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Colicin V-Treated *Escherichia coli* Does Not Generate Membrane Potential

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Colicin V-treated *Escherichia coli* was inhibited in its capacity to carry out active transport of proline and was unable to generate a membrane potential. Colicin V also prevented membrane potential formation by isolated cytoplasmic membrane vesicles. We conclude that a primary effect of this colicin involves the cytoplasmic membrane as a target.

Although studies on bacteriocins have been initiated after Gratia's discovery of colicin V (7, 8), this colicin displays several properties which distinguish it from colicins which have been subsequently described. In particular, colicin V is of a small size, having a molecular weight of ca. 4,000 (5); furthermore its synthesis is not inducible (9). These characteristics are in contrast to those of other colicins, which range in size from 27,000 to 80,000 daltons and syntheses of which are induced by conditions that activate SOS repair (11, 21). These differences suggest that colicin V may be more closely related to a class of antibiotic polypeptides, termed microcins, which are produced by some *Escherichia coli* strains (1, 2).

Characterization of colicin V has been hampered by several factors, including instability of colicin V, the low amounts produced by colicinogenic cells, difficulty in purification, and concomitant production of colicin I in cells that produce colicin V (17, 23). We have previously reported the cloning of colicin V structural and immunity genes originating from plasmid pColV-B188 (5). On the basis of the availability of this clone and our ability to stabilize colicin V activity, we decided to initiate studies on the mode of action of colicin V despite its having so far proven refractory to purification.

Colicin V was prepared from the supernatant of a tryptone broth culture of *E. coli* K-12 strain 294 carrying plasmid pBQ41. This plasmid carries the colicin V structural gene derived from plasmid pColV-B188 (5). A late-log culture (100 Klett units as measured in a Klett-Summerson colorimeter, blue filter) grown at 37°C with shaking was harvested, and the supernatant fraction (50 ml) was placed in a steam oven for 30 min. The steam treatment was found to substantially stabilize the preparation. Whereas unsteamed samples often lost 90% of their activity in 1 week, steamed samples retained full killing activity over 2 to 3 months when stored at 5 to 10°C. After being steamed, the preparation was allowed to come to room temperature and was then mixed with an equal volume of 1.0 M potassium phosphate buffer (pH 6.6). The sample was next passed over a hydrophobic column (5 ml of phenyl-Sepharose CL-4B; Sigma Chemical Co.) which had been equilibrated with 0.5 M potassium phosphate buffer (pH 6.6). Elution of the colicin was by application of a step-wise gradient consisting of 15-ml washes of 0.5, 0.25, 0.125, 0.0625, and 0.03125 M potassium phosphate buffer (pH 6.6). Activity eluted at 0.0625 M potassium phosphate and was determined by the spot-test dilution assay (14). This preparation was not pure; analysis by high-pressure liquid chromatography (mentioned below) revealed that it contained many components. Because none of the systems tried led to the elution of substantial activity, we were unable to estimate the upper limit of purity obtained. Unfortunately, our efforts to further purify this material by several high-pressure liquid chromatography systems, including gel permeation on a Waters I-60 column, reverse-phase chromatography (Waters C18 Bondapak) with a variety of solvents, or ion-exchange chromatography (Waters Protein-Pak DEAE 5pw) have been unsuccessful. Throughout this paper, the concentration of partially purified colicin V is presented in terms of protein concentration (15).

Initial experiments showed that colicin V inhibited the incorporation of exogenously added [^{3}H]proline into cellular protein (data not shown). To ascertain whether this finding reflected an effect of the colicin on active transport, we examined the ability of treated cells to take up [^{3}H]proline into chloramphenicol-treated cells. Cells which had been treated with colicin V 10 min before the addition of proline were drastically diminished in their ability to transport the amino acid, relative to the control untreated culture (Fig. 1). The addition of the colicin to cells which had accumulated proline for 17.5 min led to substrate efflux (Fig. 1).

