Aspartate and Maltose-Binding Protein Interact with Adjacent Sites in the Tar Chemotactic Signal Transducer of Escherichia coli

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The Tar protein of Escherichia coli is a chemotactic signal transducer that spans the cytoplasmic membrane and mediates responses to the attractants aspartate and maltose. Aspartate binds directly to Tar, whereas maltose binds to the periplasmic maltose-binding protein, which then interacts with Tar. The Arg-64, Arg-69, and Arg-73 residues of Tar have previously been shown to be involved in aspartate sensing. When lysine residues are introduced at these positions by site-directed mutagenesis, aspartate taxis is disrupted most by substitution at position 64, and maltose taxis is disrupted most by substitution at position 73. To explore the spatial distribution of ligand recognition sites on Tar further, we performed doped-primer mutagenesis in selected regions of the tar gene. A number of mutations that interfere specifically with aspartate taxis (Asp⁷⁻), maltose taxis (Mal⁻), or both were identified. Mutations affecting residues 64 to 73 or 149 to 154 in the periplasmic domain of Tar are associated with an Asp⁻ phenotype, whereas mutations affecting residues 73 to 83 or 141 to 150 are associated with a Mal⁺ phenotype. We conclude that aspartate and maltose-binding protein interact with adjacent and partially overlapping regions in the periplasmic domain of Tar to initiate attractant signalling.

The Tar protein of Escherichia coli is one of a family of homologous chemotactic sensory transducers (4, 6, 19). These proteins are found in the cytoplasmic membrane and enable the bacterium to monitor its chemical surroundings. Four such transducers mediate responses to particular sets of attractants and repellents (24). Transducer monomers consist of 535 to 553 residues and contain an N-terminal periplasmic domain of about 160 residues and a C-terminal cytoplasmic domain of more than 300 residues (19, 25). The polypeptide spans the membrane twice. The first transmembrane region (TM₁) precedes the periplasmic domain and anchors it in the membrane, and the second transmembrane region (TM₂) connects the periplasmic and cytoplasmic domains. A model based on analysis of the amino acid sequence depicts the periplasmic domain of Tar from Salmonella typhimurium as a four-helix bundle aligned perpendicular to the membrane and surmounted by two apical loops (35). The transducers are dimers in their native state (9, 32).

Attractants and some repellents bind to the periplasmic domain of the transducers (18). Ligand binding may induce conformational changes in the periplasmic domain that propagate through the membrane to the cytoplasmic domain. The cytoplasmic domain regulates the activity of the CheA protein, a histidine kinase that phosphorylates the CheY protein (3, 5, 23, 28, 45). Changes in the concentration of phosphorylated CheY modulate frequency control of the flagellar motor. Cells adapt to attractants and repellents through methylation and demethylation, respectively, of specific glutamate residues in the cytoplasmic domain of the transducers. (For reviews, see references 24 and 45).

The Tar protein of E. coli mediates attractant responses to both aspartate and maltose (43). Aspartate binds directly to the periplasmic domain of Tar (18, 37), but maltose binds to the periplasmic maltose-binding protein (MBP) (10). MBP is a soluble protein of 370 amino acids that functions in both maltose transport and chemotaxis. Upon binding maltose, MBP assumes a conformation in which it can interact with Tar (44). Substitutions at residues Thr-53, Asp-55, and Thr-345 of MBP cause specific defects in maltose taxis but do not interfere with maltose transport (16, 27).

Arginine residues 64, 69, and 73 of Tar constitute a motif that is conserved in all transducers that bind amino acids (7, 50). The primary effect of mutations altering these residues is to reduce affinity for aspartate (37). Replacing Thr-154 of Tar with isoleucine interferes with aspartate sensing (21), and altering the corresponding Thr-156 residue in the Tar transducer disrupts serine sensing (22).

Alterations of the three arginine residues of Tar affect maltose chemotaxis to various degrees (16, 50). Substitution of Arg-73 by tryptophan and, to a lesser extent, other changes at Arg-69 and Arg-73 suppress defects in maltose taxis caused by mutations that alter residues Thr-53 and Asp-55 of MBP (16, 27). Since E. coli cells respond to aspartate or maltose when one of these attractants is added in the presence of a saturating concentration of the other (36, 49), aspartate and MBP cannot bind to the identical site. However, the genetic data indicate that Arg-73 of Tar interacts with both of these ligands.

