Concentration of a Major Outer Membrane Protein at the Cell Poles in *Escherichia coli*

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Autoradiography of cell envelope ghosts obtained from a strain of *Escherichia coli* which lacks two major outer membrane proteins has been used to demonstrate the polar concentration of another major outer membrane protein, *ompA* protein. The β -lactam antibiotic cephalixin prevents the insertion of newly synthesized *ompA* protein into the poles but removal of the antibiotic allows the randomly dispersed protein to migrate to the polar and possibly the septal areas of the cell. Labelling of whole cells with bacteriophage K3 has confirmed a polar concentration of *ompA* protein.

INTRODUCTION

The outer membrane of *Escherichia coli* contains a few major species of proteins which are present in large numbers of copies per cell (Henning *et al.*, 1973*a,b*; Hindennach & Henning, 1975), together with many other proteins present in much smaller amounts. In a previous communication (Begg, 1978) experiments were described which showed that matrix proteins (*ompC*, *ompF*) (Endermann *et al.*, 1978) were inserted into the outer membrane of *E. coli* at random locations and that they did not remain fixed at the original sites of insertion. This report is concerned with the distribution of another major outer membrane protein of *E. coli*, *ompA* protein (Endermann *et al.*, 1978). In contrast to matrix protein, this protein appears normally to be concentrated at the cell poles.

Henning *et al.* (1973*a*) have described a technique for obtaining from *E. coli* cell ghosts which consist largely of only four major outer membrane proteins: I (matrix proteins), II (derived from the action of trypsin on *ompA* protein), III (see Garten *et al.*, 1975; Hindennach & Henning, 1975) and IV (lipoprotein; Braun & Rehn, 1969). This technique has been slightly modified to facilitate the production of ghosts from strains of *E. coli* which lack either matrix proteins or matrix proteins and *ompA* protein.

Lipoprotein does not contain histidine (Braun & Rehn, 1969). Consequently, using radioactive histidine labelling of the appropriate mutants, cell ghosts can be prepared which contain only one or two labelled major outer membrane proteins. Ghosts of this kind were used to study the localization of *ompA* protein under various conditions.

An alternative experimental approach, using bacteriophage K3, was applied to whole cells with normal outer membrane proteins. Bacteriophage K3, which uses *ompA* protein as the protein part of its receptor (Alphen *et al.*, 1977), was used as a 'label' for *ompA* protein in electron microscopy studies.

METHODS

Strains and growth conditions. The *E. coli* strains were: P400 (Skurray *et al.*, 1974), F^- *thi argE proA thr leu mtl xyl ara galK lacY rpsL supE non*; P530H, a variant of P400 lacking matrix proteins, F^- *thi argE proA thr leu mtl xyl ara galK lacY ompB*; P530HK3, a variant of P530H resistant to bacteriophages K3 and TuII* lacking *ompA* protein. Cells were grown in minimal medium (M9 salts; Adams, 1959) plus 0.2% glucose plus Casamino acids at 0.4%, or required amino acids at 20 mg ml⁻¹, in a shaking water bath at 37 °C.

Fig. 1. Stained polyacrylamide gel after electrophoresis of proteins from cell ghosts of: (a, b) P530H cells treated with cephalixin; (c, d) P530H cells untreated; (e) P400 cells untreated. The positions of matrix proteins (which run together in this gel system), of the *ompA* protein fragment, and of protein III, are indicated.

Ghost preparation. The technique used was essentially that described by Henning *et al.* (1973*a*) as procedure II. The sacculus, however, was kept intact by omitting the lysozyme procedure because strains lacking matrix proteins and *ompA* protein will not form stable rod-shaped ghosts after the mucopeptide has been removed (unpublished observation).

