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Outer Membrane-Dependent Transport Systems in *Escherichia coli*: Effect of Repression or Cessation of Colicin Receptor Synthesis on Colicin Receptor Activities

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Proteins in the outer membrane of gram-negative bacteria serve as general porins or as receptors for specific nutrient transport systems. Many of these proteins are also used as receptors initiating the processes of colicin or phage binding and uptake. The functional activities of several outer membrane proteins in *Escherichia coli* K-12 were followed after cessation or repression of their synthesis. Cessation of receptor synthesis was accomplished with a thermolabile suppressor activity acting on amber mutations in *btuB* (encoding the receptor for vitamin B_{12}, the E colicins, and phage BF23) and in *fepA* (encoding the receptor for ferric enterochelin and colicins B and D). After cessation of receptor synthesis, cells rapidly became insensitive to the colicins using that receptor. Treatment with spectinomycin or rifampin blocked appearance of insensitive cells and even increased susceptibility to colicin E1. Insensitivity to phage BF23 appeared only after a lag of about one division time, and the receptors remained functional for B_{12} uptake throughout. Therefore, possession of receptor is insufficient for colicin sensitivity, and some interaction of receptor with subsequent uptake components is indicated. Another example of physiological alteration of colicin sensitivity is the protection against many of the *tonB*-dependent colicins afforded by provision of iron-supplying siderophores. The rate of acquisition of this nonspecific protection was found to be consistent with the repression of receptor synthesis, rather than through direct and immediate effects on the *tonB* product or other components of colicin uptake or action.

Uptake of colicins is a complex process involving the interaction of numerous cell surface components. Colicins bind first to specific receptor proteins in the outer membrane. There appear to be two separate routes for the subsequent transmission of bound colicin to their sites for lethal action, the route used being a characteristic of the receptor rather than of the colicin. The group A colicins (including colicins A, E1, E2, E3, and K) seem to require the integrity of the outer membrane or proper arrangement of outer membrane components, since many of the mutants tolerant to these colicins exhibit deranged function of the outer membrane as a permeability barrier (reviewed in reference 10). In contrast, action of the group B colicins (including colicins B, D, I, and M) requires only the *tonB* product and is unaffected by the mutational alterations of outer membrane structure which result in tolerance to the group A colicins (5).

Colicin receptors bind other ligands. For example, the *btuB* protein is the receptor for the E colicins as well as for phage BF23, whose action is not impaired in mutants tolerant to the E colicins, and vitamin B_{12}, whose uptake requires *tonB* function but is also unaffected in colicin-tolerant mutants (1). The functional interaction of the *btuB* receptor protein with subsequent components required for uptake of its various ligands was investigated by measuring its receptor activities remaining after receptor synthesis had been halted in growing cells. Specific cessation of *btuB* receptor synthesis was achieved both by conjugal introduction of a mutant *btuB* allele (2) and by thermal inactivation of a temperature-sensitive suppressor allele [SupD(Ts)] necessary for expression of a *btuB* amber mutation (3). In both cases, receptor activity for colicins E2 and E3 (both of which are nucleases active on cytoplasmic constituents) was lost more rapidly than was receptor function for phage BF23. Receptor remaining retained full activity for B_{12} uptake, suggesting that the rapid loss of colicin receptor activity was not the result of disappearance of the receptor from the cell surface. It was proposed that the interaction of receptor with subsequent uptake components was possible only with newly synthesized receptor molecules (3).

To gain some insight into the meaning of these findings, it was necessary, first, to repeat these

studies in a strain allowing a greater degree of suppression activity than was possible in the previous study; second, to compare loss of receptor activity for colicins E2 and E3 with that of E1, which may not have to enter the cell for its lethal action (13); and finally, to see whether these responses were unique to this receptor system. For these purposes, colicin sensitivity was determined in a strain with a more efficient temperature-sensitive suppression system. In addition to the *btuB* system, analogous studies were made with the *fepA* product, which is considered to be the outer membrane receptor for ferric enterochelin and colicins B (an energy poison) and D (an inhibitor of protein synthesis) (16, 18). In this way, we hoped to determine whether the apparent requirement for recent synthesis of receptor for colicin lethality was a reflection of colicin entry into the cytoplasm, or was a general requirement for colicin passage across the outer membrane.

