Communication

Isolation and Characterization of an Escherichia coli Clone Overproducing Prolipoprotein Signal Peptidase*

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Masao Tokunaga, Judith M. Loranger, and Henry C. Wu†

From the Department of Microbiology, Uniformed Services University of the Health Sciences, Bethesda, Maryland 20814

Most, if not all, outer and periplasmic proteins in Escherichia coli are first synthesized as precursor forms containing signal peptides. These signal peptides are required for the correct localization of nascent secretory proteins across the membrane.

Based on the rationale that Escherichia coli cells containing increased levels of pro lipoprotein signal peptidase would be highly resistant to globomycin, a specific inhibitor of the pro lipoprotein signal peptidase, we have isolated a clone from the Carbon-Clarke collection, plasmid pLC3-13, which is globomycin-resistant and contains an increased level of pro lipoprotein signal peptidase activity. The plasmid pMT521, a subclone of pLC3-13 in pBR322, conferred on its host cells approximately 20 times overproduction of pro lipoprotein signal peptidase and an extremely high level of resistance against globomycin. The overproduced pro lipoprotein signal peptidase was completely inhibited by the presence of globomycin in the in vitro assay, and the overproduced activity was found in the cell envelope fraction. Several lines of biochemical and genetic evidence suggest that the gene contained in pLC3-13 and its derivative clones is most likely the structure gene (isp) for pro lipoprotein signal peptidase.

The processing of pro lipoproteins is specifically inhibited by a cyclic antibiotic globomycin, and the accumulation of modified pro lipoproteins occurs in globomycin-treated cells in vivo (12). Furthermore, pro lipoprotein signal peptidase activity is completely inhibited by globomycin in in vitro (6). We reasoned therefore, that E. coli variants containing increased levels of pro lipoprotein signal peptidase can be selected by virtue of their increased resistance to globomycin. Indeed, we have isolated one globomycin-resistant mutant of E. coli K12 (strain SM31-2B4) which was found to contain 3–4 times more pro lipoprotein signal peptidase activity in the crude extracts. We therefore employed globomycin selection in an attempt to isolate E. coli strains containing multiple copies of pro lipoprotein signal peptidase gene encoded on plasmids.

In this paper, we describe the isolation of a pro lipoprotein signal peptidase overproducing clone in the Carbon-Clarke collection (13), the subcloning of the pro lipoprotein signal peptidase gene (isp) into pBR322, and preliminary characterization of E. coli strains containing the amplified isp gene.

MATERIALS AND METHODS

Bacterial Strains and Medium—Bacterial strains, JA200 (F' ΔtrpE5 recA thr leu lacY), SM31 (F' supE tonA thr leu rK' mk' recBC), KL200 (F' met pro his trp lac wrpL naiY) E609 (HfrC pps), E610 (HfrC pps mlpA), and JE6565 (F' lpp pps hisA proA argE thi gal lac mtl tex) were used. L Broth and M9 minimal medium containing 0.2% glucose were used throughout this study. Ampicillin (30 µg/ml) and tetracycline (10 µg/ml) were used for the selection or identification of plasmid containing strains.

Scanning of Globomycin-resistant Clones among the Carbon Collection—Individual clones of the Carbon collection (generous gift of H. Tabor, National Institutes of Health) were grown in 50 µl of L broth containing colicin E1 in microtitre plates at 37 °C overnight. Approximately 106 cells of overnight culture were inoculated into 50 µl of L broth containing 50 µg/ml of globomycin and incubated overnight at 37 °C. Globomycin-resistant clones were further screened by the in vitro pro lipoprotein signal peptidase assay described below.

In Vitro Prolipoprotein Signal Peptidase Assay—E. coli cells grown in 500 µl of L broth containing colicin E1 were harvested in a Microfuge and suspended in 50 µl of lysis buffer (0.1% saponin, 10 mM EDTA, 20% sucrose, 50 mM Tris-HCl buffer (pH 8.0), and 10 µg each of DNase and RNase/ml). After a 15-min incubation at 37 °C, 270 µl of 50 mM Tris-HCl buffer (pH 7.4) containing 0.25% Nikkol was added and the tube was mixed by brief vortexing and sonication. The crude homogenate (7 µl) was then added to 3 ml of a reaction mixture containing glyceralde-modified pro lipoprotein (20,000 cpm) (prepared according to Ref. 7). 0.25% Nikkol, 50 mM Tris-HCl buffer (pH 7.4) and 0.25% β-mercaptoethanol and the incubation was continued for 90 min at 37 °C. Termination of enzyme reaction, gel electrophoresis, and calculation of pro lipoprotein signal peptidase activity were described previously (7).

Plasmid Preparation and Subcloning of isp Gene—Plasmid DNA purified by a NaOH-sodium dodecyl sulfate rapid method (14) was utilized for plasmid screening, restriction endonuclease analysis, and transformation. The procedures described by Maniatis et al. (15) were employed for large-scale plasmid DNA purification by cesium chloride

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† To whom correspondence should be addressed.

