Physical Interaction between the Phage λ Receptor Protein and the Carrier-immobilized Maltose-binding Protein of Escherichia coli*

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When Triton X-100/EDTA extracts of the outer membrane of Escherichia coli K12 were passed through a column containing maltose-binding protein covalently linked to Sepharose 6MB beads, the phage λ receptor protein or LamB protein was quantitatively and specifically adsorbed to the column and was eluted with a solution containing 1 mM NaCl, but not with that containing 0.5 mM maltose. The binding did not take place when columns containing inactive Sepharose beads alone, or Sepharose bound to histidine-binding protein of Salmonella typhimurium, were used. This interaction is consistent with the hypothesis that the periplasmic maltose-binding protein interacts with the part of the LamB protein exposed on the inner surface of the outer membrane, thereby increasing the specificity of the solute penetration process through the LamB channel.

Several transport systems in Gram-negative bacteria require the presence of periplasmic binding proteins, in addition to components located in the cytoplasmic membrane (1). It is now well established that these proteins play an essential role both in solute transport and chemotaxis, and this is thought to involve the physical interaction between the binding proteins and cytoplasmic membrane-associated proteins (2, 3).

It has been suggested, furthermore, that some binding proteins, such as maltose-binding protein of Escherichia coli, may additionally interact with components of the outer membrane. In E. coli, maltose diffuses across the outer membrane mainly through the channel produced by the phage λ receptor protein, or LamB protein, especially when the external concentration of maltose is low (4). Although the LamB channel allows the diffusion of solutes other than maltose and maltooligosaccharides both in intact cells (5) and in liposomes reconstituted with purified LamB protein (6), there are important quantitative differences. In the liposome system, glucose and lactose diffuse through the LamB channel at 290 and 9% of the rate for maltose (6); in contrast, in intact cells, the corresponding rates were about 0.5 and 0.3% of that of maltose, when calculations are made on the basis of published data (4, 5) by the procedure previously described (7). Since the intact cells, but not the liposomes, contain MBP, it seems possible that MBP may directly or indirectly modify the specificity of the LamB channel.

A more specific model involving physical interaction between MBP and the LamB protein has been proposed by other workers. To the best of our knowledge, such a model was proposed first by Szmelcman, and later by Endermann et al. (8). More recently, Heuzenroeder and Reeves (9) reached the same conclusion by studying transport of solutes in mutants lacking porins and containing or not containing MBP. Furthermore, the groups of Schwartz and Boos also proposed a similar hypothesis (10, 11) on the basis of the behavior of a strain producing altered MBP. However, the evidence presented so far has been rather indirect.

In this study, we describe the direct evidence for the specific physical interaction between LamB protein and carrier-immobilized MBP.

MATERIALS AND METHODS

Purified Proteins Used—MBP was purified from HN94 (12) grown in minimal medium M63 (13) containing 0.5% maltose, according to Ferenci and Klitz (14). The protein eluted from the affinity column was dialyzed against 10 mM Tris-Cl, pH 7.2, for at least 5 days. The sample appeared to be at least 95% pure on the basis of SDS-polyacrylamide gel electrophoresis followed by the scanning of the stained gel, one of the contaminating proteins corresponded in apparent molecular weight to amylomaltase.

The LamB protein, as well as porins 1a ("OmpF protein") and 1b ("OmpC protein") were purified from strains AB2894-T19 (5), JF701 (15), and JF703 (15), respectively, essentially as described by Tokunaga et al. (16), except that a Sephacryl S-200 column was used instead of Sepharose 4B. The pooled fractions were dialyzed against 3 mM sodium azide for at least 14 days at room temperature in order to remove most of SDS. Before application to the affinity column, the proteins were diluted in 2% Triton X-100/10 mM Tris-Cl, pH 7.2.

Purified histidine-binding protein from a hisJ5625 mutant of Salmonella typhimurium LT2 (2) was a gift of K. Nikaido and G. F.-L. Ames. Preparation of Outer Membrane Extracts—E. coli K12 strain HN94 (12) grown in Medium 83 containing 0.5% maltose and 40 μg/ml of L-arginine. Outer membranes were prepared from exponentially growing cultures according to Smit et al. (17).

