

Aspartate Taxis Mutants of the *Escherichia coli* Tar Chemoreceptor

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The Tar protein of *Escherichia coli* belongs to a family of methyl-accepting inner membrane proteins that mediate chemotactic responses to a variety of compounds. These transmembrane signalers monitor the chemical environment by means of specific ligand-binding sites arrayed on the periplasmic side of the membrane, and in turn control cytoplasmic signals that modulate the flagellar rotational machinery. The periplasmic receptor domain of Tar senses two quite different chemoeffectors, aspartate and maltose. Aspartate is detected through direct binding to Tar molecules, whereas maltose is detected indirectly when complexed with the periplasmic maltose-binding protein. Saturating levels of either aspartate or maltose do not block behavioral responses to the other compound, indicating that the detection sites for these two attractants are not identical. We initiated structure-function studies of these chemoreceptor sites by isolating *tar* mutants which eliminate aspartate or maltose taxis, while retaining the ability to respond to the other chemoeffector. Mutants with greatly reduced aspartate taxis are described and characterized in this report. When present in single copy in the chromosome, these *tar* mutations generally eliminated chemotactic responses to aspartate and structurally related compounds, such as glutamate and methionine. Residual responses to these compounds were shifted to higher concentrations, indicating a reduced affinity of the aspartate-binding site in the mutant receptors. Maltose responses in the mutants ranged from 10 to 80% of normal, but had no detectable threshold shifts, indicating that these receptor alterations may have little effect on maltose detection sensitivity. The mutational changes in 17 mutants were determined by DNA sequence analysis. Each mutant exhibited a single amino acid replacement at residue 64, 69, or 73 in the Tar molecule. The wild-type Tar transducer contains arginines at all three of these positions, implying that electrostatic forces may play an important role in aspartate detection.

Many of the chemotactic responses exhibited by *Escherichia coli* are mediated by a family of inner membrane proteins known as methyl-accepting chemotaxis proteins (MCPs) (for reviews, see references 16 and 32). Four different MCP species have been identified in *E. coli*. Each monitors a different set of chemoeffector compounds through specific ligand-binding (chemoreceptor) sites arrayed on the periplasmic side of the membrane. Changes in receptor occupancy trigger cytoplasmic signals that alter the cellular pattern of flagellar rotation, eliciting a chemotactic response. Subsequent changes in MCP methylation state cancel the excitatory flagellar signals, resulting in sensory adaptation. These sensory transducers offer useful models for investigating stimulus detection and transmembrane signaling events at the molecular level.

MCP transducers are organized into two discrete structural domains with a relatively simple transmembrane organization (3, 4, 15, 27). The N-terminal half of the molecule comprises a periplasmic domain that is flanked at each end by a hydrophobic membrane-spanning segment. The C-terminal half of the molecule is located on the cytoplasmic side of the membrane and contains the glutamic acid residues that serve as substrates for the methylation and demethylation enzymes (4, 15, 33). The cytoplasmic domain is highly conserved in sequence (4, 15, 27) and appears to mediate flagellar signaling, a function common to all MCP transducers. In contrast, the more variable periplasmic domain appears to carry the chemoreceptor sites specific to each transducer species (20). The manner in which the receptor and signaling domains communicate across the membrane is still very much a mystery, but presumably

involves conformational alterations induced by changes in binding site occupancy (8).

To better understand receptor site structure and the initial events of transmembrane signaling, we sought to isolate transducer mutants with specific defects in chemoreceptor function. A few such mutants have been described for the Tsr and Trg transducers (11, 22). The Tar transducer was chosen for this work because it handles two very different chemoeffector inputs, aspartate and maltose (28, 31). Aspartate molecules are detected by direct binding to the transducer (34), whereas maltose is detected indirectly by maltose-binding protein (MBP) (9, 13, 18, 26), a periplasmic protein also involved in maltose transport (12). Competition experiments indicate that saturation of either the aspartate or maltose-MBP receptor site in Tar does not block subsequent responses to the other compound, demonstrating that the sites are not identical (21; C. Wolff, Diploma thesis, University of Konstanz, Konstanz, Federal Republic of Germany, 1983). We reasoned, therefore, that it should be possible to isolate *tar* mutants that still respond to aspartate or to maltose, but no longer respond to both compounds. Such mutants should enable us to identify amino acid residues critical to each ligand-binding site in the Tar molecule. In this report, we describe the isolation and properties of *tar* mutants with defects in aspartate chemotaxis.

