Two Modes of Ligand Binding in Maltose-binding Protein of *Escherichia coli*

FUNCTIONAL SIGNIFICANCE IN ACTIVE TRANSPORT*

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In the preceding two papers (Hall, J. A., Gehring, K., and Nikaido, H. (1997) *J. Biol. Chem.* 272, 17605–17609; Hall, J. A., Thorgeirson, T. E., Liu, J., Shin, Y.-E., and Nikaido, H. (1997) *J. Biol. Chem.* 272, 17610–17614), we showed that ligands that bind to the *Escherichia coli* maltose-binding protein (MBP) without producing the closure of its two lobes are not transported into the cytoplasm. Here, we examine various combinations of ligands, MBPs, and membrane-associated transporters, by utilizing reconstituted proteoliposomes, right side-out membrane vesicles, and intact cells. Closed forms of wild type MBP, complexed with maltose or maltodextrins, interacted with wild type transporter complex to stimulate the hydrolysis of ATP by MalK ATPase located on the other side of the membrane, as shown earlier for the maltose-MBP complex (Davidson, A. L., Shuman, H. A., and Nikaido, H. (1992) *Proc. Natl. Acad. Sci. U. S. A.* 89, 2360–2364). In contrast, open forms of liganded MBPs, such as the complex containing wild type MBP and reduced, oxidized, or cyclic maltodextrins or the complex containing the mutant MBP MalE254 and unmodified maltodextrins, did not stimulate ATP hydrolysis, suggesting that the proper interaction between the ligand-MBP complex and the external surface of the transporter requires the former to be in the closed conformation. However, when a mutant transporter containing MalG511 was used, the already significant basal level of ATP hydrolysis was further stimulated not only by ligand MBPs in the closed form but also by those in the open form (except containing β-cyclodextrin). Data suggesting that the mutant transporter does not always require the closed MBP complex presumably because of its exceptionally strong affinity to MBP, described earlier (Dean, D. A., Hor, L.-I., Shuman, H. A., and Nikaido, H. (1992) *Mol. Microbiol.* 6, 2033–2040). Furthermore, this mutant transporter was able to transport reduced maltodextrins, and cells expressing the transporter were able to grow by using reduced maltodextrin, if the periplasmic concentrations of MBP were kept low so as not to inhibit the transport process.

Maltose and maltodextrins are transported in *Escherichia coli* by an ATP-dependent process, which requires the interaction between soluble, liganded maltose-binding protein (MBP)¹ and a membrane-associated transporter (MalFGK₂) composed of one copy each of MalF and MalG channel proteins and two copies of MalK ATPase (1, 2). The participation of MBP is absolutely needed for this transport process, and one of the reasons may be that the liganded MBP, by binding to the external surface of the transporter, sends a transmembrane signal so that the MalK ATPase can become activated on the opposite, internal surface of the membrane (3). The requirement for MBP, however, can be circumvented in “MBP-independent” transporter mutants, such as the one containing mutant MalG511 (MalFG511K₂) (4), which constitutively hydrolyze ATP even in the absence of the liganded MBP (3).

We have shown in the preceding papers (5, 6) that MBP can bind its ligands in two different ways. Maltose and linear maltodextrins bind to MBP in a way that produces a slight red shift of the intrinsic fluorescence emission spectrum of the protein (called R mode (for red shift)) (5, 7) and a characteristic hypochromatic trend in the <265-nm region of UV absorbance spectrum (5, 8). An earlier study showed that the binding of maltose and α-anomers of maltodextrins produced a large upfield shift of the NMR resonance of ²H on the anomeric carbon of the reducing glucose residue ("end-on" mode) (9), and the R and end-on modes appear to refer to the same manner of ligand binding. In contrast, when the MBP binds β-cyclodextrin, or reduced or oxidized derivatives of maltodextrins, the fluorescence emission spectrum is blue-shifted (B mode (for blue shift)), and the UV absorbance differential spectra show no hypochromatic trend in the <265-nm region (5, 8). Since β-cyclodextrin and the modified dextrins just mentioned do not contain a reducing sugar residue with its anomeric carbon, we hypothesize that this mode corresponds to the binding mode that does not involve the tight interaction of the hydrogen on the anomeric carbon with the binding site of MBP, i.e. the middle mode, earlier observed by ²H NMR for β-anomers of maltodextrins (9). Interestingly, a mutant MalE254 MBP, which allows the transport of maltose but not of maltodextrins (10), binds unaltered maltodextrins exclusively via the B mode (5). By using representative ligands, we showed further that the two lobes of MBP become closed when the R mode binding occurs, whereas there is little or no closing of the lobes when the binding occurs through the B mode (6).

