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Role of the Receptor for Bacteriophage Lambda in the Functioning of the Maltose Chemoreceptor of *Escherichia coli*

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Chemotaxis towards maltose is specifically defective in many strains of *Escherichia coli* carrying mutations affecting *lamB*, the gene coding for the outer membrane receptor for bacteriophage lambda. However, with one exception, the most extreme effect of *lamB* mutations on the maltose response as determined in the capillary assay is a shift to higher sugar concentrations and a reduction in the number of bacteria accumulated to about 25% of the wild-type level. The severity of the taxis defect is strongly correlated with reduced ability of the cells to take up the maltose present at 1 and 10 μM. Evidence presented here and in the accompanying paper indicates that the lambda receptor is involved in the transport of maltose at these concentrations. The effects of *lamB* mutations on maltose taxis can be explained by postulating that the high-affinity maltose transport system in which the lambda receptor participates transfers maltose from the surrounding medium across the outer membrane and into the periplasmic space. If the maltose chemoreceptor detects sugar present in the periplasmic space, and not molecules external to the outer membrane, then defective transport of low concentrations of maltose into the periplasm would result in the observed apparent reduction in the sensitivity of the maltose receptor. Thus, the lambda receptor protein would participate in maltose chemoreception only indirectly through its role in maltose transport.

It was recently observed in this laboratory that some strains of *Escherichia coli* that carry mutations affecting the gene *lamB* are defective in chemotaxis towards maltose but not towards other attractants (9). Thus, there was reason to suspect that the receptor protein for bacteriophage lambda, which is the product of *lamB* (20), might be a component of the maltose chemoreceptor. The lambda receptor is a protein of about 55,000 daltons, which is located exclusively in the outer membrane of *E. coli* (20). It is tightly associated with that membrane and requires the presence of detergent to remain in aqueous solution (20).

The results presented here indicate that the lambda receptor is involved in the functioning of the maltose chemoreceptor only through its role in a high-affinity uptake system for maltose. The apparent affinity of the maltose chemoreceptor is reduced 10- to 100-fold by *lamB* mutations which cause defects in high-affinity maltose uptake. The high-affinity system functions over a range of sugar concentrations from approximately 0.1 to 10 μM, a range similar to that over which the β-methylgalactoside transport system transports galactose (4). The role of the lambda receptor in the uptake of maltose is considered in detail in the preceding paper (22).

A study of the maltose chemoreceptor has been published recently (9) and can be summarized as follows. A maltose-binding protein, the product of *malE* (13), serves as the recognition component of the maltose chemoreceptor, as well as functioning in the transport of maltose. The products of other maltose genes that function in metabolism of maltose (*malP* and *malQ*) or transport of the sugar (*malF* and *malK*) do not appear to be involved in maltose chemoreception. As indicated above, there was preliminary evidence for an involvement of the *lamB* product in maltose chemoreception.

The *malB* region consists of two divergent operons, one containing *malE* and *malF* and the other containing *malK* and *lamB* (11). The observations that the lambda receptor is part of an uptake system for maltose and can influence the functioning of the maltose chemoreceptor provide an explanation for the inclusion of *lamB* in a maltose operon (21).

MATERIALS AND METHODS

Bacteria. All bacterial strains except the maltose taxis revertants were obtained from M. Hofnung at

the Institut Pasteur, Paris. The parent of the 100 series of *lamB* mutants is *E. coli* K-12 strain pop 1021, a *metA, trp, galE, galy* (a mutation in the *gal* region which confers resistance to galactose on the *galE* mutant [21]), *rif*^{r} derivative of Hfr G6 (20a). The parent of the 1-100 series and the 200 series is pop 1048, a *metB, lacZ*^{am} derivative of Hfr G6 (M. Hofnung, A. Jezierska, and C. Braun, manuscript in preparation). The *malB* deletions are derivatives of Hfr G6 (12). A motile derivative of each strain was selected from a swarm on a tryptone semisolid plate (1) and, if that derivative was poorly motile when grown on minimal media, a second selection was carried out on a minimal medium-galactose semisolid plate (18).