Polyacrylamide gel analysis (Lutkenhaus, 1977) of wild-type cell ghosts obtained by this procedure showed one dense band at mol. wt approx. 36000 (matrix proteins), a dense band at mol. wt approx. 19000 (the tryptic fragment of *ompA* protein), a third, less-dense band at mol. wt approx. 18000 (protein III), and several minor bands (see Fig. 1). Protein IV, lipoprotein, being covalently bound to the intact sacculus, would not enter the gel. The appearance of the tryptic fragment of *ompA* protein at mol. wt 19000 was surprising, as the original report gave a molecular weight of approx. 28000 (Henning *et al.*, 1973*b*) and more recent reports suggest a molecular weight of 24000 (Muhlradt & Golecki, 1975). The identity of the band at mol. wt 19000 was checked by analysing cell envelope preparations, before and after trypsin treatment, on polyacrylamide gels. In addition, Henning's ghost preparations were analysed in similar fashion before and after trypsin treatment. In both cases the dense band at mol. wt 33000 (*ompA* protein) disappeared after treatment with trypsin and a new dense band appeared at mol. wt 19000 (see Fig. 2).

Ghosts prepared from strain P530H lacked the dense band at mol. wt 36000 and ghosts from strain P530HK3 lacked the dense bands at mol. wt 36000 and mol. wt 19000. Protein III and the minor bands were still present in ghosts prepared from both these strains (see Fig. 3).

Labelling with [3H]histidine and autoradiography. The techniques used have been described previously (Begg, 1978).

Autoradiograms were examined in a Zeiss photo-microscope and photographs were taken using Pan-F film (Ilford). Prints were made and the position of the grains along each cell were measured. Grain positions were expressed as fractional distances from the nearer of the two cell poles. Fractions of 5% of cell length were used for filamentous cells and fractions of 10% for cells of normal length.

Fig. 2. Stained SDS polyacrylamide gel showing: (a) cell envelopes of P400; (b) cell envelopes of P400 after treatment with trypsin; (c) a ghost preparation of P400 immediately after trypsin treatment; (d) the same preparation immediately before trypsin treatment; and (e) molecular weight standards, in descending order from the top, 68000, 42000, 18000, 14000, 11700. The positions of matrix proteins (which migrate together) and the *ompA* protein are indicated, together with the 18000 molecular weight standard.

Cephalexin was a generous gift from Eli Lilly & Co., and was used at $40 \mu\text{g ml}^{-1}$. Nalidixic acid was used at $20 \mu\text{g ml}^{-1}$.

K3 labelling technique. Cells were grown to a density of approx. $2 \times 10^8 \text{ ml}^{-1}$ in Oxoid Nutrient Broth no. 2 and 2 vols culture were mixed with 1 vol. K3 phage lysate (approx. $8 \times 10^{10} \text{ p.f.u. ml}^{-1}$) to give a phage multiplicity of 200 per bacterium. Adsorption was stopped by adding 3 vols glutaraldehyde fixative [5 ml glutaraldehyde (TAAB Laboratories, Reading, UK), 20 ml 0.1 M-phosphate buffered saline pH 7.4]. Cells were pelleted and resuspended in glutaraldehyde fixative for 1 h. This procedure was followed by washing three times in distilled water.

RESULTS

Fig. 4(a) shows the grain distribution obtained when ghosts were prepared from *E. coli* P400 cells which had been pulsed with [^3H]histidine for 10 min immediately before ghost preparation. Strain P400 contains all major outer membrane proteins. The distribution shows label over the whole cell surfaces with a slight excess at the poles. Fig. 4(b) shows the grain distribution obtained in the same way with P530HK3 cells, which lack matrix proteins (*ompC* and *ompF*) and *ompA* protein. The pattern is very similar to that obtained with P400. Fig. 4(c) shows the grain distribution obtained when P530H cells were treated in similar fashion. P530H lacks matrix proteins (*ompC* and *ompF*), due to a mutation in the regulatory gene *ompB*, but does contain *ompA* protein. *ompA* protein is responsible for 40% of the radioactive label in the ghosts (data not shown). The distribution of grains obtained with strain P530H is clearly non-random,

Fig. 3. Stained SDS polyacrylamide gel showing: cell envelope proteins of (a) P400, (b) P530H, (c) P530HK3; cell ghosts from (d) P400, (e) P530H, (f) P530HK3; and molecular weight standards (g) in descending order 68 000, 42 000, 18 000, 14 000, 11 700. The positions of matrix proteins, *ompA* protein, the tryptic fragment of *ompA* protein and the 18 000 molecular weight standard, are indicated.