MATERIALS AND METHODS

Bacterial and plasmid strains. Bacterial strains used in this study were isogenic derivatives of RP437, an *E. coli* K-12 strain that is wild type for chemotaxis (25). Plasmid pMK1, a pBR322 derivative, was kindly provided by Mike Manson (Texas A&M University). It contains a 3.3-kilobase *EcoRI*-*AvaI* fragment carrying the 3' end of the *cheA* locus, all of

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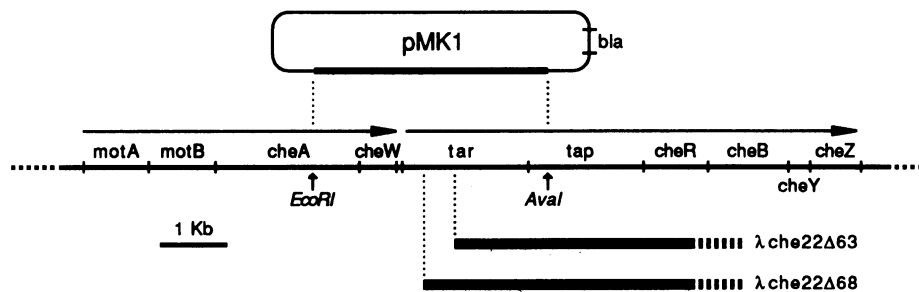


FIG. 1. Physical and genetic map of the *tar* region. Receptor mutations were isolated on plasmid pMK1, which carries an *EcoRI*-*AvaI* fragment of the *E. coli* chromosome spanning the *tar* locus and its promoter. The *bla* (β -lactamase) locus confers resistance to ampicillin. The mutations described in this report were located between the deletion endpoints of the indicated λ transducing phages. Kb, Kilobase.

cheW, an intact *tar* gene and its promoter, and the 5' portion of the *tap* locus (Fig. 1).

Media and growth conditions. Tryptone and H1 minimal media were used throughout this work and have been described previously (23). Plasmid pMK1 was maintained in strains by selection for ampicillin resistance, using 50 to 100 μ g of ampicillin per ml in solid or liquid media and 25 μ g/ml in semisolid swarm plates. Unless otherwise noted, all incubations were done at 35°C.

Plasmid mutagenesis and screening for *tar* chemoreceptor mutants. Approximately 400 ng of pMK1 DNA was treated with 0.4 M hydroxylamine in 0.5 M potassium phosphate buffer (pH 6.0) containing 5 mM EDTA for 36 h at 37°C. Mutagenesis was terminated by overnight dialysis at 4°C against at least 1,000 volumes of H₂O. RP4372 [Δ (*tar-tap*)5201 *tsr-1*] and RP5854 [Δ (*tar-tap*)5201] were transformed with the treated plasmid DNA. Ampicillin-resistant colonies were then picked to minimal aspartate and maltose swarm plates and scored for chemotactic ability after overnight incubation.

Transfer of plasmid-borne mutations to the host chromosome. Mutant plasmids were transformed into RP4338 (*tar-235*) and RP4362 (*tar-226*), which carry polar *tar* mutations (29). The resulting strains were generally nonchemotactic owing to the polar block on expression of downstream *che* genes in the *tar* operon (Fig. 1). Recombinants in which the polar *tar* mutation in the chromosome had been replaced with the nonpolar *tar* allele from the plasmid were isolated by selecting for chemotactic colonies on tryptone swarm plates. Plasmid-free derivatives were then obtained by several rounds of single-colony isolation on nonselective medium and tested on swarm plates to confirm that they retained the expected mutant chemotaxis phenotype. Representative chromosomal *tar* mutations were then transferred by P1 cotransduction with the *eda* locus to RP6538, a *malT1*(Con) derivative of RP437. This strain constitutively expresses MBP and other maltose regulon genes (7) and precluded the need to induce these functions by growth on maltose, thereby avoiding the possibility of catabolite repression effects on other chemotaxis and flagellar operons.

Deletion mapping. Chromosomal *tar* mutations causing an aspartate-blind phenotype were mapped with λ che22 deletion derivatives essentially as described previously (30), except that *tar*⁺ recombinants were scored on minimal aspartate plates.

Capillary assays of chemotactic ability. Assays were performed essentially as described by Adler (1), with the following modifications. Cells were grown to the mid-log phase in H1 medium supplemented with each required amino acid (1 mM) and 0.5% glycerol. Cells were washed by

centrifugation three times in KEP buffer (23) and resuspended at a final concentration of 5×10^6 cells per ml in KEP buffer containing 10 mM sodium lactate and 0.1 mM methionine. Except for maltose, which was purchased from Calbiochem-Behring (La Jolla, Calif.), all attractant compounds used in the assays were obtained from Sigma Chemical Co. (St. Louis, Mo.).

DNA sequence determinations. DNA sequence changes in the *tar* mutations were determined directly from double-stranded plasmid DNA, using custom primers and the dideoxy sequencing technique (6).

