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Maltose Chemoreceptor of *Escherichia coli*: Interaction of Maltose-Binding Protein and the Tar Signal Transducer†

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The maltose chemoreceptor in *Escherichia coli* consists of the periplasmic maltose-binding protein (MBP) and the Tar signal transducer, which is localized in the cytoplasmic membrane. We previously isolated strains containing *malE* mutations that cause specific defects in the chemotactic function of MBP. Four of these mutations have now been characterized by DNA sequence analysis. Two of them replace threonine at residue 53 of MBP with isoleucine (MBP-TI53), one replaces an aspartate at residue 55 with asparagine (MBP-DN55), and the fourth replaces threonine at residue 345 with isoleucine (MBP-TI345). The chemotactic defects of MBP-TI53 and MBP-DN55, but not of MBP-TI345, are suppressed by mutations in the *tar* gene. Of the *tar* mutations, the most effective suppressor (isolated independently three times) replaces Arg-73 of Tar with tryptophan. Two other *tar* mutations that disrupt the aspartate chemoreceptor function of Tar also suppress the maltose taxis defects associated with MBP-TI53 and MBP-DN55. One of these mutations introduces glutamine at residue 73 of Tar, the other replaces arginine at residue 69 of Tar with cysteine. These results suggest that regions of MBP that include residues 53 to 55 and residue 345 are important for the interaction with Tar. In turn, arginines at residues 69 and 73 of Tar must be involved in the recognition of maltose-bound MBP and/or in the production of the attractant signal generated by Tar in response to maltose-bound MBP.

We are studying maltose taxis in the enteric bacteria as a model for the function of complex chemoreceptors. Maltose-binding protein (MBP) (15), a soluble protein localized in the periplasmic space, is the recognition component of this receptor (13). MBP is also necessary for active uptake of maltose into the cell (15, 35). MBP is synthesized as a precursor from which 26 amino acids at the N terminus (the signal sequence) are removed to form mature MBP (3). Mature MBP consists of 370 amino acids (10).

The signalling element of the maltose chemoreceptor is the Tar (taxis to aspartate and away from some repellents) protein (39). Tar mediates chemotactic responses to maltose, to aspartate (and chemically related amino acids), and to the repellents Ni^{2+} and Co^{2+}. The protein is located in the cytoplasmic membrane (31). The amino acid sequences of Tar from *Escherichia coli* (16) and *Salmonella typhimurium* (32) have been derived from the DNA sequences of the respective *tar* genes.

Chemotactic signal transducers contain four structural domains (16). The N terminus begins with a short sequence containing several basic residues followed by a stretch of hydrophobic residues. This structure generally resembles the signal sequence of exported proteins. In the transducers, however, this sequence is apparently not removed from the mature polypeptide (25) and is thought to anchor the N terminus in the membrane (18; J. Gebert, M. D. Manson, and W. Boos, unpublished data). The next 150 amino acids form a hydrophilic domain in the periplasm, where binding of chemoeffector occurs. The periplasmic region is followed by a second hydrophobic sequence that spans the membrane. Finally, there is a large cytoplasmic domain of about 300 amino acids. This region initiates the signal that ultimately controls the direction of flagellar rotation (27). It also contains the glutamic acid residues that are methylated during adaptation to a chemotactic stimulus (16). Tar from *E. coli* contains a total of 553 amino acids (16).

We previously described a series of *malE* mutations that specifically impair the chemoreceptor function of MBP (20). Some of these mutations can be suppressed in an allele-specific fashion by certain mutations in *tar*. We report here the identification of these *malE* and *tar* mutations and one other *malE* mutation by DNA sequence analysis. Together with the findings presented in the accompanying paper (41), these results provide information about how substrate-bound MBP interacts with the Tar transducer.

