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Relationship Between the Transport of Iron and the Amount of Specific Colicin Ia Membrane Receptors in *Escherichia coli*

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Strains of *Escherichia coli* K-12 defective in their ability to utilize exogenously supplied iron due to genetic defects in the *entF*, *tonB*, *fes*, or *fep* gene exhibited elevated levels of the specific outer-membrane receptor for colicin Ia when compared with parental strains. Although *entF*, *fes*, and *fep* strains showed a higher degree of Ia sensitivity than did the parental strains, *tonB* strains were resistant to colicin action. The colicin insensitivity of *tonB* strains was not due to hyperproduction of enterochelin. Growth in medium containing 101.8 μM Fe^{2+} led to a lowering of receptor levels in all the above strains and resulted in decreased colicin Ia sensitivity in all strains except *tonB*, which was already at maximal resistance. Growth in citrate plus iron (1.8 μM) or in ferrichrome resulted in a substantial reduction in both receptor levels and Ia sensitivity in *ent*, *fes*, and *fep* strains but had no effect on receptor levels in *tonB* strains. Growth in citrate did not lead to an alteration in receptor levels in a mutant specifically defective in citrate-mediated iron transport. The presence of enterochelin during growth led to a reduction in the number of receptors in the parental and *ent* strains but not in *tonB*, *fes*, or *fep* strains. Thus, in all cases examined, there was an inverse relationship between the number of colicin receptors per cell and the ability of the strain to take up iron from the growth medium. This suggests that under conditions of iron limitation there is a derepression of colicin Ia receptor biosynthesis. These results may point to a role of the colicin I receptor in iron uptake.

An early step in the sequence of events whereby colicins kill sensitive *Escherichia coli* strains is the adsorption of a colicin protein molecule to a specific cognate receptor. In those cases studied, it has been shown that colicin receptors are protein components of the bacterial outer membrane (1, 13, 22, 26).

Within the last few years, evidence has accumulated that points to a relationship between cell sensitivity to colicins of the B type (3) and the functioning of the various iron utilization systems operating in *E. coli* K-12 strains (3, 6, 11, 17, 24, 25). Selection of mutants for insensitivity toward colicins of the B group (B, D, G, H, Ia, Ib, M, Q, S1, and V) can lead to the isolation of mutants that are indistinguishable from classical *tonB* mutants (3, 9). Since *tonB* mutants were found to hyperexcrete enterochelin (enterobactin, the cyclic trimer of 2,3-dihydroxybenzolserine) (9) and since the addition of enterochelin to cells unable to synthesize the catechol led to protection against colicin B by seemingly preventing colicin adsorption, Guterman (8) suggested a major direct role for enterochelin in the mechanism of colicin resistance in *tonB* strains. It has recently become apparent that the proper functioning of *E. coli* K-12 iron transport systems utilizing enterochelin, citrate, or hydroxamate siderochromes for iron uptake depends upon a functional $tonB^+$ allele (6). This leads to the possibility that the colicin insensitivity in *tonB* strains may derive from an alteration in iron utilization rather than from a direct effect of enterochelin. Support for such a proposal is obtained from studies showing that *tonB* mutants that are unable to synthesize enterochelin retain insensitivity toward colicin (8, 10).

Wayne et al. (24) have recently 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. In the case of ferrichrome-mediated protection against colicin M and enterochelin-mediated protection against colicin B, it was suggested that protection is mediated via competition of colicin and siderochrome for a common receptor. This would be consistent with the finding that the $tonA^+$ gene

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product serves as the receptor for phage T5, colicin M, and ferrichrome (11, 17, 25). In contrast to the mechanism of protection against colicins M and B described above, Wayne et al. (24) described a second class of siderophore-mediated protection that leads to protection against colicins B, V, and Ia. In this case, protection required the availability or metabolism of siderophore iron.

We have previously reported that an *E. coli* mutant defective in heme biosynthesis exhibits a reduced capacity for the biosynthesis of colicin I receptors (7). This finding together with the results described above suggested a relationship between iron availability and regulation of receptor synthesis. In the present communication, we show that such a relationship does indeed exist.

MATERIALS AND METHODS

Bacterial strains and medium. All the strains used (Table 1) were derived from *E. coli* K-12. In all experiments described, cultures were grown at 37 C with aeration by shaking in medium 63 (20) supplemented with the appropriate growth requirements, shikimic acid (40 μg/ml), Casamino Acids (0.15%), and glucose (0.15%). Medium 63 contains 1.8 μM $FeSO_4$ as added iron. Where appropriate, cultures were grown in medium 63 for at least 10 generations in the presence of the siderophore to be tested.