Medium. The growth medium, containing mineral salts (H1 medium), glycerol (50 mM), and maltose (5 mM), and minimal medium-sugar swarm plates (18) have been described previously (3, 18).

Chemicals. Maltose used for the chemotaxis assays was purified by the procedure described previously (1). D-Galactose (Sigma Chemical Co.) was "substantially glucose free." [^{14}C]maltose (Radiochemical Centre, Amersham, England; universally labeled) at 10 mCi/mmol was used for uptake assays. Essentially all radioactivity in this material is maltose (9). Glass-distilled water was used in making all solutions.

Revertant selection. Washed cells of a *lamB* mutant (approximately 4×10^9) were suspended in 0.4 ml of chemotaxis medium and spread in a streak on four minimal medium swarm plates containing 25 μM maltose plus any required nutrients. At this sugar concentration, wild-type cells form a distinct, fast-moving chemotactic ring. The plates were incubated at 35 C in a humid incubator. At 24 h there is usually some growth but no visible swarms or rings. At 48 h there are often indistinct areas of swarming and sometimes very light chemotactic rings. Samples of cells from these areas were streaked out, and individual colonies were tested for the ability to form chemotactic rings on a maltose swarm plate.

Chemotaxis assay. The procedures used for growth, preparation, and assay of the bacteria were essentially those described by Adler (3). These procedures, along with the specific conditions used in this study, can be summarized as follows. Bacteria were grown at 35 C and washed at room temperature. A capillary (1-μliter disposable micropipette) containing a solution of attractant was placed into a 0.3-ml suspension of bacteria (1×10^6 to 3×10^6 cells per ml) on a glass plate. The chemotaxis medium (10 mM potassium phosphate at pH 7 and 0.1 mM ethylenediaminetetraacetate) allows motility and chemotaxis but not growth; the bacteria rely on their endogenous energy sources. After incubation at 32 C for 45 min, the capillary was removed and the number of bacteria inside was determined by plating the contents. Under these conditions, the relation between number of motile bacteria in the suspension and accumulation in the capillary is linear (3). The number of bacteria accumulating in a capillary containing only buffer (blank value) was considered an assay of the number of motile bacteria in the suspension and of the degree of motility. Taxis was quantiated by measuring the area under the response curve (after subtracting blank values) and normalizing to that same blank value. The derived value was expressed as the percentage of the wild-type value. The relation between the responses is not altered significantly if accumulation of bacteria at the peak is used instead of the area value, nor if normalization is accomplished by use of the magnitude of the response to an attractant other than maltose. The usual range of variation in these assays was approximately 20%. All points in an assay were done at least in duplicate, and the average value was plotted. Examples of tactic response to maltose are shown in Fig. 1 and 2.

Maltose uptake assay. The initial rate of uptake of ^{14}C-labeled maltose was determined by filtering 0.5-ml samples of a suspension of three-times-washed cells (5×10^7 cells per ml) on 0.45-μm pore cellulose filters (Schleicher and Schüll, Dassel). The procedure was to incubate 3.5 ml of cells for 20 min at 30 C, to add [^{14}C]maltose as a 100×-concentrated solution, and to take samples every 5 s for 30 s. The use of automatic pipettes and a Millipore sampling manifold made possible rapid sampling and immediate washing with 5 ml of chemotaxis medium. All solutions were at 30 C. Previous studies have shown that the presence of 10 mM glycerol does not alter the initial uptake rate (9). Filters were dried, and the retained radioactivity was determined by liquid scintillation counting. Values for nonspecific retention of maltose by the cells and filter were determined by using cells that had been first treated with 20% formaldehyde. Those values were subtracted from experimental values; they were less than 10% of the radioactivity retained by the wild-type cells 20 s after maltose addition.