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gradient centrifugation, digestion of DNA with restriction endonucleases, purification of DNA fragments by the spin column procedure, ligation with T4 DNA ligase, and agarose gel electrophoresis.

**Chemicals**—Restriction endonucleases and T4 DNA ligase were purchased from New England Biolabs. pBR322 DNA was obtained from Bethesda Research Laboratory. [35S]Methionine (1266.7 Ci/mmol) and [35S]Cysteine (942.2 Ci/mmol) were obtained from New England Nuclear. Nikkol (octaethylene glycol mono-n-dodecyl ether) was obtained from Nikko Chemical (Tokyo).

Globomycin and colicin E1 were generous gifts from M. Arai (Saakyo, Tokyo), S. Omoto (Meiji Seika, Tokyo), and E. Creeger (University of Connecticut Health Center, Farmington, CT), respectively.

**RESULTS**

**Strains Carrying pLC3-13 Overproduce Prolipoprotein Signal Peptidase**—Since globomycin is a specific inhibitor of prolipoprotein signal peptidase, we assumed that overproduction of prolipoprotein signal peptidase would overcome the effect of globomycin and that *E. coli* strains with an amplified *isp* gene would be globomycin-resistant. Among the approximately 2200 clones of the Carbon collection, the clone carrying plasmid pLC3-13 was found to be globomycin-resistant (Fig. 1A, lane 1). This was confirmed by the *in vitro* prolipoprotein signal peptidase assay which revealed that this clone, JA200 (pLC3-13), indeed contained 4–5 times higher prolipoprotein signal peptidase activity (Fig. 1B, lane 1) as compared with the parental strain (Fig. 1B, lane 4). When strain JA200 was cured of the pLC3-13 plasmid, both globomycin sensitivity and prolipoprotein signal peptidase activity were restored to normal levels (Fig. 1, A and B, lane 3). Other *E. coli* strains transformed by plasmid pLC3-13 became globomycin-resistant and contained increased levels of prolipoprotein signal peptidase (Fig. 1, A and B, lanes 2 and 5).

**Subcloning of Prolipoprotein Signal Peptidase Gene into Plasmid pBR322**—The restriction map of pLC3-13 is shown in Fig. 2. Plasmid pLC3-13 DNA was digested by restriction endonuclease EcoRV yielding two fragments (10.4 and 9.0 kb). EcoRV-digested pLC3-13 DNA was ligated with pBR322 DNA which had been digested with the same enzyme, and the ligated DNA was used to transform strain SM31. SM31 was used as the host for transformation with subcloned plasmids because of the high efficiency of transformation in this strain. Amp'Tet' transformants were selected and further screened for resistance to globomycin (50 μg/ml). One of these clones (containing plasmid pMT503) was found to have an insertion of the larger fragment (10.4 kb) of pLC3-13 into the EcoRV site of pBR322 (Fig. 2). The *E. coli* strain containing pMT503 showed the expected phenotypes, Amp', Tet', and Gmb' and contained increased levels of prolipoprotein signal peptidase activity as determined by the *in vitro* assay.

The plasmid pMT503 DNA was totally digested by NruI and religated by T4 DNA ligase. Although the religation at the NruI site was incomplete, about half of the Amp'Tet'Gmb' transformants obtained by transformation of strain SM31 with the religated DNA were found to contain plasmid DNA of a smaller size (8.5 kb) than that of pMT503. This smaller plasmid, designated pMT521, resulted from the deletion of 6 kb of the ColE1 region and 0.8 kb of pBR322 DNA from pMT503 (Fig. 2). The strains carrying pMT521 contained approximately 20 times higher prolipoprotein signal peptidase activity than the parental strains (data not shown). The prolipoprotein signal peptidase activity overproduced in strains carrying pMT521 was completely inhibited by globomycin in the *in vitro* assay (Fig. 3). Furthermore, all prolipoprotein signal peptidase activity in JE5505 (pMT521) was found in the cell envelope fraction (Fig. 4) which corresponds to the subcellular localization of prolipoprotein signal peptidase in wild type cells (16).

pMT503 DNA was totally digested by BamHI and religated by T4 DNA ligase resulting in the construction of plasmid pMT522 which contains further deletion of 2 kb of *E. coli* DNA (Fig. 2). The deletion of the 2-kb *E. coli* DNA fragment from pMT503 resulted in the loss of both the globomycin-resistance phenotype and increased levels of prolipoprotein signal peptidase. It is clear, therefore, that this 2-kb region must contain the promoter and/or part of the prolipoprotein signal peptidase structural gene.

Globomycin Sensitivity of *E. coli* Strains Harboring Plasmid...
peptidase activity by globomycin in the reaction mixture. No enzyme control; lane 2, 6 μg of SM31 (pMT521) cell envelopes was used; lane 3, same as lane 2 plus 0.1 μg of globomycin in the reaction mixture. MPLP, modified prolipoprotein.

