Mutations in tar Suppress Defects in Maltose Chemotaxis Caused by Specific maleE Mutations

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Maltose-binding protein (MBP), which is encoded by the maleE gene, is the maltose chemoreceptor of Escherichia coli, as well as an essential component of the maltose uptake system. Maltose-loaded MBP is thought to initiate a chemotactic response by binding to the tar gene product, the signal transducer Tar, which is also the aspartate chemoreceptor. To study the interaction of MBP with Tar, we selected 14 maleE mutants which had specific defects in maltose taxis. Three of these mutants were fully active in maltose transport and produced MBP in normal amounts. The isoelectric points of the MBPs from these three mutants were identical to (maleE461 and maleE469) or only 0.1 pH unit more basic than (maleE454) the isoelectric point of the wild-type protein (pH 5.0). Six of the mutations, including maleE454, maleE461, and maleE469, were mapped in detail; they were located in two regions within maleE. We also isolated second-site suppressor mutations in the tar gene that restored maltose taxis in combination with the closely linked maleE454 and maleE461 mutations but not with the maleE469 mutation, which maps in a different part of the gene. This allele-specific suppression that MBP and Tar interact directly.

The periplasmic maltose-binding protein (MBP) of Escherichia coli is an essential component of the maltose transport system (for a review, see reference 17) and of the maltose chemotactic system (14). MBP is encoded by the maleE gene (20), which is located in the malB region (18, 32) at 91 min on the E. coli linkage map.

The product of the tar gene, the chemotactic signal transducer Tar protein (taxis to aspartate and some repellents), is also required for maltose chemotaxis (34), although the tar gene is not part of the maltose regulon. The Tar protein is located in the cytoplasmic membrane (29) and also mediates chemotactic responses to the attractant L-aspartate and the repellents CO2+ and Ni2+. Aspartate binds to the Tar protein directly (10); MBP is required for stimulation of the Tar protein by maltose (14).

Upon binding maltose, MBP undergoes a conformational change (37) that is thought to increase its affinity for the Tar protein and the inner membrane transport components. Biochemical data have suggested that MBP binds to the Tar protein; in the presence of maltose, solubilized Tar protein was retained on affinity chromatography columns containing immobilized MBP (21), and maltose-loaded MBP sedimented with energized membrane vesicles prepared from tar+ strains but not from tar−strains (28).

To study the MBP-Tar protein interaction genetically, we wanted to isolate maleE mutants that were defective in maltose taxis but normal for maltose transport. This proved to be a laborious process, since we did not succeed in developing a good selection or enrichment procedure for mutants lacking maltose taxis. However, by performing localized mutagenesis of the malB region with phage P1 and screening for the loss of maltose chemotaxis, we were able to find mutants of the type that we sought. With one of these mutants we were also able to isolate secondary mutations in tar that showed allele-specific suppression of the chemotactic defect caused by certain maleE mutations. This finding provided genetic evidence that there is physical interaction between the MBP and the Tar protein.

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MATERIALS AND METHODS

Reagents. The maltose (E. Merck AG) used for chemotaxis assays was purified by descending paper chromatography (Whatman 3MM paper). The chromatogram was developed in butanol-pyridine-water-acetic acid (15:12:10:3, vol/vol). Hydroxylamine and N-methyl-N'-nitro-N-nitosoguanidine were purchased from Sigma Chemical Co. [U-14C]Maltose (185 mCl/mmol) was obtained from Amersham Corp. All other chemicals were of reagent grade.

Bacterial strains. The bacterial strains used are listed in Table 1. All of the strains used for quantitative measurements of chemotaxis carried the malT−1 mutation (12), which allows constitutive expression of all operons of the maltose regulon. Strain MC4100 (9) is nonmotile because of an flbB mutation. We made strain MC4100 flbB+ in two steps. Using phage P1 vir, we introduced the eda mutation of strain MM515 by cotransduction with zeb-741::Tn10, selecting for tetracycline resistance (Tet′). The resulting transductant was then transduced to eda+ with a P1 vir lysate prepared on strain MM110, and the transductants were screened for motility and tetracycline sensitivity (Tet′). The flbB+ strain generated in this way produced more distinct and more rapidly expanding chemotactic rings on tryptone swarm agar plates (3) than either of the generally used chemotactically wild-type strains of E. coli K-12, strains RF437 (26) and AW405 (3). All genetic manipulations were done by using the methods of Miller (24).