There is no known non-metabolizable analogue for maltose, so that maltose transport can only be tested with the metabolizable sugar. Thus, the radioactivity accumulated in the cell includes nonmetabolized "soluble" maltose and metabolic products of the sugar but does not include ^{14}C, which has been lost as $^{14}CO_2$ during metabolism. In general, accumulation of radioactivity by wild-type strains was linear for the first 20 s (see Fig. 4) and then reached a plateau by 90 s. Within the limit of the scatter resulting from low accumulation of radioactivity, uptake by the mutant strains was linear over the 30-s sampling time. At 20 s, more than 80% of the accumulated radioactivity could be removed from wild-type cells by a wash with chemotaxis medium containing 100 μM unlabeled maltose. At this time, less than 5% of the radioactivity had been lost from the incubation solution. The accumulated exchangeable maltose represents about a 700-fold concentration at 20 s. Initial rates in Table 2 were determined from the linear portion of the uptake curves; 10^7 cells is equivalent to about 25 μg of cell wet weight.

RESULTS

Strains isolated and characterized as carrying mutations in gene *lamB* by M. Hofnung were tested for their ability to be attracted to maltose. All strains were also tested for chemotaxis

toward at least one amino acid, usually L-aspartate, and one sugar, usually D-galactose. The only significant differences observed between the taxis behavior of a wild-type parent strain and a mutant strain were for taxis toward maltose.

The maltose taxis behavior of the *lamB* mutants is summarized in Table 1. The response to maltose is characterized by two values, the normalized (see above) percentage of parental response and the maltose concentration at which the strain exhibited maximum accumulation (the "peak concentration"). Examples of plots from which such values were derived are shown in Fig. 1. For a number of the strains listed in Table 1, the rate of initial uptake of ^{14}C-labeled maltose was determined in the presence of 1 and 10 μM sugar (Table 2; see Fig. 4).

The strains carrying nonsense mutations in *lamB* should contain very defective lambda receptors or, often, none at all. The maltose response in all such strains is shifted to higher sugar concentrations as indicated by the increased peak concentrations (Table 1 and Fig. 2). Most of these strains also exhibit a reduced total response. However, no strain has lost the ability to respond to maltose. The peak concentration is a function of a number of parameters (see reference 3 for a discussion). Thus, it is also informative to determine "threshold concentrations," the lowest attractant concentration in the capillary that produces an accumulation greater than that obtained in the absence of attractant. If chemotaxis data like that shown in Fig. 1 are plotted on a double logarithmic scale (Fig. 3), a straight line can usually be drawn through the values between the threshold and the peak (2). When the background accumulation is accurately determined, the intersec-

TABLE 1. *Maltose taxis behavior of lamB mutants*

Strain	Mutation	Mutation[a] type	Sensitivity to:			Maltose taxis	
			λv	λvh	λvh*	% Parental response	Peak concn (mM maltose)
G6	None		s	s	s	100	1
pop 1715	*malBΔ1*	Deletion begins in *malK* extends into *lamB*	r	r	r	23	2.5
pop 1718	*malBΔ5*	Deletion begins in *malK* extends into *lamB*	r	r	r	16	5
pop 1730	*malBΔ17*	Deletion begins in *malK* extends into *lamB*	r	r	r	0	
pop 1048	None		s	s	s	100	1
pop 1063	*lamB1*	Missense	r	s	s	60	1
pop 1064	*lamB2*	Missense	r	s	s	43	1
pop 1066	*lamB4*	Missense	r	r	s	28	1
pop 1067	*lamB5*	Missense	r	r	s	118	1
pop 1068	*lamB6*	Missense	r	s	s	80	1
pop 1072	*lamB9*	Opal	r	r	r	75	10
pop 1073	*lamB11*	Not determined	r	r	r	49	10
pop 1051	*lamB201*	Amber	r	r	r	16	10
pop 1053	*lamB203*	Amber	r	r	r	48	5
pop 1054	*lamB204*	Amber	r	r	r	16	10
pop 1054r1		Taxis revertant (suppressed)	s	s	s	98	1
pop 1054r2		Taxis revertant	s	s	s	114	1
pop 1021	None		s	s	s	100	1
pop 1079	*lamB101*	Missense	r	s	s	83	1
pop 1080	*lamB102*	Ochre	r	r	r	62	10
pop 1084	*lamB106*	Missense	r	s	s	55	1
pop 1087	*lamB109*	Missense	r	r	s	25	10
pop 1088	*lamB110*	Missense	r	s	s	100	1
pop 1089	*lamB111*	Ochre	r	r	r	57	10
pop 1090	*lamB112*	Missense	r	s	s	88	1
pop 1091	*lamB113*	Missense	r	r	s	129	1
pop 1092	*lamB114*	Amber	r	r	r	92	5

[a] The information is from M. Hofnung, A. Jezierska, and C. Braun (manuscript in preparation).

