Effects of Colicin Ia on Transport and Respiration in Escherichia coli*

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SUMMARY

Treatment of Escherichia coli with colicin Ia leads to an inhibition in the active transport of exogenously supplied proline, thiomethyl-β-D-galactoside and potassium ion. Furthermore, the addition of colicin Ia to cells preloaded with these substances leads to their almost immediate efflux. In contrast, colicin Ia treatment enhances by as much as 10-fold the level of accumulation of α-methyl-D-glucoside. The colicin Ia-induced stimulation of glucoside accumulation is mediated by the phosphotransferase system. Cells treated with colicin Ia exhibit an increased rate of respiration when glucose is the substrate and a decreased rate when glycerol or succinate is the substrate. The decreased rate of succinate-dependent respiration is probably due to the failure of Ia-treated cells to accumulate succinate.

The effects of the antibacterial proteins, colicin Ia and Ib, on several metabolic functions of Escherichia coli have been described (1). It was demonstrated that colicin Ia or Ib treatment led to an inhibition of the incorporation of $^{32}$P into DNA and RNA by more than 95% Only partial inhibition of $^{32}$P incorporation into phospholipid and nucleotide fractions was observed. Incorporation of $^{32}$P into the terminal phosphate of ATP was reduced to approximately one-half of the control level. Protein synthesis, as measured by the incorporation of [14C]leucine, was abolished by the I colicins. In two cases a stimulatory effect was observed: first, a slight enhancement in the rate of respiration was seen; second, the level of $^{32}$P-containing non-nucleotide organic phosphate compounds in colicin I-treated cells was increased to approximately twice the level found in control cells. The results led to the suggestion that the mode of action of colicin Ia and Ib involved a primary interference with energy metabolism which subsequently led to the general effects described above.

In the present study we have extended studies on the mode of action of colicin Ia. We demonstrate that colicin Ia treatment results in an alteration in the activities of several transport systems as well as respiration.

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MATERIALS AND METHODS

Bacterial Strains—Escherichia coli K12 strains W3110 str-r (JK1) and its colicin I resistant derivative (BC3) were employed in studying the basic effects of colicin Ia on coli-sensitive and colicin-resistant strains. In some cases E. coli K12 strain JK114 was used as the sensitive strain. In those cases tested, colicin Ia treatment had identical effects on the two sensitive strains. E. coli strain 1100 (thi) and its derivative 1101 (thi, HPr), obtained from Milton Saier, University of California at San Diego, were utilized to study the effects of colicin Ia on the phosphotransferase system. Strain 1101 has reduced levels of a small, histidine-containing protein which is a phosphorylated intermediate in the group translocation process. As a result, growth on substrates taken up by the phosphotransferase system is impaired. At 2 × 10^{-5} M substrate concentration, we found the final level of α-methylglucoside accumulated by the mutant was 50% that found for the parental strain. Both strains were equally sensitive to colicin Ia. The mutant strain was routinely checked for revertants by plating on mannitol broth agar containing phenol red.

Preparation of Colicin Ia—Purified colicin Ia, prepared as previously described (2) was used for all studies of mode of action. Killing activity was measured as described previously (1).

Growth of Bacterial Strains—Cells were grown in Tris-S buffer (1), which contained 0.1 M Tris-HCl, pH 7.4; 0.08 M NaCl; 0.22 M KCl; 6.4 × 10^{-4} M KH2PO4; 1.6 × 10^{-3} M NaaSO4; 1.0 × 10^{-3} M MgCl2; 1.0 × 10^{-4} M CaCl2; and 2.0 × 10^{-4} M FeCl3. Tris-HCl buffer was supplemented with 0.15% casamino acids and the carbon sources designated with each experiment. Cells were grown at 37°C with shaking to mid-log phase, harvested at 4°C, and then washed twice with Tris-S buffer prior to suspension in the transport or respiration medium described with each experiment. For transport studies those cells were utilized immediately; in respiration studies the cell suspension was kept on ice for as long as 2 hours. Repeated measurements throughout this time period showed no effect of time of storage on the rate of oxygen uptake for either control or colicin-treated cells.

Transport Assays—Cell suspensions were prewarmed to the desired temperature in a shaking water bath. Chloramphenicol (final concentration, 100 μg/ml) was added 10 min prior to the addition of colicin or transport substrate. For measurement of uptake, aliquots of the cell suspension were removed and filtered immediately over Millipore HA filters of 0.45 micron pore size. The filters were then washed twice with 2.5 ml of Tris-S buffer; Tris-HCl buffer (0.1 M, pH 7.4) was substituted for Tris-S buffer when potassium ion uptake was measured. The washed filters were removed immediately, dried, and counted in toluene-2,5-diphenyloxazole.

