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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 (Asp^-), 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 (TM_1) precedes the periplasmic domain and anchors it in the membrane, and the second transmembrane region (TM_2) 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 reversal frequency 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 Tsr 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 *tsr*$^+$ *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*$^+$ *lacI*^{q} *lacZM15*]} (29) was used to produce single-stranded DNA for sequencing of mutant plasmids. Strain CJ236 (*dut-1 ung-1 thi-1 relA1* containing plasmid pCJ105 [Cm^r]) (14) was used to generate single-stranded plasmids for site-directed and doped-primer mutagenesis.

Plasmid pMK113 (Amp^r) contains the *E. coli tar* gene and is a derivative of pMK1 (27) that has been modified to contain the single-stranded origin of phage 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 *Bam*HI site in pMK113 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 *Nde*I site in *tar* to the downstream *Nde*I site in pMK113 and lacks the last 297 codons of *tar*. Plasmid pRKZ41 was constructed by cloning the *Eco*RI-*Bam*HI 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 $(NH_4)_2SO_4$, 1 mM $MgSO_4$] 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.

TABLE 1. Mutations obtained with doped primers

Primer	Residues covered	No. tested[a]	No. with phenotype			
			Che$^-$	Mal$^-$	Asp$^-$	Mal$^-$ Asp$^-$
PG-1	130–142	1,000	11	0	0	0
PG-2	150–159	1,000	13	0	5	3
PG-3	141–152	1,000	13	8	7	1
PG-4	73–88	1,000	13	16	1	1
PG-8	93–107	1,000	11	0	0	0
PG-9	119–132	1,000	20	0	0	0
PG-10	64–73	400	9	5	9	0

[a] Total number of transformants tested on swarm plates.

The products of in vitro DNA synthesis reactions with pMK113 and mutagenic primers were transformed into strain VB12, with selection for ampicillin resistance. Amp^r 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 taxes 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 H1-minimal glycerol. Cells were harvested at exponential phase at an optical density at 578 nm of about 0.5 (2×10^8 to 3×10^8 cells per ml). Capillary assays were carried out by the method of Adler (1), with 5×10^6 cells per ml in the chemotaxis chamber. 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

