The outer membrane of lipid A-deficient Escherichia coli mutant LH530 has reduced levels of OmpF and leaks periplasmic enzymes

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We have previously described a new Escherichia coli K-12 mutant, LH530, which has a defective outer membrane. LH530 is very sensitive to hydrophobic antibiotics, does not grow at 42 °C and synthesizes reduced amounts of lipid A. Phenotypically LH530 is very similar to the known lipid A biosynthesis mutants of E. coli and Salmonella typhimurium. Its genetic defect is not known, but the defect is suppressed by multiple copies of ORF195. Here we show that at 37 °C LH530 contains a reduced amount of the OmpF porin and that it leaks periplasmic β-lactamase at 37 °C and 42 °C. We further show that ORF195, when present at low copy number, restores the antibiotic resistance and lipid A biosynthesis of LH530 at 28 °C, but not at higher temperatures. In contrast, OmpF expression is restored at 37 °C.

Keywords: Escherichia coli, ORF195, OmpF, periplasmic enzymes, outer membrane permeability

INTRODUCTION

Lipopolysaccharide (LPS) is an essential component of the outer membranes (OMs) of Gram-negative bacteria, and mutations affecting the structure of LPS severely decrease the ability to grow in normal environments (Nikaido & Vaara, 1987). Defects in the LPS inner core region render the OM permeable to hydrophobic antibiotics and sensitize the bacteria to these agents. However, mutations affecting the early phase of lipid A biosynthesis are lethal to the bacteria. Thus, leaky or conditional, e.g. thermosensitive, lipid A mutants are the best choice for studying the lipid A biosynthesis pathway. At permissive temperatures lipid A mutants are even more sensitive to hydrophobic antibiotics than the mutants that are defective in synthesis of the inner core part of the LPS (Vaara, 1993). Moreover, at non-permissive temperatures lipid A deficiency leads to leakage of periplasmic enzymes (Vuorio & Vaara, 1992).

The proper trimerization and export of porin proteins requires a specific LPS structure (Sen & Nikaido, 1991) and deep rough mutants contain reduced amounts of OM proteins (Ames et al., 1974; for review, see Nikaido & Vaara, 1985). The relative abundance of OmpC and OmpF is a function of the temperature and osmolarity of the growth medium (Pratt et al., 1996). envZ and ompR control porin gene transcription in response to osmolarity, and the antisense RNA, micF, influences ompF translation. OmpF deficiency can also result from dsbA mutations (Pugsley, 1993) and pleiotropic tolC mutations (Misra & Reeves, 1987; Forst & Inouye, 1988). DbsA is a periplasmic disulphide oxidoreductase responsible for the formation of disulphide bonds in periplasmic proteins and in periplasmic domains of inner and outer membrane proteins as well as secreted proteins (Bardwell, 1994). TolC probably affects OmpF levels by increasing micF expression.

Recently, we isolated a new E. coli K-12 mutant, LH530 (Hirvas et al., 1997), which in many aspects resembles the known lipid A biosynthesis mutants. It is hyper-susceptible to hydrophobic antibiotics, grows in a heat-sensitive manner and has a decreased rate of lipid A biosynthesis. The defect is suppressed by multiple, but not by low copy numbers, of ORF195 (Hirvas et al., 1997), an ORF located in the 76 min region of the chromosome (Sofia et al., 1994).

In this work we show that LH530 leaks periplasmic β-lactamase and is deficient in OmpF protein at higher temperatures. Further we compare the effect of low- and high-copy expression of ORF195 in the mutant on the rate of lipid A biosynthesis and on the expression of OmpF.
METHODS

Bacterial strains and plasmids and general DNA manipulations. The OM mutant LH530 originates from JM105 (Yanisch-Perron et al., 1985) and has been described previously (Hirvas et al., 1997). DME553 is an ompF deletion mutant of E. coli K-12 (Misra & Benson, 1988). The pUC19-derived plasmids, pLH29 (containing ORF376 and ORF195) and pLH31 (containing ORF195), as well as the low-copy-number vector pACYC184-derived plasmid pLH37 (containing ORF376 and ORF195) have been described previously (Hirvas et al., 1997). General DNA manipulations were carried out as described by Sambrook et al., (1989).

