Journal of Supramolecular Structure 13:101–116 (1980)
Membrane Transport and Neuroreceptors 35–50

The Role of the Escherichia Coli λ Receptor in the Transport of Maltose and Maltodextrins

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The λ receptor is a peptidoglycan-associated integral protein that spans the outer membrane. Beside its function in phage λ adsorption it participates in transport. The latter function can be summarized as follows: 1) Receptor allows the nonspecific permeation of small molecules other than maltose and maltodextrins (in close analogy to a molecular sieve). Here the only criterion for selectivity is size and it has the properties of an unspecific pore. In this respect, it is similar to the outer membrane proteins Ia, Ib, and Ic, the porins. 2) It is a binding protein for maltodextrins. Binding affinity is low but increases by a factor of 500 as the chain length of the maltodextrins increases. In contrast, the affinity of the periplasmic maltose-binding protein for maltose and maltodextrins is similarly high (in the μM range). 3) In the in vitro system of liposomes, the λ receptor facilitates specifically the diffusion of maltodextrins that exceed the size limit given by its porin function. This clearly demonstrates that the λ receptor alone is able to specifically overcome the permeability barrier of the outer membrane for maltodextrins. 4) From the genetic and kinetic analysis of maltose and maltodextrin transport, it can be concluded that the λ receptor interacts with the periplasmic maltose-binding protein. 5) Electron microscopic studies indicate a location for the maltose-binding protein in the outer cell envelope. This location is dependent on the presence of the λ receptor.

Key words: λ receptor, maltose-binding protein, outer membrane permeability, maltodextrin transport

The active transport system for maltose and maltodextrins in E coli was discovered by Wiesmeyer and Cohn [1], and has been thoroughly studied mainly on a genetic level by Schwartz et al [2]. Recently, this transport system has become an attractive model system for membrane function and biogenesis. The entire system consists of at least five proteins that are coded for by the *malB* region containing two divergent operons [3, 4]. Three of these proteins are known. 1) The maltose-binding protein, the gene product of the *malE* gene, is a water-soluble periplasmic protein of 40,000

Received February 27, 1980; accepted March 10, 1980.

molecular weight (mol wt) that is released by the cold osmotic shock procedure [5, 5a]. It exhibits binding affinities for maltose and higher maltodextrins with dissociation constants that are in micromolar range [5–8]. The maltose-binding protein is not only essential for maltose and maltodextrin transport, but also for the chemotaxis towards these sugars [7]. A fully induced cell contains about 25,000 molecules [8]. 2) The λ receptor, the gene product of the *lamB* gene, is an integral protein of the outer membrane [9]. It has been isolated and purified by several groups [9–12]. It has a mol wt of 47,000 to 55,000 and occurs in vivo as a dimer [12a] or trimer [13]. It has been estimated that one cell contains about 100,000 copies of the polypeptide [14]. 3) The last protein identified so far is the gene product of *malF*. It is localized in the cytoplasmic membrane and has a mol wt of about 40,000. As few as 100 copies per cell may be present of this protein [15].

Both the periplasmic maltose-binding protein and the outer membrane localized λ receptor are synthesized on membrane-bound polysomes in the form of precursors [16]. The processing of these precursors is intimately connected with the in vivo assembly and the final location [17]. In addition, the synthesis of both proteins is linked to the cell cycle of the bacterium [8, 18].

Physiological studies of maltose transport in mutants that cannot metabolize maltose have shown that it is an "active" transport system. It transports its substrate against the concentration gradient, a process that is energy dependent [1, 19]. Typically, as a system involving a periplasmic binding protein, energy is provided in phosphate-bound form even though the maintenance of the electrochemical potential gradient of protons does seem to play an essential role, at least in the exit reaction [20]. The mechanism of substrate translocation across the cytoplasmic membrane remains unclear.

The early studies on maltose transport did not recognize the involvement of the receptor of phage λ even though it was curious that the gene for the λ receptor was part of a maltose regulon [21]. E.C.C. Lin was the first to suggest that this protein may participate in maltose transport at low – but not at the high – concentrations of maltose that are supplied when used as a carbon source [22]. Later, this suggestion could indeed be verified [23] and it has become clear that the λ receptor in some way facilitates the diffusion of maltose and maltodextrins through the outer membrane [19]. It is the main purpose of this publication to elucidate the role of the λ receptor in this process. However, it seems in order to first discuss briefly the permeability properties of the outer membrane for small polar molecules.

The outer membrane of the gram-negative E coli consists of phospholipids, lipopolysaccharide, and protein [24]. Morphologically, the outer membrane appears to be a typical biological membrane that is linked via the murein lipoprotein to the underlying network of the peptidoglycan [25]. In contrast to most biological membranes, the outer membrane of E coli exhibits high and largely unspecific permeability for small polar substances such as monosaccharides and disaccharides, amino acids, and ions [26]. Recently, it has become clear that this permeability of the outer membrane is due to some of the major outer membrane proteins characterized by several laboratories [27], the so-called porins [28]. The porins are typical intrinsic membrane proteins; they are peptidoglycan associated [29] and span the membrane [30]. They have a mol wt of

37,200 as subunit polypeptide chain [31] and form, in vivo and in solution, a trimeric structure [31A–31C].