There are some hints that the B mode binding may not lead to the successful transport of ligands through the MalFGK₂ complex. For example, maltodextrin derivatives that are reduced, oxidized, or substituted at their reducing glucose units bind to wild type MBP exclusively via the B mode as described above, and are not transported by the wild type *E. coli* (11). The

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¹ The abbreviations used are: MBP, maltose-binding protein; IPTG, isopropyl-β-D-galactoside.
mutant MalE254 MBP, which binds unmodified maltodextrins exclusively via the B mode (see above), does not allow the transport of these ligands (10). The failure to be transported can result either because the B mode complex fails to stimulate the ATPase activity of the transporter or because the B mode complex cannot deliver the ligand into the transport channel. The present paper examines this question and shows that the stimulation of ATP hydrolytic functions of the wild type MalFGK2 transporter is not induced by B mode ligand-MBP complex. We also show that ligands bound via the B mode to MBP are transported nevertheless through the mutant MalFG511K2 complex and that these mutant cells are indeed able to grow by using reduced maltodextrins as their carbon and energy source.

**EXPERIMENTAL PROCEDURES**

**Strains and Plasmids—**These are shown in Table I. Strains HN889, HN924, and HN930 were constructed by transferring the F' factor carrying the lacUV5 mutation and Tn5 (kanamycin) from HN596 to HN933, NT411 containing pH16, NT229 containing pH16, and HN931, respectively, using standard mating technique (17). High affinities were calculated at 160,000 × g for 1 h at 4 °C, and the supernatant was aliquoted and stored at −70 °C until use. Membrane vesicles containing the MalG511 transporter that were destined for use in the proteoliposome substrate uptake assay were solubilized as above except that sonicated E. coli acetone/ether-washed total lipids (Avanti Polar Lipids, Inc.) were added to a final concentration of 8–9 mg/ml. This mixture was then processed as above, and the supernatant fraction was used immediately.

**Binding Affinities of MBP—**The affinities were determined from the concentration dependence of the quenching of the intrinsic protein fluorescence (7), measured on a Perkin-Elmer MPF-44B spectrophotometer. The MBPs (0.2 μM) were dissolved in 10 mM KPO4, pH 7.0. Excitation was at 280 nm with a bandwidth of 7 nm, and emission was recorded at 348 and 346 nm for wild type and MalE254 MBPs, respectively, with a bandwidth of 7 nm.

**ATP Hydrolysis in Proteoliposomes—**Solubilized membrane proteins were reconstituted into liposomes via a detergent dilution method (13). In all experiments 4.5 mg of sonicated E. coli acetone/ether-washed total lipids (Avanti Polar Lipids, Inc.) in 20 mM KPO4, pH 6.2, 2 mM β-mercaptoethanol was mixed with 90 μg of solubilized proteins, and octyl-β-D-glucopyranoside was added to a final concentration of 1.1%. This mixture (0.54 ml) was then incubated on ice for 30 min, diluted into 14 ml of 20 mM KPO4, pH 6.2, 1 mM dithiotreitol, and centrifuged at 160,000 × g for 1 h at 4 °C to isolate the proteoliposomes. MBP was added prior to dilution to give a final concentration of 1.5 μM (for assays using wild type MBP) or 0.15 μM (for assays using malE254 MBP) after dilution. In these experiments, the weight ratio of proteoliposomes to MBP was 50:1:1 when wild type MBP was used and 50:1:1 when MalE254 MBP was used. Also, transport substrate was added prior to dilution to give a final concentration after dilution such that >90% of the MBP would be in the liganded form.

ATP hydrolysis by the MalFGK2 transporter was measured as described (3). Proteoliposomes were resuspended in 20 mM KPO4, pH 6.2, 3 mM MgCl2, 10 μM sugar substrate (if added when preparing proteoliposome) to a final concentration of 0.45 μM of protein/ml and incubated with 100 μM γ-[32P]ATP (50 mCi/mmol) at room temperature, and release P was determined at various time points.