The second part of this paper examines the mechanism for the protection against most group B colicins afforded by provision of siderophores capable of donating iron to the cell (16, 20). This protection is in addition to the specific protection by a siderophore, such as ferrichrome or enterochelin, against those colicins (M or B, D, respectively) which share a common receptor. We had previously shown that addition of *tonB*-dependent siderophores reversibly decreased the steady-state level of TonB activity, measured from the rate of B_{12} uptake (11). The decrease in TonB activity did not appear to be of sufficient magnitude to account for the protection against the group B colicins. Thus, the involvement of repression of receptor synthesis was investigated by following the time course for the loss of sensitivity to group B colicins after addition of siderophores. Colicin susceptibility was not measured by effects of the colicin on viability as in the previous studies (16, 20), but by a much more rapid assay for the inhibition by colicin of proline uptake activity. The results obtained indicate that repression of receptor synthesis plays an important role in the observed nonspecific protection.

MATERIALS AND METHODS

Bacterial strains. *Escherichia coli* K-12 strain MX399 carries the temperature-sensitive suppressor allele *supD43,74* along with two unlinked mutations, *sueB,C*, which increase markedly the efficiency of suppression (M. P. Oeschger, personal communication). The genotype of the strain is F^- *metB1 leu*(Am) *lacZ*(Am) *galK*(Am) *galE sueB,C tsx relA rpsL supD43,74*; the strain was obtained from M. Oeschger. Clones of the strain were mutagenized with ethyl methane sulfonate, grown overnight in L broth at 42°C, and then plated with phage BF23 or colicin D at 42°C. Survivors were purified and tested for their response to these lethal agents at 30 and 42°C; several that were resistant at 42°C but sensitive after growth at 30°C were taken. The mutation in strain RK4852 conferring temperature-sensitive resistance to phage BF23 and the three E colicins was shown to be a *btuB*(Am) mutation (*btuB459*) by its cotransduction with *argH* and by its appropriate temperature-insensitive response when the *supD43,74*(Ts) allele was replaced by $supD^+$ or *supD* alleles, introduced by cotransduction with a Tn*10* insertion near *supD*.

Strain RK4856 exhibited a temperature-sensitive response to colicins B and D, but not to any of the other colicins tested. As with the previous strain, introduction of a temperature-insensitive *supD* allele resulted in sensitivity to colicins B and D at both 30 and 42°C. The lesion in this strain was linked to *entA* by cotransduction from a strain carrying a Tn*10* insertion near this genetic region; this location is as expected for a *fepA* mutation, but does not precisely identify this mutation.

Strain RK4617 [F^- *his trp*(Am) *lacZ*(Am) *metB1 pan-6 entA*] carries the *entA* mutation preventing synthesis of enterochelin unless the intermediate 2,3-dihydroxybenzoic acid (DHB) is provided. It is otherwise wild type for iron and B_{12} transport. A *tonA* derivative of this strain was obtained as a spontaneous mutant resistant to phage T5.

Colicin-producing strains were listed previously (2, 11) and were provided by A. P. Pugsley. Colicin samples were dialyzed solutions from the ammonium sulfate precipitation step as previously.

Growth media. Growth media included medium A of Davis and Mingioli (6), medium M63 (14), and MOPS medium (15). The morpholine propanesulfonic acid (MOPS) medium was depleted of iron by passage of concentrated solutions of all ingredients over a Chelex column; in addition, the growth medium contained 100 μM nitrilotriacetic acid to suppress nonchelate-mediated iron uptake.

All media were supplemented with glucose (0.5%), required amino acids (100 μg/ml), thiamine (2 μg/ml), and pantothenic acid (2 μg/ml). When specified, DHB (20 μM) or ferrichrome (2 μM) was added along with 5 μM $FeCl_3$. Supplemented minimal medium contained, in addition to the above, a synthetic mixture of all amino acids except proline and arginine (2).

All chemicals were from Sigma Chemical Co., St. Louis, Mo., except ferrichrome, which was purchased from Porphyrin Products, Logan, Utah. Radioisotopes were from Amersham/Searle, Arlington Heights, Ill.

Effect of colicins on viability after cessation of receptor synthesis. Strain RK4852 [*btuB*(Am) SupD(Ts)] was grown at 30°C in M63-based supplemented minimal medium into midlog phase (4×10^8/ml). Portions were diluted 200-fold into the same medium at 30 or 42°C. At intervals, portions were removed and mixed with either colicin diluent (3) or diluent containing appropriate dilutions of colicin E1 or E3 or phage BF23. After 5 min of incubation at 37°C, dilutions of each incubation mixture were made in medium A and spread on L plates which were incubated overnight at 37°C. The proportion of cells at each time point surviving exposure to colicins or

phage was calculated. Susceptibility to colicins or phage remained constant during growth at 30°C.

