EXISTENCE OF SEVERAL HOMOLOGOUS SEQUENCES IN THE *ESCHERICHIA COLI* CHROMOSOME TO THE GENE FOR THE MAJOR OUTER MEMBRANE LIPOPROTEIN

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Received 16 October 1981; revision received 4 December 1981

1. Introduction

The major outer membrane lipoprotein of *E. coli* is one of the most extensively characterized membrane proteins (reviews [1,2]). It has a peculiar amino-terminal structure, a glycerylcysteine residue to which 3 fatty acid residues are covalently linked [3]. It is produced from a secretory precursor, prolipoprotein, with a signal peptide consisting of 20 amino acid residues [4]. Besides the major lipoprotein, another lipoprotein called PAL has been found in the outer membrane of Gram-negative bacteria, which has a similar amino-terminal structure to the *E. coli* major lipoprotein [5–8]. There are as many as 9 lipoproteins in the *E. coli* envelope [9]. Although the M_r-values of these lipoproteins and their amounts per cell are very different from each other, they share the following common features: (i) they are membrane proteins; (ii) they are produced from secretory precursors with signal peptides; (iii) all of them appear to have a glycerylcysteine residue at the amino-terminus [9]. The amino-terminal structure of PAL from *Proteus mirabilis* has been shown to be glycerylcysteine–Ser–Ser–Asn–, which is identical to that of the *E. coli* major lipoprotein [8].

Here we report results which suggest that there are ⩾7 more genes for 'lipoprotein-like' proteins in *E. coli* in addition to the gene for the major lipoprotein.

2. Materials and methods

2.1. *Bacterial strains*

The following strains of *E. coli* K-12 were used: JE5506 F⁻ *pps his proA argE thi gal lac xyl mtl tsx* and JE5505 F⁻ *lpp-1 pps his proA argE thi gal lac xyl mtl tsx* [10].

2.2. *Hybridization*

Total DNA from cells were prepared and digested with *Hin*dIII (Bethesda Res. Lab.) or *Eco*RI (Bethesda Res. Lab.) as in [11]. Hybridization was carried out in 5 × SSC (SSC = 0.15 M NaCl + 0.015 M sodium citrate) and 50% formamide at various temperatures according to [12]. The probe was purified from pKEN015 which is a derivative of pBR322 carrying the *lpp* promoter and a part of the coding region of the prolipoprotein. In this plasmid, an *Eco*RI linker was inserted at the *Sau*3A site at position 123 of the DNA sequence of the *lpp* gene [13] (Nakamura and Inouye, in preparation). Thus, *Xba*I and *Eco*RI double digest of pKEN015 DNA gave rise to a DNA fragment which covers position; 23–123 of the *lpp* gene plus 6 bases at the 3′-end due to the *Eco*RI linker. This probe was purified by polyacrylamide gel electrophoresis and nick-translated with d[^{32}P]XTP [14].

3. Results and discussion

It is assumed that the amino acid sequences around the signal peptide cleavage sites of all the above lipoproteins are probably quite homologous. This further suggests that DNA sequences of the lipoprotein genes corresponding to these regions are homologous and will hybridize to each other. To examine this hypothesis, we have purified a DNA fragment ~100 basepairs in length as a hybridization probe from a plasmid clone of the major lipoprotein gene, which

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Published by Elsevier Biomedical Press
00145793/82/0000–0000/$02.75

covers the entire signal peptide (60 basepairs) and 8 amino acid residues from the amino-terminus of the lipoprotein (25 basepairs). It also contains an untranslated region of 16-bases upstream of the initiation codon and an extra sequence of 6 bases at the 3′-end due to an *Eco*RI linker inserted at this position.

When *Hin*dIII digests of the total chromosomal DNAs from *E. coli* JE5506 (*lpp*$^+$) and JE5505 (*lpp*$^-$) were hybridized with the probe at 30°C in 5 × SSC and 50% formamide, the 10 kilobase fragment carrying the *lpp* gene [15] was detected for JE5506 but not for JE5505 because of the deletion of the *lpp* gene in JE5505 as in [15] (not shown). No other DNA fragments were detected for both strains under the conditions described. However, when the hybridization temperature was lowered to 20°C, two additional *Hin*dIII fragments of apparent 25 and 3.4 kilobases were shown to hybridize with the probe for JE5505 (fig.1a-1). In the case of JE5506, the 25 kilobase fragment can be clearly seen but not the 3.4 kilobase fragment because of the high background in this region (fig.1a-2). The strong band at the 10 kilobase position for JE5506 (fig.1a-2) is due to the homologous hybridization with the gene for the major lipoprotein [15].

