JOURNAL OF BACTERIOLOGY, Sept. 1971, p. 833-839

Vol. 107, No. 3
Printed in U.S.A.

Mode of Action of a Bacteriocin from *Serratia marcescens*

JOHN FOULDS

University of Connecticut Health Center, Farmington, Connecticut 06032

Received for publication 5 May 1971

The effects of bacteriocin JF246, produced by *Serratia marcescens*, on the incorporation of labeled leucine and thymidine, the synthesis of β-galactosidase, the active transport of labeled leucine and α-methyl-D-glucoside, and the cellular levels of adenosine triphosphate (ATP) in *Escherichia coli* were studied. This bacteriocin strongly inhibited the incorporation of leucine and thymidine into protein and deoxyribonucleic acid, respectively, as well as the active transport of leucine. The accumulation of α-methyl-D-glucoside, which is mediated by a phosphoenolpyruvate-dependent phosphotransferase system, was not markedly inhibited. The level of ATP in bacteriocin-treated cells rapidly fell to 10 to 15% of the control value. However, the kinetics of inhibition of macromolecular synthesis by various levels of bacteriocin was not related to the kinetics of ATP decline.

Bacteriocins are high-molecular-weight antibacterial substances, apparently protein in nature, which are synthesized by certain strains of bacteria. Bacteriocins kill cells. Bacteriocin sensitivity depends upon the presence of a specific receptor on the cell surface and bacteriocins are classified according to the specificity of adsorption onto these receptors. The most extensively studied bacteriocins are the colicins produced by *Escherichia coli* and certain other strains of the *Enterobacteriaceae* (20).

Adsorption of different bacteriocins may have different biochemical consequences for a cell. For example, colicin E3 specifically inhibits protein synthesis (21) whereas colicins E1 (13), K (19), and A (18) inhibit deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and protein synthesis as well as the active transport of β-D-galactosides and amino acids. This suggests that this class of colicins interferes with the supply of energy in the affected bacteria (5, 12).

Several authors have described bacteriocins produced by *Serratia marcescens* (8, 9, 23), but little is known about the mode of action of these bacteriocins. Hamon and Peron (9) find that certain *Serratia* strains elaborate at least two distinct bacteriocins: one, which is trypsin-resistant, and affects only *Serratia* strains, and a second which is trypsin-sensitive and affects only *E. coli* strains.

The purpose of this report is to describe the biochemical events associated with the killing of *E. coli* cells by a trypsin-sensitive bacteriocin produced by *S. marcescens* JF246 (6, 7). We find that the mode of action of this bacteriocin is identical in several respects to that of colicins A, E1, or K.

MATERIALS AND METHODS

Organisms and culture media. The bacteriocin-sensitive indicator strain, JF135 (*ilv-1*, *trpA23*, *leu*), was derived from an *E. coli* W3110 strain (PB11) obtained from Paul Berg. A spontaneous mutant, JF365, requiring thymine (*ilv-1*, *trpA23*, *leu*, *thyA*) was isolated from JF135 by a method previously described (19) using the folate reductase inhibitor, trimethoprim (Burrows-Wellcome Co., Tuckahoe, N.Y.). Wild-type *E. coli* strain W3100 was obtained from Henry Wu. *S. marcescens* JF246 (previously called JF58-12) was used to prepare the bacteriocin. Nutrient Broth, Proteose Peptone no. 3, Beef Extract, and Agar were purchased from Difco.

Strains were maintained on slants of Nutrient Broth containing 2% agar. Minimal medium (M9) contained: Na_2HPO_4, 6 g; KH_2PO_4, 3 g; NaCl, 0.5 g; NH_4Cl, 1.0 g; $MgSO_4$, 0.13 g; plus 0.2% glucose unless otherwise noted. Amino acid supplements were added where required at a concentration of 20 μg/ml. Rich medium (PPBE) contained 10 g of Proteose Peptone No. 3, 2 g of Beef Extract, and 5 g of NaCl per liter. PPBE agar contained 1.5% agar and PPBE soft agar contained 0.75% agar.

L-Leucine-*UL*-^{14}C, L-leucine-*4,5*-^{3}H, thymidine-*methyl*-^{3}H, and adenosine-*2,8*-^{3}H were purchased from New England Nuclear Corp. [*αMethyl*-^{14}C]D-glucoside and adenosine triphosphate (ATP) were from Calbiochem. Mitomycin C, methyl-β-D-thiogalactoside, chloramphenicol, Pronase, deoxyribonuclease I (DNase I), and firefly lantern extract were from Sigma Chemical Co.

