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NOTES

Bacteriophage Lambda Receptor Protein in *Escherichia coli* K-12: Lowered Affinity of Some Mutant Proteins for Maltose-Binding Protein In Vitro

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Mutant and wild-type LamB proteins (phage λ receptor proteins) were purified by affinity chromatography with immobilized maltose-binding protein, and their transport functions were tested in reconstituted liposomes. Two mutant proteins exhibited a marked decrease in affinity for immobilized maltose-binding protein, as well as altered transport rates.

Nutrient transport in gram-negative bacteria involves passage across the outer membrane, the periplasmic space, and the inner, or cytoplasmic, membrane. In *Escherichia coli*, maltose transport involves the outer membrane protein coded for by the *lamB* gene (the receptor for bacteriophage λ, called the LamB protein), a periplasmic binding protein coded for by *malE*, and three inner membrane proteins coded for by genes designated *malF*, *malG* and *malK* (3). Little is known about the exact function of the inner membrane components in active transport. The LamB protein, required for transport when the external concentration of maltose is low (15), has been shown to form transmembrane channels specific for maltose and maltodextrins in reconstituted liposomes (7, 11).

The precise role of the periplasmic maltose-binding protein (MBP) in transport is unknown, but genetic (18) and physiological (4) evidence suggests that it interacts with the LamB protein in the transport process. Recently a physical interaction between the LamB protein and immobilized MBP has been detected by affinity chromatography (1).

Missense mutations in the *lamB* gene have been classified according to their effects on infection by λ and its host range mutants and were found to reduce initial rates of maltose uptake by 20 to 94% (15). Recently the nucleotide sequence of the entire *lamB* gene has been determined (2), and the precise amino acid changes in some of the *lamB* missense mutations were identified (M. Hofnung, personal communication). Therefore, biochemical analysis of the mutant LamB proteins could be expected to allow a correlation of genetic changes with functional properties and to reveal information about the mechanism of transport.

In this study we describe the purification of mutant LamB proteins by affinity chromatography, the transport properties of these proteins in reconstituted liposomes, and the weakened interaction between two mutant LamB proteins and immobilized MBP.

Strains of *E. coli* K-12 carrying mutations in *lamB* were derived from pop1021 (HfrG6 *metA trpE thi galE galK rpoB*) by M. Hofnung (5, 15) (Table 1). Since these strains all contained porins in their outer membranes, the porin-lacking K-12 strain T19 (7) was used as another control. Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis was performed according to Lugtenberg et al. (9). Protein was determined by the Lowry et al. (6) procedure, with the addition of 1% SDS for samples containing Triton X-100 (13).

Preliminary experiments were performed with mutant strain pop1091 to compare various conditions for extraction of LamB protein. On the basis of the results, the following standard procedure was used for purification of all of the LamB mutant proteins. Bacteria were grown at 37°C in L broth (14) containing 0.8% maltose. Whole envelopes were prepared from exponential-phase cells as described previously (14) and were extracted with Triton X-100 in the presence of EDTA (1). For affinity chromatography, about 2 ml of extracts obtained from envelopes and containing 15 mg of protein was applied to a 6-ml column of Sepharose 6MB beads carrying covalently linked MBP (1), and the column was washed with 50 ml of 0.1% Triton X-100–10 mM Tris-hydrochloride (pH 7.2), followed by elution with 15 ml of 10 mM Tris-hydrochloride (pH 7.2)–0.1% Triton X-100 (Tris-Triton buffer) con-

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TABLE 1. Permeability of channels formed by LamB proteins in reconstituted liposomes[a]

Source of LamB protein	Mutation in *lamB*	Relative rates of permeation of[b]: Mal	Glc	Gal	Suc	Lac	Glc_3	Maltose permeation rates[c]	Maltose uptake by intact cells[d]
T19	None	1.00	1.89	1.31	0.10[e]	<0.03[e]	0.29	1.48	ND[f]
pop1021	None	1.00	1.79	1.53	0.07[e]	<0.03[e]	0.39	0.83	100
pop1079	*lamB101*	1.00	2.79	2.25	0.78	<0.03	0.27	0.62	24
pop1084	*lamB106*	1.00	2.57	2.40	0.22	<0.03	0.31	0.71	68.5
pop1086	*lamB108*	1.00	2.56	3.09	1.24	<0.03	0.23	1.45	21
pop1087	*lamB109*	1.00	3.12	2.46	1.19	0.12	0.21	1.64	6.5
pop1088	*lamB110*	1.00	1.92	1.49	0.14	<0.03	0.28	0.90	80
pop1091	*lamB113*	1.00	2.10	2.06	0.49	<0.03	0.28	0.64	46.5

[a] A 1/24 aliquot of liposomes reconstituted from 2 μmol of phospholipids and 0.4 μg of protein was diluted into 630 μl of sugar solution at the isotonic concentration in 5 mM Tris-hydrochloride (pH 7.4), and the initial rate of swelling was followed as a decrease in turbidity (7).