When specified, spectinomycin (100 μg/ml) was added 10 min after the temperature shift, and rifampin (10 μg/ml) was added after 40 min. There was less than 10% further increase in viable cells after antibiotic addition, and no significant loss of total viability over the course of the experiment.

The same protocol was used with strain RK4856 [*fepA451*(Am) SupD(Ts)], except that colicins B and D were used as lethal agents.

The amount of colicin or phage used in these experiments was that necessary to give 90 to 99% killing of the cells grown at 30°C in this time of incubation. The same amount of colicin was used throughout the experiment because, although the total number of viable cells increased four- to eightfold, the total amount of colicin receptor remained approximately constant. We had shown previously (3) that receptor produced in the presence of a temperature-insensitive *supD* allele was stable and that such cells retained sensitivity to phages or colicins whether incubated at 30 or 42°C.

Transport activities after temperature shift. Strain RK4852 [BtuB(Am) SupD(Ts)] and its *btuB*$^+$ parent, MX399, were grown at 30°C in M63 medium in duplicate flasks to midlog phase. One flask of eacn pair was shifted to 42°C. At intervals, portions were removed and mixed with colicin diluent or diluent containing a dilution of colicin E1 or E3 and incubated for 10 min at 37°C. To the colicin E1-treated samples and their control, [^{14}C]proline (5 μM, 2.5 μCi/ml) was added. Portions were removed after 0.3 and 1.3 min, filtered on membrane filters (Millipore Corp., Bedford, Mass.) and washed with 5 ml of medium A. The colicin E3-treated cells received the same amount of labeled proline, but incorporation was stopped after 1 and 2 min by twofold dilution into cold 10% trichloroacetic acid containing 100 μg of unlabeled proline per ml. After 30 min at 4°C, the precipitates were collected on membrane filters, washed twice with 2 ml of cold 5% trichloroacetic acid, and then washed once with 5 ml of 1% acetic acid. All filters were air dried, and the radioactivity retained was determined in a liquid scintillation spectrometer with toluene-Omnifluor (New England Nuclear Corp., Boston, Mass.).

B_{12} uptake activity was measured as previously described (11) with uptake times of 0.3, 3, and 5 min.

Uptake activities are expressed as picomoles of substrate accumulated per microliter of cell water; rates are in these units per minute.

Effect of colicins B and Ia after siderophore addition. Cells of strain RK4617 (*entA*) were grown overnight in MOPS-minimal medium supplemented with DHB and $FeCl_3$. Cells were collected by centrifugation, washed twice, suspended in Chelex-treated MOPS medium in three flasks, and incubated for 4 h at 37°C. At that time, DHB was added to one flask, ferrichrome to another, and nothing to the third. At intervals thereafter, portions were removed from each flask and transferred to three tubes, one containing MOPS medium and the others also containing an appropriate dilution of colicins B and Ia, respectively. After 12 min at 37°C, [^{14}C]proline was added as before; then the cells were filtered at 0.3 and 1.3 min, washed with medium A, and counted. In separate experiments, the uptake of B_{12} was assayed after addition of siderophore, with uptake periods of 0.3, 3, and 5 min.

RESULTS

Characterization of BtuB(Am) SupD(Ts) strain. Strain RK4852 carries the *btuB459* amber mutation and a highly efficient but temperature-sensitive suppressor activity. Growth on plates spotted with serial colicin dilutions and incubated at 30 or 42°C revealed this strain to be fully resistant to colicin E3 at 42°C (>10^4 times more resistant than the parent). At 30°C, the mutant was almost as sensitive to E3 as the parent, within a factor of 10. Resistance to colicin E1 at 42°C was not complete. Killing was obtained with E1 levels 10^3 times higher than that needed for killing of the wild-type strain at either temperature and 10^2 times greater than the concentration needed to kill the mutant grown at 30°C. Resistance to phage BF23 was complete at 42°C, whereas at 30°C the mutant was almost as sensitive as the parent.

