GROUP A STREPTOCOCCAL BACTERIOCIN

PRODUCTION, PURIFICATION, AND MODE OF ACTION*

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The bacteriocins are an intriguing potpourri of bactericidal antibiotics, having a wide range of different genetic determinants, physicochemical properties, and killing mechanisms (1). Bacteriocinogeny seems an almost universal property of bacteria and various strains of most species have now been shown to produce bacteriocins. Although there have been several reports of bacteriocin-like inhibition produced by Group A streptococci on solid media (2–4), progress in this field has been hampered by the inability to demonstrate the active bacteriocin in broth cultures.

A streptococcal bacteriocin was recently obtained in cell-free form following "freeze-thaw" extraction of the liquor from cultures grown on Oxoid Todd-Hewitt agar (4, 5). The producer organism was a Group A beta-hemolytic streptococcus (strain FF-22) and the bacteriocin was correspondingly named streptocin A. The present communication is concerned with the isolation and purification of streptocin A from broth cultures of this same producer strain and an investigation of the mode of action of the bacteriocin against a susceptible Group A streptococcus. Several properties of the bacteriocin are compared with those of a bacteriocin produced by a strain of phage type 71 *Staphylococcus aureus*.

Materials and Methods

Bacterial Strains.—Group A streptococcus strain FF-22 (5) and *S. aureus* strain C55 (6) were the organisms used for the production of the streptococcal and staphylococcal bacteriocins. Various other strains were utilized as indicators in studies of the specificity of the range of bactericidal activity of these bacteriocins. Group A streptococcus strain PF 1643 (6) is an indicator sensitive to both streptocin A and staphylococcin. *S. aureus* strain 502A (6)

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is susceptible to staphylococcin, but not to streptocin A. *Escherichia coli* B is an organism resistant to both bacteriocins. When in regular use the bacteria were subcultured weekly on Todd-Hewitt agar and stored at 4°C. Stock cultures were maintained at −70°C or as lyophilized preparations.

Media and Chemicals.—Liquid media tested as substrates for the production of bacteriocin were Todd-Hewitt broth (Oxo Ltd., London), Trypticase soy broth (Baltimore Biological Laboratories, Baltimore, Md.), and also tryptic soy broth, brain-heart infusion, tryptose phosphate broth, and Todd-Hewitt broth from Difco Laboratories, Detroit, Mich. Unless stated otherwise, Difco rather than Oxoid Todd-Hewitt broth was used in the present studies. A dialysate of tryptic soy broth was prepared as previously described (7).

Blue dextran 2000 and Sephadex G-25 and G-100 were from Pharmacia Fine Chemicals, Inc., Piscataway, N. J. Reference proteins used to calibrate the Sephadex G-100 column were obtained from Sigma Chemical Co., St. Louis, Mo. (type III cytochrome *c*; glucagon) and from Miles Laboratories, Inc., Kankakee, Ill. (horse skeletal muscle myoglobin). Purified casein was from Difco Laboratories. [*Methyl*-^{3}H]thymidine, [5-^{3}H]uridine, L-[^{3}H]alanine, and D-[^{14}C]-glucose were purchased from New England Nuclear, Boston, Mass.

Purification of Bacteriocins.—In a typical procedure for the production and purification of streptocin A, the cells from a 500-ml overnight tryptic soy broth culture of the producer strain were collected by centrifugation, resuspended in 100 ml of fresh medium, and then reinoculated into 10 liters of sterile tryptic soy broth. This culture was incubated at 35°C without agitation for 24–36 h, then the supernatant fluid was harvested by filtration through Filter Cel (Hyflo Filter Cel, Johns-Manville, New York). After boiling the liquor for 30 min and then cooling to room temperature, the pH was adjusted to 6.5 with NaOH, and solid ammonium sulfate was added slowly to achieve 80% saturation. Stirring was continued for 6 h at 4°C before collecting the precipitate on Filter Cel and then eluting it into a minimum volume of distilled water. This preparation was desalted and fractionated by gel filtration on Sephadex G-25 to give preparations of "crude streptocin A." Further purification of the bacteriocin was by ion exchange chromatography on carboxymethyl cellulose (5). Subsequently, dialysis and concentration of the material in a Diaflo ultrafilter by use of a UM-2 membrane (Amicon Corp., Lexington, Mass.) gave the final product referred to here as "purified streptocin A."