To provide more information about how a receptor responds to molecules as diverse as aspartate and MBP, we sought to expand our characterization of the ligand interaction sites of Tar. We identified mutations that produce specific defects in aspartate or maltose taxis and found that they affect amino acid residues 64 to 83 and 141 to 154 of Tar. Alterations associated with aspartate-defective and maltose-defective phenotypes are clustered in distinct subregions within these two sequences. We also performed site-directed mutagenesis to assess the contribution of the three arginine residues and their immediate neighbors to aspartate and maltose sensing.

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MATERIALS AND METHODS

E. coli strains and plasmids. Strain VB12 is a thr+ eda+ Δtsr-7021 Δtar-tap-5201 derivative of strain RP437 (40) and was used to screen plasmid-borne tar mutations. Strain MM509, a tar+ Δtar-tap-5201 derivative of strain RP437, was used to determine how plasmid-borne tar mutations affect responses in capillary assays. Strain JM101 (supE thi Δ(lac-proAB)F' [traD36 proAB+ lacIq lacZM15]) (29) was used to produce single-stranded DNA for sequencing of mutant plasmids. Strain C236 (del-1 ung-1 thi-1 relA1 containing plasmid pCJ105 [Cm']) (14) was used to generate single-stranded plasmids for site-directed and doped-primer mutagenesis.

Plasmid pMK113 (Amp') contains the E. coli tar gene and is a derivative of pMK1 (27) that has been modified to contain the single-stranded origin of plasmid M13 from pZ150 (51). Strain VB12 containing pMK1 forms small swarms in aspartate or maltose soft agar because the multiple copies of the plasmid-borne gene lead to overproduction of Tar. A BamHI site was introduced 26 bases upstream of the tar initiation codon fortuitously decreases expression of the gene so that strain VB12 containing this plasmid forms wild-type aspartate and maltose swarms. The tar mutant control plasmid pMK2b is deleted from the NdeI site in tar to the downstream NdeI site in pMK113 and lacks the last 297 codons of tar. Plasmid pRKZ41 was constructed by cloning the EcoRI-BamHI fragment of plasmid pRK41 (41) that contains the entire tar gene of S. typhimurium into the equivalent sites in plasmid pZ150.

Media. Strains carrying plasmids were selected and maintained in Luria-Bertani (LB) broth (31) or on Luria-Bertani agar (1.5%) supplemented with 50 μg of ampicillin per ml. Minimal swarm plates contained 0.325% Difco Bacto Agar and Che salts [10 mM potassium phosphate [pH 7.0], 1 mM (NH₄)₂SO₄, 1 mM MgSO₄] supplemented with 20 μg of each of L-threonine, L-leucine, L-histidine, and L-methionine per ml and 1 μg of thiamine per ml. L-Aspartate, L-serine, maltose, and glucose were each added at 0.1 mM. Aspartate and serine swarm plates were supplemented with 0.5% NaCl and 1 mM glycerol. Swarm plates contained 25 μg of ampicillin per ml. Cultures for capillary assays were grown in H1 minimal media containing 0.5% glycerol; 0.2% Casamino Acids (Difco); 100 μg each of L-leucine, L-histidine, and L-methionine per ml; 1 μg of thiamine per ml; and 25 μg of ampicillin per ml. Reagents were purchased from Sigma Chemical Co.

Primer-directed in vitro mutagenesis. Site-directed mutagenesis with plasmid pMK113 or pRKZ41 was done by the method of Kunkel et al. (20). Mutations were confirmed by DNA sequencing. The same procedure was used for doped-primer mutagenesis. Doped primers were made using nucleotide reservoirs contaminated at 1% with each incorrect nucleotide, a ratio that should produce one or two base changes relative to the wild-type sequence per primer. The codons covered by the primers are indicated in Table 1.

