JOURNAL OF BACTERIOLOGY, May 1981, p. 804-807
0021-9193/81/050804-04$02.00/0

Vol. 146, No. 2

NOTES

Reduction in Three Iron-Regulated Outer Membrane Proteins and Protein a by the *Escherichia coli* K-12 *perA* Mutation

MICHAEL LUNDRIGAN AND CHARLES F. EARHART*

Department of Microbiology, The University of Texas at Austin, Austin, Texas 78712

Received 3 December 1980/Accepted 6 March 1981

We identified four outer membrane proteins (protein a and the iron-regulated proteins 74K, 81K, and 83K) present in reduced amounts in *Escherichia coli* K-12 *perA* strains. A comparison of the levels of enterochelin with the levels of 74K, 81K, and 83K suggested that *perA* acts posttranscriptionally.

The *Escherichia coli* K-12 *perA* mutation (16), which maps in the *ompB* region, affects a subclass of secreted proteins. The mutation results in reduced amounts of seven or more periplasmic proteins, including alkaline phosphatase, and at least three outer membrane proteins, one of which is OmpF (nomenclature system of Reeves [12]). Wanner et al. (16) provided evidence that, in the case of alkaline phosphatase, the *perA* mutation may act at a step beyond transcription initiation. More recently, *tpo* mutations have been described (15). These mutations, which are apparently identical to *perA*, were found to affect the maltose regulon as well. Thus, in cells with the *tpo* lesion, the outer membrane LamB protein and the periplasmic MalE protein are decreased in both uninduced and induced states. The cytoplasmic protein amylomaltase (MalQ) was affected only in the induced state. In contrast to the previous results with alkaline phosphatase, gene fusion studies indicated that the *tpo* mutation acts at the promoter level to influence LamB production. This discrepancy has not been explained. We show here that the *perA* mutation and some *ompB* mutants reported by Verhoef et al. (14) which are phenotypically identical to *perA* also affect the prevalence of three iron-regulated outer membrane proteins (74K, 81K, and 83K) (2) and outer membrane protein a (8).

The affect of the *perA* mutation on the outer membrane protein complement of cells grown in low-iron medium is shown in Fig. 1. (The gel system used was particularly effective in resolving the major outer membrane proteins; more definitive evidence regarding the iron-regulated polypeptides is shown in Fig. 2.) BW490.9 (*perA*) is deficient in OmpF, as previously reported, and also protein a and the iron-regulated proteins. In control experiments, it was demonstrated that the reduced levels of 74K, 81K, and 83K are not the result of low levels of OmpF; regulation of synthesis of these proteins is normal in *ompF* mutants (data not shown). UT5600 (Fig. 1, lane 3) is a deletion mutant that lacks protein a and 81K (3); its protein profile is included for identification purposes. Introduction of the F13 episome into UT5600 restores both proteins (Fig. 1, lane 4).

Verhoef et al. (14) isolated several *ompB* mutants of strain PC0479 which have low levels of OmpF on the basis of phage TuIa resistance. Wanner et al. (16) noted the similarity of these to the *perA* mutant, and Wandersman et al. (15) provided evidence that at least two of these (CE1121 and CE1123) are identical to *tpo*. This information and the data shown in Fig. 2 and 3 are consistent with the notion that the *perA*, *tpo*, and several of the *ompB* strains described by Verhoef et al. carry mutations in the same cistron. The *perA* effect on the iron-regulated polypeptides (Fig. 2) and on protein a (Fig. 3) is manifested in three (CE1121, CE1123, and CE1124) of the five *ompB* mutants tested. CE1122 and CE1126 have outer membrane profiles similar to those of their parent (PC0479) and BW490.12. CE1122 was previously noted to differ from *tpo* mutants, CE1121, and CE1123 with respect to maltose regulon control. All five of the *ompB* strains maintained their resistance to phage TuIa; in each case the efficiency with which phage TuIa plated on these strains was reduced approximately 100-fold. In contrast, *perA* had no effect on the plating efficiency of LP81, a phage which uses protein a for at least part of its receptor (3). The reduced level of protein a in *perA* strains is apparently still adequate to permit infection by LP81.