Crude staphylococcin was obtained by the method described elsewhere (7). Sterilization of the staphylococcin or streptocin A preparations was achieved either by boiling for 30 min or by filtration through a 0.45-μm pore size filter (Millipore Corp., Bedford, Mass.). Storage was at 4°C.

Demonstration of Inhibitory Activity.—The titer of inhibitory activity of both the streptococcal and staphylococcal bacteriocins was determined by use of a standard surface-spotting dilution test on lawn cultures of the susceptible indicator streptococcus (strain PF 1643) (5). The highest twofold dilution of the test preparation to give definite inhibition of the indicator lawn was defined as containing 1 arbitrary unit (A.U.)[1] of bacteriocin per milliliter. Specific activities were expressed in terms of A.U. per milligram protein, after quantitation of the amount of protein in the samples by the method of Lowry et al. (8).

The bactericidal activity of the bacteriocins was determined by measurement of the decrease with time in the number of colony-forming units (CFU) of a suspension of the sensitive indicator streptococcus after mixing the cells with a bacteriocin preparation. Twice-washed cells from an exponential Todd-Hewitt broth culture of Group A streptococcus strain PF 1643 were resuspended in 0.067 M phosphate buffer (pH 6.5) to the original culture volume. Portions of the bacteriocin or of phosphate buffer (control) were then mixed with an equal volume of the washed cell suspension and incubation was continued at the appropriate temperature. OD readings of the control and test cell suspensions were made at 600 nm in a Coleman Junior

[1] *Abbreviations used in this paper*; A.U., arbitrary units of bacteriocin; CFU, colony-forming units; V_E, elution volume; V_O, void volume.

Spectrophotometer (Coleman Instruments Div.; Perkin Elmer Corp., Maywood, Ill.) using blanks of phosphate buffer and of a 1:1 mixture of bacteriocin:buffer, respectively. Survivors were determined at intervals by plating suitable 10-fold dilutions (in cold Todd-Hewitt broth) of the test and control mixtures on Todd-Hewitt agar and incubating at 35°C for 18 h. Viable counts were expressed as the total number of CFU per milliliter.

Streptococcal Proteinase Assay.—The assay utilized was essentially as described by Liu et al. (9) and is based upon the digestion of casein by the activated proteinase. One unit of proteinase activity corresponded to the solubilization of 0.1 absorbancy unit at 280 nm in a 30-min assay.

Molecular Weight Estimation.—A 2.5 × 90-cm column of Sephadex G-100 was equilibrated with sodium phosphate buffer (0.02 M; pH 7.0) at 4°C. All samples were applied in 20% (wt/vol) sucrose. Blue dextran (0.2% wt/vol) was used to estimate the void volume (V_O) and myoglobin, cytochrome *c*, and glucagon were the reference proteins used to construct the standard curve. Eluted fractions were collected and assayed for absorbance at 280 nm and for streptocin A activity. The elution volume (V_E) of the standards and of the bacteriocin were determined, and from the standard curve of V_E/V_O against the logarithm of the molecular weight, the molecular weight of streptocin A could be estimated.

Incorporation of Radioactive Materials.—Protein, DNA and RNA synthesis, and the formation of acid-insoluble glucose products were determined by measurement of the incorporation of the radioactive precursors [^{3}H]alanine, [^{3}H]thymidine, [^{3}H]uridine,and [^{14}C]glucose into trichloroacetic acid (TCA)-precipitable material. An exponentially growing culture of the test strain in Todd-Hewitt broth was added in 3.5-ml volumes to paired tubes, each containing 0.1 ml of the labeled precursor (final activity 3.0 μCi/ml). At 5 and 20 min, 0.3-ml samples were removed and added to 3.0 ml of cold 5% TCA. At 30 min, each pair of tubes received 3.0 ml of either the bacteriocin or 0.067 M phosphate buffer (pH 6.5). Samples of 0.6 ml were taken at intervals into cold TCA. Each time, before withdrawing a sample, the cultures were mixed briefly. The material precipitated by TCA after 30 min was collected on 0.45-μm Millipore filters, washed twice with 5-ml aliquots of ice-cold 5% TCA, and then once with 2 ml of 95% ethanol. Finally, the filters were dried before counting.

