Interaction of the Maltose-Binding Protein with Membrane Vesicles of *Escherichia coli*

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The interaction of the radioactively labeled purified maltose-binding protein of *Escherichia coli* with membrane vesicles was studied. The maltose-binding protein bound specifically to the vesicles, in the presence of maltose, on few sites. Under conditions in which a potential was imposed across the membrane, the specific binding was (i) increased, (ii) dependent on maltose, and (iii) abolished in a mutant defective in the tar gene product, one of the methyl-accepting chemotaxis proteins. At least 1,300 binding sites were present in the membrane fraction of logarithmically growing cells.

The maltose-binding protein (MBP) of *Escherichia coli* is an essential element in maltose transport (13) and maltose chemotaxis (5). It is a periplasmic protein that can be released from bacteria by the osmotic shock procedure of Neu and Heppel (21). It binds maltose reversibly with a dissociation constant of 1 \( \mu \)M (13, 37). Maltose binding induces an alteration of the intrinsic fluorescence of the protein (37), which reflects ligand-induced conformational changes. Malto transport requires, in addition to the MBP coded for by gene malE, the products of four genes: malF, malG, malK, and lamB (24, 31). The product of lamB facilitates the diffusion of malto and maltodextrins across the outer membrane and acts as a receptor for phage \( \lambda \) (25, 36). The products of malF and malK are associated with the cytoplasmic membrane (2, 30). The nature of the malG product still remains unknown.

In bacterial chemotaxis, the chemical stimuli are detected by specific chemoreceptors; more than 20 chemoreceptors have been identified, each specific for one chemical or a group of closely related chemicals. The MBP is the chemoreceptor specific for malto (5). Information about receptor occupancy is transmitted to the chemotactic machinery via a set of methyl-accepting chemotaxis proteins (MCPs) (33). These cytoplasmic membrane proteins are products of the *tar* (MCPI), *tar* (MCPII), and *trg* (MCPIII) genes, each specific for mediating signals from a different set of stimuli. The *tar* gene product is involved in chemotaxis toward aspartate, maltose, and some repellents (32). Information from receptors is ultimately integrated and used to control the direction of rotation of the flagella; the che gene products are implicated in this final pathway. The role of binding proteins in transport and chemotaxis suggests a specific interaction of these periplasmic proteins with integral cytoplasmic membrane proteins. Some genetic studies support this hypothesis (1, 34), but biochemical evidence for such an interaction is scarce. The interaction of the MBP bound to Sepharose with several membrane proteins solubilized with Triton has been reported (14), but the authors raised the possibility that immobilized MBP may act as an ion-exchange group or as a hydrophobic group causing nonspecific adsorption of some proteins as well as biologically specific binding.

We report in this study a specific binding of purified MBP to *E. coli* membrane vesicles in the presence of malto. Under conditions in which a potential is imposed across the membrane, the specific binding of MBP to vesicles is: (i) increased; (ii) dependent on malto; and (iii) abolished in a mutant defective in MCPII, the *tar* gene product.

**MATERIALS AND METHODS**

*Chemicals.* D-[\(^{14}\)C]maltose (360 mCi/mmol) was obtained from C.E.A., Saclay; \(^{35}\)S)sulfate (25 to 40 Ci/mg) was from Amersham Corp.; sucrose and maltose (less than 0.1% glucose) were purchased from Merck & Co. Valinomycin, lysozyme, DNase, and RNase were from Sigma Chemical Co. All other chemicals were reagent grade.

*Bacterial strains.* All strains used were derivatives of *E. coli* K-12. Strain HfrG6 (His), the wild-type mal* strain, and POP3325, malto constitutive, were obtained from M. Schwartz and M. Hofnung, Institut Pasteur, Paris. Strains AW518 (tar, taxi defects to serine and repellents) and AW539 (tar, taxi defects to malto, aspartate, and repellents) were isolated by Mesibov and Adler (19).