Characterization of tar mutations. Strain VB12 is smooth swimming and generally nonchemotactic because it lacks the two major transducers, Tsr and Tar (43). Plasmid pMK113 restores normal alternation of runs and tumbles to strain VB12 and enables it to form chemotactic swarms in soft agar containing aspartate or maltose. This plasmid also allows strain VB12 to form swarms in soft agar containing attractants, such as glucose, whose response is mediated by other transducers (2, 11, 15, 48). The ability to swarm well in glucose soft agar indicated that cells containing a mutant Tar protein could run and tumble normally. Since strain VB12 containing pMK113 swarms well in the presence of 0.1 mM glucose, catabolite repression of motility or chemotaxis by glucose does not interfere with swarm formation under these conditions.

The products of in vitro DNA synthesis reactions with pMK113 and mutagenic primers were transformed into strain VB12, with selection for ampicillin resistance. Amp' transformants were screened on maltose, aspartate, and glucose swarm plates and assigned to one of five categories based on the diameter and sharpness of the swarm rings: (i) wild-type; (ii) defective for aspartate taxis (Asp-); (iii) defective for maltose taxis (Mal-); (iv) defective for aspartate and maltose but normal for glucose taxis (Mal- Asp+); (v) defective for chemotaxis for all three attractants (Che-). Plasmid DNA from transformants exhibiting an Asp', Mal', or Asp- Mal' phenotype was isolated and retransformed into strain VB12. The resulting colonies were examined on aspartate, maltose, and glucose swarm plates to confirm that mutant chemotaxis phenotypes were associated with the plasmid. DNA from plasmids conferring the desired mutant phenotypes was sequenced in the region corresponding to the mutagenic primer.

Efficiency of doped-primer mutagenesis. About 95% of the plasmids obtained with each doped primer confer a Tar+ phenotype. Randomly selected Tar+ plasmids obtained with primers PG-2, PG-3, PG-4, and PG-8 were sequenced. Of these, 0 of 10, 1 of 12, 3 of 13, and 1 of 10, respectively, contained mutations in the mutagenized region, for an average of about 10%. Since the combined Che- Asp-, and Mal- mutation frequency was 1 to 4% (Table 1), about 10 to 15% of the plasmids from the doped-primer mutagenesis contain one or more tar mutations.

Chemotaxis assays. For quantitative swarm assays a fresh colony from Luria-Bertani–ampicillin agar was inoculated into maltose, aspartate, and glucose swarm plates. Plates were incubated at 30°C, and the swarm diameter was measured hourly. Plasmids were transformed into strain MM509 for analysis in capillary assays so that the serine taxis mediated by Tsr could be used to correct for any differences in motility or chemotactic behavior. Cultures were grown with vigorous swirling in 125-ml Erlenmeyer flasks at 32°C in 10 ml of HI-minimal glycerol. Cells were harvested at exponential phase at an optical density at 578 nm of about 0.5 (2 × 10⁹ to 3 × 10⁹ cells per ml). Capillary assays were carried out by the method of Adler (1), with 5 × 10⁷ cells per ml in the capillary and washed serum. Results were normalized to the response to 1 mM serine which attracted 20,000 to 40,000 cells per capillary.

DNA sequencing. Single-stranded DNA for sequencing was produced by infecting strain JM101 containing plasmid.
A.

\[
\begin{array}{cccccccccc}
\text{C} & \text{G} & \text{H} & \text{C}^* & \text{W} & \text{W}^* & \text{I} & \text{K} & \text{K} & \text{K} \\
\text{S} & \text{I} & \text{K} & \text{I} & \text{K} & \text{I} & \text{K} & \text{I} & \text{K} & \text{I}
\end{array}
\]\n
(64) R I N L S R S A V R N M M D S S N Q Q S (83)

B.

\[
\begin{array}{cccccccccc}
\text{K} & \text{S} & \text{L} & \text{I}^* \\
\end{array}
\]\n
(140) Y L D Y G N T G A Y F A Q P T Q (155)

\[
\begin{array}{cccccccccc}
\text{R} & \text{C} & \text{K} & \text{R} & \text{S} & \text{S} & \text{N}
\end{array}
\]

