Dependence of Maltose Transport and Chemotaxis on the Amount of Maltose-binding Protein*

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Maltose-binding protein (MBP) is essential for maltose transport and chemotaxis in *Escherichia coli*. To perform these functions it must interact with two sets of cytoplasmic membrane proteins, the MalFGK transport complex and the chemotactic signal transducer Tar. MBP is present at high concentrations, on the order of 1 mM, in the periplasm of maltose-induced or malT constitutive cells. To determine how the amount of MBP affects transport and taxis, we utilized a series of malE signal-sequence mutations that interfere with export of MBP. The MBP content in shock fluid from cells carrying the various mutations ranged from 4 to 23% of the malE* level. The apparent Kₘ for maltose transport varied by less than a factor of 2 among malE* and mutant strains. At a saturating maltose concentration 9% (~90 μM) of the malE* amount of MBP was required for half-maximal uptake rates. Transport exhibited a sigmoidal dependence on the amount of periplasmic MBP, indicating that MBP may be involved in a cooperative interaction at some stage of the transport process. The chemotactic response to a saturating maltose stimulus exhibited a first-order dependence on the amount of periplasmic MBP. Thus, interaction of a single substrate-bound MBP with Tar appears sufficient to initiate a chemotactic signal from the transducer. A half-maximal chemotactic response occurred at 25% of the malE* MBP level, suggesting that in vivo the Kₐ for binding of maltose-loaded MBP to Tar is quite high (~250 μM).

The proteins needed for maltose uptake in *Escherichia coli* are encoded by genes of two divergent operons in the malB region, located at 91 min on the chromosome (Fig. 1). The malE gene codes for maltose-binding protein (MBP), which is localized in the periplasmic space (2). MalF and MalG are integral proteins of the cytoplasmic membrane (3–5), and MalK is apparently attached to the inner surface of the cytoplasmic membrane via MalG (5). The lamB gene codes for the subunit of maltoporin, which exists as a trimeric pore in the outer membrane (6). Maltoporin specifically facilitates permeation of maltose and longer maltodextrins through the outer membrane (7, 8) and also serves as the receptor for phage λ (9).

The malT gene, mapping at 74 min, codes for a positive regulator required for expression of all other mal operons, including those of the malB region (10). Growth on maltose induces expression of MalT-dependent operons; malT* mutations lead to constitutive expression of the mal regulon (11). When maltose binds to MBP the protein undergoes a change in conformation (7, 12). Maltose transport is thought to be initiated by interaction of substrate-loaded MBP with the inner membrane transport components (13, 14). The apparent Kₘ for maltose transport, about 1 μM in lamB* cells, increases 100-fold in mutants lacking maltoporin (7) because maltose entry into the periplasm becomes rate limiting at low maltose concentrations. The MBP-dependent system is the only significant pathway for maltose uptake in *E. coli* under normal conditions (13).

In addition to its role in transport, MBP functions as the maltose chemoreceptor (15). A second protein, the chemotactic signal transducer Tar (taxis to aspartate and some repellents), is required to generate the chemotactic response to maltose (16). Tar is located in the cytoplasmic membrane (17) and also serves as the receptor for the potent attractant L-aspartate and the repellents Co²⁺ and Ni²⁺. Whereas aspartate binds directly to Tar (18) maltose binds indirectly in the form of maltose-loaded MBP (19, 20). Receptor and signal transducer functions in chemotaxis have been recently reviewed (21).

Maltoporin, with 10⁶ copies of monomer/cell (22), and MBP, with 2 to 4.5 × 10⁴ copies/cell (23, 24), are major components of the cell envelope in maltose-induced or malT* strains. The periplasmic MBP concentration has been estimated to be around 1 nM (23). In contrast, maltose-induced cells contain much lower amounts of *malF*, *G*, and *K* gene products, probably about 1000 copies/cell (5). Tar, which is not part of the mal regulon, also is present in about 1000 copies/cell (20, 25).