RESULTS

Isolation of Tar chemoreceptor mutants. To identify regions of the Tar molecule specifically involved in aspartate or maltose sensing, we looked for chemotaxis mutants that no longer responded to one of these compounds, but still responded to the other. To focus our search on the Tar transducer, we subjected a plasmid carrying the wild-type *tar* gene to random mutagenesis with hydroxylamine in vitro. This mutagen induces predominantly G·C-to-A·T transitions and was expected to generate a variety of missense defects in *tar*. Treated plasmids were tested for Tar function by transformation of a Δ *tar* host strain and subsequent screening on aspartate (ASP) and maltose (MAL) swarm plates. The parental plasmid, pMK1, expresses Tar function, but did not fully complement Δ *tar* recipients owing to gene dosage effects (Fig. 2). Nevertheless, it proved superior to other *tar* plasmids and was used as the *tar*⁺ parent in all mutant isolations. Fourteen independent mutagenesis and screening experiments were done, yielding about 6% mutants from approximately 16,500 tested transformants. Five different chemotaxis phenotypes were observed among the mutants. Examples of each are shown in Fig. 2 and discussed briefly below.

(i) **Large swarmers.** Approximately 0.4% of the tested colonies formed larger swarms on both MAL and ASP plates than the parental controls did. We confirmed that the mutation(s) responsible for this behavior was present on the plasmid, but did not investigate these mutants further. It seems likely that they carry mutations that alter the level of expression of the *tar* gene on the plasmid.

(ii) **Nonchemotactic.** Nearly 4% of the tested colonies exhibited partial or complete swarming defects on both MAL and ASP plates. These mutants presumably represent Tar null defects and dominant Tar signaling defects analogous to previously described Tsr mutants (5, 24).

(iii) **Maltose-blind.** Approximately 0.5% of the tested colonies formed normal-sized swarms on ASP plates, but small

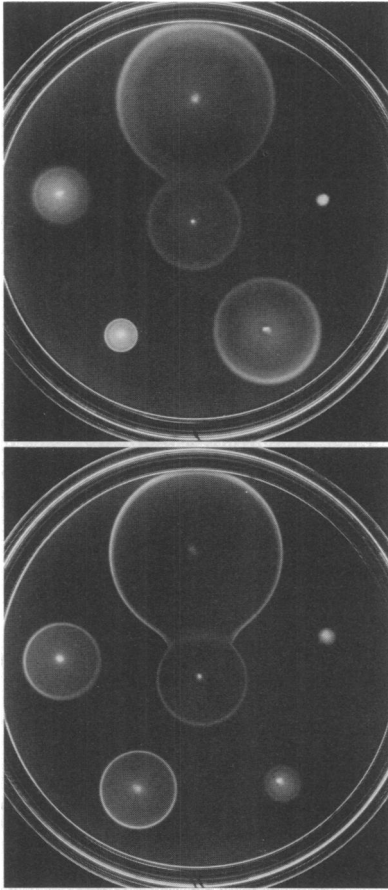


FIG. 2. Swarm plate phenotypes of *tar* mutants. RP4372 strains [$\Delta(tar-tap)5201\ tsr-1$] carrying mutant derivatives of pMK1 were used to initiate colonies on minimal semisolid agar plates containing either aspartate (upper panel) or maltose (lower panel) as the attractant source. Plates were photographed after overnight incubation at 32°C. The parental pMK1 control (*tar*⁺) is shown in the center of each plate, with the five classes of mutant plasmids arranged in the following order (reading clockwise from the top): (i) large swarmers; (ii) generally nonchemotactic; (iii) maltose-blind; (iv) aspartate sensitive; (v) aspartate-blind.

or aberrant swarms on MAL plates. These mutants may include ones with specific defects in the Tar receptor site for maltose and will be described in a subsequent report.

(iv) **Aspartate sensitive.** Approximately 0.8% of the tested colonies formed essentially normal swarms on MAL plates, whereas on ASP plates they formed small colonies with no evidence of even oxygen taxis rings. Since an inability to sense aspartate should not preclude oxygen taxis (see below), it appeared that taxis toward oxygen was actually inhibited by the presence of aspartate. Subsequent swarm plate tests indicated that approximately 0.2 mM aspartate was sufficient to block chemotactic responses toward a variety of other attractants in these strains. These mutant transducers may be capable of generating receptor signals that lock the flagellar machinery in a nonresponsive state. However, unlike constitutive signaling mutants of the *Tsr* transducer (5, 24), these Tar defects depend on attractant to initiate and maintain the aberrant signaling condition.

(v) **Aspartate-blind.** Approximately 0.3% of the tested colonies exhibited near-normal MAL plate swarms, but no chemotactic bands on ASP plates. Unlike the aspartate-

sensitive strains described above, these mutants formed dome-shaped swarms indicative of oxygen taxis. (The cells metabolize aspartate aerobically, reducing the oxygen supply around the growing colony. Oxygen supply is only partly replenished by diffusion from the atmosphere, leading to a lateral oxygen gradient that is steepest at the bottom of the agar and progressively shallower toward the surface of the plate). These mutants seemed most likely to have specific defects in aspartate detection and are the subjects of the remainder of this article. To simplify subsequent discussion, we denote their phenotype as Asp⁻ Mal⁺ (or aspartate-blind) and their genotype as *tar* (Asp).

