JOURNAL OF BACTERIOLOGY, Nov. 1968, p. 1700–1703
Vol. 96, No. 5

Printed in U.S.A.

Mode of Action of Colicins of Types E_1, E_2, E_3, and K

PETER REEVES

Department of Microbiology, University of Adelaide, Adelaide, South Australia

Received for publication 3 September 1986

The effect of colicins on deoxyribonucleic acid and protein synthesis, and also their effect on the ability of T4 phage to replicate in *Escherichia coli* K-12, were studied. Colicins of type K inhibited deoxyribonucleic acid synthesis, protein synthesis, and phage growth. Among colicins of type E, there was an absolute correlation between mode of action and subdivision into types E_1, E_2, and E_3.

Studies on the mode of action of colicins have been confined to only a few examples and these have yielded some very interesting results. Colicins ML-E_1, K30-E_1, and K235-K are all thought to affect energy metabolism (5, 8); P9-E_2 and CA42-E_2 both affect deoxyribonucleic acid (DNA) metabolism (6, 8; Renolds and Reeves, *in press*), and CA38-E_3 inhibits protein synthesis (6, 8). On the basis of this evidence, it is not really justifiable to generalize on the modes of action of colicins E_1, E_2, E_3, or K, since only one or two of each have been studied. I have studied several colicins of each of these types for their effect both on DNA and protein synthesis and on the ability of T4-infected bacteria to produce progeny phage. Although only a limited range of effects was studied, sufficient colicins of each type were included to determine whether there was any correlation between colicin type and its mode of action.

MATERIALS AND METHODS

Materials. Broth was the Oxoid (CM67) dehydrated medium prepared as directed, and nutrient agar was Difco Blood Agar Base prepared as directed without addition of blood. Chloramphenicol was chloramphenicol sodium succinate, sold as Synthomycetin by Lepetit (Pharmaceuticals) Pty. Ltd., Melbourne, Australia. Tritiated thymine and $^{35}SO_4$ were obtained from the Radiochemical Centre, Amersham, England (TRK.15 and SJS.1).

Bacterial strains. Escherichia coli P503 and P109, derivatives of *E. coli* K-12, were used as the sensitive organisms for this study. P503 has the genetic constitution *thy, met*, λ^-; P109 has various nutritional requirements (10) which are probably of no importance in this study, and is also λ^-. The colicinogenic strains and colicin-resistant and colicin-immune indicator strains are listed in Table 1.

Colicins. The colicins used were prepared by exposing an overnight culture of the colicinogenic strain in broth to ultraviolet irradiation (a constant dosage was used but the source was not calibrated), adding an equal volume of broth, and then incubating at 37 C for 4 to 5 hr. The bacteria were removed by centrifugation, and the supernatant fluid was used as a crude solution of colicin. The colicin was dispensed and stored at −20 C, a new ampoule being used each day. The colicin was normally assayed by spotting loopfuls of serial dilutions onto a nutrient agar plate seeded with a sensitive strain, the highest dilution to give a completely clear zone being taken as the end point and its reciprocal taken as the titer (in arbitrary units/milliliter). When needed, the multiplicity of infection (MOI) was calculated from the proportion of surviving bacteria (6).

The colicins used are known by the strain designation, followed by the colicin type as defined by Fredericq (1); thus, K235-K refers to a colicin of type K produced by *E. coli* K235 (9). Colicins of type E_1 were usually produced only to low titer (20 to 80 units/ml), whereas the colicins of other types were produced at 250 to 1,000 units/ml. (Strains producing less were not included in this study.)

