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Normal Iron-Enterochelin Uptake in Mutants Lacking the Colicin I Outer Membrane Receptor Protein of *Escherichia coli*

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The outer membranes of two independent colicin Ia-resistant mutants of *Escherichia coli* K-12 lack the colicin Ia receptor protein. Such mutants exhibit normal capacity for enterochelin (enterobactin)-mediated iron uptake. It is concluded that the colicin Ia receptor is not involved in iron-enterochelin uptake.

Evidence is accumulating that suggests a relationship between iron transport functions in *Escherichia coli* K-12 and the ability of cells to bind certain colicins. Crucial to these seemingly unrelated phenomena are proteins found in the *E. coli* outer membrane.

The *tonA*$^+$ gene product of *E. coli* determines the production of an outer membrane protein that serves as the receptor for bacteriophages T1, T5, and ϕ80, and for colicin M, as well as ferrichrome. *tonA*$^-$ mutants are resistant to these phages and the colicin and are defective in ferrichrome-mediated iron transport (7). There is direct competition between ferrichrome and phage T5 for binding to the partially purified T5 receptor (11), which has been shown to be the receptor for colicin M (2). In separate experiments, Wayne and Neilands (168th ACS National Meeting, MICR 3, 1974; 15) showed that ferrichrome and bacteriophage ϕ80 bind to a common site in the cell envelope. A common binding site for colicin M and ferrichrome is supported by the finding that ferrichrome protects against colicin M in a mutant able to bind ferrichrome but defective in its utilization (14).

Guterman was the first to suggest an interaction between enterochelin (enterobactin), colicin B, and the sensitive cell (3, 4). She found that the addition of enterochelin to cells leads to protection against colicin B and provided evidence that enterochelin prevents colicin B adsorption. Wayne et al. (14) observed that enterochelin specifically protects cells against colicin B in a bacterial mutant able to accumulate but unable to utilize enterochelin, providing evidence for an earlier suggestion that the colicin B receptor functions as a component in enterochelin binding (15). Support of this notion derives from the finding that a class of mutants (*feuB*) defective in ferric enterochelin uptake are both unable to adsorb colicin B and lack an outer membrane protein (6).

There is indirect evidence pointing to a role of the colicin I receptor in iron uptake. Wayne

TABLE 1. *Colicin Ia adsorption to sensitive and resistant strains*[a]

Strain	Molecules of Ia per cell	
	Ia added	Ia adsorbed
CA274 (sensitive)	2,750	360
	12,000	508
	279,000	996
JK258 (resistant)	2,750	0
	12,000	33
	27,900	0
JK254 (resistant)	2,750	0
	12,000	0
	27,900	0

[a] Various amounts of ^{125}I-labeled colicin Ia (3.44×10^7 cpm/nmol) were incubated with 8.7×10^7 cells for 40 min at 37°C. The amount of colicin Ia adsorbed was determined as previously described (8).

TABLE 2. *Enterochelin-mediated iron uptake by colicin Ia-sensitive and -resistant cells*[a]

Strain	$^{55}Fe^{3+}$ uptake (pmol/10^8 cells)		
	Minus enterochelin	Plus enterochelin	Plus/minus
CA274 (sensitive)	0.15	3.0	20.0
JK258 (resistant)	0.17	3.3	19.4
JK254 (resistant)	0.16	2.6	16.2

[a] Cells were grown in M56 medium supplemented with 5 mM sodium citrate. Nitrilotriacetic acid, to 0.2 mM, was added to transport assays 2 min before the addition of ^{55}Fe and enterochelin (final concentrations, 6.2 nM and 4.5 nM, respectively). The specific activity of iron was 887 cpm/pmol. The values given represent 2 min of transport activity, since enterochelin-mediated uptake was essentially complete by that time.

