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Relationship Between the OmpC and LamB Proteins of *Escherichia coli* and Its Influence on the Protein Mass of the Outer Membrane

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Cultures of *Escherichia coli* K-12 which possessed varied amounts of the LamB protein were found to contain reduced amounts of the OmpC protein. This process was regulated in part at the level of transcription. However, additional controls were inferred from anomalously high levels of the OmpC protein present at low levels of the LamB protein. Both proteins were present at increased levels, and this led to an increase in the total outer membrane protein mass per cell. The absolute amount of outer membrane protein per cell was found not to be a constant as had been tacitly assumed.

It is a prevailing concept that *Escherichia coli* is capable of adjusting for reductions or losses in major outer membrane proteins by a compensatory increase in other major outer membrane proteins. These changes in the levels of individual proteins may be environmentally induced or may arise from the mutational loss of proteins (i.e., 1, 7, 8, 11, 14, 15, 17). It is implied from these observations that the culture endeavors to maintain a constant outer membrane protein mass per cell.

A few studies have approached quantitatively the phenomenon of protein compensation (6, 16). However, the conclusions have been based on relative changes among the major outer membrane proteins. In this communication, we report on the relationship between the OmpC and LamB proteins and the effect of this relationship upon the absolute outer membrane protein mass.

MATERIALS AND METHODS

Bacterial strains and cultivation. The *E. coli* K-12 strains used in this study were: CS138 (W1485F$^-$*gyrA*) (1), MC4100 (F$^-$Δ*lacU169 araD139 rpsL relA thiA*) (6), and MH225 [MH110 λp1(209) ΔMuΦ(*ompC-lacZ*$^+$)10–25] (6).

Rotory shaken cultures were grown at 36°C and harvested in exponential phase at a density of 2.8 × 10^8 to 3.6 × 10^8 cells per ml. Cell counts were determined with a Coulter Counter (5). Cultures were grown in L-salts minimal medium (2) with either 0.5% (wt/vol) glucose or 0.5% (wt/vol) K lactate for the control cultures. Cultures containing various levels of the LamB protein were obtained by growth of the cultures in a variety of maltose concentrations which were determined empirically for each strain. Maltose and maltotriose were used as sole carbon sources at 0.1% (wt/vol) to generate cultures with high levels of the LamB protein. Intermediate and low levels of the LamB protein were induced by cultivation in media containing 0.5% K lactate (wt/vol) supplemented with maltose from 0.005% to 0.3%.

Cultures were radioactively labeled with [4,5-^{3}H]leucine (ICN) at 0.4 μCi per ml (specific activity: 55 Ci/mmol) and 20 μg of carrier L-leucine per ml.

Analytical methods. Triton-insoluble outer membranes were prepared as previously described (2). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed as previously described (1, 2), with the exception that 10- by 16-cm gels were used. Staining and destaining was by the method of Fairbanks et al. (4).

Stained bands of the LamB, OmpF, OmpC, and OmpA proteins were excised from the gels and destained completely with two washes of 2-propanol–acetic acid–water (13:5:32). The destained gel slices were incubated for 3 h at 60°C in Soluene 350 (Packard Instrument Co.) and counted in a liquid scintillation spectrometer after the addition of a toluene-based counting fluid.

The radioactivity in each protein was expressed as a percentage of the total protein radioactivity applied to the gel. This was found to yield the same results as control experiments in which the remainder of the gel was sliced and counted to arrive at a sum of the total radioactivity applied to the gel.

The absolute amount of outer membrane protein per cell was measured as a glycine/diaminopimelate ratio by amino acid analysis. Triton-insoluble outer membrane preparations (2 mg) were hydrolyzed for 18 h in 6 N HCl in vacuo. Amino acid analysis was performed on a Glenco automated amino acid analyzer by using a citrate buffer system through a 0.325- by 40-cm column of Aminex HP-C (Bio-Rad Laboratories). A digital integrator (Spectra-Physics) was fitted to the ninhydrin detector.