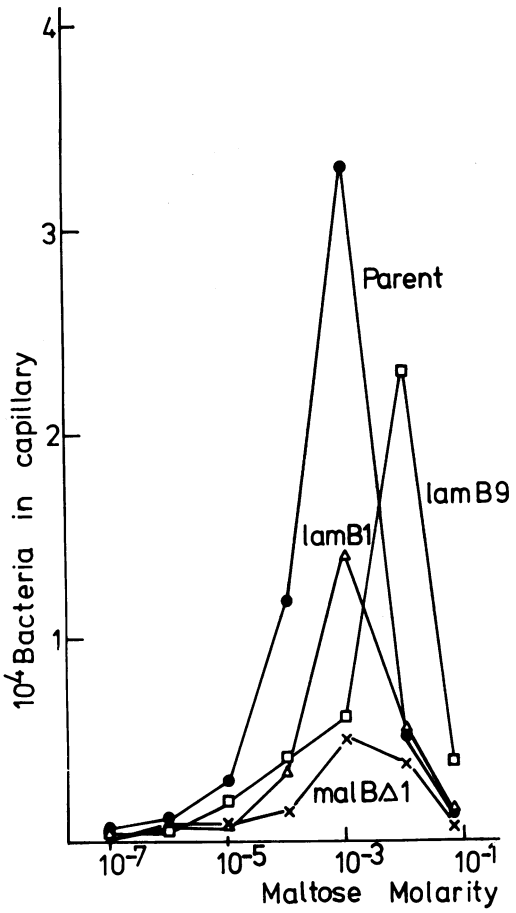

FIG. 1. *Maltose taxis of parent and mutant strains. The data have not been normalized. Blank accumulations: pop 1048, 350; malBΔ1, 300; lamB9, 300. Symbols: ●, pop 1048; ×, malBΔ1; △, lamB1; □, lamB9.*

tion of the line with the background value defines the threshold concentration. These plots are best done with data from several replica determinations to minimize the effect of the 20% variation in the assay. Since the line is determined by values that are large relative to background, as well as those close to background, much of the error inherent in the small numbers is avoided. In Fig. 3, accumulation values representing the average of at least four separate determinations are plotted for a wild-type parent and two *lamB* mutants. Threshold values for other nonsense mutants are similar to the value shown here for the amber mutant *lamB201*, i.e., about 100-fold higher than that of wild type.

The inability of nonsense mutants in *lamB* to respond to gradients of low concentrations of maltose can be visualized in another way. Motile bacteria form "chemotactic rings" when spotted on a swarm plate (1, 18). On a plate containing a relatively low concentration of a metabolizable attractant as the carbon source (e.g., 0.1 mM maltose), the initial inoculum will metabolize all the attractant in the immediate area, thus creating a gradient of attractant at the periphery of growth. The bacteria will move out in response to that gradient, consuming the attractant as they go, forming a chemotactic ring. Wild-type strains will form a ring on a plate containing 25 μM maltose, but *lamB* nonsense mutants will not. At 250 μM maltose, both mutants and wild type form rings. At intermediate concentrations, the mutants spread from the point of inoculation without forming distinct rings. As has been pointed out previously (18), the inability to form chemotactic rings can be due to defects in metabolism, transport, or chemotaxis. The *lamB* mutants have no maltose metabolism defects, but, as documented in the preceding paper (22) and here (Table 2 and Fig. 4), *lamB* nonsense mutants are vastly defective in the uptake of 1 μM and often of 10 μM maltose. This transport defect is sufficient to explain why *lamB* nonsense mutants do not form chemotactic rings at low maltose concentrations.