Incorporation of [^{14}C]glucose into glycogen-like material was measured essentially according to the method of Abraham and Hassid (10). Samples were withdrawn from the test and control mixtures, placed in 2.0 ml of KOH and then boiled for 20 min. After adding one drop of 10 M LiCl and cooling to 4°C, glycogen-like polymer material was precipitated by addition of 4.0 ml of cold 95% ethanol. The precipitate was collected by filtration, washed twice with 5-ml volumes of a 1:2 mixture of cold buffer:ethanol, dried, and counted. Total uptake of labeled glucose was determined by chilling samples of the test and control preparations in 3.0 ml of cold phosphate buffer (pH 7.1), collecting the cells on membrane filters, washing twice in 5-ml aliquots of cold buffer, drying and then counting. All samples in these experiments were counted in a Beckman (Beckman Instruments, Inc., Electron Instruments Div, Shiller Park, Ill.) LS-150 liquid scintillation counter using a toluene-based fluor.

RESULTS

Recovery of Streptocin A from Broth Cultures.—Attempts were made to recover active bacteriocin from the supernatant fluid of cultures of Group A streptococcus strain FF-22 grown in various liquid media, under different physical conditions. Parameters varied were temperature (22°, 30°, 35°, 39°C), time (4, 8, 12, 24, 36, 48, 60, 72 h), and the degree of mixing (gently shaken or still cultures).

Active bacteriocin could be recovered from cultures in tryptic soy broth, a dialysate of tryptic soy broth and Difco Todd-Hewitt broth, but not from Oxoid Todd-Hewitt broth, Trypticase soy broth, tryptose phosphate broth, or

brain-heart infusion. The yield of streptocin A from tryptic soy broth cultures (inhibitory titer of up to 4 A.U./ml) was considerably greater than that obtained from cultures in tryptic soy broth-dialysate or Difco Todd-Hewitt broth (titer not greater than 1 A.U./ml). Active bacteriocin could not be recovered from shaken cultures. Table I shows the inhibitory titer of streptocin A in samples withdrawn at intervals from tryptic soy broth cultures of the producer strain incubated at various temperatures. Greatest recovery was from cultures incubated at 35°C for 24–48 h. No activity could be detected until at least 12 h incubation and the yield decreased beyond 48 h. There did not appear to be any production of active streptocin A during growth at 39°C.

Factors Influencing the Yield of Streptocin A.—Significant fluctuations in the yield of streptocin A have sometimes been observed to occur in different tryptic

TABLE I

Titer of Streptocin A in Tryptic Soy Broth Cultures*

Incubation period	Temperature (°C) of incubation			
	22	30	35	39
h				
4	0	0	0	0
8	0	0	0	0
12	0	1	2	0
24	1	1	2	0
36	2	2	4	0
48	2	2	4	0
60	2	2	2	0
72	2	1	2	0

* Measured in arbitrary units of streptocin A per milliliter.

soy broth cultures, apparently incubated under identical conditions. These findings emphasize the possible role of ill-defined factors in the in vitro production of the bacteriocin. The failure to recover active streptocin A from producer strain cultures in certain types of liquid media may be interpreted as being due either to specific deficiencies in the growth medium or to the concomitant synthesis of streptocin A-inactivating substances (such as streptococcal proteinase) in these media.

Proteinase activity was measured in culture supernatants obtained from samples taken during the growth (at 35°C) of the streptocin A producer strain in different liquid media (Table II). Media failing to support the production of active bacteriocin, such as Oxoid Todd-Hewitt broth and brain-heart infusion, had the highest levels of proteinase activity in 24- or 48-h cultures. Addition of 2% (wt/vol) neopeptone to Oxoid Todd-Hewitt broth suppressed proteinase production and also facilitated the recovery of detectable bacteriocin.

Supporting, albeit indirect, evidence for a bacteriocin-inactivating role of

streptococcal proteinase was accrued from the observation that, although the production of active bacteriocin appeared significantly suppressed by repeated (10-fold) subculture of the producer strain on Oxoid Todd-Hewitt agar, production could be restored to former levels by passing the strain through mice, a procedure previously shown to result in decreased proteinase formation by Group A streptococci (11).