The permeability properties of porins have been demonstrated by several different means. 1) In vivo by mutations in porin resulting in the pleiotropic impairment in the uptake of several solutes [32–37]; 2) in vitro by the reconstitution of permeability in liposomes due to the addition of porins [38]; and 3) in vitro by following the increase in electrical conductivity of black lipid membranes due to the flow of ions after the addition of minute amounts of porins [39, 40].

Porins exhibit for small polar substances, if at all, a low degree of specificity [37] and function essentially as molecular sieves with a cutoff point for the transported solute at about 600–700 mol wt [41–43]. Even though the synthesis of the different porins are regulated to some extent by the composition and osmolarity of the growth medium [44, 45], the cell tends to maintain a sufficient number of porins under different growth conditions and mutational loss of a particular porin [56]. How strong the selection pressure for a permeable outer membrane is, can be demonstrated by the appearance of "novel outer membrane proteins" in mutants that are devoid of the usual porins [32, 35, 37, 46, 47].

In addition to porins, there exists in the outer membrane a different class of transport-related and mostly inducible outer membrane proteins that exhibit a rather high degree of specificity for certain substrates that exceed the size limit of porin. Examples for these are the recognition sites for iron chelator complexes [48] and vitamin B_{12} [49]. These outer membrane receptors do not exhibit porin properties, since they are unable to relieve mutants missing all porin proteins of their permeability problems. Moreover, they seem to belong to a certain class of transport systems that are dependent on a functional *tonB* gene product [50]. Most transport-related outer membrane proteins – porins as well as specific receptors – appear to have multiple functions in as much as they can operate (alone or together) with the other components of the outer membrane as specific phage and colicin receptors [30, 51–56].

As will become clear from the following paragraphs, the properties of the λ receptor cannot simply be classified as a specific receptor protein or as a porin alone. In addition, its proper function in the maltose and maltodextrin transport machinery also appears to require the interaction with another protein of the maltose transport system, the maltose-binding protein.

THE DIFFERENT ASPECTS OF THE λ RECEPTOR AS TRANSPORT PROTEIN

1. Role of the λ Receptor in Maltose and Maltodextrin Uptake and Growth

The involvement of the λ receptor in maltose transport was discovered by Szmelcman and Hofnung [23]. They could demonstrate that λ-resistant strains exhibit an impairment of maltose transport and growth at low but not at high concentration. From the kinetic analysis of this phenomenon it was clear that the K_S of maltose transport was increased by a factor of 100 from 1.0 μM to 0.1 mM, while the V_{max} remained identical (Fig. 1) [19]. When higher dextrins were tested, it was found that wild-type cells could transport maltodextrins up to maltohexaose with an apparent K_S in the μM range (Table I) [57]. In contrast, mutants missing the λ receptor were severely defective in transporting malto-

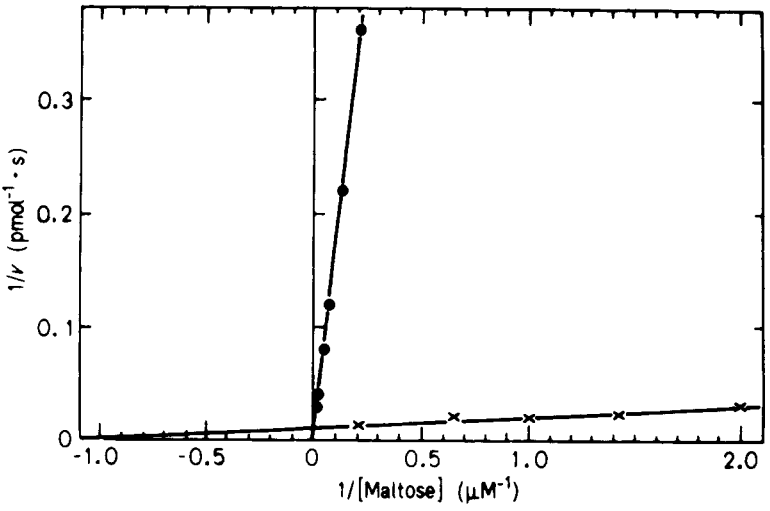


Fig. 1. The effect of a *lamB* mutation on maltose transport. Lineweaver-Burk plots of the initial rates of maltose uptake into strains with (x) and without λ receptor (•) indicate a shift in K_S for maltose from 1.0 μM to 100.0 μM as a result of the mutation; the V_{max} is unaltered [19].

triose (the K_S could not be determined [19], while maltotetraose and higher maltodextrins were not accumulated at all [57]. These transport patterns were clearly reflected by the ability of wild-type and λ-receptor mutant to grow on maltodextrins of different chain length at high concentration (far above their transport K_S) (Fig. 2) [58]. As can be seen, the wild-type strain grows equally well on maltose and maltodextrins up to maltohexaose. In contrast, the strain missing the λ-receptor grows equally well as the wild-type only on maltose. Severe impairment of growth already occurs with maltotriose and no growth can be seen with maltotetraose and above. From this analysis one might conclude that the λ-receptor specifically overcomes the permeability barrier of the outer membrane for maltose and maltodextrins very much in analogy to the highly specific outer membrane receptors of the vitamin B_{12} type. However, this is clearly an oversimplification. Even though the λ-receptor is in fact a binding protein for maltodextrins [59], its binding affinities are not in agreement with the respective overall transport parameters (Table I). In addition, as discussed in the next paragraph, λ-receptor also exhibits properties that are compatible with an unspecific porin activity.