When approximately 10^9 cells of a *lamB* nonsense mutant are spread on a swarm plate containing 25 μM maltose, some areas of swarming occur on the border of the inoculum

TABLE 2. *Uptake of* [^{14}C]*maltose*

Maltose mutation	% Parental initial uptake rate[a]	
	10^{-6} M maltose	10^{-5} M maltose
None (pop 1048)	100	100
lamB1	25	44
lamB2	57	49
lamB4	37	35
lamB5	50	66
lamB9	0	4
lamB11	4	8
lamB204	5	85
lamB204r2	62	64
lamB101	16	72
lamB106	66	66
lamB109	0	7
lamB110	68	75
lamB112	20	65
lamB113	56	113

[a] Rates were determined from linear accumulation of [^{14}C]maltose during approximately the first 20 s after addition of the sugar to cells at 30 C. The wild-type rate is approximately 50 pmol/min per 10^7 cells.

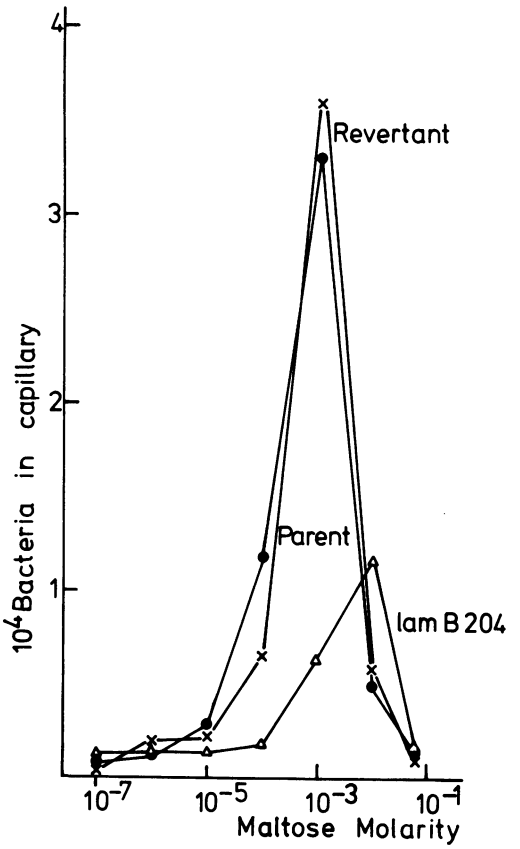

FIG. 2. *Maltose taxis of a lamB amber mutant strain, its parent, and a taxis revertant. The data have not been normalized. Blank accumulations: pop 1048, 350; lamB204, 600; lamB204r2, 500. Symbols: ●, pop 1048; Δ, lamB204; ×, lamB204r2.*

after 24 to 48 h. Clones isolated from such areas are chemotactic revertants, which respond to maltose as well as the original parent. Maltose responses of a representative parent-mutant-revertant set are shown in Fig. 2. All taxis revertants isolated from a number of different nonsense mutants have also reverted to lambda sensitivity. Amber mutants derived from strain pop 1048 carry the *lac* amber mutation. Thus, it is possible to classify the chemotactic revertant of these strains into a class of suppressed amber mutants and a class reverted in some other fashion. An example of each is listed in Table 1. One nonsuppressed revertant was tested for maltose uptake and found to be close to normal (Table 2).

Three deletions that begin in *malK*, the gene immediately proximal to *lamB*, and end in *lamB* (M. Hofnung, personal communication) all result in defective maltose taxis. The response of strains carrying *malBΔ1* and *malBΔ5* are similar to the responses of strains carrying *lamB* nonsense mutations. The behavior of these deletion mutants strengthens the observation that absence of the lambda receptor alters the response to maltose but does not eliminate it. Since the defective taxis phenotype is expressed in the absence of lambda receptor protein, it is difficult to argue that defective taxis results from a sequestering of the maltose-binding protein by defective lambda receptor, making it unavailable to the maltose chemoreceptor.