**Substrate Uptake in Membrane Vesicles—**Strains HS338 and NT411 were grown at 37 °C overnight in medium 63 containing 0.4% glycerol and 10 μg/mL thiamine. The culture was diluted 1:20 into 200 ml of the same medium and grown to a cell density of 2 × 109 cells. Membrane vesicles were prepared by a modification (20) of the Kacback procedure (21), except that vesicles were separated from unlysed spheroplasts and whole cells by two centrifugations at 2000 × g for 10 min and then collected by centrifugation at 35,000 × g for 15 min. Vesicles were resuspended in 20 mM KPO4, pH 6.2, 3 mM MgCl2, to a final concentration of 1.5–2.0 mg of protein/ml, electron donors (10 mM ascorbate, 100 μM phenazine methosulfate) were then added as indicated, and the preparation was used immediately.

**TABLE I**

| Strain or Plasmid | Genotype | Reference or Source |
|-------------------|----------|---------------------|
| HS2019            | F' araI39 ΔlacU169 rpsL thi ΔmalE444 | 12 |
| HN741             | argH his rpsL1 malT1Δ malI13Δ malC1Δ lacF1Δ Tn10F' lacF1Δ Tn5 | 3 |
| HS3386            | F' ΔargF-lacU169 rpsL150 relAI1 ribB1 ribB5301 ptsF25 thi-1 deoC1 malT-1 Δ(malF-lacZ')/lacY1 F9 | H. A. Shuman |
| NT229             | ΔargF-lacU169 araD139 rpsL150 thi-1 ribB5301 ptsF25 relAI1 ΔmalE444 malG511 | 4 |
| NT411             | NT229 malT1Δ ΔmalF-lacZ' | 4 |
| HN596             | malT1Δ araD1 lacF1Δ F1Δ lacF1Δ; Tn5 proA' proB' | 13 |
| HN889             | NT411 (pIF2'; F' lacIΔ lacZ::Tn5 proAB') | This work |
| HN892             | NT411 (pLH16' F' lacIΔ lacZ::Tn5 proAB') | This work |
| HN924             | NT229 (pLH16' F' lacIΔ lacZ::Tn5 proAB') | This work |
| HN930             | NT229 (pIF2'; F' lacIΔ lacZ::Tn5 proAB') | This work |
| HN931             | NT229 (pIF2') | This work |
| HN932             | NT229 (pPDI) | This work |
| HN933             | NT411 (pIF2') | This work |
| HN934             | NT411 (pPDI) | This work |
| pEH1              | pBluescript S/K with malE254 insert | This work |
| pFG23             | malF malG bla | 13 |
| pLH33             | malF malG511 bla | 3 |
| pMR11             | malK cat | 14 |
| pPDI              | malE (under pmarB control) bla | 15 |
| pP2              | malE (under plocUV5 control) bla | 16 |
| pLH16             | malE632 (under plocUV5 control) bla | H. A. Shuman |
Substrate Uptake into Proteoliposomes—The accumulation of maltose and maltotetraose inside proteoliposomes was measured as described (13). Proteoliposomes were prepared as above (see "ATP Hydrolysis in Proteoliposomes") except that neither MBP nor substrate was added. Instead, ATP was added prior to dilution to give a final concentration of 5 mM after dilution. Proteoliposomes were resuspended to a final concentration of 0.45 μg of protein/ml in 20 mM KPO₄, pH 6.2, 3 mM MgCl₂ with or without 1 μM MBP and incubated with 10 μM [³H]maltose (150 μCi/μmol), or 10 μM [³H]maltotetraose (120 μCi/μmol) at room temperature. At specified times, 25–40 μl of the reaction mixture was diluted 1:10 with 20 mM KPO₄, pH 6.2, 3 mM MgCl₂, filtered through a Millipore filter (0.22-μm GTSF), and washed with 5 ml of 50 mM LiCl. Filters were dried and counted as described above.