FIG. 1. Mutations in \textit{tar} that result in specific Asp\textsuperscript{−} and Mal\textsuperscript{−} phenotypes. The predicted amino acid sequence of Tar in the vicinity of residues implicated in aspartate taxis is shown. Residue substitutions that cause an Asp\textsuperscript{−} phenotype are given above the sequence, those that cause a Mal\textsuperscript{−} phenotype are given below the sequence, and those that cause an Asp\textsuperscript{−} Mal\textsuperscript{−} phenotype are shown both above and below the sequence. Substitutions described by Lee and Imae (21) or Wolff and Parkinson (50) are marked with asterisks. Substitutions generated by site-directed mutagenesis are underlined. Unmarked substitutions were obtained with doped-primer mutagenesis. The region near the arginine cluster is shown in panel A. The region near Thr-154 is shown in panel B.

pMK113 with M13 phage K07 (47), whose DNA competes poorly for packaging into virions. Double-stranded templates were generated directly from VB12 strains by using alkaline lysis. The dideoxynucleotide chain termination method (42) was used for both single- and double-stranded DNA sequencing (17). Sequencing kits, including Sequenase Version 2.0, were purchased from United States Biochemical.

RESULTS

Site-directed mutagenesis within the arginine cluster. The sequence of the Tar protein in the vicinity of the arginine cluster is shown in Fig. 1A. To investigate the interaction of the Arg-64, Arg-69, and Arg-73 residues with aspartate or MBP, we substituted each arginine residue with lysine and the two serine residues flanking Arg-69 with isoleucine. Plasmids carrying these mutations were transformed into strain MM509, and the transformants were examined in aspartate and maltose capillary assays (Fig. 2). The RK64 mutation (substitution of Arg-64 with lysine) disrupted aspartate taxis severely (Fig. 2A), even at the highest concentration of aspartate tested (0.1 M). This result agrees with previous evidence that Arg-64 is crucial for aspartate taxis (37, 50). The RK69, RK73, Sl68, and S170 mutations caused less severe defects, with peak accumulations reduced by 25 to 40% and shifted to a 10-fold-higher aspartate concentration. Other mutations altering Arg-69 and Arg-73 have been shown to decrease the affinity for aspartate without substantially affecting the generation of attractant signal (37, 50). The RK73 mutation affected maltose taxis most (Fig. 2B), decreasing accumulation at 1 mM maltose to 30% of the wild-type value. The RK64 mutation decreased accumulation at 1 mM maltose to 60% of the wild-type value, whereas the remaining mutations allowed accumulation at 75% or more of the wild-type level.

Rationale for doped-primer mutagenesis of \textit{tar}. Mutagenesis with primers PG-1, PG-2, and PG-10 was targeted to the arginine cluster or conserved sequences (7) at residues 136 to 138 and 152 to 155. The latter region contains Thr-154, which is important in aspartate sensing (21). Mutations obtained with PG-2 and PG-10 had a distinct Asp\textsuperscript{−} or Mal\textsuperscript{−} phenotype. The altered residues were located in the apical loops predicted by the Moe and Kosolich model for the periplasmic domain of Tar (35). Mutagenesis was subsequently targeted at other portions of the loops or at other structural elements present in the model. PG-3 was directed at the loop between helices 3 and 4, PG-4 was directed at the loop between helices 1 and 2, and PG-8 and PG-9 were directed at helices 2 and 3, respectively.

Pattern of mutations obtained with doped-primer mutagenesis. One thousand transformants from reactions with each mutagenic primer (only 400 with PG-10) were screened on aspartate, maltose, and glucose swarm plates (Table 1). Transformants having a Che\textsuperscript{−} phenotype were obtained with all seven primers (about 1.5% of the total transformants), but only primers PG-2, PG-3, PG-4, and PG-10 produced mutations with a specific Asp\textsuperscript{−} or Mal\textsuperscript{−} phenotype. Primers PG-1, PG-8, and PG-9, covering residues 93 to 107 and 119 to 142, produced mutations with nonchemotactic phenotypes or slight defects in aspartate and/or maltose chemotaxis. When the mutations associated with either an Asp\textsuperscript{−} or Mal\textsuperscript{−} phenotype were analyzed by DNA sequencing, both single and multiple base substitutions were found (Table 2). A number of identical mutations were isolated. In one in-
stance, the insertion of a codon was observed. Only single mutations were characterized further.