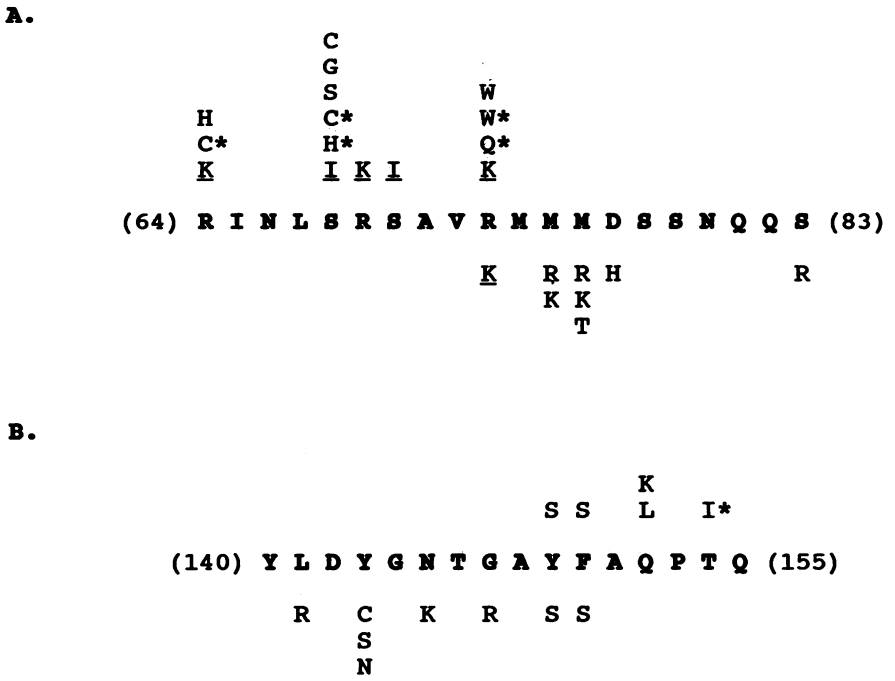

FIG. 1. Mutations in *tar* that result in specific Asp$^-$ and Mal$^-$ phenotypes. The predicted amino acid sequence of Tar in the vicinity of residues implicated in aspartate sensing is shown. Residue substitutions that cause an Asp$^-$ phenotype are given above the sequence, those that cause a Mal$^-$ phenotype are given below the sequence, and those that cause an Asp$^-$ Mal$^-$ 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, SI68, and SI70 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 *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$^-$ or Mal$^-$ phenotype. The altered residues were located in the apical loops predicted by the Moe and Koshland 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$^-$ 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$^-$ or Mal$^-$ 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$^-$ or Mal$^-$ 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-

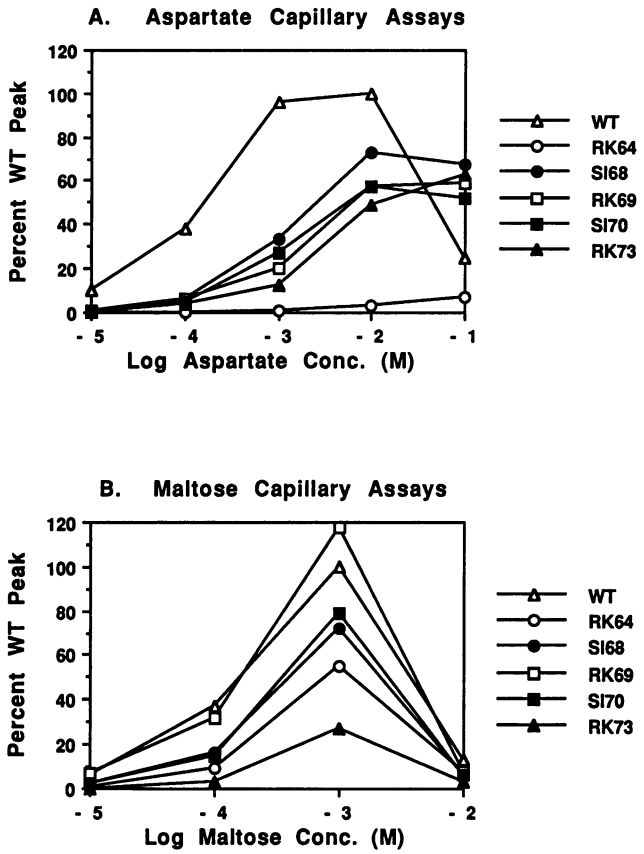

FIG. 2. Chemotaxis of *tar* mutants with alterations in the arginine cluster. Strain MM509 (*tsr*$^+$ Δ*tar-tap-5201*) transformed with pMK113 carrying site-directed mutations was tested in the capillary assay for responses to aspartate (A) or maltose (B). Responses were normalized to the response to 1 mM serine and are 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.

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$^-$, or 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. Strain VB12 carrying *tar* deletion plasmid pMK2b swarmed at rates less than 10% of the wild-type rate.

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

TABLE 2. Pattern of doped-primer mutagenesis

Primer	No. of plasmids[a]	No. of unique mutations[b]	No. of repeat mutations[c]	No. of multiple mutations[d]
PG-2	5	3	2 (1)	0
PG-3	14	9	4 (2)	1
PG-4	26	9	7 (2)	10
PG-10	23	5	15 (6)	3

[a] Number of plasmids with mutations that confer an Asp$^-$, Mal$^-$, or Asp$^-$ Mal$^-$ chemotaxis phenotype and for which the mutation has been identified by DNA sequencing.
[b] Number of plasmids with mutations that appear only once.
[c] Number of plasmids with a single mutation that appears on different plasmids. The total numbers of residues affected are given in parentheses.
[d] Number of plasmids containing two or three mutations.

