JOURNAL OF BACTERIOLOGY, May 1980, p. 521-526
0021-9193/80/05-0521/06$02.00/0

Vol. 142, No. 2

Lambda Receptor in the Outer Membrane of *Escherichia coli* as a Binding Protein for Maltodextrins and Starch Polysaccharides

THOMAS FERENCI,* MARINA SCHWENTORAT, SUSANNE ULLRICH, AND JEANNINE VILMART

Department of Biology, University of Konstanz, D-7750 Konstanz, West Germany

The starch polysaccharides amylose and amylopectin are not utilized by *Escherichia coli*, but are bound by the bacteria. The following evidence supports the view that the outer membrane λ receptor protein, a component of the maltose/maltodextrin transport system is responsible for the binding. (i) Amylose and amylopectin both inhibit the transport of maltose into *E. coli*. (ii) Both polysaccharides prevent binding of non-utilizable maltodextrins by the intact bacterium, a process previously shown to be dependent on components of the maltose transport system (T. Ferenci, Eur. J. Biochem., in press). (iii) A fluorescent amylopectin derivative, *O*-(fluoresceinyl thiocarbamoyl)-amylopectin, has been synthesized and shown to bind to *E. coli* in a reversible, saturable manner. Binding of *O*-(fluoresceinyl thiocarbamoyl)-amylopectin is absent in mutants lacking the λ receptor, but mutations in any of the other components of the maltose transport system do not affect binding as long as λ receptor is present. (iv) Using the inhibition of λ receptor-dependent *O*-(fluoresceinyl thiocarbamoyl)-amylopectin binding as an assay, the affinities of the λ receptor for maltodextrins and other sugars have been estimated. The affinity for dextrins increases with increasing degree of polymerization (K_d for maltose, 14 mM; for maltotetraose, 0.3 mM; for maltodecaose, 0.075 mM). Maltose and some other di- and trisaccharides are inhibitory to amylopectin binding, but only at concentrations above 1 mM.

The outer membrane protein known as the λ receptor (11) has been shown to be involved in bringing maltose and maltodextrins into *Escherichia coli* (13, 17) and is one of at least five proteins specifically involved in this process (10, 12). Absence of the λ receptor in *lamB* mutants results in an inability to grow on maltohexaose, maltopentaose, and maltotetraose, as well as a defect in growth on maltose and maltotriose at low extracellular concentrations (13, 17). The λ receptor has been isolated (11), and reconstitution of this protein into artificial bilayer membranes (1) and into outer membrane vesicles (9) resulted in the formation of aqueous pores in these membranes through which ions and various sugars could pass. Other outer membrane proteins called porins have been previously shown to have comparable pore properties in allowing passage of oligosaccharides up to trisaccharides, or other compounds generally smaller than about 600 molecular weight, through the outer membrane (8). Based on assays in black lipid films, it has been claimed that the pores formed by λ receptor are bigger than porin pores (1), and this could explain the ability of maltooligosaccharides up to maltoheptaose to get through the outer membrane. However, λ receptor pores in reconstituted outer membrane vesicles were found to be no larger than porin pores, and excluded tetrasaccharides similarly, though the saccharides tested were not maltooligosaccharides (9). A very recent claim has been that the rate of permeability through the λ receptor is higher for maltose and maltotriose than for other oligosaccharides (6, 15).

In studies of the substrate specificity of the maltodextrin transport system, we have shown that the outer membrane does not prevent the access of maltodextrins larger than maltoheptaose to the periplasm, even though these large dextrins are not further transported or utilized (T. Ferenci, Eur. J. Biochem., in press). Dextrins of 2,500 molecular weight can reach the periplasmic maltose-binding protein through the outer membrane when the λ receptor is intact (Ferenci, in press). The affinity for these long maltodextrins in the intact cell is as high as the affinity of the periplasmic maltose-binding protein itself (K_d 1 to 3 μM). Using [^{14}C]maltodextrins, the high-affinity binding of maltodextrins by *E. coli* was shown to depend on both λ receptor and maltose-binding protein (Ferenci, in press).

