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Preferential Selection of Deletion Mutations of the Outer Membrane Lipoprotein Gene of *Escherichia coli* by Globomycin

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Globomycin is an antibiotic which inhibits the processing of the prolipoprotein. Eighty globomycin-resistant mutants were independently isolated from *Escherichia coli* K-12 which had a deletion mutation in chromosomal lipoprotein gene (*lpp*), but contained a plasmid carrying the wild-type *lpp* gene. Twenty-six of the mutants did not have the lipoprotein in the membrane fractions. From the analysis of the plasmids of these mutants, all of the lipoprotein-deficient mutations were found to be due to deletion mutations around the *lpp* gene.

Globomycin is a cyclic peptide antibiotic consisting of five amino acids and one fatty acid (9, 11) and has been shown to specifically inhibit the processing of a secretory precursor of the outer membrane lipoprotein of *Escherichia coli*, the prolipoprotein (10). As a result, the prolipoprotein is accumulated in the cytoplasmic membrane, and this accumulation is lethal to the cell (6, 10). This is evident from the fact that a mutant missing the lipoprotein is resistant to globomycin (M. Inkai, unpublished data).

Globomycin provides an excellent system to study the mechanism of biosynthesis and assembly of the lipoprotein, which is one of the most extensively investigated membrane proteins (see reviews in references 4 and 7). In the present study, we attempted to characterize the lipoprotein gene (*lpp*) of lipoprotein-deficient mutants isolated as globomycin-resistant mutants.

E. coli K-12 strain JE5505 (F⁻*lpp-2 pps his proA argE thi gal lac xyl tsx*) (5) was transformed with plasmid pKEN111 DNA. Plasmid pKEN111 was constructed by insertion of a 2.8-kilobase pair (kb) *Hae*III fragment of hybrid phage λ *lpp*Ec-1 carrying the *E. coli lpp* gene (12) into an *Eco*RI site of plasmid pSC101 (conferring tetracycline resistance) by using *Eco*RI linker oligonucleotide (N. Lee, K. Nakamura, and M. Inouye, manuscript in preparation). Tetracycline-resistant transformants thus obtained produced a large amount of the lipoprotein and became sensitive to globomycin (Lee et al., in preparation). Independent transformants (130) were grown overnight at 37°C in 0.5-ml cultures of L-broth medium (8) containing 10 μg of tetracycline per ml. All of the transformants were globomycin sensitive. An amount of 2 μl of each culture was spotted on L-broth plates containing 25 μg of globomycin and 10 μg of tetracycline per ml. Eighty spots (about 60%) gave rise to globomycin-resistant colonies. One colony from each spot was isolated and used for further examination.

Membrane fractions were prepared from 10-ml cultures of mutant cells grown in L-broth medium containing 10 μg of tetracycline per ml (8) and analyzed by sodium dodecyl sulfate-polyacrylamide (17.5%) gel electrophoresis as described previously (1). From this analysis, 26 of 80 mutants (33%) were found to have no lipoprotein in the membrane fractions (data not shown).

To characterize the mutations causing lipoprotein deficiency, sizes of the plasmid DNA from these mutants were compared with that of the original pKEN111 DNA by the rapid alkaline extraction method of Birnboim and Doly (2). Figure 1 shows a result of such analysis for nine lipoprotein-deficient mutants. For each analysis, both open circular (upper band) and closed circular (lower band) plasmid DNA molecules were observed. It can be clearly seen that all of the plasmid DNAs isolated from mutant cells migrated faster than did pKEN111 DNA, indicating that all of these plasmid DNAs contained deletion mutations. The sizes of these DNAs were estimated by using closed circular plasmid DNAs of known molecular weights as standards. The nine mutant plasmids carried deletion mutations of 2.9- to 8.2-kb DNA fragments. The same examination was carried out for the remaining 17 lipoprotein-deficient mutants, and it was found that all of them also had similar deletion mutations as described above. From another independent experiment, 41 lipoprotein-deficient mutants were isolated in the way described above, and it was found that all of them were also caused by deletion mutations.

Further detailed characterization was performed for mutant plasmid no. 1, which had the smallest deletion (2.9 kb) among the mutant plasmids examined (Fig. 1). Figure 2 shows restriction enzyme analysis of plasmid no. 1 DNA.

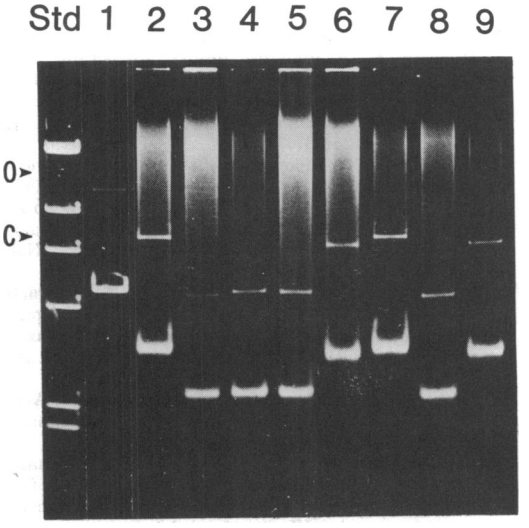

FIG. 1. *Analysis of alkaline-extracted plasmid DNAs on 0.7% agarose gel. Nine independently isolated mutant plasmids were characterized in lanes 1 through 9. Std indicates an HindIII restriction digest of phage λ DNA. The sizes of these fragments are (from top to bottom): 27.5, 23.0, 9.8, 6.6, 4.5, 2.5, and 2.2 kb. Arrows with O and C represent positions of open circular and closed circular molecules of pKEN111 DNA (12.2 kb), respectively.*