Protein was estimated by the method of Lowry et al.

(10). β-Galactosidase was determined by the method of Miller (12).

RESULTS AND DISCUSSION

We observed that the sodium dodecyl sulfate-polyacrylamide gel electrophoresis profiles of two K-12 strains of *E. coli* possessed reduced levels of the OmpC protein when the LamB protein was present in the outer membrane. This phenomenon was quantitated to establish a stoichiometry for this apparent inverse relationship. The quantitation was performed by measuring the [4,5-^{3}H]leucine radioactivity in each of the major outer membrane proteins and determining the contribution of each protein to the total outer membrane protein radioactivity applied to the gel. The approach was to empirically generate cultures containing different amounts of the LamB protein and to harvest these at the same cell density. We have not inferred that the measured levels of individual proteins were steady-state measurements.

Figure 1 shows the relationship between the OmpC and LamB proteins in two *E. coli* K-12 strains. Strain CS138 (open circles) contained proportionally reduced levels of the OmpC protein when the level of the LamB protein increased from 4 to 13% of the total outer membrane protein. Strain MC4100 (filled circles) displayed the same relationship from 2% to slightly over 9% LamB protein. The highest levels of the LamB protein shown in Fig. 1 were the highest levels achieved in this study.

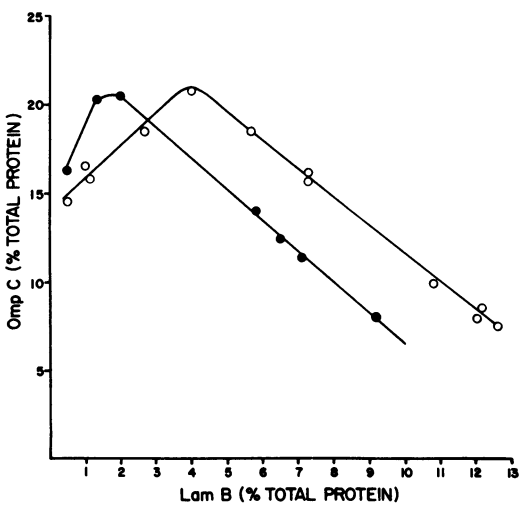

FIG. 1. Influence of the level of the LamB protein on the level of the OmpC protein in two strains of *E. coli* K-12. The levels of the two proteins were measured by determining the amount of [4,5-^{3}H]leucine in each band from sodium dodecyl sulfate-polyacrylamide gel electrophoresis and expressing this as a percentage of the total protein radioactivity applied to the gel. Symbols: ○, strain CS138; ●, strain MC4100.

The mole percentages of leucine in the LamB protein and the OmpC protein are nearly identical at 6.2% (3) and 6.6% (2), respectively. A value of 6.4% can be calculated for the OmpC protein from Ichihara and Mizushima (9). Thus, a comparison of the radioactivity possessed by each of the proteins is an accurate comparison of their relative mass contributions to the outer membrane. If the two strains were replacing the OmpC protein with the LamB protein by a mass stoichiometry, the slope of the curves in Fig. 1 should be −1.0. Alternatively, if the OmpC protein was being replaced by the LamB protein with a molar stoichiometry, the theoretical slope should be −0.78. This slope reflects the difference in molecular weights between these proteins by applying a molecular weight for the OmpC protein of 36,500 g mol^{-1} (2) and a molecular weight of 47,000 g mol^{-1} (3) for the LamB protein. The experimentally determined negative slopes in Fig. 1 for both strains were found to be −1.3. This calculation reveals that the mass lost from the outer membrane by the decreased level of the OmpC protein is not precisely compensated by an increase in the LamB protein.