*Preparation of membrane vesicles.* *E. coli* HfrG6 was grown at 35°C in 20 liters of minimal salts medium M63 (22) containing 0.01% histidine, 1% glycerol, and 0.1% maltose and harvested at the end of the exponential phase by centrifugation in a Sharples centrifuge. Mem-
brane vesicles were prepared by osmotic lysis of spheroplasts, as described by Kaback et al. (10, 29); vesicles that had been washed twice at 4°C were suspended in 0.1 M potassium phosphate buffer, pH 6.6. Membrane vesicles of mutants tsr and tar were prepared by sonic disruption of bacteria: 2 g of wet cells was washed with 10−2 M Tris (pH 8) and suspended in 50 ml 0.1 M potassium phosphate (pH 6.6) containing 10 mM MgSO4 and 10 μg each of DNase and RNase per ml and then sonicated at 10 kc for 5 min (10 times for 30 s each) at 0°C; undisrupted cells were removed by centrifugation at 2,000 × g for 20 min. The membrane pellet was washed once with 0.1 M potassium phosphate (pH 6.6) containing 10 mM EDTA and finally suspended in 0.1 M potassium phosphate (pH 6.6).

Preparation of MBP. Strain POP3325 was used for MBP extraction. The MBP was purified as described previously (13). One kilogram of bacteria produced 100 mg of pure MBP. The radioactively labeled MBP (100 mCi/mmol) was obtained from cells grown in 2 liters of minimal salts medium containing 20 μCi of 35S-labeled inorganic sulfate at a final concentration of 0.2 mM. Both labeled and unlabeled MBPs produced only one band when analyzed by sodium dodecyl sulfate-gel electrophoresis. Protein concentrations were determined by the technique of Lowry et al., using bovine serum albumin as a standard (17). Maltose-binding activity was measured by equilibrium dialysis or, more conveniently, by the filtration technique described for the galactose-binding protein (26): 20 volumes of a saturated ammonium sulfate solution at 0°C was added to 1 volume of reaction mixture (MBP plus [14C]-maltose). The samples were filtered on nitrocellulose filters and rinsed three times with saturated ammonium sulfate at 0°C. The filters were counted in a dioxane-based scintillation mixture. Detection of [35S]MBP in the first steps of the purification was made with specific antibodies, prepared as described elsewhere (13).

Binding of MBP to membrane vesicles. The MBP-binding capacity of membrane vesicles was determined by an assay which utilizes the ligand saturation gel filtration principle devised by Hummel and Dreyer (8). A plastic column of Bio-Gel P100 (100 to 200 mesh; 10-ml bed volume) was preequilibrated at 22°C with the column buffer: 10 mM Tris (pH 8.1), 2 mM MgSO4, 1 mM 2-mercaptoethanol, 0.1 mM phenylmethylfluorosulphonate, 10 mg of bovine serum albumin per ml, 10−4 M maltose, and 10−7 M [35S]MBP. 0.2 ml of membrane vesicles (6 mg of protein), pretreated with DNase and RNase (10 μg/ml each), were incubated in the same buffer, applied to the gel, and developed with 20 ml of the buffer. [35S]MBP bound to the membrane vesicles was accumulated as a peak of radioactivity at the void volume where the vesicles emerged. The peak of radioactivity was followed by an equivalent trough caused by depletion of labeled MBP from the column by the membrane vesicles. The amount of MBP bound to the vesicles could be calculated by measuring the amount of radioactivity in the peak and trough. This technique has three advantages: high amounts of membrane can be used in a single experiment; equilibration of the column with labeled MBP prevents dissociation of bound MBP from receptor sites; and nonspecific binding is lowered.

Membrane energization and binding of MBP to energized membranes. Energization of membrane vesicles was realized by a membrane potential produced artificially, as described by Hirata et al. (6): membrane vesicles were first loaded with K+ by incubation at 37°C in 0.5 M potassium phosphate and then transferred to K+-free medium. Addition of the potassium ionophore valinomycin elicited rapid efflux of K+ down the concentration gradient, with the generation of a membrane potential interior negative. Membrane vesicles (5 mg of protein) were incubated 1 h at 37°C in 10 ml of 0.5 M potassium phosphate (pH 6.6). They were then transferred to ice, and MgSO4 was added to 10 mM. After centrifugation, vesicles were rinsed and washed once with 50 ml of 0.4 M sucrose–10 mM MgSO4 at 0°C. They were centrifuged and finally suspended in 0.5 ml of 0.4 M sucrose–10 mM MgSO4 at 0°C. A portion (40 μl) of this membrane suspension was added at 0°C to a mixture (135 μl) containing 0.4 M sucrose, 10 mM MgSO4, 2 × 10−4 M maltose, and 10−7 M [35S]MBP in an airfuge centrifugation tube. The tube was allowed to set at 22°C (4 min), and valinomycin was added (1 μl, 4 × 10−3 M in ethanol). Centrifugation was started at different times after valinomycin addition, as indicated in the figures. After centrifugation (2 min at 160,000 × g), the supernatant was aspirated; 200 μl of cold water was carefully layered over the tightly packed pellet and aspirated as quickly as possible. The pellet was suspended in 0.1 ml of water and counted for radioactivity in a dioxane-based scintillation mixture. As binding is dependent on a transient membrane potential which lasts less than 2 min after valinomycin addition, the vesicles must be pelleted in a very short time (it takes 15 s to start the airfuge and a few seconds to pellet these membrane vesicles at 160,000 × g in an A100 rotor with a clear factor of 11), and radioactivity should not leak out of the membrane pellet once it is packed. (The pellet is very tightly packed, and this is probably the case except for the surface in contact with the supernatant).

