JOURNAL OF BACTERIOLOGY, July 1983, p. 97–106
0021-9193/83/070097-10$02.00/0

Vol. 155, No. 1

Reconstitution of Maltose Transport in *Escherichia coli*: Conditions Affecting Import of Maltose-Binding Protein into the Periplasm of Calcium-Treated Cells

JOHANN M. BRASS,* ULRIKE EHMANN, AND BERND BUKAU
Department of Biology, University of Konstanz, D-7750 Konstanz, West Germany

Received 7 January 1983/Accepted 19 April 1983

The reconstitution of active transport by the Ca^{2+}-induced import of exogenous binding protein was studied in detail in whole cells of a *malE* deletion mutant lacking the periplasmic maltose-binding protein. A linear increase in reconstitution efficiency was observed by increasing the Ca^{2+}- concentration in the reconstitution mixture up to 400 mM. A sharp pH optimum around pH 7.5 was measured for reconstitution. Reconstitution efficiency was highest at 0°C and decreased sharply with increasing temperature. The time necessary for optimal reconstitution at 0°C and 250 mM Ca^{2+} was about 1 min. The competence for reconstitution was highest in exponentially growing cultures with cell densities up to 1×10^9/ml and declined when the cells entered the stationary-growth phase. The apparent K_m for maltose uptake was the same as that of wild-type cells (1 to 2 μM). V_{max} at saturating maltose-binding protein concentration was 125 pmol per min per 7.5×10^7 cells (30% of the wild-type activity). The concentration of maltose-binding protein required for half-maximal reconstitution was about 1 mM. The reconstitution procedure appears to be generally applicable. Thus, galactose transport in *Escherichia coli* could also be reconstituted by its respective binding protein. Maltose transport in *E. coli* was restored by maltose-binding protein isolated from *Salmonella typhimurium*. Finally, in *S. typhimurium*, histidine transport was reconstituted by the addition of shock fluid containing histidine-binding protein to a *hisJ* deletion mutant lacking histidine-binding protein. The method is fast and general enough to be used as a screening procedure to distinguish between transport mutants in which only the binding protein is affected and those in which additional transport components are affected.

It is often desirable to test the biological function of proteins and nucleic acids by introducing them into cells. Since gram-negative cells are surrounded by an outer and an inner membrane, much effort has been expended to develop conditions for making these membranes permeable to such large molecules (21, 22, 34). The best-known example is transformation, during which gram-negative bacteria take up DNA by passive diffusion after Ca^{2+} treatment combined with a temperature shift (6, 22, 30). Until recently, Ca^{2+} treatment was only used for DNA import.

We have shown that pretreatment with Tris and Ca^{2+} is a simple and gentle method for making the outer membrane permeable to small molecules like that of maltose (8). Under these conditions, even proteins can be imported into the periplasm, as demonstrated by the reconstitution of maltose transport in the nonpolar deletion strain HS3018 (Δ*malE malT*^{c}) by the addition of maltose-binding protein (MBP). Previously, the reconstitution of binding protein-dependent transport had only been achieved by the addition of binding protein to spheroplasts or isolated membrane vesicles of mutant strains that were free of this protein (4, 15, 16, 20, 28). Reconstitution experiments with whole cells, which are much easier and highly reproducible, reflect a more natural situation. The technique allows testing not only of the binding protein interaction with inner membrane transport proteins, but also of the interaction of binding protein with outer membrane porins (λ-receptor [maltoporin]) in the case of maltose transport. Here we present a detailed study of the conditions affecting this process to characterize this reconstitution procedure more closely. We also describe experiments showing the general applicability of this procedure to the reconstitution of other binding protein-dependent transport systems.

TABLE 1. Bacterial strains and plasmids used

Strain/plasmid	Genotype/phenotype	Origin
E. coli		
pop1080	HfrG6 *lamB102 metA trpE*(Am) *galE galY*	Hofnung et al. (19)
pop1740	HfrG6 *malB112 his*	Hofnung et al. (18)
HS3018	F⁻ Δ*malE444 malT^c-1 araD139* Δ(*argF-lac*)*U169 relA1 rpsL150 flbB5301 deoC1 ptsF25*	Shuman (30a)
JB3018-2	F⁻ *malT^c-1 araD139* Δ(*argF-lac*)*U169 relA1 rpsL150 flbB5301 deoC1 ptsF25*	Brass, this study—*mal*⁺ transductant of HS3018
LA5539	F⁻ *lacY galE arg ptsF zee-700*-Tn*10 fpk*	W. Boos, personal communication
LA6021	*mgl-501*	Independent, spontaneous *mgl* mutants of LA5539, GBP defective, selected by W. Boos
LA6022	*mgl-502*	
LA6028	*mgl-503*	
pHG4	Tet^r *mgl*⁺	pACYC184 derivative harboring the 6-kilobase *Eco*RI fragment containing the *mgl* region as described by Müller et al. (24)
S. typhimurium		
LT2	Wild type	Ames and Lever (2)
TA271	*hisF645 dhuA1*	Ames and Lever (2)
TA2918	Δ*hisJ6776*	Ames et al. (3)
TA1835	Δ(*ubiX-dhuA-hisJ-hisP*)*5651*	Ames et al. (3)

MATERIALS AND METHODS

Bacterial strains and growth medium. The bacterial strains used are listed in Table 1. Overnight cultures were grown in minimal medium A (MMA) (23) plus 0.4% glycerol and the appropriate supplements. Cells grown on nutrient broth were also competent for reconstitution. The efficiency of reconstitution, however, was lower than that of the cells grown in minimal medium.

Binding protein purification. Shock fluid (25) and purified MBP (14) were prepared as described elsewhere. All protein preparations were centrifuged for 20 min at 40,000 × *g* before lyophilization. Binding protein activity was measured by the substrate binding test as described previously (27).