**Effect of mutations on swarm rates.** Quantitative swarm assays were performed with strain VB12 carrying plasmids derived from site-directed or doped-primer mutagenesis (Table 3). A mutation was assigned an Asp+ Mal− phenotype if the swarm expansion rate in aspartate or maltose soft agar was 70% or less of the rate achieved with plasmid pMK113 and if the expansion rate in glucose soft agar was normal. A few mutations caused smaller decreases in swarm rate (pseudo-wild-type phenotype) or impaired glucose taxis. We observed that the Tar− phenotype of plasmid pMK113 contributed to a slower rate of swarm expansion in the absence of Tar gene activity.

### Table 2. Pattern of doped-primer mutagenesis

<table>
<thead>
<tr>
<th>Primer</th>
<th>No. of plasmids</th>
<th>No. of unique mutations</th>
<th>No. of repeat mutations</th>
<th>No. of multiple mutations</th>
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<tr>
<td>PG-2</td>
<td>5</td>
<td>3</td>
<td>2 (1)</td>
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<td>PG-3</td>
<td>14</td>
<td>9</td>
<td>4 (2)</td>
<td>1</td>
</tr>
<tr>
<td>PG-4</td>
<td>26</td>
<td>9</td>
<td>7 (2)</td>
<td>10</td>
</tr>
<tr>
<td>PG-10</td>
<td>23</td>
<td>5</td>
<td>15 (6)</td>
<td>3</td>
</tr>
</tbody>
</table>

* Number of plasmids with mutations that confer an Asp+, Mal−, or Asp− Mal− phenotype and for which the mutation has been identified by DNA sequencing.
* Number of plasmids with mutations that appear only once.
* Number of plasmids with a single mutation that appears on different plasmids. The total numbers of residues affected are given in parentheses.
* Number of plasmids containing two or three mutations.

### Table 3. Relative swarm rates of strain VB12 containing plasmids with tar mutations

<table>
<thead>
<tr>
<th>Phenotype and amino acid change</th>
<th>Malose swarm rate</th>
<th>Aspartate swarm rate</th>
<th>Ratio of malose/aspartate rates</th>
<th>Glucose swarm rate</th>
</tr>
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<tr>
<td>Asp+ Mal− phenotype</td>
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<tr>
<td>Asp+ phenotype</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>RH64</td>
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<td>0.29</td>
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<td>1.0</td>
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<tr>
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<td>0.43</td>
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<tr>
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<td>0.46</td>
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<tr>
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<td>0.57</td>
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<td>3.1</td>
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<tr>
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<td>1.0</td>
<td>0.60</td>
<td>1.7</td>
<td>1.5</td>
</tr>
<tr>
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<tr>
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<td>Mal− phenotype</td>
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<td>1.0</td>
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<td>0.70</td>
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<td>0.54</td>
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<td>Asp− Mal− phenotype</td>
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<tr>
<td>RK73</td>
<td>0.65</td>
<td>0.32</td>
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<td>0.47</td>
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<tr>
<td>Impaired glucose taxis</td>
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<tr>
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<td>DN142</td>
<td>0.83</td>
<td>1.2</td>
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</table>

* The rate at which the swarm diameter expanded was measured as described in Materials and Methods. For each attractant, rates are expressed as a percent of the rate obtained with the tar+ plasmid. Mean expansion rates (in millimeter per hour) for the tar+ control were 0.88, 1.7, and 0.97 for maltose, aspartate, and glucose, respectively.
* This plasmid had a fourth ATG codon inserted in the region normally encoding methionine residues at positions 74 to 76 of Tar.
* Mutations decreasing glucose swarm rates significantly.
* Mutations that did not decrease swarm rates to 70% of the control rate but still produced distinctive swarm morphologies.