Purification of Streptocin A.—Since crude tryptic soy broth producer strain cultures rarely contained greater than 4 A.U. of streptocin A per milliliter, purification of the bacteriocin involved the processing of large volumes of broth culture. Boiling the crude filtrate for 30 min achieved the dual function of sterilizing the fluid and of inactivating heat-labile streptococcal enzymes. Fractionation of crude filtrates with ammonium sulfate indicated that the greatest specific activity of bacteriocin was obtained in the range of 60–80% saturation. However, since significant quantities were also recovered in the range 30–60%, routine processing involved the collection of all of the material precipitated by addition of ammonium sulfate to 80% saturation. Results of the purification of streptocin A are summarized in Table III. Although a 139-

TABLE II

Relationship between Proteinase and Streptocin A Activity in Broth Cultures

Growth medium	Units of proteinase activity*		Streptocin A titer‡	
	24 h	48 h	24 h	48 h
Tryptic soy broth	2.1	3.2	2	4
Todd-Hewitt broth (Difco)	2.9	3.5	1	1
Todd-Hewitt broth (Oxoid)	7.6	10.4	0	0
Todd-Hewitt broth (Oxoid) + 2% neopeptone	3.4	4.6	0	1
Brain-heart infusion	6.7	7.6	0	0

* Per milliliter of culture supernatant.

‡ In arbitrary units per milliliter of culture supernatant.

TABLE III

Purification of Streptocin A

	Volume	Bacteriocin/ml	Protein	Specific activity	Recovery	Times purified
	ml	*A.U.*	*mg*	*A.U./mg*	%	
Crude filtrate	10,000	20,000	91,000	0.22	100	1
Ammonium sulfate precipitate 0–80%	200	6,400	16,600	0.39	32	1.8
Sephadex G-25 chromatography (crude streptocin A)	1,010	4,040	8,680	0.47	20	2.1
Carboxymethyl cellulose chromatography	126	2,016	76	26.53	10	120
Diaflo concentrate (purified streptocin A)	22	1,408	46	30.61	7	139

fold purification was achieved, there appeared to be considerable loss of total activity during purification, with only 7% of the original amount recovered.

Physical Properties of Streptocin A.—Investigations of the elution characteristics of streptocin A on a calibrated column of Sephadex G-100 (Fig. 1) gave an apparent molecular weight of approximately 8,000. The bacteriocin was eluted from the column as a single peak of activity, indicating that the streptocin A in the preparation was probably homogeneous with respect to molecular size.

Other properties investigated were the sensitivity to temperature and to change in pH and the susceptibility of the bacteriocin to the action of proteolytic enzymes. Streptocin A was stable to heating, with no detectable loss of activity on boiling for 60 min. Although the bacteriocin was stable to pH adjustment

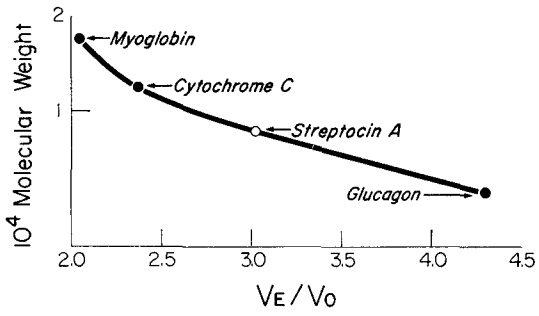

FIG. 1. Estimation of the molecular weight of streptocin A, using a calibrated column of Sephadex G-100 as outlined in the text. The migration of marker proteins and streptocin A is expressed relative to the migration of Blue dextran (V_E/V_O). A molecular weight of 8,000 can be extrapolated from the relative migration of streptocin A.

within the range of 2–7, it was inactivated by alkali, with no detectable activity after adjustment to a pH of 11 or higher. Treatment with 1 mg/ml of either trypsin or pronase inactivated the bacteriocin during incubation for 60 min at 37°C. In all of these properties the bacteriocin purified from tryptic soy broth cultures appeared similar to the product obtained in previous investigations from Oxoid Todd-Hewitt agar cultures of the producer strain (5).