Determination of colicin receptors. The use of iodinated colicin Ia to measure colicin Ia receptors was described previously (12). In the experiments reported here, cultures were harvested at a Klett reading of 70 to 100 (no. 42 filter in a Klett colorimeter), washed twice with TBM [0.1 M tris(hydroxymethyl)aminomethane (pH 8.0)–0.2% bovine serum albumin–β-mercaptoethanol], and resuspended in TBM to a Klett reading of 70 to 75. Such suspensions were used directly in receptor assays. For all strains, one Klett unit corresponded to 3×10^6 to 5×10^6 cells/ml.

Resistant mutants. Spontaneous mutants resistant to colicin Ia were isolated by cross-streaking cells with purified colicin Ia. Colonies appearing in the zone of inhibition were picked and purified. Each independent isolate was checked for the presence of parental genetic markers.

Chemicals. Chemicals were of the highest chemical purity available. Enterochelin (enterobactin) was prepared from the culture supernatant fraction of *Aerobacter aerogenes* 62-1 (25). Ferrichrome and ferrichrome A were kindly supplied by J. B. Neilands.

RESULTS

Colicin Ia receptor levels in strains defective in iron utilization. The use of colicin Ia or Ib for the direct selection of *E. coli* mutants insensitive to these colicins leads to the isolation of several distinct mutant classes (3). One of these is composed of mutants that are both genotypically and phenotypically indistinguishable from *tonB* mutants. When we determined the capacity of several independent *tonB* mutants from several different strains to adsorb [^{125}I]colicin Ia, we found that all *tonB* strains tested bound 5- to 20-fold higher levels of iodinated colicin when compared with the appropriate parental strain.

Since it is known that *tonB* mutants hyperexcrete enterochelin (9), it became necessary to examine the possibility that the apparent increased binding of [^{125}I]Ia to *tonB* strains was, in fact, an artifact of the assay procedure resulting from the enterochelin-mediated retention of Ia on the assay filter. To rule out this possibility, it was desirable to examine Ia binding to a *tonB* strain unable to produce enterochelin. For this purpose, we obtained appropriate strains (see Table 1) that had been used previously to study certain aspects of iron uptake (6). When the ability of these strains to bind [^{125}I]Ia was compared, it was found that *tonB* strains exhibited excess colicin binding when compared with the parental strains whether or not the *tonB* strain carried the *entF* allele. Since *entF* strains are defective in enterochelin biosynthesis (18), it may be concluded that the high binding observed in *tonB* mutants does not derive from interference of the assay by enterochelin.

TABLE 1. *Strains of E. coli K-12 used*

Strain	Relevant genotype[a] or comment	Reference
AN92	Parental	
AN260	AN92 *fep*, defective in enterochelin-dependent iron transport	2
AN272	AN92 *fes*, defective in ferric enterochelin esterase, blocked in post-transport hydrolysis of ferric enterochelin	14
AN299	AN92, defective in citrate-mediated iron uptake	5
AN441	AN92 *entF*, blocked in conversion of 2,3-dihydroxybenzoate to enterochelin	18
AN409	Parental	
AN408	AN409 *tonB*, defective in citrate-, enterochelin-, and ferrichrome-mediated iron transport	6
AN442	AN409 *entF*, *tonB*; see above	
AN443	AN409 *entF*; see above	

[a] Genetic nomenclature is that used by Taylor and Trotter (23). For description of full genotypes, see references 2, 5, 6, and 14. All strains were kindly supplied by H. Rosenberg.

As can be seen in Fig. 1, both strains AN443 *(entF)* and AN408 *(tonB)* bound substantially more colicin Ia then did the parental strain AN409. Since both AN443 and AN408 are defective in enterochelin-mediated iron uptake (6), we decided to examine mutants blocked in two other steps of iron-enterochelin utilization. Both strain AN260 *(fep;* 2) and AN272 *(fes;* 14) bound levels of [^{125}I]Ia that were substantially higher than those found with the parental strain AN92 (Fig. 2). Thus, the presence of any one of four mutant alleles, each of which renders a strain defective in enterochelin-mediated iron uptake via a distinct block in the transport pathway (see Table 1), is sufficient to lead to elevated colicin Ia receptor levels.

It should be noted that the parental strains AN92 and AN409 differ in receptor levels. Since these strains differ only in the state of the *trp* gene, differences in receptor levels may result from a secondary effect of the *trp* mutation on the levels of enterochelin. This is likely since both tryptophan and enterochelin share chorismic acid as a precursor.