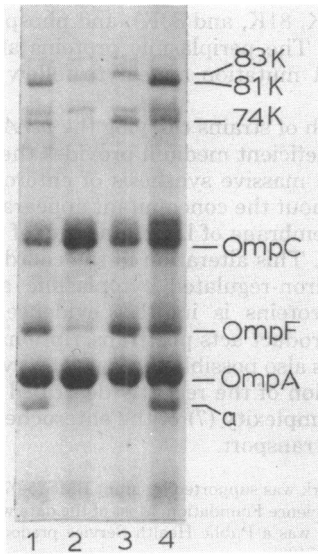

FIG. 1. *Effect of the perA mutation on the outer membrane protein composition of E. coli K-12. Outer membranes were isolated (5) from overnight cultures grown in iron-depleted M9 medium (10). The electrophoresis procedure was a modification of gel system D of Pugsley and Schnaitman (11). The separation gel (slabs, 1.5-mm thick by 11-cm long) consisted of 7% acrylamide, 0.17% bisacrylamide, 0.006% ammonium persulfate, 0.12% N,N,N',N'-tetramethylethylenediamine, 0.1% sodium dodecyl sulfate, and 8 M urea in a 0.375 M Tris-hydrochloride buffer, pH 8.8. The stacking gel and chamber buffer were according to Lugtenberg et al. (8); samples (15 μg of protein) were dissolved as described by Laemmli (6). Electrophoresis was carried out at 5 W of constant power for 3 h, and proteins were stained with Coomassie brilliant blue (4). Strains studied were: (1) BW490.12 (perA$^+$) (16); (2) BW490.9 (perA) (16); (3) UT5600 (a$^-$; 81K$^-$) (3); (4) F13/UT5600 (prepared by standard techniques for this work).*

The syntheses of the iron transport molecule enterochelin and that of 74K, 81K, and 83K are coordinately regulated (10). Indeed, *fep*, the structural gene for 81K, maps in the enterochelin biosynthetic gene cluster. The excretion of enterochelin into the growth medium by BW490.9 and the cognate *perA*$^+$ strain BW490.12 was monitored by the Arnow assay (1) over their entire growth cycles. The design of these experiments was similar to that described previously (10). In two different iron-deficient media (iron-depleted M9 supplemented with either 0.6 μM $FeCl_3$ or 10 μM $FeCl_3$-1 mM α,α'-dipyridyl), the rate of appearance of enterochelin in the medium and the amount of enterochelin produced were essentially the same in both strains. The

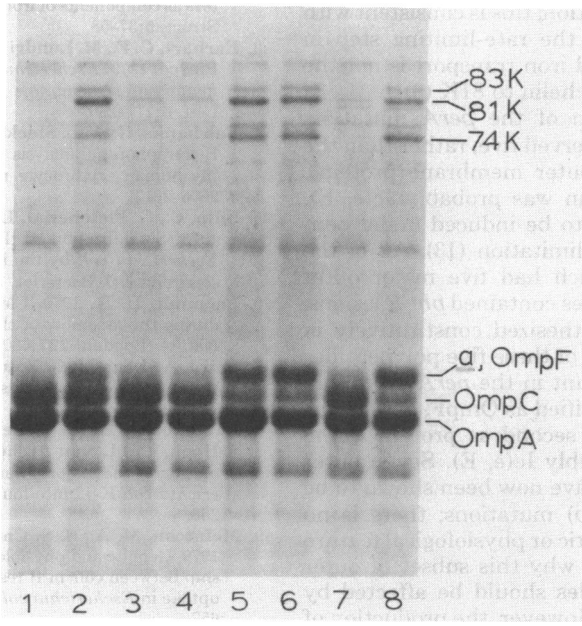

FIG. 2. *Comparison of the iron-regulated outer membrane proteins of selected ompB strains with that of the perA mutant. Electrophoresis of outer membrane samples containing 20 μg of protein was carried out as described by Lugtenberg et al. (8). E. coli K-12 strains, except BW490.12 and BW490.9, are described by Verhoef et al. (14). Strains examined were: (1) CE1121 (ompB); (2) CE1122 (ompB); (3) CE1123 (ompB); (4) CE1124 (ompB); (5) CE1126 (ompB); (6) PC0479 (ompB$^+$); (7) BW490.9 (perA); (8) BW490.12 (perA$^+$).*

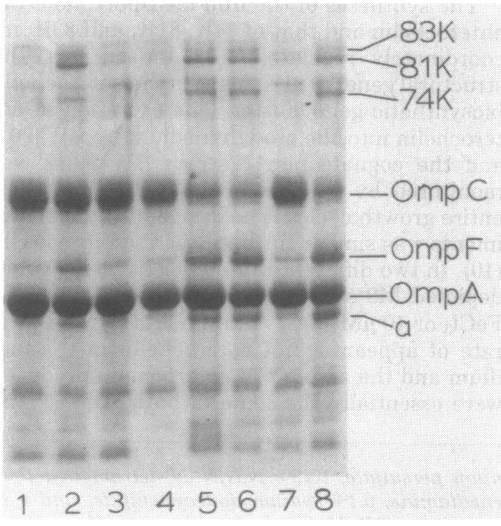

FIG. 3. *Effect of the perA and selected ompB mutations on outer membrane protein composition. Electrophoresis was carried out as in Fig. 1, and the strains examined are as in Fig. 2.*

simplest interpretation of these results is that the *perA* mutation affects the iron-regulated outer membrane polypeptides at a posttranscriptional step. The decreased levels of 81K in the *perA* mutation apparently did not impose premature iron starvation; this is consistent with the observation that the rate-limiting step in enterochelin-mediated iron transport is not the binding of ferrienterochelin to 81K (9).

