EFFECT OF IRON ON THE RELATIVE ABUNDANCE OF TWO LARGE POLYPEPTIDES OF THE ESCHERICHIA COLI OUTER MEMBRANE

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SUMMARY: The relative abundance of two polypeptides of the Escherichia coli outer membrane is affected by the growth medium. The polypeptides have molecular weights of 85,000 and 95,000 and, in cells grown in medium containing low concentrations of iron, are dominant outer membrane proteins.

The outermost layer of the envelope of Escherichia coli is a membrane consisting of lipopolysaccharide, protein, and phospholipid. This outer (L) membrane contains approximately 60% of the total membrane protein of the cell (1) and the vast majority of L membrane protein is accounted for by only four or five different polypeptides. These polypeptides, which will be referred to as "dominant" outer membrane proteins, include a lipoprotein of molecular weight 7,000 (2) and a group (the major protein complex [3]) of three or four polypeptides which have molecular weights ranging from 35,000 to 44,000. At least several of the dominant proteins are present in at least 100,000 copies per cell (2, 4) and all are readily observed in profiles of both total and outer membrane proteins. In contrast, many of the outer membrane proteins for which specific functions have been demonstrated are present in such minor quantities that they are discernable in gel electropherograms only after partial purification (5, 6).

We observed that the amounts of two large membrane polypeptides varied with culture conditions. We show here that two outer membrane proteins (85p and 95p) are present in quantities approaching those of the dominant proteins

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85p and 95p are 85,000 and 95,000 dalton outer membrane proteins, respectively.

when E. coli B cells are grown in media containing low concentrations of iron. To our knowledge, this is the first demonstration that a specific inorganic component of growth medium can affect the polypeptide complement of the outer membrane.

MATERIALS AND METHODS

Strains. E. coli B and ER22 were from the culture collection of this laboratory; B/r was kindly supplied by H. E. Kubitschek.

Media and reagents. Tryptone broth (7) and M9 medium (8) have been described. T medium is a Tris-buffered minimal salts medium (9) modified to contain 10^{-4} M Na_2SO_4. M9 and T media were supplemented with 0.4% glucose and, where indicated, CAA or $FeCl_3$. SDS was obtained from Matheson, Coleman and Bell (source 1), from Sigma Chemical Company (source 2), and from Nutritional Biochemicals Corporation (source 3). Sarkosyl NL-30 was a gift of Geigy Chemical Corporation, Ardsley, N. Y., and carrier-free [^{35}S]H_2SO_4 was purchased from New England Nuclear Corporation.

Isolation of membrane. Total membrane was isolated by the procedures of Inouye and Guthrie (10) and Osborn et al. (1). Outer and cytoplasmic membrane fractions were obtained by isopycnic sucrose density gradient centrifugation (1). Outer membranes were also prepared by treating total membrane with 0.5% vol/vol Sarkosyl NL-30 for 20 min at 23 C (11).

Polyacrylamide gel electrophoresis. Membranes were solubilized at 100 C in the digestion mix described by Laemmli (12). Electrophoresis was carried out on SDS-polyacrylamide slab gels in a discontinuous buffer system (12). Electrophoresis was initiated at 60 V until the marker dye entered the separating gel. After that time, 10% gels were run at 120 V for 2.5 h and 10-20% gradient gels were run at 200 V for 3 h. Gels were stained by the procedure of Fairbanks et al. (13). Radioactive gels were dried and then contact exposed to Dupont Cronex 2DC medical X-ray film at room temperature. Extracts prepared from E. coli B cells which had been infected with T4 amber or deletion mutants in genes 43, 46, and rIIA and then labeled with [^{35}S] provided molecular weight standards for radioactive gels (14).

Chemical analyses. Protein determinations were performed by the procedure of Lowry et al. (15) and iron was assayed according to Stookey (16).

RESULTS

Among the factors which affect the profiles observed after polyacrylamide gel electrophoresis of E. coli membrane proteins are (i) the growth medium (3), (ii) the purity of the SDS employed (17), (iii) the temperature at which the membranes are digested (18, 19, 20) and (iv) the E. coli strain itself (3). The results of an experiment in which the influence of three of these factors on the appearance of polypeptides with molecular weights of 85,000 and 95,000 in total membrane preparations are shown in Fig. 1. There is a clear correlation between the growth medium and the abundance of these

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CAA, casamino acids; SDS, dodecyl sodium sulfate.