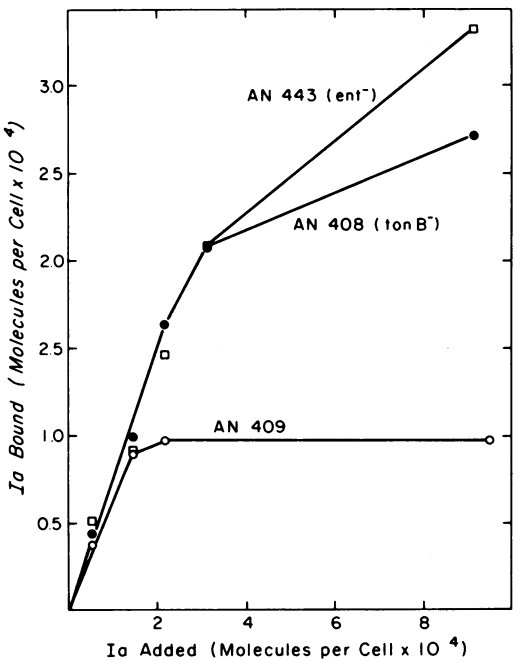

FIG. 1. *Adsorption of ^{125}I-labeled colicin Ia. Various amounts of ^{125}I-labeled Ia (9.23 × 10^6 counts/min per nmol) were incubated for 40 min at 37 C with 2.7 × 10^8 cells of each strain grown in medium 63. The amount of colicin Ia adsorbed was determined by the filter paper method previously described (12). Symbols: ○, AN409; ●, AN408; □, AN443.*

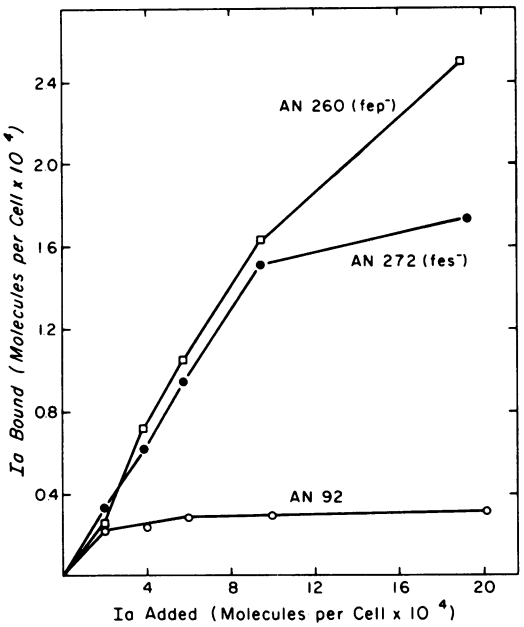

FIG. 2. *Adsorption of ^{125}I-labeled colicin Ia. The procedure was identical to that used for Fig. 1. Symbols: ○, AN92; ●, AN272; □, AN260.*

Effect of growth in iron-supplemented medium on levels of colicin receptors. It is possible that the elevated levels of Ia binding found in the above-described mutants are (i) a result of an alteration in the enterochelin system specifically or (ii) a general result of altered iron utilization caused by an inoperative iron uptake system. Since *tonB* mutants are unimpaired in the low-affinity uptake of uncomplexed iron, it is possible to distinguish these two possibilities by circumventing the enterochelin-dependent iron uptake system by supplementation of the growth medium with $FeSO_4$ (101.8 M, final concentration). The level of [^{125}I]Ia binding was lower in all strains grown in the presence of higher iron (Fig. 3 and 4). These effects of iron were seen only in those cases where iron was included in the culture medium during growth. The presence of 101.8 M $FeSO_4$ in a colicin-binding assay mixture containing cells grown in normal medium 63 (1.8 M $FeSO_4$) had no effect on the amount of colicin bound to cells. These data suggest that the high level of colicin receptors found in the above mutants results from general iron limitation. The iron-mediated reduction in receptors seen in strain AN92 was small and was not always observed. This may indicate that the receptor level in this strain is already at some basal level not subject to further reduction.