To determine whether the effect of colicin V on proline transport resulted from a specific inhibition of active transport or from a more generalized disruption of membrane integrity, we examined its effect on the accumulation of α-methylglucoside, which is a substrate of the phosphoenolpyruvate phosphotransferase system and whose accumulation is not directly coupled to the transmembrane proton motive force (20). Addition of colicin V to cells which had accumulated this substrate induced a dramatic increase in the amount of substrate accumulated (Fig. 2). As expected, a similar effect was observed in the case of colicin Ia-treated cells (6). In contrast, neither colicin had an effect on an isogenic strain carrying a mutation in the structural gene for the colicin I receptor. Such *cir* mutants are resistant to colicins Ia and Ib and insensitive to colicin V (3, 4). Finally, it was shown that substrate accumulation was stimulated by colicin Ia, but not by colicin V in a strain harboring a plasmid which contains the cloned colicin V immunity gene. The apparent, less-dramatic effect of colicin Ia on the colicin V-immune strain (Fig. 2C) when compared with the effect on the strain harboring neither plasmid (Fig. 2A) was partly due to the higher level of α-methylglucoside accumulated in this strain before colicin addition. We have obtained comparable

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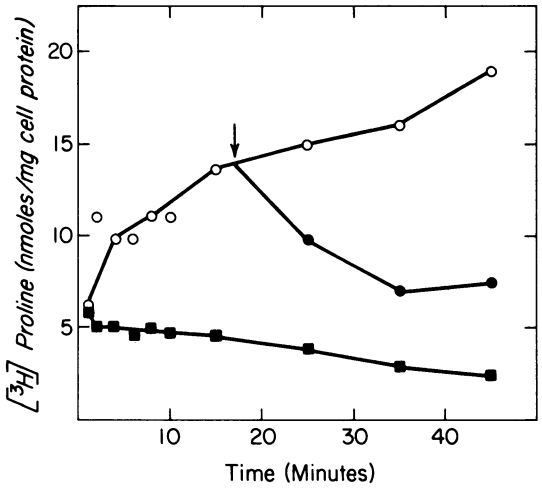

FIG. 1. Effect of colicin V on proline accumulation. *E. coli* JK362 was grown to 35 Klett units (ca. 10^8 cells per ml) at 37°C in medium 63 (16) containing glycerol (0.4%) as carbon source. Chloramphenicol (100 μg/ml) was then added, and the culture was incubated for 20 min at 25°C. At zero time, [^{3}H]proline (8.7×10^{-5} M, 11.5 Ci/mol) was added. At the indicated times, 0.4-ml portions were withdrawn, and the amount of proline which had accumulated in the cells was determined (6). The data have not been corrected for the zero time sampling. When used, colicin was added to a final concentration of 0.4 μg/ml. Symbols: ○, control; ■, colicin added at −10 min; ●, colicin added at 17.5 min.

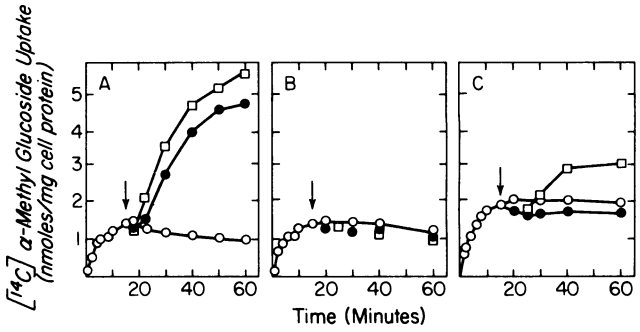

FIG. 2. Effect of colicins V and Ia on α-methylglucoside accumulation. Cells grown as described in the legend to Fig. 1 were harvested, washed in medium 63 (no added glycerol), and resuspended in medium 63 (no added glycerol) containing chloramphenicol (100 μg/ml). After a 10-min incubation at 25°C, *methyl*-D-glucopyranoside ([^{14}C]glucose [U]) (2.3×10^{-6} M, 52.2 Ci/mol) was added (zero time). At the indicated times, 0.2-ml portions were withdrawn, and the amount of substrate which had accumulated in the cells was determined (6). Addition of colicin is indicated by the arrow. (A) *E. coli* JK362. Symbols: ○, control; ●, 0.4 μg of colicin V per ml; □, 0.3 μg of colicin Ia per ml. (B) *E. coli* JK381 (-JK362 *cir*). Symbols: ○, control; ●, 1.0 μg of colicin V per ml; □, 0.3 μg of colicin Ia per ml. (C) *E. coli* JK487 (colicin V-immune *E. coli* JK362(pKF1) [5]). Symbols: ○, control; ●, 1.0 μg of colicin V per ml; □, 0.3 μg of colicin Ia per ml.

higher levels of stimulation by colicin Ia in these strains in other experiments. These results show that the inhibition of active transport by colicin V does not result from a generalized leakiness of the cellular membrane.