Purified outer membranes containing 4 mg of protein were extracted in 10 mM Tris-Cl, pH 7.2/10 mM EDTA/2% Triton X-100 (Sample A, 0.6 ml) at 4 °C for 30 min with intermittent sonications (1 min each) in a Bransonic 12 bath-type sonicator. The extract was obtained by centrifugation of the suspension at 20,000 × g for 1 h at 4 °C.

Preparation of Affinity Columns—MBP or histidine-binding protein (10 mg) in 0.1 M NaHCO3, pH 8.1/0.5 M NaCl was cross-linked to 2 g (dry weight) of Sepharose 6MB beads as follows. The beads were washed on a glass filter with 0.1 M NaHCO3, pH 8.1, after each of the following 3 steps. (i) The beads were first swollen and washed several times in 1 mM HCl, then added to 30 ml of the protein-containing solution and incubated for 2 to 24 h with shaking at 4 °C. (ii) Remaining binding sites were then blocked by incubating the beads for 1 h in 1 M ethanolamine, pH 9, at 24 °C. (iii) Noncovalently bound material was then removed by 2 successive washes in 0.1 M acetate buffer, pH 4/0.5 M NaCl and 0.1 M borate buffer, pH 8/0.5 M NaCl. This washing step was repeated 4 times.

The protein-bead complexes were then packed in a Pharmacia Sepharose 4B column and stored in 10 mM Tris-Cl, pH 7.2, containing 0.1% Triton X-100 and 0.01% NaN3 at 4 °C. The column volume was about 6 ml.

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1 The abbreviations used are: MBP, maltose-binding protein; SDS, sodium dodecyl sulfate.

2 S. Szmelcman, (1976) Ph.D. thesis, Université de Paris.
Other Methods—SDS-polyacrylamide electrophoresis was performed according to Laemmli et al. (18). Protein was determined by the Lowry method (19). With eluates from the column, scanning of the Coomassie blue-stained gels was used to estimate the fraction of proteins eluted.

RESULTS

Selective Adsorption of LamB Protein to MBP Affinity Columns—Lucky and Nikaido (6) have shown that Triton X-100/EDTA mixture effectively extracts LamB protein from E. coli outer membranes. This observation was confirmed in this study. As they found, the procedure was somewhat selective, and much of porins and the OmpA protein remained in the insoluble fraction whereas the LamB protein was mostly extracted into the soluble fraction (Fig. 1).

Such Triton X-100/EDTA extracts were applied onto an affinity column made of purified MBP covalently bound to Sepharose 6MB beads. Fig. 2 shows the typical elution profile obtained in such an experiment: all proteins were eluted from the column uniformly in the flow-through fractions with the exception of the LamB protein which remained almost totally adsorbed on the column. Although a few proteins other than the LamB protein were also bound to the column in some experiments (such as the one shown in Fig. 2), this was not reproducible. For example, small fractions of the porins were found to bind to the column especially when the sample was the crude extract of the outer membrane and the column had accumulated bound LamB proteins through several previous runs without elution with 1 M NaCl (see below). In contrast, when purified porin 1a or 1b was applied to a freshly made column, there was never any adsorption of these proteins.

These results suggest that the occasional, weak adsorption of non-LamB proteins is probably caused by the interaction of these proteins with the LamB protein already bound to the column.

Washing the column with up to 3 column volumes of 0.1% Triton X-100/10 mM Tris-HCl, pH 7.2, did not elute any of the bound LamB protein. Continued washing with larger amounts of the same buffer sometimes resulted in a very slow release of a small fraction of the LamB protein initially bound. Washing the column with 0.5 M maltose/0.1% Triton X-100/10 mM Tris-HCl, pH 7.2, produced very similar results, and efficient elution was not obtained (results not shown). However, washing with 1 M NaCl/0.1% Triton X-100/10 mM Tris-HCl, pH 7.2, eluted all (90-120%) of the bound LamB protein in a fraction close to the solvent front (Fig. 3).

To demonstrate the specificity of the binding of the LamB protein to MBP, two new columns were made: (a) a "blank" column in which the Sepharose 6MB beads were treated as described under "Materials and Methods" but no protein was added in the reaction mixture; (b) a column in which the Sepharose 6MB beads were cross-linked to a protein of similar properties as those of MBP, i.e. cellular location, function, isoelectric point (20), etc. Histidine-binding protein from a mutant strain of S. typhimurium was used in this control experiment (see "Materials and Methods"). Figs. 4 and 5 show the protein elution profiles obtained when Triton X-100/EDTA extracts of outer membranes were applied onto these two columns. As can be seen, all proteins including the LamB protein were eluted without significant retardation.