2. The Porin Activity of the λ-Receptor

When purified λ-receptor was incorporated in black lipid membranes the electrical conductivity across the membranes was dramatically increased [60]. At very low protein concentrations the conductivity increase could be observed in single steps that were due to pores formed by the λ receptor (Fig. 3). Based on the average single-channel conductivity, a membrane thickness of 4.0 nm, and the shape of a cylindrical hydrophilic channel, a pore diameter of 1.1 nm could be estimated. The channel exhibits a slight cation specificity. These properties were very similar to the pore-forming activity of porin when used in the same experimental setup [39, 40].

TABLE I. Transport Kinetics Into Intact Bacteria

Substrate	Mol wt	Utilized as sole C-source	Transported	λ Receptor K_D (M)	Maltose binding protein K_D (M)	Transport kinetics into intact bacteria	
						K_S (M)	K_I (inhibition of maltose transport) (M)
Maltose	342	+	+	1.4×10^{-2}	1.9×10^{-6}	8.0×10^{-7}	
Maltotetraose	666	+	+	3.0×10^{-4}	1.6×10^{-6}	1.6×10^{-6}	5.0×10^{-7}
Maltohexaose	990	+	+	7.5×10^{-5}	2.8×10^{-6}	1.2×10^{-6}	Not determined
Maltodecapentaose	2,448	–	–	5.7×10^{-5}	1.1×10^{-6}		2.3×10^{-6}
Amylose	$\geqslant 10^4$	–	–	Tightly bound	Tightly bound		Strong inhibition
Amylopectin	$> 10^4$	–	–	TIghtly bound (1.3 mg/ml)	Tightly bound		Strong inhibition
Cyclohexaamylose	972	–	–	$\geqslant 10^{-2}$ M	3.4×10^{-6}		No inhibition

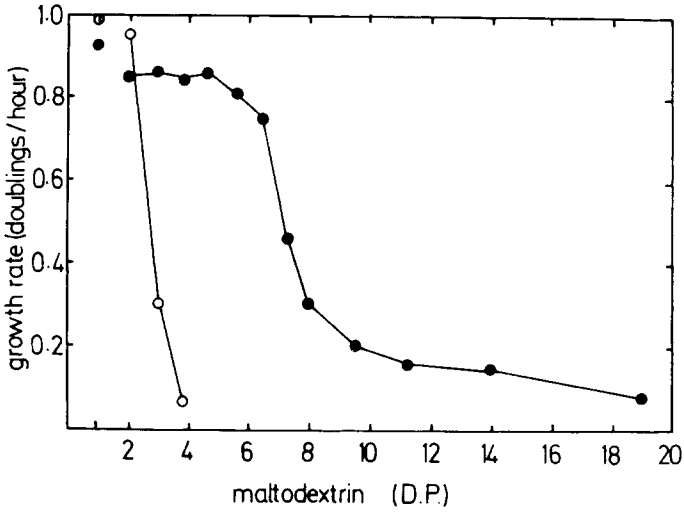


Fig. 2. The effect of a *lamB* mutation on the growth of E coli on maltodextrins. The growth rates of a wild-type (•) and a *lamB* mutant (○) were tested on maltodextrins containing the number of glucose residues given on the abscissa. The growth rate on glucose (DP = 1) is shown as reference. The substrate concentration in each experiment was 0.5 mM [58].

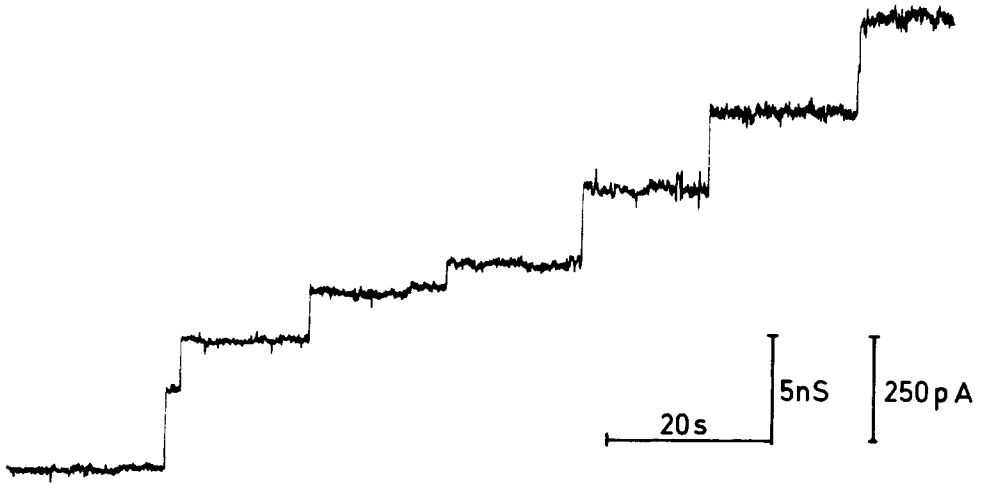


Fig. 3. The pores formed in black lipid membranes by the λ receptor. The addition of isolated λ receptor (20 ng/ml) to the aqueous solutions bathing the bilayer results in a stepwise increase in membrane conductance. The applied voltage was 50 mV and the current before addition of λ receptor 1.8 pA [60].