Standard procedure for reconstitution of maltose transport. The optimized conditions for reconstitution were as follows. Cultures of strains HS3018 (Δ*malE444 malT^c*; nonpolar deletion in *malE*, constitutive expression of the remaining *malB* genes) and pop1740 (Δ*malB112*, lacking all five *malB* genes) were grown overnight at 37°C in MMA plus 0.4% glycerol (HS3018) or 0.4% glucose (pop1740) in such a way that exponentially growing cells could be harvested the next morning (optical density at 578 nm ≤ 1). A total of 10^9 cells were spun down, the supernatant was decanted, and the remaining drops of medium were removed with a paper towel. The pellet was washed once with ice-cold 100 mM Tris-hydrochloride buffer (pH 7.2) containing 10 mM glycerol, 50 μg of chloramphenicol per ml, and 250 mM $CaCl_2$. (Washing with Ca^{2+}-free buffer resulted in a complete loss of the reconstitution competence of the cells.) After centrifugation, the cells were suspended in 50 μl of the same buffer (0°C) and transferred to a new test tube containing 1 mg of purified (14) and lyophilized MBP (final concentration, 20 mg/ml) or appropriate amounts of lyophilized crude shock fluid. The suspension was shaken at 0°C from 30 min to 2 h; varying the incubation time over this range did not influence reconstitution efficiency. The initial rate of maltose uptake was determined after the cells were washed free of MBP with 1 ml of 0.9% NaCl at room temperature.

For the determination of the initial rate of maltose uptake, 1×10^9 Δ*malE* cells treated as described above or 5×10^8 wild-type cells were suspended at room temperature in 1 ml of MMA containing 10 mM glycerol and 50 μg of chloramphenicol per ml. At time zero, [^{14}C]maltose (specific activity, 5.9 mCi/mmol; final concentration, 6.75 μM) was added. Samples (150 μl) were withdrawn at 10, 25, 40, 60, and 80 s, and the cells were filtered onto membrane filters (0.65-μm pore size; Millipore Corp.) and washed three times with 5-ml portions of MMA at room temperature. The radioactivity of the dried filters was counted in a toluene-based scintillation fluid.

We found that the ability of MBP for restoration and substrate binding could be differentiated by denaturation. Although MBP easily renatures for its maltose-binding activity after treatment with 4 M guanidine hydrochloride and subsequent dialysis against buffer, it remained entirely inactive with respect to transport restoration.

Reconstitution of other binding protein-dependent transport systems. For the reconstitution of galactose transport, the cells were grown in LB (23) plus 10^{-3} M fucose and subjected to the standard reconstitution procedure, except that shock fluid (40 μg/ml) from a *mgl* strain harboring plasmid pHG4 (galactose-binding protein [GBP] overproducer; GBP content in this shock fluid ≥ 50% of the total protein) was added to the cells (Table 2). The initial rate of galactose uptake was determined as described for maltose uptake except that [^{14}C]galactose (specific activity, 50 mCi/

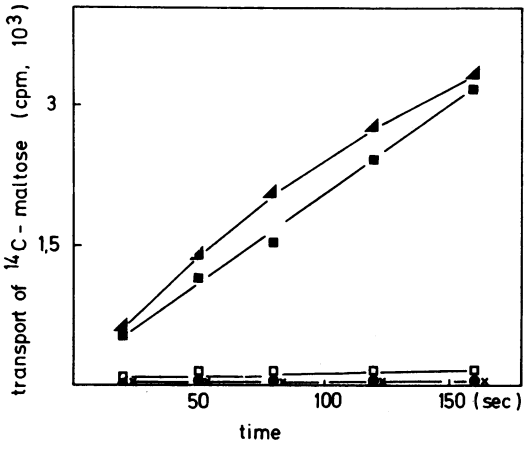

FIG. 1. Reconstitution of active maltose transport in whole Δ*malE* cells with purified MBP and crude shock fluid. A total of 1×10^9 cells from an exponentially growing culture of strain HS3018 (Δ*malE malT*^{c}) in MMA plus 0.4% glycerol (or 0.4% glucose in the case of strain pop1740 [Δ*malB*]) were subjected to the standard reconstitution procedure. After the cells were washed free of Ca^{2+} and MBP, maltose uptake was measured. Symbols: ●, HS3018 without protein in the reconstitution mixture; ◢, HS3018 plus 40 mg of crude shock fluid per ml containing 20 mg of MBP per ml; ■, HS3018 plus 20 mg of MBP per ml; □, HS3018 plus 20 mg of MBP per ml in the reconstitution mixture and 20 mM NaN_3 in the uptake medium; ×, pop1740 plus 20 mg of MBP per ml. The results are given as the amount of maltose taken up by 7.5×10^7 cells.

mmol; final concentration, 0.4 μM) was added to the uptake mixture, which contained only 1×10^8 cells per ml.

For the reconstitution of histidine transport in *Salmonella typhimurium*, cells were grown in Vogel-Bonner minimal medium (23) containing 0.4% glucose and 40 μg of histidine per ml and subjected to the standard reconstitution procedure, except that shock fluid (40 mg/ml) from strain TA271 (constitutive expression of histidine-binding protein) was added to the cells (Table 2). The initial rate of histidine uptake was measured by the protein incorporation assay described by Ames (1).

RESULTS

Reconstitution of active maltose transport with MBP. The calcium-induced permeability increase of the outer membrane allowed the reconstitution of maltose transport in the nonpolar deletion strain HS3018 (Δ*malE malT*^{c}). This strain lacks the periplasmic MBP but constitutively expresses the remaining *malB* genes responsible for the active transport of maltose (30a). Strain HS3018 had virtually no maltose transport, whereas maltose transport could be reconstituted in the same cells after pretreatment with Tris buffer plus calcium containing purified MBP (1 mg/50 μl of incubation mixture) or crude shock fluid (2 mg/50 μl; MBP content, about 1 mg/50 μl) from a maltose-induced *lamB* strain (pop1080) (Fig. 1).

In earlier reconstitution experiments under suboptimal conditions, purified MBP was five times less efficient than was crude shock fluid containing equivalent amounts of MBP (7, 8). With the new, optimized standard procedure described below, purified MBP and crude shock fluid had virtually the same activity (Fig. 1). Therefore, in many of the following experiments, crude shock fluid was used instead of purified MBP.

MBP did not restore maltose transport in the control strain (pop1740), which carries a deletion covering all five genes necessary for maltose uptake (Fig. 1).

