Cloning and expression of a gene coding for the prolipoprotein signal peptidase of *Escherichia coli*

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An *Escherichia coli* mutant, Y815, has a temperature-sensitive prolipoprotein signal peptidase. IPTG-induced synthesis of the major outer membrane prolipoprotein (PLP) results in the inhibition of cell growth because of accumulation of PLP in its envelope [J. Bacteriol. (1982) 152, 1163–1168]. The 2000 *E. coli* strains of Clarke and Carbon's collection were screened for the presence of a plasmid complementing the IPTG-sensitivity of the growth of Y815. One plasmid, pLC3-13, complemented the IPTG-sensitivity. The envelope fraction prepared from Y815 transformed by pLC3-13 showed high activity of the PLP signal peptidase in vitro at high temperature. A 4 kb *Acc*I fragment subcloned onto plasmid pHY001 was shown to carry the gene for the PLP signal peptidase.

Cloning *Prolipoprotein signal peptidase* *Clarke and Carbon's collection* *In vitro assay*
E. coli *outer membrane* *Temperature-sensitive signal peptidase*

1. INTRODUCTION

In *Escherichia coli*, outer membrane and periplasmic proteins are synthesized in a precursor form with a signal peptide at the NH_2-terminus [1]. Signal peptidases which cleave off signal peptides are considered to play a key role in the process of protein secretion across the cytoplasmic membrane. No signal peptidase except that for M13 phage coat protein [2] has been purified. Among *Escherichia coli* proteins, the major outer membrane lipoprotein (LP) is the most abundant in terms of the number of molecules and has many unique features [3]. The signal peptidase that cleaves off the signal peptide of the secretory precursor of LP (PLP, prolipoprotein) and minor membrane lipoproteins has been studied [4–7].

Abbreviations: IPTG, isopropyl-β-D-thio-galactopyranoside; LP, the major lipoprotein of the outer membrane; PLP, prolipoprotein; SDS, sodium dodecyl sulfate; kb, kilobase pairs

An *E. coli* mutant, Y815, that has a temperature-sensitive signal peptidase for PLP (PLP signal peptidase) has been isolated [8]. Mutant Y815 harbors plasmid pHY001 carrying the *lpp* gene that is fused to the *lac* UV5 promoter–operator region. Upon addition of isopropyl-β-D-thio-galactopyranoside (IPTG) to the medium, growth of the mutant was inhibited because of the accumulation of PLP in the envelope [8]. Using the PLP accumulated in the mutant, an efficient and convenient assay system for PLP signal peptidase has been established [9].

Taking advantage of this IPTG-sensitivity of mutant Y815, we have screened Clarke and Carbon's collection [10] of 2000 *E. coli* strains. This paper describes isolation from the above collection and characterization of a plasmid carrying the gene for PLP signal peptidase, and its subcloning.

2. MATERIALS AND METHODS

2.1. *Plasmid and bacterial strains*

Plasmid pHY001 and *Escherichia coli* strains

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Y8, Y815 and JE5506 (parental strain of Y8 and Y815) were described in [8]. Y815 has a temperature-sensitive PLP signal peptidase [9]. Y8 was also shown to lack the PLP signal peptidase activity at high temperature (unpublished). A collection of 2000 *E. coli* strains harboring ColE1 plasmids joined to random segments of the *E. coli* chromosome DNA was described [10].

2.2. *Media*

Difco penassay broth medium no.3 (M3) and the same medium supplemented with 20% (w/v) sucrose (M3su) [8] were used as nutrient media. Solid media contained 1.35% agar. Peptide broth contained 1% polypeptone (Daigo Eiyo Kagaku Co.) and 0.5% NaCl. The pH was adjusted to 7.2. The PT plates contained peptone broth, 1% agar, and 8 μg/ml of tetracycline hydrochloride.

2.3. *Assay of PLP signal peptidase*

The in vitro assay of PLP signal peptidase was described in [9]. The PLP-containing envelope fraction prepared from Y815 cells grown in the presence of 5 μCi/ml of [^{35}S]methionine and 6 mM IPTG was used as the substrate envelope fraction.

2.4. *Screening of Clarke and Carbon's collection*

The two thousand strains of Clarke and Carbon's collection were inoculated in micro test dishes (Falcon Plastics) containing one drop (~0.05 ml) of peptone broth in each well via a block with 48 needles and grown overnight at 37°C. One drop of a suspension of Y815 cells in peptone broth (5×10^8 cells/ml) was added to each well of the test dishes and grown overnight at 30°C. One drop of a mixture (1 : 1) of crude colicin E1 prepared as in [11] and phage T6 (10^{11} plaque forming units/ml) was added to each of the wells (Y815 is resistant to T6). After incubation at 37°C for 30 min, cells were spotted by means of the block with needles onto PT plates and PT plates supplemented with 0.2 mM IPTG. Strains which showed the same growth on both an IPTG-containing plate and an IPTG-free plate were picked and analyzed for membrane proteins.

2.5. *Other methods*

Envelope fractions were prepared as in [12]. SDS–polyacrylamide gel electrophoresis and fluorography were performed as in [9]. Transformation was done as in [13]. M3su plates or M3 plates supplemented with appropriate antibiotics were used for selection of transformants.

