SHORT COMMUNICATIONS

The Isolation of a New Outer Membrane Protein from the Parent Strain of *Neisseria gonorrhoeae* P9

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INTRODUCTION

The surface components of gonococci are of current interest as potential vaccines and in the search for antigens for use in the serological diagnosis and epidemiological studies of gonorrhoea. The outer membrane, composed of lipopolysaccharide, phospholipid and relatively few proteins (Johnston & Gotschlich, 1974), is potentially useful in both systems. A complex derived from it has been used to subdivide gonococci into serologically distinct groups based on the antigenic specificity of a major outer membrane protein (Johnston, Holmes & Gotschlich, 1976). A similar preparation has been found to produce strain-related immunity to gonococcal challenge of subcutaneous chambers implanted in guinea pigs (Buchanan *et al.*, 1977). Previous work in this laboratory has involved the isolation of individual gonococcal surface antigens in a pure form as a prelude to biological studies. Thus lipopolysaccharide (Stead *et al.*, 1975), pili (Robertson, Vincent & Ward, 1977) and two major outer membrane proteins (Heckels, 1977) have been purified from *Neisseria gonorrhoeae* P9. In this communication we describe the isolation and purification of a new major outer membrane protein from the parent strain of P9 which had been subjected to fewer subcultures *in vitro*.

METHODS

Strains and growth conditions. *Neisseria gonorrhoeae* strain P9 had been maintained in this laboratory for some years with repeated subcultures and storage in liquid nitrogen. The parent strain P9 (PS) had been maintained as a freeze-dried sample with minimum subculture. Bacteria were grown overnight on trays (27 × 37 cm) of Bacto GC medium base (Difco) supplemented as described by White & Kellogg (1965) in a humid atmosphere containing 5% CO₂.

Isolation of outer membrane complex. Bacteria were harvested and the outer membrane complex was extracted into 0.2 M-lithium acetate pH 6.0 as described previously (Heckels, 1977).

SDS-polyacrylamide gel electrophoresis. Samples were subjected to SDS-polyacrylamide gel electrophoresis using the discontinuous system of Laemmli (1970). Staining and destaining was as described previously (Heckels, 1977). Gels were recorded with a Pye Unicam SP1809 scanning densitometer.

RESULTS

Comparison of outer membranes

The outer membrane isolated from strain P9 showed, on SDS-polyacrylamide gel electrophoresis, the presence of two major proteins with molecular weights of 36000 and 24000 (proteins I and II), whereas strain P9 (PS) contained a third major protein with a molecular weight of 60000 (protein III, Fig. 1a).

Fig. 1. Densitometer scans of SDS-polyacrylamide gels: (a) Outer membrane fraction from *N. gonorrhoeae* P9 (ps); (b) Purified fraction obtained by ion-exchange chromatography on DEAE-cellulose in 6 M-urea.

Extraction of proteins into urea

Outer membrane from strain P9 (ps) was suspended in 6 M-urea in sodium acetate buffer, 0.2 M in acetate, pH 6.0 (3 ml) at a protein concentration of 10 mg ml⁻¹. After 30 min at 25 °C the suspension was centrifuged at 100 000 *g* and the supernatant solution, which contained 26% of the original protein, was retained. SDS-polyacrylamide gel electrophoresis of the soluble fraction showed that protein III and some minor proteins were readily solubilized but proteins I and II were only present in trace amounts.

Purification of protein III

Gel filtration. The urea-soluble material was applied to a column (15 × 300 mm) of Sephadex G-200 and eluted with 6 M-urea in acetate buffer. Fractions were collected and analysed for protein (Lowry *et al.*, 1951). A single peak was observed eluting at the void volume of the column. Fractions were bulked together and examined by SDS-polyacrylamide gel electrophoresis, which showed the presence of protein III together with trace amounts of proteins I and II.