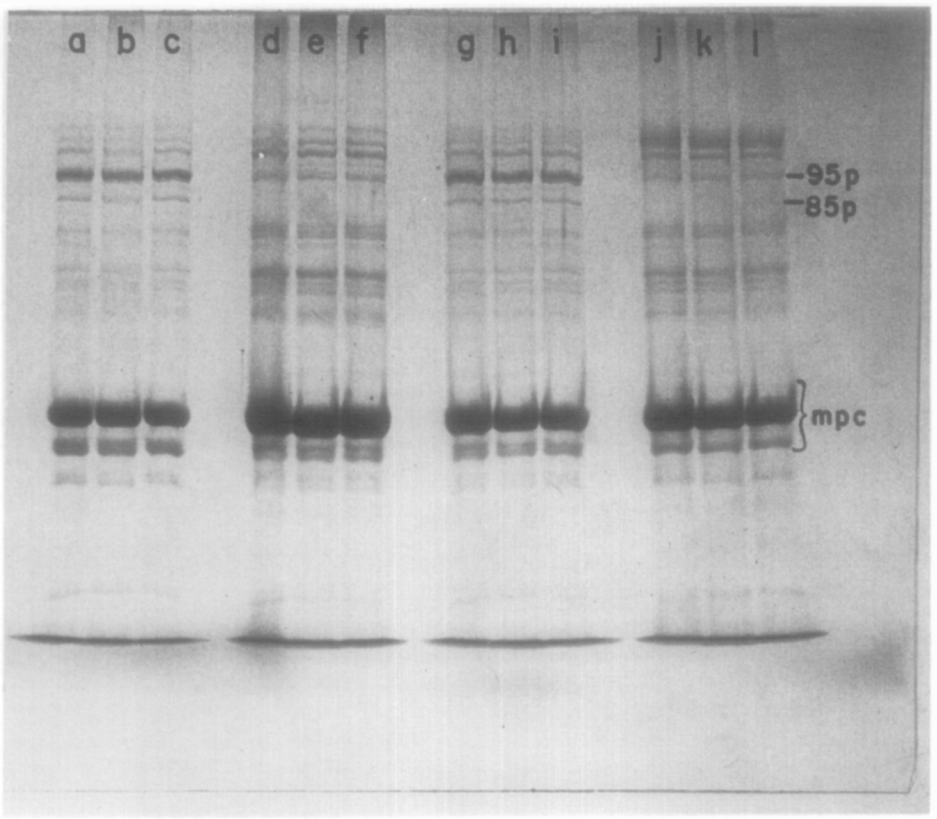

Fig. 1. Effect of media on membrane protein profiles of E. coli B and B/r. E. coli B was grown in T medium supplemented with 100 µg/ml CAA (a-c) or broth (d-f) to a concentration of 10^8 cells/ml. Total membranes were isolated (10) and their protein complement analyzed on a 10% polyacrylamide gel (40 µg/well) after staining. Similar preparations from B/r grown in supplemented T medium (g-i) or broth (j-l) are also shown. Samples were solubilized in digestion mix made up with SDS from source I (a, d, g, j), source II (b, e, h, k), or source III (c, f, i, l). The major protein complex is identified as mpc.

high molecular weight polypeptides. The polypeptides of interest are seen in membranes isolated from cells grown in T medium supplemented with 100 µg/ml CAA (a-c, g-i) and are absent, or present in greatly reduced amounts, in the membrane protein from broth-grown cells (d-f, j-l). Our laboratory strain of E. coli B does not appear to be atypical; 85p and 95p can be observed in membranes from B/r and in ER22 (data not shown), a derivative of the B strain of Eigner (21). Similarly, the appearance of these polypeptides was not affected by the source of SDS and other experiments (data not shown) demon-