Determination of Periplasmic MBP Concentration—Cells were grown in M63 medium containing 1 μg/ml thiamine, 0.4% glycerol, any necessary antibiotics, and 250 μM IPTG (if required), at 37 °C to a density of 2 × 10⁹ cells/ml and centrifuged, and osmotically shocked as described above. The shock fluid containing periplasmic proteins was cleared of cells and cellular debris by passage through Whatman 1 filter paper. Proteins were then separated via SDS-polyacrylamide gel electrophoresis (2) and either visualized by Coomassie Blue staining or transferred to a nitrocellulose membrane for immunoblot analysis. The latter procedure was carried out by using an anti-MBP rabbit antisera (Sigma) or another rabbit antisera (Sigma) (22). The total amount of MBP loaded for immunoblot analysis. The latter procedure was carried out by using an anti-MBP rabbit antiserum and alkaline phosphatase-conjugated anti-rabbit IgG antibody (Sigma) (22). The total amount of MBP loaded for immunoblot analysis. The latter procedure was carried out by using an anti-MBP rabbit antiserum and alkaline phosphatase-conjugated anti-rabbit IgG antibody (Sigma) (22).

Substrate Uptake and Metabolism in Whole Cells—Strains to be used in all experiments were grown overnight at 37 °C in M63 medium (17) containing 1 μg/ml thiamine, 0.4% glycerol, and any necessary antibiotics. Overnight cultures were diluted 1:20 into fresh medium of the same composition, except for the addition of 250 μM IPTG (if required), and grown at 37 °C to a density of approximately 5 × 10⁹ cells/ml. Cells were washed twice with M63 and then resuspended in the same medium at a density of 2 × 10⁹ cells/ml. The assay was initiated by the addition of labeled substrate ([³H]maltose (75–150 μCi/μmol), [³H]maltotetraose (200 μCi/μmol), or [³H]maltotetraose (200 μCi/μmol)) to a final concentration of 10 μM. Portions of 50 μl each were removed, diluted 1:10 with cold M63, and filtered through a 0.45-μm HAWP Millipore filter. Filters were then washed with 5 ml of 50 mM LiCl, dried, and counted as described above. All values were corrected for background counts on filters.

To assay the ability of strains to transport and metabolize various substrates, overnight cultures were diluted 1:200 into 2.0 ml of fresh M63 media containing 1 μg/ml thiamine, any necessary antibiotics, 250 μM IPTG (if required), and maltose, maltotetraose, or maltotetraose at the indicated concentration. Cultures were grown at 37 °C with continuous shaking, and growth was scored at various times.

Protein Determination—Either the BCA protein assay (Pierce) or the method of Brown et al. (25) was used.

RESULTS

ATP Hydrolysis by the Wild Type Transporter—The wild type complex, MalFGK₂, required the presence of both maltose and MBP inside the vesicles to hydrolyze, rapidly, ATP added from the outside, as noted earlier (3) (data not shown). When MBP and various ligands were added inside proteoliposomes at concentrations that would make MBP more than 95% liganded, only those ligands that bound to MBP via the R mode stimulated ATP hydrolysis by the MalFGK₂ complex (Table II). Interestingly, maltotriose and maltotetraose both stimulated the transporter to a slightly higher degree than did maltose and maltotetraose. In contrast, ligands that bound to MBP exclusively via the B mode, such as reduced, oxidized, or cyclic maltodextrin derivatives, caused little stimulation (Table II). A marginal stimulation seen with maltotriitol and maltotetraitol could have been caused by traces of unmodified maltodextrins remaining in these preparations.

Substrate Transport by the Wild Type Transporter—Transport by the wild type MalFGK₂ transporter was examined in various systems. Fig. 1A shows the accumulation of various substrates into proteoliposomes. In these experiments, ATP was trapped inside the proteoliposomes, and wild type MBP and substrate were added to the outside. Clearly, both maltose and maltotetraose (binding to MBP via the R mode) were transported quite well, but maltotetraose (binding via the B mode) was not accumulated to a significant level.

Similar results were obtained when right side-out membrane vesicles from HS3368 (containing the wild type transporter) were used; the addition of maltose, maltotetraose, and maltotetraose (in addition to MBP) resulted in the uptake of 0.38, 0.18, and 0.02 nmol of substrate/mg of protein/min, respectively. Without MBP, the addition of these sugars produced a residual uptake value of 0.02–0.03 nmol/mg of protein/min. There were some differences between the membrane vesicles and proteoliposomes. First, the uptake rates of maltose and maltotetraose were much higher in proteoliposomes; this is most likely due to the partial purification of the transporter via selective solubilization. Second, maltotetraose was transported into proteoliposomes better than maltose, whereas in membrane vesicles the opposite was true. A possible explanation is that many of the inner membrane vesicles were surrounded by residual outer membranes, which could have hindered the access of maltotetraose.