Genetic characterization of aspartate-blind mutants. Attempts to map the plasmid-borne *tar* (Asp) mutations with λ transducing bacteriophage were unsuccessful, presumably because the multicopy nature of the pMK1 plasmid prevented detection of *tar*⁺ recombinants in the crosses. This might happen, for example, if formation of one functional *tar* gene by recombination was insufficient for correction of the mutant phenotype in the presence of a vast excess of mutant genes. The Asp⁻ Mal⁺ transducers could conceivably compete for limited membrane sites or in some other way mask the function of a few wild-type Tar molecules. Thus, we transferred the *tar* (Asp) mutations into the bacterial chromosome for all subsequent genetic and phenotypic studies. The transfer scheme was predicated on the assumption (subsequently validated) that *tar* (Asp) mutations would not have polar effects on expression of downstream genes in the *tar* operon. Mutant plasmids were transferred into host strains with polar *tar* point mutations that caused a generally nonchemotactic phenotype. Recombinants in which the polar defect had been alleviated by replacement with the plasmid-borne allele were selected on tryptone swarm plates. After growth under nonselective conditions to permit segregation of the plasmid, the recombinants were tested for the Asp⁻ Mal⁺ phenotype, and the chromosomal *tar* (Asp) mutations were subsequently transduced to other backgrounds for further study.

Once the *tar* (Asp) mutations had been introduced into the chromosome, they could be readily mapped with λ transducing phage deletions. Seventeen mutations were examined, and all of them fell between the deletion endpoints of λ che22 Δ 63 and λ che22 Δ 68 (Fig. 1). The approximate physical positions of the mutations within the *tar* coding sequence could be determined by the restriction sites known to be present or missing in the deletions (26). The mutations appeared to be located within the first half of the periplasmic domain of the Tar molecule, consistent with our expectation that they represented receptor site alterations.

DNA sequence changes in aspartate-blind mutants. Based on the deletion mapping results, appropriate oligonucleotide primers were synthesized and used to determine the DNA sequence changes in 17 *tar* (Asp) plasmid isolates (Table 1). At least 11 of these mutations were independent isolates, but some of the others may be clonally derived. All the sequenced mutations arose through G · C-to-A · T transitions, consistent with the mutagenic specificity of hydroxylamine. Surprisingly, only five different mutational changes affecting only three different codons were observed. Those codons specify amino acids 64, 69, and 73, all of which are arginine residues in the wild-type Tar protein. Each of the sequenced mutations had a missense change in one of these arginine codons. To facilitate subsequent discussion, we will refer to these mutants, not by their allele numbers, but by their amino acid replacements. For example, mutant RC64 has an arginine-to-cysteine change at residue 64. These findings,

TABLE 1. Mutational changes in aspartate receptor mutants

Mutant ^a	Codon or residue	Codon change	Residue change	Alleles ^b
RC64	64	CGC → TGC	Arg → Cys	242, 244
RC69	69	CGT → TGT	Arg → Cys	(250, 252, 253)
RH69	69	CGT → CAT	Arg → His	246, 249, (247, 254), (241, 255, 256, 257)
RW73	73	CGG → TGG	Arg → Trp	243, 245
RQ73	73	CGG → CAG	Arg → Gln	248, 251

^a Mutants are designated by their amino acid replacements. The letters denote, respectively, the wild-type and mutant amino acids (in one-letter code), and the number indicates the position of the altered residue in the protein.

^b Alleles in parentheses could be clonally related and may not represent independent mutational events.

particularly the fact that the critical residues are located close together in the primary structure of Tar, suggest that this region of the Tar molecule forms part of the receptor site for aspartate.

Chemotactic behavior of aspartate-blind mutants. One representative allele from each of the five mutational classes shown in Table 1 was transferred to strain RP6538 to evaluate its effects on chemotactic responses in the quantitative Pfeffer assay described by Adler (1). The unstimulated swimming behavior and flagellar rotation patterns of these strains exhibited frequent reversal episodes, characteristic of the wild-type controls (data not shown). Moreover, all strains exhibited similar capillary responses to serine stimuli, further indicating that the *tarA* mutations had no generalized effect on chemotactic behavior. In the tests discussed below, serine controls were used to normalize other responses, thereby facilitating comparison of different strains.

All the mutant strains exhibited residual chemotactic responses to aspartate, but with elevated detection thresholds (Fig. 3A). The RQ73 and RW73 strains had response thresholds of approximately 10^{-5} M aspartate, in contrast to a 10^{-7} M threshold in the wild-type control. The RC69 mutant exhibited a threshold at 10^{-4} M, and the RC64 and RH69 strains had thresholds at about 10^{-3} M. Thus, the mutant strains are capable of detecting aspartate stimuli, but with reduced sensitivity. These findings indicate that the mutations affect the affinity of the aspartate receptor for ligand, but do not eliminate its ability to generate flagellar signals.

The specific nature of the affinity changes in these mutants was borne out in tests with other attractants from the aspartate receptor family, including α -methyl aspartate (a nonmetabolizable analog of aspartate), methionine, glutamate, and succinate (19). The aspartate-blind mutants showed substantial threshold increases to nearly all these compounds (methionine responses are shown in Fig. 3B). The inferred shifts in receptor affinity varied from mutant to mutant, but for any one mutant they were generally consistent across the entire set of attractants. The methionine response in the RW73 mutant provides a notable exception to this rule. This strain sensed methionine with wild-type affinity, whereas all other aspartate-family compounds were detected with reduced affinity.