TABLE 3. Relative swarm rates of strain VB12 containing plasmids with *tar* mutations[a]

Phenotype and amino acid change	Maltose swarm rate	Aspartate swarm rate	Ratio of maltose/aspartate rates	Glucose swarm rate
Asp$^-$ phenotype				
RH64	0.86	0.29	3.0	1.0
RK64	0.95	0.43	2.2	1.2
SI68	0.97	0.70	1.4	1.1
RG69	1.1	0.46	2.4	1.2
RK69	0.95	0.57	1.7	1.0
RS69	1.1	0.35	3.1	1.2
SI70	1.0	0.60	1.7	1.5
RW73	1.1	0.43	2.3	1.4
QK152	1.1	0.68	1.6	1.2
QL152	1.1	0.56	2.0	1.1
TI154	0.95	0.37	2.6	1.0
Mal$^-$ phenotype				
MR75	0.47	1.1	0.43	1.3
MK75	0.48	1.4	0.34	1.2
MK76	0.45	1.3	0.35	1.2
MT76	0.68	1.3	0.52	1.3
MR76	0.45	1.2	0.38	1.3
Insertion M[b]	0.40	1.0	0.40	1.2
DH77	0.68	1.4	0.49	1.3
SR83	0.40	1.0	0.40	1.2
LR141	0.34	0.84	0.40	0.87
YS143	0.61	1.0	0.61	1.1
YC143	0.47	1.1	0.43	0.88
YN143	0.50	1.0	0.50	0.94
NK145	0.49	1.4	0.35	1.0
GR147	0.70	1.3	0.54	1.2
Asp$^-$ Mal$^-$ phenotype				
RK73	0.65	0.32	2.0	1.3
YS149	0.40	0.47	0.85	1.1
FS150	0.52	0.47	1.1	1.2
Impaired glucose taxis[c]				
QL155	0.76	0.51	1.5	0.71
QL158	0.42	0.43	0.98	0.63
Pseudo-wild type[d]				
SG83	0.88	1.2	0.73	1.2
DN142	0.83	1.2	0.69	1.1

[a] The rate at which the swarm diameter expanded was measured as described in Materials and Methods. For each attractant, rates are expressed as a fraction 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.
[b] This plasmid had a fourth ATG codon inserted in the region normally encoding methionine residues at positions 74 to 76 of Tar.
[c] Mutations decreasing glucose swarm rates significantly.
[d] Mutations that did not decrease swarm rates to 70% of the control rate but still produced distinctive swarm morphologies.

