Translocation of colicin E1 through cytoplasmic membrane of *Escherichia coli*

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The product of the *malE–lacZ* gene fusion was reported to compete with some proteins including outer membrane lipoprotein in the protein translocation across the *Echerichia coli* membrane. The fusion product also inhibited colicin E1 export. Furthermore, globomycin, which accumulated prolipoprotein in the membrane, inhibited the translocation of colicin E1 in the wild-type cells, but not in lipoprotein-negative mutant cells. Since colicin E1 contains the internal signal-like sequence [Proc. Natl. Acad. Sci. USA (1982) 79, 2827–2831], these results suggest that colicin E1 is exported by the aid of this sequence at a common site for maltose-binding protein and lipoprotein translocation.

Colicin E1 *Signal sequence* *Protein export* *Maltose-binding protein* *Lipoprotein* *Globomycin*

1. INTRODUCTION

Synthesis of colicin E1, an antimicrobial protein, is induced when the *Escherichia coli* cells harboring ColE1 are treated with agents such as mitomycin C or ultraviolet light [1,2]. The protein thus synthesized is exported to the periplasmic space or to the outer membrane [3]. In *E. coli* cells, some proteins that are transported across the inner membrane are synthesized as a precursor form containing a signal sequence in the NH_2-terminal portion which is later removed during translocation [4]. From the DNA sequence analysis of the structural gene for colicin E1, the protein has no such signal sequence in the NH_2-terminal portion. However, we found a region near the COOH-terminus that is homologous to the NH_2-terminal signal sequences in the precursors of outer membrane lipoprotein of *E. coli* and β-lactamase of *Bacillus licheniformis* [5].

To clarify the role of this sequence, we analyzed the effects of the *malE–lacZ* hybrid protein and prolipoprotein on the translocation of colicin E1. Lines of evidence are presented which suggest that the region of colicin E1 homologous to the NH_2-terminal signal sequence acts as an internal signal for the protein translocation.

2. EXPERIMENTAL

Detailed experimental conditions are given in appropriate figure legends.

3. RESULTS

3.1. *Effect of a hybrid protein, the product of the* malE–lacZ *gene fusion*

A fusion of the *malE* and *lacZ* gene encodes a hybrid protein consisting of the NH_2-terminal part of the maltose-binding protein and the enzymatically active COOH-terminal part of β-galactosidase [6]. The synthesis of the hybrid protein is induced by adding maltose to the cells carrying the gene fusion. The hybrid protein becomes bound and stuck to the cytoplasmic membrane, probably because β-galactosidase part of the protein is unable

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to pass through the membrane even when the maltose-binding protein part has penetrated to the membrane via the hydrophobic signal sequence. Thus some of the periplasmic and outer membrane proteins including lipoprotein are accumulated inside the cells as precursor forms with unprocessed signal sequences [7].

If the internal sequence of colicin E1 homologous to the NH_2-terminal signal sequence of lipoprotein plays a role in translocation of the protein and the proteins are transported at a common site on the membrane, colicin E1 would not be exported upon accumulation of the hybrid protein. The amount of colicin E1 exported were determined from the colicin E1 activity extractable with 1 M NaCl from the cells, while those of the protein inside the cells were measured by the activity in sonic extracts of the residual cells (see legend to fig. 1).

After the induction treatment for the *malE–lacZ* hybrid protein with maltose for 3 h, colicin E1 synthesis was induced by mitomycin C. In the strain MC4100 (ColE1:Tn*5*), colicin E1 synthesized was first accumulated in the cells and then gradually exported. Concomitantly, the amount of colicin E1 inside the cells decreased as the protein was exported. However, in the strain MC4100 (ColE1:Tn*5*,λ*malE–lacZ*), colicin E1 export but not synthesis was inhibited to a great extent.

Although the *malE–lacZ* prophage could be also induced by mitomycin C, the degree of induction was extremely low under our experimental conditions; plaque-forming units of the phage in the culture 5 h after addition of mitomycin C was $\sim 5 \times 10^6$/ml, while the cell number was 5×10^9/ml. Furthermore, the lysis of the cells appeared seldom to occur during these experiments. Absorbance at 660 nm of the culture of MC4100 (ColE1:Tn*5*,λ*malE–lacZ*) was >95% of that of MC4100 (ColE1:Tn*5*) 5 h after mitomycin C addition. In addition, the β-galactosidase activity of the hybrid protein detected in the culture medium was <1% even 5 h after mitomycin C addition. It is, therefore, unlikely that colicin E1 observed in the NaCl extracts in the present experiments is due to lysis of the cells after λ phage induction.