We also assayed substrate accumulation in whole cells of strain HS3368 (Fig. 1B). Interestingly, maltose was transported approximately 25 times more rapidly than maltotetraose. Maltodextrin transport in whole cells is most likely limited by diffusion through the LamB channel (26, 27). Maltotetraose accumulation was close to the base-line level.

ATP Hydrolysis by the Wild Type Transporter in the Presence of MalE254 MBP—The MalE254 allele was isolated by Wandersman et al. (10) and is a member of a class of “maltodextrin-negative” malt mutants, which are unable to utilize maltodextrins although their MBPs have good affinity for these substrates. The MalE254 MBP, unlike wild type MBP, binds both maltodextrins and their nonreducing derivatives exclusively by the B or open mode (5). However, it binds maltose (at high concentrations) by the R mode (5). The MalE254 MBP was trapped inside proteoliposomes containing the wild type MalFGK₂ complex together with various ligands (at concentrations that would assure at least 90% saturation of MalE254 MBP), and ATP hydrolysis was measured. We found that this
mutant MBP could stimulate ATP hydrolysis only with maltose (Fig. 2). The maltotetraose-MalE254 complex showed no stimulation of ATP hydrolysis (Fig. 2), in contrast to the strong stimulatory activity of maltotetraose-wild type MBP complex (Table II).

ATP Hydrolysis by the MalFG511 Transporter—The malG511 allele is a member of a class of mutant malF and malG alleles that allow a cell to transport maltose in the absence of MBP (4). The MalFG511K2 transporter, when reconstituted into proteoliposomes, hydrolyzed ATP in the absence of MBP and transport substrates (not shown), reproducing earlier results (3). Its ATP hydrolytic activity was not stimulated significantly by the incorporation of either MBP alone or substrate alone into the intravesicular space. However, ATP hydrolysis was stimulated when proteoliposomes were reconstituted so that they contained any substrates (except α-cyclodextrin) in addition to the wild type MBP (Table II). In other words, even those ligands that appeared to bind exclusively via the B mode, such as maltotetraol and maltohexaol, showed enhancement almost as strong as the unmodified parent compounds.

Substrate Transport by the MalFG511 Transporter—In the early experiments with proteoliposomes, the mutant transporter was found to be unstable, when extracted, and showed no apparent uptake activity. However, when solubilized in the presence of E. coli total lipids (see “Experimental Procedures”), it showed good transport activity upon reconstitution. (The presence of total lipid extract during MalG511 transporter solubilization also resulted in higher ATP hydrolysis rates (not shown)). Proteoliposomes containing the MalFG511K2 complex accumulated maltose, maltotetraose (both R mode binders), and maltotetraol (an exclusive B mode binder), at a rate of 0.06, 0.12, and 0.14 nmol/mg of protein/5 min, respectively. In the absence of MBP there was little uptake of these substrates (<0.01 nmol/mg of protein/5 min).

The same pattern of transport was seen with NT411 right side-out membrane vesicles containing the same mutant transporter, with rates of 0.51, 0.12, and 0.13 nmol/mg of protein/5 min for maltose, maltotetraose, and maltotetraol, respectively. Thus, these vesicles transported maltotetraol quite well in the presence of MBP. We also note that maltose was transported better than maltotetraose by these membrane vesicles, whereas in the proteoliposomes the opposite was true.

Substrate Transport by Intact Cells in the Presence of Wild Type MBP and the MalG511 Transporter—Maltotetraol that bound via the B mode was transported into proteoliposomes and membrane vesicles containing MalG511 transporter. Since active transport of modified maltodextrins has not been seen in E. coli (see Ref. 11), we examined whether the transport occurred in intact cells. However, the MalG511 mutant transporter, as well as other “MBP-independent” mutant transporters, cannot transport any ligands when wild type MBP is present at high concentrations that are usually found in induced cells, presumably because transport is inhibited by the excessively tight binding of MBP-ligand complex to the mutant transporters (3, 28). We therefore constructed strains that expressed MBP to different levels and studied their capacity to transport and metabolize various substrates.

