Export-Defective LamB Protein Is a Target for Translational Control Caused by OmpC Porin Overexpression

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Overexpression of OmpC protein from an inducible plasmid vector reduced the amount of the precursor form of LamB protein in LamB signal sequence mutants. The stability of the precursor form of LamB protein was not affected, indicating that the effect of OmpC overexpression was on the synthesis of the precursor rather than on degradation. These results indicate that a functional signal sequence is not required on an outer membrane protein for it to be a target for translational control.

The rate of synthesis of bulk outer membrane protein is regulated in exponentially growing Escherichia coli cells so that the concentration of protein per unit surface area remains nearly constant independent of the growth rate or the induction of new surface proteins (2, 6). We studied the mechanism of this regulation by examining the consequences of induction of a very high level of expression of the OmpC porin from a multicopy plasmid expression vector (4). Overexpression of OmpC protein resulted in a rapid inhibition of the synthesis of other outer membrane proteins, such as OmpA and LamB, but had no effect on the synthesis of periplasmic proteins, such as maltose-binding protein (MBP) or β-lactamase (4; E. M. Click, unpublished data). Since there was no accumulation of precursors of the inhibited proteins and no significant reduction in transcription, we proposed that the inhibition was at the level of translation. A similar inhibition was caused by the ompC(Td) mutation, a six-base in-frame deletion near the 3' end of the OmpC-coding region. The ompC(Td) inhibition was relieved by introducing a signal sequence mutation which prevented secretion of the ompC(Td) product (3). This observation, together with the specificity of the inhibition of outer membrane proteins, led us to propose that there is a coupling between the translation of outer membrane proteins and their secretion (4).

To study the mechanism of this coupling in more detail, we asked whether a functional signal sequence must be present on an outer membrane protein for its expression to be inhibited by overexpression of OmpC protein. As in the previous study (4), we used the expression plasmid pEMC1, in which overexpression of OmpC protein is induced upon the addition of isopropyl-β-D-thiogalactoside (IPTG). This plasmid was introduced into strains which carried lamB mutations that altered or deleted the LamB signal sequence, thus causing the accumulation of precursors of LamB protein (pLamB) in the cytoplasm. The effect of induction of OmpC overexpression on the rate of synthesis of these precursors was measured by methionine pulse-labeling followed by immunoprecipitation.

Multicopy plasmid pEMC1 expresses the ompC 5' untranslated leader and coding region from a hybrid trp-lac (tac) promoter. Its construction and properties were described previously (4). The background strain in which wild-type LamB protein was expressed was a pEMC1 transformant of CS1389, a malPQ ompR151 ∆ompC178 derivative of JM101 (4). The 19R signal sequence mutation was introduced into CS1389 by cotransduction with linked transposon Tn10. The other strains carrying lamB mutations were constructed beginning with a ∆ompC178 malF::Tn10 derivative of JM101. Pl phage grown on strains carrying the desired lamB mutations were used to transduce this strain to Mal+ Tet+. After being screened for lamB mutations by λ sensitivity, these strains were transduced to ompR151 malPQ::Tn10 and transformed with pEMC1. The malPQ mutation, which prevents maltose metabolism, was necessary in strains which are both porin and maltoporin deficient to ensure efficient maltose induction and to prevent possible changes in growth rate caused by increased maltose uptake following induction of the OmpC porin.

To facilitate accurate quantitation, we measured pLamB relative to MBP. Proteins from pulse-labeled extracts were simultaneously immunoprecipitated with anti-LamB and anti-MBP sera and analyzed by gel electrophoresis (4, 11). Control lanes 1 and 2 show, respectively, immunoprecipitates from a lamBΔ106 mutant which produces a truncated precursor and from a lamB+ strain. Lanes 3 to 7 show immunoprecipitates from a lamBΔ60 signal sequence mutant which was labeled at various times after the induction of OmpC overexpression with 0.5 mM IPTG. Induction times are indicated in minutes above each lane.

FIG. 1. Autoradiograph showing the effect of IPTG-induced OmpC overexpression on the relative amounts of pLamB, MBP, and pMBP produced by a LamB signal sequence mutant. Cells were grown on glycerol-containing minimal medium and induced for one generation with maltose. Cells were pulse-labeled for 30 s with [35S]methionine, followed by a 15-s chase with unlabeled methionine, after which the total labeled protein was simultaneously immunoprecipitated with anti-LamB and anti-MBP sera and analyzed by gel electrophoresis (4, 11). Control lanes 1 and 2 show, respectively, immunoprecipitates from a lamBΔ106 mutant which produces a truncated precursor and from a lamB+ strain. Lanes 3 to 7 show immunoprecipitates from a lamBΔ60 signal sequence mutant which was labeled at various times after the induction of OmpC overexpression with 0.5 mM IPTG. Induction times are indicated in minutes above each lane.