3. RESULTS AND DISCUSSION

3.1. *Plasmid pLC3-13 carries the gene for PLP signal peptidase*

Plasmids harbored by each of the two thousand strains of Clarke and Carbon's collection were introduced to Y815 through mating and examined for their ability to complement IPTG-sensitivity of the mutant. A plasmid, pLC3-13, rendered Y815 IPTG-insensitive. When the IPTG-insensitive strain thus obtained was grown in the presence of [^{35}S]methionine and IPTG, the envelope fraction contained a large amount of mature LP and almost no PLP, indicating that the processing of PLP was normal in this strain (fig.1). IPTG-insensitive cells were also obtained when Y815 was transformed with pLC3-13 DNA prepared from the original Clarke and Carbon's strain. The envelope fraction prepared from the transformant showed high activity of PLP signal peptidase at high temperature in the in vitro assay system (fig.2f,g, where PLP was cleaved efficiently into mature LP), while the envelope fraction of Y815 showed no signal peptidase activity (lanes d,e). Thus, it is clear that plasmid pLC3-13 donated PLP signal peptidase activity to Y815. These results strongly indicate that the plasmid carries the gene coding for PLP signal peptidase. This conclusion was also supported by the finding that Y815 transformed by pLC3-13 showed appreciably higher PLP signal peptidase activity than that of the wild-type, JE5506, probably because of the gene dosage effect (fig.2). A restriction map of pLC3-13 is shown in fig.3.

3.2. *Location of the gene for PLP signal peptidase on pLC3-13*

To locate the gene for PLP signal peptidase, plasmids carrying various portions of pLC3-13 DNA were constructed: Plasmids pKD1 to pKD8 were constructed by subcloning of the restriction fragments of the pLC3-13 DNA (pKD1, *Bam*HI fragment; pKD2 and pKD5, *Hin*dIII fragments; pKD3, pKD4, pKD6 and pKD7, *Pst*I fragments;

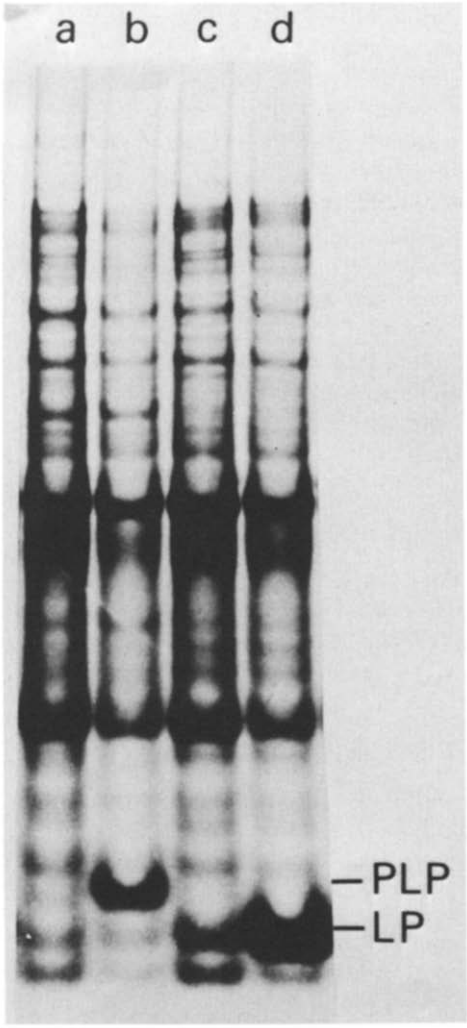

Fig.1. Processing of PLP in Y815 harboring pLC3-13. Cells were labeled with [^{35}S]methionine in the presence (lanes b,d) or the absence (lanes a,c) of 6 mM IPTG as described [8] and the envelope proteins were analyzed by SDS–polyacrylamide gel electrophoresis and fluorography: (a,b) Y815; (c,d) Y815 harboring pLC3-13; about 5000 cpm of the sample was loaded on each well of the gel.