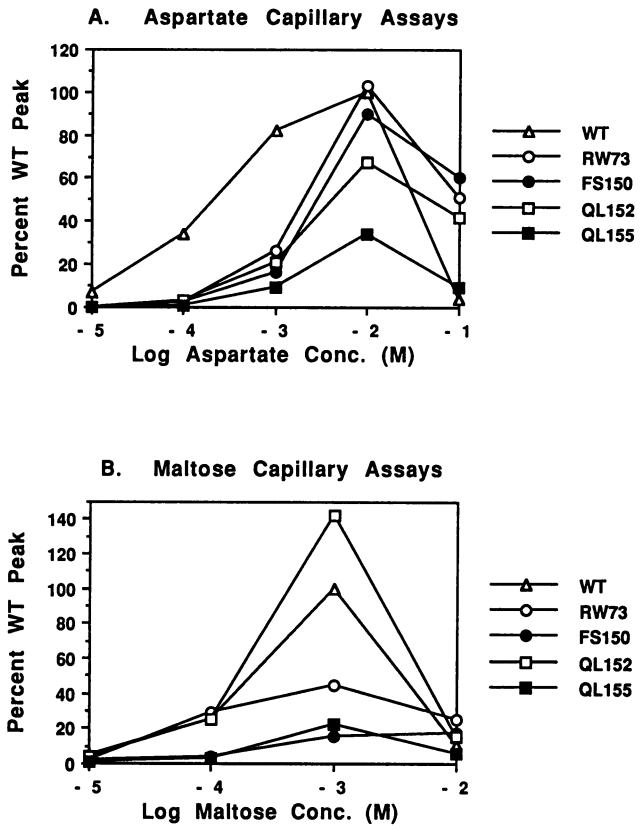

FIG. 3. Chemotaxis of Asp$^-$ and Asp$^-$ Mal$^-$ mutants obtained by doped-primer mutagenesis (Table 3). Strain MM509 transformed with pMK113 or mutant plasmids was tested for responses to aspartate (A) or maltose (B) as described in the legend to Fig. 2. WT, wild type.

maltose and aspartate taxes. In addition to changes in the arginine cluster, Asp$^-$ mutations occur at Gln-152 (QK152 and QL152), 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-73 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 catagories were transformed into strain MM509, and the transformants were tested in capillary assays (Fig. 3 and 4). The Asp$^-$ (RW73 and QL152) and Asp$^-$ Mal$^-$ (FS150) mutations 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*$^+$ 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* (Tar_E) and *S. typhimurium* (Tar_S) are 68% identical (19, 41). Tar_S does not mediate maltose taxis with MBP from either *E. coli* or *S. typhimurium*, whereas Tar_E interacts productively with MBP from either species (8, 34). The predicted amino acid sequences of Tar_E and Tar_S have nine mismatches in the regions of Tar_E that have produced Mal$^-$ mutations (Fig. 5). Since we had found that amino acid substitutions (YN143 and GR147) in Tar_E 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 Tar_E to its counterpart in Tar_S. 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 Tar_S from sensing maltose, the NY143 substitution was made in Tar_S encoded by pRKZ41, but no improvement in maltose taxis was observed.

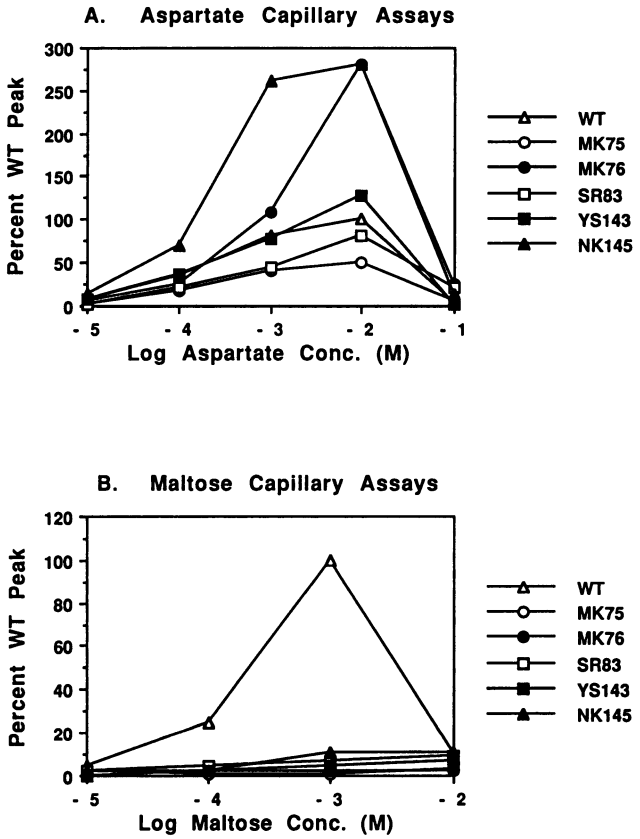

FIG. 4. Chemotaxis of Mal$^-$ mutants obtained by doped-primer mutagenesis (Table 3). Strain MM509 transformed with pMK113 or mutant plasmids was tested for responses to aspartate (A) or maltose (B) as described in the legend to Fig. 2. WT, wild type.