For the measurement of the zero time point, an aliquot of the cell suspension to which no isotope had yet been added was transferred to the filter chamber. The proper volume of isotope was added to this cell suspension and vacuum filtration was initiated. The filters were then washed as described for the experimental time points. The value of the zero time point (less than 10% of the experimental value at any time points) was subtracted from each of the experimental time points. The in vivo rate of hydrolysis of...
**Results**

**Effect of Ia on Active Transport.**—Fig. 1 shows that cells treated with colicin Ia for 10 min prior to the addition of I-[H]proline accumulate substantially less substrate than the untreated control cells. Reference to the dashed line which represents the level of isotope predicted if no concentrative effect were occurring and was calculated using the cell water volume values employed by Kusche (4). Control, O; colicin Ia-treated, •.

The effects of colicin Ia on accumulation and efflux of a substrate of the \( \beta \)-galactoside permease system thiomethyl-\( \beta \)-galactoside were similar to the effects observed with proline. Fig. 2 shows the induction of the efflux of \([H]TMG\) by the action of colicin Ia on cells which have previously accumulated the substrate. Similarly, treatment with colicin Ia for 10 min prior to the addition of \([H]TMG\) resulted in a reduction in the amount of isotope accumulated. As with proline, the cells retained a residual capacity for concentrating TMG at high levels of colicin treatment where less than 1% of the cells survived. At 90 min after colicin treatment, when the intracellular level of TMG was stabilized, the level of isotope in colicin treated cells was 40 to 50% of the level in control cells. The level of \([H]TMG\) accumulated by the colicin I resistant strain was not affected by the addition of colicin Ia.

In the experiments described in Figs. 1 and 2 chloramphenicol was used to inhibit protein synthesis in both control and colicin-treated cells. Therefore, the reduction in transport capacity following colicin treatment cannot be attributed to decreased numbers of transport proteins due to lowered levels of protein synthesis in the colicin-treated cells. However, colicin treatment might induce the destruction or release of carrier proteins from the membrane. In view of this possibility the effect of colicin I on the in vivo rate of hydrolysis of ONPG was determined.

Colicin Ia treatment for 10 min prior to the addition of ONPG reduced the in vivo rate of ONPG hydrolysis by only 8%.
Fig. 3. Effect of colicin Ia on uptake of potassium ion. Cells grown on Tris-S medium with 0.15% glucose as carbon source were washed and resuspended in Tris-S from which KCl was omitted. To this cell suspension 4K+ was added to a final concentration of 1.44 X 10^5 cpm/ml. The final suspension consisted of Tris-S containing 1.6 X 10^-3 M potassium ion and 0.15% glucose. The cell concentration was adjusted to 102 Klett units (No. 42 filter). After 50 min, chloramphenicol was added to a final concentration of 100 &.ug/ml. Ten minutes later colicin was added to a final concentration of 0.089 &ml. Survivors assayed after 10 min exposure to colicin -

Fig. 4. Effect of colicin Ia on a-methylglucoside accumulation. Cells were grown as described with 0.5% glycerol as a carbon source. The experiment is as described in Fig. 1 except that a-methyl[14C]glucoside (25 &.Ci/ &mol, 2 X 10^-4 M) was allowed to accumulate for 20 min before colicin Ia addition (final concentration 0.1 &g/ml). Survivors were 0.001%. Control, 0; colicin Ia-treated, •.

assuming that the membrane carriers are a homogeneous population with respect to energy coupling, this slight reduction in membrane carrier activity is insufficient to account for the pronounced reduction in levels of accumulation observed with TMG.

In addition to its effect on proline and TMG, colicin Ia treatment reduces the intracellular levels of potassium ion. Fig. 3 illustrates the effect of colicin Ia on 4K+ loading by colicin sensitive cells. At the termination of the experiment the level of potassium ion in the colicin-treated cells was approximately one-seventh the level in control cells. No reduction in potassium ion content was observed when the colicin resistant strain was exposed to colicin Ia.