Preparation and assay of bacteriocin. The purification and characterization of the bacteriocin from mitomycin-induced cells of *S. marcescens* JF246 will be described elsewhere. The purified material migrates as

a single band on electrophoresis in polyacrylamide gels containing 2% sodium dodecyl sulfate, and has an apparent molecular weight of 60,000 daltons.

The bacteriocin was assayed by the spot test method previously described (6). One unit of bacteriocin activity was defined as the lowest concentration which completely inhibited the growth of the indicator strain. The specific activity was defined as the number of units of bacteriocin activity per milligram of protein.

The amount of bacteriocin adsorbed by *E. coli* JF135 cells was measured by using cells which were first grown to a concentration of 5×10^8 cells/ml in minimal medium. The culture was divided into two flasks. One portion was diluted with an equal volume of 0.16 M NaCl (control cells) and the second with a solution containing 0.1 M NaF and 0.06 M NaN_3. After a 20-min incubation with aeration at 37 C, bacteriocin JF246 was added at various concentrations. The amount of bacteriocin not absorbed to the cells after a further 10 min of incubation at 37 C was determined by first removing the cells by centrifugation (10,000 × *g* for 5 min at 4 C) followed by measurement of bacteriocin in the supernatant solution.

Incorporation of labeled substrates. The incorporation of ^{3}H-labeled leucine and ^{3}H-labeled thymidine into an acid-insoluble form by bacterial cultures was measured by placing 0.2-ml samples of culture into 3.0 ml of ice-cold 7% trichloroacetic acid containing 1 mg of unlabeled carrier per ml. After 20 min at 0 C, the precipitate was collected on glass fiber filters (Whatman GF/C), washed six times with 2-ml portions of ice-cold 7% trichloroacetic acid and once with 5 ml of 95% ethanol. The filters were dried and counted in a scintillation counter using a toluene based fluor.

Active transport of α-methylglucoside and L-leucine. Intracellular accumulation of α-methyl-β-D-glucopyranoside was measured in JF135 cells which were first grown to a concentration of 2×10^8 cells/ml in minimal medium supplemented with the required amino acids and glucose. The cells were washed three times and resuspended in minimal medium containing 100 μg/ml chloramphenicol with no additional supplements. After 30 min of incubation at 25 C, bacteriocin JF246, NaF (final concentration, 0.05 M), or bacteriocin JF246 and NaF (final concentration, 0.05 M) was added. After a 5-min incubation period at 37 C, α-methyl-β-D-glucopyranoside was added (final concentration, 10^{-6} M; 50 Ci/mole). Samples of 0.5 ml were removed periodically and the cells were collected on membrane filters (Millipore Corp., New Bedford, Mass., type HA) and washed twice with 2-ml portions of ice cold minimal medium. The filters were dried and counted in a scintillation counter by using a toluene-based fluor.

The accumulated radioactivity in the control cells was identified as α-methylglucoside phosphate by chromatography on Silica Gel G followed by treatment with bacterial alkaline phosphatase as described by Kaback (14).

The intracellular accumulation of L-leucine was measured in cells which were first grown and treated with chloramphenicol as described above except that the chloramphenicol incubation was at 37 C. Next, ^{3}H-L-leucine was added (final concentration, 10^{-6} M; 250 Ci/mole); after 5 min of incubation at 37 C, bacteriocin JF246 was added to a portion (final concentration, 10 units/ml). At 9 min after the addition of the labeled leucine, unlabeled leucine was added to another portion (final concentration; 8×10^{-4} M). Samples were removed, washed, and counted as described above. The radioactivity accumulated in the control cells was identified as leucine as follows. The radioactivity was extracted three times with 2-ml portions of boiling water, and the extracts were pooled and brought to dryness in vacuo. The residue was dissolved in 0.05 ml of water, and a portion was applied to a paper chromatogram. The chromatogram was developed for 16 hr by using butanol, glacial acetic acid, and water (120:30:50, v/v/v) as the solvent. The radioactivity co-chromatographed with authentic leucine.

Estimation of cellular levels of ATP. Cellular ATP was measured by counting the flashes of light emitted by mixtures of cell extracts and firefly lantern extracts. The dried firefly lantern extracts were rehydrated, by following the directions of the supplier, to a concentration of 10 mg/ml in 0.05 M potassium arsenate (*p*H 7.4) and 0.02 M magnesium sulfate. The suspension was clarified by centrifugation and stored at room temperature until used. Under these conditions, the firefly lantern extract appeared to be stable for at least 3 hr. In this procedure, ATP was first extracted from 0.5-ml samples of culture fluid by heating with 4.5 ml of water at 100 C for 10 min as described by Fields and Luria (4).