Measurement of leakage of periplasmic enzymes and lipid A biosynthesis rate. The leakage of β-lactamase and glucose-6-phosphate dehydrogenase from bacteria was measured essentially as described by Vuorio & Vaara (1992) using the methods of O’Callaghan et al. (1972) and Langdon (1966), respectively.

The ratio of LPS to phospholipid biosynthesis in LH530 and its derivatives was assayed (Vuorio & Vaara, 1995; Hirvas et al., 1997) after labelling their fatty acids with radiolabelled acetate in vivo at 28 °C, 37 °C and 42 °C.

Analysis of major OM proteins. LH530, JM105 and DME553 (ompF−) were grown on L-plates at 28 °C and 37 °C. Plasmid-containing derivatives of LH530 were grown on L-plates containing 100 μg ampicillin ml−1 or 30 μg chloramphenicol ml−1. Approximately 10¹⁰ bacteria were suspended in 10 ml 0-9% NaCl and collected by centrifugation. Bacterial cell envelopes were isolated (Hirvas et al., 1991a) and their protein profiles analysed in 10% SDS-PAGE (Laemmli, 1970). Gels were stained with Coomassie Blue, scanned by an Agfa Arcus II scanner and edited by Canvas 3.5 using a Macintosh 7500/100 computer.

RESULTS

Release of periplasmic enzymes

We have previously shown that the OM of LH530 is permeable to hydrophobic agents (Hirvas et al., 1997). Here we studied whether its OM leaks periplasmic proteins, as does the OM of certain lipid A mutants. We therefore introduced pUC19 (encoding periplasmic β-lactamase) into LH530. As a control we introduced plasmid pLH31 into LH530. pLH31 is a pUC19 derivative and contains wild-type ORF195. A temperature shift from 28 °C to 37 °C or 42 °C induced approximately 39% and 45% leakage of the periplasmic β-lactamase from LH530(pUC19), respectively. With pLH31 the leakage was only 3-5% and 1-0%, respectively (Fig. 1a, b). At 28 °C the leakage was less than 12% (data not shown). The cytoplasmic marker enzyme, glucose-6-phosphate dehydrogenase was not released (Fig. 1c, d).

Fig. 1. Release of periplasmic β-lactamase and cytoplasmic glucose-6-phosphate dehydrogenase from derivatives of LH530. LH530 carrying either pUC19 ( ), or ORF195 ( ), was shifted from 28 °C to 37 °C (a, c) or to 42 °C (b, d) at time zero. β-Lactamase (a, b) and glucose-6-phosphate dehydrogenase (c, d) activities in the growth medium ( ), and the corresponding cellular activities ( , ) are shown.
The protein composition of LH530

Fig. 3 shows the OM protein profile of LH530 and its derivatives. At 37 °C the profile of LH530 (lane 4) differed markedly from that of JM105 (lane 2) and resembled the profile of the OmpF-deficient strain DME553 (lane 9). The OmpC content was compensatorily increased. Both the multi-copy-number plasmid (pLH29) and low-copy-number plasmid (pLH37) restored the protein profile to one similar to JM105. At 28 °C the OM protein profiles of JM105 (lane 1), LH530 (lane 3), and pLH29- (lane 5) and pLH37- (lane 7) containing derivatives of LH530, were identical.

We have previously shown that high-copy expression of ORF195 (i.e. as in pLH29) is needed to suppress the defect of LH530 at all temperatures. Low-copy expression (pLH37) suppresses the defect totally at 28 °C, and almost totally at 37 °C, but does not restore the ability of LH530 to grow at 42 °C. Fig. 3 shows that both pLH29 and pLH37 were able to restore the OmpF content of LH530 at 37 °C (lanes 6 and 8, respectively).