Bactericidal Action of Streptocin A.—Preliminary experiments demonstrated that the lethal effect of streptocin A, as demonstrated by loss of colony-forming ability, was more pronounced against cells in the exponential phase of growth than those in stationary phase. The lethal action of streptocin A at different concentrations was investigated to determine an appropriate level of activity for use in subsequent studies. It was found that preparations of crude streptocin A of titer 16 were lethal for over 99% of the CFU of exponential Todd-Hewitt broth cultures of Group A streptococcus strain PF 1643 in 4 h at 37°C. All streptocin A preparations utilized in subsequently described experiments had a titer of 16 A.U./ml.

Investigation of the effect of the bacteriocin on the optical density of suspensions of the susceptible indicator strain at 37°C indicated that, in spite of a considerable decrease in the number of CFU, there was no apparent change in the optical density of the preparations and hence, that the lethal effect was not accompanied by cell lysis (Fig. 2). It can also be seen that the temperature of incubation had a marked effect on the bactericidal action, with less killing at lower temperatures.

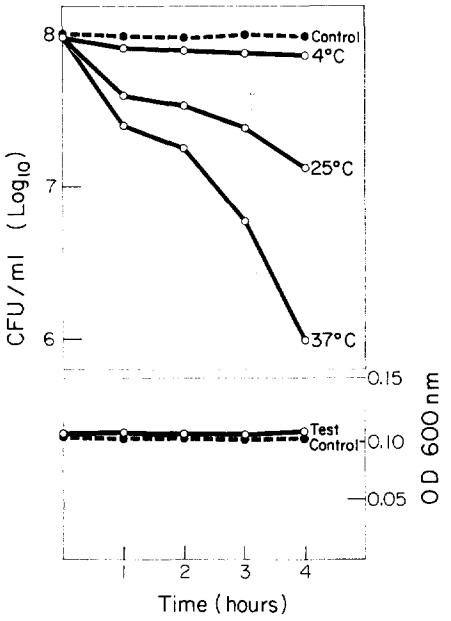

FIG. 2. The effect of crude streptocin A (O---O) or 0.067 M phosphate buffer (●---●) on the optical density (OD 600 nm) at 37°C and viability (CFU/milliliters) of a suspension of cells of Group A streptococcus strain PF 1643. The bactericidal effect of streptocin A was determined at 37°, 25°, and 4°C.

Streptocin A had no significant bactericidal effect against cultures of *S. aureus* strains C 55 and 502A or against *E. coli* B. The bacteriocin producer (strain FF-22) showed some sensitivity to streptocin A preparations of titer greater than 16, indicating incomplete immunity to the homologous bacteriocin.

Effect of Streptocin A on Macromolecule Synthesis.—Since other bacteriocins have been shown to inactivate particular biochemical targets in susceptible bacteria, efforts were made to determine the effect of streptocin A on various macromolecular syntheses in a sensitive indicator streptococcus.

Treatment of cells of Group A streptococcus strain PF 1643 with crude or purified preparations of streptocin A resulted in rapid shutdown of the biosynthesis of protein and DNA, as demonstrated by the inhibition of the in-

corporation of the appropriate radioactive precursors into acid-insoluble material. As can be seen in Figs. 3 and 4, both protein and DNA synthesis appeared to be stopped shortly after the addition of streptocin A while continuing steadily in control preparations. Streptocin A was inactivated by adjustment to pH 11 for 24 h and then readjustment to pH 6.5 (4). This preparation was non-inhibitory when assayed on a lawn culture of strain PF 1643 and moreover, it was shown to have only a minimal effect on the uptake of acid-insoluble [^{3}H] thymidine by this strain (Fig. 5).

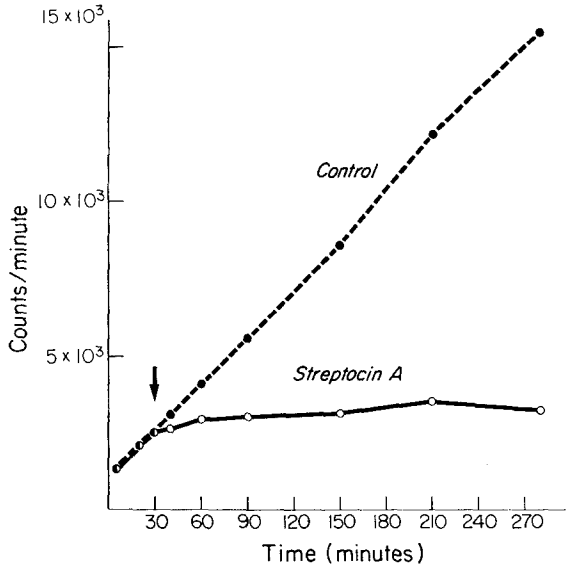

FIG. 3. The uptake of L-[^{3}H]alanine by Group A streptococcus strain PF 1643 and the effect of addition (↓) of purified streptocin A (O---O) or 0.067 M phosphate buffer (●---●) on incorporation.