Although the *tar* (Asp) mutants showed near-normal rates of colony expansion on MAL swarm plates, they exhibited reduced maltose responses in capillary assays, ranging from 10 to 80% of wild-type responses (Fig. 4). Unlike the aspartate results, the response thresholds were not demonstrably shifted to higher concentrations, suggesting that the affinity for maltose-MBP is not substantially altered in the mutants. The reduced responsiveness to maltose raises doubts about the specificity of the receptor defects in these mutants.

DISCUSSION

Isolation of aspartate receptor mutants. The Tar chemoreceptor of *E. coli* mediates chemotactic responses to aspartate and maltose. Aspartate is detected by direct binding, whereas maltose is sensed through interactions with the periplasmic MBP. We isolated *tar* mutants with a greatly

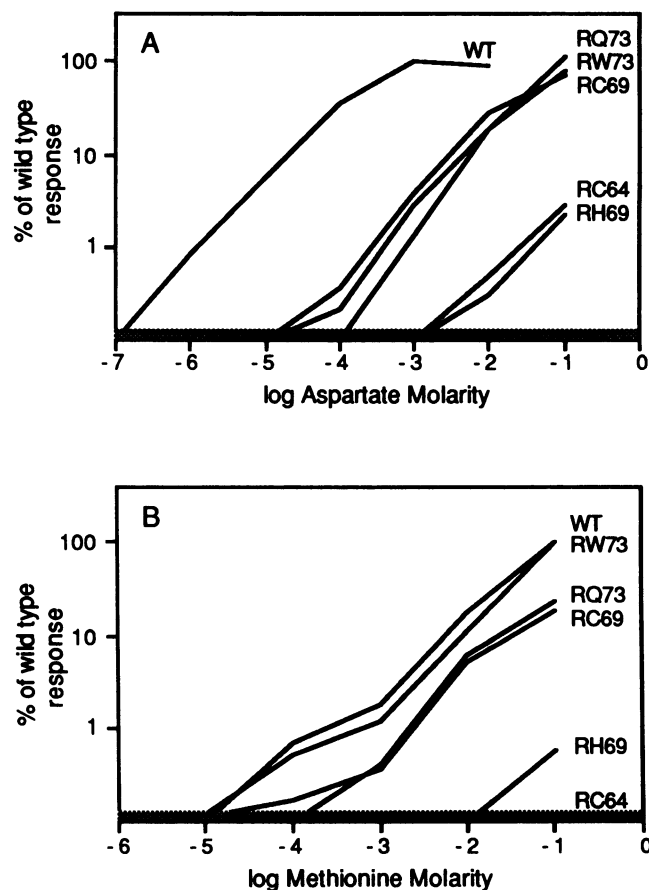


FIG. 3. Response thresholds of aspartate-blind mutants. Iso-genic strains bearing the indicated *tar* mutations (see text) were tested for residual aspartate (A) and methionine (B) responses in capillary assays. At least four data points (omitted for clarity) were taken at each of the indicated attractant concentrations. Response values, normalized to a 1 mM serine control, are expressed as the percentage of the wild-type (WT) response to 1 mM aspartate (A) or 100 mM methionine (B). Background accumulations, measured with buffer-filled capillaries, fell within the range indicated by the shaded bar at the bottom of each figure. In each panel, the mutant strains are listed from top to bottom in order of increasing detection threshold (i.e., from highest to lowest receptor affinity).

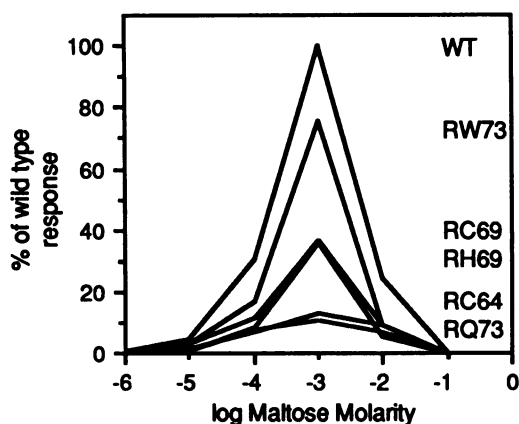


FIG. 4. Maltose responses of aspartate-blind mutants. Strains bearing the indicated *tar* mutations were tested for maltose chemotaxis in capillary assays as described in the legend to Fig. 3. (Note that the responses are plotted on a linear, rather than logarithmic, scale, as in Fig. 3.) Strains are listed from top to bottom in order of decreasing accumulation at 1 mM maltose. WT, Wild type.

reduced ability to detect aspartate and its attractive analogs, but with relatively minor deficits in maltose sensing. These mutants failed to make chemotactic rings on ASP swarm plates, but some still exhibited detectable responses to aspartate-family compounds (aspartate, α -methyl aspartate, glutamate, methionine, and succinate) in more sensitive capillary assays. The detection thresholds for these residual responses were shifted to higher concentrations, indicative of a decreased receptor affinity for ligand. In contrast, the flagellar signaling efficiency of the mutant receptors appeared to be unaltered. These properties suggest that the mutants have specific defects in the aspartate-binding site of the Tar transducer.