Swarm plates. Tryptone swarm plates contained 0.3% Bacto-Agar (Difco Laboratories), 1% tryptone (Difco), and 0.8% sodium chloride. Minimal swarm plates contained 0.3% Bacto-Agar, motility salts [100 mM potassium phosphate, pH 7.0, 10 mM (NH4)2SO4, 1 mM MgSO4, 0.5% NaCl, and 0.5 μg of FeCl3 per ml], and either 100 μM maltose or 100 μM L-aspartate. Aspartate swarm plates also contained 1 mM glycerol. Tetracycline was added to swarm plates to a final concentration of 5 μg/ml. The plates were scored after 12 to 16 h of incubation at 37°C.

Growth of cells. Overnight cultures grown in minimal medium H1 (3) containing 0.5% glycerol, 0.2% Casamino Acids, and 1 μg of thiamine per ml were diluted 100-fold into
the same medium and grown with vigorous swirling at 32°C. The media used for strains having amino acid requirements were supplemented with the needed amino acids at concentrations of 100 μg/ml. Cells were harvested in exponential phase at an optical density at 578 nm of 0.5 to 0.6.

Localized mutagenesis. (i) Treatment of phage P1 with hydroxylamine. Our procedure for treating phage P1 with hydroxylamine was based on the method of Hong and Ames (19). P1vir lysates were prepared by using strains carrying a Tn10 transposon that was cotransducible with the gene to be mutagenized. Nine parts of L-broth (24) containing 450 mM hydroxylamine, 10 mM CaCl₂, and 2 mM sodium EDTA and adjusted to pH 6.0 with 1 M NaOH was mixed with 1 part of a high-titer phage lysate. This mixture was incubated at 37°C for 12 to 18 h and then centrifuged at 4°C for 1 h at 29,000 x g to pellet the phage. The bulk of the supernatant was decanted, and the phage were suspended in the residual liquid (several hundred microliters) during gentle shaking of the pellet overnight at 4°C. L-broth was added to one-tenth of the original volume, and particulate matter was removed by centrifugation at 0.6 x g for 20 min at 4°C. When this treatment was used, the plaque-forming titer of the lysate fell by a factor of 10 to 100. The suspended phage were stored over chloroform at 4°C and used for transduction within 1 month.

(ii) Isolation of malE mutants. A P1vir lysate was grown on strain MM110 and treated with hydroxylamine as described above. Strain MM110 is malE+ and contains the zjb-729::Tn10 insertion, which is between malK and lamB and is 85 to 90% cotransducible with malE. The zjb-729::Tn10 insertion allows MalT-independent transcription of lamB. The mutagenized lysate was used to transduce strains MM106 (malE+), MM113 (ΔmalE444; Δ31) to Tet-. With strain MM106, transductants were selected on double-strength YT agar (24) containing 5 μg of tetracycline per ml; with strain MM113, transductants were selected on minimal medium A plates (24) containing 5 μg of tetracycline per ml, required amino acids at concentrations of 20 μg/ml, 1 μg of thiamine per ml, and 0.2% maltose. The malE taxis of the transductants was then determined on swarm plates.

Using strain MM106 (malE+) as the recipient, we determined the efficiency of mutagenesis from the frequency of Mal+ transductants. This frequency was about 1 to 5% of the total number of Tet+ colonies; the frequency varied from one phage lysate to the next. Since mutations in four of the genes in the malB region (malE, malF, malG, and malK) can result in a Mal+ phenotype, the frequency of Mal+ mutations per gene was about 1%. The majority of malE mutants were obtained when strain MM113 was used as the recipient.

We also mutagenized malE with N-methyl-N'-nitro-N-nitrosoguanidine. Parallel cultures of strain MM110 were treated as described by Miller (24), and a P1vir lysate was grown on the mutagenized cells. These lysates were used to transduce strain MM106 to Tet-, and the transductants were screened on maltose swarm plates. This method of nitrosoguanidine mutagenesis was less effective than hydroxylamine mutagenesis.