MATERIALS AND METHODS

Reagents. [α-^{32}P]dATP and deoxyadenosine 5′-[α-^{35}S]thiotriphosphate were obtained from Amersham Corp. (Arlington Heights, Ill.), as was [U-^{14}C]maltose (specific activity, 185 mCi/mmol). Deoxynucleotides and dideoxynucleotides, restriction endonucleases, T4 DNA ligase, and *E. coli* DNA polymerase (Klenow fragment) were purchased from Boehringer Mannheim Biochemicals (Indianapolis, Ind.). Maltose (analytical grade, >99.0%) was supplied by E. Merck AG (Darmstadt, Federal Republic of Germany) or Fluka AG (Buchs, Switzerland), and L-aspartic acid was from Sigma Chemical Co. (St. Louis, Mo.). All other chemicals were of reagent grade.

Swarm plates. Minimal swarm plates contained 0.3% Bacto-Agar (Difco Laboratories, Detroit, Mich.) and motility salts (10 mM potassium phosphate [pH 7.0], 1 mM $(NH_4)_2SO_4$, 1 mM $MgSO_4$, 0.5% NaCl, 1 μg of thiamine per ml, 0.5 μg of $FeCl_3$ per ml). Minimal maltose swarm plates contained 100 μM maltose; minimal aspartate swarm plates contained 1 mM glycerol and 100 μM L-aspartate.

Bacterial and bacteriophage strains. Strain MM314 (20) is an *flbB*$^+$ Δ*malE444* (34) *malT*^{c}*-1* (9) derivative of *E. coli* MC4100 (7). *malE* mutations were introduced into strain MM314 by phage P1 *vir* transduction, selecting for growth on

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† Dedicated to our many friends in Konstanz, Federal Republic of Germany.

minimal medium A (23) agar containing 0.2% maltose. *tar* mutations were introduced with phage P1 by cotransduction with the *zeb-741*::Tn*10* insertion (20), with selection for resistance to 5 μg of tetracycline per ml and subsequent testing of the swarm phenotype of the transductants in aspartate soft agar. (About 90% of the transductants receiving the *tar* mutations swarmed poorly on tryptone swarm agar. We chose isolates that gave normal swarms for further analysis. The mutation causing the poor swarming was probably present in the strains used for crossing the *tar* mutations from plasmids into the chromosome [41; J. S. Parkinson, personal communication].) Strain MM140 is a derivative of strain AW405 (2) that carries the *tar-tap-5201* deletion (38). Strain KK2186, used for propagation of M13 phage, is identical to strain JM103 (21) except that it is cured of phage P1.

λpK3 is a plaque-forming Lac$^+$ *malE*$^+$ transducing phage (37). Phage λche22 (30) contains the entire *mocha* and *meche* operons of *E. coli* and the λ *c*I ts857 gene. Phage M13mp8 (22) and M13mp18 (42) were used as cloning vectors for producing single-stranded template DNA for sequencing reactions.

Nomenclature for mutations. The precise location of *malE* or *tar* mutations was determined by DNA sequence analysis. Each mutation will be referred to by the amino acid substitution it generates, using the single-letter code for the amino acids. Thus, a mutation leading to the replacement of the arginine at residue 73 of Tar by tryptophan will be designated as Tar-RW73. (For MBP, position 1 is defined as the amino-terminal residue of the mature polypeptide, after cleavage by leader peptidase.)

Quantitative swarm assays. Maltose swarm plates were inoculated from single fresh colonies grown on nutrient agar and incubated at 32°C. The swarm diameter was measured every 2 h after the initial 10 h of incubation. The rate of swarm ring expansion was determined from a plot of swarm diameter versus time. These plots were linear for all strains and yielded a value of 2.14 mm/h for the *malE*$^+$ *tar*$^+$ wild-type strain.

Capillary assays. The capillary assay developed by Adler (1) was performed at 32°C as described previously (19). Assays were done in duplicate with two or three repetitions. All attractants were filter sterilized to avoid thermal degradation. Accumulations in capillaries containing chemotaxis buffer alone were subtracted as background from the accumulations in capillaries containing attractants. To correct for day-to-day variations in cell motility, we expressed maltose and aspartate accumulations as percentages of the accumulations in capillaries containing 1 mM L-serine. At the peak concentration of 1 mM maltose, the response of our *malE*$^+$ *tar*$^+$ strain was 234% of the serine response.