To understand why MBP is present in 20- to 40-fold excess relative to its membrane partners, it is necessary to know how transport and chemotaxis depend on the periplasmic MBP concentration. Maltose-induced cells have higher transport and chemotactic activity than uninduced cells (26, 27). For maltose, galactose, and ribose, the increase in chemotactic activities toward each sugar was roughly proportional to the respective binding activities in the periplasm of uninduced and induced cells (24). These experiments, however, permitted the comparison of only two levels of binding protein and, for

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1 The abbreviation used is: MBP, maltose-binding protein.
transport, induction altered the amount of all components involved, not just binding protein.

The resolution to these difficulties lay with a series of malE mutations that affect secretion of MBP (28, 29). MBP is synthesized as a precursor from which 26 amino acids at the NH₂ terminus, the signal peptide, are removed to form mature periplasmic MBP (30, 31). The mutations alter the signal peptide so as to interfere in varying degrees (from 4 to 23% of malE periplasmic MBP levels) with secretion of MBP (32). Unexported MBP remains as precursor in the cytoplasm, while the reduced amount of MBP that does reach the periplasm is found in the normal, mature form.

We measured the periplasmic MBP level and correlated this value with maltose chemotactic and transport activities in malT strains carrying five different malE signal sequence mutations. With these data, we determined in vivo affinities for the interaction of MBP with Tar and the MalFGK complex. The maltose chemotactic response depended on the first power of the MBP concentration. Using 1 µM as the periplasmic MBP concentration in malT malE+ cells, the Kₐ for MBP binding to Tar was determined to be 250 µM. Maltose transport showed a sigmoidal dependence on the MBP concentration, with half-maximal transport occurring at 90 µM MBP. The apparent Kₚₐ of maltose transport remained essentially constant over the range of MBP levels tested. The implications of these findings for the mechanisms of maltose transport and chemotaxis are discussed.

EXPERIMENTAL PROCEDURES

Materials—[1-¹⁴C]maltose (185 mCi per mmol) was purchased from Amersham Corp. Highly purified maltose for chemotaxis assays was prepared by descending paper chromatography (Whatman 3MM paper). The chromatogram was developed in butanol-pyridine-water-acetic acid (15:12:10:3, v/v/v/v). All other chemicals are of reagent grade.

Bacterial Strains—The bacterial strains used in this study are described in Table 1. The construction of strains carrying malE signal sequence mutations, malF::Tn10 and lamB102(Am), is explained in Fig. 2. All genetic manipulations were performed according to Miller (33).

Preparation of Osmotic Shock Fluid—Strains MM209 through MM215 were grown at 37 °C in 1 l of H₁ minimal medium (39) containing 0.2% glycerol and 0.1% casamino acids. The cells were harvested at an A₀₋₀₋₀ = 0.05 and resuspended in 100 ml of 10 mM Tris-HCl, pH 7.3, containing 0.03 mM NaCl. Osmotic shock was done according to Neuf and Heppel (40) with a shock volume of 100 ml. The crude shock fluid was lyophilized, resuspended in 2.5 ml of 10 mM Tris-HCl, pH 7.3, and dialyzed against the same buffer. The slightly turbid solution was cleared by centrifugation at 13,000 x g for 5 min and used for the determination of MBP.

Polyacrylamide Gel Electrophoresis in Urea—Analysis of MBP by polyacrylamide gel electrophoresis in sodium dodecyl sulfateproved unsatisfactory, since another protein in shock fluid from our strains co-migrated with MBP (data not shown). In contrast, MBP formed a unique band during polyacrylamide gel electrophoresis in 8 M urea performed according to Fuglsang and Schneitman (41).

Equilibrium Dialysis—Three times 200-µl aliquots of crude shock fluid were transferred into Visking dialysis tubing and dialyzed at 4 °C against 100 ml of Tris-HCl, pH 7.3, containing 3 x 10⁻⁷ M CaCl₂, 10⁻⁷ M chloramphenicol, and ampicillin (50 µg/ml). Maltose was added to a concentration of 1 mg/ml, equilibrium was reached after 20 h of dialysis. After 36 h the dialysis bags were drained and the volume of the content determined by weighing. Volume changes of up to ±20% occurred during dialysis. To determine the amount of maltose bound to MBP, the radioactivity in the bags was measured and the radioactivity of the corresponding volume of the external solution was subtracted. The total MBP concentration (Pt) was determined by the equation,