A different experimental approach to demonstrate the porin qualities of λ receptor was taken by Nakae [12]. He studied the permeability properties of liposomes in which λ receptor was incorporated. He could demonstrate that λ receptor behaves very similarly to porin [28] by allowing the permeation of different unrelated sugars up to a certain size (< 600 mol wt) (Fig. 4). Moreover, the porin property of λ receptor could also be

demonstrated in vivo. Mutants that exhibit a pleiotropic transport defect due to missing porin [32] could be suppressed to a normal phenotype by introducing an intact λ receptor [60A].

From these reconstitution studies one might conclude that λ receptor is nothing but a porin-type protein. Indeed, it was speculated that the obvious specificity of the λ receptor for maltodextrins seen in vivo was just due to the somewhat larger pore size of the λ receptor in comparison to the classical porins [39, 60]. However, as will be discussed in the next paragraph, λ receptor is not just a porin but a specific porin.

3. The λ Receptor is a Specific Facilitator for Maltodextrins

Nakae et al [61] and Nikaido et al [62A] developed two methods to measure the rate of diffusion through porin and λ receptor in reconstituted liposomes. The first was to measure the release of glucose from intracellular maltodextrins via entrapped α-glucosidase after the addition of exogeneous maltodextrins. The other was to measure the osmotic swelling properties after the addition of the permeable carbohydrate. Both studies arrive at the conclusion that maltodextrins of mol wt that exceed the porin limit of the λ receptor still diffuse easily via the λ receptor but not the porins Ia/Ib. Thus,

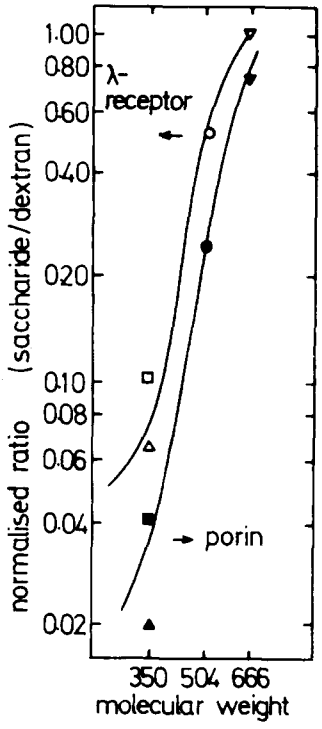


Fig. 4. The permeability of reconstituted vesicles to oligosaccharides. Vesicles reconstituted with equivalent amounts of λ receptor (open symbols) or porin (closed symbols) were prepared in the presence of (^{14}C)-maltose (^{3}H)-dextran (△,▲), (^{14}C)-lactose (^{3}H)-dextran (□,■), (^{3}H)-raffinose (^{14}C)-dextran (○, ●) or (^{3}H) stachyose (^{14}C)-dextran (▽ ▼). The normalized ratio saccharide/dextran (mol wt 20,000) is a measure of the permeability of the vesicles for small molecules (modified according to [12].

it is clear that the λ receptor specifically catalyzes the diffusion of maltodextrins. However, the rate of diffusion for maltose through the λ receptor seems not to be significantly different from that through porin.

4. The λ Receptor is a Maltodextrin-Binding Protein

The selectivity of the λ-receptor pores for maltodextrins could be most easily explained if the pores had a binding specificity for α-1→4-linked glucose residues. Such a binding site has recently been demonstrated in binding studies using intact bacteria [59]. In fluorescence labeling studies, the binding of O-(fluoresceinylthiocarbamoyl)-amylopectin (FITC-amylopectin) was shown to be dependent on the presence of the λ receptor in the outer membrane. Maltose and maltodextrins were able to inhibit the λ-receptor-dependent binding of FITC-amylopectin and, from competition studies, the K_D's for these substrates were determined (Fig. 5; Table I). The affinity of the λ receptor is clearly higher for increasingly long maltodextrins while the affinity for maltose is extremely low. Indeed, at the level of disaccharide, isomaltose is as good as maltose in inhibiting FITC-amylopectin binding even though isomaltose is not recognized by the maltose transport system or the periplasmic maltose-binding protein [5]. In contrast, the periplasmic protein has a higher affinity than the λ receptor for all maltodextrins tested (Table I) and the affinity for the different maltodextrins is nearly constant, in the μM range.

There is no evidence, as yet, that the binding site of the λ receptor is in the transmembrane pore itself. Even if the binding site were within the pore, it is difficult to see that the binding would play a significant role in maltose permeability as the affinity for this substrate is so poor. For maltotriose and longer maltodextrins, however, the binding