To measure transport, NT411 was used as a parent strain because it is unable to metabolize maltose and maltodextrin due to the absence of amylomaltase and maltodextrin phosphorlyase. Detectable amounts of maltose were not transported into NT411 cells (Fig. 3A). This result may seem unexpected,
since strain NT411 expresses the “MBP-independent” MalG511 transporter. However, since the transport assays were done using a concentration of maltose (10 μM) far below the K_m of this mutant transporter (~2.0 mM), any significant transport is not expected. As has been shown (4), maltose transport was readily detected if maltose was present at 500 μM (not shown). When MBP was present at a very low concentration in the periplasm (HN889, uninduced), maltose transport into the cytoplasm was enhanced greatly in comparison with NT411 (Fig. 3A). This stimulatory effect of MBP continued up to MBP concentrations of at least 250 μM (HN889 and HN933, both induced). As the periplasmic concentration of MBP approached that found in the periplasm of induced cells (~1.0 mM; HN934), MBP showed an inhibitory effect instead (Fig. 3A). This is in stark contrast to the system containing the wild type MalFGK2 transporter (HS3368, Fig. 3A), in which the fully induced level of MBP supported maltose transport at a nearly maximal rate.

The transport of maltotetraose followed a somewhat similar pattern (Fig. 3B). As shown earlier (4), strain NT411 could not transport maltotetraose in the absence of MBP. Maltotetraose transport in uninduced HN889 cells was strongly stimulated by the presence of a low level of MBP (Fig. 3B). At MBP concentrations around 250 μM, however, maltotetraose transport became inhibited (Fig. 3B) in contrast to the strong stimulation seen for maltose transport under similar conditions (Fig. 3A), suggesting that the MalFG511K2 transporter became inhibited more strongly by the maltotetraose-MBP complex than by the maltose-MBP complex.

Maltotetraitol, although it binds to MBP via the normally inactive B binding mode, was transported (Fig. 3C), generally much more rapidly than maltotetraose (compare with Fig. 3B), when MBP was present up to concentrations of 250 μM. As periplasmic MBP concentrations approached 1.0 mM, maltotetraitol transport also became somewhat inhibited. It was not transported by either HS3368 (producing wild type MalFGK2 transporter) or NT411 (not producing any MBP) (Fig. 3C).

The growth on various substrates was also tested (Table III). Strain NT229 (malP− malQ−) was used as the parent strain producing the mutant MalFG511K2 transporter as well as varying levels of MBP. All such strains were capable of growing upon media containing 6 mM maltose, although HN932, with a high level of MBP, could not grow in 1, 2, or 4 mM maltose, confirming the results of Treptow and Shuman (4). At lower maltose concentrations, those strains that produced lower levels of MBP (uninduced HN930 and IPTG-induced HN931 and HN932, both induced), maltose transport was readily detected if maltose was present at 500 μM (not shown). When MBP was present at a very low concentration in the periplasm (HN889, uninduced), maltose transport into the cytoplasm was enhanced greatly in comparison with NT411 (Fig. 3A). This stimulatory effect of MBP continued up to MBP concentrations of at least 250 μM (HN889 and HN933, both induced). As the periplasmic concentration of MBP approached that found in the periplasm of induced cells (~1.0 mM; HN934), MBP showed an inhibitory effect instead (Fig. 3A). This is in stark contrast to the system containing the wild type MalFGK2 transporter (HS3368, Fig. 3A), in which the fully induced level of MBP supported maltose transport at a nearly maximal rate.

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Maltotetraitol, although it binds to MBP via the normally inactive B binding mode, was transported (Fig. 3C), generally much more rapidly than maltotetraose (compare with Fig. 3B), when MBP was present up to concentrations of 250 μM. As periplasmic MBP concentrations approached 1.0 mM, maltotetraitol transport also became somewhat inhibited. It was not transported by either HS3368 (producing wild type transporter) or NT411 (not producing any MBP) (Fig. 3C).

