Mode of Action of Two *Streptococcus faecium* Bacteriocins

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The mechanism of action of enterocins E1A and E1B, bacteriocins produced by *Streptococcus faecium* E1, was studied. The enterocins killed susceptible cells rapidly, but cell lysis does not appear to be involved directly. Susceptible cells could be rescued from the lethal damage by trypsin treatment only within 2 to 3 min after addition of enterocin E1A. Enterocins E1A and E1B inhibited protein synthesis and drastically reduced biosynthesis of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) but did not cause degradation of DNA or RNA. Enterocin E1A strongly inhibited the accumulation of isoleucine and caused rapid exit of previously accumulated isoleucine.

Two bacteriocins (enterocins E1A and E1B) are produced without induction by *Streptococcus faecium* E1. The purification and partial characterization of enterocins E1A and E1B were described in earlier reports (12; J. Krämer, habilitation paper, University of Bonn, Bonn, West Germany; 1972; J. Krämer and H. Brandis, J. Gen. Microbiol., in press). Enterocin E1A, a basic protein with a molecular weight of approximately 10,000, is the predominant species in the supernatant fluid; enterocin E1B, with particle weight exceeding 10^4, represents more than 90% of the total activity in the cell-free extract. Despite this high molecular weight, no distinct morphological features of the enterocin E1B could be detected. These enterocins have been shown to be active only against certain strains of enterococci, *Streptococcus salivarius* and *Listeria monocytogenes*. The present investigation describes the biochemical events associated with the inhibition of *S. faecium* cells by enterocins E1A and E1B.

MATERIALS AND METHODS

Cultures. The enterocin-producing strain was *S. faecium* E1. *S. faecium* 158 was used as the enterocin-susceptible strain. Both strains were isolated from human urine. The organisms were maintained in tryptose phosphate broth (Difco) and grown in tryptose phosphate broth or in a chemically defined medium (15).

Preparation of enterocins. The production, purification, and assay of enterocins E1A and E1B have been described previously (Krämer, habilitation paper). Enterocin E1A was isolated from the supernatant fluid and purified 400-fold; enterocin E1B was isolated from the cell-free extract and purified 100-fold.

Incorporation of radioactive substrates. Deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and protein synthesis in enterocin-sensitive bacteria were measured by the incorporation of radioactive precursors, namely, [3H]thymidine, [3H]uracil, and [3H]isoleucine, into trichloroacetic acid-precipitable material. Samples (0.5 ml) of the cell suspension were added to 0.5 ml of 10% trichloroacetic acid containing 50 μg of the unlabeled substrate per ml. After 30 min at 10 C, the acid-precipitated material was collected on membrane filters (0.22-μm pore size; Millipore Corp.) and washed twice with 2 ml of cold 5% trichloroacetic acid solution supplemented with 50 μg of the unlabeled substrate per ml. The filters were dried at 60 C, and the radioactivity was determined by liquid scintillation counting.

RESULTS

Killing of *S. faecium* cells. When enterocin E1A or E1B was added to a culture of *S. faecium* 158, a marked reduction in the number of colony-forming units was noted in a short period of time, whereas no change in the number of viable enterococci was found in the control without enterocin over the same 1-h period of incubation (Fig. 1). However, comparison of the optical densities of the cell suspensions showed no appreciable difference between the test and the control specimen, thus suggesting that no significant lysis of enterococci occurred despite the marked reduction in colony-forming units caused by both enterocins.

Effect of enterocins E1A and E1B on protein, RNA, and DNA synthesis. The incorporation of radioactive precursors into acid-precipitable material was taken as a measure of synthesis. Treatment of *S. faecium* 158 cells with enterocins resulted in a total cessation of protein synthesis after a lag time of 8 min (Fig. 2A). The influence of enterocins on RNA and DNA synthesis is recorded in Fig. 2B and C. Immediately after addition of enterocins, incor-
grown in flasks. Samples in phosphate buffer (1 ml) (control).

The content of RNA, DNA, and other substances was demonstrated for several bacteriocins (2, 14, 17). The accumulation and efflux of isoleucine was selected as a parameter for investigating possible alterations in the membrane system of S. faecium 158 after addition of enterocins. A marked reduction of [14C]isoleucine accumulation by cells of S. faecium 158 was noticed with the addition of enterocins ElA and E1B.

**Effect of enterocins ElA and E1B on substrate accumulation.** Effects on permeability to amino acids, ions, and other substances were demonstrated for several bacteriocins (2, 14, 17). The accumulation and efflux of isoleucine was selected as a parameter for investigating possible alterations in the membrane system of S. faecium 158 after addition of enterocins. A marked reduction of [14C]isoleucine accumulation by cells of S. faecium 158 was noticed with the addition of enterocins ElA and E1B.

