannitol. The inclusion of the *lamB* mutation results in an even further decrease in uptake ability of an *ompB* strain. This result demonstrates that the restorative effect of the *malE* mutation is dependent upon the presence of the λ receptor pore, which presumably is acting as nonspecifically in the absence of the maltose-binding protein.

TABLE 3. *Inhibition by toxic peptides*

Strain group	Genotype	Mean zone of inhibition (mm ± SD)[a]	
		GGV	TOR
1	*malE ompB*	34.0 ± 2.10	23.3 ± 0.49
2	*malE*	37.7 ± 2.40	24.4 ± 1.08
3	*ompB*	30.3 ± 1.70	19.6 ± 0.86
4	Wild type	35.4 ± 1.20	24.5 ± 1.33
5	*malE ompB lamB*	30.4 ± 1.94	19.6 ± 1.12

[a] Each value represents the pooled data for four strains of similar genotype as presented in Table 2. SD, Standard deviation; GGV, glycylglycyl-L-valine; TOR, tri-L-ornithine.

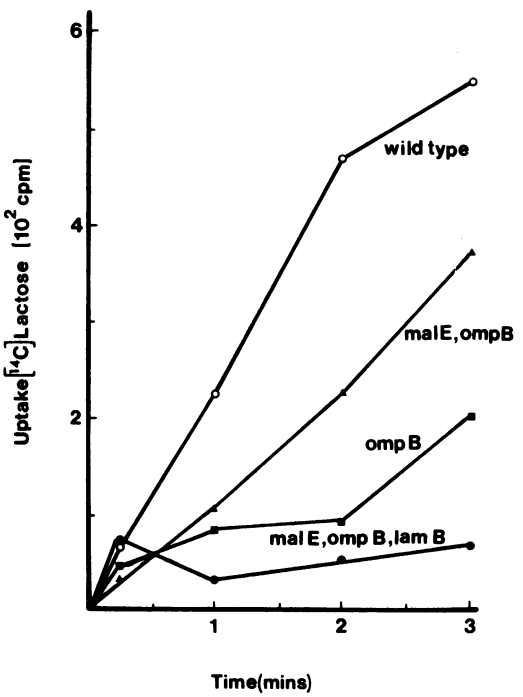

FIG. 1. *Uptake of [^{14}C]lactose by strains P2421 malE ompB lamB, P2341 malE ompB, P2342 ompB, and P2343 wild type. The uptake was carried out at 37°C with a final concentration of lactose of 1 μM in the uptake vessel.*

DISCUSSION

It has been known for some time that the λ receptor can partially alleviate the permeability

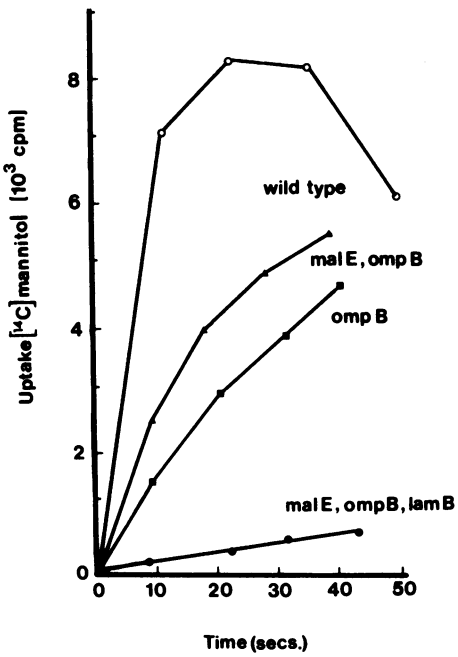

FIG. 2. *Uptake of [^{14}C]mannitol by strains P2421 malE ompB lamB, P2341 malE ompB, P2342 ompB, and P2343 wild type. The uptake was carried out at 25°C with a final concentration of 2 μM of mannitol in the uptake vessel.*

defect of a mutant lacking porins. Von Meyenberg and Nikaido (31) measured the uptake K_m in porin-deficient strains lacking the λ receptor and in similar strains having the λ receptor present. It appeared that lactose and glucose could diffuse through the λ receptor, although much less effectively than through the usual porins. It was also suggested by similar criteria that histidine and 6-aminopenicillanic acid did not cross the outer membrane via the λ receptor pore. Independently in our own laboratory, V. Sarma (Ph.D. thesis, University of Adelaide, Adelaide, Australia, 1978) was able to demonstrate a similar phenomenon with a wide range of substances. The K_m of the porin-deficient λ receptor-positive strains was slightly lower than that of the porin-deficient λ receptor-deficient strains, although in both cases the K_m was much higher than that for strains with porin.

The experiments in this paper confirm these earlier results. The λ receptor has been shown to partially alleviate an *ompB* defect, for the two peptides tested as well as for mannitol and lactose; however, this effect is greatly amplified when the maltose-binding protein is absent as in a *malE* mutant, indicating that the maltose-binding protein, perhaps by acting as a specific gate, impedes the passage of molecules other

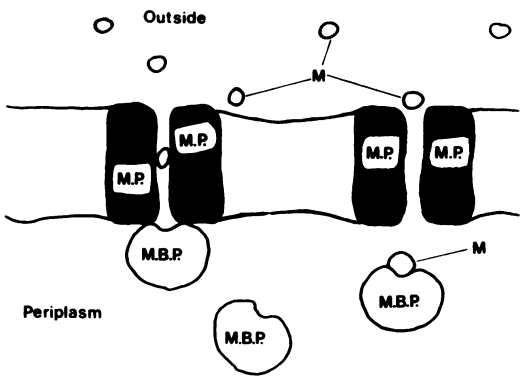

FIG. 3. *Model for interaction between the maltose pore (M.P.) in the outer membrane and the maltose-binding protein (M.B.P.) in the periplasm. The maltose-binding protein associates with the pore protein so as to block the pore, but dissociates after binding maltose (M).*

than maltose through the receptor pore. However, other experiments (data not shown) show that neither the *lamB* or *malE* mutation affects the uptake of some amino acids and another tripeptide. Presumably these other substances are unable to diffuse even through the ungated pore. We are currently investigating this phenomenon. The specificity for maltose permeability is hypothesized to be due to a physical association between the λ receptor protein and maltose-binding protein such that the maltose affinity site is available to exogenous maltose through the pore, while the protein at the same time blocks free diffusion through the pore. The maltose-binding protein, upon binding maltose, would presumably release from the λ receptor due to an allosteric conformational change, and thus make the maltose available to the permease in the cytoplasmic membrane. The model is illustrated in Fig. 3. The results obtained by Von Meyenberg and Nikaido (31) with lactose and glucose can perhaps be explained by postulating that at any given time a small number of the λ receptor pores can be unblocked even in a wild-type (*malE*$^+$) strain. We believe our model could be applied in modified form to other specific uptake systems in *E. coli*.

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