(iii) Isolation of tar mutants. A P1 vir lysate was grown on strain MM115, which is tar+ and contains the zeb-741::Tn10 insertion, which lies between tar and eda and is about 50% cotransducible with tar and eda. Lysates mutagenized with hydroxylamine were used to infect strains containing various malE mutants. Sets of 100 to 300 Tet+ transductant colonies were pooled, grown in L-broth overnight, and then examined for maltose taxis-positive revertants on swarm plates as described below. (A large majority of the sets of transductants contained no revertants. Swarms that did contain a revertant moved more rapidly than swarms of the original mutant and formed a sharp edge. No isolated fans of more rapidly swarming cells were observed, presumably because a revertant, if present, constituted 0.3 to 1% of the total population of cells on a plate.)

Isolation of maltose taxis-positive revertants. On a maltose-tetracycline swarm plate, a 20-μl portion of a concentrated cell suspension (about 2 x 10⁶ cells) was evenly distributed with an Eppendorf pipette in a shallow dish that was 5 cm long and 2 to 4 mm deep. The plates were incubated at 37°C for 16 h, held at 4°C for 8 h, and then incubated again at 37°C for 16 h. Revertants usually appeared as more rapidly spreading fans of growth along the slowly moving front of the maltose taxis-negative swarm (see above). Single colonies were isolated by picking cells out of these fans with a toothpick and streaking them onto double-strength YT agar containing 5 μg of tetracycline per ml. The two-step incubation procedure proved to be more effective than a single longer incubation period, perhaps because chemotaxis still occurred at 4°C while growth was not, allowing revertants to migrate to the edge of the swarm at 4°C.

Preparation of osmotic shock fluid. Cold osmotic shock was performed by the method of Neu and Heppel (25), using exponentially growing cells and incorporating the modifications described previously (23).

Isoelectric focusing. Isoelectric points were determined on analytical LKB ampholine polyacrylamide gel electrophoresis plates (pH range, 4.0 to 6.5) according to the recommendations of the manufacturer and as described by Boos et al. (6).

Maltose transport. Transport assays were performed with cells harvested in exponential phase. Initial rates of radioactive maltose uptake were measured as described previously (23).

Capillary assay. The capillary assay developed by Adler (2) was performed exactly as described previously (23). Growth of cells during the assay was prevented by leaving required amino acids out of the chemotaxis buffer.

Tethered cell assay. The tethered cell assay was performed as previously described (23), with the following modifications. The strains were motile derivatives of strain MC4100 and were not tsr. The tethering buffer contained 10 mM potassium phosphate (pH 7.0), 100 mM NaCl, 100 μM sodium EDTA, 10 μM L-methionine, 30 mM DL-lactate

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**TABLE 1. Bacterial strains**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Known markers</th>
<th>Reference(s) or source</th>
</tr>
</thead>
<tbody>
<tr>
<td>AW405</td>
<td>F′ thr-1(Am) leuB6 his-4 thi-1 rpsL136 ara-14 lacY1 mtl-1 xyl-1 tonA31 tss-78</td>
<td>3</td>
</tr>
<tr>
<td>MC4100</td>
<td>F′ araD130 [argF-lac]U169 rpsL150 relA1 ptsF25 deoC1 rbsR tbbB301</td>
<td>9</td>
</tr>
<tr>
<td>RP437</td>
<td>F′ thr-1(Am) leuB6 his-4 metF159(Am) eda50 thi-1 rpsL136 ara-14 lacY1 mtl-1 xyl-1 tonA31 tss-78</td>
<td>26</td>
</tr>
<tr>
<td>MM106</td>
<td>AW405 malT-1</td>
<td>8</td>
</tr>
<tr>
<td>MM110</td>
<td>AW405 malT-1 zjb-729::Tn10</td>
<td>8</td>
</tr>
<tr>
<td>MM113</td>
<td>AW405 malT-1 ΔmalE444</td>
<td>8</td>
</tr>
<tr>
<td>MM316</td>
<td>MC4100 ΔmalB11 malF::Tn10</td>
<td>18, 23; tbbB</td>
</tr>
<tr>
<td>MM515</td>
<td>RP437 zeb-741::Tn10</td>
<td>this study</td>
</tr>
</tbody>
</table>

* C. Wolff, unpublished data.
(about 10 mM d-lactate, the racemate utilized by *E. coli*), and 50 μg of chloramphenicol per ml. Chloramphenicol prevented synthesis of flagellar filaments, which had been sheared to stubs by exposing 25 ml of a cell suspension in a 50-ml blender cup to the highest speed of a Waring blender. Cells were tethered to acid-cleaned cover slips with antiserum against flagellar filament. The flow chamber used in the assay has been described previously (4). Response times were measured as the period of exclusively counterclockwise rotation from the end of the 15-s flow of attractant-containing buffer until the first reversal. Other authors have referred to this interval as the transition time (5) or recovery time (35).