**Fig. 1. Bactericidal effect of enterocins ElA and E1B.** S. faecium 158 was grown in tryptose phosphate broth to the early exponential growth phase. Cells were centrifuged, washed, and resuspended in 0.05 M phosphate buffer (pH 7.2). A 10-ml amount of the suspension was treated with enterocin ElA (1 ml; titer 1:2,000), enterocin E1B (1 ml; titer 1:5,000), or with phosphate buffer (1 ml) (control). Samples were taken at intervals and the number of colony-forming units and the absorbance at 550 nm were determined.

**Fig. 2. Inhibition of macromolecular biosynthesis by enterocins ElA and E1B.** S. faecium strain 158 was grown in chemically defined medium with 5 μg of isoleucine, uracil, or thymidine per ml to early log phase. The culture was divided into two portions, one of which received enterocin ElA (●) and E1B (△), respectively, at a concentration that gave 0.1% survivors. Control cells were not treated with enterocins (○). After 3 min, (A) [14C]isoleucine (1 μCi/ml), (B) [14C]uracil (10 μCi/ml), or (C) [3H]thymidine (50 μCi/ml) was added to both flasks. Samples were removed at intervals and cold trichloroacetic acid was added for determination of insoluble radioactivity.
Enterocin ElA (Fig. 3A), whereas enterocin ElB had no effect on the uptake of isoleucine. The inhibitory effect of enterocin ElA could be demonstrated also by adding this enterocin to bacteria that had previously accumulated substrate; enterocin ElA caused a rapid release of isoleucine from the cells (Fig. 3B). A similar effect was observed by the addition of unlabeled isoleucine.

**Trypsin rescue of enterocin-treated cells.** Enterocin ElA has been shown to be sensitive to the action of trypsin (Krämer, habilitation paper, University of Bonn, Bonn, West Germany, 1972; J. Krämer and H. Brandis, J. Gen. Microbiol., in press). Therefore, the possibility of rescuing cells by trypsin treatment after addition of enterocin ElA was investigated. Exponentially growing *S. faecium* 158 cells were treated with enterocin ElA and then incubated with trypsin. A rapid killing of the cells occurred after the addition of enterocin ElA.

Enterocin-treated cells subjected to the action of trypsin could be rescued within 4 min after the addition of enterocin (Fig. 4). At times later than 4 min, enterocin ElA induced a irreversible lethal response in the actively growing cells.

This rescue of enterocin-treated cells was not due to the digestion of a trypsin-susceptible enterocin binding site, as demonstrated by the addition of enterocin ElA to exponentially growing *S. faecium* 158 cells pretreated for 20 min at 37°C with 300 μg of trypsin per ml. Trypsin-pretreated cells were as susceptible as untreated cells to the action of enterocin ElA.

**DISCUSSION**

Enterocins ElA and ElB have been shown to be active only against certain strains of enterococci and *L. monocytogenes*, with no effect on gram-negative bacteria (Krämer, habilitation paper, University of Bonn, Bonn, West Germany, 1972; Krämer and Brandis, J. Gen. Microbiol., in press). Rapid decrease in the number of colony-forming units occurred upon the addition of enterocin ElA or ElB to susceptible cells. Despite the rapid killing of the bacteria, no reduction in optical density of the susceptible cell suspension occurred, thereby...
suggesting that enterocins E1A and E1B had no significant bacteriolytic activity. These data indicated clearly that enterocins E1A and E1B are distinct from other bacteriocin-like substances from enterococci with bacteriolytic and hemolytic action (2, 3, 5, 10).

Studies on the mode of action of various colicins have revealed that certain colicins stay at the receptor site and act from there (9, 19). Support for this hypothesis came from the observation that it is possible to rescue susceptible cells which have already absorbed colicins by the addition of trypsin. Although the activity of some bacteriocins can be rescued by trypsin treatment for a long period (4, 6, 18, 20), enterocin-treated cells were rescued only within a few minutes after the addition of enterocin E1A, resembling different colicins (21, 22) and other bacteriocins (7, 11, 16). This is of interest since the free enterocin E1A is inactivated by trypsin. This might suggest that the enterocin E1A is rapidly taken up by susceptible cells or is no longer susceptible to trypsin bound to the outside of the cell.

The biochemical events which were observed in cells treated with enterocin were similar to those found in cells treated with colicins El, K, A, Ia, and Ib (13, 17, 19) and with bacteriocins from *Pseudomonas aeruginosa* (12) and *Serratia marcescens* (8). Like these bacteriocins, enterocins inhibited DNA, RNA, and protein synthesis, and enterocin E1A was found to inhibit the accumulation of isoleucine and to induce the efflux of already accumulated isoleucine. These effects on biochemical processes may indicate an inhibition of energy supply or an alteration of the membrane permeability. The present studies showed that the cessation of DNA or RNA synthesis in cells treated with enterocin was not correlated with a degradation of nucleic acid. However, preliminary studies suggest a correlation with structural changes consisting of extensive condensation of nuclear material.

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**LITERATURE CITED**