At 1 mM maltose, the response of strain MM314 (Δ*malE*) was 4% of the serine response, or about 1.7% of the response of the *malE*$^+$ *tar*$^+$ strain at 1 mM maltose. The response of strain MM314 increased with higher maltose concentrations: 10% of the serine response at 3 mM, 18% at 10 mM, and 28% (the peak value) at 30 mM. This response is not mediated by MBP, since the Δ*malE* strain lacks MBP. Thus, to determine the MBP-dependent response to maltose, we made a further correction of the data from the capillary assay by subtracting the accumulation of strain MM314 from the accumulations of the test strains at each maltose concentration. For most strains for which an MBP-dependent accumulation in maltose capillaries is recorded in Table 3, a distinct peak was seen at 0.3 or 1 mM maltose, even if a second, MBP-independent peak was seen at higher maltose concentrations. The exception is the very small MBP-dependent maltose response seen with the Tar-RC64 *malE*$^+$ strain, for which the peak at 1 mM maltose appeared only as a shoulder on the 30 mM peak. (The response of the Δ*malE* strain to high concentrations of maltose may be due to glucose or other attractants contaminating our maltose solutions, although we did use the highest grade of maltose available commercially.)

Maltose transport assays. Cells were grown at 32°C in minimal medium A containing 0.5% glycerol, 1 μg of thiamine per ml, and 0.2% Casamino Acids (Difco). The *malT*^{c}-*1* allele (9) in strain MM314 causes constitutive high-level expression of the maltose transport system. The K_m and V_{max} values for maltose transport were determined as described previously (19).

Cloning of *malE* and *tar* genes containing specific mutations. *malE* mutations (20) were crossed onto phage λpK3 by homologous recombination, which occurs during insertion and excision of the phage DNA into the *malB* region of mutant strains. Phage released spontaneously from λpK3 lysogens during overnight growth at 37°C in LB medium (23) were used to lysogenize strain MM314. Lac$^+$ lysogens were identified as red colonies in the center of plaques on MacConkey lactose plates (23) and purified on the same agar. Recombinant phage containing *malE* mutations were isolated by screening the lysogens on maltose swarm plates and recovering phage from lysogens that did not produce normal maltose swarm rings.

The 1.7-kilobase (kb) *Eco*RI-*Stu*I fragment (10) containing the entire *malE* gene and the *malB* control region was subcloned from the recombinant phage into the *Eco*RI and *Pvu*II sites of plasmid pBR322 (4). The resulting 4.0-kb plasmids (Ap^r) were used as sources of DNA fragments for cloning regions of *malE* into phage M13mp8. The plasmid containing the wild-type *malE* gene was called pMK3.

tar mutations were crossed onto λche22 after isolating lysogens of this phage in *tar* strains at 30°C on tryptone agar plates (23) spread with about 10^9 λ *c*I857 *h*80 *int*9 phage. Lysogens were then purified on the same plates. λche22 phage released from these lysogens during overnight growth at 37°C were used to lysogenize strain MM140 Δ(*tar-tap*)*5201*. Recombinant phage carrying *tar* mutations were isolated by screening the lysogens of strain MM140 at 30°C on aspartate swarm plates and recovering the phage from lysogens incapable of forming aspartate swarm rings.

The 3.3-kb *Eco*RI-*Ava*I fragment (38) carrying the 3′ end of *cheA*, all of *cheW*, the *meche* operon promoter, *tar*, and the 5′ end of *tap* was subcloned from the recombinant phage into the *Eco*RI and *Ava*I sites of pBR322 (the *Ava*I sites in *tap* and pBR322 are compatible). The resulting 6.2-kb plasmids (Ap^r) were used as sources of DNA for cloning into phage M13mp18. The plasmid containing the *tar*$^+$ gene was called pMK1. Manipulations of DNA were done by the methods of Maniatis et al. (17) and Silhavy et al. (36) or as recommended by the manufacturer.