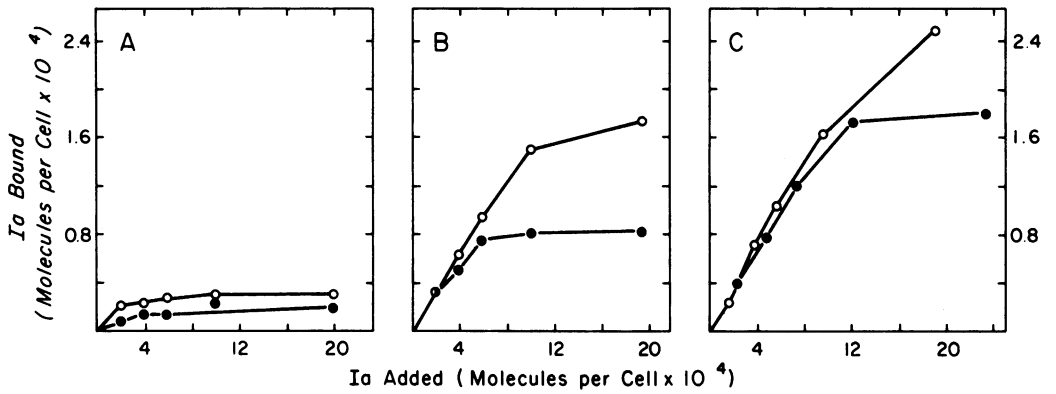

FIG. 3. *Adsorption of ^{125}I-labeled colicin Ia to cells grown in medium containing normal (1.8 μM) or high (101.8 μM) $FeSO_4$. Adsorption was determined as for Fig. 1 with the exception that the colicin specific activity was 8.9 × 10^6 counts/min per nmol and the number of cells per assay was 1.8 × 10^8. (A) AN92; (B) AN272; (C) AN260. Symbols: ○, Strains grown in 1.8 μM $FeSO_4$; ●, strains grown in 101.8 μM $FeSO_4$.*

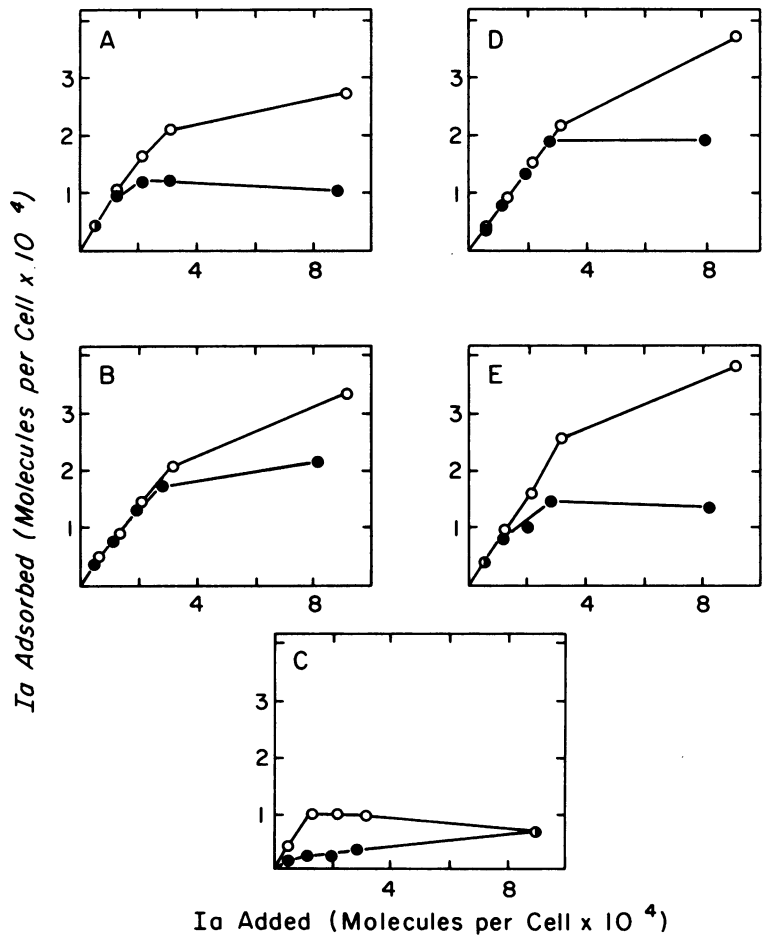

FIG. 4. *Adsorption of ^{125}I-labeled colicin Ia to strains grown in M63 medium containing normal (1.8 μM) or high (101.8 μM) $FeSO_4$. The procedure was as in Fig. 3. (A) AN408; (B) AN443; (C) AN409; (D) AN442; (E) AN441. Symbols: ○, Strains grown in 1.8 μM $FeSO_4$; ●, strains grown in 101.8 μM $FeSO_4$.*

Of those metals tested, only growth in the presence of Fe^{2+} led to reduction in colicin I receptor levels (Table 2). In some cases (Cu^{2+}, Al^{3+}, Zn^{2+}, and Cr^{3+}), a higher level of Ia receptors was found in the parental strain AN92. Although the reason for these increases is not understood, in some cases it may result from competition with iron leading to subsequent iron limitation.