The growth on various substrates was also tested (Table III). Strain NT229 (malP− malQ−) was used as the parent strain producing the mutant MalFG511K2 transporter as well as varying levels of MBP. All such strains were capable of growing upon media containing 6 mM maltose, although HN932, with a high level of MBP, could not grow in 1, 2, or 4 mM maltose, confirming the results of Treptow and Shuman (4). At lower maltose concentrations, those strains that produced lower levels of MBP (uninduced HN930 and IPTG-induced HN931 and HN932, both induced). As the periplasmic concentration of MBP approached that found in the periplasm of induced cells (~1.0 mM; HN934), MBP showed an inhibitory effect instead (Fig. 3A). This is in stark contrast to the system containing the wild type MalFGK2 transporter (HS3368, Fig. 3A), in which the fully induced level of MBP supported maltose transport at a nearly maximal rate.

The transport of maltotetraose followed a somewhat similar pattern (Fig. 3B). As shown earlier (4), strain NT411 could not transport maltotetraose in the absence of MBP. Maltotetraose transport in uninduced HN889 cells was strongly stimulated by the presence of a low level of MBP (Fig. 3B). At MBP concentrations around 250 μM, however, maltotetraose transport became inhibited (Fig. 3B) in contrast to the strong stimulation seen for maltose transport under similar conditions (Fig. 3A), suggesting that the MalFG511K2 transporter became inhibited more strongly by the maltotetraose-MBP complex than by the maltose-MBP complex.
HN930 grew more rapidly than those that had high periplasmic MBP concentrations (HN932) or produced no MBP at all (NT229) (Table III and results not shown). These results agree well with the active transport data and again indicate that high levels of MBP inhibit transport through the MalG511 transport complex.

Maltotetraose was not utilized by strain NT229 (Table III), confirming the earlier report (4). On the other hand, strains producing MBP at noninhibitory levels were capable of growing not only on maltotetraose but also on the alcohol maltotetraitol (see uninduced and induced HN930 as well as HN931 in Table III).

**Substrate Transport in Intact Cells in the Presence of MalE632 MBP and the MalG511 Transporter—**The malE632 allele was isolated due to its ability to allow a malG511 mutant strain to grow on maltotetraose as a sole carbon source in the presence of fully induced levels of MBP (29). We studied the interaction between the MalE632 suppressor MBP and the MalG511 transporter to see whether the substrate specificity of this MalFG511K2 system, which allowed the transport of ligands binding via the B mode, was restored to the more restrictive pattern found in the wild type system.

Ligand binding affinity of MalE632 was not grossly different from that of wild type MBP, as assessed by the fluorescence quenching assay (data not shown). Binding of maltose, maltotetraitol, or β-cyclodextrin to MalE632 produced the same shifts of fluorescence emission spectra as seen with the wild type MBP (not shown). Maltotetraose, however, caused a marked blue shift (5 nm) in MalE632, in contrast to the very slight blue shift (0.7 nm) observed with the wild type MBP (5). This suggests that maltotetraose uses mainly the B binding mode with the MalE632.

Transport data are shown in Fig. 4. IPTG-induced HN892, which had a periplasmic MalE632 MBP concentration of approximately 300 μM, transported both maltose and maltotetraose at rates considerably higher than that found in the ΔmalE strain NT411. This strain also transported maltotetraitol, but the initial rate (0.17 nmol/mg at 1 min) was much lower than in the strains expressing the wild type MBP at similar levels (1.4 and 0.7 nmol/mg at 1 min for IPTG-induced HN933 and HN889, respectively, in Fig. 3C).

Strain NT229 grew only on maltose, while IPTG-induced strain HN924, producing MalE632, grew well on both maltose and maltotetraose (Table III). Interestingly, it seemed to grow only marginally on maltotetraitol, much more slowly than the strain producing the wild type MBP (HN930). The transport and growth data are thus consistent with the hypothesis that MalE632 MBP restores, at least partially, the wild type substrate specificity to the MalG511-containing cells.

**DISCUSSION**

Previous studies showed that MBP may bind its ligands in one of the two ways. Thus, the wild type MBP binds α-anomers of maltodextrins in the end-on or R mode, which is accompanied by the closure of its two lobes, whereas it binds reduced, oxidized, or cyclic maltodextrins exclusively in the middle or B mode, which leaves its two lobes essentially open (5, 6). In contrast, the mutant MalE254 MBP binds even unmodified maltodextrins entirely via the middle mode (5). It is known that ligands that are now known to be bound in the open or middle mode fail to be transported (e.g. reduced, oxidized, or cyclic maltodextrins in wild type cells or unmodified linear maltodextrins in malE254 cells (see Introduction)). We examined why the middle binding does not lead to transport.

