SHORT COMMUNICATION

Antigenic Alteration of Contaminating Lipopolysaccharide during Extraction of *Escherichia coli* Outer-membrane Proteins from Polyacrylamide Gels

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An antiserum raised to the ferric enterobactin receptor protein of *Escherichia coli*, isolated from SDS-polyacrylamide gels, contained high-titre antibodies to the lipopolysaccharide (LPS) of *E. coli* O111. This antiserum was used to show that proteins dissected from polyacrylamide gels can be contaminated with comigrating LPS at levels below those detectable by very sensitive silver staining methods. Using this antiserum it was also shown that the procedures used to extract proteins from polyacrylamide gels can alter the molecular structure and, consequently, the antigenic properties of the contaminating LPS.

INTRODUCTION

Lipopolysaccharide (LPS) forms a major component of the outer membrane of Gram-negative bacteria and, being located at the bacterial cell surface, has direct contact with the external environment. The O-polysaccharide portion of LPS consists of carbohydrate polymers and constitutes one of the major bacterial surface antigens. Antisera raised to LPS appear to be highly specific and have been used to demonstrate considerable strain variation in LPS structure. Indeed they have been used to establish the present-day serotyping schemes for bacterial species such as *Escherichia coli* (Ørskov *et al.*, 1977).

The migration of LPSs during PAGE is due to their content of lipid A and phosphate, but the relative mobility of the individual species depends on the length of the O-polysaccharide chains (Jann *et al.*, 1975). LPS often comigrates with outer-membrane proteins during electrophoresis and it is very difficult to prevent the O-polysaccharide from contaminating protein preparations obtained by dissecting protein bands from SDS-PAGE gels. Since LPS is very antigenic, antisera raised to a protein isolated in this way may also contain high-titre antibody to the LPS. In a recent publication we described how a protein preparation, considered to be a purified iron-regulated outer-membrance protein from *E. coli* O111, was used to raise antibodies to the protein. Although this protein appeared free from LPS according to the established silver staining procedures for carbohydrate, the resultant antiserum was found to contain high-titre antibodies to the LPS of *E. coli* O111. Surprisingly, these antibodies also reacted with the LPS from an *E. coli* strain of a different serotype (Chart & Griffiths, 1985). In the present communication we present data which suggest that the molecular configuration and thus the antigenic properties of LPS can be altered by procedures used for extracting proteins from polyacrylamide gels.

METHODS

Bacteria. Isolates of *Escherichia coli* O111 : K58 : H2 and *E. coli* O18 : K1 : H7 were obtained from stocks held at this laboratory.

Bacterial surface antigens. LPS was prepared from *E. coli* O111 : K58 : H2 and *E. coli* O18 : K1 : H7 by the method of Fitzgerald & Rogers (1980). LPS from *E. coli* O111 was kindly donated by Dr H. J. Rogers, National Institute for Medical Research, Mill Hill, London, UK.

Media and culture conditions. Strains were stored in brain heart infusion broth (Difco) containing 10% (v/v) glycerol. They were grown in trypticase soy broth (BBL) at 37 °C for 6 h.

SDS-PAGE. This was done as described previously (Chart & Griffiths, 1985).

Silver staining of LPS. LPS was prepared from whole cells and electrophoretically separated as described previously (Chart *et al.*, 1984); it was detected in SDS-PAGE profiles using the silver staining method of Tsai & Frasch (1982). Essentially, preweighed bacterial cell pellets were digested with proteinase K (Sigma) at a cell mass/enzyme ratio of 20 : 1 in SDS-PAGE solubilization buffer (60 °C, 1 h). After digestion the preparation was incubated at 100 °C for 10 min before electrophoresis.

Immunoblotting. LPS profiles were electrophoretically transferred from SDS-PAGE gels to nitrocellulose papers and reacted with antisera as described previously (Stevenson & Griffiths, 1985). Phenylmethylsulphonyl fluoride (PMSF) was added to blotting reagents to give a final concentration of 10 mM in order to inhibit any residual proteinase K activity.