Effect of Colicin Ia on α-Methylglucoside Accumulation—The colicin Ia-induced alteration in the function of the various transport systems described above could be due to a specific effect on active transport or to the induction by colicin of a generalized leakiness in the cell membrane. If the cell becomes leaky, then substrates of the phosphotransferase system might be expected to exhibit altered transport properties. However, if colicin treatment affects some component involved in the energization of active transport and common to the proline, TMG, and potassium ion transport systems, then substrates of the phosphotransferase system would be expected to be transported normally.

We, therefore, examined the effect of colicin Ia on accumulation of a substrate of the phosphotransferase system, α-methylglucoside. As can be seen in Fig. 4 colicin Ia treatment failed to reduce the level of accumulated α-methylglucoside. Instead, colicin Ia addition induced a pronounced elevation in the levels of accumulated substrate. Colicin Ia treatment did not affect the levels of accumulated substrate in the resistant strain, BC3. When the extracellular level of α-methylglucoside was adjusted to 2.2 mM by the addition of [14C]-α-methylglucoside, the [14C-α-methyl]-glucoside which had been accumulated by colicin treated cells was released. Thus, the additional isotope accumulated after colicin treatment is present inside the cells in a form which is susceptible to release. The fact that α-methylglucoside accumulation is not diminished by colicin treatment and the further observation that the accumulated isotope is exchangeable indicate that colicin Ia does not induce a generalized cell leakiness.

It was of interest to determine whether colicin Ia was actually affecting the phosphotransferase system or whether the enhanced level of α-methylglucoside accumulation was due to the activation of some alternate mode of uptake. Two approaches were applied to this problem. In the first, we established that the α-methylglucoside taken up following colicin Ia treatment was phosphorylated. In the second, we determined that a mutant possessing a defect in the phosphotransferase system was less affected than its parent in the stimulation by colicin Ia of α-methylglucoside uptake.

Phosphorylation of substrates of the phosphotransferase system occurs during the transport process (5). However, the nonmetabolizable substrate, α-methylglucoside, accumulates intracellularly both in phosphorylated and nonphosphorylated forms. It has been shown that the proportion of α-methylglucoside that remains phosphorylated decreases with increasing time after exposure of the cells to the transport substrate (6). Therefore, even if the increased level of α-methylglucoside accumulated...
in colicin Ia treated cells were taken up by the phosphotransferase system one would not expect to find the accumulated substrate exclusively in a phosphorylated form. On the other hand, if one were to find a virtual absence of phosphorylated substrate, one would have little confidence that substrate accumulation was mediated by the phosphotransferase system. With use of paper chromatography to separate phosphorylated and nonphosphorylated α-methylglucoside (see "Materials and Methods"), we were able to determine that 60% of the isotope accumulated by colicin-treated cells occurred as the phosphorylated derivative. This constitutes presumptive evidence in favor of the conclusion that the enhanced levels of α-methylglucoside accumulation are not due to an activation by colicin Ia of an alternate mode of transport.

To further substantiate the conclusion that colicin Ia effects a stimulation of α-methylglucoside uptake by specifically affecting the phosphotransferase system, a second approach was employed utilizing a mutant defective in the phosphotransferase system. The mutant strain possesses low levels of HI+, a small cytoplasmic protein which is a phosphorylated intermediate in the phosphotransferase system (5). As a result the mutant shows a reduced rate of hexose uptake. A finding that colicin Ia was less effective in stimulating the accumulation of α-methylglucoside in the mutant than in the parent, would constitute indirect evidence that colicin Ia affects the phosphotransferase system.

On the other hand, if it were found that colicin Ia was equally effective in stimulating α-methylglucoside in the mutant and in the parent, it would appear likely that some alternate mode of uptake was activated by the colicin. Although both strains were found to be equally colicin Ia-sensitive, we found that colicin Ia was much less effective in enhancing the levels of α-methylglucoside accumulated by the mutant (1101) than it was for the parent (1100). In a representative case in which the parent was stimulated by colicin Ia to accumulate 9-fold greater levels of α-methylglucoside, the mutant was only stimulated to accumulate 2-fold greater levels. Whether the 2-fold stimulation observed in the mutant is due to leakiness of the mutation or to a second mode of substrate transport resistant to colicin Ia action is unclear. In any event, the decreased effect on the accumulation of α-methylglucoside by the mutant indicates that the increased level of α-methylglucoside accumulation following colicin treatment is a result of the activity of the phosphotransferase system. This result, in combination with the data showing that the α-methylglucoside is phosphorylated, strongly suggests that the colicin Ia effect on α-methylglucoside accumulation is mediated specifically by the phosphotransferase system.