Effect of enterochelin on receptor levels. If the higher levels of colicin receptors seen in the various mutants described above result from defects in various stages of the iron-enterochelin utilization system, the addition of exogenous enterochelin to the growth medium of such mutants should lead to predictable results. Thus, growth in the presence of enterochelin should lead to reduced receptor levels in those strains unable to synthesize enterochelin *(entF)*, but have no effect on receptor levels in those strains unable to utilize iron-enterochelin (that is, strains *fep*, *fes*, *tonB*, and *ent tonB)*. As can be seen (Table 3), the results were exactly as predicted. The presence of enterochelin in the receptor assay of all strains grown without added enterochelin had no effect on the amount of colicin adsorbed.

Effect of growth in citrate on receptor levels. *E. coli* K-12 possesses an inducible citrate-dependent iron transport system that is genetically and physiologically distinct from the enterochelin system (5). It has recently been shown that the functioning of the citrate system is also dependent on the presence of the $tonB^+$ allele (6). In addition, strain AN299, which was derived from strain AN92, has been shown to have a specific defect in citrate-mediated iron uptake (5). When the various strains were grown in the presence of citrate and subsequently tested for their ability to adsorb [^{125}I]Ia, the results shown in Fig. 5 were obtained. It can be seen that growth in the presence of citrate led to a reduction in the number of Ia receptors in all strains except AN408 *(tonB)* and AN299. Growth of AN299 in 101.8 M $FeSO_4$ led to a reduction in receptor levels (data not shown). The presence of citrate in receptor assays of all the above strains grown without added citrate had no effect on the amount of colicin bound. These results show that the effect of growth in citrate-supplemented medium on colicin Ia receptor levels depends on a functioning iron-citrate transport system.

Effect of growth in ferrichrome and ferrichrome A on receptor levels. Mutants of *E. coli* specifically defective in enterochelin biosynthesis maintain the ability to obtain iron for growth via an uptake system that utilizes the hydroxamate siderochrome, ferrichrome (6, 24).

TABLE 3. *Effect of growth in enterochelin on receptor levels*[a]

Strain	Molecules of Ia/cell			
	−Enterochelin		+Enterochelin	
	Added	Bound	Added	Bound
AN92	95,000	1,008	91,800	1,320
AN260 *(fep)*	98,200	61,700	95,000	57,300
AN272 *(fes)*	96,200	59,800	91,700	59,200
AN441 *(ent)*	90,500	51,500	91,800	7,750
AN409	82,700	10,700	81,800	4,170
AN408 *(tonB)*	87,500	34,300	82,800	29,600
AN442 *(ent, tonB)*	84,500	60,800	80,700	60,800
AN443 *(ent)*	85,800	52,500	84,500	8,620

[a] Each strain was grown in medium 63 ± 0.368 μM enterochelin. Colicin adsorption was determined as described in Materials and Methods.

TABLE 2. *Effect of growth in various divalent and trivalent cations on receptor levels*[a]

Expt	Cation	Molecules of Ia/cell in:					
		AN92		AN272 *(fes)*		AN260 *(fep)*	
		Added	Bound	Added	Bound	Added	Bound
1	None	5,780	388	6,070	1,950	7,650	4,380
	$CuSO_4$	5,970	1,040	6,820	4,420	6,580	4,300
	$MnCl_2$	5,880	357	6,270	3,550	7,500	4,780
	$ZnCl_2$	5,630	3,370			6,580	4,250
	$CoCl_2$	7,650	293				
2	None	6,420	453	6,420	3,050	7,070	6,450
	$Al_2(SO_4)_3(NH_4)_2SO_4$	6,620	8,730	6,230	4,230	6,230	4,200
	$CrK(SO_4)_2 \cdot 12H_2O$	5,970	3,580			7,570	4,120
	$FeSO_4$	5,970	560	6,130	467	6,330	406

[a] Each strain was grown in medium 63 supplemented with the indicated cation (100 μM, final concentration). Colicin adsorption was determined as described in Material and Methods.