**RESULTS**

Identification of *malE* mutants defective for maltose chemotaxis. Mutants lacking maltose taxis were identified after localized phage P1 mutagenesis of the *malB* region of the *E. coli* chromosome. Figure 1 shows a representative sample of the swarms made by various mutants identified by this method. Mutants were isolated from cells that were mutagenized with either hydroxylamine or nitrosoguanidine. Between 30,000 and 40,000 transductants were screened for defects in swarm formation on maltose swarm plates. About 80% of these came from the hydroxylamine mutagenesis. Of the mutants listed in Table 2, only *malE*456 and *malE*458 were obtained with nitrosoguanidine.

Characterization of mutants. Mal* transductants exhibiting defects in maltose taxis were purified on minimal maltose plates containing tetracycline and then subjected to a series of tests to identify mutants of the type that we wanted. The first criterion was that the isolates formed normal-size colonies on the minimal maltose plates; the isolates forming smaller colonies were discarded. The second test was a test for resistance to phage λ, which was determined by cross-streaking colonies against a virulent derivative of the phage. The resistant isolates, which presumably had mutations in *lamB*, were discarded: it is known that *lamB* mutants are defective in maltose taxis (15). Such resistant strains constituted a major class among the Mal* mutants with reduced maltose taxis.

Isolates that cleared these first two hurdles were tested for their ability to transport 1 μM maltose. We set an arbitrary limit of 20% of the *malE*+ transport rate as the dividing line between adequate and seriously impaired maltose uptake and discarded all isolates falling under this level. Of the mutants listed in Table 2, *malE*460 was the only exception to this rule.

A total of 14 mutants passed through this screening procedure. An analysis of maltose transport and taxis in these strains is shown in Table 2. We divided the mutants into the following five classes based on their responses in the maltose capillary test and their rates of transport at low (1 μM) and high (100 μM) maltose concentrations: class I, chemotaxis strongly reduced at all maltose concentrations, transport like *malE*+; class II, chemotaxis strongly reduced at all maltose concentrations, transport somewhat reduced at maltose concentrations of 1 and 100 μM; class III, good chemotactic response but shifted to higher maltose concentrations, transport reduced only at a maltose concentration of 1 μM (these mutants could have a MBP with an elevated *Kd* for maltose binding); class IV, chemotaxis strongly reduced and shifted to higher maltose concentrations, transport reduced mainly at a maltose concentration of 1 μM; class V, good chemotactic response but shifted to lower maltose concentrations, transport strongly reduced at both 1 and 100 μM maltose (the primary defect in this mutant may be in maltose transport, which would then have a secondary effect on its behavior on maltose swarm plates).

Mapping of the mutations causing defects in maltose taxis. Mapping of the mutations was done by using a set of λ

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**TABLE 2. Maltose chemotaxis and transport in *malE* mutants**