B_{12} binding and uptake in strain RK4852 was also dependent upon the growth temperature, being 20 to 25% of the parental level when cells were grown at 30°C, but less than 4% of the parental level when grown at 37°C. This level of suppression of receptor activity is very close to the degree of suppression of β-galactosidase synthesis in these strains at the two temperatures. Suppression in these strains was 8- to 10-fold more efficient than in the SupD(Ts) strains we had previously used (3).

Loss of receptor activity after cessation of receptor synthesis. Colicin E sensitivity in strain RK4852 was measured at intervals after shift of a culture from 30 to 42°C, using as assay the inhibition of proline incorporation after 5-min exposure to colicin E1 or E3 (Fig. 1). Strain RK4852 grown at 30°C was less sensitive to these colicins than was the parental strain MX399. In the mutant strain, sensitivity to both colicins was lost after the shift to 42°C without apparent lag in the case of E1 and at the same rate but after a lag for E3. B_{12} uptake activity per milliliter of culture remained constant after the shift for at least 2 h.

Receptor activity was also determined by the loss of viability after exposure of cells for 10 min to enough colicin E1 or E3 or phage BF23 to kill 95 to 99% of cells grown at 30°C. The proportion of cells surviving this treatment at each time was plotted against the time after the shift to 42°C (Fig. 2). Sensitivity to both colicins declined with no apparent lag after the shift. Differences between the two colicins were noted at higher colicin concentrations than used here because this strain remained somewhat sensitive

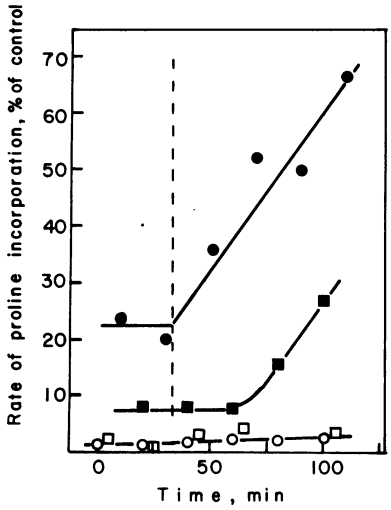

FIG. 1. *Effect of colicins E1 and E3 on proline incorporation after cessation of colicin receptor synthesis. Strains MX399 (open symbols) and RK4852 [BtuB(Am) SupD(Ts)] (filled symbols) were grown in M63-based minimal medium at 30°C and assayed for the effect of colicins E1 (circles) and E3 (squares) on the rate of proline uptake, as described. At 32 min, the cultures were shifted to 42°C. Data are expressed as rates of proline uptake in colicin-treated cultures relative to those without colicin.*

to E1 even when grown at 42°C. Appearance of E3-insensitive cells followed exponential kinetics, whereas that of E1-insensitive cells was better described by the linear kinetics drawn in Fig. 2A. Insensitivity to phage BF23 appeared with a similar rate, but after a 40- to 60-min lag, in agreement with our previous results (3) and showing that receptor activity for all E colicins was lost more rapidly than that for the phage.

Treatment with spectinomycin or rifampin after the temperature shift resulted in an extensive and exponential increase in sensitivity to colicin E1 (Fig. 2). There was a much less marked, although reproducible, increase in sensitivity to colicin E3 after spectinomycin treatment. Exposure to rifampin did block the increase in resistance to E3 after the shift, but did not result in increased sensitivity to this colicin. Both antibiotics halted the appearance of phage BF23-insensitive cells without increasing sensitivity to the phage. We had previously shown that the increased sensitivity to colicins E2 and E3 after inhibition of growth following the temperature shift was still dependent on the *btuB*-coded receptor and that the colicin action was still blocked by B_{12} (3).

Treatment with chloramphenicol or nalidixic acid also appeared to result in increased sensitivity to the E colicins, but the lethality produced by these antibiotics prevented a definite statement on their effect. Thus, the lethal action of the E colicins appears to require newly synthesized receptor, although "old" nonfunctional receptors could, at least in some cases, be reactivated by some process after inhibition of protein synthesis or cell growth.

Turnover of receptor activity for colicins B and D. Strain RK4856 carries the high-efficiency temperature-sensitive suppressor activity acting on an amber mutation presumably in *fepA*, encoding the receptor for colicins B and D (16, 18). As with the E colicins, shift of a culture of this strain from 30 to 42°C resulted in the immediate, extensive, and exponential loss of sensitivity to both colicins B and D (Fig. 3). The rate of appearance of colicin-insensitive cells was approximately equal to the rate of increase in cell mass after the shift.