NaN_3 (20 mM) completely inhibited maltose uptake in reconstituted cells as it did in wild-type cells. This demonstrates that the maltose uptake in reconstituted cells is due to active transport.

Calcium dependence of reconstitution. The calcium concentration was a critical factor in the reconstitution of maltose transport. Cells incubated with shock fluid at 0°C in Tris buffer (100 mM) alone were completely incompetent for reconstitution. Reconstitution efficiency increased linearly with increasing Ca^{2+} up to 400

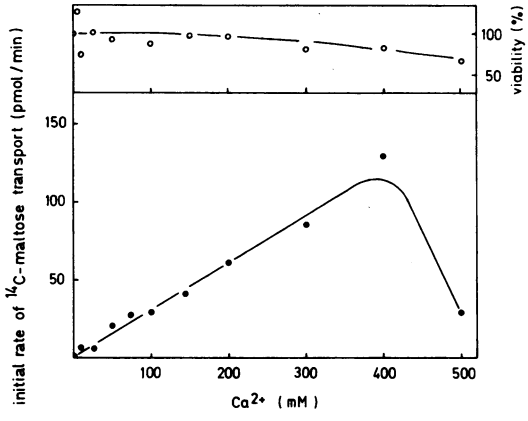

FIG. 2. Calcium dependence of reconstitution of maltose transport. Samples of 1×10^9 cells from strain HS3018 were subjected to the standard reconstitution procedure except that different Ca^{2+} concentrations from 10 to 500 mM were used during washing and in the reconstitution mixture. Shock fluid, containing 10 mg of MBP per ml was added to the reconstitution mixture. After the cells were washed in 0.9% NaCl, the viability (○) (100% = viability of cells in 100 mM Tris, pH 7.2) and the initial rate of maltose uptake per 7.5×10^7 cells (●) were determined.

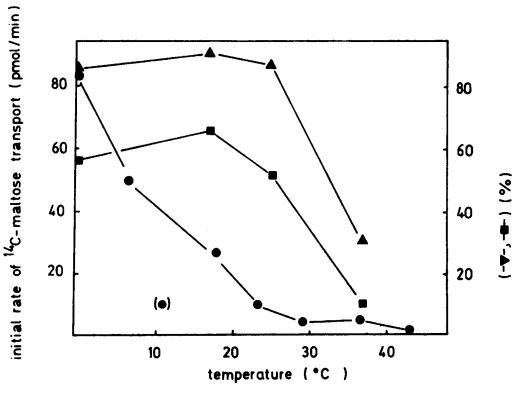

FIG. 3. Influence of temperature on reconstitution and viability. Samples of 2×10^9 cells from the Δ*malE* strain (HS3018) were subjected to the standard procedure except that the cells were kept at the indicated temperature during washing in Tris-Ca^{2+} buffer and during incubation in the same buffer with shock fluid containing 10 mg of MBP per ml. As the control, cells from a *malE*$^+$ derivative (JB3018-2) were treated in the same way except that shock fluid was omitted. After the cells were washed in 0.9% NaCl, the efficiency of reconstitution in HS3018 (●) was determined and expressed as picomoles of maltose taken up per minute by 7.5×10^7 cells. In the control experiment with JB3018-2 cells, the percent relative viability (▲) and the percent relative maltose transport activity (■) were compared with those of JB3018-2 cells treated with MMA (100%).

mM. The viability of the cells remained high at all Ca^{2+} concentrations (Fig. 2).

In control experiments with cells from a *malE*$^+$ derivative (JB3018-2) of strain HS3018, the amount of MBP leaking out through the outer membrane of Ca^{2+}-treated cells was determined with anti-MBP-antibodies in Ouchterlony double-diffusion plates. In contrast to cells treated for 2 h under suboptimal conditions (37°C, 25 mM Ca^{2+}), where no leakage of MBP in wild-type cells was observed (8), the exit of around 5% of total cellular MBP into the supernatant of *malE*$^+$ cells treated for 2 h under optimal conditions (0°C, 250 mM Ca^{2+}) was measured (data not shown).

The possibility that the inner membrane of the cells was also permeabilized by the Ca^{2+} treatment was tested by adding [^{14}C]maltose to cells of strain pop1740, which is defective in maltose uptake due to a *malB* deletion. The cytoplasm of these cells did not equilibrate with external [^{14}C]maltose (150 μM) during 2 h of treatment at 0°C in the presence or absence of 250 mM Ca^{2+}. The internal maltose concentration reached only 7% of the external maltose concentration (data not shown). This shows that the inner membrane is not susceptible to permeabilization by the standard reconstitution procedure.

Temperature dependence of reconstitution. The temperature at which the cells were kept during the incubation with Ca^{2+} and shock fluid strongly influenced the reconstitution efficiency. The efficiency of reconstitution increased dramatically with decreasing temperature of the reconstitution mixture (Fig. 3).

Control experiments were performed to clarify whether this temperature effect was due to a higher Ca^{2+}-induced permeabilization of the outer membrane at low temperatures or to other factors, such as different viability of the cells at different temperatures. The viability and the rate of maltose transport of cells from a *malE*$^+$ derivative (JB3018-2) of strain HS3018 were measured after a pretreatment of the cells for 2 h with 250 mM Ca^{2+} at different temperatures and compared with those of cells pretreated with MMA at room temperature. In the range from 0 to 25°C, we found no great difference in viability or maltose transport between the Ca^{2+}-treated