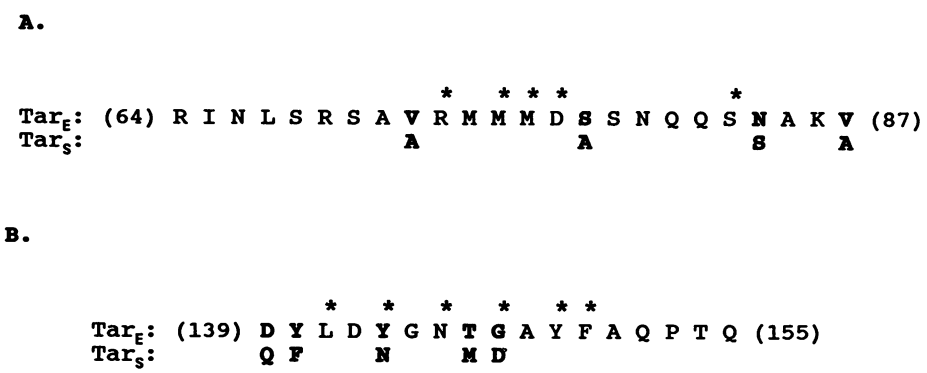

FIG. 5. Predicted amino acid sequences of Tar_E and Tar_S in the regions containing residues 63 to 87 (A) and 139 to 155 (B). Residues not shown for Tar_S are identical to those in Tar_E. Residues in Tar_E altered by Mal^- mutations (Table 3) are marked with asterisks.

DISCUSSION

Moe and Koshland (35) proposed a structure for the periplasmic domain of bacterial signal transducers based on the deduced amino acid sequence of the Tar protein of *S. typhimurium* (Tar_S). The model predicts that the domain consists of four long alpha-helices packed in a left-handed helical bundle and surmounted by two antiparallel loops. X-ray crystallographic analysis has confirmed the predicted overall structure (30). The mutations described here that cause specific defects in aspartate or maltose chemotaxis all affect residues within the apical region of the periplasmic domain (Fig. 6). The residues are distributed in a distinctive spatial pattern; residues affected by Asp^- mutations fall on the distal face of Tar as depicted in Fig. 6, residues affected by Mal^- mutations fall on the proximal face, and residues associated with an Asp^- Mal^- phenotype are located at intermediate positions within each apical segment (AS_1 and AS_2).

One consequence of the mutations could be to decrease binding of a particular ligand. Mowbray and Koshland (37) showed that the primary effect of mutations altering the Arg-64, Arg-69, and Arg-73 residues (50) is to reduce the affinity for aspartate. The K_d for aspartate is increased by a factor of at least 10^4 by the RC64 substitution and by a factor of 10^2 by substitutions at Arg-73. The RC64 substitution also destabilizes the Tar protein (37).

The cumulative genetic data strongly suggest that the three arginine residues in AS_1 and residues 149 to 155 in AS_2 comprise the aspartate-binding site. Lee and Imae (21) proposed that the alpha-amino group of an amino acid ligand interacts with Thr-154 of Tar (or Thr-156 of Tsr) (22) and that the alpha-carboxyl group of the ligand interacts with Arg-64. Binding specificity for a particular amino acid 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 Tar_S (30), Arg-64, Tyr-149, Gln-152, and Thr-154 of one subunit of Tar 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 taxis 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 taxis defects associated with the DN55 and TI53 mutations in MBP are partially suppressed by the RW73 substitution in Tar (16). The RK64 substitution impairs maltose taxis 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 taxis. This distribution of mutations is consistent with the antiparallel configuration of AS_1 and AS_2 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 conferring 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 taxis (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 (30) 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, Tar_S (which is incapable of mediating maltose taxis) and Tar_E are identical in the regions associated with a Mal^- phenotype. Conversion of these residues in Tar_E to the residues found at the equivalent positions in Tar_S produced only one substitution, YN143, which disrupts maltose taxis. The reverse substitu-