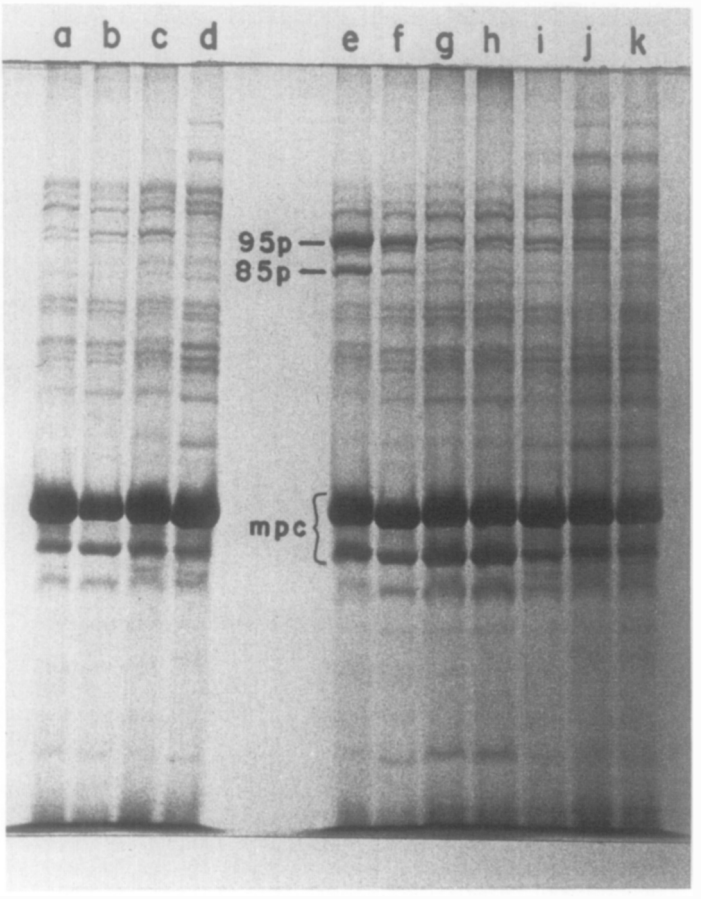

Fig. 2. Effect of iron and CAA on the protein composition of total membrane. Total membrane preparations from E. coli B cells grown in supplemented or unsupplemented M9 or T medium were isolated and analyzed as described in the legend for Fig. 1. Protein profiles are shown for cells grown in the following media: M9 (a); M9 + 1 mg/ml CAA (b); M9 + 10 μM $FeCl_3$ (c); M9 + 100 μM $FeCl_3$ (d); T (e); T + 100 μg/ml CAA (f); T + 5 mg/ml CAA (g); T + 10 mg/ml CAA (h); T + 10 μM $FeCl_3$ (i); T + 50 μM $FeCl_3$ (j); T + 100 μM $FeCl_3$ (k).

strated that the migration rate of the two polypeptides was unaltered by the temperature of digestion.

To investigate further the affect of medium on the presence of 85p and 95p, total membrane protein from cells grown in minimal media supplemented with various amounts of CAA or $FeCl_3$ was examined (Fig. 2). Membranes from cells grown in M9 medium do not contain large amounts of 85p and 95p and the quantity of these polypeptides is relatively unaffected by addition of either

CAA or $FeCl_3$ (a-d). In contrast, cells grown in T medium contain large amounts of these proteins (e) and addition of either CAA or $FeCl_3$ diminishes the quantity of these polypeptides (f-k). [It was also noted that at concentrations of $FeCl_3$ of 50 μM or greater, three new very high molecular weight polypeptides appear in membrane fractions (d, j, k).] These results suggested that 85p and 95p are repressed in media which contain high concentrations of iron. Accordingly, the content of iron in the several media was determined. Freshly-prepared T medium contained the lowest concentration (1.9 μM) of measurable iron and this value decreased with time; after one week, only 0.19 μM iron was detectable. M9 contained 3 μM iron and addition of 1 mg/ml CAA to media increased the iron concentration by 0.34 μM.

Two procedures were employed to determine with which E. coli membrane (outer or cytoplasmic) 85p and 95p are associated. Outer and cytoplasmic membranes were isolated by isopycnic sucrose density gradient centrifugation (1) and outer membrane was also isolated on the basis of its resistance to solubilization by Sarkosyl (11). Gel profiles shown in Fig. 3 demonstrate that both 85p and 95p are localized in the outer membrane (b, d, g, h).

