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A Mutant Form of Maltose-Binding Protein of *Escherichia coli* Deficient in Its Interaction with the Bacteriophage Lambda Receptor Protein

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In one *malE* mutant known to be deficient in the transport of maltose and maltodextrins across the outer membrane, the altered MalE protein was shown to be defective in its interaction with the phage lambda receptor, or LamB protein, of the outer membrane.

Maltodextrins larger than maltotriose have sizes beyond the exclusion limit of the *Escherichia coli* porin channel (13) and therefore must cross the outer membrane through a specific channel produced by the phage lambda receptor protein, or LamB protein (14, 16). Maltose can diffuse through the porin channel (13, 15) at sufficient rates if the external concentration of maltose is high, e.g., higher than 1 mM. At low (e.g., less than 10 μM) external concentration of maltose, however, the rate of its diffusion through the porin channel will become insufficient for the maintenance of maximal growth rate, because the narrow diameter of the *E. coli* porin channel severely retards the diffusion of disaccharides such as maltose, and because the rate of diffusion through the porin channel is expected to decrease in proportion to the concentration difference of maltose across the outer membrane, that is, roughly in proportion to the external concentration of maltose (13, 15). Thus, under such conditions the presence of the LamB protein becomes essential for the rapid uptake of maltose through the outer membrane and eventually into the cytoplasm (16).

The LamB protein shows distinct specificity for facilitation of the diffusion of oligosaccharides of the maltose series when purified and reconstituted into phospholipid bilayers (10). Nevertheless, the LamB channel in intact cells appears to show significantly different discriminatory properties than those shown in the reconstituted system (17). Intact cells contain maltose-binding protein, or MalE protein, in the periplasmic space (9), that is, on the inner side of the outer membrane, whereas this protein is not present in reconstituted vesicles. Several workers thus proposed that the MalE protein directly interacts with the LamB protein and that this interaction has physiologically significant consequences (5, 7, 18). Two of us have presented a strong piece of evidence favoring this hypothesis. Thus, some missense mutations in the *malE* gene were found to produce strains that are apparently incapable of transporting maltodextrins across the outer membrane in spite of the presence of intact LamB protein (18). A priori, the MalE protein may be assisting in the influx of maltodextrins across the outer membrane, either (i) indirectly by binding to the maltodextrin molecules that have already reached the periplasmic space or (ii) directly by association with the LamB protein, thereby affecting the transmembrane flux process itself. The latter hypothesis was favored because the results of competition experiments suggested that maltotetraose could not cross the outer membrane in these mutants, in spite of the fact that the dissociation constant of the mutant MalE protein-maltotetraose complex was still in the range of 2×10^{-5} to 8×10^{-5} M (18).

These dissociation constant values, however, were at least an order of magnitude higher than the dissociation constant of the wild-type MalE protein. Thus, it was difficult to exclude completely the possibility that this change was affecting the behavior of the system, especially because the binding protein conceivably could behave in a different manner in the periplasmic space in comparison with its behavior in a dilute in vitro environment.

We therefore thought that a more direct piece of evidence for the postulated interaction between the MalE and LamB proteins would be desirable. In this note, we show by using the direct assay for the physical interaction between

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these proteins developed by Bavoil and Nikaido (2) that, at least in one of the mutants previously studied (18), there is indeed a drastic decrease in the ability of the MalE protein to associate with the LamB protein.

As in previous studies (2), the interaction was studied by examining the binding of the LamB protein to a column of Sepharose 6MB coupled to MalE protein. In this study, however, the MalE protein was purified not only from the wild-type cells but also from strain pop1151 (18), which carries the *malE88* mutation presumed to affect the interaction between the MalE and LamB proteins (18). The MalE proteins were purified according to published procedures (6) from cells grown in L broth (3) containing 0.25% maltose instead of glucose or in medium 63 (4) containing 0.5% maltose and coupled to Sepharose 6MB (Pharmacia) as described earlier (2), except that in both cases 7.25 mg of protein was coupled to 2 g (dry weight) of Sepharose 6MB. The coupling reaction went to completion, as judged by the absence of visible MalE protein bands upon the sodium dodecyl sulfate-polyacrylamide slab gel electrophoresis of the supernatant fluid of the coupling mixture.