\[ P_t = \frac{P_L}{L} K_d \]

where PL represents MBP complexed with maltose, L the free maltose concentration, and Kₚ the dissociation constant. This equation is valid at free maltose concentrations far below Kₚ (42). A calibration curve for PL/L was obtained with known amounts of MBP ranging from 0.01 to 1.0 mg/ml. A Kₚ of 3 µM at 4 °C (34) was used, and one binding constant for MBP monomer was assumed. PL/L values obtained from the six different MBP preparations ranged from 4 (wild type) to 0.2 (malE-18). The corresponding value for a preparation from ΔmalE mutant was 0.01. Triplicate determinations showed, at most, 10% variation. The values obtained were normalized to the number of cells from which the shock fluid was obtained.

Immunodiffusion Assay—Shock fluid was tested with antiserum against MBP on Ouchterlony double-diffusion plates (44) at 37 °C. The central wells contained 10 µl of antiserum and the six peripheral wells 10 µl of 2-fold serial dilutions of crude shock fluid.

Immunoprecipitation of MBP from Shock Fluid—To ensure that MBP in the shock fluid was in the mature form and not precursor, samples were precipitated with anti-MBP antiserum followed by adsorption of immunoglobulins to glutaraldehyde-treated Staphylococcus aureus. The cells were extensively washed. MBP and antibodies were removed with sodium dodecyl sulfate and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis as previously described (45).

Maltose Transport—Strains MM186 through MM191 were grown at 32 °C in minimal medium A (38) containing 0.2% glycerol and 0.1% casamino acids. The cells were harvested at A₀₋₀₋₀ = 0.5 (1 to 2 x 10⁹ cells/ml), washed three times with minimal medium A, and resuspended to A₀₋₀₋₀ = 1.0. To 2.9 ml of cells 100 µl of [¹⁴C]maltose was added. 0.5-ml samples were withdrawn at 15, 30, 60, and 90 s and filtered through Millipore filters of 0.45-µ pore size; the filters were washed with 10 ml of minimal medium A, dried at 120 °C, and counted in a scintillation counter after addition of a toluene-based cocktail.

To determine the Kₚₐ of maltose transport, a constant amount of radioactive maltose and varying amounts of unlabeled maltose were mixed in the 100-µl substrate solution added to the cell suspension. Depending on the total substrate concentration, the cell density was adjusted so that the initial rate of uptake was linear over a period of 2 min. For instance, the malE+ strain at 0.1 µM substrate concentration was used at a density of 0.01 (578 nm) and at a density of 1.0 at 0.1 mm maltose. With these precautions, at most 10% of the total radioactivity was accumulated by the cell suspension after 2 min.

Capillary Assay—Cells were grown as for maltose transport assays and harvested at A₀₋₀₋₀ = 0.5 and then washed three times with 10 mM of chemotaxis buffer (10 mM potassium phosphate, pH 7.0, containing 10⁻⁴ M EDTA and 10⁻⁵ M l-methionine). Cells were resuspended very gently to avoid damaging the flagella. The capillary assay was done according to Adler (39) and run for 40 min at 32 °C with cells resuspended in chemotaxis buffer to A₀₋₀₋₀ = 0.005 (1.2 x 10⁹ cells/ml). The number of cells entering the capillary was determined by diluting the capillary contents and plating on nutrient agar.

Tethered Cell Assay—Strains used in this assay were made tsr to minimize possible thermotactic responses (46). Cells were grown and harvested as for the capillary assay, except that the cells were washed twice in tethering buffer (10 mM potassium phosphate, pH 7.0, containing 0.1 M NaCl, 10⁻⁴ M EDTA, and 10⁻⁵ M l-methionine). Flagellar filaments were sheared with a Waring blender; a 25-ml cell suspension in a 50-ml blender cup was exposed to the highest speed for 45 s. After shearing, cells were washed once more and resuspended in tethering buffer to A₀₋₀₋₀ = 0.05. Cells were tethered with anti-