Obviously, it would be interesting to under-

stand in more detail the nature of the permeability specificity exerted by the λ receptor. We have previously proposed that part of the maltodextrin specificity of the λ receptor is derived from an interaction with the periplasmic maltose-binding protein (17). In this study, evidence is presented that the λ receptor is not just a pore but also a maltodextrin-binding protein.

MATERIALS AND METHODS

Materials. Maltodextrins were prepared and purified as previously described (17), and ^{14}C-labeled maltodextrins were synthesized also as described (Ferenci, in press). Amylopectin was coupled to fluorescein isothiocyanate by an adaptation of a method described for dextrans (2). Amylopectin (100 mg, dissolved in 5 ml of dimethyl sulfoxide) was mixed with 5 drops of pyridine and 100 μl of dibutyltin dilaurate solution (10%, vol/vol, in dimethyl sulfoxide). The solution was mixed with 500 mg of 10% (wt/wt) fluorescein isothiocyanate adsorbed on Celite and kept in a sealed tube for 2 h at 95°C. The Celite was removed by low-speed centrifugation, and the supernatant was treated with 3 volumes of cold ethanol. The precipitate formed was collected after 10 min by centrifugation. The precipitate was redissolved in dimethyl sulfoxide and reprecipitated with 3 volumes of ethanol. These precipitations were repeated four times in total to remove unbound fluorescein. The final precipitate containing *O*-(fluoresceinyl thiocarbamoyl)-amylopectin (FITC-amylopectin) was dried at 60°C overnight and redissolved in water. The amylopectin content was assayed by the anthrone method (2), and a standard curve of relative fluorescence versus amylopectin concentration was established by fluorescence measurements in minimal medium A as buffer (7). For all fluorescence measurements, the excitation wavelength was 470 nm, and emission was measured at 515 nm (uncorrected, Perkin-Elmer 650-10S fluorescence spectrophotometer).

All other sugars and polysaccharides used were from commercial sources.

Bacterial strains and growth of organisms. The strains of *E. coli* K-12 used in this study are described in Table 1. All the maltodextrin transport mutants map in the *malE-malF-malG* and *malK-lamB* operons (10, 12). The media for the growth experiments have been described (17).

Transport studies. For all experiments, bacteria were grown on maltose minimal medium, and late-exponential-phase cells were harvested and prepared as described (3). The cell density in the experiments was 3×10^8 bacteria per ml. To study the effect of inhibitors, [^{14}C]maltose (2.5 μM, 0.2 μCi/ml) was added to bacteria preincubated for 1 min with the inhibitors at the given concentrations. The sampling techniques and evaluation of results have been previously described (3).

Binding of [^{14}C]maltodextrins. The procedures for these binding assays have been described (Ferenci, in press). The substrate in all tests was 12 μM [^{14}C]-maltodecaose in the presence of the inhibitors indicated, using strain pop6434 at a cell density of 8×10^{10} bacteria per ml.

TABLE 1. *Bacterial strains*

Strain	Genetic marker	Source or reference
Hfr G6	Hfr G6 *his*	(17)
pop1021	Hfr G6 *metA trpE*	(17)
pop1080	Hfr G6 *metA trpE lamB102*	(17)
pop3325	F$^-$ *malT*^{c} *araD* Δ*lacU169* *rpsL*	M. Schwartz
pop6430	F$^-$ *malT*^{c} *malK* (Mu *cts72*) *araD*	M. Schwartz
pop6432	F$^-$ *malT*^{c} *malE* (Mu *cts64*) *araD rpsL*	M. Schwartz
pop6434	F$^-$ *malT*^{c} *malF* (Mu *cts60*) *araD rpsL*	M. Schwartz