Since it is known that agents which disrupt the proton motive force enhance α-methylglucoside accumulation (18), a similar finding in the case of this colicin suggested a similar effect on cell metabolism. To examine this possibility, we determined the capacity of colicin V-treated cells to generate a membrane potential by assaying their capacity to accumulate the membrane permeant, triphenylmethylphosphonium cation ($TPMP^+$) (19). Treatment of sensitive cells with either colicin V or the uncoupler, carbonyl cyanide *m*-chlorophenylhydrazone, prevented the generation of a membrane potential (Fig. 3A). An isogenic strain carrying a plasmid containing the cloned colicin V immunity gene was fully able to generate a membrane potential after treatment with colicin V, but was inhibited by colicin Ia (Fig. 3B). In contrast, a strain carrying a plasmid containing the cloned colicin Ia immunity gene was able to generate a membrane potential after treatment with colicin Ia but not after treatment with colicin V.

We have previously described conditions whereby it has been possible to demonstrate the in vitro action of colicin Ia and Ib on membrane vesicles prepared from sensitive or resistant (receptorless) strains (24, 27). These experiments and those with liposomes and planar membranes (10, 22, 25, 26) have led to the conclusion that the primary target of colicins which are known to disrupt the membrane potential in whole cells is the cytoplasmic membrane. We, therefore, examined the effect of colicin V on the ability of membrane vesicles prepared from sensitive and insensitive (*cir*) strains to generate a membrane potential. Addition of colicin V to vesicles prepared from either strain inhibited ascorbate-phenazine methosulfate-driven $TPMP^+$ uptake (Fig. 4). These results taken together with our in vivo observations strongly suggest that the primary target of colicin V is the cytoplasmic membrane. It should be noted that a suggestion has been made that colicin V inhibits cells by affecting the cellular energy supply (13).

That vesicles prepared from colicin V-insensitive cells were sensitive to the colicin in vitro was not surprising in view of previous studies dealing with other colicins (12). Although the *cir* gene product is known to be the outer membrane receptor for colicin Ia and Ib (3), its exact role in

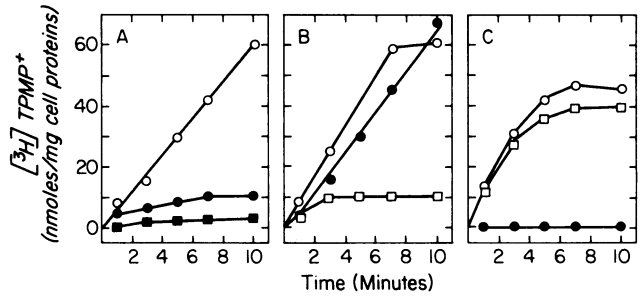

FIG. 3. Effect of colicins V and Ia on $TPMP^+$ accumulation in whole cells. Cells grown to a 100 Klett units as described in the legend to Fig. 1 were treated with colicin for 15 min at 25°C, harvested, treated with EDTA as previously described (27), and finally washed and resuspended in 1/10 of the original culture volume in 50 mM potassium phosphate buffer (pH 6.6). At zero time, potassium ascorbate (20 mM) and phenazine methosulfate (0.1 mM) were added, followed immediately by [^{3}H]$TPMP^+$ (0.4 mM, 2.5 Ci/mol). This mixture was incubated at 25°C under an atmosphere of pure O_2. At the indicated times, samples were removed, and the amount of accumulated $TPMP^+$ was determined (27). (A) *E. coli* JK362. Symbols: ○, control; ●, 0.5 μg of colicin V per ml; ■, 100 μM carbonyl cyanide *m*-chlorophenyhydrazone. (B) *E. coli* JK487 (colicin V-immune *E. coli* JK362(pColKF1 [5]). Symbols: ○, control; ●, 0.5 μg of colicin V per ml; □, 0.3 μg of colicin Ia per ml. (C) *E. coli* JK434 (colicin Ia-immune *E. coli* JK362(pAR29) [28]). Symbols: ○, control; ●, 0.3 μg of colicin V per ml; □, 0.3 μg of colicin Ia per ml.