**DISCUSSION**

In this work we have further characterized the thermosensitive and lipid A-deficient mutant, LH530, and showed that at 37 °C its OM is deficient in OmpF perin and leaks periplasmic β-lactamase. We also demonstrated that at 37 °C the low-copy expression of ORF195 (the high-copy suppressor gene of LH530) only partially restores the rate of lipid A biosynthesis of LH530 to normal levels. However, it does restore its OmpF level.

In many respects LH530 resembles the well-characterized lipid A mutants lpxA and lpxD (Galloway & Raetz, 1990; Hirvas et al., 1991b; Vaara, 1993; Helander et al., 1992, 1993; Vuorio & Vaara, 1992) in being sensitive to hydrophobic antibiotics, in producing decreased amounts of LPS (75% at 28 °C, 35-40% at 37 °C and 42 °C; Hirvas et al., 1997) and in leaking periplasmic proteins. In addition we have demonstrated that the lpxD mutant is OmpF deficient at 37 °C (unpublished observation) similar to LH530. However, LH530 does not harbour any mutation in lpxA or lpxD (Hirvas et al., 1997).

Our results indicate that the synthesis, assembly, or translocation of OmpF is dependent on certain level of LPS synthesis. Although some studies show that OmpA assembly differs from that of OmpF and OmpC (Ried et al., 1990; Bocquet-Pagès et al., 1981), it is difficult to explain why only OmpF is affected in LH530. A similar situation exists in certain deep rough mutants (for review, see Nikaido & Vaara, 1985). However, another explanation for the OmpF deficiency could be that, among other periplasmic components, LH530 also leaks membrane-derived oligosaccharides as well as disulphide oxidoreductase encoded by dsbA. Mutations in dsbA have been shown to decrease OmpF production in E. coli (Pugsley, 1993), and membrane-derived oligosaccharides have been proposed to have a role in recognition of the growth medium osmolarity (Pratt et al., 1996).

The OmpF deficiency is unlikely to be responsible for the antibiotic hypersensitivity of LH530, as it is sensitive even at 28 °C, where its OmpF level is normal. Furthermore, E. coli and S. typhimurium mutants lacking one or more of the porins or the OmpA protein, have an...
almost unaltered sensitivity to hydrophobic antibiotics (Chai & Foulds, 1977; Lounatmaa & Nurminen, 1977; van Alphen et al., 1977). The sensitivity of LH530 to hydrophobic antibiotics could be similar to that of deep rough LPS mutants, in which the relative lack of LPS in OM results in the compensatory appearance of glycerophospholipids in the outer leaflet of OM (for review, see Nikaido & Vaara, 1985). As LH530 is also sensitive to large hydrophilic antibiotics, transient rupturing (and resealing) of the OM may be involved, as is also suggested for some lipid A biosynthesis mutants (Vaara, 1993).

Obviously, LH530 has a mutation in a gene essential in OM biosynthesis. Phenotypically the mutant resembles the known lipid A biosynthesis mutants of E. coli and S. typhimurium (Galloway & Raetz, 1990; Hirvas et al., 1991b; Helander et al., 1992, 1993; Vuorio & Vaara, 1992; Normark et al., 1969; Karow & Georgopoulos, 1991, 1992; Karow et al., 1991). In contrast to mutants deficient in late acyl transferases (Lee et al., 1995; Clementz et al., 1995), there is no detectable change in the fatty acid composition of lipid A of mutant LH530 (Hirvas et al., 1997).

The mutation in LH530 does not necessarily involve any of the so far uncharacterized structural genes of lipid A biosynthesis. Thus, the characterization of mutant LH530 and the role of its suppressor gene, ORF195, in membrane assembly might give new insights into the regulation of the biosynthesis pathway for lipid A or into the translocation and biogenesis of OM components.

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REFERENCES


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