![FIG. 1. The malB region of E. coli. Five genes whose products are known to be involved in maltose uptake are arranged into two divergent operons. In one operon malE codes for maltose-binding protein, and malF and malG for proteins found in the cytoplasmic membrane. In the other operon malK codes for a protein that is associated with the cytoplasmic membrane and lamB codes for the subunit of maltoporin, an outer membrane protein that also serves as receptor for phage λ. Two positive regulators, the MalT protein and cyclic AMP-receptor protein (CAP), bind to the promoter region to stimulate transcription of both operons (1).](image)
sulfate-polycrylamide gels, indicating that periplasmic MBP existed only in the mature form (data not shown).

MBP was quantitated by measuring maltose binding with equilibrium dialysis and by the Ouchterlony immunodiffusion test with antiseraum against MBP. The results obtained with the two methods were in good agreement (Table II). According to the binding data, the MBP levels in the mutants ranged from a high of 23% of the malE* level in malE16-1 to 3.6% in malE19-1. We could not calculate absolute periplasmic MBP concentrations since we did not measure the periplasmic volume. The MBP concentration in maltose-induced E. coli cells has been estimated to be 1 mM (23). In what follows, therefore, we will accept 1 mM as the MBP concentration in the periplasm of malE* cells, also.

**MalTose Transport in Signal-Sequence Mutants**—The initial rate of maltose transport was determined at maltose concentrations ranging from 0.1 to 33 μM in malT*-1 derivatives of the wild-type and mutant strains. All were malF" G*K", lamB*, and tar*). The curves in Fig. 4 provided the best fit to the data, assuming Michaelis-Menten kinetics. The V_max and K_m values (given in the figure legend) were estimated directly and confirmed by double reciprocal plots (not shown). The estimated V_max values ranged from 91% of the malE* value for malE16-1 to 8% for malE18-1. The K_m values were relatively constant, being around 0.8 μM for malE* and malE16-1 and rising only to 1.3 and 1.4 μM for malE18-1 and malE19-1.

To test whether the reduced transport rates in the mutants

### TABLE I

**Bacterial strains**

| Strain      | Known markers | Constructions | Source                  |
|-------------|---------------|--------------|-------------------------|
| HfrG6       | his           |              | Hofnung (33)            |
| pop2629     | amEΔB11       |              | Hofnung (33)            |
| MC4100      | araD139 Δ(argF-lac)169 |              | Casadaban (34)         |
| TST7        | MalE4100 F::Tn10 |              | T. Silhavy (personal communication) |
| pop2629R    | HfrG6 ΔmalE11 malF::Tn10 | P1 on TST7 into pop2629; select Te', screen Mal' and λ' | This study |
| AW405       | F- thr-1(Am) leuB6 his-4 rpsL136 thi-1 ara-14 lacY1 mtl-1 xyl-1 tonA31 tss-78 |              | Armstrong et al. (35) |
| MM112       | AW405 malT-1 rpsB(rij) metA | P1 on strains carrying the various malE alleles into MM185; select Met*, screen Mal*, Te', and λ' | This study |
| MM122       | AW405 malT-1 tsr-1 thr+ | P1 on MM185 into MM186 through MM191; select Te', screen Met', Mal', and λ' | This study |
| MM186       | AW405 metA malT-1 malF::Tn10 ΔmalE11 | P1 on pop2629R into MM112; select Te', screen Met*, Mal*, and λ' | This study |
| MM186 through MM191 | AW405 malT-1 malF::Tn10 containing malE*, malE10-1, malE14-1, malE16-1, malE18-1, and malE19-1, in that order | P1 on strains carrying the various malE alleles (29) into MM185; select Met*, screen Mal*, Te', and λ' | This study |
| MM192 through MM197 | AW405 malT-1 malF::Tn10 containing malE*, malE10-1, malE14-1, malE16-1, and malE19-1, in that order | Multistep construction introducing ΔmalE444 into a matT-1 strain | This study |
| MM207       | AW405 malT-1 ΔmalE444 lamB102(Am) tar-1 Δtar201 thr+ | P1 on MM192 through MM197 into MM207; select Te', screen λ'. See Fig. 2 for further explanation | This study |
| MM218       | AW405 malT-1 thr-1 malF::Tn10 lamB102(Am) tar-1 Δtar201 containing ΔmalE444, malE*, malE10-1, malE14-1, malE16-1, malE18-1, and malE19-1, in that order | P1 on MM209 through MM215 into MM122; select Te', screen for λ' and presence of MBP | This study |

serum against flagellar filament; as described previously (37), except that acid-cleaned rather than siliconized coverslips were used.