Adsorption of the Purified LamB Protein—In the experiments using Triton X-100/EDTA extracts of outer membranes, there remained a possibility that the LamB protein did not adsorb directly to the column and that the adsorption was mediated by some other components of the extract. However, when we applied to the column a highly purified preparation of the LamB protein, which was more than 94% pure on the basis of the scanning of the Coomassie blue-stained SDS-polyacrylamide gel, more than 90% of the LamB protein was adsorbed (not shown). Furthermore, the adsorbed LamB protein could not be eluted by washing with 3 column volumes of 0.5 M maltose/0.1% Triton X-100/10 mM Tris-HCl, pH 7.2, and a nearly complete adsorption took place when the purified LamB protein was applied to the column in this solution containing 0.5 M maltose (Fig. 6). This is significant because it rules out the possibility that the interaction between LamB protein and MBP is mediated by strands of protein and porins are indicated.

Fig. 1 (left). Preparation of outer membrane extracts. Outer membranes from HN94 were extracted as described under "Materials and Methods." Lane A, purified outer membranes; Lanes B and C, 2% Triton X-100/10 mM EDTA soluble and insoluble fractions, respectively. The bands of the LamB, OmpF, OmpC, and OmpA proteins are indicated.

Fig. 2 (center). Interaction of outer membrane proteins with MBP-Sepharose. Triton X-100/EDTA extraction was performed as described under "Materials and Methods". A portion of the supernatant fluids (0.4 ml) was applied onto the column. It was washed at 4 °C with 10 mM Tris-HCl, pH 7.2/0.1% Triton X-100 at a flow rate of 0.6 ml/min. Fraction size was 0.185 ml. SDS-polyacrylamide gel electrophoresis was performed as follows: Lane A, 10 μl of sample applied to the column; Lanes B through J, 35 μl of fractions 16, 17, 18, 19, 20, 21, 22, 30, and 40, respectively. The positions of the LamB protein and porins are indicated.

Fig. 3 (right). Interaction of outer membrane proteins with MBP-Sepharose and elution of LamB protein with 1 M NaCl. This experiment is composed of 2 phases: (a) Triton X-100/EDTA extracted material was applied to the column, the column was washed as described in Fig. 2, and fractions 1 through 80 (0.185 ml each) were collected; (b) the column was then eluted with 10 mM Tris-HCl, pH 7.2/0.1% Triton X-100/1 M NaCl, and fractions 81 through 96 (0.925 ml each) were collected. SDS-polyacrylamide gel electrophoresis was performed as follows: Lane A, 10 μl of sample applied to the column; Lanes B through E, 40 μl of fractions 15, 20, 25, and 30, respectively; Lanes F through M, 230 μl of fractions 82, 84, 86, 88, 90, 92, 94, and 96, respectively (fractions 81 through 96 were dialyzed overnight against 10 mM Tris-HCl, pH 7.2/0.1% Triton X-100 and concentrated against Ficoll 400 before gel electrophoresis).
maldodextrins, which are known to bind both to the LamB channel (21, 22) and to MBP (4); the very high concentration of maltose used would obviously have competed against such interactions.

**DISCUSSION**

Our results demonstrate the existence of a rather strong, physical interaction between the LamB protein and the Sepharose-immobilized MBP. The specificity of this process is suggested by the following observations. (i) The adsorption is not due to the physical trapping of large protein aggregates, because a column of inactivated Sepharose 6MB did not produce any binding (Fig. 4). (ii) Although 1 M NaCl results in the dissociation of the complex, the association is unlikely to be due to the nonspecific electrostatic interaction between MBP and LamB proteins, because both are acidic proteins and because the column containing another periplasmic binding protein with a similar PI (20) did not retard the LamB protein (Fig. 5). (iii) Furthermore, the efficiency of the binding process was strikingly high. In a similar work using an affinity column containing MBP, Koiwai and Hayashi (3) found that only a few per cent of the methylated cytoplasmic membrane proteins were adsorbed, in spite of the fact that the Tar protein, which was expected to interact with MBP, must comprise a large fraction of such methylated proteins. In our case, practically complete adsorption was observed in every experiment (Figs. 2, 3, and 6), and these results were reproducible with columns made with three different batches of the MBP. (iv) Although porins share some of the gross physicochemical properties of the LamB protein in being acidic, in existing as stable oligomers that cannot be dissociated by SDS at moderate temperatures, and in associating noncovalently to peptidoglycan (8), they do not become bound to the MBP column. Although these lines of evidence are rather strong, we must emphasize that it is impossible to rule out the possibility that the observed association is an *in vitro* artifact. Demonstration of association between unmodified MBP and LamB protein and observation of the expected alteration of the properties of the LamB channel through this association are ultimately needed for proving unequivocally the physiological role of the presumed interaction phenomenon.