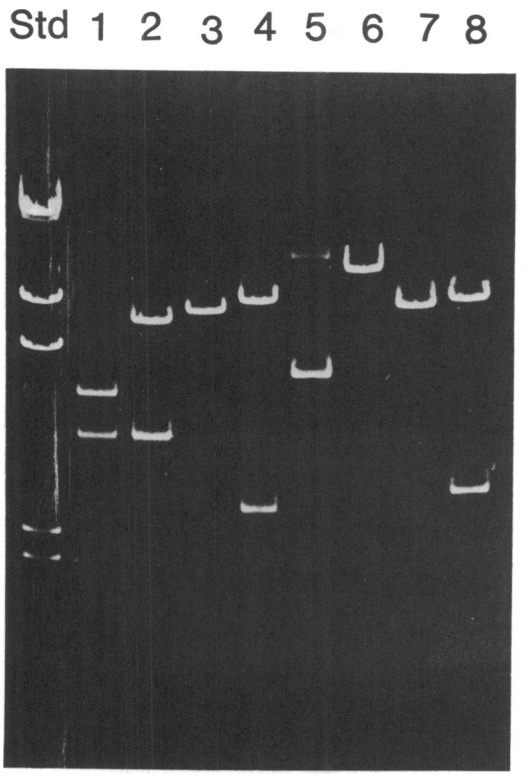

FIG. 2. *Restriction enzyme analysis of plasmid no. 1 DNA on 0.7% agarose gel. Plasmid DNA was isolated by the method of Clewell and Helinski (3). Lanes 1, 3, 5, and 7 represent HpaI, PvuII, XbaI, and EcoRI digestions of plasmid no. 1, respectively. Lanes 2, 4, 6, and 8 represent HpaI, PvuII, XbaI, and EcoRI digestions of pKEN111 DNA, respectively. Std indicates an HindIII digestion of phage λ DNA.*

*Hpa*I digestion of plasmid no. 1 and pKEN111 DNAs (Fig. 2, lanes 1 and 2) indicated that a deletion of a 2.9-kb DNA fragment occurred within the 8.4-kb *Hpa*I fragment (upper band in lane 2) of pKEN111 DNA which carries the *lpp* gene (Lee et al., in preparation). Figure 2 also shows that plasmid no. 1 DNA lost one of the two *Pvu*II sites (lanes 3 and 4), one *Xba*I site (lanes 5 and 6), and one of the two *Eco*RI sites (lanes 7 and 8). Figure 2 also shows that plasmid no. 1 DNA was constructed as shown in Fig. 3. This indicates that the mutant plasmid contained a deletion of a 2.9-kb fragment consisting of about 1.2 kb from pSC101 DNA and about 1.7 kb from the cloned *E. coli* chromosomal DNA fragment in pKEN111, which encompasses the entire lipoprotein gene.

Preferential selection of deletion mutations of the *lpp* gene by globomycin is rather surprising, and its mechanism is not known at present. However, it is possible that many of the point mutants in the *lpp* gene still could be lethal by causing the accumulation of the mutant proli-

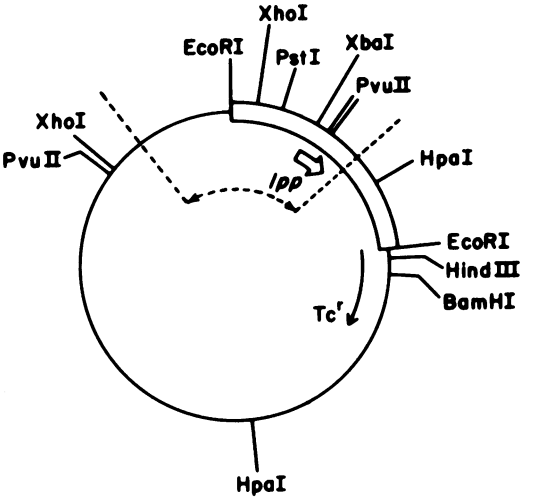

FIG. 3. *Restriction enzyme map comparing pKEN111 and plasmid no. 1. The solid line represents the 9.4-kb pSC101 DNA, and the blocked segment represents the 2.8-kb chromosomal DNA fragment carrying the lpp gene. The location and the direction of transcription of the lpp gene and the tetracycline resistance gene are indicated by a thick arrow (⇨) and a thin arrow (→), respectively. There are two PvuII sites within the lpp gene. The dotted line indicates the extent of the deletion in plasmid no. 1.*

poprotein in the cytoplasmic membrane. In this regard, it is interesting to note that many spontaneous lipoprotein-deficient mutants have been isolated from JE5527 carrying pKEN221 (13) without using globomycin, and that these mutants have been shown to be derived either by point mutations, by insertion sequences, or by deletion mutations (unpublished data).

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