<table>
<thead>
<tr>
<th>Mutant</th>
<th>Class</th>
<th>Malotose chemotaxis with the following maltose concn in the capillary:</th>
<th>Malotose transport (nmol/min per 2.5 × 10⁵ cells) with:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.1 μM</td>
<td>1 μM</td>
</tr>
<tr>
<td><em>malE</em>+</td>
<td>I</td>
<td>10.0 25.0 2.0</td>
<td>3.5 4.7</td>
</tr>
<tr>
<td>Δ<em>malE</em></td>
<td></td>
<td>0.0 0.4 1.3</td>
<td>0.0 0.0</td>
</tr>
<tr>
<td><em>malE</em>454</td>
<td>I</td>
<td>0.4 2.7 2.4</td>
<td>3.5 5.4</td>
</tr>
<tr>
<td><em>malE</em>461</td>
<td>I</td>
<td>0.4 1.3 1.3</td>
<td>2.9 4.1</td>
</tr>
<tr>
<td><em>malE</em>469</td>
<td>I</td>
<td>1.3 3.2 1.3</td>
<td>3.2 4.2</td>
</tr>
<tr>
<td><em>malE</em>451</td>
<td>I or II</td>
<td>1.5 3.9 3.1</td>
<td>2.5 3.6</td>
</tr>
<tr>
<td><em>malE</em>452</td>
<td>II</td>
<td>0.9 3.0 1.9</td>
<td>1.7 3.3</td>
</tr>
<tr>
<td><em>malE</em>458</td>
<td>II</td>
<td>2.9 4.2 1.3</td>
<td>1.4 2.2</td>
</tr>
<tr>
<td><em>malE</em>464</td>
<td>II</td>
<td>3.8 4.6 1.7</td>
<td>1.4 2.6</td>
</tr>
<tr>
<td><em>malE</em>467</td>
<td>II</td>
<td>1.9 9.0 0.7</td>
<td>1.8 5.0</td>
</tr>
<tr>
<td><em>malE</em>470</td>
<td></td>
<td>2.8 2.6 1.4</td>
<td>1.0 2.0</td>
</tr>
<tr>
<td><em>malE</em>456</td>
<td>III</td>
<td>3.2 26.0 31.0</td>
<td>1.3 4.1</td>
</tr>
<tr>
<td><em>malE</em>453</td>
<td>IV</td>
<td>2.1 14.0 11.0</td>
<td>1.5 4.7</td>
</tr>
<tr>
<td><em>malE</em>466</td>
<td>III or IV</td>
<td>0.1 1.0 4.3</td>
<td>1.3 3.6</td>
</tr>
<tr>
<td><em>malE</em>453</td>
<td>IV</td>
<td>0.3 2.9 6.6</td>
<td>0.8 4.2</td>
</tr>
<tr>
<td><em>malE</em>460</td>
<td>V</td>
<td>16.0 16.0 2.8</td>
<td>0.5 1.5</td>
</tr>
</tbody>
</table>

* All of the strains used were isogenic derivatives of strain MM110 (see Table 1). See text for description of classes.

* Maltose responses were normalized by dividing the accumulation in maltose-containing capillaries by the accumulation in capillaries containing 1 mM L-aspartate (23). The normalized responses are expressed as percentages of the aspartate response.

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FIG. 1. Maltose swarms of *malE* mutants. A minimal maltose swarm plate was incubated for 18 h at 37°C. All of the strains were isogenic derivatives of strain MM110 (see Table 1) containing different *malE* alleles or, in one instance, *tar* alleles. Swarm a, *malE*+; swarm b, Δ*malE*; swarm c, *malE* Δtar-5201; swarm d, *malE*454; swarm e, *malE*+ Δtar-5201; swarm f, *malE*469; swarm g, *malE*452; swarm h, *malE*456; swarm i, *malE*460. The mutants in swarms d through i are described in Table 2.
transducing phage carrying various portions of the malE gene (32). Since these phage carry imm + and protein fusions of the lacZ gene to genes of the malB region, lysogens can be identified by their Lac + phenotypes on MacConkey lactose plates (24) and their immunity to phage λ cI857 h80 at 42°C.

The malE mutations were first crossed into strain MM316, an fliB + derivative of strain MC4100, which is tonA + and carries the Δ(argF-lac)U169 deletion. Strain MM316 contains a malI: Tn10 insertion and the ΔmalB11 deletion (18), which covers the entire malB control region and prevents transcription of both malB operons. Phage P1 vir lysates grown on the original isolates were used to transduce strain MM316 to Mal +. Mal + Tet + transductants should, with high probability, have received the entire donor malE gene.

When lysogenized with malE + phage λ pK3 (32), all of the mutants showed complementation to wild-type maltose chemotaxis when they were tested on maltose swarm plates. Thus, the mutations were in malE, since malE + is the only intact mal gene carried by this phage.