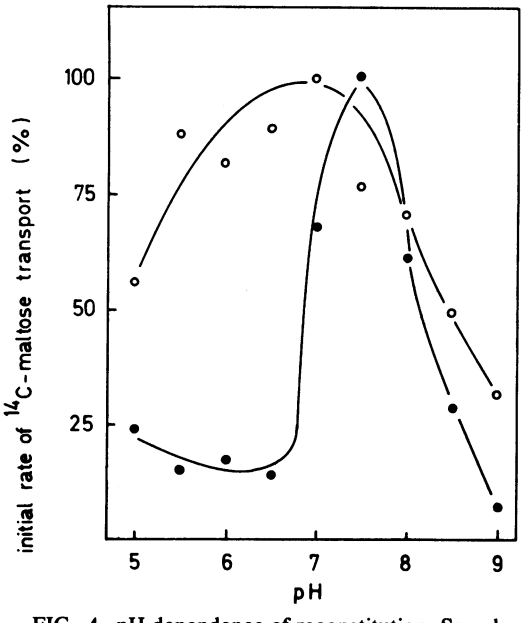

FIG. 4. pH dependence of reconstitution. Samples of 1×10^9 cells from the Δ*malE* strain (HS3018) were subjected to the standard reconstitution procedure except that the pH of the washing buffer and of the reconstitution mixture was varied as indicated. Shock fluid containing 10 mg of MBP per ml was added to strain HS3018. The pH in the uptake buffer was kept at 7.1. As the control, cells from a *malE*$^+$ derivative (JB3018-2) were treated in the same way except that shock fluid was omitted. After the cells were washed in 0.9% NaCl, the initial rates of maltose transport were determined in both strains. The values are given as relative rates (in percent). Symbols: ●, HS3018, 100% = 80 pmol/min per 7.5×10^7 cells; ○, JB3018-2, 100% = 450 pmol/min per 7.5×10^7 cells.

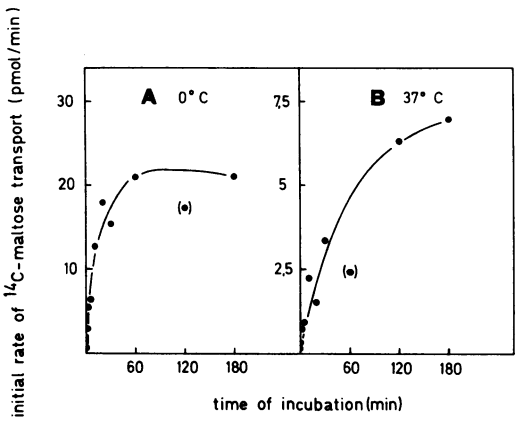

FIG. 5. Time dependence of reconstitution of maltose transport at 0 and 37°C. Cells (2×10^{10}) from strain HS3018 were subjected to the standard reconstitution procedure except that the cells were suspended in 750 μl of 10 mM Tris buffer (pH 7.2), containing 25 mM Ca^{2+}, 10 mM glycerol, and 50 μg of chloramphenicol per ml. Shock fluid containing 5 mg of MBP per ml was added to the cells. After being shaken at 0°C (A) or 37°C (B) for the indicated times, samples of 50 μl of the reconstitution mixture containing around 2×10^9 cells were withdrawn. After the cells were washed in 0.9% NaCl, the initial rate of maltose transport was determined.

malE$^+$ cells and *malE*$^+$ cells treated with MMA. Above 25°C, however, both viability and transport in Ca^{2+}-treated cells decreased dramatically relative to MMA-treated cells. Thus, the low efficiency of the procedure in the temperature range between 25 and 45°C was due to a loss of viability of the cells treated in 250 mM Ca^{2+}. In contrast, the low efficiency of reconstitution in the temperature range from 10 to 25°C could be due to suboptimal permeabilization of the outer membrane. (At 25 mM Ca^{2+}, no loss of viability and only a slight decrease in maltose transport activity [from 83 to 61%] were observed upon increasing the temperature from 0 to 37°C [data not shown].)

pH dependence of reconstitution. A sharp pH optimum around 7.5 was measured for reconstitution (Fig. 4). Wild-type cells showed nearly normal maltose transport activity after pretreatment in Tris buffer plus Ca^{2+} in the pH range between 5.5 and 8. *malE* cells, however, showed a very poor reconstitution competence in the pH range between 5.5 and 6.5.

Time dependence of reconstitution. Under optimal conditions (0°C and 250 mM Ca^{2+}), reconstitution proceeds rapidly. The kinetics of this process could not be resolved, since the cells were fully reconstituted within 1 min after the addition of shock fluid (data not shown). Therefore, we measured the process using a suboptimal Ca^{2+} concentration (25 mM). To test whether the more efficient reconstitution observed at 0°C was due to a more extensive permeabilization of the outer membrane, we compared the kinetics of reconstitution at 0 and 37°C. (As mentioned above, at 25 mM Ca^{2+} the viabilities of these cells were identical.) Reconstitution increased much more rapidly in cells treated at 0°C (Fig. 5A) than at 37°C (Fig. 5B). The slope of the curve at 0°C was 10 times steeper than that of the curve at 37°C, indicating a 10-fold-higher rate of permeabilization. We also noticed a striking difference in the plateau level of transport after 3 h of reconstitution (three times lower in the case of the 37°C cells).

Stability of maltose transport in reconstituted cells. To determine the stability of maltose transport, we subjected the *malE* cells to the optimized standard reconstitution procedure but measured transport after shaking the cells for different times at room temperature in MBP-free MMA containing glycerol (10 mM) and chloramphenicol (50 μg/ml). A twofold increase in the initial rate of maltose uptake was observed after incubation for 60 min in MMA, followed by a slow decrease in activity during the next 2 h.

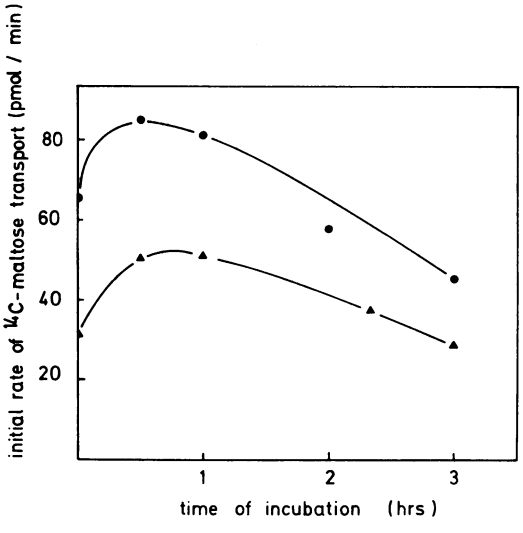

FIG. 6. Stability of maltose transport in reconstituted cells. Cells (7.5×10^9) from strain HS3018 were subjected to the standard reconstitution procedure of applying shock fluid containing 10 mg (▲) or 50 mg (●) of MBP per ml. After the cells were washed in 0.9% NaCl, however, they were suspended in 5 ml of uptake medium and shaken at room temperature for the indicated time periods. Samples of 1×10^9 cells were then withdrawn and transferred to new uptake medium, and the initial rates of maltose transport were determined.