There is a class of *lamB* mutations that confers resistance to wild-type lambda but not to one or more host range mutants of that phage (Table 1). These mutations are probably all missense (Hofnung et al., manuscript in preparation). Since the lambda receptor protein of such strains still functions as a receptor for some types of lambda, probably the protein produced by those mutants is only slightly altered, either by an amino acid change directly in the "absorption site" or by an alteration in the conformation of that site resulting from an alteration in a different part of the molecule.

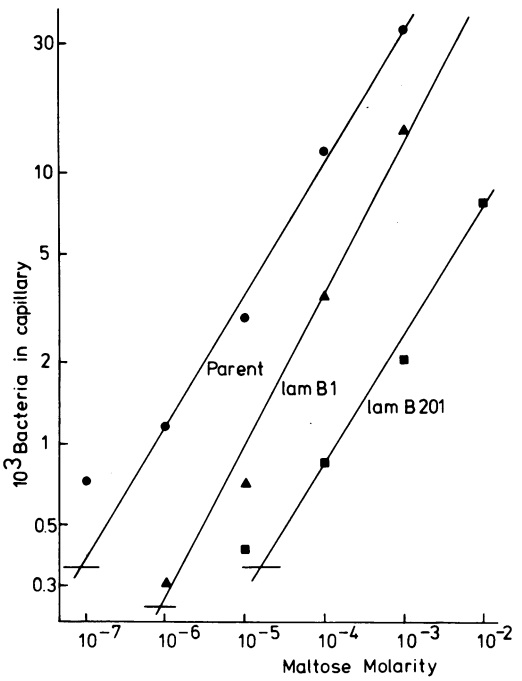

FIG. 3. *Double logarithmic "threshold" plot of maltose response by parent and lamB mutant strains. The points represent averages of at least four capillaries. The horizontal lines represent the average accumulation into at least four capillaries containing only buffer. Symbols: ●, pop 1048; ▲, lamB1; ■, lamB201.*

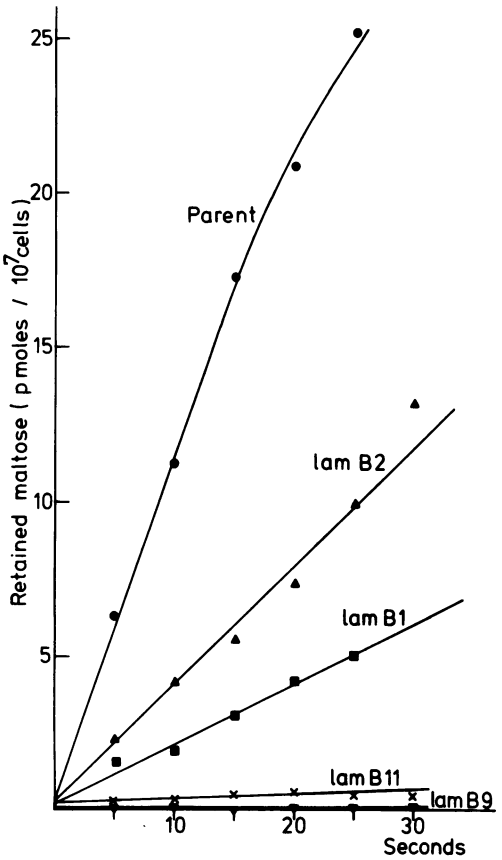

FIG. 4. *Initial uptake of 1 μM [¹⁴C]maltose by parent and lamB mutant strains. Symbols: ●, pop 1048; ■, lamB1; ▲, lamB2; ▼, lamB9; ×, lamB11.*

Thus, it is not surprising that most of these mutants are only partly defective in maltose uptake (Table 2 and Fig. 4) and only slightly defective in taxis towards maltose (Table 1). In only one lambda host range-sensitive mutant, *lamB109*, is maltose uptake as defective as in the nonsense mutants (Table 2). This uptake defect is reflected in an altered threshold and peak concentration of the response to maltose. There are a few lambda host range-sensitive mutants (*lamB1*, *lamB101*, and *lamB113*) that take up maltose better than the nonsense mutants but still are significantly defective. These defects are not reflected in a shift in peak concentration but are reflected in the formation of "fuzzy" or dim rings on maltose swarm plates and, when enough data have been collected, in a maltose threshold concentration shifted to a value intermediate between the wild type and the totally defective mutants (Fig. 1 and 3).