Coverslips were sealed with Apiezon L grease onto a flow chamber (47) mounted on the stage of a Nikon Optiphot reverse-phase-contrast microscope. Cells were viewed at a magnification of 400×. Recordings were made with a video camera (Panasonic WV-1850/G) and cassette recorder (Panasonic AG-6200) and analyzed by playback on a video monitor (Panasonic WV-5410). Solutions were drawn through the chamber with vacuum; 99% replacement of the contents occurs within 5 s (47). Flow times were 15 s. Maltose and L-aspartate were added at 1 mM, a concentration that saturates the respective chemoreceptors (47). Flow times were 15 s. Maltose and L-aspartate were added of

### RESULTS

**Determination of Periplasmic MBP Levels**—We determined MBP levels in shock fluid prepared from malE* strains carrying the malE signal-sequence mutations, a malE* gene, or the nonpolar malE444 deletion. Since binding of MBP to maltoporin, Tar, and the MalFGK complex could hinder the release of MBP by cold osmotic shock, all strains contained an amber mutation in lamB, a deletion of tar, and a Tn10 insertion in malE (see Table I). MBP formed a unique band when shock fluid was analyzed by polyacrylamide gel electrophoresis in the presence of 8 M urea (Fig. 3). The intensity of the band decreased in the order malE*, malE16-1, malE10-1, malE14-1, and malE18-1 and malE19-1, with the last two being very similar. No MBP was seen with shock fluid from a strain containing the nonpolar deletion malE444. Material precipitated from malE* and mutant shock fluids with antisera against MBP showed only one band on sodium dodecyl
were solely the consequence of reduced MBP levels, we increased the amount of MBP by lysogenizing with phage λK3 (48). This phage carries a malE* gene but no other mal genes. Maltose transport rates in lysogens of malE*, malE444 deletion, and malE18-1 strains were identical to each other and to the nonlysogenic malE* strain (data not shown). Thus, in the signal-sequence mutant with the lowest transport rate and presumably in the other mutants as well, the cause of the transport defect was the inadequate supply of MBP.

Maltose Chemotaxis in Signal-Sequence Mutants—All of the mutants formed smaller chemotactic swarms than a malE* strain in soft agar plates containing 100 μM maltose. The swarm diameter became progressively smaller in the

![Fig. 2](image)

**Fig. 2.** Construction of strains carrying malF:Tn10, lamB102mbcr, and malE signal-sequence mutations. In the first step, not illustrated, malF:Tn10 was transduced with phage Plvi into a strain containing the deletion malB11 (33), which confers resistance to phage λ (λ') by removing the malK-lamB promoter. Tetracycline-resistant (Tc') transductants were screened for λ'. A Plvi lysate grown on one such isolate was used in a second step (A) to transduce Tc' into strains carrying the malF:Tn10 and the malE signal-sequence mutations; these were identified by their sensitivity to phage λ. In the final step (B) P1vir lysates grown on isolates from A were used to transduce Tc' into strains containing the nonpolar deletion malE444 and the lamB102 amber mutation. Tc' transductants, after screening for λ', were assayed for MBP with the Ouchterlony immunodiffusion test. For each shock fluid the dilution factor at which the last visible precipitation band formed was determined. If the last band was very faint the shock fluid was assigned the mean value of the last two dilution factors giving visible bands. The results are expressed as fractions of the malE* dilution factor.