Cells treated with high (100 mg/ml) and low (20 mg/ml) concentrations of shock fluid were equally stable (Fig. 6).

Influence of growth phase of the recipient cells on reconstitution. Cells growing in MMA plus 0.4% glycerol (Fig. 7B) exhibited a rapid decrease in competence for reconstitution (Fig. 7A) as soon as the exponential growth of the culture slowed at cell densities higher than 10^9/ml (optical density at 578 nm = 1). This was not due to a decrease in maltose transport per se in stationary-phase cells, since stationary-phase *malE*$^+$ cells with and without Ca^{2+} treatment are fully active in maltose transport (data not shown).

Fidelity of reconstitution as judged by the K_m of the maltose transport system. MBP introduced into the periplasm during reconstitution appeared to function normally in transport. Ca^{2+}-treated *malE* cells exposed to MBP in crude shock fluid (20 mg/ml [equivalent to 10 mg of MBP per ml]) exhibited a K_m of 1.89 μM (Fig. 8) as compared with a K_m of 1 to 2 μM for maltose transport in wild-type cells (32).

MBP concentration optimum for reconstitution of maltose transport in *malE* cells. The concentration optimum for MBP in the reconstitution mixture was determined by adding various amounts of purified MBP to Δ*malE* cells. A concentration of 45 mg of MBP per ml, equivalent to 1 mM, was necessary for half-maximal reconstitution of maltose transport at a saturating concentration of maltose (6.75 μM) (Fig. 9). The extrapolated maximal rate of transport as a function of the MBP concentration (Fig. 9) was 128 pmol per min per 7.5×10^7 cells, a value which is around 30% of the rate of Ca^{2+}-treated cells from a *malE*$^+$ *malT*^{c} derivative of strain HS3018 (Table 2).

General applicability of the Ca^{2+}-dependent reconstitution procedure for whole cells. The calcium-dependent reconstitution procedure with whole cells, described here in detail, appears to be generally applicable (Table 2). (i) *E. coli* HS3018 could be reconstituted with shock fluid from maltose-induced *S. typhimurium* cells. The *S. typhimurium* and *E. coli* shock fluids were equally effective. (ii) The galactose transport of *E. coli* LA6021 and LA6022, which lack GBP, could be reconstituted by the addition of shock fluid containing GBP. Strain LA6028, also lacking GBP, could not be reconstituted. (iii) The procedure is also applicable to *S. typhimurium*. Histidine transport in *S. typhimurium* TA2918, which lacks the histidine-binding protein, was restored by the addition of shock fluid from strain TA271, constitutively expressing histidine transport genes (3). A deletion mutant covering also the *hisP* gene (TA1835) could not be reconstituted.

DISCUSSION

In a previous paper (8), we introduced a novel approach to successful binding protein-dependent restoration of transport by adding binding protein to whole cells that had been treated with Ca^{2+} to increase the permeability of the outer membrane to large molecules. We showed that cells of a nonpolar *malE* deletion strain, which lacks periplasmic MBP but constitutively expresses the remaining *malB* genes (30a), were partially restored for maltose transport activity by the import of MBP into the periplasm. Ac-

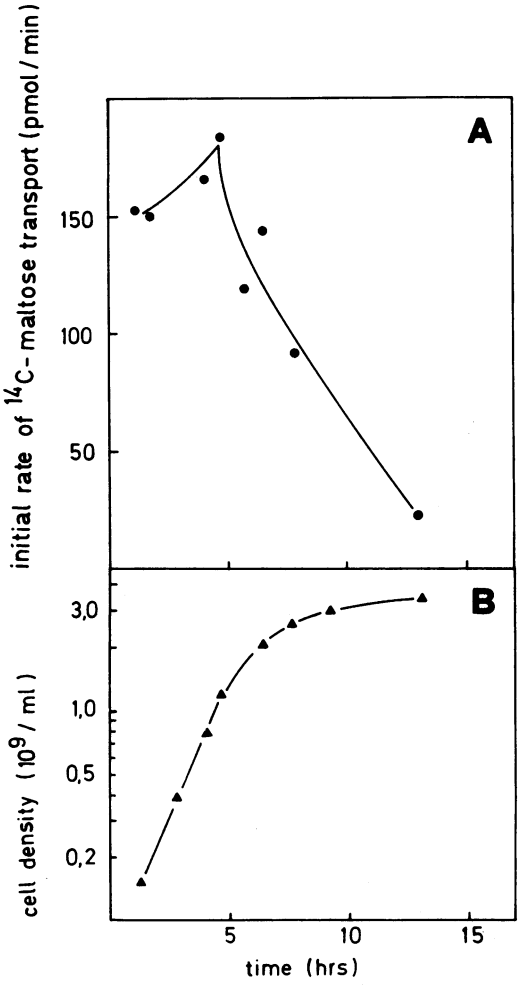

FIG. 7. Influence of growth phase of the recipient HS3018 cells on reconstitution. Samples of 2.5×10^9 cells from strain HS3018 were harvested at different stages of the growth curve (B) and subjected to the standard reconstitution procedure of applying shock fluid containing 10 mg of MBP per ml. The efficiency of reconstitution was determined by measuring the initial rates of maltose uptake (A).