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![Graphs showing induction time for LamB protein and precursors](image)

**FIG. 2.** Relative synthesis of LamB by the wild type and various signal sequence mutants after IPTG induction of OmpC overexpression. Panel B shows the structure of the signal sequence in the wild type (WT) and the mutants and is adapted from the data of Emr et al. (8). The data in panel A were obtained by densitometric analysis of autoradiographs like that shown in Fig. 1., and for each strain ○ and ● represent separate experiments. For each time point the density of the LamB or pLamB band was corrected for background density and expressed relative to the density of the MBP band. For each labeled culture these values were normalized to the value at induction time zero. bp, Base pairs.

ti-MBP sera, and gels were loaded with a constant number of $^{35}$S counts in each well. A representative gel is shown in Fig. 1. On each gel, an immunoprecipitate from mutant $\Delta$106, which produces a truncated pLamB, was included to provide background subtraction for densitometry in the region of the gel in which the LamB or pLamB band migrated. This was necessary to compensate for a low level of contamination of the immunoprecipitates. A small amount of containing OmpC protein appeared in immunoprecipitates from IPTG-induced cultures (Fig. 1, lanes 4 to 7).

A summary of the effect of OmpC overexpression on the synthesis of wild-type LamB and pLamB from the various signal sequence mutants is shown in Fig. 2. Panel B shows the structure of the signal sequence of wild-type LamB protein and mutant LamB proteins. The mutants were chosen to represent a range of secretion phenotypes in terms of the kinetics of secretion of the mutant proteins and the extent of suppression of the secretion defects by prl mutations (7, 11).

A consistent decrease in the rate of synthesis of pLamB after the induction of OmpC overexpression was observed in all of the signal sequence mutants. This included mutant $\Delta$106, which is missing about one-third of the N-terminal end of the mature LamB protein as well as most of the signal sequence. The extent of this decrease was substantial and in some experiments approached that which was seen with
null-type LamB protein. No consistent significant differences were apparent among the various mutants. There was considerable variation in the kinetics of inhibition from experiment to experiment. In some experiments an increase in the labeling of the LamB protein relative to MBP was seen at the first time point after IPTG addition or there was a delay in the decrease in the labeling of the LamB protein. The reason for this variability is not known.

A small amount of the precursor form of MBP (pMBP) was observed in the double immunoprecipitates from the LamB signal sequence mutants, as noted in Fig. 1. This is likely to have resulted from sequestering of the SecB protein by pLamB which accumulated in the cytoplasm (1, 5). It was observed even in mutant Δ106, a result consistent with the localization of the SecB-binding site at the C-terminal ends of precursor proteins (5). Although the pMBP band could not be measured accurately by densitometry, it could be readily seen on the original radioautographs that the intensity of this band decreased relative to that of the mature MBP band following the induction of OmpC overexpression. Since the amount of pMBP reflects the size of the pool of pLamB in the cytoplasm, this result provides an independent demonstration that the synthesis of pLamB is reduced by OmpC overexpression.

It could be argued that the decrease in pLamB was due to increased degradation occurring as a consequence of OmpC overexpression and not to a reduction in the synthesis of the precursor. To address this question, we measured the stability of pLamB both in the absence of induction and 10 min after the induction of OmpC overexpression. Mutant Δ60 was chosen for this experiment, since essentially no precursor is secreted by this mutant (7, 9) and, thus, any decrease in pLamB during the chase period would be due to degradation. The results are shown in Fig. 3. Under both induced and uninduced conditions, the half-life of Δ60 pLamB was just under 2 min, and no significant difference in stability was detected, even though the induction of OmpC overexpression for 10 min decreased the amount of Δ60 pLamB by more than 50% (Fig. 2).

The data presented above argue against proteolytic degradation of precursors as a major component of the regulation of bulk outer membrane protein synthesis. The observation that the stability of pLamB was not affected by OmpC overexpression indicates that at least on a brief time scale, proteolysis of a precursor during the cytoplasmic stage of secretion is not increased by a high level of induction of a gene for an outer membrane protein. The observation that such induction led to a substantial decline in both the rate of synthesis of pLamB, as detected by pulse-labeling, and the level of accumulation of pLamB in the cytoplasm, as seen by the accumulation of pMBP, also rules out proteolysis of a precursor during the membrane stage of secretion, since the mutant signal sequences prevent entry into the membrane. Taken together, these results support the proposal (4) that the major mechanism of inhibition of other outer membrane proteins by OmpC overexpression is at the level of translation.

The combined data from the various signal sequence mutants indicate that in the case of LamB protein, a functional signal sequence is not required for inhibition of the translation of this protein by overexpression of another outer membrane protein. This result is similar to that obtained by Hengge-Aronis and Boos (10) in a study of translational control of periplasmic proteins; they showed that a functional signal sequence was not required for inhibition of the synthesis of MBP by overexpression of truncated GlpQ protein. Thus, the expression of these two classes of exported proteins may be regulated at the translational level by mechanisms which are class specific but otherwise similar.

In addition, the data from mutant Δ106 suggest that the sequence at the N-terminal end of mature LamB protein is not required for inhibition of the translation of this protein by OmpC overexpression. Thus, at least one target for inhibition may lie in the C-terminal two-thirds of the protein or in its mRNA. The specificity of the inhibition suggests that this region may also include information which uniquely identifies the target as a potential outer membrane protein.

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