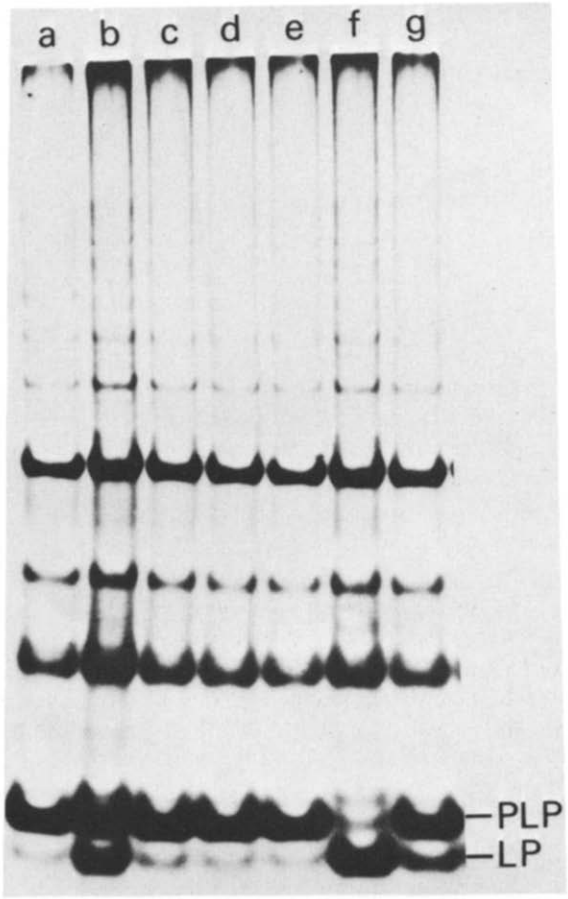

Fig.2. In vitro assay of PLP signal peptidase of mutant cells transformed by pLC3-13. Envelope fractions were examined for PLP signal peptidase activity using the PLP-containing substrate envelope fraction labeled with [^{35}S]methionine. Lane: (a) control, substrate envelope fraction only; (b–g) envelope fractions (8 μg protein for lanes b,d,f; 2 μg protein for lanes c,e,g) prepared from JE5506 (lanes b,c), Y815 (lanes d,e), or Y815 harboring pLC3-13 (lanes f,g) were mixed with the substrate envelope fraction. The reaction was performed at 60°C for 2 h and proteins were analyzed by SDS–polyacrylamide gel electrophoresis and fluorography.

pKD8, *Bgl*II fragment) into respective site of plasmid pBR322 (the *Bgl*II fragment was cloned into the *Bam*HI site). Plasmids pHY11 and pHY12 were constructed by insertion of *Acc*I 4 kb fragments (shown by a hatched bar in fig.4) into the *Eco*RI site of pHY001 [8] using synthetic *Eco*RI linkers after filling in cohesive ends with the aid of the Klenow fragment of *E. coli* DNA polymerase I (pHY11 and pHY12 have the insertion in opposite directions). Fig.4 shows regions of the *E. coli* chromosomal DNA carried by these plasmids. Y815 or Y8 cells were transformed by these plasmids and the envelope fractions were examined for their PLP signal peptidase activity in

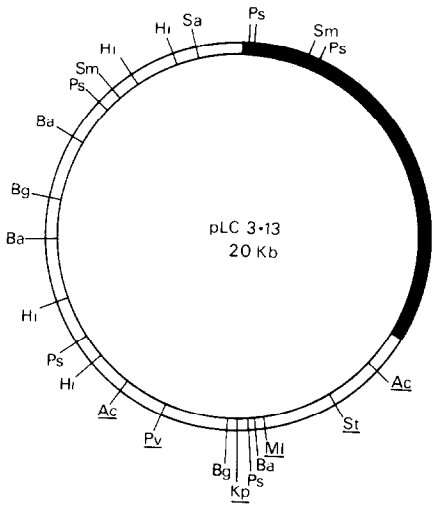

Fig.3. Restriction map of pLC3-13. Restriction sites: Ba, *Bam*HI; Hi, *Hin*dIII; Ps, *Pst*I; Sa, *Sal*I; Sm, *Sma*I; Bg, *Bgl*II; Kp, *Kpn*I; Ml, *Mlu*I; Pv, *Pvu*II; St, *Stu*I; Ac, *Acc*I. Only those adjacent to the gene for PLP signal peptidase are shown for the underlined sites. The open bar and closed bar indicate the *E. coli* chromosomal DNA and the ColE1 DNA, respectively.

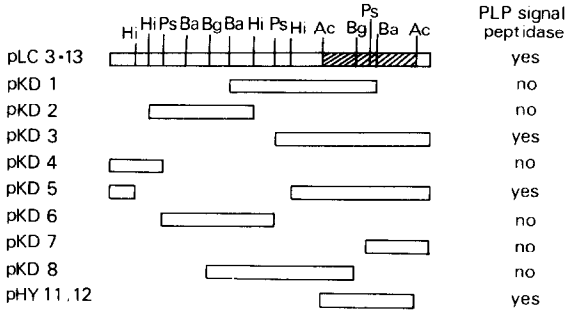

Fig.4. Location of the PLP signal peptidase gene on plasmid pLC3-13. Bars indicate *E. coli* chromosomal DNA carried by the plasmids constructed. For abbreviations of restriction sites, see the legend to fig.3. The presence or absence of PLP signal peptidase in Y815 harboring these plasmids is shown by 'yes' or 'no', respectively.

vitro at 60°C. Plasmids carrying the *E. coli* chromosomal DNA covering the 4 kb *Acc*I fragment always gave the mutant cells the PLP signal peptidase activity (fig.4). From these results, the gene coding for the PLP signal peptidase was concluded to be located within the region covered by the *Acc*I 4 kb fragments. Finally, it should be noted that the mutation responsible for the temperature-sensitive PLP signal peptidase in Y815 has been mapped genetically near the *rpsT* locus on the *E. coli* chromosomal map and pLC3-13 has been shown to carry the *rpsT* and *dnaJ* loci (in preparation). Identification of a polypeptide specified by the cloned gene is in progress.

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