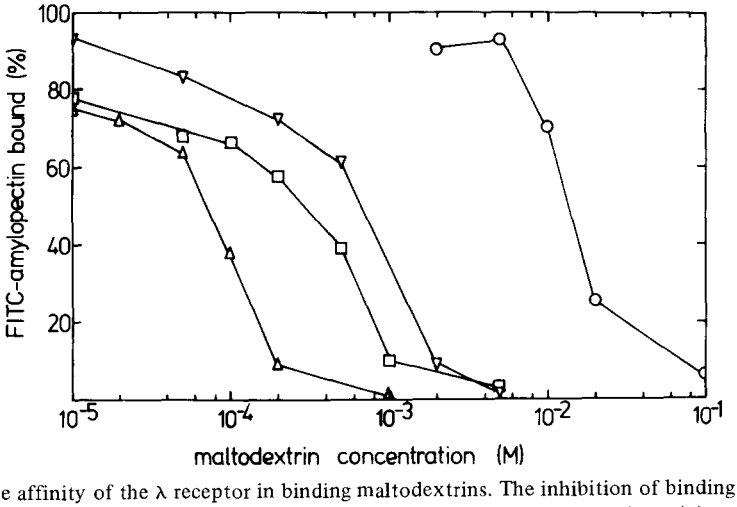


Fig. 5. The affinity of the λ receptor in binding maltodextrins. The inhibition of binding of FITC-amylopectin in a strain lacking maltose-binding protein is shown for maltose (○), maltotriose (▽) maltotetraose (□), and maltodecaose (△). The 100% bound value for FITC-amylopectin in these experiments was 0.55–0.65 μg bound/10^9 bacteria, well below the K_D for amylopectin, Therefore, the concentration of dextrin at which 50% inhibition of binding occurs is reflected in the K_D for the given dextrin [59].

site may turn the pore into a relatively specific transporter for maltodextrins. As yet, no evidence has been obtained using reconstituted vesicles that the λ-receptor pore is indeed a facilitated diffusion system according to the usual kinetics criteria, and whether the kinetic constants are consistent with the measured binding affinities.

5. Do the Pore Properties of the λ Receptor Explain Maltose and Maltodextrin Transport Across the Outer Membrane?

Although the studies with reconstituted membranes have described the pore properties and selectivity of the λ receptor in some detail, there are several observations that suggest that these properties do not fully explain the mechanism of transport of maltose and maltodextrins across the outer membrane. Three observations will be discussed below that suggest that the specificity of transport across the outer membrane in an intact bacterium is not solely dependent on the selectivity of the λ receptor as shown in reconstituted membranes.

A. Transport of maltose into *lamB* mutants. A longstanding observation is that the effect of a mutation removing the λ receptor is to leave intact the maximal rate of maltose transport into the bacterium but to result in a hundredfold increase in the apparent K_S for the substrate (Fig. 1) [19]. This effect is observed despite the presence of porin pores in the outer membrane of the *lamB* mutants. The change in kinetics of maltose transport cannot be explained by differences in pore specificities, as the λ receptor and porin have the same size specificity for oligosaccharides [12], and the rates of maltose permeability through porin and λ-receptor pores into vesicles are similar [61]. The drastic change in affinity of maltose transport into *lamB* mutants would suggest that the λ receptor is involved in maintaining the high affinity towards maltose in normal bacteria (K_S 1.0 μM; Table I) in a way that cannot be explained by the pore specificity or binding specificity (K_S 14 mM) of the λ receptor towards maltose. Obviously, porin cannot substitute for λ receptor in maintaining the high affinity of the transport system towards maltose.

B. Kinetics of maltodextrin transport. Using ^{14}C-labeled maltodextrins, the transport affinities for maltose, maltotetraose, and maltohexaose have been measured (Table I). For each substrate, the affinity is of the same order (0.8–1.6 μM) but the maximal rate of transport for the various sugars decreases with chain length; maltodextrins above maltoheptaose are not utilized [57, 58] or transported (Fig. 6). An important finding, however, is that longer maltodextrins are still able to interact with the maltose transport system and can act as competitive inhibitors of the system [57]. As shown in Table I, the K_I for a maltodecapentaose fraction with an average mol wt of 2,448 daltons is also comparable to the K_I for the shorter dextrins such as maltotetraose. Interestingly, all the transport affinities are also comparable to the binding affinity of the maltose-binding protein and are much higher than that of the λ receptor for the same substrates (Fig. 6; Table I). These results suggest that the λ receptor is not a kinetic barrier even for molecules of over 2,000 mol wt, which can readily reach the maltose-binding protein. How is it possible to explain access of such large molecules across an outer membrane 7.0 nm wide through a pore narrower than the Stokes' radius of the substrate? Clearly, such access is difficult to explain by a conventional pore model of maltodextrin permeability across the outer membrane.