The inverse relationship between the OmpC and LamB proteins does not hold true for either strain at low levels of the LamB protein (Fig. 1). Neither strain achieves the level of the OmpC protein predicted by extrapolation of the negative slope portion of the curves to zero concentration of the LamB protein. Both curves display a portion with a positive slope where both the OmpC and LamB proteins are increasing. When strain CS138 has an outer membrane with 4.0% LamB protein, it additionally possesses a 6.5% increase in the level of OmpC. If this observation is correct, there should be an increase of approximately 10% in the total outer membrane protein per cell. This assumes that there is not a compensatory decrease in the level of other outer membrane proteins.

The other major outer membrane proteins were examined to assess their contribution to the total outer membrane protein radioactivity applied to the gel. Although there were relative changes in the levels of the OmpF and OmpA proteins, the sum of the radioactivity in these two proteins yielded a remarkable constant percentage of the total radioactivity regardless of the levels of the OmpC and LamB proteins. The sum of the OmpF and OmpA proteins for strain CS138 was 42.0% ± 1.8% of the total Triton-insoluble [^{3}H]leucine radioactivity. Strain MC4100 possessed a contribution for OmpF plus OmpA of 45.4% ± 1.1%.

Since the OmpF and OmpA proteins did not decrease in cultures containing low levels of the LamB protein, we tested the prediction from

Fig. 1 that the increases observed for both the OmpC and LamB proteins should be additive to the outer membrane protein mass per cell. This was done by amino acid analysis of Triton-insoluble outer membranes. Triton-insoluble outer membranes contain the peptidoglycan of the cell, so that a measurement of protein mass per unit of peptidoglycan should be a measurement of the total outer membrane protein mass per cell. Since the peptidoglycan content of *E. coli* has been shown to be present at a constant amount per unit of surface area (18), there should be no errors introduced as a result of differences in cell size. We chose to measure the outer membrane protein mass per cell as a glycine/diaminopimelate ratio.

The glycine/diaminopimelate ratio increases as a function of the LamB protein content of strain CS138 to a level of 4% LamB protein (Fig. 2). LamB protein levels beyond 4% resulted in a relatively constant outer membrane protein mass as would be expected from Fig. 1. The increase in the protein mass of the outer membrane was calculated from Fig. 2 to be 19%. This was approximately twice the increase expected from Fig. 1 and suggests that other outer membrane proteins also increased. The observation that the OmpF plus OmpA protein sum is a constant suggests that these proteins additionally must be increased for them to provide a constant percentage to membranes which possess increased amounts of the OmpC and LamB proteins. Thus, cultures containing low levels of the LamB protein probably contain increased

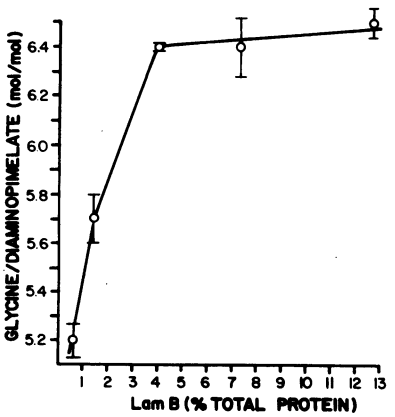

FIG. 2. Influence of the level of the LamB protein on the total outer membrane protein per cell in strain CS138. Total protein per cell was measured by determining the glycine/diaminopimelate molar ratios by amino acid analysis of hydrolysates of Triton-insoluble outer membranes from cultures containing various levels of the LamB protein.

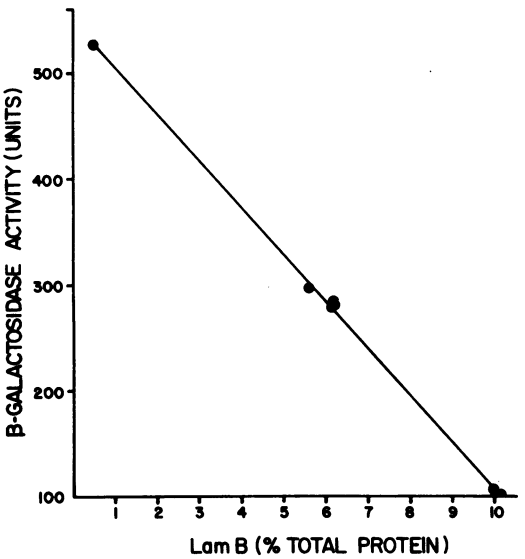

FIG. 3. Influence of the level of the LamB protein on the transcription of *ompC* by the amount of β-galactosidase produced in the *ompC-lacZ* promoter fusion strain MH225.

absolute levels of the OmpC, OmpF, and OmpA proteins.