The assays were performed by first mixing the sample in a scintillation vial with 2.4 ml of buffer (0.05 M KH_2AsO_4, 0.02 M $MgSO_4$, 0.001 M ethylenediaminetetraacetic acid, *p*H 7.4). At time zero, 0.5 ml of firefly lantern extract at 25 C was added, and the contents of the vial were mixed. The vial was then lowered into the counting chamber of a Beckman model 100 scintillation counter which had been adjusted to a gain of 10.0 and a window of 0.5 to 9.9. This operation was completed within 6 sec. At exactly 7 sec after the addition of the lantern extract, counting was begun. Each sample was counted for exactly 6 sec, and the amount of ATP in the sample was estimated from the counts obtained with known amounts of ATP. The addition of known amounts of ATP to culture fluid before boiling gave the expected additive results. ATP levels in the culture supernatant solutions were assayed after removal of the cells by centrifugation. They were negligible, both in control and bacteriocin-treated cultures.

Other procedures. β-Galactosidase activity was measured by the method of Pardee, Jacob, and Monod (22) in bacterial extracts prepared by shaking 5.0 ml of culture fluid with a mixture of 0.1 ml of toluene and 0.05 ml of 0.1% sodium deoxycholate. One unit of β-galactosidase activity was defined as the hydrolysis of 1 μmole of orthonitrophenyl-β-D-galactopyranoside per min at 28 C. Protein concentrations were determined by the method of Lowry (16) with bovine albumin (A grade, Calbiochem) used as the standard.

RESULTS

Killing of E. coli cells by bacteriocin. The killing of *E. coli* JF135 by bacteriocin JF246 followed single-hit kinetics, suggesting that a single active molecule of bacteriocin can kill a cell.

Figure 1A shows that, when the percentage of survivors after exposure of cells to bacteriocin for 10 min was plotted on a logarithmic scale against the concentration of bacteriocin, the points fell on a straight line which extrapolated to 100.

Pretreatment of cells with NaN_3 and NaF markedly reduced both the amount of bacteriocin adsorbed and the amount of killing of these cells. However, pretreatment of cells with chloramphenicol had no effect either on the amount of bacteriocin adsorbed or on the amount of killing (Fig. 1B and Table 1). This reduction in killing was not due to the irreversible inactivation of the bacteriocin because, when the NaN_3 and NaF were removed by dialysis from the bacteriocin-treated culture, the cells were killed as effectively as in the control.

Effect on protein and DNA synthesis. Treatment of sensitive cells with bacteriocin JF246 resulted in the rapid inhibition of incorporation of labeled leucine and thymidine into acid-insoluble material (Fig. 2). The time required for the cessation of ^{14}C-L-leucine and ^{3}H-thymidine incorporation was dependent on bacteriocin concentration. When sonic extracts prepared from control and bacteriocin-treated cultures were treated with Pronase (^{14}C-L-leucine experiments) or DNase I (^{3}H-thymidine experiments), the

TABLE 1. *Adsorption of bacteriocin JF246 by cells of Escherichia coli in the presence of NaN_3 and NaF*

Bacteriocin added (units/ml)	Bacteriocin not adsorbed (units/ml)	
	Control cells (NaCl)[a]	Treated cells (NaN_3, NaF)[a]
1	0[b]	1
4	0[b]	4
16	0[b]	16
64	2	64
256	128	256

[a] Final concentrations: NaCl, 0.08 M; NaN_3, 0.03 M; NaF, 0.05 M.

[b] Zero means no bacteriocin could be detected in supernatant solutions.