![Fig. 3](image)

**Fig. 3.** MBP levels in shock fluid from malE signal-sequence mutants. Electrophoresis in 8 M urea was performed according to Pugsley and Schnaitman (41); the gel was stained with Coomassie Brilliant Blue. Sample volumes were adjusted to contain shock fluid from an equal number of cells. Lane 1, strain MM215 (malE19-1); lane 2, strain MM224 (malE18-1); lane 3, strain MM213 (malE16-1); lane 4, purified wild type E. coli MBP; lane 5, strain MM212 (malE14-1); lane 6, strain MM211 (malE10-1); lane 7, strain MM210 (malE*); lane 8, strain MM209 (ΔmalE444). The arrow indicates the position of MBP. The band immediately above the MBP band, most obvious in lane 3, is apparently not MBP precursor (see text) and also appears in the ΔmalE strain.
order malEl6-1, malElO-1, malEl4-1, and malEl9-1, the last two hardly swarming at all. Also, none of the mutants formed sharp chemotactic rings, although malEl6-1 showed a faint ring. However, since establishment of a maltose gradient in the swarm plate requires maltose uptake and metabolism, swarming behavior could not distinguish between defects in maltose transport and chemotaxis.

Chemotactic responses can be analyzed without the complicating factor of transport by utilizing the capillary assay (39), in which chemotactic gradients are created by diffusion. Our assays were performed with malT* strains containing a Tn10 insertion in malF. This Tn10 insertion completely blocks maltose transport. The results of the capillary test are presented in Fig. 5. To correct for possible differences in motility and Tar content, accumulations in maltose-containing capillaries were normalized by dividing by the accumulation in aspartate-containing capillaries for each strain. The malE* strain and all of the mutants showed a peak accumulation in capillaries containing 100 μM maltose, but the magnitude of the accumulation varied markedly among the mutants. For malEl6-1, malElO-1, malEl4-1, malEl8-1, and malEl9-1 the peak accumulations were 64, 36, 18, 8, and 4% of the malE* accumulation, respectively.

When E. coli cells are exposed to a large, rapid increase in attractant concentration, they respond by totally suppressing clockwise rotation of their flagella for a time proportional to the change in the fraction of receptor bound with attractant (49, 50). The period of exclusively counterclockwise rotation that ensues, called the transition time (49) or recovery time (55) by different authors, is here referred to as the response time. For a maltose stimulus, the response time should be determined by the degree to which Tar is occupied by maltose-bound MBP. Thus, the in vivo binding of MBP to Tar can be investigated by recording response times to maximal maltose stimuli in cells with different periplasmic MBP content.

Response times were measured in a flow chamber (47) with cells tethered to a glass coverslip with antiseraum against flagellar filament. The strains were all malT* tsr derivatives. To prevent possible competition with Tar for binding of MBP, the strains contained a Tn10 insertion in malF and an amber mutation in lamB. The maltose concentration was quickly shifted from zero to 1 mM maltose, a change that elicits the maximal response to maltose even in lamB mutants (37). At 1 mM maltose, MBP should be saturated with substrate.

The response time data are given in Table III. As in the capillary test, the maltose response times were normalized by dividing by the response time to a 1 mM aspartate stimulus. The mean response times for malEl6-1, malElO-1, malEl4-1, malEl8-1, and malEl9-1 were 60, 36, 20, 11, and 8% those of isogenic malE* cells, respectively. The correspondence of these results to those of the capillary test (above) was striking.

**Dependence of Transport and Chemotaxis on Periplasmic MBP Concentration**—The relationship between periplasmic MBP levels, transport, and taxis was examined by plotting transport V_{max} or tactic response against the amount of MBP (Fig. 6). The dependence of transport rate on MBP level did not follow simple Michaelis-Menten kinetics (Fig. 6A). To identify possible cooperativity we constructed a Hill plot from the transport data. The points fell on a straight line with a slope of 2.7 (not shown), suggesting that the rate-limiting step of transport involves MBP in a cooperative interaction. Nine per cent (90 μM) of the malE* MBP concentration was required for half-maximal transport.