Fine-structure deletion mapping was carried out with the three class I mutations and three other mutations. Several hundred Lac + lysogens of each λ malE transducing phage were tested on maltose swarm plates for the presence of maltose taxis-positive transductants (Fig. 2). The mutations mapped in one of two deletion segments. There was no obvious correlation between the mutant class and the deletion segment in which the corresponding mutation mapped.

Analysis of MBP made by class I mutants. The mutants in class I seemed to be the mutants most likely to have specific alterations in portions of MBP which interacted with the Tar protein, since their transport properties, and by extension their maltose-binding properties, were identical to those of malE + strains. To determine how similar the mutant binding proteins were to wild-type MBP, we examined osmotic shock fluids prepared from mutant and wild-type strains on isoelectric focusing gels (Fig. 3). Two facts emerged from this analysis. First, all three mutants produced normal amounts of MBP. Second, the isoelectric points of the mutant proteins were similar to the isoelectric point of wild-type MBP; the MBPs from the malE461 and malE469 mutants had pI values identical to the pI of wild-type MBP (5.0), and MBP from the malE454 mutant had a pI value of 5.1.

Maltose response times of class I mutants. In order to measure chemotaxis quantitatively, we examined the maltose responses of cells carrying the class I mutations. The cells were tethered in a flow chamber (4) and stimulated with step gradients from zero maltose to different maltose concentrations. To normalize the responses of different strains (23), cells were also stimulated with a saturating concentration of L-aspartate (1 mM).

The results of these experiments are shown in Table 3. The response times of the three class I mutants were substantially reduced at all maltose concentrations. At 100 μM maltose, the highest concentration examined, the mutants had response times that ranged from 16 to 21% of the response time of the malE + control strain. In contrast, the dependence of the response time on the maltose concentration did not vary substantially in the mutants and the malE + strain, confirming that the Kd for maltose binding of the mutant proteins was not significantly altered. As anticipated, the malE mutations did not affect aspartate response times.

Reversion analysis of the class I mutants. We believed that the most likely explanation for the defects in maltose taxis of the class I mutants was an ineffective interaction of the mutant MBP with the Tar protein. Therefore, among the revertants with restored maltose taxis we expected to find strains containing compensating mutations in the tar gene.

We found spontaneous or ethyl methanesulfonate-induced (24) revertants of all three class I mutants. However, all of the reverting mutations proved to be in malE. The five revertants obtained with malE454 and the two revertants obtained with malE461 behaved like a malE + strain on maltose swarm plates, whereas the two revertants of malE469 exhibited only partially restored maltose taxis.

We next tried localized hydroxylalanine mutagenesis of tar. Although we tested about 106 transductants for each of the class I mutants, we found only three independent isolates with improved maltose taxis; all three were isolated from malE461. These revertants behaved abnormally on aspartate swarm plates; in fact, their swarms were indistinguishable from swarms made by a strain carrying the Δtar-5201 deletion (33). The mutations that restored maltose taxis (tar-451, tar-452, and tar-453) all mapped in tar; when lysogenized with phage λche25-5 (33), which carried tar + as its only functional chemotaxis-related gene, the revertants formed normal aspartate swarms. The tar mutations did not interfere with chemotaxis mediated by the Tar signal transducer (10, 16), since strains containing the tar mutations formed normal serine rings on tryptone swarm plates (1).
Chemotactic responses in strains containing tar-451, tar-452, and tar-453. The tar mutations were crossed into strains carrying different malE alleles, selecting for the Tet' conferred by zeb-741::Tn10. The presence of the tar mutation was detected by the formation of defective aspartate swarms. All three tar mutations gave identical results. Strains containing the malE454 and malE461 mutations formed larger, sharper-edged swarms on maltose swarm plates in combination with the tar mutations than in combination with tar"; strains containing the malE469 mutation formed even poorer swarms with the tar mutations than with tar"; and the malE+ strain formed significantly smaller, more diffuse swarms in combination with the tar mutations than in combination with tar".