Rabbit antiserum. The antiserum used in these experiments was raised to the ferric enterobactin receptor protein of *E. coli* O111 which had been isolated from SDS-PAGE gels (Chart & Griffiths, 1985). This antiserum also contained a high antibody titre to the LPS of *E. coli* O111 as confirmed by an enzyme-linked immunosorbent assay (Chart & Griffiths, 1985). An antiserum was also raised in a rabbit to the outer membrane of *E. coli* O111 as described previously (Griffiths *et al.*, 1985).

RESULTS AND DISCUSSION

The silver-stained LPS profiles of *E. coli* O111 and *E. coli* O18 shown in Fig. 1 show the typical 'ladder' appearance produced by O-polysaccharide chains separating during SDS-PAGE. Identical profiles were used for reaction with specific antisera by immunoblotting and are shown in Fig. 2. When blots were reacted with an antiserum raised to the outer membrane of *E. coli* O111, the results demonstrated the expected serotype specificity of the antiserum, which reacted only with the LPS of *E. coli* O111 (Fig. 2, lane *a*) and not with the LPS of *E. coli* O18 (Fig. 2, lane *b*). However, the antiserum raised to the isolated iron-regulated outer-membrane protein of *E. coli* O111 contained antibodies which recognized the LPS of both *E. coli* O111 and *E. coli* O18 (Chart & Griffiths, 1985; see also Fig. 2, lanes *c* and *d*). Preimmune serum from the immunized rabbit failed to recognize the LPS of either strain (data not shown). By adding purified LPS from *E. coli* O111 to the cross-reacting antiserum, it was possible to absorb out the antibodies which reacted with the LPS of *E. coli* O111 (Fig. 2, lane *e*). The absorbed antiserum still contained antibodies which reacted with the LPS of *E. coli* O18 (Fig. 2, lane *f*). Similarly, the addition of purified *E. coli* O18 LPS to the cross-reacting antiserum absorbed antibodies recognizing the LPS of *E. coli* O18; this absorbed antiserum reacted with the LPS of *E. coli* O111 (Fig. 2, lane *g*) but not the LPS of *E. coli* O18 (Fig. 2, lane *h*).

We have no explanation for this interesting phenomenon, although an examination of the structure of the respective O-polysaccharides suggests one possibility. The repeating subunit of LPS from both strains contains glucose (Fig 3). However, the glucose present in the O-polysaccharide of *E. coli* O111 is masked by colitose, which is linked to the glucose by 1,6 linkages. It is possible that some of the colitose-glucose linkages may have been cleaved during the extraction procedures described above, however mild, unmasking glucose and generating epitopes which may be similar to those found in the *E. coli* O18 serotype. Nevertheless, whatever the mechanism involved, the work suggests that not only should traces of LPS be considered as possible contaminants of proteins isolated from SDS-PAGE gels, but also that antisera raised to the protein/LPS preparations may give unexpected reactions with O-polysaccharide chains.

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Fig. 1

Fig. 2

Fig. 1. Silver-stained SDS-PAGE profiles of LPS prepared by proteinase K digestion of whole cells of *E. coli* O111 (lane a) and *E. coli* O18 (lane b), showing the typical ladder appearance of O-polysaccharide chains separating due to the polysaccharide chain lengths.

Fig. 2. Immunoblots showing the reaction of rabbit antibodies with the LPS of *E. coli*. A rabbit antiserum raised to the outer membrane of *E. coli* O111 reacted with the LPS of *E. coli* O111 (lane a) but not with the LPS of *E. coli* O18 (lane b). However, the antiserum raised to the isolated ferric enterobactin receptor protein of *E. coli* O111 contained antibodies which recognized the LPS of both *E. coli* O111 (lane c) and *E. coli* O18 (lane d). Adding purified LPS from *E. coli* O111 to this cross-reacting antiserum absorbed antibodies recognizing the LPS of *E. coli* O111 such that no reaction with the LPS of *E. coli* O111 could be detected (lane e); however, the absorbed antiserum still contained antibodies recognizing the LPS of *E. coli* O18 (lane f). In contrast, adding purified LPS from *E. coli* O18 to the cross-reacting antiserum failed to absorb antibodies to the LPS of *E. coli* O111 (lane g) but did absorb the antibodies recognizing the LPS of *E. coli* O18 (lane h).

Fig. 3. Subunit structures of LPS from *E. coli* O111 and *E. coli* O18. Both LPSs contain glucose, although the glucose in the LPS of *E. coli* O111 is masked by colitose.

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