Binding of FITC-amylopectin; standard assay. Exponentially growing bacteria in the specified media were harvested and washed twice in minimal medium A without carbon source. Bacteria in minimal medium A (1 ml) at the appropriate cell density (0.5×10^9 to 2.0×10^9 bacteria per ml) were mixed with 40 μg of FITC-amylopectin for 5 min at room temperature. Bacteria plus bound ligand were sedimented by centrifugation in an Eppendorf 3200 micro-centrifuge, and the supernatant was discarded. The bacteria were washed once with minimal medium A and again sedimented. The washed bacteria were then suspended in excess amylopectin (5 mg/ml) in minimal medium A to remove bound fluorescent ligand, incubated for a further 5 min and again centrifuged. The amount of fluorescence in this final supernatant was determined and quantitated by reference to a set of standard FITC-amylopectin concentrations diluted in minimal medium A.

To determine the effect of competing substrates on FITC-amylopectin binding, various concentrations of the competing substrates were substituted for the 5-mg/ml amylopectin in the standard assay. The fluorescence released by these various concentrations into the supernatant after centrifugation was measured. The FITC-amylopectin still bound to bacteria was also determined by collecting the bacteria and releasing bound FITC-amylopectin by washing with excess amylopectin (5 mg/ml). The sum of the FITC-amylopectin released by the two wash steps was constant in all assays.

RESULTS

Effect of amylose and amylopectin on maltose transport. The largest linear maltodextrin previously shown to inhibit maltose transport contained 15 glucose residues (Ferenci, in press). To test whether macromolecular α-1 → 4-linked glucans also inhibited maltose transport, amylose, amylopectin, and glycogen, as well as cyclic maltodextrins, were tested as possible inhibitors. Although none of these substrates can be used as sole carbon source by *E. coli* (data not shown), the data in Fig. 1 show that amylose and amylopectin are effective inhibitors of maltose transport, whereas glycogen and cyclohexaamylose are not. This transport

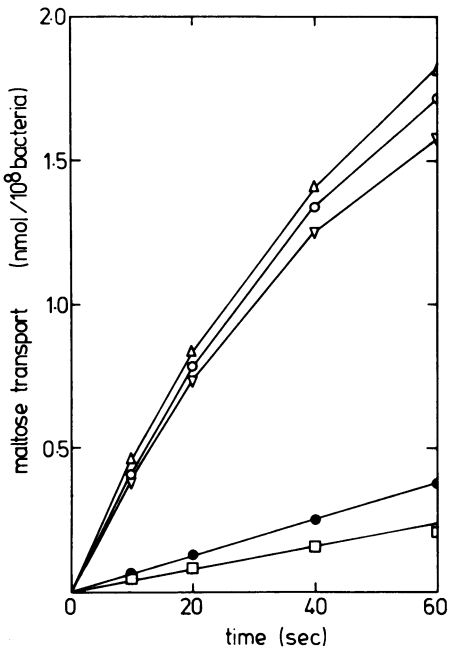

FIG. 1. *Transport inhibition by amylose and amylopectin. Maltose transport was assayed at 2.5 μM substrate concentration as described in the text. Maltose-grown pop1021 was used at a cell density of 5 × 10⁸ bacteria per ml. The transport of maltose is shown, without treatment* (○) *and in the presence of 1-mg/ml glycogen* (△), *1-mg/ml amylose* (□), *1-mg/ml amylopectin* (●), *and 1 mM cyclohexaamylose* (▽). *The same results were obtained with dialyzed polysaccharides; hence, the inhibitions by amylose and amylopectin were not due to low-molecular-weight contaminants.*

inhibition by polysaccharides could be correlated with inhibition of [^{14}C]maltodextrin binding by *E. coli*. The high-affinity binding of maltodecaose is dependent on two of the components of the maltose transport system, the periplasmic maltose-binding protein and the λ receptor (Ferenci, in press). As shown in Table 2, binding of 12 μM [^{14}C]maltodecaose was inhibited by amylose and amylopectin but not by glycogen or cyclohexaamylose. These results indicate not only that the two starch polysaccharides interact with the maltose transport system, but also that an extensive content of linear α-1 → 4-linked residues is required for recognition by the transport system. Cyclic maltodextrin or glycogen, a highly branched polysaccharide, is not recognized.