### Distribution of mutations found with doped-primer mutagenesis

Amino acid substitutions associated with Asp− and Mal− phenotypes are shown in Fig. 1. The Asp− mutations alter residues 64 to 73 and 149 to 154, and Mal− mutations alter residues 75 to 83 and 141 to 150. Substitutions at residues Arg-73, Tyr-149, or Phe-150 can interfere with both

![Graph A: Aspartate Capillary Assays](image)

**FIG. 2.** Chemotaxis of tar mutants with alterations in the arginine cluster. Strain MM509 (tar− tar-tap-5201) transformed with pMK113 carrying site-directed mutations was tested in the capillary assay for responses to aspartate (A) or maltose (B). Responses were normalized to the response to 1 mM serine and expressed as a percent of the accumulation at 1 mM aspartate or maltose seen with tar+ pMK113. Each datum point represents the mean value for two duplicate assays. WT, wild type.
maltoolose and aspartate taxis. In addition to changes in the arginine cluster, Asp amino-acid mutations occur at Gln-152 (QL152 and QL155), the first residue of the Q-P-T-Q motif conserved in all known amino acid transducers (7). The QL155 and QL158 substitutions partially disrupt both aspartate and maltose taxis and impair glucose taxis. These mutations may affect signalling properties of Tar, although QL155 decreases aspartate taxis more than maltose taxis and may specifically interfere with aspartate binding.

Just C-terminal to Arg-75 is a run of three methionine residues. Mutations in this region, including substitutions at Met-75 and Met-76, produced a Mal phenotype. Insertion of a fourth consecutive methionine codon also generated a Mal phenotype, as did the DH77 and SR83 substitutions. A Mal phenotype was also associated with alterations of Leu-141, Tyr-143, Asn-145, and Gly-147. These are the first identified amino acid replacements in Tar that cause specific defects in maltose taxis, although tar mutations causing similar phenotypes have been described previously (50).

Behavior in capillary assays. Mutant plasmids representing the different phenotypic categories were transformed into strain MM509, and the transformants were tested in capillary assays (Fig. 3 and 4). The Asp mutation (RW73 and QL152) and Asp Mal mutations (FS150) greatly decreased responses at aspartate concentrations below 1 mM, although peak accumulations were only moderately reduced. The Asp mutation RW73 also reduced the peak accumulation in the maltose capillary assay by 50%. All of the Mal and Asp Mal mutations drastically reduced the maltose response, but the Mal mutant plasmids supported aspartate responses that were about as good as, or, in some cases, even better than those supported by the tar Mal plasmid.

Site-directed mutagenesis in regions producing Mal mutations. The predicted amino acid sequences of the periplasmic regions of the Tar proteins of E. coli (TarE) and S. typhimurium (TarG) are 68% identical (19, 41). TarG does not mediate maltose taxis with MBP from either E. coli or S. typhimurium, whereas TarE interacts specifically with MBP from either species (8, 34). The predicted amino acid sequences of TarE and TarG have nine mismatches in the regions of TarE that have produced Mal mutations (Fig. 4). Since we had found that amino acid substitutions (YN143 and GR147) in TarE at two of these mismatched residues produced a Mal phenotype, we examined whether others among the mismatched residues might be important in interaction with MBP.

Site-directed mutagenesis converted each of the nine residues in TarE to its counterpart in TarG. Mutant plasmids were transformed into strain VB12, and the transformants were tested for swarming in maltose soft agar. Only the plasmid carrying the YN143 substitution failed to support maltose taxis. To test whether this difference prevented TarG from sensing maltose, the YN143 substitution was made in TarG encoded by pRZ41, but no improvement in maltose taxis was observed.
perfect
be
aspartate-bound
of
binding
of
Thr-154
Typhimurium (Tars).

The
cumulative
genetic
data
strongly
suggest
the
that
three
arginine
residues
in
AS1
and
residues
149
to
155
in
AS2
comprise
the
aspartate-binding
site.
Lee
and
Imae
proposed
the
alpha-amino
group
of
an
aspartate
ligand
interacts
with
Thr-154
of
Tars
and
Thr-156
of
Tar
(22)
and
that
the
alpha-carboxyl
group
of
the
ligand
interacts
with
Arg-64.

Binding
specificity
for
a
particular
aspartate
ligand
would
be
determined
by
the
interaction
of
its
R
group
with
other
residues
in
the
two
segments.
The
observation
that
the
RK64
substitution
disrupts
aspartate
taxis
more
than
RK69
or
RK73
confirms
the
crucial
role
of
Arg-64.