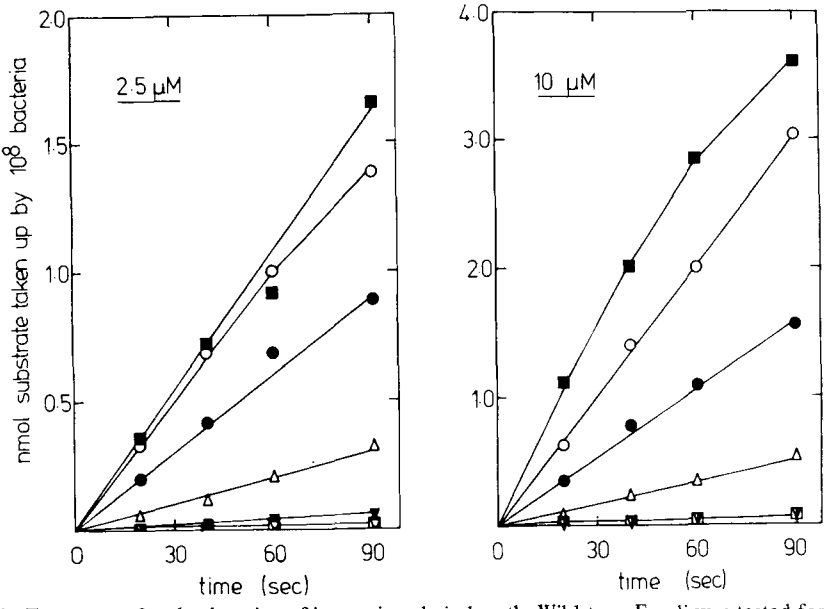


Fig. 6. Transport of maltodextrins of increasing chain length. Wild-type E coli was tested for transport activity towards maltotetraose (■), maltopentaose (○), maltohexaose (●), maltoheptaose (Δ), maltooctaose (▼), maltodextrin DP 9.5 (□), and maltodextrin DP 11.2 (▽). Substrates were present at 2.5 μM (left) and 10.0 μM (right) [57].

C. Properties of *malE* mutants with a maltose$^+$ maltodextrin$^-$ phenotype. The transport properties of a class of mutants altered in the periplasmic maltose-binding protein also suggest that transport of maltodextrins across the outer membrane is not solely dependent on the λ receptor. These *malE* mutants have the same phenotype as *lamB* mutants and are unable to grow on maltodextrins longer than maltotriose. The mutant binding proteins have been isolated and their binding properties characterized. The phenotype of the mutants is not explicable by a defect in binding along maltodextrins; on the contrary, the affinity for long maltodextrins that are not transported is higher than the affinity for maltose that is transported. The defect in the mutants is likely to be a lack of access of longer maltodextrins across the outer membrane, as these mutants are also resistant to the inhibition of maltose transport by maltotetraose [58]. Because the isolated mutant maltose-binding protein binds tetraose in preference to maltose, the resistance to inhibition suggests the binding protein is not accessible to the longer dextrin even in the presence of an intact λ receptor. A model of maltodextrin transport that takes these observations into account is presented below.

6. An Interaction Model of Maltodextrin Transport Across the Outer Membrane

The proposed model is shown in Figure 7. The model differs from a porin pore in two important aspects. Firstly, the proposed pore is considerably shorter than the width of the outer membrane and secondly, the λ-receptor component of the outer membrane interacts closely with the periplasmic maltose-binding protein. The advantages of the model in explaining maltodextrin transport can be considered in terms of the two novel features of the model. The shorter pore would help to explain two previously inexplicable findings; namely, the ready accessibility of the maltose-binding protein to large substrates (section 5B) and why the dimensions of the λ-receptor pore in ion flux

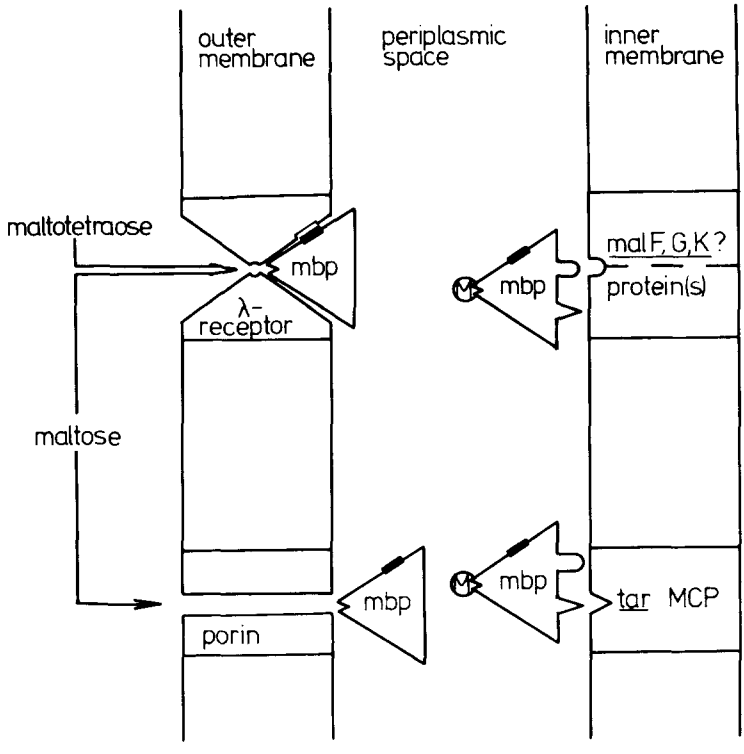


Fig. 7. A schematic model of maltose and maltodextrin transport across the outer membrane. It is not known which of the *malF*, *malG*, or *malK* products is involved in recognition of maltose-binding protein in the cytoplasmic inner membrane. The interaction of maltose-binding protein containing bound ligand has been shown with the *tar* gene product, one of the methylated chemotaxis proteins involved in modulating the chemotactic response towards maltose [65].

experiments with black lipid membranes were estimated as being larger than porin pores, assuming the same pore length [60]. If the λ-receptor pore were actually shorter than the width of the outer membrane, the pore diameter may be considerably smaller which would explain the size selectivity as measured with oligosaccharides [12].

As discussed above (section 5), it is not clear whether the maltodextrin binding site of the λ receptor is actually in the pore or not, but it has been assumed in Figure 7 that the binding site is indeed part of the pore. In favor of such an assumption are the findings of Lukey and Nikaido [62] who showed that maltoheptaose can inhibit glucose transport in a porin-dependent mutant which would suggest the dextrins can, in fact, block the λ-receptor pore.