DNA sequencing. The 0.8-kb *Bgl*II-*Eco*RI fragments (10) from the pMK3 (*malE*) plasmid series were cloned into the unique *Bam*HI and *Eco*RI sites in the polylinker region of phage M13mp8. The *Bgl*II site is at a position corresponding to amino acid 119 of mature MBP, and the *Eco*RI site is early in *malK*. This fragment was sequenced in the direction *Bgl*II toward *Eco*RI.

The 0.7-kb *Bgl*II-*Hin*fI fragments (10) from the pMK3 plasmid series were cloned into the unique *Bam*HI and *Hin*cII sites of the phage M13mp8 polylinker after filling in

the *Hin*fI end. The *Hin*fI site is very late in *malE*. This fragment was sequenced in the direction *Hin*fI toward *Bgl*II.

The 1.6-kb *Eco*RI-*Xba*I fragments (38) from the pMK1 plasmid series were cloned into the unique *Eco*RI and *Xba*I sites of the phage M13mp18 polylinker. The *Xba*I site is in the region of *tar* that codes for the periplasmic portion of the protein, and the *Eco*RI site is in *cheA*. This fragment was sequenced in the direction *Xba*I toward *Eco*RI.

DNA sequencing was done by the dideoxynucleotide chain termination method (33) as described in the *M13 Cloning "Dideoxy" Sequencing Manual* and the *M13 Cloning and Sequencing Handbook* of Bethesda Research Laboratories, Inc. (Gaithersburg, Md.) and Amersham Corp., respectively. The universal 15-base-pair *lacZ* primer (2 μg/ml; Bethesda Research Laboratories) was used in sequencing reactions; products were labeled with [α-^{32}P]dATP or deoxyadenosine 5′-[α-^{35}S]thiotriphosphate.

RESULTS

Identification of *malE* mutations causing specific defects in maltose chemotaxis. We previously described 14 *malE* mutations that specifically interfere with maltose chemotaxis without significantly affecting maltose transport (20). The chemotactic defects of the *malE454* and *malE461* mutations could be partially reversed by three independently isolated *tar* mutations. To extend this analysis, we tested the ability of one of these *tar* alleles (*tar-453*) to suppress all 14 of the original *malE* mutations. Except for strains containing *malE454* and *malE461*, only the *malE467* mutant produced a swarm ring in maltose soft agar that was sharper and had a larger diameter in the presence of *tar-453*. In particular, strains containing the *malE469* mutation, which exhibited wild-type maltose transport in preliminary assays (20), exhibited even weaker swarm rings in combination with *tar-453* than with *tar*$^+$.

To establish unequivocally that mutant *malE* genes encode MBP molecules that support normal maltose transport, we lysogenized strain MM314 (Δ*malE444*) with λpK3 transducing phage carrying the *malE*$^+$, *malE454*, *malE461*, *malE467*, and *malE469* alleles. All these lysogens had very similar apparent K_m values and V_{max} values for maltose uptake (data not shown).

From deletion mapping data (20), we knew the approximate locations of the *malE454*, *malE461*, *malE467*, and *malE469* mutations in *malE*. This information dictated our DNA sequencing strategy and enabled us to identify each of the mutated sites in a single sequencing run. In the regions that we sequenced, corresponding to the first 119 and the last ca. 100 residues of mature MBP, only a single nucleotide substitution was found in each mutant *malE* gene relative to the *malE*$^+$ control (Table 1).

Identification of suppressor mutations in *tar*. We assumed that the suppressor mutations would affect the periplasmic portion of Tar. Therefore, we began sequencing toward the 5′ end of the gene from the *Xba*I site of *tar* (38). All three of our independently isolated suppressor mutations were in this region and proved to be identical; they converted the CGG triplet that codes for Arg-73 of Tar to TGG, which codes for tryptophan. This result was consistent with our observation that the changes in swarm phenotype produced by *tar-451*, *tar-452*, and *tar-453* are indistinguishable (20).