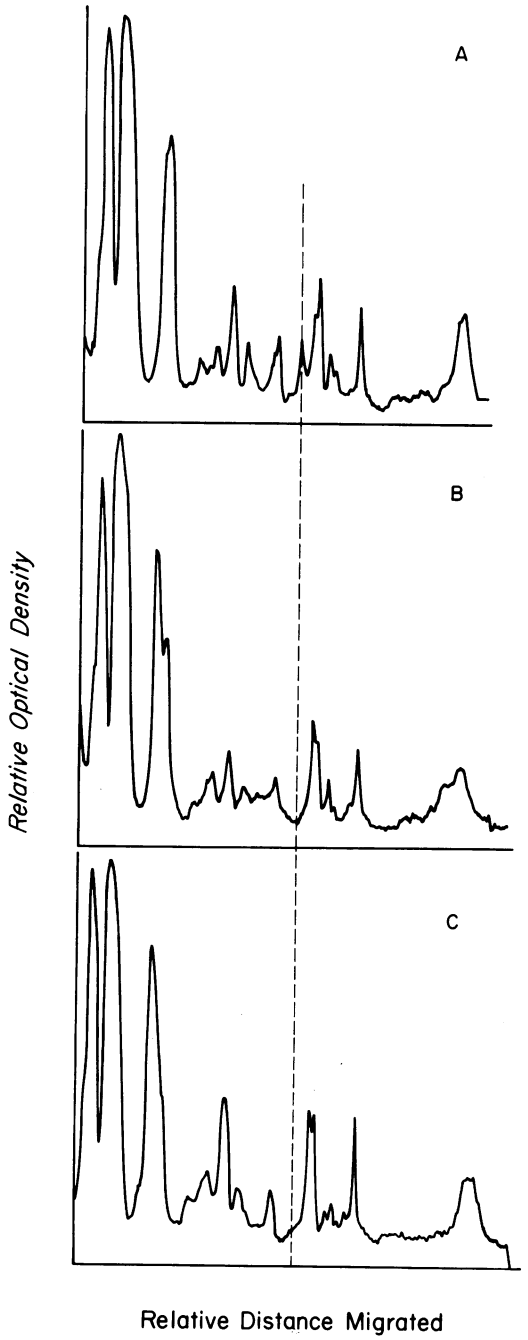

FIG. 1. *Microdensitometer analysis of SDS-polyacrylamide electrophoretic patterns of outer membrane proteins from sensitive and resistant cells. Each sample was electrophoresed in a separate well of a common slab, stained with Coomassie brilliant blue, and photographed; the resulting negative was traced on an EC Apparatus Model 910 microdensitometer. To optimize resolution in the region of interest, electrophoresis was prolonged until the time required for migration of the colicin Ia receptor (dashed line, approximately 74K daltons) to a position midpoint in the slab. Migration was right to left. (A) Strain CA274 (I sensitive), (B) strain JK258 (I resistant), and (C), strain JK254 (I resistant).*

et al. (14) demonstrated that inclusion of various natural or synthetic siderophores in the growth medium of an *E. coli* K-12 strain renders the strain insensitive to colicins B, V, and Ia. Protection required the availability or metabolism of siderophore iron. The basis for this finding was provided by experiments from this laboratory, which demonstrated an inverse relationship between the number of colicin Ia receptors per cell and the ability of *E. coli* K-12 to take up iron from the growth medium (9). These results raise the possibility that the Ia receptor functions in iron transport.

Recently, Hancock and Braun (5) reported the isolation and characterization of a mutant, designated *feuA*, which lacked the ability to bind colicin I and was reported to be deficient in ferric enterochelin transport. Further examination on polyacrylamide slab gels of outer membrane proteins extracted from *feuA* mutants revealed the absence of a protein of approximately 74,000 daltons (74K). The authors concluded that both the *feuA* function and colicin I binding require this outer membrane protein. Wayne and Neilands (Fed. Proc. 35:1453, 1976), however, examined the response of colicin-resistant mutants exposed in low-iron medium to a variety of siderophores. Their results not only linked the colicin B receptor with enterochelin uptake but also failed to uncover any relationship between colicin I resistance and the ability of cells to utilize any of the six tested siderophores. The data presented here extend the observations of Wayne and Neilands by showing that colicin Ia-resistant mutants, which do not bind colicin and are lacking an outer membrane protein, the Ia receptor, retain normal capacity to take up ferric enterochelin.