Although it is not possible from these data to assess if there is any significance to a 19% increase in the total outer membrane protein mass per cell, the experiment shown in Fig. 2 clearly demonstrates that *E. coli* outer membranes are not at bulk saturation for outer membrane proteins. This conclusion was illustrated more dramatically in our studies on phenotypic revertants of *ompB151* mutants which exhibited the $OmpF^+$ $OmpC^-$ phenotype (unpublished data). These revertants possessed very high levels of the OmpF protein, and they were found to have a glycine/diaminopimelate ratio of 7.3 ± 0.2. This was a 31% increase of outer membrane protein above the level observed in the parent strain. The inordinately high levels of the OmpF protein resulted in an undetectable level of the OmpA protein by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. This reduction in the level of the OmpA protein further illustrates the effort by the cell to maintain a constant contribution of OmpF plus OmpA.

The complexity of the regulation of the OmpC protein in the presence of the LamB protein led to experiments with the *ompC-lacZ* fusion strain (MH225) of Hall and Silhavy (6) in an effort to determine the level at which the OmpC protein is regulated. Strain MH225, a promoter fusion derivative of strain MC4100, was used in an experiment similar to the one shown in Fig. 1. This experiment is shown in Fig. 3, and it shows

the influence of the LamB protein content on the level of *ompC* transcription (measured by β-galactosidase production).

This experiment revealed that the decreased levels observed for the OmpC protein in the presence of increased levels of the LamB protein occurred in part at the level of transcription. In contrast to Fig. 1, there was no discontinuity to the curve at low LamB protein levels. This observation suggests that transcriptional control is not a sufficient explanation for the anomalous levels of the OmpC protein observed in Fig. 1. At 4.0% LamB protein, the transcription of *ompC* was reduced by 29%; however, there was 6.5% more OmpC protein residing in the outer membrane.

Alternatively, it was possible that the disparity between Fig. 1 and Fig. 3 resulted from the shedding of outer membrane protein into the medium. We examined culture supernatants after ultrafiltration for outer membrane proteins (data not shown). Additionally, we subjected washed cell pellets to glycerol gradient purification and examined on gels all of the gradient fractions above the band of cells (data not shown).

We found in cultures fully induced for the LamB protein that 8.6% of the LamB protein could be recovered from the culture fluid and 1.3% of the total LamB protein could be recovered from the glycerol gradient fractions above the band of cells. The relative levels of major outer membrane proteins were identical in the Triton-insoluble outer membrane and in the outer membrane material recovered from the medium. Thus, neither the LamB or OmpC protein values given in Fig. 1 would be influenced more than 1% by the protein present in the medium. The glycine/diaminopimelate ratios would be influenced less than 2% by the small fraction of culture fluid material recovered with the cells.

The contrast between Fig. 1 and Fig. 3 shows that the results derived from fusion strains, where transcription is measured in the absence of the *ompC* gene product, should not be extrapolated to imply a quantitation of the level of the OmpC protein in the cell envelope. Additionally, we have shown that measurements of the relative amounts of outer membrane proteins may be misleading. The ability of *E. coli* to display variations in the absolute levels of outer membrane protein (Fig. 2) further complicates studies on the organization and assembly of the outer membrane. The increase in the protein mass of the outer membrane observed in this study would be predicted to exert an influence on the absolute levels or packing densities of the phospholipid and lipopolysaccharide. However, our results are not sufficiently extensive to permit speculation on their consequences. Since this study was not designed to reveal regulatory mechanisms, we have refrained from interpreting our data in this context.

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