DNA and protein synthesis. A log-phase culture of *E. coli* P503 was grown with aeration in a medium containing tris(hydroxymethyl)aminomethane (0.1 M), NaCl (0.08 M), KCl (0.2 M), NH_4Cl (0.02 M), KH_2PO_4 (6.4×10^{-4} M), Na_2SO_4 (1.6×10^{-4} M), $MgCl_2$ (10^{-3} M), $CaCl_2$ (10^{-4} M), $FeCl_3$ (2×10^{-6} M), methionine (20 μg/ml), thymine (3 μg/ml), glucose (0.2%), at *p*H 7.4. When it had reached an optical density value (at 650 nm) of about 0.4, it was washed on a Millipore HA membrane and suspended in the same medium (but containing only 1 μg of thymine per ml). This cell suspension was dispensed in 1-ml amounts into flasks maintained at 37 C and aerated. After 15 to 27 min, 0.1 ml of colicin was added; 3 min later, 2 ml of the medium including (per ml) 2 μc of tritiated thymine and 5 μc of ^{35}S (as sulfate). Samples (0.1 ml) were taken at intervals and placed on small discs of glass fiber paper (Whatman GP83; previously soaked in 1 M Na_2SO_4 and then dried, to prevent adsorption of $^{35}SO_4$ to the glass fiber). The discs were put into cold 5% trichloroacetic acid containing 0.2 M Na_2SO_4; they were washed in the same solution, followed by washing in cold 5% trichloroacetic acid and then alcohol. After the final wash, the alcohol was removed in a vacuum oven, and the samples were counted in a Packard liquid scintillation counter (independent counts for ^{35}S and ^3H).

Effect of colicins on bacteriophage-infected cells. The technique used was essentially that of Nomura (6); P109 and phage T4 were used.

RESULTS

Specificity of the colicins used. Each colicinogenic strain was streaked on nutrient agar, incubated overnight, and killed by chloroform vapor; a second nutrient agar layer was added, and the indicator strains P109, K-12 (Col K30-E_1), K-12 (Col K317-E_2), P170, and P173 were cross-streaked. Sensitive indicator strains showed a gap in growth where the streak crossed the underlying streak of colicinogenic bacteria. Each colicin was tested by assaying it on the same five indicator strains and comparing the assay on each with that on P109 (Table 1).

All colicinogenic strains and their colicins behaved as expected on P109 and the two receptor mutants P170 and P173, with the exception of colicin K216-K which had some (3%) activity on P173, perhaps due to a second colicin. There were, however, some anomalies in the patterns of sensitivity of the colicinogenic indicators. K-12 (Col K30-E_1) was only slightly sensitive to colicin preparations of types E_2 and E_3, whereas it was fully immune to colicins of types E_1 and fully sensitive to those of type K. By the cross-streak method, the same strain was clearly sensitive to all E_2 and E_3 producers. K-12 (Col K317-E_2) was generally fully sensitive to colicins of types E_1, E_3, and K and fully immune to colicin E_2 by both tests. Strain K321, described by Fredericq (2) as producing colicin E_3, behaved as though it produced E_2 and a trace of another colicin of type E; K-12 (Col K317-E_2) was slightly sensitive to the colicin of K321, but not to the extent that would be expected if K321 produced E_3.

DNA and protein synthesis. Since the colicins used were crude preparations in broth, it was first shown that the addition of 0.1 ml of broth did not appreciably alter the incorporation of either tritiated thymine or $^{35}SO_4$. In all cases, the addition of colicin ultimately affected incorporation of both isotopes, but the pattern varied.

Only two colicins of type E_1 were obtained in sufficient yield to use, but both of these [from N104 and K-12 (Col K30-E_1)] affected both syntheses equally, confirming and extending earlier work on ML-E_1 and K30-E_1 (3, 6). The data for N104-E_1 are given in Fig. 1.