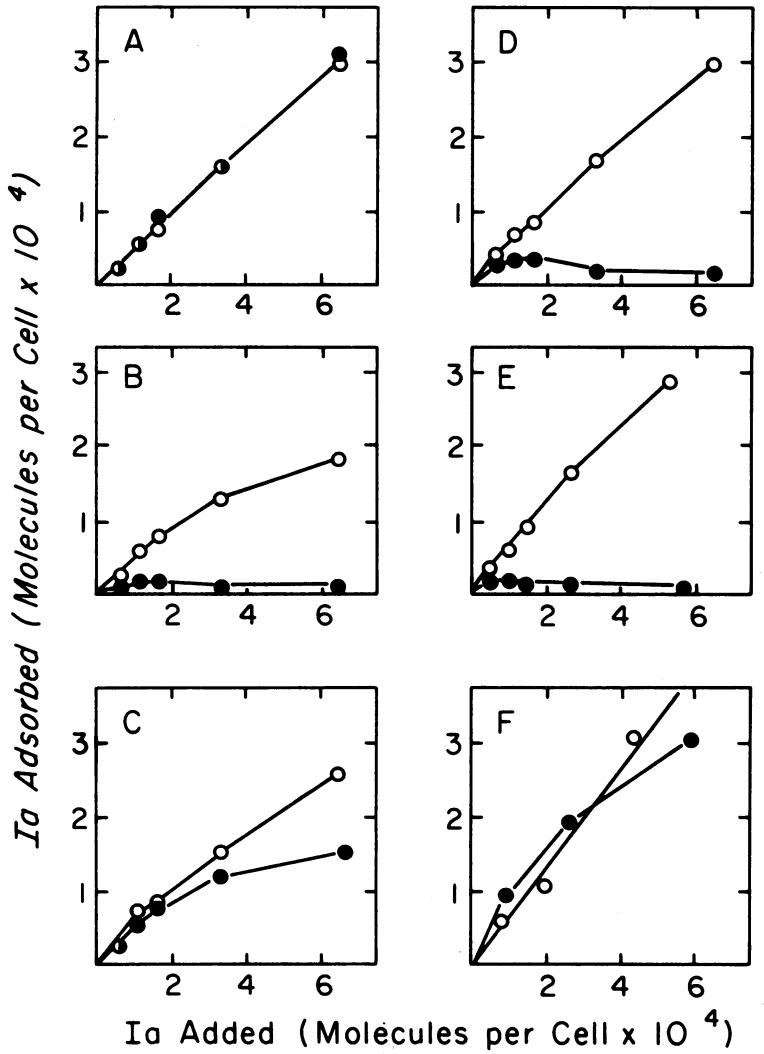

FIG. 5. *Adsorption of ^{125}I-labeled colicin Ia to strains grown in the presence of citrate. Adsorption was determined as in Fig. 1 with the exception that the colicin specific activity was 8.3 × 10^6 counts/min per nmol and number of cells per assay was 1.8 × 10^8. (A) AN408; (B) AN409; (C) AN260; (D) AN441; (E) AN272; (F) AN299. Symbols: ○, Strains grown in medium 63; ●, strains grown in medium 63 supplemented with 10 mM sodium citrate.*

This uptake system is inoperative in *tonB*$^-$ mutants (6).

Growth of strain AN409 (*tonB*$^+$) in medium supplemented with ferrichrome led to a reduction in the number of colicin Ia receptors per cell (Fig. 6). The degree of reduction was dependent upon ferrichrome concentration. In contrast, growth in the presence of the siderochrome had no significant effect on the level of Ia binding to strain AN408 (*tonB*). The response of strains AN92, AN260 (*fep*), AN272 (*fes*), AN441 (*ent*), AN443 (*ent*), and AN299 was similar to that of AN409. Receptor levels in AN442 (*entF, tonB*) did not respond to the presence of ferrichrome. Ferrichrome had no effect on Ia binding when included in a receptor assay using any strain grown without ferrichrome supplementation.

The ferrichrome analogue ferrichrome A is unable to deliver its iron for cell utilization (24). The presence of ferrichrome A in the growth medium of strain AN409 (*tonB*$^+$) had a much less drastic effect on receptor levels than did ferrichrome (Fig. 6). Since the ferrichrome A preparation used here is known to contain small amounts of ferrichrome (as assayed by