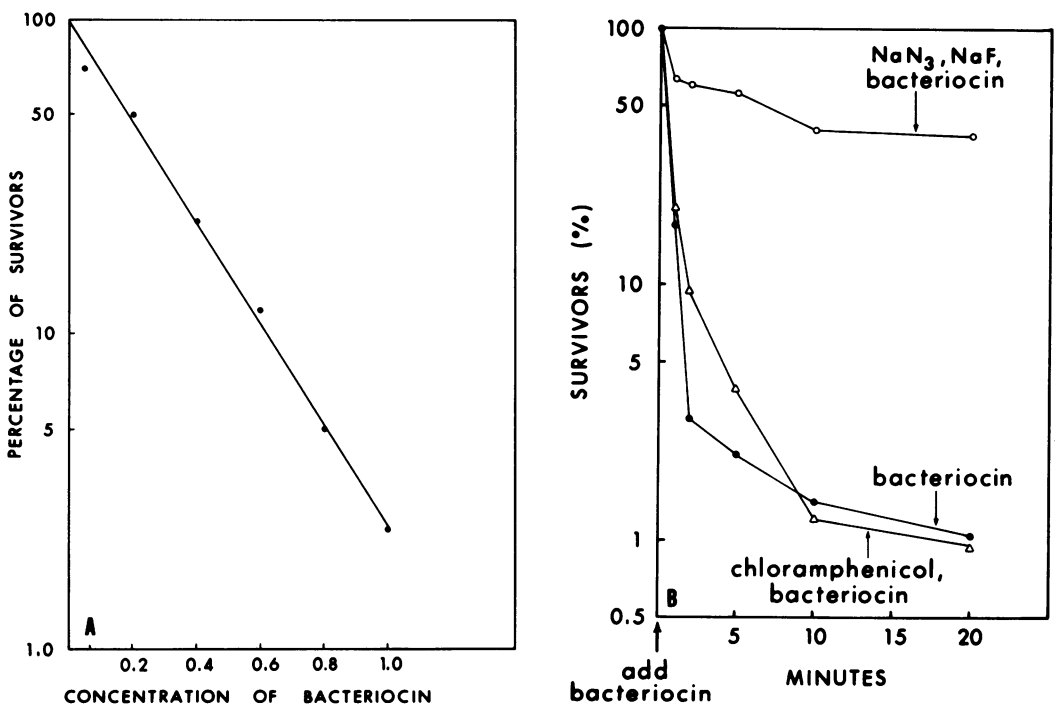

FIG. 1. *A. Survival of cells treated with various levels of bacteriocin. Bacteriocin (0.1 ml) at various concentrations was added to 0.9 ml of E. coli JF135 cells grown in minimal medium. After 10 min at 37 C, the number of viable cells was determined. Saline was added to the control culture which contained 3.1 × 10^8 cells. B. Effect of NaN_3 and NaF on the survival of bacteriocin-treated cells. A culture of E. coli JF135 cells grown in minimal medium was divided into three flasks. NaN_3 and NaF were added to one flask at final concentrations of 0.03 M and 0.05 M, respectively. Chloramphenicol was added to another flask at a final concentration of 50 μg/ml. The third flask (control) contained 0.08 M NaCl. After 20 min of incubation at 37 C, 2 units of bacteriocin per ml was added to all cultures. Samples were removed as indicated, and the number of viable cells was determined.*