One of the essential functions of liganded MBP is to interact with the periplasmic face of the MalFGK2 transporter and to send a transmembrane signal so that ATP hydrolysis by the MalK ATPase, located on the opposite, cytosolic face of the membrane, could be initiated (3). The middle mode binding may not lead to transport because the liganded MBP in the open form may fail to stimulate this ATPase activity. Our results with the wild type MalFGK2 complex showed clearly, both with the wild type MBP and with the mutant MalE254 MBP, that little ATP hydrolysis occurred when any ligand was bound exclusively via the B or middle mode (Fig. 2 and Table II, columns labeled “By MalFGK2”). In addition, MBP-ligand complexes of this type did not cause the transport of ligands, confirming the previously published data (10, 11). In contrast, all ligands that bind to MBP via the R or end-on mode, which produces the closure of the two lobes of MBP, stimulated ATP hydrolysis and substrate uptake activities of the MalFGK2 transporter (Fig. 2; Table II). The simplest hypothesis then is that the open form of MBP cannot produce ligand transport simply because it cannot produce activation of the ATPase. We note that this hypothesis is also consistent with the conclusion, from genetic suppression studies, that the N-terminal and C-terminal lobes of the MBP interact, respectively, with the external surfaces of MalG and MalF (30, 31), since the closure of MBP may then bring the two lobes to the correct distance for their interaction with these subunits of the transporter complex (see Fig. 5).

We also examined a mutant transporter complex, MalFG511K2, which transports maltose in the absence of MBP, with an affinity 1000-fold less than the affinity shown by the wild type in the presence of MBP (4, 30). Most significantly, such transporters hydrolyze ATP in the absence of liganded MBP, i.e. in a partially constitutive manner (3). According to the hypothesis above, such transporters should be able to transport reduced or oxidized maltodextrins, since they do not require closed MBP to start the ATP hydrolytic cycle. However, as shown earlier by Treptow and Shuman (4), cells of this mutant cannot transport even unmodified maltodextrins in the absence of MBP. Proteoliposomes containing the mutant transporter complex could not transport maltodextrins by themselves, confirming these intact cell data (Fig. 3; see “Results”). Interestingly, the MalFG511K2 complex could transport maltodextrins when MBP was present (Fig. 3; see “Results”), suggesting that MBP must play an additional role in the transport of maltodextrins, perhaps by positioning the ligands correctly in the transport channel. It has been shown earlier (3) that the
The inhibition of maltose transport caused by the tight binding of MBP to MalFG511K₁ transporter can be relieved by suppressor mutations in MBP (30). Such suppressor MBPs most likely act by decreasing the affinity of the liganded MBP complex for the MalG511 transporter (28). If the middle binders are transported by the mutant transporter because of the very high affinity of the transporter for MBP, it may be expected that a suppressor MBP will not allow the transport of middle mode binders such as maltotetraose. We tested this possibility by using a suppressor MBP MalE632. The strain expressing both MalFG511K₁ transporter and the MalE632 MBP indeed had difficulty in growing on maltotetraose (Table III) and transported maltotetraose more slowly than did the strains expressing the wild type MBP (compare Fig. 4 with Fig. 3C). However, rigorous comparison between the strains is difficult, because there were too many variables. Crystallization of wild type maltose transporter has been achieved (33), and the solution of its structure may eventually solve some of these uncertainties.

Finally, this study provided an interesting glimpse of the relationship between the length of a sugar substrate and its transport efficiency. The wild type transporter hydrolyzes ATP to a greater degree when interacting with MBP complexed with maltotriose and maltotetraose than when MBP is complexed with either maltose or maltotetraose. This is true of the MalG511 transporter (Table II). This variation appears to be correlated with the subsequent transport of the sugar. Thus, in reconstituted proteoliposomes, maltotetraose (and maltotetraose in MalFG511K₁-containing proteoliposomes) is transported more rapidly than maltose (Fig. 1A; see “Results”). In contrast, in whole cells maltose is transported more efficiently than any of the maltodextrins (Fig. 1B). The flux of maltodextrins through the LamB channel, however, is far slower than that of maltose (26, 27), and it seems possible that the interaction between liganded MBP and the transport complex would favor transport of maltodextrins over maltose to counterbalance this effect of the outer membrane barrier.

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