[b] Mal, Glc, Gal, Suc, Lac, and Glc_3 denote maltose, D-glucose, D-galactose, sucrose, lactose, and maltotriose, respectively.

[c] Initial rate of maltose transport in liposomes calculated as optical density units per minute per microgram of LamB protein (average of at least two determinations).

[d] Initial rate of maltose uptake in whole cells, determined by Szmelcman and Hofnung (15) using 3.5 μM maltose. The values are relative rates normalized to the rate in pop1021.

[e] Similar results obtained with T19 and pop1021 preparations indicate that the LamB proteins purified by affinity chromatography are essentially free from porins, which would allow the diffusion of sucrose and maltose at rates similar to that of maltose (12).

[f] ND, Not determined.

taining 0.2 M NaCl. Eluted fractions were dialyzed overnight at 4°C against 10 mM Tris-hydrochloride (pH 7.4)–0.1% Triton X-100–0.01% NaN_3 and then stored at 4°C.

When the Triton-EDTA extract from pop1091 was chromatographed on MBP-Sepharose in Tris-Triton buffer and eluted with NaCl, the LamB protein appeared as a doublet on SDS-polyacrylamide gels. Inclusion of 2 mM phenylmethylsulfonyl fluoride (PMSF), a protease inhibitor, in the extraction mixture and elution buffer prevented doublet formation. Therefore, PMSF was routinely added in the extraction and chromatography of mutant proteins. Comparison of the pop1091 LamB doublet with the pop1091 protein obtained in the presence of PMSF and also with wild-type LamB protein purified with either Triton X-100 and cholate (7) or SDS (10) indicated that the modified protein has a slightly retarded mobility on SDS-polyacrylamide gels.

Figure 1 shows the SDS-polyacrylamide gel electrophoresis profile of the mutant LamB proteins obtained by this procedure. The purity of most of the LamB proteins was quite high as seen in the figure, but two mutants, pop1086 and pop1087, had LamB proteins that were eluted from the immobilized MBP with considerably less purity. Furthermore, a much larger fraction of the LamB proteins from these two strains eluted in the flow-through fractions from the column, and a much smaller fraction of these proteins was recovered in the NaCl-eluted fractions, in comparison with the wild type, pop1021 (Fig. 2). This indicates that the two mutant LamB proteins adhere to immobilized MBP much less than does wild-type LamB protein.

Liposomes were reconstituted from the purified LamB proteins and *E. coli* phospholipids as described previously (7), and the rates of penetration of various sugars through the protein channel were determined from the swelling rates of these liposomes (7). The specificity for most of the mutant proteins reflected that of wild-type LamB protein (Table 1). Within the maltose series, the rate of glucose diffusion was two to three times the rate of maltose permeation, while the rate for maltotriose was around one-third the rate for maltose, in agreement with our previous findings with the wild-type protein (7). For most LamB strains, lactose permeation was too slow to detect, and sucrose diffusion occurred at very low rates. However, two mutant proteins, those from pop1086 and pop1087, allowed the diffusion of sucrose at high rates, comparable to those for maltose. These two proteins also gave higher relative permeation rates for the monosaccharides, glucose and galactose, than observed with the wild-type proteins. In addition, LamB proteins from pop1079 and pop1091 gave somewhat higher relative permeation rates for sucrose and galactose than observed with the wild-type LamB protein.

A comparison of the absolute rates of maltose permeation through the reconstituted channels formed by most of the various LamB proteins

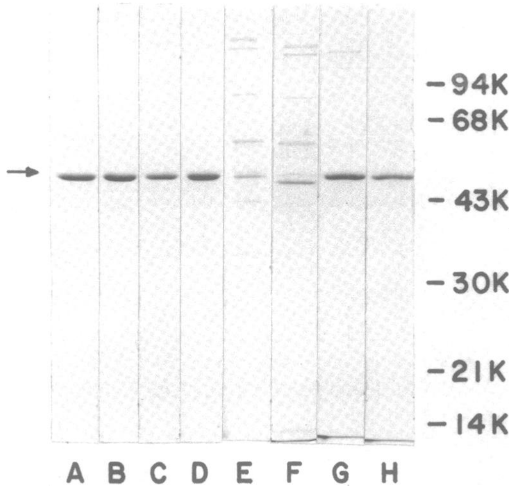

FIG. 1 LamB proteins purified by affinity chromatography from eight *E. coli* K-12 strains. Triton-EDTA extraction and chromatography on MBP-Sepharose 6MB were performed as described in the text with the addition of 2 mM PMSF throughout the procedure. SDS-polyacrylamide gel electrophoresis was performed with 10 μl of the extracts and approximately 2 μg of LamB protein from the NaCl-eluted fractions of the MBP column. The figure shows LamB proteins purified from T19 (lane A), pop1021 (lane B), pop1079 (lane C), pop1084 (lane D), pop1086 (lane E), pop1087 (lane F), pop1088 (lane G), pop1091 (lane H). The positions of molecular weight standards are also shown. The position of the LamB protein is indicated by the arrows. The protein from pop1087 always migrated slightly more rapidly than the proteins from other strains. The figure was made by placing together portions from a photograph of a gel containing many more lanes. The slightly faster migration of the LamB protein from pop1087 (lane F) was observed consistently even in gels of crude outer membrane proteins.

showed only slight differences, except that significantly higher rates were obtained with proteins from pop1086 and pop1087 (Table 1).