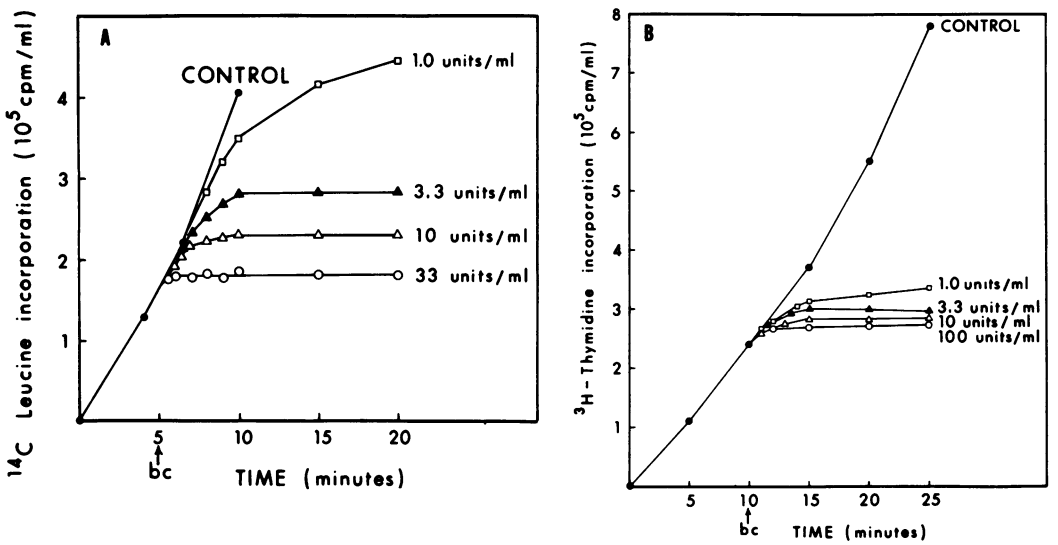

FIG. 2. *A. Effect of bacteriocin on incorporation of ^{14}C-leucine. E. coli JF135 was grown in minimal medium to a concentration of 5 × 10^8 cells/ml. The cells were washed once and resuspended in fresh medium lacking leucine. The culture was incubated at 37 C for 10 min, and at time zero ^{14}C-leucine was added (5 μg/ml, 0.25 μCi/ml). The culture was divided into five equal portions; and at 5 min after the addition of ^{14}C-leucine, bacteriocin was added at various concentrations. An appropriate amount of minimal medium was added to the control culture. The number of viable cells was determined 10 min after the addition of bacteriocin. Relative to the control culture, the survival was 4.6% in the culture containing 1 unit/ml, 0.08% at 3.3 units/ml, and less than 0.01% in the cultures containing 10 and 33 units/ml. B. Effect of bacteriocin on incorporation of ^{3}H-labeled thymidine. E. coli JF365 was grown in minimal medium to a concentration of 5 × 10^8 cells/ml. The cells were washed once and resuspended in fresh medium containing ^{3}H-thymidine (2 μg/ml, 0.5 μCi/ml). After 10 min, the culture was treated with bacteriocin as described in Fig. 1. The number of viable bacteria relative to the control culture 10 min after the addition of bacteriocin was 8.1% for 1 unit/ml, 0.3% for 3.3 units/ml, and less than 0.01% for 10 and 100 units/ml.*

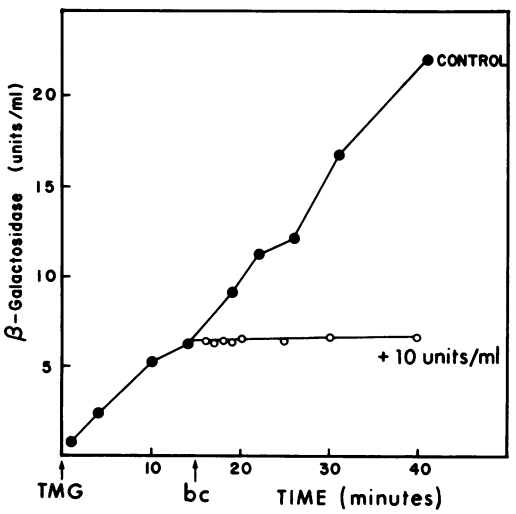

FIG. 3. *Effect of bacteriocin on synthesis of β-galactosidase. E. coli W3100 cells were grown in minimal medium supplemented with 0.1% Casamino Acids and 0.4% glycerol to a concentration of 2 × 10^8 cells/ml. Methyl thiogalactoside was added to a final concentration of 10^{-3} M, and the culture was incubated for 15 min at 37 C. The culture was divided into equal portions, and 10 units of bacteriocin per ml was added to one portion of the culture. At intervals, samples were removed and assayed for β-galactosidase. The bacteriocin treatment reduced the viable count to less than 0.01% within 5 min. The arrows indicate the time at which methylthiogalactoside (TMG) and bacteriocin (bc) were added.*

incorporated radioactivity was rendered acid-soluble, indicating that the labeled precursors were incorporated into protein and DNA, respectively. The inhibition of the incorporation of radioactive leucine into protein by bacteriocin may have been due to its effect on the active transport of leucine (*see below*). Therefore, the effect of bacteriocin on the synthesis of β-galactosidase in a wild-type *E. coli* strain was also measured. Figure 3 shows that the synthesis of β-galactosidase in cells induced with methyl thiogalactoside was inhibited by bacteriocin.

Effect on active transport. Figure 4 shows that the addition of either bacteriocin or cold leucine resulted in a rapid loss of the ^{3}H-L-leucine that accumulated in chloramphenicol-treated cells. However, bacteriocin had little effect on the active transport of ^{14}C-α-methylglucoside, because