Perturbation
of
aspartate
taxis
by
substitution
of
isoleucine
for
the
serine
residues
flanking
Arg-69
may
be
due
to
steric
hindrance
by
the
long
R
group
of
isoleucine
or
to
the
loss
of
serine
and
its
hydroxyl
group.
In
the
recently
determined
structure
of
the
aspartate-bound
form
of
Tars
and
Arg-64,
Tyr-149,
Glu-152,
and
Thr-154
of
one
subunit
of
Tars
and
Arg-69
and
Arg-73
of
the
second
subunit
make
direct
contacts
with
the
ligand,
in
perfect
accord
with
the
genetic
data.

The
RK73
substitution
affects
maltose
taxi
more
than
the
RK64
or
RK69
substitution.
This
result
suggests
that
Arg-73
interacts
more
directly
with
MBP
than
Arg-64
or
Arg-69,
as
does
the
finding
that
maltose
taxi
defects
associated
with
the
DN55
and
TI53
mutations
in
MBP
are
partially
suppressed
by
the
RW73
substitution
in
Tar
(16).
The
RK64
substitution
affects
maltose
taxi
somewhat,
but
mutations
affecting
Arg-64
may
have
pleiotropic
effects
on
Tar
(37).
The
Asp-
Mal
phenotype
caused
by
substitutions
at
Arg-73,
Tyr-149,
and
Phe-150
may
imply
that
these
residues
are
in
close
proximity
in
the
three-dimensional
structure
of
Tar
within
a
region
that
interacts
with
both
aspartate
and
MBP.

Mutations
affecting
residues
73
to
83
and
141
to
150
of
Tar
can
specifically
disrupt
maltose
taxi.
This
distribution
of
mutations
is
consistent
with
the
antiparallel
configuration
of
AS1
and
AS2
and
suggests
that
these
residues
are
closely
associated
to
form
a
contiguous
array
of
sites
for
interaction
with
MBP.
To
identify
other
regions
of
Tar
that
might
interact
with
MBP,
we
mutagenized
sequences
encoding
extensive
portions
of
helices
2
and
3
adjacent
to
the
regions
associated
with
Mal
Tar
mutations.
Since
no
mutations
affecting
a
specific
Mal
phenotype
were
found,
we
conclude
that
this
region
of
Tar
probably
does
not
contact
MBP.
Alternatively,
this
region
may
interact
with
MBP,
but
mutations
affecting
the
MBP-Tar
interaction
may
also
cause
general
structural
or
functional
defects
in
Tar.

It
is
not
known
whether
an
MBP
molecule
contacts
one
or
both
subunits
of
the
Tar
dimer.
MBP
consists
of
two
globular
domains
joined
by
a
hinge,
with
maltose
bound
in
a
cleft
between
the
two
domains
(44).
The
N-terminal
domain
of
MBP
may
interact
with
the
apical
segments
of
Tar
so
that
residues
Thr-53
and
Asp-55
of
MBP
are
positioned
near
Arg-73
of
Tar.
Mutations
affecting
the
C-terminal
domain
of
MBP
also
disrupt
maltose
taxi
(16,52).
Since
the
Mal
mutations
found
thus
far
in
tar
affect
only
residues
73
to
83
and
141
to
150,
the
N-terminal
and
C-terminal
domains
of
MBP
could
interact
with
different
subsets
of
these
residues
in
the
two
Tar
subunits.
The
observation
that
a
single
aspartate
molecule
binds
to
residues
in
both
Tar
subunits
is
consistent
with
this
possibility.
In
the
maltose
transport
system,
each
domain
of
MBP
probably
contacts
a
different
subunit
of
the
heterodimer
composed
of
the
membrane-bound
MalF
and
MalG
proteins
(46).

With
the
exception
of
nine
residues,
Tars
(which
is
incapable
of
mediating
maltose
taxi)
and
Tars
are
identical
in
the
regions
associated
with
a
Mal
phenotype.
Conversion
of
these
residues
in
Tars
to
the
residues
found
at
the
equivalent
positions
in
Tars
produced
only
one
substitution,
YN143,
which
disrupts
maltose
taxi.

The
reverse
substitu-
tion in TarS, NY143, does not restore maltose taxis. We conclude that either (i) MBP contacts TarE elsewhere in addition to the apical region; (ii) the other eight residues that differ between TarS and TarE in this region have a cumulative contribution to the interaction between TarE and MBP; or (iii) a potentially different tertiary structure of TarS relative to TarE precludes a productive interaction of TarS with MBP.