In their description of the *perA* mutation, Wanner et al. (16) observed five, rather than the normal four, major outer membrane proteins. The additional protein was probably Ic(e, E), which is now known to be induced under conditions of phosphate limitation (13). All of the strains examined which had five major outer membrane polypeptides contained *phoR* lesions, and protein Ic is synthesized constitutively in *phoR* mutants. Three of these five polypeptides were reduced in amount in the *perA* mutation. One protein was identified as OmpF, and in this work we identified a second as protein a; the third protein is probably Ic(e, E). Seven outer membrane proteins have now been shown to be affected by *perA* (*tpo*) mutations; there is no obvious common genetic or physiological feature which would indicate why this subset of outer membrane polypeptides should be affected by one regulatory gene. However, the production of each of the proteins affected can be influenced by various aspects of the environment, such as temperature (protein a), osmolarity (OmpF), or the levels of nutrilites, such as maltose (LamB), iron (74K, 81K, and 83K), and phosphate (protein Ic). The periplasmic proteins affected by the *perA* mutation appear to follow the same pattern.

Growth of strains carrying the *perA* mutation in iron-deficient medium provides the first case in which massive synthesis of enterochelin occurs without the concomitant appearance in the outer membrane of large amounts of 74K, 81K, and 83K. This alteration in the coordinate control of iron-regulated cytoplasmic and membrane proteins is indirect evidence that the *perA*$^+$ product acts posttranscriptionally. However, it is also possible that our observations are a reflection of the recently described transcriptional complexity (7) of the enterochelin system for iron transport.

This work was supported by grant BMS75-07693 from the National Science Foundation. Most of the data were collected while M.L. was a Public Health Service predoctoral trainee (T32 GM-07126).

We thank B. Wanner and B. Lugtenberg for making their strains available.

LITERATURE CITED

1. **Arnow, L. E.** 1937. Colorimetric determinations of the components of 3,4-dihydroxyphenylalanine-tyrosine mixtures. J. Biol. Chem. **118**:531–537.
2. **Braun, V., R. E. W. Hancock, K. Hantke, and A. Hartmann.** 1976. Functional organization of the outer membrane of *Escherichia coli*: phage and colicin receptors as components of iron uptake systems. J. Supramol. Struct. **5**:37–58.
3. **Earhart, C. F., M. Lundrigan, C. L. Pickett, and J. R. Pierce.** 1979. *Escherichia coli* K-12 mutants that lack major outer membrane protein a. FEMS Microbiol. Lett. **6**:277–280.
4. **Fairbanks, G., T. L. Steck, and D. F. H. Wallace.** 1971. Electrophoretic analysis of the major polypeptides of the human erythrocyte membrane. Biochemistry **10**: 2606–2617.
5. **Filip, C., G. Fletcher, J. L. Wulff, and C. F. Earhart.** 1973. Solubilization of the cytoplasmic membrane of *Escherichia coli* by the ionic detergent sodium-lauryl sarcosinate. J. Bacteriol. **115**:717–722.
6. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
7. **Laird, A. J., and I. G. Young.** 1980. Tn5 mutagenesis of the enterochelin gene cluster of *Escherichia coli*. Gene **11**:359–366.
8. **Lugtenberg, B., J. Meijers, R. Peters, P. van der Hoek, and L. van Alphen.** 1975. Electrophoretic resolution of the major outer membrane protein of *Escherichia coli* K-12 into four bands. FEBS Lett. **58**:254–258.
9. **McIntosh, M. A., S. S. Chenault, and C. F. Earhart.** 1979. Genetic and physiological studies on the relationship between colicin B resistance and ferrienterochelin uptake in *Escherichia coli* K-12. J. Bacteriol. **137**:653–657.
10. **McIntosh, M. A., and C. F. Earhart.** 1977. Coordinate regulation by iron of the synthesis of phenolate compounds and three outer membrane proteins in *Escherichia coli*. J. Bacteriol. **131**:331–339.
11. **Pugsley, A. P., and C. A. Schnaitman.** 1978. Identifi-

cation of three genes controlling production of new outer membrane pore proteins in *Escherichia coli* K-12. J. Bacteriol. **135**:1118–1129.

12. **Reeves, P.** 1979. The genetics of outer membrane proteins, p. 255. *In* M. Inouye (ed.), Bacterial outer membranes: biogenesis and functions. John Wiley & Sons, Inc., New York.
13. **Tommassen, J., and B. Lugtenberg.** 1980. Outer membrane protein e of *Escherichia coli* K-12 is co-regulated with alkaline phosphatase. J. Bacteriol. **143**:151–157.
14. **Verhoef, C., B. Lugtenberg, R. van Boxtel, P. de Graaf, and H. Verheij.** 1979. Genetics and biochemistry of the peptidoglycan associated proteins b and c of *Escherichia coli* K12. Mol. Gen Genet. **169**:137–146.
15. **Wandersman, C., F. Moreno, and M. Schwartz.** 1980. Pleiotropic mutations rendering *Escherichia coli* K-12 resistant to bacteriophage TP1. J. Bacteriol. **143**:1374–1383.
16. **Wanner, B. L., A. Sarthy, and J. Beckwith.** 1979. *Escherichia coli* pleiotropic mutant that reduces amounts of several periplasmic and outer membrane proteins. J. Bacteriol. **140**:229–239.