Cells treated with spectinomycin or rifampin after the temperature shift became insensitive to these colicins to the same degree and at approximately the same rate as untreated cells. However, exposure of cells at 30°C to these antibiotics also resulted in decreased sensitivity to these colicins, although the untreated cells at

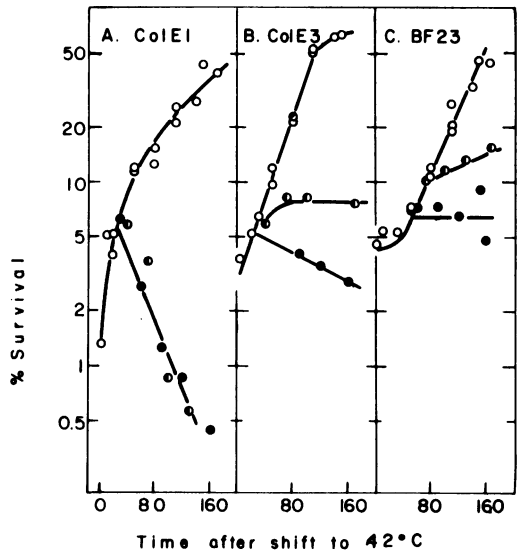

FIG. 2. *Effect of colicins E1 and E3 and phage BF23 on cell viability after cessation of receptor synthesis. Strain RK4852 [(BtuB(Am) SupD(Ts)] was grown in supplemented minimal medium at 30°C and then diluted, shifted to 42°C, and tested for killing by colicin E1 (A), E3 (B), and phage BF23 (C). Data are presented as percentage of cells surviving exposure to the lethal agents for 5 min as a function of the time after the shift to 42°C. To one of triplicate flasks, spectinomycin was added at 10 min (●); to another, rifampin was added at 40 min (◐). The line drawn in (A) is that expected for a linear increase of E1-insensitive cells with time. This figure contains results from three separate experiments.*

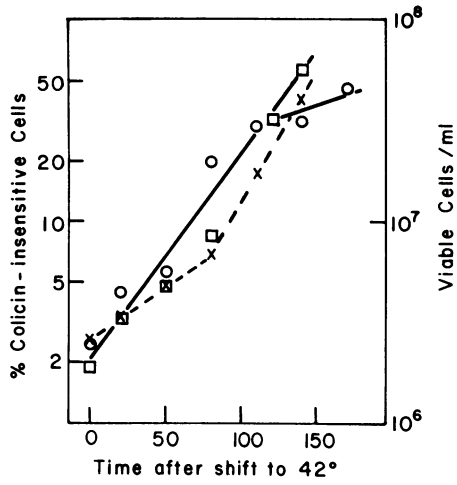

FIG. 3. *Effect of colicins B and D on viability after cessation of receptor synthesis. Strain RK4856 [FeuB(Am) SupD(Ts)] was grown in supplemented minimal medium at 30°C. At time zero, the culture was diluted and shifted to 42°C. At intervals, the proportion of cells killed by 5-min exposure to colicin B (○) or D (□) was determined. The number of viable cells is shown (×).*

30°C remained sensitive. This was probably not a result of receptor turnover, but represented the previously described loss of TonB activity after the inhibition of protein synthesis (11). Thus, the requirement for newly synthesized receptors for colicin action appears to also extend to those receptors dependent on *tonB* function.

Repression of receptor activity. We had previously shown that sensitivity to the E colicins declined markedly after growth of cells in the presence of B_{12}, resulting from the repression of synthesis of the *btuB*-coded receptor (8). Levels of the group B colicin receptors, some of which are clearly involved in iron transport, are low in cells grown with excess iron and high in iron-starved cells (12, 17). In addition, TonB activity, measured by the rate of B_{12} uptake, was depressed by the presence of other *tonB*-dependent transport substrates, such as DHB or ferrichrome (11). These regulatory effects might be involved in the nonspecific protection against the group B colicins that siderophores provide (20).

The effect of the addition of siderophores (DHB and ferrichrome) on the colicin susceptibility of strain RK4617 (*entA*) growing in iron-depleted minimal medium was assayed by the effect of a 10-min incubation with appropriate dilutions of colicins B and Ia on the rate of proline uptake. Cells grown for many generations in the presence of these siderophores were markedly less sensitive to these colicins than were cells grown in iron-poor medium, although even the iron-supplemented cells were fully sensitive to sufficiently high colicin concentrations (Fig. 4). Growth in the presence of citrate also resulted in decreased colicin sensitivity. About 100 times more colicin B was required for 50% inhibition of proline uptake in DHB-supplemented cells than in cells grown in iron-poor medium. For colicin Ia, this difference in susceptibility was only about 10-fold.