Effect of aspartate-negative *tar* mutations on maltose chemotaxis. The accompanying paper (41) describes the isolation and characterization of *tar* mutants with defective aspartate taxis. (The amino acid substitutions introduced by these mutations are incorporated into Tables 2 and 3 of this paper.) When present on a multicopy plasmid, these *tar* mutations support a maltose-taxis-positive phenotype on swarm plates (41), but elevated amounts of a partially defective transducer could mask defects in maltose taxis. Therefore, we wanted to look at maltose taxis in strains in which the mutant *tar* alleles were present in the chromosome in combination with different *malE* mutations.

We transduced chromosomal *malE* (20) and *tar* (41) mutations into strain MM314 with phage P1 and then examined the chemotactic behavior of the transductants. The *malE* mutations did not affect aspartate chemotaxis in a *tar*$^+$ strain, but all the *tar* mutations seriously impaired aspartate taxis. Our data (not shown) from aspartate capillary assays were essentially the same as those reported in the accompanying paper (41).

The results of maltose swarm plate (Table 2) and capillary (Table 3) assays yielded the same basic conclusions, although quantitative differences among strains were more obvious from the capillary assay data. The *tar* mutations disrupted maltose taxis to various degrees in *malE*$^+$ strains (Table 3), with the Tar-RW73 mutant having the largest residual maltose response, followed in order by Tar-RC69, Tar-RQ73, Tar-RH69, and Tar-RC64.

In *tar*$^+$ strains containing MBP-TI53 or MBP-DN55, there was no detectable MBP-dependent maltose response. The Tar-RW73, Tar-RQ73, and Tar-RC69 mutations restored maltose responses in combination with either of these mutant MBP molecules. Strains containing the Tar-RC64 or Tar-RH69 alleles and MBP-TI53 or MBP-DN55 did not show any detectable MBP-dependent maltose response.

TABLE 1. Identification of *malE* mutations by DNA sequence analysis

Mutation	Wild-type configuration		Mutant configuration	
	Codon (no.)[a]	Amino acid	Codon	Amino acid
malE461	ACT (53)	Thr	ATT	Ile
malE467	ACT (53)	Thr	ATT	Ile
malE454	GAT (55)	Asp	AAT	Asn
malE469	ACT (345)	Thr	ATT	Ile

[a] Codon number refers to the position of the encoded amino acid relative to the N terminus of the mature form of MBP.

DISCUSSION

To identify residues of MBP that interact with the Tar signal transducer, we analyzed the DNA sequence of *malE* from four strains defective for maltose chemotaxis. The *malE461* and *malE467* mutations, isolated separately, gener-

TABLE 2. Maltose swarm rates of *malE* and *tar* mutants[a]

tar Allele	*malE* wild type	*malE* TI53	*malE* DN55
Wild type	1.00 (+++)	0.40 (−)	0.46 (+)
RC64	0.54 (+)	0.41 (−)	0.42 (−)
RC69	0.55 (+)	0.46 (+)	0.47 (+)
RH69	0.47 (+)	0.42 (−)	0.36 (−)
RQ73	0.50 (+)	0.44 (+)	0.44 (+)
RW73	0.59 (++)	0.60 (++)	0.61 (++)

[a] Rates are expressed as a fraction of the rate for the wild-type (*malE*$^+$ *tar*$^+$) strain. The symbols in parentheses indicate the phenotype of the swarm rings, grading from +++ (the densest, sharpest ring) to − (the complete absence of a ring).

TABLE 3. Maltose capillary assays with *malE* and *tar* mutants

tar allele	*malE* wild type		*malE* TI53		*malE* DN55	
	Peak concn (mM)[a]	% Wild type[b]	Peak concn (mM)	% Wild type	Peak concn (mM)	% Wild type
Wild type	1	100	No accumulation		No accumulation	
RC64	1	3	No accumulation		No accumulation	
RC69	1	27	1	5	0.3	5
RH69	1	12	No accumulation		No accumulation	
RQ73	1	18	0.3–1	8	0.3	5
RW73	1	41	1–3	11	0.3	7

[a] Peak concentration refers to the concentration of maltose in capillaries for which the highest normalized MBP-dependent accumulations for each strain were recorded (see Materials and Methods). Assays were run with capillaries containing 10 μM, 30 μM, 100 μM, 300 μM, 1 mM, 3 mM, 10 mM, 30 mM, and 100 mM maltose.