Short duration of transient potential and partial leak of bound radioactivity may explain some variability in our results.

RESULTS

We had to distinguish a binding interaction of MBP with specific sites, related to its biological functions, from nonspecific binding (e.g., to test tubes and nonreceptor membrane structures). We expected that MBP at a high concentration would saturate fully all binding sites related to its biological action. Consequently, it was assumed in binding studies that the amount of [35S]MBP not displaced from the membrane vesicles by such high concentrations of unlabeled MBP was bound in a nonspecific manner. Conversely, the radioactivity displaced by unlabeled MBP was considered to reflect specific binding. Such an experimental strategy is currently used to distinguish specific binding from nonspecific binding in studies of hormone binding to hormone receptors (3). Furthermore, as we thought that maltose might be the signal for a specific binding of MBP to a membrane component, we tested in each experiment the binding
in the presence and in the absence of maltose.

**Binding of MBP to unenergized membrane vesicles.** Membrane vesicles were applied to three exclusion chromatography columns equilibrated with buffer containing, respectively, \[^{35}\text{S}]\text{MBP}\) and maltose, \[^{35}\text{S}]\text{MBP}\) maltose and unlabeled MBP, and \[^{35}\text{S}]\text{MBP}\) without maltose. In the experiment with \[^{35}\text{S}]\text{MBP}\) and maltose (Fig. 1), a peak of radioactivity appeared at the void volume of the column, where the membrane vesicles emerged, followed by a trough which formed as radioactive MBP was extracted from the column buffer by the membrane vesicles. This reflected an interaction of membrane vesicles with MBP. In the experiment with \[^{35}\text{S}]\text{MBP}\), maltose, and unlabeled MBP, there was no radioactivity uptake by the membrane fractions, and the eluate remained at a constant level. This was the result of a complete displacement of \[^{35}\text{S}]\text{MBP}\) binding to the membrane vesicles by unlabeled MBP at a \(4 \times 10^{-6}\) M concentration; the binding sites for MBP on the vesicles were fully saturated at this MBP concentration. Since the uptake of radioactivity shown in the first experiment, when vesicles were incubated with \[^{35}\text{S}]\text{MBP}\) and maltose, was abolished when an excess of unlabeled MBP was included in the column buffer as shown in the latter experiment, this radioactive uptake should reflect a specific binding of MBP to membrane vesicles. In a third experiment, with \[^{35}\text{S}]\text{MBP}\) alone, there was no peak of radioactivity associated with the membrane fractions: MBP did not interact with membrane vesicles in the absence of maltose. These results show that, in the presence of maltose, the MBP interacted with the membrane of *E. coli* in an apparently specific manner on a limited number of sites fully saturated at an MBP concentration of \(4 \times 10^{-6}\) M.

As binding increased linearly with increasing \[^{35}\text{S}]\text{MBP}\) concentrations in a range of \(10^{-8}\) to \(10^{-7}\) M (not shown), and as a concentration of \(4 \times 10^{-6}\) M unlabeled MBP was sufficient to compete completely for labeled MBP binding, an estimation in the range \(10^{-7}\) to \(10^{-6}\) M for the dissociation constant of MBP for the membrane, in the presence of maltose, seems reasonable. The binding capacity of the membrane vesicles was calculated from the radioactivity in the column eluate containing the membranes corrected for the basal radioactivity in the column buffer. Calculation was made on \(2 \times 10^{-11}\) mg of inner membrane protein per cell. In the conditions described for the first column, the number of binding sites per cell revealed in this experiment was around 10.