The time course for the reduction in colicin susceptibility was determined after the addition of DHB or ferrichrome. Addition of the siderophores increased the growth rate from 63 to 49 min; all cultures exhibited logarithmic growth throughout the experiment. Colicin susceptibility gradually declined to the level expected for cells grown in excess iron (Fig. 5B, C). The time course for the loss (line drawn in Fig. 5B, C) is that predicted for exponential dilution by growth. This suggests that addition of siderophores, i.e., iron supplementation, results in a reduced rate of receptor synthesis and that the lower level of receptor results in lower colicin susceptibility. Whether the receptors existing before repression retain activity cannot be determined without a direct correlation of the rate of receptor synthesis with the loss of colicin susceptibility. Susceptibility in the unsupplemented culture remained constant.

Presence of the siderophores also depressed the level of B_{12} uptake activity approximately 35% (Fig. 5A). Uptake activity in the sidero-

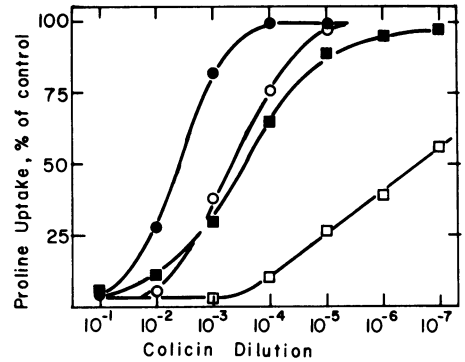

FIG. 4. *Effect of colicins B and Ia on proline uptake in cells grown with excess or limiting iron. Strain RK4617 (entA) was grown overnight and then into log phase in either Chelex-treated MOPS medium (open symbols) or that medium supplemented with DHB and $FeCl_3$ (filled symbols). Cells were incubated for 10 min with dilutions of colicin B (circles) or Ia (squares) and then assayed for the rate of proline uptake. Data are expressed as rates of proline uptake in the presence of colicin relative to that without colicin; the control rate of proline uptake in the iron-limited culture was 84 to 90% of that in the iron-supplemented culture.*

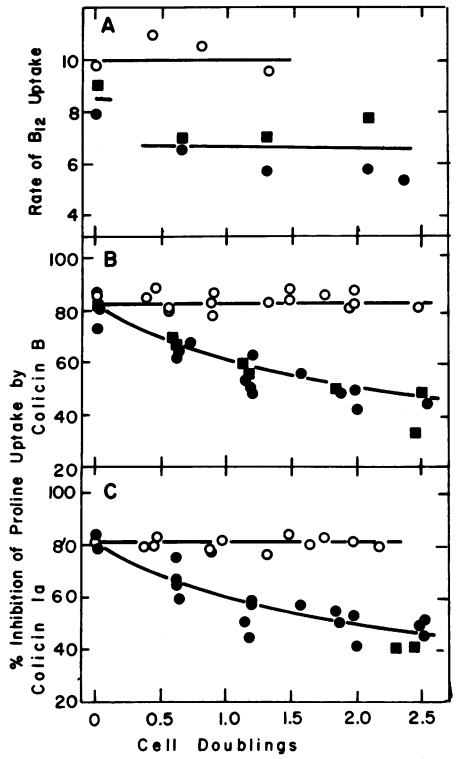

FIG. 5. *Effect of supplementation with siderophores on B_{12} uptake and inhibition by colicins B and Ia of proline uptake. Strain RK4617 was grown in Chelex-treated MOPS medium in triplicate flasks. At time 0, one flask received 2 μM ferrichrome (●), another received 20 μM DHB (■), and the third received no addition (○); incubation at 37°C was continued. At intervals, samples were withdrawn and assayed for the rate of B_{12} uptake (A), for the relative inhibition of proline uptake after a 5-min exposure to colicin B (B), or for the inhibition of proline uptake by colicin Ia (C). The data presented are compiled from five separate experiments.*

phore-supplemented cultures remained essentially constant after a rapid initial decline. This supports our previous suggestion that the level of TonB activity was not subject to repression, but was depressed by the presence or operation of TonB-dependent reactions. Thus, the decreased colicin susceptibility observed after addition of siderophores appears to be the result of a repression, presumably of receptor synthesis.