DISCUSSION

The defective uptake of micromolar concentrations of maltose by *lamB* mutants indicates that the protein first identified as the receptor for the phage lambda (20) is a component of a high-affinity system for the transport of maltose (21). Thus, the outer membrane protein can be grouped with the vitamin B_{12}-E colicins receptor (5) and the iron bacteriophage T1 receptor (6) as a cell surface protein that is part of a transport system and also the receptor for an invading particle. The outer membrane has been characterized as a barrier to medium and large-size molecules (8, 14, 18, 22). The discovery that in that membrane there are several apparent transport systems for small molecules implies that, at least in certain cases, the outer membrane also limits the passage of such molecules. The observed involvement of lambda receptor-related transport of maltose in the chemoreception of that sugar can be explained by postulating an outer membrane barrier to free entry of maltose. The possibility is developed below.

Mutations in *malF* or in *malK* (except those with polar effects on *lamB*) have little effect on maltose taxis, although those mutations, as well as ones in *malE*, eliminate maltose transport at concentrations from 1 μM to 10 mM (9, 12). Most *malE* strains, mutant in the maltose-binding protein, are defective in both chemotaxis and transport. However, some *malE* mutants exhibit nearly normal maltose taxis, although they have the usual defective transport (9). It was possible to demonstrate that some of these mutants release a normal maltose-binding activity (9). Presumably, these mutations affect parts of the maltose-binding protein that are necessary for the transport function but are not necessary for binding of maltose or for the chemotaxis function. Thus, any of the three genes previously identified with maltose transport can be mutated in such a way that maltose transport is vastly defective, without affecting taxis towards maltose. This leads to the conclusion that transport of maltose is not necessary for the function of the maltose chemoreceptor (9).

However, in the case of *lamB* mutants, defects in the high-affinity maltose transport system alter the apparent affinity of the maltose chemoreceptor. In the presence of 1 μM maltose, a wild-type cell takes up maltose at an initial rate of about 5×10^4 molecules per s. After 90 s, a plateau level is reached which is equivalent to a 500- to 1,000-fold concentration of exchangeable maltose in the cell volume. The

gene products of *malE*, *malF*, *malK*, and *lamB* are all necessary for this accumulation (9, 21; Table 2). Completely transport-defective *lamB* mutants exhibit an initial rate of uptake of 1 μM maltose that is less than 10% that of the wild-type. These strains also reach a plateau level less than 10% of normal, although this level still represents a significant concentration of maltose from the surrounding medium (data not shown). Only the products of *malE*, *malF*, and *malK* are required for maltose transport sugar concentrations above 0.1 mM (9, 12). It is possible to explain these observations by postulating that the products of these three genes function to transport maltose across the cytoplasmic membrane, i.e., from the periplasm to the cell interior. At high concentrations of maltose, the sugar enters the periplasm by "unassisted diffusion" but, at concentrations in the order of 1 μM, maltose enters the periplasm by a mechanism in which the lambda receptor participates. If that mechanism does not function, less maltose enters the periplasm and entry is at a slower rate.

The effect of complete *lamB* defects on the extent as well as the rate of maltose entry can be interpreted to indicate that there is a component in the outer membrane transport system that, in the mutant strains, has an altered affinity for maltose, thus limiting the absolute amount of sugar that enters. If the outer membrane became less permeable to maltose in some nonspecific manner, then the sugar should finally accumulate to the usual level, particularly with the cytoplasmic membrane system clearing the periplasm of maltose.