A double reciprocal plot of normalized maltose response time versus MBP level (not shown) was linear at the four highest MBP concentrations. From the linear region of the plot, we determined that the hypothetical maximum response, at infinite MBP concentration, would be 0.34. Our malE* cells had a maltose response of 0.27, about 80% of the theoretical maximum response. The amount of MBP giving half of the hypothetical maximum response, equivalent to the K_{D} for the MBP-Tar interaction in vivo, was 25% (250 μM) of the malE* level. The curve in Fig. 6B was derived using these values for the maximum response and K_{D}, assuming a first-order dependence on the MBP concentration. The fit to the data was good except at the two lowest concentrations of MBP.

Strains with the lowest MBP levels had briefer responses than expected from first-order dependence on MBP level. This deviation from linearity could be an artifact introduced by subtracting the response of a ΔmalE strain from the maltose responses of all other strains (Table III). This correction was made assuming that the brief responses of ΔmalE were elicited by small amounts of contaminating sugars in our purified maltose. If the responses to these contaminants and maltose were not fully additive (51) we would overcorrect by subtracting the ΔmalE response. This source of error would be greatest for mutants with low MBP levels.

**DISCUSSION**

We studied the dependence of maltose transport and chemotaxis on periplasmic MBP concentration using strains carrying various malE signal-sequence mutations. Under these conditions MBP was the only component of the transport or taxis systems whose amount varied. Our results demonstrated that more MBP is required for chemotaxis than for transport. For example, malEl6-1 strains, with 23% of the malE* amount of MBP, transported maltose at 96% of the malE* rate but had only 60% of the malE* chemotactic response to maltose. This finding is consistent with the report that 5- or 10-fold overproduction of Tar, the signal transducer for maltose taxis, did not inhibit maltose transport in uninduced cells (52). The data also suggest that the reported proportionality of maltose transport and MBP content in uninduced and induced E. coli (24) is fortuitous, simply re-
The MBP-Tar interaction was determined from the linear portion of the Hill coefficient of the square root of the motactic stimulation with function (see text). In against relative MBP levels. The standard error (1 S.D. divided by 4) contained within the data points as they are drawn. The responses was reflecting the coregulation of MBP with the MalF, proteins. MBP should always be in excess for transport in maleE+ cells.

The sigmoidal curve represents the best fit to the data and reflects the coregulation of MBP with the MalF, G, and K proteins. MBP should always be in excess for transport in maleE+ cells.

The rates of transport at saturating maltose concentrations appeared to increase in a sigmoidal fashion with the amount of MBP (Fig. 6A). A Hill plot of this data yielded a straight line with a slope of 2.7. We do not want to emphasize this numerical value, but we do believe our data present evidence for some degree of cooperativity. The half-maximal transport rate occurred at 9% (90 μM) of the maleE+ MBP level, which we have taken as 1 mM (23). Since there are 1000 copies each of MalFGK (5) and Tar (20, 25) per cell, the amount of free MBP could be reduced by 20 to 25% through binding to membrane components. Thus, 90 μM is an upper estimate for the concentration of MBP needed to maintain half-maximal transport rates at saturating maltose concentrations.

The apparent maltose Km for transport varied less than a factor of 2 (from 0.8 to 1.4 μM) over a 25-fold range of MBP concentrations (Fig. 4). A model in which MBP exists only as monomer and only substrate-bound MBP interacts with membrane components would predict that the apparent Km would increase in mutants with reduced MBP levels. Since such an increase in Km was not observed, this model is inadequate to explain how binding protein functions in transport.

Published reports that binding proteins form dimers (53-55) or larger aggregates (56) may have a bearing on our observation of cooperativity. Richarme (53) reported isolation of dimers of MBP or galactose-binding protein that could be converted to monomers by addition of substrate. According to his scheme, substrate-loaded monomers would interact with membrane transport components, then dimerize, causing release of substrate. Dimers would subsequently dissociate from the membrane components and re-enter a monomer-dimer equilibrium. A transport mechanism of this type, or variations thereof, might well involve cooperative interactions of MBP.