Investigation of the effect of the bacteriocin on RNA synthesis (Fig. 6) showed a significant decrease in the amount of acid-precipitable labeled RNA after treatment of the sensitive indicator with streptocin A. This finding indicated RNA breakdown in the treated cells. In other experiments similar RNA degradation was detected after treatment of the bacteria with streptocin A in the presence of 300 μg/ml of chloramphenicol, a concentration shown to be sufficient to stop protein synthesis. This indicated that the effect of streptocin A on RNA synthesis was independent of protein synthesis by the cells.

The effect of streptocin A on RNA synthesis in strain PF 1643 was compared with that of a staphylococcal bacteriocin which has previously been shown to bring about the degradation of RNA in a susceptible Group A streptococcus (12). Both bacteriocin preparations used in this experiment had inhibitory

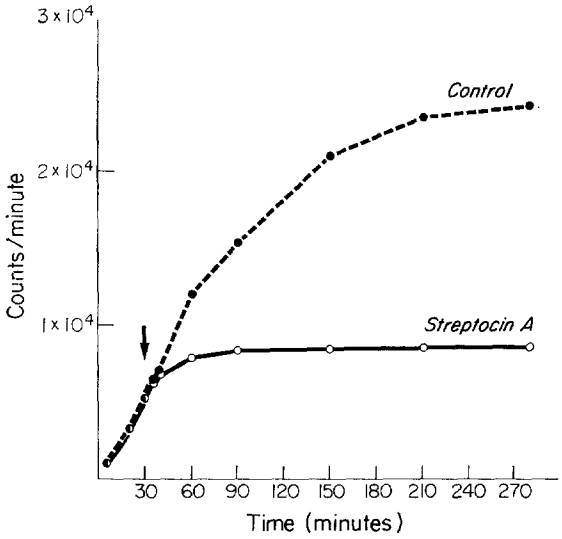

FIG. 4. The incorporation of [*methyl*-^{3}H]thymidine by Group A streptococcus strain PF 1643 showing the effect of the addition (↓) of purified streptocin A (○---○) or 0.067 M phosphate buffer (●---●).

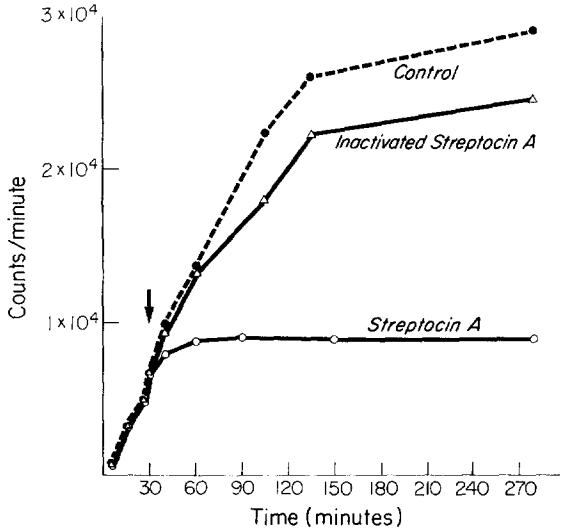

FIG. 5. Effect on the uptake of [*methyl*-^{3}H]thymidine by Group A streptococcus strain PF 1643 of the addition (↓) of purified streptocin A (○---○), the "inactivated" bacteriocin (▽---▽) or 0.067 M phosphate buffer (●---●).

titers of 16 when assayed on a lawn culture of the indicator strain. In spite of this, the staphylococcal bacteriocin had a significantly more potent bactericidal effect against suspensions of the indicator cells, no viable CFU being detected after 3 h exposure. Moreover, as can be seen in Fig. 7 the rate of RNA degrada-