There are two ways in which the *lamB* maltose transport defects could reduce the apparent affinity of the maltose chemoreceptor. The defects would eliminate part of the concentration range over which there would be sufficient maltose available in the periplasm to bind to the binding protein and generate a minimal "chemotaxis signal." Second, even if maltose were present in the periplasm at concentrations that would allow a detectable amount of association with the binding protein, the rate of entry of maltose as the exterior concentration rose might be too slow relative to the time constant of the process by which the chemoreceptor detects temporal increases in attractant concentration; i.e., the gradient would appear too shallow. The present data do not distinguish between these two effects. Probably both of them are involved in the taxis defects of *lamB* mutants. In both cases the defects would be expressed not only in the responses to gradients centered around 1 μM (an initial capillary concentration of 100 μM produces a concentration of about 1 μM at the capillary mouth [7]), but also in the total response. For any initial attractant concentration in the capillary, there is a region of the formed gradient with concentrations in the micromolar range. Normally these regions are areas of "recruitment," resulting in the movement of cells up the gradient towards the capillary mouth (16). Transport-defective *lamB* mutants would be insensitive to recruitment in these regions. The insensitivity would account for the generally lower total response of *lamB* mutants to maltose.

The complete absence of response to maltose by *malBΔ17* is not explained by the above considerations. Either the total absence of both the *malK* and *lamB* products results in an apparently inactive maltose receptor or the deletion extends into an unknown gene, distal to *lamB*, whose product is necessary for maltose taxis. The strains carrying *malBΔ1* or *malBΔ5*, deletions which begin in the distal third of *malK* and extend into *lamB* (12), are maltose transport defective; thus, they have no active *malK* product but respond to maltose in the same way as mutants lacking solely the lambda receptor. Such observations make the second possibility seem more likely. However, it appears that deletion *malBΔ17* ends within *lamB* (M. Hofnung, personal communication); thus, another explanation, perhaps the existence of a second mutation in the strain, must be sought.

The outer membrane transport system for maltose would not necessarily require a source of energy. The maltose-binding protein has dissociation constants for maltose in the micromolar range (13) and is probably present at 1 to 10 mM in the periplasm (20a). Thus, molecules of maltose entering the periplasm would most probably immediately bind to binding protein sites (20a) and could then be funneled to cytoplasmic membrane transport components. No binding of maltose to the lambda receptor protein has been detected by straightforward equilibrium dialysis (unpublished data).

The β-methylgalactoside transport system exhibits an apparent affinity for galactose and glucose, which is similar to that exhibited by the high-affinity system for maltose (4). In each system, a shock-released binding protein serves both transport and chemoreception (9, 10). However, the galactose-binding protein is involved only in a high-affinity system, whereas the maltose-binding protein is necessary for all maltose transport (9, 13). There are two complementation groups of mutants in the β-methylgalactoside system (18), besides gene

mglB, which produce the galactose-binding protein (4). Mutations in *mglA* and *mglC* usually affect galactose taxis only by shifting the response to lower sugar concentration (19). This shift is most probably a result of less destruction of sugar gradients by cells unable to take up and metabolize the attractant (19). Mutations in *malK* often have a similar effect on the response to maltose (9). A few *mglA* and *mglC* mutants are significantly defective in galactose taxis but, since the genetic organization of the *mgl* genes is not defined, it is not clear whether these defects are direct results of the mutations or the results of effects of the mutations on production of other gene products. In any case, no galactose transport mutation shifts the apparent affinity of the galactose receptor to higher sugar concentrations, as is the case for maltose taxis affected by *lamB* mutations.

All identified chemoreceptor components are localized in the cytoplasmic membrane of the periplasmic space (3a). There are indications that the state of bulk lipid of the cytoplasmic membrane is important for chemotaxis (15). It appears that attractants do not have to enter the cytoplasm to elicit chemotaxis (3a). Thus, probably the whole of the chemotactic process occurs through components located in or at the cytoplasmic membrane. The suggestion made here that the maltose chemoreceptor is sensitive to attractant entering the periplasm, and not to the sugar external to the outer membrane, is consistent with that concept.

There is always a possibility that the apparent affinity of a receptor system, as determined by the response of intact biological material, is influenced by barriers to the free access of ligand to the binding site and is not simply a reflection of the affinity of receptor for ligand exhibited in vitro. The functioning of the maltose chemoreceptor in transport-defective *lamB* mutants appears to be an example of such a possibility.

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