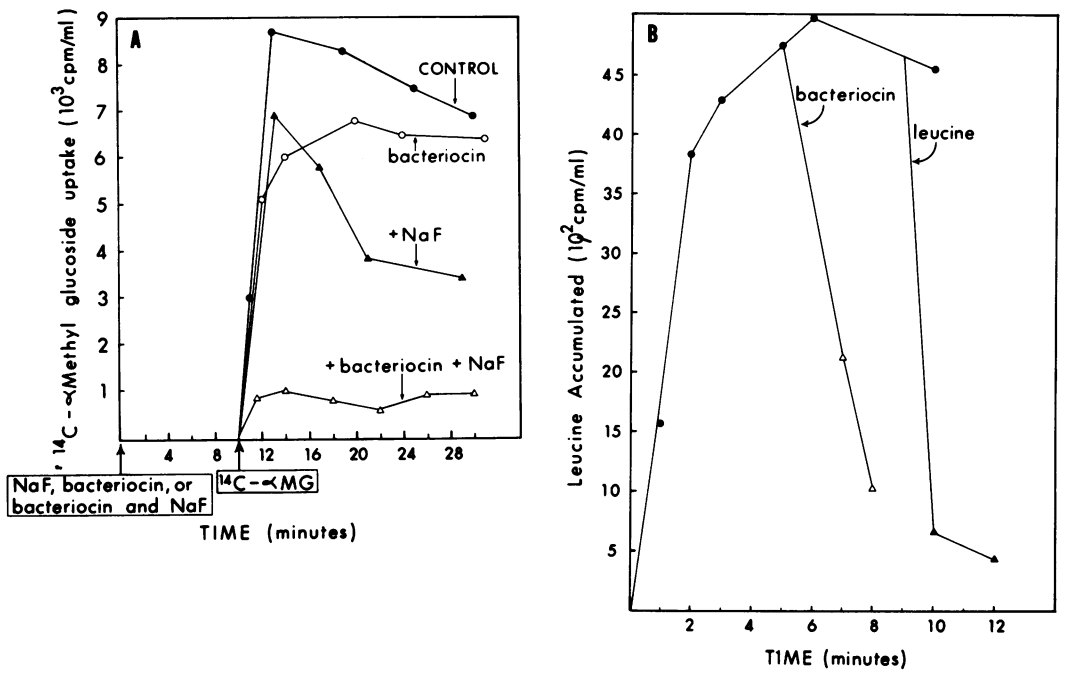

FIG. 4. *Effect of bacteriocin on the active transport of α-methylglucoside and leucine. A. A suspension of E. coli JF135 cells, grown in minimal medium and treated with chloramphenicol as described in Materials and Methods, was divided into four flasks. Bacteriocin (2 units/ml) was added to one flask, bacteriocin (2 units/ml) and NaF (0.05 M) were added to a second flask, NaF (0.05 M) was added to a third flask, and saline was added to the control flask. After a 10 min of incubation at 37 C, ^{14}C-α-methylglucoside (10^{-6} M, 50 Ci/mole) was added to all four flasks. Samples were removed at intervals, and the amount of radioactivity accumulated in the cells was determined. The arrow at time zero indicates the addition of bacteriocin, NaF, or bacteriocin and NaF. The arrow at 10 min designates the addition of ^{14}C-α-methylglucoside. The number of viable cells in the flask treated with bacteriocin alone was reduced to 0.05%. B. ^{3}H-L-leucine was added to chloramphenicol-treated cells as described in Materials and Methods. Bacteriocin (10 units/ml) was added to a sample of the culture 5 min after the addition of the ^{3}H-L-leucine. Unlabeled L-leucine was added to another sample at 9 min. At intervals, samples were removed and the radioactivity accumulated in the cells was determined.*

bacteriocin-treated cells accumulated nearly as much α-methylglucoside phosphate as did control cells. The addition of both bacteriocin and NaF was required to markedly inhibit the active transport of α-methylglucoside.

Effects on ATP levels. The firefly assay for the estimation of intracellular levels of ATP in bacteriocin-treated cells was extremely sensitive and gave a linear response over a 10-fold range of ATP concentrations (Fig. 5). In addition, the presence of a threefold molar excess of adenosine diphosphate did not interfere with this measurement.

The results obtained with bacteriocin-treated cells showed that the level of ATP was reduced to approximately 10 to 15% of that found in the untreated control cells within 10 min (Fig. 6). The total decrease in level of ATP was independent of the concentration of bacteriocin used, and the rate of decrease was not significantly more rapid in cultures treated with higher concentrations.

DISCUSSION

The killing of *E. coli* cells by bacteriocin JF246 followed single-hit kinetics, suggesting that a single active molecule of bacteriocin has a definite probability of killing a cell. Similar killing kinetics have been observed with a number of other bacteriocins (12, 20). The biochemical events which were observed in cells treated with bacteriocin JF246 were similar to those found in cells treated with colicins E1, K, or A. Like these three colicins, bacteriocin JF246 completely inhibited DNA and protein synthesis. It also abolished the active transport of leucine. However, it had little effect on the transport of α-methylglucoside, whose active transport is mediated by the phosphoenolpyruvate-dependent phosphotransferase system (15). This suggested that the bacteriocin may have had an effect on the energy (ATP?) supply required to transport