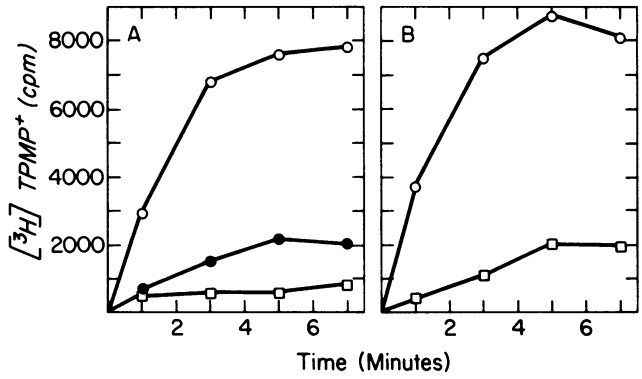

FIG. 4. Effect of colicin V on $TPMP^+$ accumulation in membrane vesicles. Membrane vesicles (5 mg/ml, final concentration), prepared as previously described (28), were incubated with colicin V (0.2 μg/ml, final concentration) at 25°C for 20 min. At zero time, potassium ascorbate (20 mM) and phenazine methosulfate (0.1 mM) were added, followed immediately by [^{3}H]$TPMP^+$. This mixture was incubated at 25°C under an atmosphere of pure O_2. At the indicated times, 45-μl samples were removed, and the amount of $TPMP^+$ which had accumulated was determined (28). Accumulation of solute was entirely dependent on the presence of the artificial electron donor pair. (A) Vesicles prepared from *E. coli* JK362. Symbols: ○, control; ●, colicin V; □, carbonyl cyanide *m*-chlorophenylhydrazone. (B) Vesicles prepared from strain JK381 (colicin V-insensitive *E. coli cir* mutant). Symbols: ○, control; □, colicin V.

colicin V sensitivity has not been established. If it does serve as the colicin V receptor, it would be expected that membranes prepared from such cells would be subject to colicin action. This would be in keeping with current notions that receptors function in vivo to facilitate translocation of colicin molecules across the outer membrane (12). Direct access of colicin to isolated cytoplasmic membrane vesicles would, thus, obviate any need for outer membrane receptors.

There is now much evidence that those colicins which act by depolarizing the bacterial energy transducing membrane in vivo are able to form aqueous channels in both closed liposomes as well as in planar membranes (12). In the case of colicin V, however, we have found that under conditions in which we can demonstrate colicin Ia- or E1-induced leakiness of asolectin liposomes toward phosphate or chloride, colicin V has no effect (C.-C. Yang, J. Konisky, V. L. Davidson, and W. A. Cramer, unpublished data). It remains to be established whether these differences reflect a real difference in the in vivo mode of action.