DISCUSSION

We have presented evidence which indicates that the relative abundance of two outer membrane polypeptides is affected by the iron concentration of the medium. We suggest that tryptone broth, media containing 1 mg/ml or more of CAA, and M9 medium, probably because of iron contamination in the salts of the phosphate buffer, contain iron in sufficient quantities to inhibit the appearance of 85p and 95p in membrane. The concentration of iron in T medium is below that necessary for repression of synthesis of 85p and 95p; increasing the iron concentration directly or by adding CAA reduces the levels of 85p and 95p to amounts similar to those observed in M9. The minimal concentration of iron necessary for repression cannot be accurately stated because of the time-dependent decrease in the detectable iron concentration of T medium. (In T medium [pH 7.4], Fe^{+3} is probably gradually converted to $FeOH^{+2}$.) Although

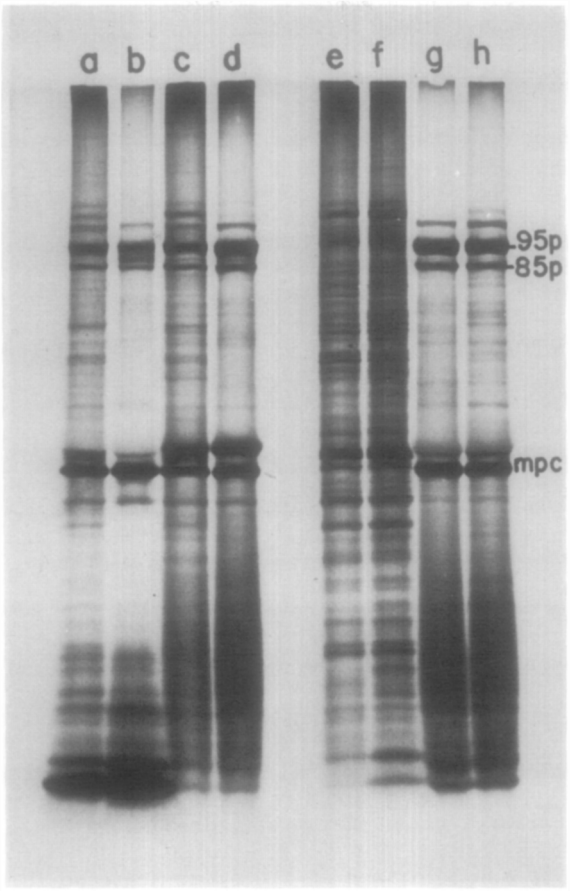

Fig. 3. Localization of 85p and 95p. Membrane preparations from E. coli B cells grown in T medium supplemented with 100 μg/ml CAA and 5 μCi/ml $[^{35}S]H_2SO_4$ were isolated by the procedures of Inouye and Guthrie (a, b) or Osborn et al. (c-h). The protein complements of the membrane fractions were analyzed on 10-20% polyacrylamide gradient gels (50,000 cpm/well). Membrane fractions are as follows: total membrane (a); Sarkosyl-resistant membrane (b); total membrane (c); Sarkosyl-resistant membrane (d); cytoplasmic membrane (e); Sarkosyl-sensitive proteins (f); Sarkosyl-resistant outer membrane proteins (g); outer membrane proteins (h).

85p and 95p are present in large quantities under conditions of low iron supply, they are unlike most of the "dominant" outer membrane proteins in at least two respects; (i) their cognate mRNAs have normal half-lives (McIntosh, unpublished results) and (ii) their migration rates in SDS gels are unaffected by the temperature of digestion.

The findings described in this report may be related to three previous general observations. Examination of the electropherograms of James (22) indicates that 85p and 95p may be identical to a group of three or four pro-

teins (protein "D") whose rate of appearance is increased by treatment with the antibiotic FL1060. Also, a previous report from our laboratory (23) demonstrated that the appearance in membrane of a large outer membrane protein of E. coli B is inhibited immediately upon T4 infection. Lastly, we have tentative evidence that the regulatory effect of iron occurs in K-12 as well as B strains. If this proves to be the case, the medium-dependent alterations in membrane protein profiles described by Lentzen (Ph.D. thesis, 1974, U. of Texas at Austin) in lon$^+$ and lon$^-$ strains of E. coli K-12 is very probably a manifestation of the iron effect.

The tonB product affects all known active transport systems for iron (24, 25) and at least one of these is repressed by high iron concentrations (24). In coordination with the tonA polypeptide, the tonB protein is necessary for cellular sensitivity to phages T1 and Ø80 and colicin M (6). The tonA protein binds ferrichrome (26), T5, and colicin M and is an 85,000 dalton polypeptide of the outer membrane (27). The tonA polypeptide and 85p may be identical; this idea and the possibility that the tonB product and 95p are related are being tested.

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