Effect of Colicin Ia on Respiration—It was previously reported that colicin Ia stimulated respiration by 5 to 10% (1). Since electron transport is known to play an important role in active transport (7), it was of interest to re-examine this subject in more detail. As shown in Fig. 5, the rate of oxygen uptake in the presence of glucose is increased 50% by the addition of colicin Ia. In contrast to the stimulation encountered when glucose was substrate, a reduction in the rate of oxygen uptake was observed when succinate or glycerol was utilized. In succinate medium the rate of oxygen uptake was reduced to nearly zero within 15 min after colicin addition. However, in glycerol the rate of oxygen uptake was reduced to 30% of the original rate and no further reduction in rate occurred following prolonged colicin treatment.

It seemed reasonable that the inhibition of succinate-dependent respiration was a secondary effect caused by the inability of colicin Ia-treated cells to accumulate succinate. That this was indeed the case is suggested by the results described in Fig. 6 where it can be seen that colicin Ia-treated cells are inhibited in their ability to take up succinate.

DISCUSSION

It is of interest to determine which of the many biochemical consequences of colicin Ia treatment are primary effects of colicin action. Some of the effects are probably secondary, occurring as
a result of some new physiological state induced by the initiation of the primary event. Though it may be the sum of several events which ultimately leads to cell death, it is the primary set of events which will elucidate the molecular mechanism of colicin Ia.

As a result of the demonstration of the inhibitory effect of colicin Ia on the transport of several substances it is necessary to comment on previous results (1) in which macromolecular synthesis was measured by examining the incorporation of exogenously supplied radioactive substrates. In view of the present findings demonstrating that colicin Ia treatment leads to an inhibition of several transport systems, we feel that it is likely that the effects of this colicin on macromolecular syntheses may be the result of a combination of lowered ATP levels (1) and inability to maintain sufficient intracellular levels of substrates and cofactors.

A likely primary target of the colicin is the cytoplasmic membrane which is the site of respiration, oxidative phosphorylation, and active transport (7). It is also known that some of the proteins of the phosphotransferase system are located in the cytoplasmic membrane (5). If the cytoplasmic membrane is, indeed, the primary target of the colicin, then transport effects may initiate all other effects and ultimately lead to cell death. It should be noted that we have recently obtained evidence that colicin Ia treatment does lead to a structural change in the bacterial membrane. Using the fluorescent probe, N-phenylnaphthalene, it has been shown that colicin Ia treatment leads to a change in probe fluorescence which most likely reflects structural changes in the membrane. This effect is immediate and mimics the effect of the energy poisons, azide, cyanide, anaytal, and carbonyl cyanide m-chlorophenyl hydrate.

It is of interest to ask just how colicin Ia might be inhibiting active transport. Since several systems are inhibited, it is likely that colicin Ia acts at a level which is common to all systems. Therefore it is unlikely that specific membrane carriers per se are affected directly by colicin Ia. Since the mechanisms of generating energy and of coupling this energy to active transport may be common to the active transport of many substances (8), energy generation and energy coupling are potential targets of colicin Ia. Inhibiting them or destroying their efficacy would lead to a reduced capacity for the active transport of all of the substances which share the energy source.

The postulate that colicin Ia inhibits transport by interfering at the level of energy coupling is a difficult one to test at this time since the mechanism of coupling energy to transport has not been established. Although it is not clear whether chemical coupling by alteration of membrane constituents or chemiosmotic coupling by establishment of a proton gradient provides the energy input necessary to drive active transport (see, for example, Refs. 9 and 10), the sources of energy generation are known. Energy input for active transport is derived from two sources, electron transport and ATP hydrolysis (11-15). Therefore, it is more fruitful at this time to ignore the prospect of how colicin Ia might interfere with energy coupling. Instead, we will address the question of whether the sources of energy input, ATP hydrolysis and respiration, are equally susceptible to colicin Ia. In other words, does colicin Ia affect the energization of the membrane via respiration, via ATP, or via both energy sources. In experiments in which we examined TMG and proline uptake, we found that very high levels of colicin did not completely inhibit active transport. Considerable residual capacity for active transport was retained by the population of cells although less than 1% eventually survived colicin Ia treatment. One possible interpretation is that colicin Ia interferes with only one of the two means of generating the energized membrane state. Residual concentrative effects could then be attributed to the unimpeded system. One approach to this problem could involve the study of the effect of colicin Ia on membrane vesicles in which active transport is driven by respiration derived energy (16). Unfortunately, we have not found conditions to demonstrate an inhibitory effect of colicin Ia on transport in membrane vesicles. However, it has been found that other colicins similar to colicin Ia in mode of action inhibit transport in membrane vesicles (17-19). These results suggest that respiration derived energy input is susceptible to colicin action but do not eliminate the possibility that both ATP and respiration derived energy input or the energized membrane state itself might be the target of colicin action. A second approach to determine whether colicin Ia inhibits transport by affecting energy input via respiration or via ATP is to utilize whole cells. Studies with mutants and various inhibitory conditions would be very useful in this regard.