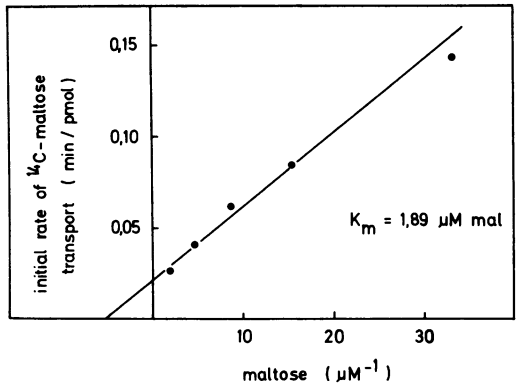

FIG. 8. Determination of the K_m of maltose transport in reconstituted cells. Samples of 1×10^9 cells from strain HS3018 were subjected to the standard reconstitution procedure of applying shock fluid containing 10 mg of MBP per ml. The K_m of maltose transport was determined by measuring the initial rates of maltose uptake at maltose concentrations between 3×10^{-7} and 5.15×10^{-6} M.

cording to a recent proposed model of maltose transport (30a), a buried maltose-binding site of the MalF, G, K protein complex which is inaccessible in the absence of MBP becomes accessible after interaction with maltose-loaded MBP. In the present paper, we describe the optimized reconstitution procedure (250 mM Ca^{2+}, 0°C) that drastically improves the efficiency of the technique and present data that indicate that the method can be extended to study the interaction of other binding proteins with membrane-bound proteins involved in the substrate transport.

With the new procedure (Fig. 1), the efficiency of purified MBP (20 mg/ml) in the reconstitution of maltose transport was increased by a factor of 50 compared with previously published data (7, 8). The efficiency of crude shock fluid containing the same concentration of MBP was increased by a factor of 10. Thus, both preparations were now found to be equally effective because of improved permeabilization of the outer membrane. This result clearly shows that the import of MBP alone is sufficient for the reconstitution and rules out the possible involvement of additional components in this type of reconstitution experiment.

The improved permeabilization of the outer membrane under optimized conditions was, in part, due to higher Ca^{2+} concentrations (250 mM) in the reconstitution mixture (Fig. 2). A similar concentration optimum for Ca^{2+} (80 mM) was measured for transformation in *E. coli* (33). The other important factor responsible for the improved reconstitution was the incubation of the cells at a reduced temperature during the reconstitution procedure. The efficiency of reconstitution increased dramatically upon decreasing the temperature to 0°C. A very similar temperature dependence was observed in the transformation of *E. coli* (33). A temperature upshift to 42°C, however, which is usually applied in transformation, drastically decreased the efficiency of reconstitution (data not shown). The data shown in Fig. 3 indicate that the low efficiency of the reconstitution procedure in the temperature range between 25 and 45°C was due to a loss of viability of the cells treated in 250 mM Ca^{2+}. In contrast, the low efficiency of reconstitution in the temperature range between 10 and 25°C, where no loss of viability or maltose transport activity in the wild type was observed, could be due to suboptimal permeabilization of the outer membrane.

Δ*malE* cells showed a high reconstitution competence in the pH range between 7.0 and 8.0. Reconstitution was poor in the pH range from 5.5 to 6.5 (Fig. 4). In contrast, maltose transport in *malE*$^+$ cells was normal after pretreatment with Ca^{2+} at this pH. This may indicate that the Ca^{2+}-binding sites, which are responsible for outer membrane permeabilization, are available only at neutral pH. The binding of Ca^{2+} to this site at acidic pH may be inhibited by protonation. A very similar pH optimum was measured for the transformation of *E. coli* (26, 33).

As in the transformation of *E. coli* (33), a strong decay of the reconstitution competence of the Δ*malE* cells was observed at the end of the exponential growth phase (Fig. 7). In con-

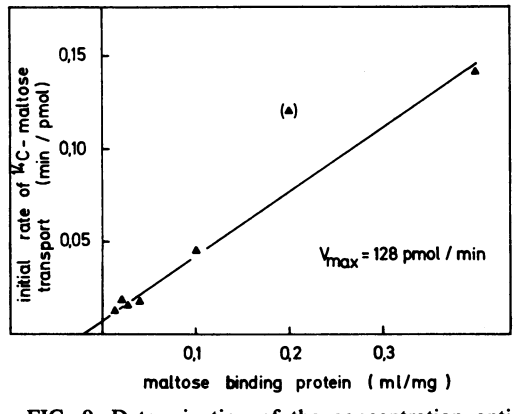

FIG. 9. Determination of the concentration optimum for purified MBP in reconstitution. Samples of 1×10^9 cells from strain HS3018 were subjected to the standard reconstitution procedure except that different concentrations of purified MBP (from 2.5 to 75 mg/ml) were added to the cells in the reconstitution mixture. The efficiency of reconstitution was determined by measuring the initial rates of maltose uptake.

TABLE 2. General applicability of the Ca^{2+}-dependent reconstitution procedure with whole cells

Strain	Relevant genotype	Treatment[a]	Concn (mg/ml) of shock fluid (strain)	Initial rate of transport[a] (pmol/min per 7.5×10^7 cells) of:		
				Maltose (6×10^{-6} M)	Galactose (5×10^{-7} M)	Histidine (3×10^{-8} M)
E. coli						
JB3018-2	*malE*$^+$	MMA	—[b]	597		
		Ca^{2+}	—	423		
HS3018	Δ*malE*	Ca^{2+}	—	0.22		
		Ca^{2+}	20 (pop1080)	94.8		
		Ca^{2+}	20 (LT2)	79.8		
LA5539	*mgl*$^+$	MMA	—		81.1	
		Ca^{2+}	—		6.7	
LA6021	*mgl*	Ca^{2+}	—		0.16	
		Ca^{2+}	40 [LA5709(pHG4)]		17.5	
LA6022	*mgl*	Ca^{2+}	—		0.00	
		Ca^{2+}	40 [LA5709(pHG4)]		20.4	
LA6028	*mgl*	Ca^{2+}	—		0.00	
		Ca^{2+}	40 [LA5709(pHG4)]		0.02	
S. typhimurium						
TA271	*hisJ*$^+$ *dhuA*	Vogel-Bonner				0.80
		Ca^{2+}				0.56
TA1835	*hisJ hisP*	Ca^{2+}	—			0.014
		Ca^{2+}	40 (TA271)			0.00
TA2918	*hisJ*	Ca^{2+}	—			0.29
		Ca^{2+}	40 (TA271)			0.80

[a] Reconstitution and transport assays were done as described in the text.
[b] —, None.

trast, in *malE*$^+$ cells the rate of maltose transport is highest in early stationary phase (data not shown). The low reconstitution competence in these cells could be due to the higher degree of murein cross-linking observed in late exponential cultures (11).