Each of these aspartate-blind mutants contained a single base substitution in the *tar* gene at one of three closely spaced arginine codons. These arginine residues are located in the periplasmic receptor domain of Tar (Fig. 5) and evidently play critical roles in aspartate detection. On the one hand, they could be directly involved in binding aspar-

tate molecules, for example, through electrostatic interactions with the negatively charged carboxyl groups of aspartate. On the other hand, they could be responsible for maintaining proper conformation of the aspartate-binding site, which might be located elsewhere in the receptor domain. Neither of these alternatives alone can account for the properties of the mutant receptors, and circumstantial evidence summarized below suggests that the arginine segment plays key roles in several aspects of receptor function.

Arginine segments may comprise amino acid-binding sites in Tar and Tsr receptors. In general, the receptor domains of different MCP transducers vary in primary structure. It is striking, therefore, that the Tsr transducer, which serves as the chemoreceptor for serine, alanine, and glycine (11, 19), contains an arginine segment very similar to that of Tar (Fig. 5). Arginine residues are found at the same positions in both transducers, and many of the flanking residues are identical or chemically similar as well. Tap and Trg, transducers that interact exclusively with periplasmic binding proteins (2, 10, 17), show little sequence similarity to either Tar or Tsr in this portion of the receptor domain, suggesting that the arginine segment motif is specifically involved in amino acid sensing. Consistent with this view, Y. Imae and co-workers (15a) have found that several *tsr* mutants with specific defects in serine detection (11) have amino acid replacements at arginine residue 64.

Mesibov and Adler (19), in an extensive study of amino acid chemotaxis in *E. coli* showed that the Tar and Tsr receptors sense all four constituent groups on the α -carbon of amino acids. We propose that the conserved residues in the Tar and Tsr arginine segments are directly responsible for detecting determinants common to the aspartate and serine attractant families. For example, one or several of the arginine residues could participate in charge interactions with the α -carboxyl group. Similarly, the conserved aspartic acid residue at position 77 (Fig. 5) might participate in detection of the α -amino group through hydrogen bonding. Sensing of the R group unique to each amino acid could in principle be done either by a distant portion of the receptor domain or by residues within or near the arginine segment.

Ligand-induced changes in receptor conformation. Falke and Koshland (8) recently showed that Tar molecules un-

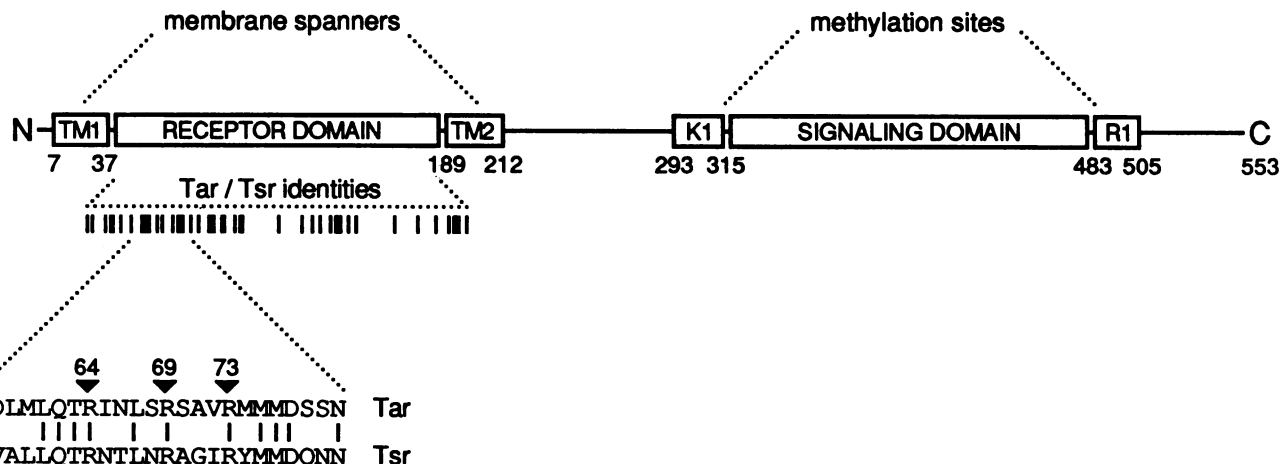


FIG. 5. Conserved residues in the periplasmic portions of the Tar and Tsr chemoreceptors. The domain organization and structural landmarks are given in Tar coordinates (residue positions); Tsr coordinates are nearly identical through the R1 region near the C terminus. Residue identities between the two transducers are indicated by vertical lines. The indicated arginine residues may form a general amino acid-binding site in both receptors (see text).

dergo substantial conformational changes upon ligand binding. It seems likely that changes in receptor site occupancy first trigger conformational shifts in the periplasmic domain, which in turn are propagated to the cytoplasmic domain to modulate its signaling activity. Although communication between the chemoreceptor and flagellar signaling domains of MCP transducers is still poorly understood, the magnitude of the resultant signal is probably proportional to the extent of the induced conformational change (21).