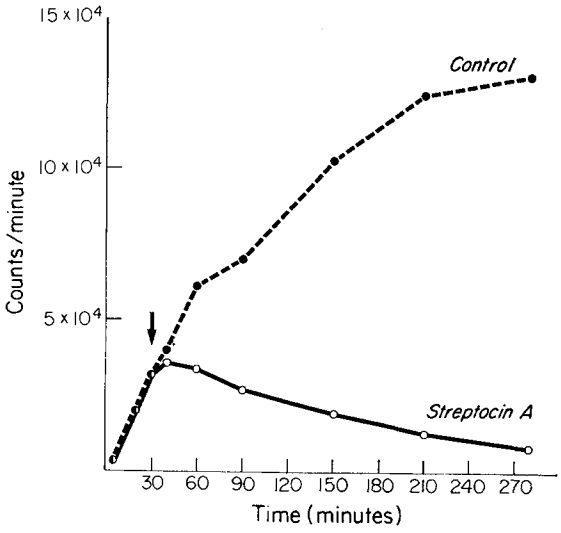

FIG. 6. The effect of the addition (↓) of purified streptocin A (○—○) or 0.067 M phosphate buffer (●—●) on the incorporation of [5-^{3}H]uridine by Group A streptococcus strain PF 1643.

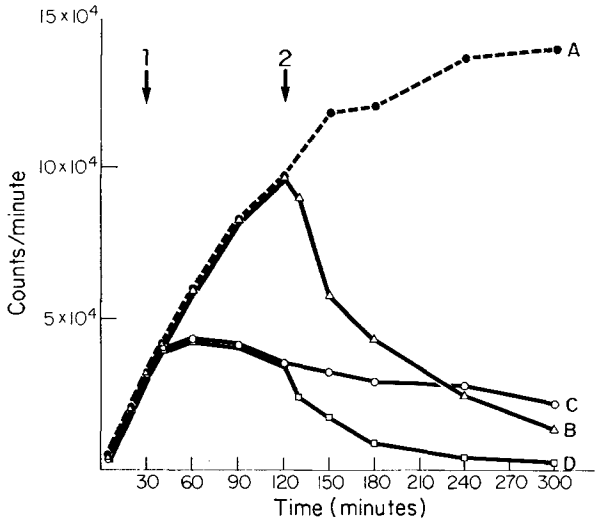

FIG. 7. Comparison of the effect of crude streptocin A, staphylococcin, or 0.067 M phosphate buffer on RNA synthesis by Group A streptococcus strain PF 1643, when exposed 30 min (arrow 1) or 120 min (arrow 2) after the addition of [5-^{3}H]uridine. The preparations added in each experiment were: curve A, 0.067 M phosphate buffer (30 min) and 0.067 M phosphate buffer (120 min); curve B, 0.067 M phosphate buffer (1) and staphylococcin (2); curve C, streptocin A (1) and 0.067 M phosphate buffer (2); curve D, streptocin A (1) and staphylococcin (2).

tion was considerably greater after treatment of the indicator cells with the staphylococcal bacteriocin.

Streptocin A was also found to prevent the incorporation of [^{14}C]glucose into TCA-insoluble material (Fig. 8). Furthermore, total [^{14}C]glucose uptake and also its incorporation into glycogen-like polymers were rapidly shut off after addition of the bacteriocin.

DISCUSSION

Our previous studies of bacteriocin production by Group A streptococci failed to demonstrate the recovery of active material from broth cultures (5). It has been repeatedly demonstrated that the production of bacteriocins may be influenced considerably by the composition of the growth medium and conditions of incubation (1, 5, 13). Strains producing bacteriocin on solid media may show little or no activity in broth cultures (14–16). Moreover, it has been shown that some bacteriocinogenic organisms may also produce substances which are inhibitory to the homologous bacteriocin. Guterman and Luria (17) described strains of *E. coli* excreting a neutralizing lipopolysaccharide material that decreased the apparent yield of colicin.