Amylopectin binding by intact bacteria. To study the interaction of the polysaccharides in more detail, a labeled amylopectin derivative was synthesized by a method described for the fluorescein labeling of dextrans (2). FITC-amylopectin binding to bacteria could then be quantitated. The binding of the labeled polysaccharide was linearly cell-concentration dependent up to 2 × 10^9 bacteria in the standard assay (not shown). The binding activity was highly inducible by maltose with 60-fold-higher amounts of FITC-amylopectin bound by maltose- rather than glucose-grown bacteria. To show even more conclusively that amylopectin binding was due to component(s) of the maltose transport system, the binding of FITC-amylopectin was tested in mutants missing the various components of the system. As shown in Table 3, any mutation leading to a loss of the λ receptor resulted in a loss of ability to bind FITC-amylopectin. Mutants lacking the *malE*, *malF*, or *malG* gene products were unaffected in amylopectin binding. The periplasmic maltose-binding protein (the *malE* gene product), the only previously identified binding component of the transport system, was clearly not essential for amylopectin binding.

Substrate specificity of the amylopectin binding site. The λ receptor-dependent binding of amylopectin was reversible and saturable (Fig. 2). Nonspecific binding of amylopectin was negligible, as shown with a *lamB* mutant at all concentrations. Binding was half-maximal at about 1.3 mg of amylopectin per ml. To test the substrate specificity of the amylopectin binding site, the inhibition of FITC-amylopectin binding by a range of sugars was investigated (Table 4). Maltotriose was the only sugar tested that gave over 50% inhibition of amylopectin binding at 1 mM concentration, though a number of other sugars, including maltose, could inhibit at higher concentrations. Interestingly, isomaltose and isomaltotriose were inhibitors at least as effective as maltose. Consistent with the transport inhibition data, the polysaccharide amylose at 1

TABLE 2. *Inhibition of [^{14}C]maltodecaose binding by polysaccharides*[a]

Inhibitor (concn)	Maltodextrin bound (nmol/10^{12} bacteria)	Inhibition %
None	41.1	0
Amylopectin (1 mg/ml)	19.2	53
Amylose (1 mg/ml)	17.6	58
Glycogen (1 mg/ml)	42.4	0
Cyclohexaamylose (1 mM)	40.8	1

[a] Strain pop6434 (*lamB*$^+$ *malE*$^+$ transport$^-$) grown on nutrient broth was used for these tests at a cell density of 8 × 10^{10} bacteria per ml in the binding test described (Ferenci, in press). The maltodecaose concentration was 12 μM, and substrate was added to the bacteria simultaneously with the potential inhibitors.

TABLE 3. *Binding of FITC-amylopectin by maltose transport mutants*[a]

Strain	Carbon source for growth	Maltose transport genes not expressed	FITC-amylopectin bound (μg per 10^{10} bacteria)	% Wild type
pop1021	Maltose	Wild type	5.46	100
pop1080	Maltose	*lamB*	0.11	2
pop3325[b]	Nutrient broth	Wild type	4.92	90
pop6430[b]	Nutrient broth	*malK, lamB*	0.05	1
pop6432[b]	Nutrient broth	*malE, malF, malG*	5.90	108
pop6434[b]	Nutrient broth	*malF, malG*	4.15	76

[a] The binding tests were carried out as described for the standard binding assay in the text. The cell density in the tests was 10^9 bacteria per ml.

[b] These strains constitutively express the maltose transport system (*malT*[c]).