The arginine segment of the Tar transducer may represent a major conformational control point in the receptor domain that is capable of amplifying or propagating local structural changes triggered by ligand binding. For example, ligand-free receptor might be held in a strained conformation by electrostatic repulsion between the closely spaced arginine residues. If aspartate detection occurred primarily through interactions with the positively charged arginines, it could serve to alleviate some of those forces. Thus, aspartate binding might enable the receptor site to assume a less strained conformation, which in turn could precipitate conformational changes throughout the molecule.

Interaction between aspartate and maltose detection sites in Tar. Saturating concentrations of either aspartate or maltose do not block chemotactic responses to the other compound, demonstrating that these stimuli are not detected by identical receptor sites in the Tar molecule (21; C. Wolff, Diploma thesis). However, the magnitude of the response is reduced under these conditions, suggesting that conformational changes at one receptor site can influence the signaling efficiency of the other site. This implies that the entire receptor domain has a limited range of conformational states and that the sensory adaptation process does not simply reverse ligand-generated conformational changes. Thus, saturation of either receptor site could consume some of the shared conformational potential of the transducer molecule, affecting its subsequent signaling properties. This type of conformational coupling between the maltose and aspartate sites could account for the reduced maltose responses in our aspartate-blind mutants.

Conformational coupling between the aspartate and maltose-MBP receptor sites implies that they might be in physical proximity. We argued in this report that the arginine segment of Tar comprises part or all of the aspartate-binding site. In the companion article (14), Kossman et al. discuss evidence that this region of Tar is also involved in interaction with maltose-bound MBP. They found that RW73, RQ73, and RC69 mutations partially suppressed the maltose chemotaxis defects of certain MBP mutants which still bound maltose, but had low affinity for wild-type Tar molecules. These receptor mutations appeared to have enhanced affinity for the mutant MBP, suggesting that the MBP interaction site had been altered by the amino acid replacement. This could mean either that arginines 69 and 73 are directly involved in MBP interactions or that they indirectly control the conformation of the MBP interaction site. Additional mutant studies should serve to clarify the structural relationship between the aspartate and maltose-MBP receptor sites in the Tar transducer.