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LITERATURE CITED

1. **Asensio, C., J. C. Perez-Diaz, M. C. Martinez, and F. Baquero.** 1976. A new family of low molecular weight antibiotics from enterobacteria. Biochem. Biophys. Res. Commun. **69**:7–14.
2. **Baquero, F., D. Bouanchaud, M. C. Martinez-Perez, and C. Fernandez.** 1978. Microcin plasmids: a group of extrachromosomal elements coding for low-molecular-weight antibiotics in *Escherichia coli*. J. Bacteriol. **135**:342–347.
3. **Cardelli, J., and J. Konisky.** 1974. Isolation and characterization of an *Escherichia coli* mutant tolerant to colicins Ia and Ib. J. Bacteriol. **119**:379–385.
4. **Davies, J. K., and P. Reeves.** 1975. Genetics of resistance to colicins in *Escherichia coli* K-12: cross-resistance among colicins of group B. J. Bacteriol. **123**:96–101.
5. **Frick, K. K., R. L. Quackenbush, and J. Konisky.** 1981. Cloning of immunity and structural genes for colicin V. J. Bacteriol. **148**:498–507.
6. **Gilchrist, M. J. R., and J. Konisky.** 1975. Effects of colicin Ia on transport and respiration in *Escherichia coli*. J. Biol. Chem. **250**:2457–2462.
7. **Gratia, A.** 1925. Sur un remarquable exemple d'antagonisme entre souches de colibacille. C.R. Soc. Biol. **93**:1040–1041.
8. **Gratia, A., and P. Fredericq.** 1946. Diversite des souches antibiotiques de *E. coli* et etendue variable de leur champ d'action. C.R. Soc. Biol. **140**:1032–1033.
9. **Herschman, H. R., and D. R. Helinski.** 1967. Comparative study of the events associated with colicin induction. J. Bacteriol. **94**:691–699.
10. **Kayalar, C., and S. E. Luria.** 1979. Channel formation by colicin K on liposomes, p. 297–306. *In* C. P. Lee, G. Schatz, and L. Ernster (ed.), Membrane energetics. Addison-Wesley, Publishing Co., Inc., New York.
11. **Konisky, J.** 1978. The bacteriocins, p. 11–136. *In* L. N. Ornston and J. R. Sokatch (ed.), The bacteria. Academic Press, Inc., New York.
12. **Konisky, J.** 1982. Colicins and other bacteriocins with established modes of action. Annu. Rev. Microbiol. **36**:125–144.
13. **Kucharzewska, T., H. Stepilen, and E. Gasior.** 1975. Isolation and some properties of colicin V preparations. Acta Microbiol. **3**:169–177.
14. **Levisohn, R., J. Konisky, and M. Nomura.** 1968. Interactions of colicins with bacterial cells. IV. Immunity breakdown studied with colicin Ia and Ib. J. Bacteriol. **96**:811–821.
15. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein determination with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
16. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
17. **Nagel de Zwaig, R., and D. N. Anton.** 1964. Interactions between colicinogenic factors and fertility factors. Biochem. Biophys. Res. Commun. **17**:358–362.
18. **Reider, E., E. F. Wagner, and M. Schweiger.** 1979. Control of PEP-dependent PTS-mediated sugar transport in *Escherichia coli* by energization of the cell membrane. Proc. Natl. Acad. Sci. U.S.A. **76**:5529–5533.
19. **Rottenberg, H.** 1979. The measurement of membrane potential and pH in cells, organelles, and vesicles. Methods Enzymol. **55**:547–569.
20. **Saier, M. H., Jr.** 1977. Bacterial phosphoenolpyruvate:sugar phosphotransferase systems: structural, functional, and evolutionary relationships. Bacteriol. Rev. **41**:856–871.
21. **Schaller, K., R. Dreher, and V. Braun.** 1981. Structural and functional properties of colicin M. J. Bacteriol. **146**:54–63.
22. **Schein, S. J., B. L. Kagan, and A. Finkelstein.** 1978. Colicin K acts by forming voltage-dependent channels in phospholipid bilayer membranes. Nature (London) **276**:159–163.
23. **Smith, H. W.** 1974. A search for transmissible pathogenic characters in invasive strains of *Escherichia coli*: the discovery of a plasmid-controlled toxin and a plasmid-controlled lethal character closely associated or identical with colicin V. J. Gen. Microbiol. **83**:95–111.
24. **Tokuda, H., and J. Konisky.** 1978. *In vitro* depolarization of *Escherichia coli* vesicles by colicin Ia. J. Biol. Chem. **253**:7731–7737.
25. **Tokuda, H., and J. Konisky.** 1979. Effect of colicins Ia and E1 on ion permeability of liposomes. Proc. Natl. Acad. Sci. U.S.A. **76**:6167–6171.
26. **Uratani, Y., and W. A. Cramer.** 1981. Reconstitution of colicin E1 into dimyristoylphosphatidylcholine membrane vesicles. J. Biol. Chem. **256**:4017–4023.
27. **Weaver, C. A., B. L. Kagan, A. Finkelstein, and J. Konisky.** 1981. Mode of action of colicin Ib: formation of ion-permeable channels. Biochim. Biophys. Acta **645**:137–142.
28. **Weaver, C. A., A. H. Redborg, and J. Konisky.** 1981. Plasmid-determined immunity of *Escherichia coli* K-12 to colicin Ia is mediated by a plasmid-encoded membrane protein. J. Bacteriol. **148**:817–828.