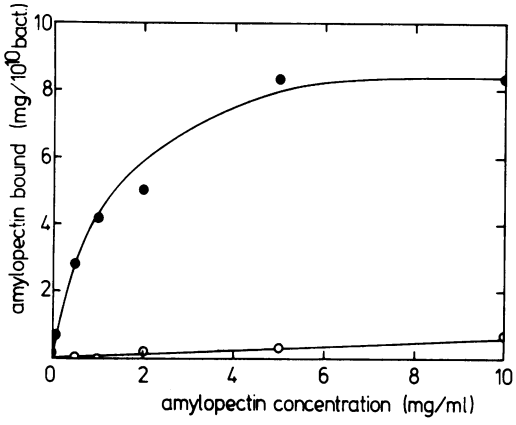

FIG. 2. *Substrate concentration dependence of amylopectin binding. The strains pop6432 (lamB⁺ malE; ●) and pop6430 (lamB malE⁺; ○) were prepared for the standard binding test as described in the text. The FITC-amylopectin concentration in all assays was 0.67 μg/μl, and its binding to the bacteria, at a cell density of 10^9/ml, was measured in the presence of the given concentrations of unlabeled amylopectin. The total amylopectin bound was calculated on the assumption that the fluorescent label binds with the same site and with the same affinity as unlabeled amylopectin.*

mg/ml also caused an 80% inhibition of amylopectin binding. The inhibition of amylopectin binding was investigated in more detail for maltose, maltotriose, and longer maltooligosaccharides. As shown in Fig. 3, the concentration of maltodextrin needed to give 50% inhibition of FITC-amylopectin binding decreased with increasing chain length, from about 14 mM for maltose to 0.65 mM for maltotriose to 0.3 mM for maltotetraose to 0.075 mM for maltodecaose. The values are probably close to the true K_d values for these dextrins, because these determinations were carried out at a fixed concentration of FITC-amylopectin well below the K_d for amylopectin.

DISCUSSION

Starch polysaccharides are bound at the outer membrane of *E. coli* by the λ receptor, a component of the maltose transport system. The genetic evidence rules out the involvement of the periplasmic maltose-binding protein, the only component of the transport system previously recognized to have a maltodextrin binding site (4, 5). The specificities of the two proteins are also very different: the maltose-binding protein has the same high affinity for maltose, maltooligosaccharides, and cyclic maltodextrins (K_d of 1 to 3 μM) and no affinity for isomaltose (4, 17), whereas the λ receptor has a poor affinity for maltose, isomaltose, and cyclic dextrins and an increasingly better affinity for longer linear maltodextrins.

It is interesting to consider in more detail

TABLE 4. *Effect of various sugars on the binding of FITC-amylopectin*[a]

Sugar	% Bound FITC-amylopectin released by sugar concn:	
	1 mM	10 mM
Maltose	5	31
Maltotriose	65	100
Isomaltose	20	53
Isomaltotriose	18	69
Cyclohexaamylose	10	27
Glucose	0	0
Lactose	0	0
Sucrose	0	9
Cellobiose	3	24
Trehalose	0	0
Raffinose	0	9

[a] The given concentrations of the above sugars were substituted for the 5-mg/ml amylopectin in the standard binding assay as described in the text. Strain pop6432, lacking the maltose-binding protein, was used in the tests at a cell density of 10^9 bacteria per ml. The amount of bound FITC-amylopectin in the test (100%) was 5.1 μg per 10^{10} bacteria.