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LITERATURE CITED

1. Adler, J. 1973. A method for measuring chemotaxis and use of the method to determine optimum conditions for chemotaxis by *Escherichia coli*. *J. Gen. Microbiol.* **74**:77-91.
2. Aksamit, R. R., and D. E. Koshland, Jr. 1974. Identification of the ribose binding protein as the receptor of ribose chemotaxis in *Salmonella typhimurium*. *Biochemistry* **13**:4473-4478.
3. Bollinger, J., C. Park, S. Harayama, and G. L. Hazelbauer. 1984. Structure of the Trg protein: homologies with and differences from other sensory transducers of *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* **81**:3287-3291.
4. Boyd, A., K. Kendall, and M. I. Simon. 1983. Structure of the serine chemoreceptor in *Escherichia coli*. *Nature (London)* **301**:623-626.
5. Callahan, A. M., and J. S. Parkinson. 1985. Genetics of methyl-accepting chemotaxis proteins in *Escherichia coli*: *cheD* mutations affect the structure and function of the Tsr transducer. *J. Bacteriol.* **161**:96-104.
6. Chun, S. Y., and J. S. Parkinson. 1988. Bacterial motility: membrane topology of the *Escherichia coli* MotB protein. *Science* **239**:276-278.
7. Débarbouillé, M., H. A. Shuman, T. J. Silhavy, and M. Schwartz. 1978. Dominant constitutive mutations in *malT*, the positive regulator gene of the maltose regulon in *Escherichia coli*. *J. Mol. Biol.* **124**:359-371.
8. Falke, J. J., and D. E. Koshland, Jr. 1987. Global flexibility in a sensory transducer: a site-directed cross-linking approach. *Science* **237**:1596-1600.
9. Hazelbauer, G. L. 1975. Maltose chemoreceptor of *Escherichia coli*. *J. Bacteriol.* **122**:206-214.
10. Hazelbauer, G. L., and J. Adler. 1971. Role of the galactose binding protein in chemotaxis of *Escherichia coli* toward galactose. *Nature (London) New Biol.* **230**:101-104.
11. Hedblom, M. L., and J. Adler. 1980. Genetic and biochemical properties of *Escherichia coli* mutants with defects in serine chemotaxis. *J. Bacteriol.* **144**:1048-1060.
12. Kellermann, O., and S. Szmelcman. 1974. Active transport of maltose in *Escherichia coli* K12. Involvement of a "periplasmic" maltose-binding protein. *Eur. J. Biochem.* **47**:139-149.
13. Koiwai, O., and H. Hayashi. 1979. Studies on bacterial chemotaxis. IV. Interaction of maltose receptor with a membrane-bound chemosensing component. *J. Biochem.* **86**:27-34.
14. Kossman, M., C. Wolff, and M. D. Manson. 1988. The maltose chemoreceptor of *Escherichia coli*: interaction of maltose-binding protein and the Tar signal transducer. *J. Bacteriol.* **170**:4516-4521.
15. Krikos, A., N. Mutoh, A. Boyd, and M. I. Simon. 1983. Sensory transducers of *E. coli* are composed of discrete structural and functional domains. *Cell* **33**:615-622.
- 15a. Lee, L., T. Mizuno, and Y. Imae. 1988. Thermosensing properties of *Escherichia coli* *tsr* mutants defective in serine chemoreception. *J. Bacteriol.* **170**:4769-4774.
16. Macnab, R. M. 1987. Motility and chemotaxis, p. 732-759. In F. C. Neidhardt (ed.), *Escherichia coli* and *Salmonella typhimurium*. American Society for Microbiology, Washington, D.C.
17. Manson, M. D., V. Blank, G. Brade, and C. F. Higgins. 1986. Peptide chemotaxis in *E. coli* involves the Tap signal transducer and the dipeptide permease. *Nature (London)* **321**:253-256.
18. Manson, M. D., W. Boos, P. J. Bassford, Jr., and B. A. Rasmussen. 1985. Dependence of maltose transport and chemotaxis on the amount of maltose-binding protein. *J. Biol. Chem.* **260**:9727-9733.
19. Mesibov, R., and J. Adler. 1972. Chemotaxis toward amino acids in *Escherichia coli*. *J. Bacteriol.* **112**:315-326.
20. Mowbray, S. L., D. L. Foster, and D. E. Koshland, Jr. 1985. Proteolytic fragments identified with domains of the aspartate chemoreceptor. *J. Biol. Chem.* **260**:11711-11718.
21. Mowbray, S. L., and D. E. Koshland, Jr. 1987. Additive and independent responses in a single receptor: aspartate and maltose stimuli on the Tar protein. *Cell* **50**:171-180.

22. **Park, C., and G. L. Hazelbauer.** 1986. Mutations specifically affecting ligand interaction of the Trg chemosensory transducer. *J. Bacteriol.* **167**:101-109.
23. **Parkinson, J. S.** 1978. Complementation analysis and deletion mapping of *Escherichia coli* mutants defective in chemotaxis. *J. Bacteriol.* **135**:45-53.
24. **Parkinson, J. S.** 1980. Novel mutations affecting a signaling component for chemotaxis of *Escherichia coli*. *J. Bacteriol.* **142**: 953-961.
25. **Parkinson, J. S., and S. E. Houts.** 1982. Isolation and behavior of *Escherichia coli* deletion mutants lacking chemotaxis functions. *J. Bacteriol.* **151**:106-113.
26. **Richarme, G.** 1982. Interaction of the maltose-binding protein with membrane vesicles of *Escherichia coli*. *J. Bacteriol.* **149**: 662-667.
27. **Russo, A. F., and D. E. Koshland, Jr.** 1983. Separation of signal transduction and adaptation functions of the aspartate receptor in bacterial sensing. *Science* **220**:1016-1020.
28. **Silverman, M., and M. I. Simon.** 1977. Chemotaxis in *Escherichia coli*: methylation of *che* gene products. *Proc. Natl. Acad. Sci. USA* **74**:3317-3321.
29. **Slocum, M. K., and J. S. Parkinson.** 1983. Genetics of methyl-accepting chemotaxis proteins in *Escherichia coli*: organization of the *tar* region. *J. Bacteriol.* **155**:565-577.
30. **Slocum, M. K., and J. S. Parkinson.** 1985. Genetics of methyl-accepting chemotaxis proteins in *Escherichia coli*: null phenotypes of the *tar* and *tap* genes. *J. Bacteriol.* **163**:586-594.
31. **Springer, M. S., M. F. Goy, and J. Adler.** 1977. Sensory transduction in *Escherichia coli*: two complementary pathways of information processing that involve methylated proteins. *Proc. Natl. Acad. Sci. USA* **74**:3312-3316.
32. **Stewart, R. C., and F. W. Dahlquist.** 1987. Molecular components of bacterial chemotaxis. *Chem. Rev.* **87**:997-1025.
33. **Terwilliger, T. C., and D. E. Koshland, Jr.** 1984. Sites of methyl esterification and deamidation on the aspartate receptor involved in chemotaxis. *J. Biol. Chem.* **259**:7719-7725.
34. **Wang, E. A., and D. E. Koshland, Jr.** 1980. Receptor structure in the bacterial sensing system. *Proc. Natl. Acad. Sci. USA* **77**: 7157-7161.