The parent strain for these studies, CA274 (*trp-49 rel-1 lacZ125* λ^-, suppressor-free), was kindly supplied by B. Bachmann, *E. coli* stock center. Strain JK254 was selected for suppressible colicin Ia resistance after hydroxylamine mutagenesis. Colicin resistance can be suppressed in this mutant by lysogenization with phage ϕ80 carrying either *supF* or *supU*. Strain JK258 is a spontaneous nonsuppressible colicin Ia-resistant mutant. The possibility of *tonB*$^-$-determined colicin resistance was eliminated by confirming that the mutants were sensitive to ϕ80 and/or colicin B.

For all studies, cells were grown in M56 salts (10), without added iron, containing 0.15% glu-

cose (or 0.4% glycerol for Ia adsorption procedures), 40 μg of tryptophan per ml, 1 μg of thiamine per ml, and 0.15% Casamino Acids (Difco). Iron transport was assayed according to the method of Langman et al. (10), except that no effort was made to rid glassware of contaminating iron. The procedure for determining the number of cell-associated colicin Ia molecules has been reported (8). *E. coli* outer membranes were isolated by the method of Osborn et al. (12), and the proteins therein were extracted with Triton X-100-ethylenediaminetetraacetic acid (13). Electrophoretic separation of outer membrane proteins was accomplished using the sodium dodecyl sulfate (SDS)-polyacrylamide slab gel system of Ames (1), except that *N,N'*-methylene-bisacrylamide was present to 0.3% (vol/vol). Enterochelin (enterobactin) was provided by K. Frick and was prepared from the culture supernatant fraction of *Aerobacter aerogenes* 62-1 (15). Thin-layer chromatography confirmed the purity of the preparation.

The results presented in Tables 1 and 2 demonstrate that uptake of ferric enterochelin is not related to resistance or sensitivity of cells to colicin Ia. Although the parent strain, CA274, binds noticeably fewer molecules of Ia per cell than is usually observed for *E. coli* K-12 strains, no binding occurs to the Ia-resistant derivatives. Enterochelin-mediated iron uptake, on the other hand, proceeds to the same extent in all strains.

Figure 1 shows that the loss of Ia-binding ability corresponds to the lack of a specific outer membrane protein of approximately 74K. This polypeptide is probably the same as that to which Hancock and Braun assigned the *feuA* function. That this protein is the colicin Ia receptor is supported by the following evidence: (i) isolated Ia receptor, recently purified to homogeneity in this laboratory, migrates on SDS-polyacrylamide gels as a 74K protein; (ii) when lysogenized with phage φ80 carrying *supF*, strain JK254 becomes colicin Ia sensitive. Outer membranes of this lysogen, subsequently examined on SDS-polyacrylamide gels, contain the 74K protein (data not shown).

As previously reported (9) the state of cellular iron transport substantially influences the number of Ia receptors in the *E. coli* outer membrane. Indeed, the Ia receptor molecule may be involved in some aspect of iron uptake. The data, however, eliminate a direct relationship between the colicin Ia receptor and ferric enterochelin uptake. These results can be reconciled with the earlier conclusions of Hancock and Braun (5), since it is now known that certain aspects of those experiments were in error (Braun, personal communication).

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ADDENDUM IN PROOF

A. P. Pugsley and P. Reeves (Biochem. Biophys. Res. Commun. 74:903-911, 1977) have recently reported that colicin Ia-resistant mutants isolated in their laboratory show normal enterochelin-mediated iron transport.

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