Isoelectric focusing. A sample of the urea-soluble protein (1 mg) was subjected to isoelectric focusing using a column of 20 ml capacity essentially as described by Robertson *et al.* (1977) but with an equal mixture of ampholines of pH 3.5 to 5.0 and pH 5.0 to 7.0 and incorporating 2 M-urea throughout the pH gradient. After focusing, a single peak of protein was observed centred at pI 5.0. SDS-polyacrylamide gel electrophoresis of samples taken across the peak showed a strong band at 60 000 molecular weight with trace amounts of a protein at 55 000.

Ion-exchange chromatography. A sample of the urea-soluble protein (5 mg) in 6 M-urea in 10 mM-acetate buffer pH 6.5 was applied to a column (6 × 100 mm) of DEAE-cellulose (chloride form). The column was washed with similar urea buffer (10 ml) and then eluted with a linear gradient (100 ml) of 0 to 0.5 M-NaCl in urea buffer. Fractions (2 ml) were collected and analysed for protein. One major peak of protein was eluted at a concentration of 0.1 M-NaCl and two minor peaks were eluted at higher concentrations; the latter were not studied further. The fractions comprising the major protein peak were combined and dialysed to remove urea.

The purified fraction contained protein with no detectable lipopolysaccharide. SDS-polyacrylamide gel electrophoresis revealed a single band with a molecular weight of 60000 (Fig. 1*b*). This value was independent of the temperature of derivatization with SDS. With heavily loaded gels, a minor component of molecular weight 55000 was also detected, but not the other major outer membrane proteins I and II.

DISCUSSION

Recent studies on the outer membrane proteins of gonococci have attempted to correlate specific biological properties with the presence or absence of particular proteins. The major contribution to serological specificity has been identified with a principal outer membrane protein in the molecular weight range 32000 to 39000 (Johnston *et al.*, 1976). Association with leucocytes depends on the presence of a protein of 28000 to 29000 molecular weight, and susceptibility to proteolytic enzymes on one of 26000 to 28000 (Swanson, 1977). This last protein, which is also responsible for colony forms of different colouration and aggregation characteristics, appears identical to protein II isolated in our previous study (Heckels, 1977), displaying the same mobility change on SDS-polyacrylamide gels after derivatization in SDS at 37 or 100 °C (Walstead, Guymon & Sparling, 1977; J. Swanson, personal communication).

Little information exists on the presence or function of proteins outside this rather narrow molecular weight range, but in the current study outer membranes isolated from *N. gonorrhoeae* parent strain P9 (ps) clearly differ from those of the same strain P9 obtained after repeated subculture, in possessing a third major protein of molecular weight 60000 (protein III, Fig. 1*a*) in addition to proteins I and II. In the previous study only minor proteins were removed by extraction into 6 M-urea which was used as a preliminary step in the purification of proteins I and II (Heckels, 1977). Protein III, however, is readily extracted into urea and this provided a convenient means of further purification. Ion-exchange chromatography on DEAE-cellulose produced a fraction which was free from other major outer membrane components. In heavily loaded SDS-polyacrylamide gels, however, small amounts of a protein of 55000 molecular weight could be detected. This protein, which could not be further removed, was also present in all fractions obtained by gel filtration and isoelectric focusing and may be a structurally related fragment of protein III. Isoelectric focusing showed protein III to have an isoelectric point of 5.0 which is consistent with its position in the outer membrane since the surface pI of whole gonococci (5.3) is similar (Heckels *et al.*, 1976).

The loss of a major outer membrane protein on repeated subculture provides a further example of the variation in properties of gonococci grown *in vitro* and of the desirability of using relatively fresh isolates for the production of material for possible vaccines. The reappearance of such components may account for reports of new surface antigens on gonococci after growth *in vivo* of laboratory strains in guinea pig chambers (Penn *et al.*, 1976). Nevertheless, variants lacking particular outer membrane proteins should be valuable in comparative experiments to correlate biological properties with particular surface components.

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