Maltose taxis showed a first-order dependence on the MBP concentration (Fig. 6B). Thus, a single substrate-bound MBP molecule can apparently elicit a chemotactic signal from the Tar transducer with which it interacts. Half the hypothetical maximum response to a saturating maltose stimulus (a jump from 0 to 1 mM) occurred at 25% (250 μM) of the maleE+ MBP level. Since the response time to an attractant is proportional to the per cent change in occupied chemoreceptor (49, 50), 250 μM should represent the Ko of MBP binding to Tar in

### Table III

**Chemotactic response times after temporal maltose stimulation of maleE signal-sequence mutants**

Chemotactic response times were determined at 20 °C with tethered cells of maleT-maleE:Tn10 lamB102(Am) tar strains (MM216 through MM222), as described under "Experimental Procedures." Mean response times, ±1 S.D., for the cell ensemble are shown for stimulation with 1 mM maltose or 1 mM L-aspartate. The number of cells analyzed is shown in parentheses.

| maleE allele | Mean response time to maltose | Mean response time to aspartate | Ratio of maltose and aspartate responses of individual cells* | Corrected ratios of maltose and aspartate responses* |
|--------------|-------------------------------|-------------------------------|-------------------------------------------------------------|-------------------------------------------------|
| ΔmaleE444    | 13 ± 8 (70)                   | 206 ± 49 (51)                 | 0.034 ± 0.019 (48)                                          | 0 (0%)                                          |
| maleE+       | 102 ± 17 (42)                 | 338 ± 39 (24)                 | 0.31 ± 0.034 (23)                                          | 0.275 (100%)                                   |
| maleE10-1    | 54 ± 17 (117)                 | 394 ± 50 (84)                 | 0.14 ± 0.041 (82)                                          | 0.106 (39%)                                    |
| maleE14-1    | 40 ± 17 (49)                  | 475 ± 39 (33)                 | 0.088 ± 0.032 (32)                                         | 0.054 (20%)                                    |
| maleE16-1    | 71 ± 16 (67)                  | 342 ± 52 (44)                 | 0.20 ± 0.063 (40)                                          | 0.168 (60%)                                    |
| maleE18-1    | 24 ± 10 (41)                  | 375 ± 52 (27)                 | 0.061 ± 0.029 (26)                                         | 0.027 (10%)                                    |
| maleE19-1    | 18 ± 9 (31)                   | 314 ± 57 (23)                 | 0.088 ± 0.024 (22)                                         | 0.024 (9%)                                     |

*The ratio of the maltose to aspartate response times of individual cells was determined. The value shown is the mean of these ratios, ±1 S.D., for the cell ensemble. The number of cells analyzed is given in parentheses.

†The mean ratios of the maltose to aspartate response times for each strain after subtraction of the mean ratio for the ΔmaleE strain. This correction was made because the brief response of the ΔmaleE strain to maltose stimuli was probably caused by residual contaminants, such as glucose, in our purified maltose. Without this correction we would tend to overestimate maltose response times. The percentages in parentheses are expressed relative to the maleE+ strain.

![Fig. 6. Maltose transport and chemotaxis as functions of MBP concentration.](image-url)
vivo. This value is substantially higher than the $K_D$, in the micromolar range, estimated for binding of maltose-loaded MBP to enzynized membrane vesicles containing Tar (20). The vesicle studies, however, were performed with dilute MBP solutions. The protein may behave differently in the periplasm, which may be a gel (57), and in which MBP may interact with other components of the cell envelope.

Sugar-binding proteins are present in the cell in vast molar excess relative to chemotactic signal transducers, up to 40-fold in the case of MBP and Tar. Given this ratio, why do binding in vitro and the chemotactic response in vivo have a similar dependence on the substrate concentration, as has been shown for ribose-binding protein (58)? For a cell to respond to changes in sugar concentration near $K_D$, where chemotactic sensing is most sensitive, an increase in substrate-bound binding protein must lead to an increase in the fraction of occupied signal transducer. This condition will be met if the affinity of substrate-loaded binding protein for the transducer is low, as we observe for the MBP-Tar interaction in vivo (Fig. 6B).

Note added in proof—The malE signal-sequence mutations could potentially reduce expression of malF and malE. This possibility was examined by assaying β-galactosidase coded by a malF-lacZ operon fusion. No polarity was observed; β-galactosidase activities were similar when the fusion was preceded by a malE" gene or a malE" gene containing any one of the signal-sequence mutations.

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