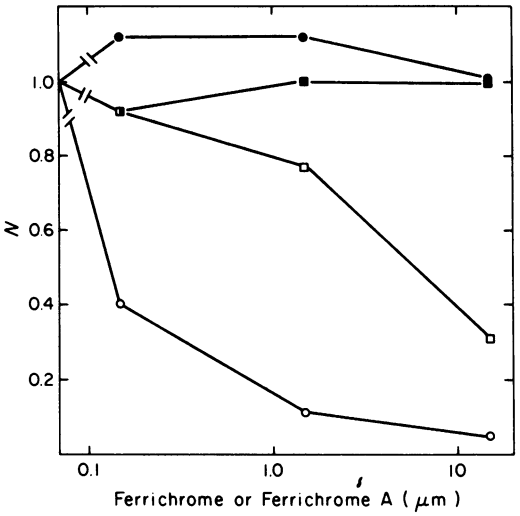

FIG. 6. *Effect of growth in medium containing ferrichrome or ferrichrome A on receptor levels. Strains AN409 or AN408 were grown in medium 63 supplemented with various amounts of ferrichrome or ferrichrome A. Adsorption of ^{125}I-labeled colicin Ia to such cells was determined as in Fig. 1. For the purpose of data normalization, the ordinate, N, is equal to the amount of Ia adsorbed per cell grown in the presence of ferrichrome or ferrichrome A divided by the amount of Ia adsorbed per cell grown in the absence of ferrichrome or ferrichrome A. All determinations were carried out at a ratio of 2,300 colicin molecules added per cell. Symbols: ○, AN409 plus ferrichrome; ●, AN408 plus ferrichrome; □, AN409 plus ferrichrome A; ■, AN408 plus ferrichrome A.*

thin-layer chromatography), the decrease in colicin Ia receptor levels seen in cells grown in the presence of high levels of ferrichrome A was most likely due to contaminating ferrichrome and not free iron. The fact that ferrichrome A had no effect on receptor levels in strain AN408 *(tonB)* supports this interpretation.

State of iron utilization systems affects the levels of active colicin Ia receptors. In previous sections the amount of [^{125}I]Ia bound to cells was taken as a measure of specific colicin Ia receptors. Strictly speaking, this assumption is unwarranted by the data. For example, it might be argued that the altered binding in the various strains is due to the iron-mediated regulation of nonspecific binding sites. The following experiments demonstrate that the state of iron utilization systems does indeed affect the levels of specific colicin Ia receptors.

The sensitivity to colicin Ia of various AN strains grown in normal medium 63 (1.8 μM Fe^{2+}) and medium supplemented with citrate or high Fe^{2+} is shown in Table 4. In the case of all *tonB*$^+$ strains, the level of Ia sensitivity exactly paralleled the levels of [^{125}I]Ia binding. Thus, mutant *tonB*$^+$ strains bind more colicin molecules (see Fig. 1 and 2) and are more sensitive than the appropriate parental strain. Furthermore, growth in the presence of citrate or high Fe^{2+} led to both a reduction in the number of Ia molecules bound (see Fig. 3 and 4) and decreased colicin sensitivity. When grown in citrate, strain AN299 exhibited no decrease in colicin sensitivity. In the case of the two *tonB* strains, which were Ia insensitive under all conditions, there was no correlation between receptor levels and strain sensitivity. Although the mechanism for *tonB* resistance to B-type colicins is unknown, it clearly does not require the production of enterochelin.

The relationship between receptor levels and strain sensitivity is qualitative. This may derive, in part, from the fact that colicin Ia-specific receptors are heterogeneous with respect to activity (12). It is not known how the extent of heterogeneity is altered in the various experiments described above.

If the altered levels of colicin Ia binding reflect alterations in specific Ia adsorption sites, these levels should be affected in colicin Ia-resistant derivatives of these strains. When such derivatives were isolated and examined for their capacity to adsorb [^{125}I]Ia, it was found that all insensitive mutants examined exhibited substantially lower levels of colicin binding (Table 5).

DISCUSSION

The finding that the state of iron uptake and utilization systems affects the levels of specific colicin Ia receptors provides an explanation for

TABLE 4. *Colicin Ia sensitivity of AN strains grown with or without citrate or with or without $FeSO_4$*

Expt	Strain	Killing units[a]		
		No additions	Citrate (10 mM)	$FeSO_4$ (101.8 μM)
1	AN92	43	11	21
	AN272 *fes*	171	11	85
	AN260 *fep*	85	13	85
	AN409	85	21	43
	AN441 *ent*	171	11	85
	AN443 *ent*	171	11	128
	AN408 *tonB*	0	0	0
	AN442 *ent tonB*	0	0	0
2	AN92	182	11	23
	AN299	182	182	23

[a] The data are expressed as the reciprocal of the maximum colicin dilution yielding a clear zone when spotted on an agar surface seeded with a lawn of sensitive indicator cells. Experiments 1 and 2 were carried out with different colicin Ia preparations.

TABLE 5. *Adsorption of ^{125}I-labeled colicin Ia to sensitive and resistant strains*[a]

Strain	Ia molecules bound/cell	% Parental
AN409	7,670	100.0
AN409 IR-1[b]	1,630	21.3
AN409 IR-2	78	1.0
AN441 *ent*	16,000	100.0
AN441 *ent* IR-1	0	0.0
AN441 *ent* IR-2	0	0.0
AN443 *ent*	26,200	100.0
AN443 *ent* IR-1	0	0.0
AN443 *ent* IR-2	0	0.0
AN92	3,990	100.0
AN92 IR-1	1,210	30.4
AN92 IR-2	537	13.4
AN272 *fes*	17,700	100.0
AN272 *fes* IR-1	733	4.2
AN272 *fes* IR-2	1,800	10.2
AN260 *fep*	21,200	100.0
AN260 *fep* IR-1	1,760	8.3

[a] Colicin adsorption was assayed as described in the legend to Fig. 3. The data show adsorption values determined at an arbitrary input concentration of Ia at which the amount of colicin bound was increasing linearly with the amount of colicin added. The amount of colicin added per cell was the same for all strains.