To detect less homologous genes, the hybridization temperature was further lowered to 12°C, and the hybridization was carried out for 4 days. As shown by arrows in fig.1b-1, as many as 7 *Hin*dIII fragments became hybridized with the probe. The extents of hybridization judged from band density were different from band to band. This is considered to be due to the extent of sequence homology in each band to the probe used. It is also possible that denser bands may carry >1 homologous sequence. However, it is unlikely that fainter bands such as those found at 15 and 9.7 kilobases are due to partial digestion, since electrophoretic analysis of the *Hin*dIII digest showed no indication of partial digestion (not shown). In the case of the *Eco*RI digest of the JE5505 DNA, only 4 bands were detected by the probe (fig.1b-2). It is possible that *Eco*RI digestion may generate very small fragments <1 kilobase carrying homologous sequences which are not detectable under the conditions used here.

Since the probe used is only ~100 basepairs long, it is reasonable to assume that each of the homologous sequences detected in fig.1b-1 was derived from an independent gene. This suggests that in addition to the gene for the major outer membrane lipoprotein

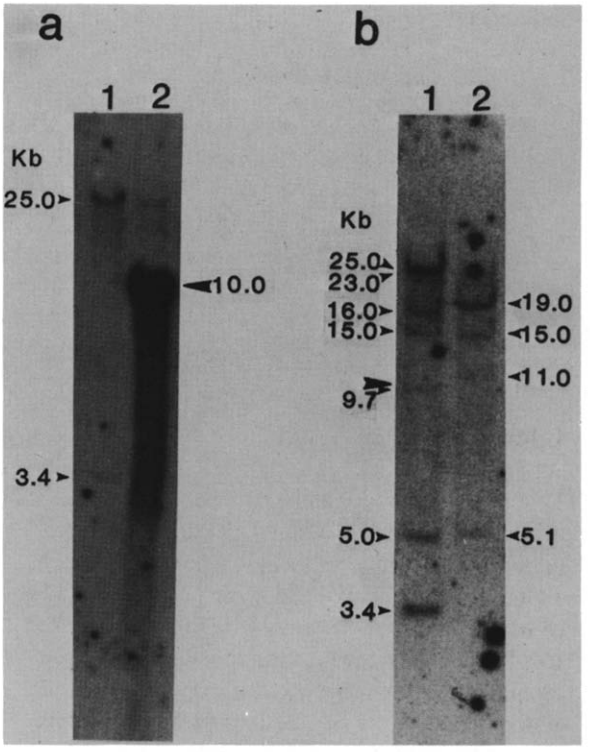

Fig.1. Hybridization of the *Hin*dIII or *Eco*RI digests of total DNA from *E. coli* JE5505 (*lpp*-1) and JE5506 (*lpp*$^+$) with a ^{32}P-labeled probe ~100 basepairs long containing the coding region from amino acid residues 1–28 of the prolipoprotein of the *E. coli* outer membrane. Hybridization was carried out in 5 × SSC in 50% formamide according to [12]. (a) Hybridization was performed at 20°C for 2 days: (1) *Hin*dIII digest of JE5505 DNA; (2) *Hin*dIII digest of JE5506 DNA. (b) Hybridization was performed at 4°C for 4 days: (1) *Hin*dIII; (2) *Eco*RI digests of JE5505 DNA. An arrow indicates the position of the 10 kilobase *Hin*dIII fragment carrying the *lpp* gene.

there are ⩾7 more genes for 'lipoprotein-like' proteins. It is highly possible that these genes code for the new lipoproteins found in [9]. Cloning and characterization of these DNA fragments may substantiate this prediction. Furthermore, these experiments will provide important information as to the functions and properties of these new lipoproteins as well as the evolutionary relationship between these genes.

Acknowledgements

This research was supported by grants from the US Public Health Service (GM-19043) and the American Cancer Society (BC-67).

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