The effect of siderophores on colicin susceptibility in a *tonA* derivative of strain RK4617, which is unable to use ferrichrome as iron source, was determined (data not shown). Supplementation with DHB resulted in the identical loss of sensitivity to colicins B and Ia as described above for the parental strain. In contrast, addition of ferrichrome had no effect on the growth rate, the rate of B_{12} uptake, or the susceptibility to these colicins.

DISCUSSION

This paper investigated the functional interaction between two colicin receptors and subsequent uptake components and also the regulation of colicin sensitivity. Synthesis of the *btuB*-coded receptor for the E colicins and the *fepA*-coded receptor for colicins B and D was specifically terminated through thermal inactivation of a suppressor activity. The receptors remaining on the growing cell surface retained their activity for B_{12} binding and uptake and for effective binding of phage BF23. However, the ability of these receptors to mediate the lethal adsorption of colicins declined immediately. This affected the action of colicins E1 and B, which act as energy poisons and may not have to enter the cell, and colicins E2, E3, and D, which inhibit intracellular processes and must enter the cell. Loss of sensitivity to phage BF23 began to appear about one cell division time after the onset of loss of colicin sensitivity. This may represent the time necessary for the appearance of progeny cells lacking receptor, indicating that receptor molecules are not free to distribute within the outer membrane (4). Otherwise, at least four divisions would be required before the appearance of receptorless progeny.

The apparent reactivation of colicin E1 (and to a lesser degree that for colicin E3) receptor activity after treatment with spectinomycin or rifampin further supports the contention that receptors can be present on the cell surface but nonfunctional with respect to transmission of colicin to the lethal target. The possibility that only newly synthesized receptors were in the proper location with respect to the cytoplasmic membrane to allow colicin entry into the cell is not supported by the finding that colicins B and E1 also require newly synthesized receptors. Rather, it may be that only newly synthesized receptors are in the proper location to allow the colicins bound to them to be acted upon by cellular components necessary for the activation of the colicins into an active form or a form able to be transmitted to its target. Possibly related to this is the finding that colicins E1, E2, E3, and K, when bound to their specific receptors, are subject to a specific proteolytic cleavage by an enzyme activity present in cellular membranes (19).

If this protease activity were preferentially located at the site of export of outer membrane proteins, this could explain the observed rapid loss of receptor activity as continued protein synthesis and export either drove the existing receptors away from the protease or vice versa.

Blockage of protein synthesis could disrupt specialized sites of protein export to the outer membrane, thereby freeing protease activity and allowing it to act on colicins bound to any receptor present on the cell surface. There has been a recent demonstration that the enterochelin receptor (*fepA* product) is subject to a proteolytic cleavage by outer membrane protein a or 3B (7); the function of this processing is still unclear and was reported not to affect colicin binding.

Another example of the physiological alteration of colicin susceptibility is the protection against most group B colicins afforded by siderophores capable of supplying iron (16, 20). Previous descriptions of this phenomenon demonstrated overnight growth of sensitive cells in the vicinity of a disk containing siderophores on a colicin-containing plate. The assay reported here was much more rapid, allowing measurement of the time required for appearance of this protective effect. By this assay, iron-supplemented cells were still sensitive to the action of colicins B and Ia on proline uptake, although significantly less so than cells grown with limiting iron. Transition from the sensitive to the less sensitive state required growth for several generations in the presence of siderophores capable of providing iron to the cell. The kinetics of the decline in colicin susceptibility were consistent with the partial repression of some component necessary for colicin action. Since TonB activity did not appear to be subject to repression and declined with different kinetics, it is more likely that the component repressed by iron is the colicin receptor. Synthesis of several of the receptors for group B colicins has been shown to be repressed by excess cellular iron (10, 12, 17).

There may also be a minor degree of protection provided by the depression of TonB activity by the presence of TonB-dependent siderophores. We found that inhibition of *tonB* expression or of general protein synthesis results in the rapid loss of all *tonB*-dependent activities and that the rate of this loss is accelerated by the presence of *tonB*-dependent siderophores (9, 11). Whether this loss of activity reflects the actual decay of the *tonB* protein or its inactivation or movement is not now known. Future experiments are directed to the mode of action and location of the *tonB* product and to the modification of colicins during their entry into the cell.

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