Eight colicins of type E_2 had a greater effect on

TABLE 1. *Colicinogenic strains used, their activity spectra on indicator strains, and their mode of action*

Characteristic	Colicinogenic strains[a]			
	E. coli K47,[b] K53,[b] N104, K-12 (Col K30-E_1)	*E. coli* CA42, GEI288, GEI554, GEI602, K317,[b] K321; *S. typhimurium* M5092, M5120, LT2 (Col P9-E_2)	*E. coli* CA38, K365	*E. coli* K40, K216, K235, K283, K297, K319
Activity[c] on indicator strains[d]				
P109	100(+)	100(+)	100(+)	100(+)
P170	0	0	0	100(+)
P173	100(+)	100(+)	100(+)	0[e]
K-12 (Col K30-E_1)	0	0.3–1(+)	1(+)	100(+)
K-12 (Col K317-E_2)	100(+)	0[f]	100(+)	100(+)
Inhibition of				
DNA synthesis	+	+	−	+
Protein synthesis	+	±	+	+
T4 replication	+	−	+	+

[a] *Escherichia coli* strains kindly provided by P. Fredericq, and the *Salmonella typhimurium* strains by M. Lewis and B. A. D. Stocker.

[b] Strains not used in the DNA and protein synthesis experiments.

[c] Titers of all colicins are corrected to give a titer of 100 against P109; (+) indicates positive on cross-streaking.

[d] All indicator strains are derivatives of *E. coli* K-12. P109 is sensitive to all colicins used and P170 and P173 are receptor mutants of P109 resistant to colicins E and K, respectively. K-12 (Col K30-E_1) and K-12 (Col K317-E_2) carry colicin factors originally derived from *E. coli* K30 and K317, respectively

[e] Colicin K321-E_2 was aberrant.

[f] Colicin K216-K was aberrant.

DNA synthesis than on protein synthesis, although there was always some DNA synthesis observed despite the addition of colicin 3 min before the radioactivity. The colicins were all added in considerable excess and gave fewer than 0.1% survivors after 30 min (when the experiment was terminated). Colicin K321-E_2 was studied at several concentrations (Fig. 2); at low concentrations, the effect on protein synthesis was considerably delayed.

Colicin CA38-E_3 was studied previously (6) and found to inhibit protein synthesis. Both it and K365-E_3 were examined and found to inhibit protein synthesis. Results for K365-E_3 are shown in Fig. 3.

The six colicins of type K were all found to affect both protein and DNA synthesis. K216-K and K283-K were also examined at various dilutions, and the effect was more marked on protein than on DNA synthesis (Fig. 4), particularly at low concentrations.

Replication of bacteriophage T4. The results fully confirm those of Nomura (6); i.e., colicins of type E_2 do not affect the ability of infected cells to give rise to a plaque, whereas all colicins of types E_1, E_3, and K reduce this ability markedly. Both colicins of type E_3 reduced plaque-forming ability, but with lower efficiency than that with which they killed uninfected cells; the inactivation of infected cells (expressed as plaque-forming units) by colicins of types E_1 and K was variable, on some occasions being as efficient as the killing of viable cells (Table 1).

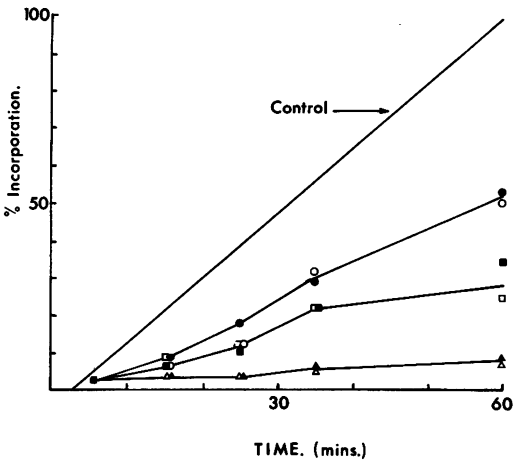

FIG. 1. *Effect of colicin N104-E_1 on DNA and protein synthesis. An exponential culture of E. coli P503 was labeled with ^3H-thymine and $^{35}SO_4$ at 3 min. At 5 min, colicin was added. Samples were removed at intervals and cold 5% trichloroacetic acid was added for determination of insoluble radioactivity.* (○) ^{35}S, *MOI 0.5;* (●) ^3H, *MOI 0.5;* (□) ^{35}S, *MOI 2.0;* (■) ^3H, *MOI 2.0;* (△) ^{35}S, *MOI 8.0;* (▲) ^3H, *MOI 8.0.*