If we assume for the moment that the observed binding indeed reflects some facets of the physiological process, it seems surprising that maltose did not have a dissociating effect and that the binding was so strong. This is because one expects that MBP will leave the LamB protein, or dissociate itself from the outer membrane, upon the binding of maltose, and because the outer membrane preparations isolated with usual procedures (such as that described in Ref. 17) do not contain significant amounts of MBP.3 One explanation of our results is that MBP normally goes through a series of conformational changes as it binds LamB protein, then maltose, and finally the cytoplasmic membrane-associated component of the transport system, but in MBP-Sepharose, some MBP molecules are “frozen” in a conformation with a maximum affinity to LamB protein owing to the covalent linkages with Sepharose. Possibly this absence of conformational flexibility is allowing us, in this system, to observe a normally transient reaction on a slow time scale.

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**REFERENCES**

1. Wilson, D. B. (1978) *Annu. Rev. Biochem.* 47, 933-965
2. Ames, G. F.-L., and Spudich, E. N. (1976) *Proc. Natl. Acad. Sci. U. S. A.* 73, 1877-1881
3. Koiwai, O., and Hayashi, H. (1979) *Biochem. (Tokyo)* 86, 27-34
4. Szmelcman, S., and Hofnung, M. (1975) *J. Bacteriol.* 124, 112-118
5. von Meyenburg, K., and Nikaido, H. (1977) *Biochem. Biophys. Res. Commun.* 78, 1100-1107
6. Luckey, M., and Nikaido, H. (1980) *Proc. Natl. Acad. Sci. U. S. A.* 77, 167-171
7. Bavoil, P., Nikaido, H., and von Meyenburg, K. (1977) *Mol. Gen. Genet.* 158, 23-33
8. Endermann, R., Hindennach, I., and Henning, U. (1978) *FEBS Lett.* 88, 71-74
9. Heuzenroeder, M. W., and Reeves, P. (1980) *J. Bacteriol.* 141, 431-435
10. Wandersman, C., Schwartz, M., and Ferenci, T. (1979) *J. Bacteriol.* 140, 1-13
11. Ferenci, T., and Boos, W. (1980) *J. Supramol. Struct.* 13, 101-116
12. Bavoil, P., Hofnung, M., and Nikaido, H. (1980) *J. Biol. Chem.* 255, 8366-8369
13. Rickenberg, H. V., Cohen, G. N., Buttin, G., and Monod, J. (1956) *Ann. Inst. Pasteur* 91, 829-857
14. Ferenci, T., and Klotz, U. (1978) *FEBS Lett.* 94, 213-217

3 P. Bavoil, unpublished results.
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15. Chai, T.-J., and Foulds, J. (1978) *J. Bacteriol.* 135, 164-170
16. Tokunaga, M., Tokunaga, H., Okajima, Y., and Nakae, T. (1979) *Eur. J. Biochem.* 95, 441-448
17. Smit, J., Kamio, Y., and Nikaido, H. (1975) *J. Bacteriol.* 124, 942-958
18. Lugtenberg, B., Meijers, J., Peters, R., van der Hoek, P., and van Alphen, L. (1975) *FEBS Lett.* 58, 254-259
19. Lowry, O. H., Rosebrough, N., Farr, A. L., and Randall, R. J. (1951) *J. Biol. Chem.* 193, 265-275
20. Lever, J. E. (1972) *J. Biol. Chem.* 247, 4317-4326
21. Luckey, M., and Nikaido, H. (1980) *Biochem. Biophys. Res. Commun.* 93, 166-171
22. Ferenci, T., Schwentorat, M., Ulrich, S., and Vilmart, J. (1980) *J. Bacteriol.* 142, 521-526