[b] The accumulation of each strain at its peak maltose concentration (0.3 or 1 mM) is expressed as a percentage of the accumulation of the $malE^+$ tar^+ strain at 1 mM maltose. No accumulation indicates that the accumulation was less than 1% of the $malE^+$ tar^+ value.

ate an ACT-to-ATT codon substitution that leads to MBP-TI53. The *malE454* mutation generates a GAT-to-AAT codon exchange that creates MBP-DN55. The *malE469* mutation converts an ACT codon to ATT, generating MBP-TI345.

The defects in maltose taxis caused by MBP-TI53 and MBP-DN55 were partially suppressed by specific mutations in *tar*. This result suggests that the Thr-53 and Asp-55 residues of MBP interact directly with Tar. If so, the conformational change that accompanies maltose binding (43) should create a recognition site for Tar by making these residues more accessible or by bringing them into the proper spatial relationship with other parts of the MBP. Alternatively, the residue 53 to 55 region may maintain MBP in a configuration in which it can interact with Tar. The MBP-TI53 and MBP-DN55 mutations could alter this configuration, and the suppressing mutations could modify Tar to enhance interaction with the mutant MBPs.

We looked without success for *tar* mutations that suppress the chemotactic defect of MBP-TI345. Perhaps the proper amino acid substitutions cannot be generated by hydroxylamine. However, the structure of MBP-TI345 could be changed so that no single amino acid substitution in Tar can restore an effective interaction with the mutant MBP. Indeed, MBP-TI345 does appear to have altered physical properties. Although the intrinsic fluorescence of wild-type MBP decreases when maltose binds (43), maltose binding increases the intrinsic fluorescence of MBP-TI345 (L. Randall, personal communication).

An extensive search (20) has yielded only a few amino acid substitutions (MBP-TI53, MBP-DN55, and perhaps MBP-TI345) that specifically alter the MBP-Tar interaction. Why not more? We selected our *malE* mutants to retain normal maltose transport. Therefore, we may have discarded mutations that affect regions of MBP that interact with both Tar and the MalF and MalG proteins, which are membrane components of the maltose transport system (35, 40). In fact, some *malE* mutations selected on the basis of altered interactions with MalF or MalG have defects in maltose taxis (40a). Also, regions comprising residues 297 to 303 and the last 7 of the 370 residues of MBP seem to be important for maltose taxis (11). Thus, several different segments of the MBP polypeptide are apt to be involved in the interaction with Tar. It must be emphasized, however, that since the MBP-Tar binding affinities have not been measured, we do not know whether the mutant MBP molecules are defective in binding to Tar or in excitation of Tar, or both.

MBP and the galactose-binding protein (GBP) are quite similar in two regions, termed A and B, of their amino acid sequences (10). The *mglB551* mutation (14), which disrupts the chemotactic function of GBP while leaving its substrate-binding and transport functions intact (28), introduces aspartate instead of glycine at residue 74 of GBP (34), which is in the middle of region A. The *malE* mutations described here do not map near region A of MBP. Thus, the data now available suggest that different regions of the GBP and MBP amino acid sequences are involved in the interaction of these two proteins with their respective transducers.