DISCUSSION

Several colicins of types E_1, E_2, E_3, and K were examined for their mode of action. As far as is known, the colicinogenic strains are of independent origin, those of type E_2 having been derived from *Salmonella* and *Shigella* strains [*Shigella sonnei* P9 is the original source of the (P9-E_2)

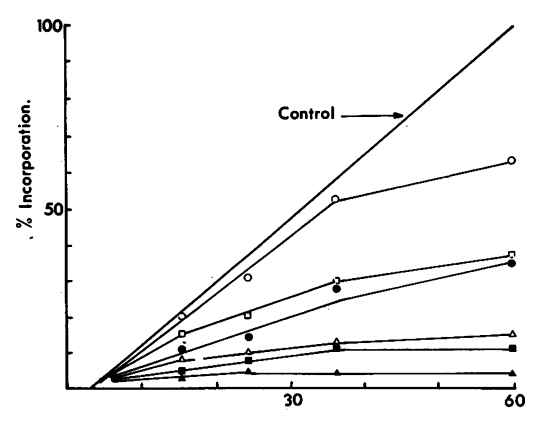

FIG. 2. *Effect of colicin K321-E_2 on DNA and protein synthesis. Techniques as for Fig. 1.* (○) ^{35}S, *colicin MOI 1;* (●) ^3H, *colicin MOI 1;* (□) ^{35}S, *colicin MOI 3;* (■) ^3H, *colicin MOI 3;* (△) ^{35}S, *colicin MOI 9;* (▲) ^3H, *colicin MOI 9.*

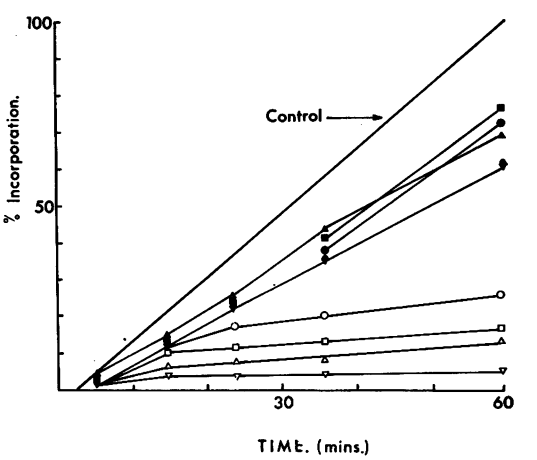

FIG. 3. *Effect of colicin K365-E_3 on DNA and protein synthesis. Techniques as for Fig. 1.* (○) ^{35}S, *colicin MOI 1;* (●) ^3H, *colicin MOI 1;* (□) ^{35}S, *colicin MOI 3;* (■) ^3H, *colicin MOI 3;* (△) ^{35}S, *colicin MOI 9;* (▲) ^3H, *colicin MOI 9;* (▽) ^{35}S, *colicin MOI 27;* (▼) ^3H, *colicin MOI 27.*