The mechanism of transmembrane signal transduction initiated by the binding of attractant is unknown. Since Tar monomers associate as homodimers (9, 32), an intermolecular mechanism is possible. There is no evidence for cooperative binding of either aspartate or MBP to Tar (26, 37). However, the periplasmic domain of Tar, artificially produced as a soluble fragment, dimerizes at a lower concentration in the presence of aspartate (33), and binding of aspartate causes appreciable shifts in the relative positions of disulfide-bonded Tar subunits (30). Maltose-loaded MBP might also bind simultaneously to both Tar subunits to stabilize a conformation in which the dimer produces an attractant signal. Alternatively, signal propagation could occur within a Tar monomer or involve both intramolecular and intermolecular conformational shifts.

The aspartate-binding site of Tar appears to overlap with elements of the signalling apparatus, since many mutations that eliminate aspartate taxis also impair maltose taxis somewhat (Fig. 2 and 3) (16, 27). In contrast, most mutations that eliminate maltose taxis have no apparent adverse effect on the aspartate response (Fig. 4), suggesting that they affect residues involved in binding MBP but not in the general signalling mechanism. Similar results (39) have been obtained with the ribose and galactose transducer Trg (12, 15). The substitution RH85, in a region of Trg analogous to the arginine cluster of Tar, appears to interfere primarily with signal generation. Another substitution in Trg, GD151, at a position approximately equivalent to Leu-141 of Tar, disrupts interaction with one of its ligands, galactose-binding protein, but not with its second ligand, ribose-binding protein.

A conformational change initiated by the interaction of ligand with the apical region of a transducer could be propagated through the four-helix bundle to the transmem-

![Diagram of TarE showing residues altered by Asp⁻⁻, Mal⁻⁻, and Asp⁻⁻ Mal⁻⁻ mutations.](http://jb.asm.org/)

**FIG. 6.** Diagram of TarE showing residues altered by Asp⁻⁻, Mal⁻⁻, and Asp⁻⁻ Mal⁻⁻ mutations. The drawing represents the periplasmic domain of TarE (based on the model of TarS proposed by Moe and Koshland [35]). Helices are labeled H 1 through H 4. Residues at which amino acid substitutions cause particular phenotypes are coded as follows: striped circles, Asp⁻⁻; shaded circles, Mal⁻⁻; solid circles, Asp⁻⁻ Mal⁻⁻. The four-helix bundle has been pulled apart for easier visualization. X-ray crystallographic analysis (30) showed that the extents of the helices are different from those predicted originally, so we have designated the regions containing the altered residues as apical segment 1 (AS1) and apical segment 2 (AS2) rather than as loops.
brane segments and thence to the cytoplasmic signalling domain (13). The helices within the bundle might slide, rotate, or change their separation with respect to one another. Although the small differences observed between the ligand-free and ligand-bound conformations of individual subunits within the crystallized Tar3 periplasmic domain give little indication of such movements (30), the intact transducer might behave quite differently.

Most of the four-helix bundle may be a generic structural component common to all transducers. Mutations that affect residues 93 to 107 and 119 to 132, which comprise most of helices 2 and 3, often have a nonchemotactic phenotype, as expected if signalling or structural properties of the transducer are disrupted. Ultimately, conformational changes in the four-helix bundle would pass on to the cytoplasmic domain via transmembrane segments 1 and 2. TM1 and TM2 appear to interact with each other as part of the signalling mechanism, since a dominant CW-signalling mutation in TM1 is suppressed by a mutation in TM2 (38).

We conclude that aspartate and MBP interact with Tar at adjacent and overlapping sites. Residues altered by phenotypically equivalent mutations are located at positions opposite one another in AS1 and AS2. This pattern may simply reflect the participation of both apical segments in ligand binding, but it may also indicate a functional relationship between AS1 and AS2 in signalling. Binding of ligand could disrupt interactions between AS1 and AS2 to trigger the initial conformational change in the periplasmic domain. Mutations that interfere with this interaction might mimic attractant binding, a possibility we are now investigating.

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REFERENCES