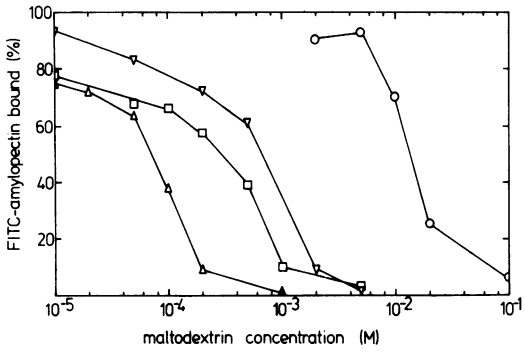

FIG. 3. *Inhibition of FITC-amylopectin binding by maltodextrins. The standard binding test with strain pop6432 (malE lamB$^+$), at a cell density of 10^9 bacteria per ml, was carried out in the presence of various concentrations of maltose* (○), *maltotriose* (▽), *maltotetraose* (□), *and maltodecaose* (△). *The 100% bound value for FITC-amylopectin was between 0.55 and 0.65 μg bound per 10^9 bacteria in each set of assays.*

what role the λ receptor binding may play in maltodextrin permeability through the outer membrane, and it may be asked whether the amylopectin binding site is a part of the transmembrane pore formed by the protein. The data are insufficient to answer the latter question, but two points suggest that the polysaccharides reach through the λ receptor pores into the periplasm. Inhibition of the high-affinity binding of [^{14}C]maltodecaose by amylose and amylopectin cannot be explained unless the polysaccharides reach the maltose-binding protein through the λ receptor pores, because this high-affinity binding is maltose-binding protein dependent (Ferenci, in press). Similarly, the inhibition of maltose transport cannot be explained by competition at the λ receptor binding site; the transport rate is more than 50% inhibited at amylopectin concentrations below the K_d of the λ receptor for amylopectin. Both these observations are explained more easily by an interaction of the polysaccharides with the periplasmic maltose-binding protein, presumably through the pores. The lack of inhibition of transport or decaose binding by cyclohexaamylose suggests that the cyclic dextrin cannot get through the pores to reach the binding protein to exert an inhibitory effect at 1 mM extracellular concentration.

The low binding affinity of the λ receptor for maltose and the previously demonstrated nonspecific pore-forming ability of the λ receptor in various membranes (1, 9) suggest that maltose (and other mono- and disaccharides) (16) can get through the λ receptor pores by a nonspecific mechanism. The increasing binding affinity for longer malto-oligosaccharides may give an increasing specificity to the pore and convert it into a facilitated diffusion system. This could explain the recently observed higher rates of maltotriose diffusion through the λ receptor than were found for another trisaccharide, raffinose (6, 15).

Maltodextrin transport across the outer membrane requires not only an intact λ receptor but also an intact maltose-binding protein (17). Mutants with the same growth phenotype as λ receptor mutants have been isolated that have altered periplasmic binding proteins. To explain these data, a specific role of the maltose-binding protein in bringing maltodextrins across the outer membrane was proposed. This interpretation was made before the binding activity of the λ receptor was recognized, and the question arises whether this interaction model is still valid considering the data in this paper. The crucial question is whether the shift in K_d of the mutant binding proteins for maltotetraose compared to the wild type (from 1.6 to 40 μM) could explain the reduction in the rate of transport across the outer membrane. Since the K_d of the λ receptor for maltotetraose is about 300 μM, it still seems necessary to postulate an interaction defect to explain the lack of transport of this substrate; simply on the basis of these binding K_d values it cannot be concluded that the maltodextrins stay tightly bound in the outer membrane. Nevertheless, further evidence is needed to clarify this point.

The binding activity of the λ receptor has not been previously detected due to its relatively low affinity for maltose, the only substrate extensively investigated (14). Tests of [^{14}C]maltodecaose binding by bacteria lacking the maltose-binding protein did show an unexplained residual binding activity at 12 μM substrate concentration (Ferenci, in press). This can now be explained on the basis of binding by the λ receptor.

An interesting result of these studies is the finding that FITC-amylopectin can be used as a specific fluorescent label for the λ receptor. This has proved useful not only in the binding studies described above but also in the labeling of bacteria containing receptors by fluorescence microscopy (unpublished data). This latter technique may have useful applications as a rapid method of detecting the presence of the receptor in comparative studies of enterobacteria and other microorganisms.