**Fig. 3. Inhibition of overproduced prolipoprotein signal peptidase activity by globomycin in the in vitro assay.** Lane 1, no enzyme control; lane 2, 6 μg of SM31 (pMT521) cell envelope was used; lane 3, same as lane 2 plus 0.1 μg of globomycin in the reaction mixture. MPLP, modified prolipoprotein.

**Table I**

<table>
<thead>
<tr>
<th>Strains</th>
<th>Globomycin sensitivity of signal peptidase overproducing strains (μg/ml)</th>
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<tbody>
<tr>
<td>E609</td>
<td>&gt;300</td>
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<tr>
<td>E609</td>
<td>20</td>
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<tr>
<td>E610</td>
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<td>E610</td>
<td>40</td>
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<tr>
<td>JE5505</td>
<td>&gt;300</td>
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<tr>
<td>JE5505</td>
<td>120</td>
</tr>
<tr>
<td>SM31-2B4</td>
<td>&gt;300</td>
</tr>
<tr>
<td>SM31-2B4</td>
<td>120</td>
</tr>
</tbody>
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* Maximum globomycin concentration in which these strains can grow.

pMT521—Plasmid pMT521 was used to transform *E. coli* strains E609, E610 (mplA), JE5505 (lp1), and SM31-2B4 (a spontaneous globomycin-resistant mutant of strain SM31). All transformants were extremely resistant to globomycin (>300 μg/ml) as compared to their parental strains regardless of the particular genetic background of these strains (Table I).

The murein lipoprotein structural gene is deleted in strain JE5505 (17). Accordingly, this strain is globomycin-resistant (120 μg/ml) since the major cause of globomycin-mediated lethality is the accumulation of modified murein prolipoprotein in treated cells (12). However, while the murein lipoprotein is the most abundant lipoprotein in *E. coli* and therefore the primary target of globomycin action, other relatively minor lipoprotein species also accumulate as their glyceride-modified precursor forms in globomycin-treated cells (5). The fact that JE5505 (pMT521) is much more resistant to globomycin than JE5505 strongly suggests that these secondary target(s) of globomycin action are responsible for the eventual globomycin-mediated lethality of JE5505. Furthermore, it provides further support that prolipoprotein signal peptidase is responsible for the processing of all these prolipoproteins. The same explanation can be applied to the results obtained with strains E610 (mplA) (18) and E610 (pMT521).

Strain SM31-2B4 was selected by globomycin treatment of SM31, and it presumably contained a spontaneous chromosomal mutation resulting in an increase level of prolipoprotein signal peptidase activity. Because of the apparent overproduction of prolipoprotein signal peptidase in mutant SM31-2B4 as compared to strain SM31, it is globomycin-resistant (120 μg/ml). Introduction of pMT521 into SM31-2B4 resulted in further overproduction of prolipoprotein signal peptidase and increased resistance to globomycin (>300 μg/ml).

**DISCUSSION**

We have identified among the Carbon-Clarke collection of *E. coli* genomic fragments a plasmid pLC3-13 which confers upon its host cell increased levels of prolipoprotein signal peptidase activity and a concomitant increase in globomycin resistance. Several lines of evidence suggest that the gene contained in pLC3-13 and its derivative clones is most likely the structural gene for prolipoprotein signal peptidase. The evidence supporting this conclusion is as follows. 1) The increased prolipoprotein signal peptidase activity in strains containing pMT521 is sensitive to globomycin *in vitro* (Fig. 3). 2) The gene product is a membrane-bound enzyme (Fig. 4). 3) The prolipoprotein signal peptidase activity in strains containing the cloned gene has the same mobility in nondenaturing gel as the enzyme present in the parental strain (data not shown). These properties are the same as observed for the prolipoprotein signal peptidase present in strains not harboring the plasmid (6, 16). 4) The crude extract of strains containing pMT521 does not contain any activity which would inactivate globomycin irreversibly (data not shown). 5) Genetic evidence indicates that the *E. coli* gene(s) in pMT521 is located approximately at 0.5 min of the *E. coli* map. This is the nearly the same location as the gene altered in the *lp1* signal peptidase mutant reported recently (19). 6) Strains harboring an F*'-plasmid (F*101) containing *E. coli* genes between *thr* and *leu* loci were found to contain 1.7 times prolipoprotein signal peptidase activity, as compared to the strains cured for the F*101* plasmid. The apparent gene dosage-prolipoprotein signal peptidase activity relationship observed among strains containing F*'-plasmid, ColE1-plasmid, and pBR322 derivatives with the *lp1* gene strongly suggests that the cloned gene is indeed the structural gene for prolipoprotein signal peptidase.

The availability of this plasmid will now permit a detailed study of the genetic organization of the *lp1* gene. In addition, the subcloning of the *lp1* gene into a runaway plasmid would facilitate the biochemical and physiological studies of this unique enzyme.

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3 M. Regue, J. Remnick, M. Tokunaga, and H. C. Wu, manuscript in preparation.

4 S. Mizushima, personal communication.
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REFERENCES
Isolation and characterization of an Escherichia coli clone overproducing prolipoprotein signal peptidase.
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