The proposed interaction of maltose-binding protein with the λ receptor during maltodextrin transport would also explain several observations on the transport system. Genetic alterations of the proteins concerned could obviously lead to defects in the proposed interaction. Two classes of mutation are already known that lead to drastic alterations in the properties of maltose and maltodextrin transport across the outer membrane. The effect on maltose transport caused by the lack of the λ receptor described in section 5A may be explained if the high affinity for maltose of the whole cell surface is dependent on the interaction of the binding protein with the λ receptor. Porin lacks the specificity to replace the λ receptor in this respect. Similarly, the high affinity for longer maltodextrins may be explained by the same mechanism; the interaction allows longer malto-

dextrins to reach the maltose-binding protein. The *malE* mutants described in section 5C may be postulated to be defective in the interaction and, therefore, lack access to longer maltodextrins sitting in the λ-receptor pore. Support for this model has been provided by Heuzenroder and Reeves [63A]. It was found that the nonspecific porin function of the λ receptor is increased by the absence of the maltose-binding protein. Thus, it was concluded that the maltose-binding protein can block the λ receptor pore by associating with it.

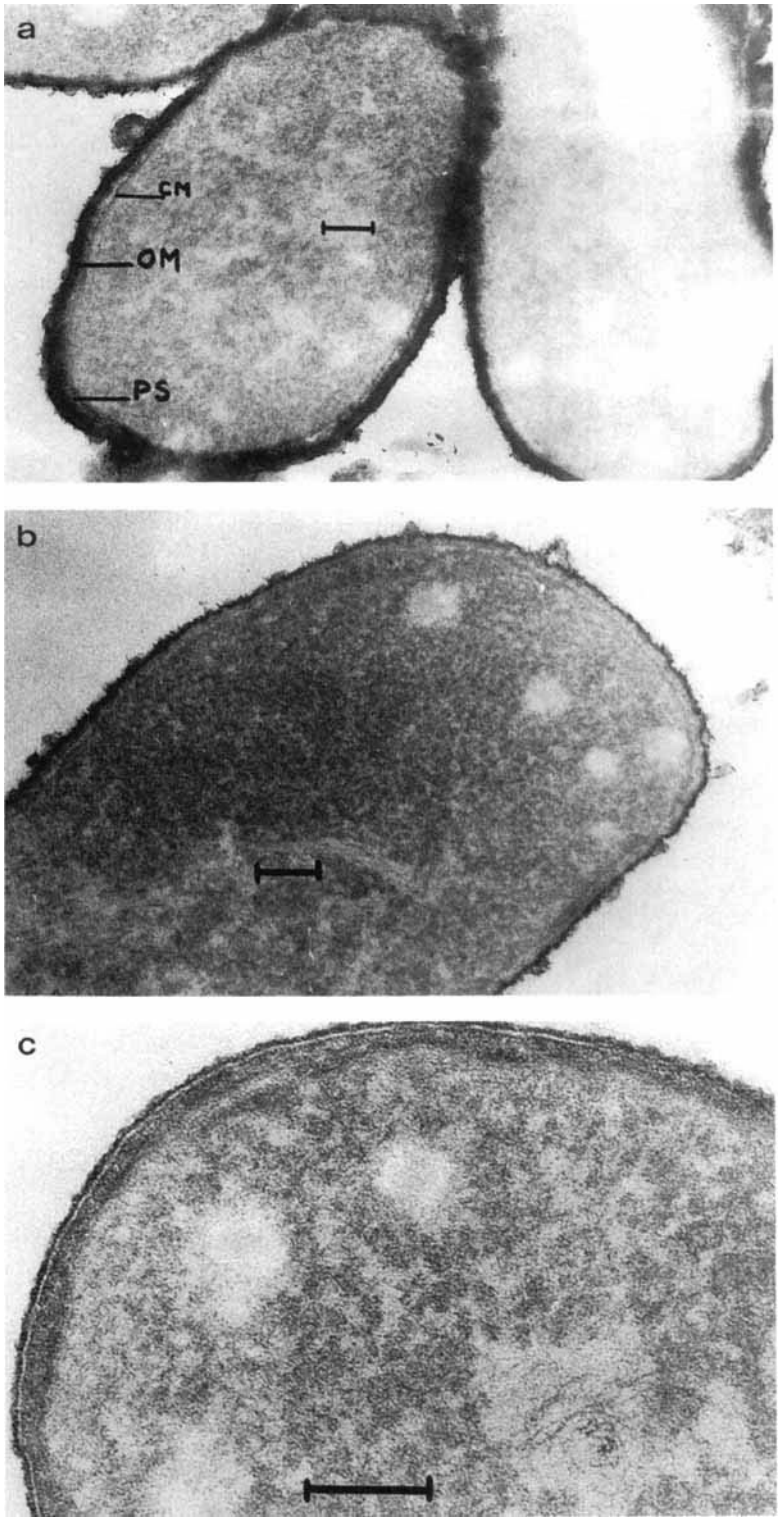
7. Localization of Maltose-Binding Protein in the Outer Envelope

The model in Figure 7 would suggest that the osmotic-shock-releasable, maltose-binding protein is closely associated with the outer membrane during its interaction with the λ receptor. There is no strong evidence to suggest that the binding protein is, in fact, an integral outer membrane protein; so what evidence is there that the localization suggested in Figure 7 is indeed correct?