Outer membrane of wild-type cells (strain HN94; see reference 1) was prepared, and Triton X-100-EDTA extracts of the outer membrane were then applied to the columns as described previously (2). The column containing immobilized wild-type MalE protein nearly completely adsorbed the LamB protein in the extract (Fig. 1A), whereas the column containing the mutant MalE protein showed very poor adsorption (Fig. 1B).

These results fully confirm the conclusion of Wandersman et al. (18). Thus, at least in one *malE* mutant that is impaired in the transport of maltodextrins across the outer membrane, there was a major decrease in the affinity of the MalE protein for the LamB protein, in addition to the alteration of its affinity to the ligands as described previously (18). It appears much easier, as discussed by Wandersman et al. (18), to explain the failure of the outer membrane diffusion process by the defective interaction between the two proteins than by the changes in the binding activity of the MalE protein.

Results that are almost a mirror image of the current study have recently been obtained by Luckey and Nikaido (11). In their analysis of *lamB* mutants with defective maltose transport across the outer membrane, originally isolated by Hofnung et al. (8), they found that the LamB proteins showed only minor changes in their channel properties, but showed more obvious decrease in their ability to become associated with the immobilized, wild-type MalE protein on the Sepharose column. Again here the more important changes seem to involve the interaction of LamB and MalE proteins. These results strongly suggest that physical cooperation of these two proteins is needed in the transport of maltose and maltodextrins across the outer membrane in intact cells and that reconstituted systems used so far (10, 12) do not give accurate models of the entire physiological process. Mo-

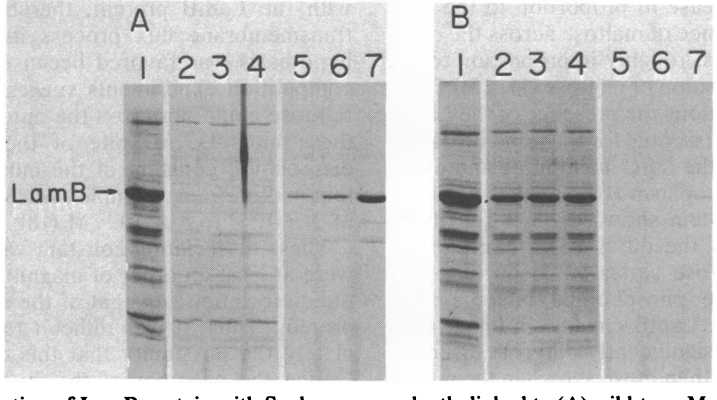

FIG. 1. Interaction of LamB protein with Sepharose covalently linked to (A) wild-type MalE protein and (B) mutant MalE protein. Triton X-100–EDTA-soluble material containing the wild-type LamB protein was applied to both columns. The columns were washed at 4°C with 10 mM Tris-hydrochloride (pH 7.2)–0.1% Triton X-100 at a flow rate of 0.6 ml/min. Fractions 1 through 80 (0.185 ml each) were collected. The columns were then eluted with 10 mM Tris-hydrochloride (pH 7.2)–0.1% Triton X-100–1 M NaCl, and fractions 81 through 96 (0.925 ml each) were collected. Samples for sodium dodecyl sulfate-polyacrylamide gel electrophoresis were as follows: lane 1, 15 μl of sample applied to the column; lanes 2 through 4, 35 μl of fractions 20, 21, and 22, i.e. the peak flow-through fractions; lanes 5 through 7, 40 μl of fractions 84, 85, and 86, containing the peak of NaCl-eluted proteins, applied after overnight dialysis against 10 mM Tris-hydrochloride (pH 7.2)–0.1% Triton X-100. The position of the LamB protein is indicated by an arrow.

lecular mechanisms of the transport by the putative LamB-MalE protein complex are subjects for future study.

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