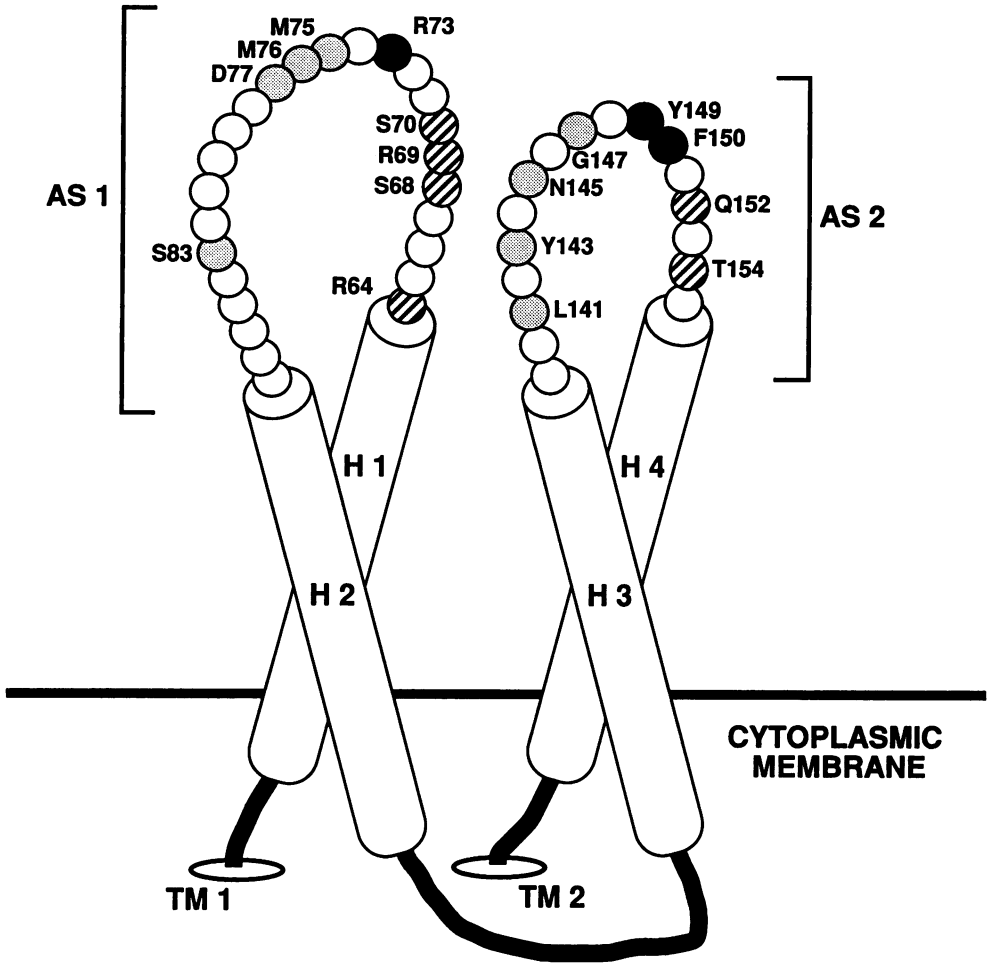

FIG. 6. Diagram of Tar_E showing residues altered by Asp^-, Mal^-, and Asp^- Mal^- mutations. The drawing represents the periplasmic domain of Tar_E (based on the model of Tar_S proposed by Moe and Koshland [35]). Helices are labeled H 1 through H 4. Residues at which amino acid substitutions cause particular 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.

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

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 Tar_S 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. TM_1 and TM_2 appear to interact with each other as part of the signalling mechanism, since a dominant CW-signalling mutation in TM_1 is suppressed by a mutation in TM_2 (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 AS_1 and AS_2. This pattern may simply reflect the participation of both apical segments in ligand binding, but it may also indicate a functional relationship between AS_1 and AS_2 in signalling. Binding of ligand could disrupt interactions between AS_1 and AS_2 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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