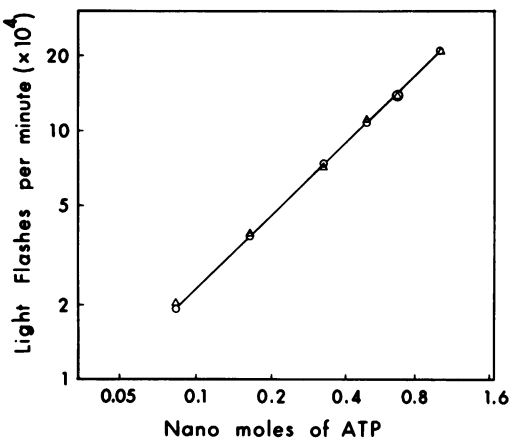

FIG. 5. *Effect of adenosine diphosphate (ADP) on the relationship between light flashes and the amount of adenosine triphosphate (ATP) in the firefly assay. Various amounts of ATP and ATP plus ADP were added to 2.4 ml of buffer (0.05 M $KAsO_4$, 0.02 M $MgSO_4$; 0.001 M ethylenediaminetetraacetic acid, pH 7.4) at 25 C. An 0.5-ml amount of firefly lantern extract were added, and the light flashes were counted in a scintillation counter. Symbols: ATP alone (○); ATP plus threefold molar excess of ADP (△).*

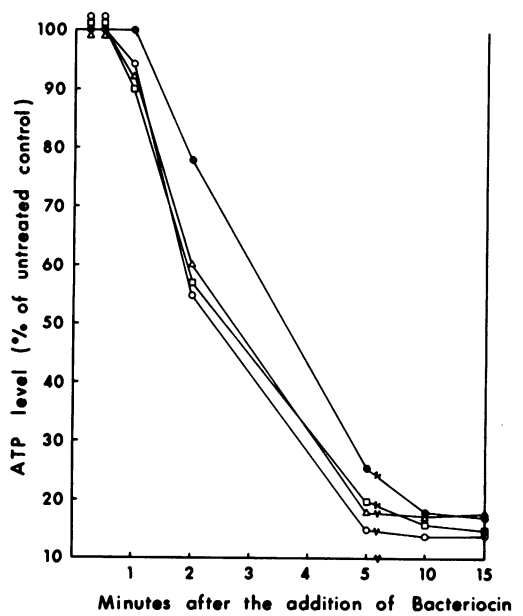

FIG. 6. *ATP levels in E. coli cells treated with various amounts of bacteriocin JF246. Various amounts of bacteriocin were added at time zero and samples were removed as indicated. The survivors at 20 min in the culture treated with 1 unit/ml were 2%. The control culture contained 770 picomoles of ATP and gave a viable count of 4.3 × 10^8 cells/ml. Symbols: 1 unit of bacteriocin/ml (●); 5 units of bacteriocin/ml (□); 10 units of bacteriocin/ml (△); 30 units of bacteriocin/ml (○).*

leucine. Further, since α-methylglucoside phosphate was concentrated in cells which had been treated with bacteriocin, it was not likely that the bacteriocin simply destroyed the cells' permeability barrier.

The cellular level of ATP was found to be rapidly reduced in bacteria treated with bacteriocin JF246. Since DNA and protein synthesis both require ATP, it was possible that the restricted supply of ATP was responsible for the inhibition of macromolecular synthesis by bacteriocin JF246. However, the amount of protein synthesis in bacteriocin-treated cells was dependent upon the amount of bacteriocin added, whereas both the rate and extent of reduction of ATP were not. Thus, β-galactosidase synthesis stopped within 30 sec after the addition of 10 units of bacteriocin per ml, whereas ATP levels dropped only 10% after 1 min. At 1 unit of bacteriocin per ml, protein synthesis continued at near the control rate for 10 min, whereas ATP levels were markedly reduced within 5 min. This indicated that low ATP levels per se were not the cause of the inhibition of protein synthesis by bacteriocin. The effects of colicin E1 on ATP levels and macromolecular synthesis have been separated by Feingold (3). He found that, in the presence of dicyclohexylcarbodiimide, an inhibitor of membrane bound adenosine triphosphatase in *Streptococcus faecalis*, the ATP levels did not fall in cells treated with colicin E1 under conditions where macromolecular synthesis was inhibited. This direct observation indicated that low ATP levels per se were not the cause of inhibition of protein or nucleic acid synthesis by colicin E1.

In a study of bacteriocin-resistant *E. coli* mutants, Hamon and Peron conclude that a trypsin-sensitive bacteriocin from *S. marcescens* Sm38 is related to colicin E1 (11). Other workers (17), again using evidence based on the bacteriocin sensitivity of colicin K-resistant *E. coli* mutants, find that bacteriocins synthesized by unnamed strains of *Serratia* are related to colicin K.