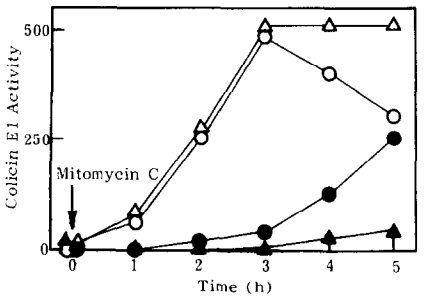

Fig. 1. Effect of a hybrid protein, the product of the *malE–lacZ* gene fusion on the translocation of colicin E1. Plasmid ColE1:Tn*5* [8] was introduced by the transformation procedure [8] into MC4100 (F⁻ *lac169 araD139 rpsL relA thiA*) [9] and MC4100 [λp72–47] [6]. The latter lysogenic strain has a *malE–lacZ* gene fusion on the prophage. The plasmid-bearing cells were aerobically grown at 30°C in the minimal medium M63 [10] supplemented with 0.5% glycerol and 1 μg thiamine–HCl/ml. Maltose was added to the culture (at final conc. 0.2%) at the early exponential phase ($\sim 4 \times 10^8$ cells/ml), and the incubation was carried out for 3 h. The induced synthesis of the hybrid protein was proved by the increased activity of β-galactosidase which was measured spectrophotometrically using *o*-nitrophenyl-β-galactoside as a substrate [10]. Then the medium was changed to the M9 salt medium [10] supplemented with 0.3% casamino acids and 0.2% maltose. Mitomycin C (1 μg/ml) was added to induce colicin E1 synthesis, and the incubation was continued at 30°C without aeration [11]. Cells were collected at each period of time, suspended in 0.1 M pottassium phosphate buffer (pH 7.0) containing 1 M NaCl [12] and stirred for 1.5 h at 4°C. After centrifugation of the cell suspension, the supernatant solution was saved. The pellet was resuspended in the phosphate buffer described above and subjected to sonication. We referred to the supernatant and the sonic extract of the pellet as NaCl extract and sonicate, respectively. Colicin E1 activity was measured as in [11]. Activity of colicin E1 in the NaCl extract was essentially that in the solution after the osmotic shock treatment which is usually used to extract periplasmic proteins [13]. Also we could not see any difference between the activity of TEM-β-lactamase, a periplasmic protein in the NaCl extract and that in the osmotic shockate. Colicin E1 activity in NaCl extract (▲) and sonicate (△) [of MC4100 (ColE1:Tn*5*,λ*malE–lacZ*); colicin E1 activity in NaCl extract (●) and sonicate (○)] of MC4100(ColE1:Tn*5*). The activity was expressed as units/absorbancy at 660 nm of the incubation mixture.

3.2. *Effect of globomycin*

Globomycin is an antibiotic of cyclic peptide. It inhibits the processing of the precursor of the outer membrane lipoprotein to accumulate the uncleaved precursor in the inner membrane [14]. Therefore, the translocation of colicin E1 would be inhibited

by the accumulated prolipoprotein in the cells treated with globomycin, if the protein was exported by the aid of the internal sequence homologous to the NH_2-terminal signal sequence of prolipoprotein.

The amount of the exported colicin E1 after induction decreased in the globomycin-treated cells (fig. 2). The concomitant amount of the protein remained inside the cells. The induced synthesis of colicin E1 itself was not inhibited by globomycin. Although the [globomycin] (20 μg/ml) used here did not efficiently inhibit the growth of the strain JE5512(lpp$^+$), longer treatment (5 h) with the agent drived the strain to gradually accumulate the precursor of lipoprotein inside the cells. On SDS–polyacrylamide gel electrophoresis, the band of lipoprotein precursor was observed in a sample prepared from the globomycin-treated wild-type cells, but not from the untreated cells (not shown; see [14]).

Next, we used a lipoprotein-negative (*lpp*$^-$) mutant JE5513 which can not produce outer membrane lipoprotein and significantly resistant to globomycin [14,15]. In the mutant strain (fig. 2), the time courses of the colicin E1 synthesis and translocation were essentially the same in both globomycin-treated and untreated cells. These results indicate that the inhibitory effect of globomycin on the translocation of colicin E1 that was observed in the *lpp*$^+$ cells is due to a secondary effect probably through the accumulation of prolipoprotein inside the cells.

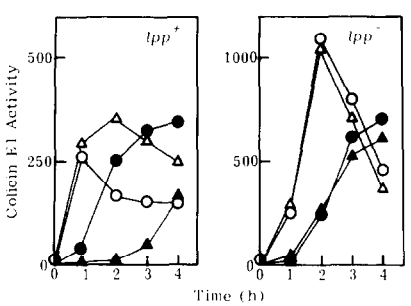

Fig. 2. Effect of globomycin on the translocation of colicin E1. ColE1 was introduced into strains JE5512 (Hfr *lpp*$^+$ *man-1 pps*) and JE5513 (same as JE5512 except *lpp-2*); a lipoprotein-negative mutant derived from JE5512 [15]. Cells of JE5512 (ColE1) and JE5513 (ColE1) were grown aerobically at 37°C in L broth till $\sim 4 \times 10^8$ cells/ml. After centrifugation of the culture, the pellet was resuspended in the M9 salt medium supplemented with 0.2% glucose, 0.5 μg mitomycin C/ml and 20 μg globomycin/ml. Incubation was continued further at 37°C without aeration. The NaCl extract and the sonicate were obtained as in fig. 1. Colicin E1 activity in NaCl extract (▲) and sonicate (△) in globomycin-treated cells; colicin E1 activity in NaCl extract (●) and sonicate (○) in untreated cells. The activity was expressed as units per absorbancy at 660 nm of the incubation mixture.

4. DISCUSSION

Export of colicin E1 was significantly inhibited by the accumulation of the *malE–lacZ* hybrid protein and prolipoprotein inside the cells. Evidence has been presented that the hybrid protein and prolipoprotein interact with the cytoplasmic membrane [6,14]. Therefore, colicin E1 seems to share a common site in the translocation process through which maltose-binding protein and lipoprotein pass across the cytoplasmic membrane. In view of the sequence homology and above results, the signal-like sequence near the COOH-terminus of colicin E1 should interact with the membrane and serve as an internal signal for protein translocation. However, colicin E1 is not cleaved at a peptide bond around this sequence during the translocation in contrast to other export proteins. Thus, the internal signal sequence of colicin E1 would be useful for analyzing the mechanism of protein translocation across the membrane.

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