The first point that needs to be made is that the maltose-binding protein is not on the outside of the outer membrane. Such a location has been suggested for the succinate-binding protein [63]. Maltose-binding protein is not accessible to antibodies against this protein, as antibodies neither inhibit maltose transport [23], nor can binding be shown of ^{125}I-labeled, antimaltose-binding protein to intact bacteria (J. Brass, unpublished communication). Also, the active site of the maltose-binding protein is not on the outside of the cell as cyclohexaaymlose (a good substrate of the protein in vitro) is unable to inhibit maltose transport (Table I). This means that this cyclic substrate of approximately 1.4 nm diameter is unable to reach the binding protein presumably due to pore limitation of the λ receptor in vivo. In contrast to the finding of Lo with the succinate-binding protein, maltose-binding protein and maltose transport are not affected by protease treatment of intact bacteria even though maltose-binding protein is degraded by the proteases in vitro (T. Ferenci, unpublished communication).

Evidence that maltose-binding protein may be associated with the outer membrane in the presence of λ receptor comes from electron microscopic localization studies [64]. When wild-type cells grown on maltose were fixed with glutaraldehyde and treated with α MBP-F_{ab}-horseradish peroxidase, peroxidase activity can be localized in thin sections close to or even within the outer membrane. In contrast, mutants missing the maltose-binding protein (and also those that only miss the λ receptor) do not show the characteristic staining pattern seen in the wild-type (Fig. 8).

Fig. 8. Ultrastructural localization of the maltose-binding protein. F_{ab} fragments of affinity purified, anti-maltose-binding-protein antibodies were coupled to horseradish peroxidase and incubated with glutaraldehyde-treated bacteria. The reaction product formed after the addition of diaminobenzidine-H_2O_2 marks the position of the complex. Subsequently the bacteria were stained with O_sO_4-uranyl-acetate, embedded, and thin sections were viewed in the electron microscope: (a) Wild-type bacteria grown on maltose; (b) *malB* mutants missing the entire *malB* region; and (c) *lamB* mutants missing the λ receptor and grown on maltose. CM, cytoplasmic membrane; OM, outer membrane. PS, periplasmic space. The wild-type exhibits smooth staining within the outer membrane that is enlarged by the deposition of the hydrophobic reaction product. Sparse granular staining is seen in the periplasm (a). This typical staining pattern is not seen in the mutant (b) missing the maltose-binding protein as well as the other *malB*-dependent components. The mutant that lacks the λ receptor but contains the maltose-binding protein exhibits mostly staining in the periplasm; ⊢—⊣ indicates 0.1 μ [64].



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CONCLUSION

As a consequence of the above studies and model proposed, the following sequence of events during maltose and maltodextrin transport may be envisaged: In the absence of substrate, the λ receptor and maltose-binding protein form a complex bringing the active site of the maltose-binding protein into the outer membrane. In the presence of exogenous maltose, passage through the λ receptor is essentially diffusion through a short pore exhibiting weak binding activity for maltose. On reaching maltose-binding protein, the binding of maltose causes a conformational change in maltose-binding protein resulting in dissociation of the maltose-binding protein–λ receptor complex. Loaded binding protein is released in the periplasm and substrate is further transported to the cytoplasmic membrane by dissociation-exchange of substrate in the periplasm, or less likely, diffusion of the protein-substrate complex to the inner membrane. Little is known of the process of maltose and maltodextrin transport across the inner membrane. As indicated in Figure 7, maltose-binding protein containing bound ligand probably has two specific interaction sites, one necessary for chemotaxis and one in transport through the inner membrane. Evidence of these different sites comes from the properties of *malE* mutants that are unimpaired in chemotaxis towards maltose but are defective in transport [7]. It has been shown recently that maltose-binding protein containing bound ligand interacts directly with the *tar* gene product, one of the methylated chemotaxis proteins involved in controlling the chemotactic response in the inner membrane [65].

For an oligosaccharide such as maltotetraose, the binding specificity of the λ receptor is more important than is the case with maltose. In this respect, the λ receptor may act as a threading mechanism, making linear maltodextrin chains (even of polysaccharides) accessible to maltose-binding protein. For increasingly large maltodextrins, especially above maltoheptaose, it may be that the rate of threading through the outer membrane is increasingly slow, even though they are readily accessible to maltose-binding protein. This would explain the decreasing V_{max} for transport of increasingly long dextrins, but the maintenance of a high affinity for these substrates. This would, in turn, explain the inability to grow on dextrins longer than maltoheptaose.

The proposed intraction of λ receptor and maltose-binding protein is testable in biochemical terms and is being investigated. However, in view of the large concentration of both binding protein and λ receptor in the cell, and the necessity of dissociation of the complex on binding substrate, such an interaction may have an elusively low affinity.

ACKNOWLEDGMENTS

We are grateful to Drs. Nakae and Nikaido for communicating their results prior to publication. The permission to reproduce published material was obtained from the European Biochemical Society, the American Society of Microbiology, and Academic Press.

The work performed in the laboratory of the authors was supported by grants from the Deutsche Forschungsgemeinschaft (Sonderforschungsbereich 138) and the Verband der Chemischen Industrie.

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