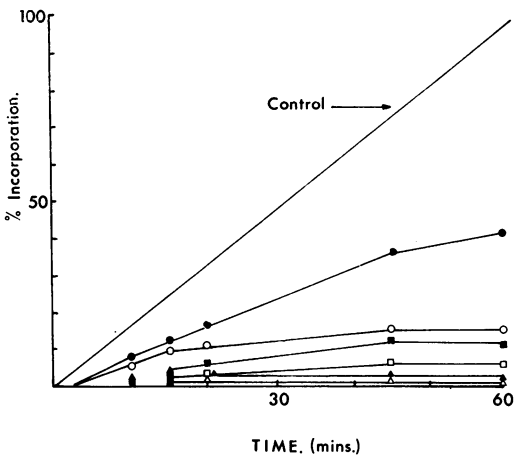

FIG. 4.[1] *Effect of colicin K216-K on DNA and protein synthesis. Techniques as for Fig. 1.* (○) ^{35}S, *3 arbitrary units per ml (AU) of colicin MOI 0.5;* (●) ^{3}H, *3 AU of colicin, MOI 0.5;* (□) ^{35}S, *50 AU of colicin, MOI 5;* (■) ^{3}H, *50 AU of colicin, MOI 5;* (△) ^{35}S, *300 AU of colicin, MOI 50;* (▲) ^{3}H, *300 AU of colicin, MOI 50.*

colicin factor] and from the C.A., G.E.I., and K series of Fredericq's *E. coli* strains isolated at different times. Lewis and Stocker (4) also showed that three of these colicins do differ in their neutralization by antiserum and in their action on *E. coli* K-12 (Col P9-E_2) and (Col P9-I). However, the strains producing E_1, E_3, and K all come from Fredericq's collection of *E. coli* strains, and none of these groups of colicin displays any known heterogeneity.

In this paper, the effects of colicins on DNA and protein synthesis and on T4-infected cells have been studied. The present work is clearly not sufficient to determine the precise mode of action of a colicin but it does enable a clear distinction to be made between the three previously described primary effects which colicins can have. An absolute correlation was observed between the type of colicin used and its effects on sensitive cells (Table 1). Thus, the heterogeneity among colicins of type E_2 does not extend to their mode of action.

Immunity to colicins is frequently not absolute (2, 7); in this study, the immunity of K-12 (Col K317-E_2) was not only absolute but it was completely specific, in contrast to the partial specificity of the immunity conferred by K-12 (Col K30-E_1) in this study and by K-12 (Col K30-E_1) and K-12 (Col P9-E_2) in a previous study (8).

The specificity of the immunity of K-12 (Col K317-E_2) suggests that, unlike the other examples of immunity to colicins, immunity may result from a cell component involved in the transmission of only the colicin E_2 effect, perhaps even modifying the system regulating DNA synthesis itself.

LITERATURE CITED

1. Fredericq, P. 1948. Actions antibiotiques réciproques chez les *Enterobacteriaceae*. Rev. Belge Pathol. Med. Exptl. **19**(suppl. 4):1–107.
2. Fredericq, P. 1956. Resistance et immunité aux colicines. Compt. Rend. Soc. Biol. **150**:1514–1517.
3. Jacob, F., L. Siminovitch, and E. Wollman. 1952. Sur la biosynthèse d'une colicine et sur son mode d'action. Ann. Inst. Pasteur **83**:295–315.
4. Lewis, M. J., and B. A. D. Stocker. 1965. Properties of some group E colicin factors. Zentr. Bakteriol. Parasitenk. Abt. I Orig. **196**:173–183.
5. Luria, S. E. 1964. On the mechanism of action of colicins. Ann. Inst. Pasteur **107**(no. 5, suppl.):67–73.
6. Nomura, M. 1963. Mode of action of colicines. Cold Spring Harbor Symp. Quant. Biol. **28**: 315–324.
7. Nomura, M. 1967. Colicins and related bacteriocins. Ann. Rev. Microbiol. **21**:257–284.
8. Nomura, M., and A. Maeda. 1965. Mechanisms of action of colicines. Zentr. Bakteriol. Parasitenk. Abt. I Orig. **196**:216–239.
9. Reeves, P. 1965. The bacteriocins. Bacteriol. Rev. **29**:24–45.
10. Reeves, P. 1966. Mutants resistant to colicin CA42-E_2: cross resistance and genetic mapping of a special class of mutants. Australian J. Exptl. Biol. Med. Sci. **44**:301–316. Erratum **45**:330.