Fig. 4. Distribution of grains as a function of relative distance from the pole in cell ghosts of *E. coli*. Cells were pulse-labelled with [^3H]histidine for 10 min immediately before ghost preparation. (a) P400 (188 grains measured), (b) P530HK3 (309 grains measured), (c) P530H (278 grains measured).

Fig. 5. (a) Distribution of grains (336 grains measured) in cells ghosts of *E. coli* P530H. Cells were pulse-labelled as in Fig. 1 then chased with non-radioactive histidine and allowed to grow for one mass doubling in the presence of cephalixin, to inhibit division. (b) Cells treated as in (a) but grown for two mass doublings in the presence of cephalixin (342 grains measured).

Fig. 6. Distribution of grains (488 grains measured) in cell ghosts of *E. coli* P530H. Cells were treated with cephalixin for 1.5 mass doublings, and pulse-labelled with [^3H]histidine in the presence of cephalixin for 10 min immediately before ghost preparation.

with a high degree of polar labelling and an indication of a second concentration at the cell centre, where new poles will form.

Fig. 5 shows the distribution of grains obtained when cells of P530H were pulsed with radioactive histidine for 10 min, chased with non-radioactive histidine and allowed to continue growing in the presence of cephalixin (Nishima & Nakazawa, 1973) to inhibit division for (a) one mass doubling or (b) two mass doublings. The labelled protein in both cases is predominantly found at the poles and at regions which would be the sites of new poles if the filaments were allowed to divide, i.e. 25% and 50% of the cell length (unpublished observation). Fig. 6 shows the result of labelling pre-formed cephalixin-induced filaments of P530H with radioactive histidine in the presence of cephalixin. This time the grains are randomly distributed over the entire surface of the ghosts.

The presence of cephalixin did not alter the protein composition of the ghosts (see Fig. 1), neither did it enhance or inhibit the incorporation of radioactive histidine (data not shown).

Fig. 7 shows the result of an experiment in which cephalixin-induced filaments of P530H were pulse-labelled with radioactive histidine in the presence of cephalixin. The cephalixin was then removed and the cells were allowed to continue growing. Samples were taken for ghost preparation at (a) 0 min, (b) 15 min and (c) 30 min after the removal of cephalixin. The observed distributions suggest that the labelled protein which is randomly distributed after incubation

Fig. 7. Cells of *E. coli* P530H were treated with cephalixin for one mass doubling, pulse-labelled in the presence of cephalixin for 10 min, washed free of cephalixin and allowed to continue growth. Ghosts were prepared as before. (a) Distribution of grains (360 grains on 112 cells) at 0 min after removal of cephalixin. (b) Distribution of grains (422 grains on 170 cells) at 15 min after removal of cephalixin. (c) Distribution of grains (422 grains on 170 cells) at 30 min after removal of cephalixin.

Fig. 8

Fig. 9

Fig. 8. Distribution of grains (460 grains measured) in cell ghosts of *E. coli* P530H. Cells were treated with nalidixic acid for one mass doubling and pulse-labelled with [^3H]histidine for 10 min in the presence of nalidixic acid immediately before ghost preparation.

Fig. 9. Distribution of bacteriophage K3 on the surface of cells of *E. coli* P400 after a 2 min adsorption period (560 phage measured).

with cephalixin can mobilize and relocate at polar and prospective polar regions after the removal of cephalixin. [Note: cell division re-starts between 30 and 40 min after the removal of cephalixin in this strain under these experimental conditions.]