The level of accumulation of α-methylglucoside is increased by treatment with colicin Ia. The finding that at least one transport substrate is not released following colicin Ia treatment is important. It indicates that generalized cell leakiness is not induced by the colicin. The increased level of glucose accumulation in colicin Ia-treated cells can provide a simple explanation for the observation that colicin Ia-treated cells contained higher levels of non-nucleotide phosphates (1) as well as the observation of increased rates of respiration reported previously (1) and confirmed here.

The finding that colicin Ia affects the level of accumulation of α-methylglucoside may be explained in two general ways. The first interpretation has been used to explain the observation that azide and 2,4-dinitrophenol treatment of E. coli cause increased levels of α-methylglucoside accumulation (20). In this view the presence of metabolic inhibitors and the resultant lowered supply of energy are responsible for decreasing the exit rate of the α-methylglucoside. As a result higher levels of accumulation are attained. The colicin Ia effect might be explained in similar terms. Alternatively, it is possible that the increased level of α-methylglucoside is due to an enhanced accumulation of phosphoenolpyruvate. However, it should be pointed out that this kind of explanation is not necessary to explain our data since recent experiments have shown that the level of phosphoenolpyruvate found in cells is not limiting with respect to α-methylglucoside accumulation (11).

Recent evidence indicates that there is a close functional association between the transport systems of sugars which do not utilize the phosphotransferase system and those which do (5). It has been shown that substrates of the phosphotransferase system interfere with the permease mediated uptake of sugars such as lactose and melibiose which are not substrates of the phosphotransferase system. This phenomenon has been called "inducer exclusion." This interaction between the two systems of transport leads us to suggest the possibility that colicin Ia may interact at the membrane level with both types of transport systems. The action of colicin Ia on the phosphotransferase system as reflected by the marked enhancement in accumulation of α-methylglucoside is accompanied by a suppression in the transport capacity for other substances. The phenomenon of inducer exclusion is primarily known to occur with sugars although there is one report of interference with induction of

1 D. Nieva Gómez, R. B. Gennis, and J. Konisky, unpublished observations.
amino acid transport (21). Since colicin Ia interferes with the uptake of potassium ion and proline as well as TMG, we do not contend that colicin directly and solely affects a membrane entity responsible for inducer exclusion. However, the existence of the phenomenon of inducer exclusion lends credibility to the tantalizing prospect that a single membrane effect might concomitantly enhance and inhibit the two types of transport systems.

As mentioned previously the colicin Ia-enhanced rate of respiration observed with glucose may possibly be explained by the occurrence of increased intracellular levels of glucose. Alternatively, it is possible that the lowering of ATP levels induced by the colicin treatment (1) leads to a stimulation in the activity of the respiratory chain. These possibilities may be interrelated. The reduced rates of oxidation of succinate and glycerol may also be due to transport effects. Succinate transport is an active process; its susceptibility to inhibitors is similar to that of TMG and proline (22-25). Indeed, we have shown here that colicin Ia inhibits succinate uptake. Thus, lowered intracellular levels of succinate might account for the observed inhibition of respiration. Glycerol uptake, shown to occur by facilitated diffusion (26), would not be expected to be inhibited by colicin Ia. Glycerol, however, is one of the many substrates whose uptake is affected in mutants deficient in the phosphotransferase system (27). The fact that glycerol uptake is somehow related to the phosphotransferase system presents the possibility, once again, that colicin Ia might coordinate enhancement and inhibition of the phosphotransferase system while inhibiting glycerol uptake. Studies of the effects of colicin Ia on cell free respiratory particles will be necessary to pursue the possibility that succinate and glycerol driven respiration per se is inhibited independent of the effects of colicin Ia on transport.

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