The effects of Ca^{2+} concentration, pH, temperature, and growth phase on the recipient cells are strikingly similar for reconstitution (Fig. 2 through 4 and 7) and transformation (26, 33) in *E. coli*. Since reconstitution depends only on the permeabilization of the outer membrane, one can conclude that in transformation these factors also mainly influence the permeation of DNA through the outer membrane.

The inner membrane is apparently not permeabilized to the same extent as the outer membrane by treatment with 250 mM Ca^{2+} at 0°C. This can be concluded from the high viability of the cells (Fig. 3) and the fact that cells of a *malB* deletion strain (pop1740) could not be loaded with [^{14}C]maltose by the Ca^{2+} treatment for 2 h. In contrast, the equilibration of the periplasmic space with MBP appeared to be complete after 60 min at 25 mM Ca^{2+} and 0°C (Fig. 5A) and after 1 min at 250 mM Ca^{2+} and 0°C (data not shown). Our earlier report about the import of the *malT* gene product into the cytoplasm (7) could not be verified.

A possible explanation for the higher susceptibility of the outer membrane to Ca^{2+}-induced permeabilization is that the outer membrane is linked covalently (via lipoprotein [9, 17]) and noncovalently (via the porins [29]) to the rigid murein layer. Microlesions in the outer membrane may result from the fact that these bonds do not allow shrinkage of the membrane after the binding of Ca^{2+} to negatively charged lipopolysaccharide or phospholipid molecules. Ca^{2+}-induced shrinkage of artificial membranes has been reported earlier (10).

MBP molecules that enter the periplasm stay active in this compartment and do not leak out upon washing and transfer into a new medium. This can be concluded from the high stability of maltose transport in these cells (Fig. 6). The increase in maltose transport which was observed in h 1 after reconstitution may be due to a better energization of the cells after this time. The fact that only very slow leakage of MBP was observed suggests that MBP is bound in the periplasm or that the outer membrane loses its leakiness after transfer to Ca^{2+}-free medium or both.

The K_m for maltose in reconstituted transport (2 μM; Fig. 8) is identical to that of wild-type cells (32). This shows that the reconstitution procedure is a gentle method allowing the normal function of all components involved in maltose transport. The high affinity clearly demon-

strates that reconstitution experiments with whole cells not only allow testing of the interaction of MBP with the inner membrane MalF, G, K protein complex but also allow testing of the interaction of MBP with the outer membrane maltoporin. This latter interaction was shown to be required for the high affinity of the maltose transport system in wild-type cells (13, 35). The physical interaction of MBP and maltoporin has been directly demonstrated recently by affinity chromatography (5). It is interesting to note that *lamB* derivatives of strain HS3018 showed a reduced (10%) competence for reconstitution (maltose transport was measured at 10^{-4} M; data not shown). Experiments are in progress to decide whether the reduced reconstitution is due to the reduced import of MBP into the periplasm of *lamB* cells or to the increased exit of MBP after transfer into MBP-free medium.

The concentration of purified MBP necessary for half-maximal reconstitution of maltose transport in Δ*malE* cells (1 mM; Fig. 9) comes very close to the estimated MBP concentration in the periplasm of maltose-induced wild-type cells (12). This high concentration may reflect the affinity of MBP for interaction with one of the other four maltose transport proteins. Preliminary experiments suggest that the component with the lowest affinity for MBP is the maltoporin.

The reconstitution procedure appears to be generally applicable, since other binding protein-dependent transport systems could also be reconstituted (Table 2). The method is fast and easy enough to be used as a screening procedure to distinguish transport mutations affecting only a periplasmic binding protein (LA6021, LA6022, and TA2918) from those also affecting additional transport components of the respective transport system (LA6028 and TA1835).

Thus, the Ca^{2+} treatment described here represents a gentle method of permeabilization of the outer membrane, allowing a variety of macromolecules access to the periplasm. The interaction of soluble binding proteins with membrane-bound proteins involved in transport or chemotaxis can now be studied in detail with the advantage of defining the K_d of this protein-protein interaction in vivo.

ACKNOWLEDGMENTS

We are grateful to W. Boos and M. Manson for critical discussions and for valuable help in preparing the manuscript. We thank G. Ames and W. Boos for providing us with strains.

The investigation was supported by grants from SFB 138 of the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie.