It could be argued that the localization of the *ompA* protein at the poles of the cell ghosts might

have arisen by loss of the *ompA* protein fragment from non-polar regions only, during ghost preparation. However, such an explanation would also imply that the presence of cephalixin during cell growth would prevent this happening, although even then only to those *ompA* protein molecules which were synthesized in the presence of cephalixin. If this were so, then ghosts of cells labelled in the presence of cephalixin should contain more label than ghosts from cells labelled in its absence. Counts of the number of grains per cell in the experiment shown in Fig. 7 showed that this is not the case: there was no significant difference between the three samples. Grains per cell values were: 0 min, 3.2; 15 min, 2.9; 30 min, 2.5. [Autoradiograms from all three samples were exposed for the same time, so the grains per cell value for each is a true reflection of the amount of labelled protein per cell.] From the numbers of grains per cell and the distribution of the grains as a function of relative distance from the pole the average number of grains per pole can be calculated. The respective values were: 0 min, 0.35; 15 min, 0.81; 30 min, 0.83 (taking the pole as the first 5% of cell length). This demonstrates that the appearance of the polar label pattern after removal of cephalixin is due to an increase in labelled protein at the poles and not to loss from other areas.

To ascertain whether or not filamentation, in general, was responsible for the random distribution observed when pulse-labelling filaments in the presence of cephalixin, filaments of P530H were induced by nalidixic acid and pulse-labelled with radioactive histidine in the presence of this antibiotic. This time the grain distribution was non-random (Fig. 8), suggesting that the action of cephalixin was responsible for the random location of a protein which otherwise appears to be concentrated in polar areas.

The bacteriophage K3 adsorbs specifically to *ompA* protein (Alphen *et al.*, 1977). It can therefore be used to reveal the location of *ompA* protein in intact cells. Fig. 9 shows the distribution of bacteriophage K3 adsorbed to the surface of P400 cells after a 2 min labelling procedure. Although the receptor sites are not confined to the poles, there is a predominance of K3 adsorbed at the poles.

DISCUSSION

The experiments outlined in this report suggest that a major outer membrane protein of *E. coli*, *ompA* protein, is predominantly located at the poles of cell envelopes. Newly synthesized *ompA* protein appears to be inserted at these sites during normal cell growth. Furthermore, the β -lactam antibiotic cephalixin, which blocks cell division (i.e. pole formation), prevents polar concentration of this protein, but upon removal of cephalixin, the randomly distributed protein can mobilize and relocate at polar sites before cell division resumes.

These results are surprising, particularly as work on other major outer membrane proteins in Gram-negative bacteria has shown that they are inserted and distributed randomly in the outer membrane (Begg, 1978; Smit & Nikaido, 1978). The data in this report are derived from the distribution of the tryptic fragment of *ompA* protein and not from *ompA* protein itself. This fragment has been studied in some detail (Schweizer *et al.*, 1978) and in the presence of lipopolysaccharide was found to exhibit the phage receptor activity of *ompA* protein. Lipopolysaccharide is only found at the outer surface of the outer membrane (Muhlradt & Golecki, 1975) so it may well be that the tryptic fragment is the outer part of the protein which has been shown to be transmembrane (Endermann *et al.*, 1978).

Cephalixin inhibits division by inhibiting cross-bridge formation by the peptidoglycan transpeptidase action of penicillin-binding protein 3, which is specifically required for septum formation (Ishino & Matsushashi, 1981). Mutants which are temperature-sensitive for the production of penicillin-binding protein 3 have been described (Spratt, 1977) and these mutants produce filaments at the restrictive temperature. Preliminary 'ghost' labelling experiments in filaments induced by such a temperature-sensitive mutation have not, however, shown the random labelling pattern observed when cephalixin was present (data not shown). The label was strongly polar as in the case of filaments induced by nalidixic acid.

Although mutant strains of *E. coli* which lack *ompA* protein can grow and divide normally

(Henning & Haller, 1975), *ompA* protein has been suggested to play a role in cell morphogenesis because cells lacking both *ompA* protein and lipoprotein lose their normal rod shape and grow as spheres (Sonntag *et al.*, 1978). The absence of lipoprotein alone does not mediate this change.

Buchanan (1981) has reported the uneven distribution of penicillin-binding protein 2 (PBP2) in the *E. coli* cell membrane, and has discussed the possibility that its specialized function in cell elongation may be determined by its uneven distribution. Similar consideration might be given to *ompA* protein. The polar concentration of *ompA* protein might be required for the shaping of the hemispherical cell poles which form at each cell division.

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