**Binding of MBP to energized membranes.** Further binding experiments were performed with vesicles energized by a transient membrane potential realized by a valinomycin-induced potassium efflux, as described in Materials and Methods. Binding of \[^{35}\text{S}]\text{MBP}\) was measured in three types of experiments: in the presence of maltose, in the presence of maltose and unlabeled binding protein, and in the absence of maltose. In all experiments, vesicles loaded with potassium were suspended in a potassium-free medium, valinomycin was added, and binding of labeled

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**FIG. 1.** Binding of \[^{35}\text{S}]\text{MBP}\) to membrane vesicles. Gel filtration of membrane vesicles (6 mg in 0.2 ml) was performed on a Bio-Gel P100 (100 to 200 mesh) column. Equilibration and elution were made in 10 mM Tris (pH 8.1), 2 mM MgSO\(_4\), 1 mM 2-mercaptoethanol, 0.1 mM phenylmethylfluorosulfonate, and 10 mg of bovine serum albumin per ml containing: (A) \(10^{-7}\) M \[^{35}\text{S}]\text{MBP}\) and \(10^{-4}\) M maltose, (B) \(10^{-7}\) M \[^{35}\text{S}]\text{MBP}\), \(10^{-4}\) M maltose, and \(4 \times 10^{-6}\) M unlabeled \[^{35}\text{S}]\text{MBP}\), and (C) \(10^{-7}\) M \[^{35}\text{S}]\text{MBP}\). Fractions of 150 \(\mu\)l were collected, and 100 \(\mu\)l was used for counting. The elution profile of membrane vesicles was obtained by measuring absorption at 280 nm. \[^{35}\text{S}]\text{MBP}\) had a specific activity of 100 mCi/mmol. Symbols: ▲, counts per minute; ○, optical density at 280 nm (100-fold dilution).
MBP was measured as a function of time before and after the addition of valinomycin. In the experiment with [35S]MBP and maltose, the radioactivity found in the membrane pellet increased upon addition of valinomycin, reached a maximum, and subsequently decayed in about 1 min to the same level as before valinomycin addition (Fig. 2). The time course of the increase in [35S]MBP binding correlated with the generation and dissipation of the membrane potential as measured by others (16). In the experiment with [35S]MBP, maltose, and unlabeled MBP, the radioactivity in the membrane pellet remained at a constant level upon valinomycin addition. Thus, the valinomycin-induced binding measured in the first experiment, with [35S]MBP and maltose, was completely displaced by competing amounts of unlabeled MBP and should reflect specific binding. Conversely, the radioactivity found in the pellet before valinomycin addition remained unchanged regardless of whether unlabeled MBP was present and would represent nonspecific binding. Since the pellet was not washed, this basal level of radioactivity was probably due to [35S]MBP trapped in the pellet. In the experiment with [35S]MBP alone, there was no increase of binding upon valinomycin addition, and the level of radioactivity in the pellet remained constant and equal to the basic level found in the two other experiments. Therefore, maltose was necessary for the valinomycin-induced specific binding of MBP to membrane vesicles. In the experiments reported in Fig. 2, no specific binding could be measured in the absence of a membrane potential. This was the consequence of the low amount of membrane vesicles used in these experiments, as compared with the amount used in the experiments reported in Fig. 1.

The binding capacity of membrane vesicles energized by the transient membrane potential was calculated on the basis of 2 × 10^{-11} mg of inner membrane protein per cell; at a [35S]MBP concentration of 8 × 10^{-6} M, 1,300 molecules were bound per bacteria. As binding increased linearly with increasing [35S]MBP concentration in a range 10^{-7} to 10^{-6} M, and as a concentration of 10^{-4} M of unlabeled MBP competed completely for labeled MBP binding, an estimation in the micromolar range for the dissociation constant of MBP for energized membrane vesicles in the presence of maltose seems reasonable.

That the transient increase of MBP binding depends on the potential was tested in the following experiment. The electrical potential induced by K^+ efflux is a function of the concentration ratios of K^+ on each side of the membrane; addition of K^+ to the external medium reduces the amplitude of the membrane potential. Addition of 0.1 M K^+ to the external medium suppressed entirely the valinomycin-induced MBP binding to the vesicles (Fig. 2). When 0.1 M Na^+ instead of 0.1 M K^+ was added to the external medium, there was no effect on the valinomycin-induced [35S]MBP binding to the vesicles (not shown).