In order to quantitate the chemotactic responses of these strains, we measured maltose and aspartate response times for cells tethered in a flow chamber (Table 4). With a stimulus of 100 μM maltose, the strain containing malE461

**TABLE 3. Maltose response times of tethered cells**

<table>
<thead>
<tr>
<th>Mutant</th>
<th>10⁻⁵ M Maltose</th>
<th>No. of cells analyzed</th>
<th>Response time (s)</th>
<th>Relative response</th>
<th>10⁻⁴ M Maltose</th>
<th>No. of cells analyzed</th>
<th>Response time (s)</th>
<th>Relative response</th>
<th>10⁻³ M Maltose</th>
<th>No. of cells analyzed</th>
<th>Response time (s)</th>
<th>Relative response</th>
</tr>
</thead>
<tbody>
<tr>
<td>malE+</td>
<td>25</td>
<td>2 ± 5²</td>
<td>1.0</td>
<td></td>
<td>24</td>
<td>36 ± 9²</td>
<td>1.0</td>
<td></td>
<td>18</td>
<td>241 ± 39³</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>ΔmalE</td>
<td>25</td>
<td>0 ± 3</td>
<td>0.0</td>
<td></td>
<td>26</td>
<td>2 ± 4</td>
<td>0.05</td>
<td></td>
<td>22</td>
<td>0 ± 3</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>malE454</td>
<td>31</td>
<td>0 ± 2</td>
<td>0.0</td>
<td></td>
<td>30</td>
<td>0 ± 3</td>
<td>0.0</td>
<td></td>
<td>28</td>
<td>21 ± 20</td>
<td>0.087</td>
<td></td>
</tr>
<tr>
<td>malE461</td>
<td>32</td>
<td>0 ± 4</td>
<td>0.0</td>
<td></td>
<td>31</td>
<td>6 ± 6</td>
<td>0.2</td>
<td></td>
<td>27</td>
<td>36 ± 16</td>
<td>0.15</td>
<td></td>
</tr>
<tr>
<td>malE469</td>
<td>29</td>
<td>1 ± 4</td>
<td>0.5</td>
<td></td>
<td>27</td>
<td>5 ± 4</td>
<td>0.1</td>
<td></td>
<td>29</td>
<td>40 ± 16</td>
<td>0.17</td>
<td></td>
</tr>
</tbody>
</table>

³ The strains used were isogenic derivatives of strain MC4100.
² Response of a mutant strain as a fraction of the mean response of the malE+ strain.
¹ Mean ± standard deviation.
TABLE 4. Response times of malE mutants containing different tar alleles

<table>
<thead>
<tr>
<th>malE Mutation</th>
<th>tar allele</th>
<th>Response time with 100 μM maltose (s)</th>
<th>Response time with 1 mM aspartate (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>malE +</td>
<td>tar +</td>
<td>245 ± 36 (14)</td>
<td>466 ± 105 (12)</td>
</tr>
<tr>
<td>∆malE</td>
<td>tar +</td>
<td>59 ± 11 (11)</td>
<td>69 ± 12 (9)</td>
</tr>
<tr>
<td>malE454</td>
<td>tar +</td>
<td>2 ± 3 (21)</td>
<td>378 ± 83 (17)</td>
</tr>
<tr>
<td>tar +</td>
<td>38 ± 18 (28)</td>
<td>523 ± 152 (24)</td>
<td></td>
</tr>
<tr>
<td>tar -</td>
<td>73 ± 15 (15)</td>
<td>82 ± 9 (15)</td>
<td></td>
</tr>
<tr>
<td>malE461</td>
<td>tar +</td>
<td>52 ± 14 (29)</td>
<td>528 ± 77 (27)</td>
</tr>
<tr>
<td>tar -</td>
<td>131 ± 14 (18)</td>
<td>91 ± 18 (18)</td>
<td></td>
</tr>
<tr>
<td>malE469</td>
<td>tar +</td>
<td>42 ± 16 (28)</td>
<td>594 ± 148 (20)</td>
</tr>
<tr>
<td>tar -</td>
<td>4 ± 5 (26)</td>
<td>103 ± 16 (22)</td>
<td></td>
</tr>
</tbody>
</table>

* All strains were isogenic fliB + derivatives of strain MC4100. The data for tar + strains are from Table 3. The mean response time ± standard deviation is given for the cell ensemble for each set of conditions. The numbers in parentheses are the numbers of cells analyzed.

and tar-453 had a response time that was 2.5-fold longer than the response time of a malE461 tar + strain. Similarly, a malE454 tar-453 strain had a response time that was about twofold longer than the response time of a malE454 tar + strain. In contrast, the response time of a malE + strain was reduced fourfold by tar-453, and the response time of a malE469 tar-453 strain was no different from that of a ∆malE tar + strain, even though the malE469 tar + strain gave a significant maltose response. Regardless of their malE configuration, all strains containing the tar-453 mutation had aspartate response times that were about sixfold shorter than those of tar + strains.