The *tar-451*, *tar-452*, and *tar-453* mutations, which suppress the defects in maltose taxis caused by MBP-TI53 and MBP-DN55, are identical: they convert a CGG codon to TGG, generating Tar-RW73. This same mutation interferes with aspartate taxis. The three arginine residues at positions 64, 69, and 73 of Tar play a crucial role in aspartate chemotaxis (41), and substitutions at these residues also impair maltose taxis in a $malE^+$ strain (Tables 2 and 3). However, the Tar-RQ73 and Tar-RC69 mutations suppress MBP-TI53 and MBP-DN55, although to a lesser extent than does Tar-RW73. The Tar-RC64 and Tar-RH69 mutations cause severe defects in aspartate taxis (41) and maltose taxis and fail to suppress MBP-TI53 or MBP-DN55. These two mutant Tar transducers may be unable to generate strong signals in response to any attractant binding to their periplasmic domains (41).

Arginine residues are found at positions 64, 69, and 73 of Tar from *S. typhimurium* LT2 (32), even though Tar from that organism does not mediate maltose taxis (8, 24). The Tsr (taxis to serine and away from some repellents) transducer also has arginines at these positions (6). Thus, although the normal interaction of MBP with *E. coli* Tar involves these arginine residues, other unique determinants in the periplasmic domain of *E. coli* Tar must be involved in its interaction with MBP.

The three arginines cannot be essential for the function of all transducers, since only one is present at the analogous position in the Trg (taxis to ribose and galactose) transducer (5), and none are present at equivalent positions in the Tap (taxis-associated protein) transducer (16). However, a *trg* mutation that diminishes responses to galactose and, especially, ribose affects an arginine residue in the same general region of Trg (29).

The arginines at positions 64, 69, and 73 may contribute to a site involved in the recognition of the constituent groups on the α-carbon of amino acids (41) and, in *E. coli* Tar, to generation of an attractant signal with maltose-loaded MBP. It is therefore noteworthy that *E. coli* cells adapted to saturating concentrations of either aspartate or maltose and then exposed to the other compound responded to the

second attractant in a temporal assay (26; C. Wolff, Diploma thesis, University of Konstanz, Konstanz, Federal Republic of Germany, 1983). However, the second response is usually shorter than the response to that attractant added alone. The methylation of Tar in response to these attractants is also partially additive. Addition of aspartate or maltose at saturating concentrations led to increased methylation of at least 78 or 54% of the total Tar molecules, respectively, present in the cells (26). If the two attractants were added together at saturating concentrations, at least 92% of the Tar molecules exhibited increased methylation.

Our genetic data suggest that the sites on Tar that interact with aspartate and MBP overlap, while the physiological data just discussed indicate that there is a partial independence of the chemotactic responses to aspartate and maltose. There are several ways to reconcile these interpretations. One way is to suppose that the sites at which Tar interacts with aspartate and MBP are distinct but that mutations at the aspartate site have an allosteric effect on the MBP site. Alternatively, free aspartate and MBP may interact with the same arginine residues of Tar to excite the transducer, but binding of MBP may rely on interactions with other regions of Tar. The net occupancy of the excitation site could increase if both aspartate and MBP bound to a single Tar monomer. Partial additivity of the aspartate and maltose responses could result from either of these mechanisms.

Another alternative is that the periplasmic domain of Tar can exist in two conformations that are in equilibrium. One conformation would favor binding of aspartate, the other binding of MBP. There is apparently only one population of Tar in *E. coli* (26). However, since Tar exists as a dimer (12), binding of aspartate to one monomer could increase the affinity of the second monomer for MBP, and vice versa. The cytoplasmic signalling domain may be formed by two monomers (16) but be activated when an attractant binds to the periplasmic receptor domain of just one monomer. If two monomers in one dimer bind aspartate and MBP concurrently, the signal generated could be greater than that generated by one attractant but less than the sum of the signals generated by the two attractants added independently, as is observed (26; C. Wolff, Diploma thesis).

With any of the models proposed above, one might expect that aspartate would have an altered affinity for Tar in the presence of maltose-loaded MBP, and vice versa. Unfortunately, the binding experiments needed to test this possibility are at present technically difficult. Additional information should come from the analysis of a wider range of mutations that affect aspartate and maltose chemotaxis. Given the complexity of the maltose chemoreceptor, future investigations of its function will require a concerted structural, biochemical, and genetic assault.

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