Binding experiments with mutants tsr and tar. Vesicles were prepared from a mutant defective in MCPI, the tsr gene product which is implicated in chemotaxis toward serine and some repellents, and a mutant defective in MCPII, the tar gene product which is implicated in chemotaxis toward maltose, aspartate, and some repellents. The valinomycin-induced binding of MBP to these vesicles was measured in the same way as for vesicles prepared from wild-type bacteria. Vesicles prepared from mutant tsr gave the same results as wild-type bacteria: a transient increase of radioactive MBP appeared in the membrane pellet upon valinomycin addition when vesicles were incubated with [35S]MBP and maltose (Fig. 3). No such increase was seen when the experiment was made in the presence of a competing potential.
amount of unlabeled MBP or in the absence of maltose. With vesicles prepared from a tar mutant impaired in the methyl-accepting protein MCPII, essential for maltose chemotaxis, the addition of valinomycin failed to induce any [35S]MBP binding, even in the presence of maltose. The tar gene product seemed necessary for the maltose-dependent specific binding of MBP to energized membrane vesicles.

**DISCUSSION**

In this study, we report a specific interaction of the purified MBP with membrane vesicles of *E. coli*. With unenergized vesicles, a specific binding was shown on a very low number of sites; binding was dependent on maltose, and the dissociation constant was estimated in a 10^{-7} to 10^{-6} M range. Since experiments with mutants was not undertaken, we do not know whether this binding is related to the transport or chemotactic function of the MBP.

With vesicles energized by a transient membrane potential, a transient specific binding was measured which followed the kinetics of generation and dissipation of the membrane potential. This specific binding occurred on a definite number of sites; at a [35S]MBP concentration of 8 \times 10^{-6} M, around 1,300 molecules were bound per bacteria. The dissociation constant was estimated in the micromolar range. This specific binding was dependent on maltose and was abolished in a tar mutant defective in MCPII, the methyl-accepting chemotaxis protein implicated in taxis toward aspartate, maltose, and some repellents. This specific binding of MBP to energized vesicles seems to be related to its chemotactic function. The induction by maltose of MBP specific binding to membrane vesicles supports the thesis that MBP, the chemoreceptor, exists in a conformation that is not attracted to the membrane and that maltose, the chemoeffector, upon binding to MBP, induces it into a new conformation that allows the association of the maltose-MBP complex with a membrane component, probably the tar gene product. Such a ligand-induced association was proposed for the association with the membrane of the galactose- and the ribose-binding proteins on the basis of a competition for chemotaxis between galactose and ribose (34). A ligand-induced association of membrane receptors with membrane enzymes has been proposed by Jacobs and Cuatrecasas, in the mobile receptor hypothesis (9).

Imposition of a membrane potential to the vesicles led to an important increase in MBP specific binding. The membrane potential was produced by a valinomycin-induced potassium efflux. In respiring vesicles or bacteria, translocation of protons across the cytoplasmic membrane generates a proton motive force composed of a chemical gradient (ΔpH) and a membrane potential (Δψ) (20). The proton motive force is able to drive energy-dependent processes, such as the synthesis of ATP, active transport, or bacterial motility, through chemosensitive, osmotic, and mechano-osmotic coupling. It realizes an energized state of the cytoplasmic membrane, which is involved in such processes as irreversible adsorption of phage T1 and φ80 to bacteria (4), phage T4 DNA injection (15), colicin K action (18, 28), and modulation of adenylate cyclase activity (23). Thus, the proton motive force, or one of its components, affects the localization, conformation, orientation, or activity of some membrane proteins. In our experiments, the membrane potential affects either the accessibility or the affinity of the membrane binding sites for MBP. In chemotaxis, Kahn and Macnab (12) have presented evidence that the proton motive force not only energizes the flagellar motor, but also regulates its sense of rotation; Szmelcman and Adler (35) have reported an increase of the membrane potential when attractants or repellents are added to the bacteria. Since formation of the maltose-MBP complex and its binding to the membrane occur first in the cascade of events that lead to the chemotactic response, the interaction of MBP with the membrane should not be dependent on this hyperpolarization for its formation.

Protein function in membrane structures can be a relatively complex phenomenon involving such different factors as the membrane potential, phospholipid methylation, which increases the efficiency of coupling between β-adrenergic
receptors and adenylate cyclase (7), protein methylation, which plays a crucial role in chemotaxis (33), receptor clustering, which may be important for hormone action (11), or GTP regulatory proteins, which have a role in signal transduction from hormone receptors to adenylate cyclase (27).

**LITERATURE CITED**