DISCUSSION

We isolated a series of malE mutants that are specifically defective in the chemotactic function of MBP. These mutants varied in the extent to which the transport activity of MBP was affected (Table 2), but all of the mutants grew normally on minimal agar plates containing 0.2% maltose as the sole carbon source. Three of these mutants were of special interest because their ability to transport maltose, even at a concentration of 1 μM, was identical to that of a malE + strain, although their chemotactic responses to maltose were seriously impaired (Table 3). The isoelectric points of the MBPs from these three mutants differed by only 0.1 pH unit (malE454) or not at all (malE461 and malE469) from the isoelectric point of the MBP from wild-type E. coli (Fig. 3). These mutants also produced MBP in normal amounts.

Six of the malE mutations, including malE454, malE461, and malE469, were localized by deletion mapping (Fig. 2). These six mutations were located in two deletion segments. Four, including malE454 and malE461, mapped fairly early in the gene, and two, including malE469, mapped in the last deletion segment. The malE454 and malE461 mutations cannot be identical, however, since the MBP produced by the malE454 mutant had a different isoelectric point than the MBP produced by the malE461 mutant.

The earlier group of mutations must be located before the BglII restriction nuclease site that occurs at a position within malE corresponding to residue 119 of the 370 amino acids of mature MBP (13). We reached this conclusion because phage λ P842 (Fig. 2), which recombined with the mutations of the early group, did not contain this BglII site, while phage λ P811, which extends only slightly farther into malE, did.

The possible clustering of mutations specifically defective for maltose taxis suggested by the mapping data indicates that the mutations affect regions of the MBP that are crucial to its chemotactic function. We are currently determining the DNA sequence alterations of the malE454, malE461, and malE469 genes in order to compare them with the published malE + sequence (13). We hope to identify the specific changes, presumably single amino acid substitutions, that lead to the loss of the chemotactic function of MBP in these mutants.

The discovery that mutations within the tar gene can at least partially suppress the chemotactic defects of some malE mutants was of particular interest. We looked for such suppressor mutants with malE454, malE461, and malE469 but found them only with malE461. All three independent tar mutations of this type had very similar properties. They stimulated maltose taxis in the malE454 and malE461 mutants and reduced maltose taxis in the malE469 mutant and in a malE + strain. They also reduced aspartate taxis in all strains. We plan to look for more tar mutations of this type, especially using the malE461 mutant. At present, we cannot say whether the malE469 mutation interferes directly with the interaction between MBP and the Tar protein. It is noteworthy, however, that the malE469 mutation maps in a different part of malE than the malE454 and malE461 mutations, whose chemotactic defects can be corrected by mutations in tar.

The pleiotropic effects of the tar mutations indicate that they may cause substantial changes of the Tar protein in its periplasmic region (22, 30), which is thought to mediate chemoreceptor function. Mutations in tar that preferentially diminish aspartate taxis, as opposed to maltose taxis, have been reported previously for E. coli (27). Also, the Tar protein from Salmonella typhimurium LT2 is defective in maltose taxis (11; Y. Imae, personal communication) but functions normally in aspartate taxis (36). Thus, various modifications of the receptor region of the Tar protein appear to be possible. It will be instructive to compare the sequences published for E. coli (22) and S. typhimurium (30) with the sequences of mutant tar genes in an attempt to identify the regions of the Tar protein that are responsible for maltose and aspartate sensing.

By using biochemical methods, other workers have found that maltose-loaded MBP binds to the Tar protein (21, 28), and it is clear that the MBP is the only gene product of the maltose regulon that is required for maltose taxis (7, 14). Our data, by demonstrating allele-specific suppression of the chemotactic defect of malE mutations by secondary mutations in tar, provide genetic evidence that MBP and the Tar protein interact physically. Characterization of the proteins encoded by the mutant malE and tar genes described here should provide an opportunity to study this interaction at the molecular level.

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