The nature of the primary event which inhibits protein and DNA synthesis in cells treated with bacteriocin JF246 is not yet clear. Any explanation must take into account the fact that only one or a few molecules of bacteriocin are required to kill a cell. It may be that adsorption of bacteriocin results in a conformational alteration of the cell membrane which results in the interruption of macromolecular synthesis. Thus, the adsorption of a single bacteriocin molecule could cause a change which could be propagated throughout the membrane. Changeaux and Thiery have proposed such a model (1). The recently reported studies on the fluorescence of 8-anilino-1-napthalenesulfonate bound to *E. coli*

treated with colicin E1 (2) have provided some experimental evidence that the adsorption of two killing units of this colicin increased both the hydrophobic environment of the 8-anilino-1-naphthalenesulfonate and the amount of 8-anilino-1-naphthalenesulfonate binding sites. Assuming that these binding sites were distributed over the cellular membrane, these results have provided some experimental support for Changeaux and Thiery's model.

ACKNOWLEDGMENTS

The skillful technical assistance of Constance Barrett is gratefully acknowledged.

This investigation was supported by Public Health Service grant AI-09055 from the National Institute of Allergy and Infectious Diseases.

LITERATURE CITED

1. Changeaux, J. P., and J. Thiery. 1967. On the mode of action of colicins: a model of regulation at the membrane level. J. Theoret. Biol. **17**:315-318.
2. Cramer, W. A., and S. K. Phillips. 1970. Response of an *Escherichia coli*-bound fluorescent probe to colicin E1. J. Bacteriol. **104**:819-825.
3. Feingold, D. 1971. The mechanism of colicin E1 action. J. Membrane Biol. **3**:372-386.
4. Fields, K. L., and S. E. Luria. 1969. Effects of colicins E1 and K on transport systems. J. Bacteriol. **97**:57-63.
5. Fields, K. L., and S. E. Luria. 1969. Effects of colicins E1 and K on cellular metabolism. J. Bacteriol. **97**:64-77.
6. Foulds, J., and D. Shemin. 1969. Properties and characteristics of a bacteriocin from *Serratia marcescens*. J. Bacteriol. **99**:655-660.
7. Foulds, J., and D. Shemin. 1969. Concomitant synthesis of bacteriocin and bacteriocin inactivator from *Serratia marcescens*. J. Bacteriol. **99**:661-666.
8. Fuller, A. T., and J. M. Horton. 1950. Marcescin, an antibiotic substance from *Serratia marcescens*. J. Gen. Microbiol. **4**:417-433.
9. Hamon, Y., and Y. Peron. 1961. Étude de la propriété bacteriocinogène dans le genre *Serratia*. Ann. Inst. Pasteur **100**:818-821.
10. Hamon, Y., and Y. Peron. 1965. Essai de classification de quelque marcescines. C. R. H. Acad. Sci. **260**:5401-5404.
11. Hamon, Y., and Y. Peron. 1966. Relations de quelques marcescines actives sur *E. coli* avec certains types de colicins. Ann. Inst. Pasteur **110**:556-561.
12. Hirata, H., S. Fukui, and S. Ishikawa. 1969. Initial events caused by colicin K infection-cation movement and depletion of ATP Pool. J. Biochem **65**:843-847.
13. Jacob, F., L. Siminovitch, and E. Wollman. 1952. Sur la biosynthèse d'une colicin et sur son mode d'action. Ann. Inst. Pasteur **83**:295-315.
14. Kaback, H. R. 1968. The role of the phosphoenolpyruvate-phosphotransferase system in the transport of sugars by isolated membrane preparations of *Escherichia coli*. J. Biol. Chem. **243**:3711-3728.
15. Kundig, W., S. Ghosh, and S. Roseman. 1964. Phosphate bound to histidine in a protein as an intermediate in a novel phospho-transferase protein. Proc. Nat. Acad. Sci. U.S.A. **52**:1067-1074.
16. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265-275.
17. Mandel, M., and F. A. Mohn. 1962. Colicins in *Serratia marcescens*. Microbial Genet. Bull. **18**:15.
18. Nagel de Zwaig, R. 1969. Mode of action of colicin A. J. Bacteriol. **99**:913-914.
19. Nomura, M. 1963. Mode of action of colicins. Cold Spring Harbor Symp. Quant. Biol. **28**:315-324.
20. Nomura, M. 1967. Colicins and related bacteriocins. Annu. Rev. Microbiol. **21**:257-284.
21. Nomura, M., and A. Maeda. 1965. Mechanism of action of colicins. Zentralbl. Bakteriol. Parasitenk. Abt. I Orig. **196**:216-239.
22. Pardee, A., F. Jacob, and J. Monod. 1959. The genetic control and cytoplasmic expression of inducibility in the synthesis of β-galactosidase by *E. coli*. J. Mol. Biol. **1**: 165-178.
23. Prinsloo, H. E. 1966. Bacteriocins and phages produced by *Serratia marcescens*. J. Gen. Microbiol. **45**:205-212.