An additional kinetic comparison was made by following the inhibition by maltoheptaose of maltose transport (8) in liposomes containing each of the mutant LamB proteins. The data obtained with increasing amounts of maltoheptaose were analyzed by a Dixon plot (8). The apparent K_i values for maltoheptaose obtained for the LamB proteins from all of the pop strains listed in Table 1 were in the range of 0.9 to 1.1 mM, except for the protein from pop1087, for which reliable measurement of K_i could not be obtained because of deviation from linearity even at low concentrations of maltoheptaose.

In conclusion, this study showed that many of the *lamB* missense mutants showing alterations in maltose transport in intact cells produced LamB proteins altered in their behavior in the liposome swelling assay (Table 1). It is particularly noteworthy that mutants showing the most severe impairment in maltose diffusion in intact cells, i.e., pop1086 and pop1087, gave very elevated rates of sucrose diffusion in vitro, suggesting a loosening of conformational discrimination in the diffusion channel. These results indicate that the liposome swelling assay does reflect certain facets of the properties of the LamB protein channel in intact cells. However, the specificity and rates observed in the swelling assay did not provide a complete explanation of the phenotype of intact cells. For example, the LamB proteins from those mutants with the lowest transport rates (pop1086 and pop1087) showed normal, or even slightly higher, rates of maltose diffusion in reconstituted liposomes (Table 1). In fact, the discrepancy between the in vitro and in vivo kinetic properties of the LamB channel has been noted before from a comparison of the rates observed with the wild-type Lamb proteins (7, 16). One possible explanation of this discrepancy is the role of MBP, which is present in intact cells and is known to interact directly with the LamB protein (1). In this connection, it is very suggestive that the two mutants with the lowest uptake rate in intact cells, pop1086 and pop1087, showed significantly weakened affinities for immobilized MBP. Thus it is possible that the interaction between the MBP and LamB protein is a prerequisite for the efficient diffusion of maltose through the

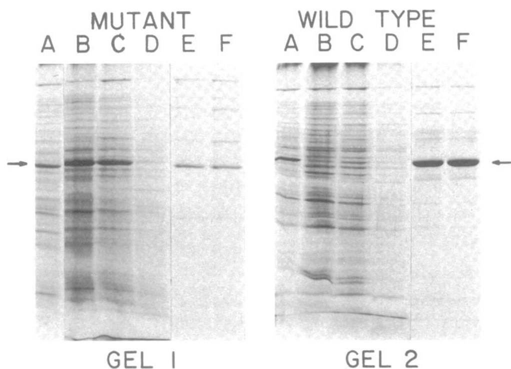

FIG. 2. Comparison of elution from MBP-Sepharose of LamB proteins from pop1086 and the parent strain, pop1021. Membranes from pop1086 (gel 1) and pop1021 (gel 2) were extracted with Triton-EDTA and chromatographed on MBP-Sepharose in Tris-Triton buffer as described in the text. A 10-μl portion of each supernatant from the extraction was applied to lane A. Larger portions (100 μl) of the fractions designated below were concentrated by evaporation at 100°C before application to the gel. Lanes B through D of both gels correspond to the peak fractions of the flow-through material. Lanes E and F correspond to the peak fractions of the NaCl eluates. The position of the LamB protein is indicated by the arrows.

LamB channel in intact cells; this model is different from an earlier one in which MBP was supposed to plug a normally open LamB channel (4). Our conclusion is also consistent with that of Wandersman et al. (18), who proposed that MBP and LamB protein cooperate in transporting maltodextrins across the outer membrane. Indeed, immobilized MBP from one of the *malE* mutants found to be unable to grow on maltodextrins (Dex^-) in that study (18) was recently shown not to bind to the wild-type LamB protein (P. Bavoil, Ph.D. dissertation, University of California, Berkeley, 1982).

The *lamB* mutants used in this study are all capable of growing on maltodextrins as the sole carbon source (Dex^+) (5). Different alterations of the transport channels can be expected in Dex^- *lamB* mutants. Such mutants, showing the Dex^- phenotype in the presence of reduced numbers of copies of the altered LamB protein, have recently been obtained by Wandersman and Schwartz (17) and are now under investigation in our laboratory.

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