ACKNOWLEDGMENTS

We thank Ulrike Ehmann for excellent technical assistance.

We also thank W. Boos, whose grant from SFB 138 of the Deutsche Forschungsgemeinschaft supported this research,

and whose useful comments on the manuscript were appreciated.

LITERATURE CITED

1. **Boehler-Kohler, B. A., W. Boos, R. Dieterle, and R. Benz.** 1979. Receptor for bacteriophage lambda of *Escherichia coli* forms larger pores in black lipid membranes than the matrix protein (porin). J. Bacteriol. **138**:33–39.
2. **de Belder, A. N., and K. Granath.** 1973. Preparation and properties of fluorescein-labeled dextrans. Carbohydr. Res. **30**:375–378.
3. **Ferenci, T., W. Boos, M. Schwartz, and S. Szmelcman.** 1977. Energy coupling of the transport system of *Escherichia coli* dependent on maltose-binding protein. Eur. J. Biochem. **75**:187–193.
4. **Ferenci, T., and U. Klotz.** 1978. Affinity chromatographic purification of the periplasmic maltose binding protein of *Escherichia coli*. FEBS Lett. **94**:213–217.
5. **Kellerman, O., and S. Szmelcman.** 1974. Active transport of maltose in *Escherichia coli* K12: involvement of a "periplasmic" maltose binding protein. Eur. J. Biochem. **47**:139–149.
6. **Luckey, M., and H. Nikaido.** 1980. Specificity of diffusion channels produced by λ phage receptor protein of *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **77**:167–171.
7. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N. Y.
8. **Nakae, T.** 1976. Identification of the outer membrane protein of *Escherichia coli* that produces transmembrane channels in reconstituted membrane vesicles. Biochem. Biophys. Res. Commun. **71**:877–884.
9. **Nakae, T.** 1979. A porin activity of purified λ receptor protein from *Escherichia coli* in reconstituted outer membrane vesicles. Biochem. Biophys. Res. Commun. **88**:774–781.
10. **Raibaud, O., M. Roa, C. Braun-Breton, and M. Schwartz.** 1979. Structure of the *malB* region in *Escherichia coli* K12. Genetic map of the *malK-lamB* operon. Mol. Gen. Genet. **174**:241–248.
11. **Randall-Hazelbauer, L., and M. Schwartz.** 1973. Isolation of the bacteriophage lambda receptor from *Escherichia coli*. J. Bacteriol. **116**:1436–1446.
12. **Silhavy, T. J., E. Brickman, P. J. Bassford, Jr., M. J. Casabadan, H. A. Shuman, V. Schwartz, L. Guarente, M. Schwartz, and J. R. Beckwith.** 1979. Structure of the *malB* region in *Escherichia coli* K12. Genetic map of the *malE, F, G*, operon. Mol. Gen. Genet. **174**:249–259.
13. **Szmelcman, S., and M. Hofnung.** 1975. Maltose transport in *Escherichia coli* K-12: involvement of the bacteriophage lambda receptor. J. Bacteriol. **124**:112–118.
14. **Szmelcman, S., M. Schwartz, T. J. Silhavy, and W. Boos.** 1976. Maltose transport in *Escherichia coli*. Eur. J. Biochem. **65**:13–19.
15. **Tokunaga, M., H. Tokunaga, and T. Nakae.** 1979. The outer membrane permeability of Gram-negative bacteria. Determination of permeability rate in reconstituted vesicle membranes. FEBS Lett. **106**:85–88.
16. **von Meyenburg, K., and H. Nikaido.** 1977. Specificity of the transport process catalysed by the λ receptor protein in *Escherichia coli*. Biochem. Biophys. Res. Commun. **78**:1100–1107.
17. **Wandersman, C., M. Schwartz, and T. Ferenci.** 1979. *Escherichia coli* mutants impaired in maltodextrin transport. J. Bacteriol. **140**:1–14.