LITERATURE CITED

1. **Ames, G. F.** 1964. Uptake of amino acids by *Salmonella typhimurium*. Arch Biochem. Biophys. **104**:1–18.
2. **Ames, G F., and J. Lever.** 1970. Components of histidine transport: histidine binding proteins and *hisP* protein. Proc. Natl. Acad. Sci. U.S.A. **66**:1096–1103.
3. **Ames, G. F., K. D. Noel, H. Taber, E. Negri Spudich, K. Nikaido, I. Afong, and F. Ardeshir.** 1977. Fine structure map of histidine transport genes in *Salmonella typhimurium*. J. Bacteriol. **129**:1289–1297.
4. **Barash, H., and Y. S. Halpern.** 1971. Glutamate-binding protein and its relation to glutamate transport in *Escherichia coli* K-12. Biochem. Biophys. Res. Commun. **45**:681–688.
5. **Bavoil, P., and H. Nikaido.** 1981. Physical interaction between the phage λ receptor protein and the carrier immobilized maltose-binding protein of *Escherichia coli*. J. Biol. Chem. **256**:11385–11388.
6. **Bergmans, H. E. N., J. M. van Die, and W. P. M. Hoekstra.** 1981. Transformation in *Escherichia coli*: stages in the process. J. Bacteriol. **146**:564–570.
7. **Brass, J. M.** 1982. Reconstitution of maltose transport in *malB* and *malA* mutants of *Escherichia coli*. Ann. Microbiol. (Paris) **133A**:171–180.
8. **Brass, J. M., W. Boos, and R. Hengge.** 1981. Reconstitution in *malB* mutants of *Escherichia coli* through calcium-induced disruptions of the outer membrane. J. Bacteriol. **146**:10–17.
9. **Braun, V., and K. Rehn.** 1969. Chemical characterization, spatial distribution and function of a lipoprotein (murein-lipoprotein) of the *E. coli* cell wall. The specific effect of trypsin on the membrane structure. Eur. J. Biochem. **10**:426–438.
10. **Dawson, R. M. C., and H. Hauser.** 1970. Binding of calcium to phospholipids, p. 17. *In* A. W. Cuthbert (ed.), Calcium and cellular function. Macmillan and Co., London.
11. **DePedro, M. A., and U. Schwarz.** 1981. Heterogeneity of newly inserted and pre-existing murein in the sacculus of *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **78**:5856–5860.
12. **Dietzel, I., V. Kolb, and W. Boos.** 1978. Pole cap formation in *Escherichia coli* following induction of maltose binding protein. Arch. Microbiol. **118**:207–218.
13. **Ferenci, T., and W. Boos.** 1980. The role of the *Escherichia coli* lambda receptor in the transport of maltose and maltodextrins. J. Supramol. Struct. **13**:101–116.
14. **Ferenci, T., and U. Klotz.** 1978. Affinity chromatographic isolation of the periplasmic-binding protein of *Escherichia coli*. FEBS Lett. **94**:213–217.
15. **Galloway, D. R., and C. E. Furlong.** 1979. Reconstitution of binding protein-dependent ribose transport in spheroplasts of *Escherichia coli* K-12. Arch. Biochem. Biophys. **197**:158–162.
16. **Gerdes, R. G., K. P. Strickland, and H. Rosenberg.** 1977. Restoration of phosphate transport by the phosphate-binding protein in spheroplasts of *Escherichia coli*. J. Bacteriol. **131**:512–518.
17. **Hirota, Y., H. Suzuki, Y. Nishimura, and S. Yasuda.** 1977. On the process of cellular division in *Escherichia coli*. A mutant of *E. coli* lacking a murein-lipoprotein. Proc. Natl. Acad. Sci. U.S.A. **74**:1417–1420.
18. **Hofnung, M., D. Hatfield, and M. Schwartz.** 1974. *malB* region of *Escherichia coli* K-12: characterization of new mutants. J. Bacteriol. **117**:40–47.
19. **Hofnung, M., A. Jezierska, and C. Braun-Breton.** 1976. *lamB* mutations in *E. coli* K-12. Growth of lambda host range mutants and effects of nonsense suppressors. Mol. Gen. Genet. **145**:207–213.
20. **Hunt, A. G., and J. S. Hong.** 1981. The reconstitution of binding protein-dependent active transport of glutamine in isolated membrane vesicles from *Escherichia coli*. J. Biol. Chem. **256**:11988–11991.
21. **Leive, L.** 1965. A non-specific increase in permeability in *E. coli* produced by EDTA. Proc. Natl. Acad. Sci. U.S.A. **53**:745–750.
22. **Mandel, M., and A. Higa.** 1970. Calcium-dependent bacteriophage DNA infection. J. Mol. Biol. **53**:159–162.

23. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratories, Cold Spring Harbor, N.Y.
24. **Müller, N., H. G. Heine, and W. Boos.** 1982. Cloning of *mglB*, the structural gene for the galactose-binding protein of *Salmonella typhimurium* and *Escherichia coli*. Mol. Gen. Genet. **185**:473–480.
25. **Neu, H. C., and L. A. Heppel.** 1965. The release of enzymes from *Escherichia coli* by osmotic shock and during the formation of spheroplasts. J. Biol. Chem. **240**:3685–3692.
26. **Norgard, M. V., K. Keem, and J. J. Monahan.** 1978. Factors affecting the transformation of *Escherichia coli* strain χ 1776 by pBR322 plasmid DNA. Gene **3**:279–292.
27. **Richarme, G., and A. Kepes.** 1974. Release of glucose from purified galactose-binding protein of *Escherichia coli* upon addition of galactose. Eur. J. Biochem. **45**:127–133.
28. **Robb, F. T., and C. E. Furlong.** 1980. Reconstitution of binding protein dependent ribose transport in spheroplasts derived from a binding protein negative *Escherichia coli* K12 mutant and from *Salmonella typhimurium*. J. Supramol. Struct. **13**:183–190.
29. **Rosenbusch, J. P.** 1974. Characterization of the major envelope protein from *Escherichia coli*. J. Biol. Chem. **249**:8019–8029.
30. **Sabelnikov, A. G., and J. V. Domaradsky.** 1981. Effect of metabolic inhibitors on entry of exogenous deoxyribonucleic acid into Ca^{2+}-treated *Escherichia coli* cells. J. Bacteriol. **146**:435–443.
30a. **Shuman, H. A.** 1982. Periplasmic binding protein independent active transport of maltose in a mutant of *Escherichia coli* K-12: evidence for a substrate recognition site in the cytoplasmic membrane. J. Biochem. **252**:5455–5467.
31. **Silhavy, T. J., and W. Boos.** 1974. Selection procedure for mutants defective in the β-methylgalactoside transport system of *Escherichia coli* utilizing the compound 2R-glyceryl-β-D-galactoside. J. Bacteriol. **120**:424–432.
32. **Szmelcman, S., M. Schwartz, T. J. Silhavy, and W. Boos.** 1976. Maltose transport in *Escherichia coli* K12. Eur. J. Biochem. **65**:13–19.
33. **Taketo, A.** 1974. Sensitivity of *Escherichia coli* to viral nucleic acid. VIII. Idiosyncrasy of Ca^{2+}-dependent competence for DNA. J. Biochem. **75**:895–904.
34. **Tilby, M., J. Hindennach, and U. Henning.** 1978. Bypass of receptor-mediated resistance of colicin E3 in *Escherichia coli* K12. J. Bacteriol. **136**:1189–1191.
35. **Wandersman, C., M. Schwartz, and T. Ferenci.** 1979. *Escherichia coli* mutants impaired in maltodextrin transport. J. Bacteriol. **140**:1–13.