[b] IR, Colicin I resistant.

siderophore-mediated protection of *E. coli* K-12 against B-type colicins (24). Based on our findings, any physiological or genetic manipulation of cells resulting in reduced ability to utilize iron from the growth medium leads to the presence of a higher level of colicin Ia receptors in the outer membrane. Conversely, increased iron utilization results in lowered receptor levels.

The modulation of receptor levels seen in our experiments would seem to be due to an effect of iron availability. Thus, although growth in high iron results in a decrease in receptor levels, the ability of growth in iron-enterochelin to lead to such a reduction requires a functioning *fes*$^+$ allele. Since *fes* strains are able to take up iron-enterochelin from the medium but are unable to utilize the iron due to a defect in enterochelin esterase (14), it may be concluded that iron-enterochelin per se is not the primary controlling element in the regulatory mechanism. In addition, although the presence of ferrichrome in the growth medium of *tonB*$^+$ cells leads to a reduction in receptor levels, the addition of the ferrichrome structural analogue ferrichrome A, which cannot deliver its iron for cell utilization (24), has no effect. These results, taken together, strongly suggest that the ability of iron transport systems to interact in the regulation of colicin Ia receptor activity requires the ability to make free iron available for cell metabolism. The mechanism whereby intracellular iron or a product of its subsequent utilization is able to affect colicin Ia receptor levels remains to be clarified.

It is known that the growth of *E. coli* in iron-deficient media leads to a derepression of synthesis of components of the iron-enterochelin uptake system, including the enterochelin biosynthetic enzymes and enterochelin esterase (4, 21). As demonstrated here, the level of colicin I receptors responds to the physiological stress of iron limitation in a manner identical to that of the cells' iron utilization system. This analogy points to the intriguing possibility that the colicin Ia receptor is involved in iron transport. Although our experiments are consistent with a role of the Ia receptor in iron uptake, this possibility must be approached with caution. It is well known that growth in iron-deficient medium elicits numerous physiological responses (for a recent review, see reference 16). Although some of these effects, such as derepression of certain aromatic amino acid biosynthetic enzymes, are probably a direct result of a derepression in the enterochelin biosynthetic pathway (19), others may derive from iron starvation per se.

By analyzing solubilized membrane proteins by polyacrylamide gel electrophoresis, Davies and Reeves have shown that the outer membranes of a *tonB* mutant exhibit two peaks that they could not detect in the solubilized protein fraction prepared from the outer membranes of the parental strain (3). Furthermore, growth of *Salmonella typhimurium* in low-iron medium results in an increase in concentration of three outer-membrane polypeptide components (L. Rothfield, personal communication). In these two cases, it remains to be established whether the genetic or physiological imposition of iron limitation has specifically led to an increase in outer-membrane components involved in iron uptake. In any case, these results point out the possibility of substantial structural changes in the outer membrane. Thus, the possibility that the observed change in colicin Ia receptor levels is nonspecific is a real one.

These results may explain our previous finding that two independent strains of *E. coli* defective in heme biosynthesis due to a mutation in δ-amino levulinic synthetase have low levels of colicin Ia receptor when compared with the same strains grown in the presence of δ-aminolevulinic acid (7). Since heme serves as one repository of internal bacterial iron, mutants defective in heme biosynthesis may have a sufficient increase in the level of intracellular free iron so as to lead to repression of colicin Ia

receptor biosynthesis. Alternatively, it is possible that some component of the heme biosynthetic pathway is a component of the Ia receptor. In this regard it is interesting to note that there is an accumulation of porphyrins under iron-deficient conditions (15).

Our studies bear on the general question of mechanisms of membrane biogenesis. We have shown that at least in the case of one outer-membrane protein component, the colicin Ia receptor, the amount of protein inserted into the membrane does not seem to be limited solely by the availability of a fixed number of recognition sites. However, we cannot at the present time rule out the possibility of a concomitant reduction of one membrane component with a subsequent replacement by colicin Ia receptors. In any case, this study points out the structural flexibility of the outer membrane of *E. coli*.

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