Other studies have indicated that an extracellular protease of *Serratia marcescens* may reduce the amount of active bacteriocin recoverable from cultures of these organisms (18). Similarly, it seems that past failures to recover streptococcal bacteriocins from broth cultures may have been due to the inactivation of

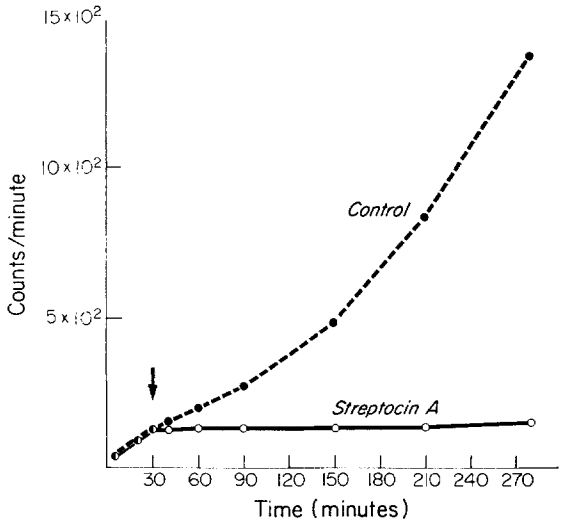

FIG. 8. The incorporation of D-[^{14}C]glucose into acid-insoluble material by Group A streptococcus strain PF 1643 and the effect of the addition (↓) of crude streptocin A (O---O) or 0.067 M phosphate buffer (●---●).

the bacteriocin by streptococcal proteinase. In the present study several different liquid media were tested as substrates for the production of streptocin A. Greatest yields were obtained from tryptic soy broth cultures. The recovery of the bacteriocin from Difco Todd-Hewitt broth, but not from Oxoid Todd-Hewitt broth, which had been utilized in our previous studies (4, 5), may be due to the presence of 2% neopeptone in the former medium only. Neopeptone has been shown to be an inhibitor of streptococcal proteinase (11). Additional indirect evidence supporting the possible role of streptococcal proteinase in the inactivation of streptocin A was the apparent enhancement of bacteriocin production after mouse passage. Furthermore, our earlier studies (5) indicated that streptocin A production was greater in serum-containing and thus, proteinase-inactivating (11) media.

From our present data, the optimal conditions for streptocin A production by strain FF-22 appear to be incubation in tryptic soy broth for 24–48 h at 35°C. The value of tryptic soy broth as a substrate for the production of bacteriocins by staphylococci has previously been demonstrated in our own (19) and other (20) laboratories. An interesting, as yet unexplained observation, is the failure to obtain active bacteriocin from freeze-thaw extracts of FF-22 cultures grown on tryptic soy agar (J. R. Tagg, unpublished data).

Studies of the killing action of bacteriocins have indicated that the adsorption of a single particle may be sufficient to kill a susceptible cell. Luria (21) termed this "quantal" killing to distinguish it from the "molar" bactericidal activity of the classical antibiotics. Sensitive bacteria are thought to be killed by bacteriocins in a two-step process. The first stage involves the adsorption of the bacteriocin to cell-surface receptors. This is then followed by the transfer of a "lethal" message to specific biochemical targets linked to the cytoplasmic membrane, via the mediation of some amplification system (22, 23). It has been suggested that a chain reaction of bacteriocin-induced conformational changes in membrane subunits may serve as the specific transmission mechanism (24).

Streptocin A was shown to be bactericidal, but not lytic, for a susceptible Group A streptococcus. The rate of this lethal effect was temperature dependent, with relatively little killing occurring at 4°C. Similar temperature dependency of the bactericidal action has been noted in investigations of other bacteriocins (6, 25) and has been taken to indicate a requirement for active cellular metabolism to effect the actual killing of the cells. In support of this, it was found in the present study that streptocin A was somewhat more lethal for cells in the exponential growth phase than those in stationary phase. Other investigators have found that sublethal concentrations of metabolic inhibitors such as dinitrophenol may serve to protect organisms from the killing action of bacteriocins (25).

Immunity of the bacteriocinogenic Group A streptococcus to streptocin A was shown to be incomplete, since preparations having titers of greater than 16 were inhibitory to the homologous producer strain. Although it is generally

stated that bacteria are insensitive to their own bacteriocins, there have been numerous other demonstrations of breakdown of this immunity, in particular to bacteriocins produced by gram-positive microorganisms (26) but also for some organisms producing colicins (27) or pyocins (28).

Treatment of a susceptible Group A streptococcus with streptocin A was shown to lead rapidly to the cessation of biosynthesis of the macromolecules, DNA, RNA, protein, and glycogen-like polymers. Furthermore, the quantity of the preaccumulated TCA-precipitable [^{3}H]uridine was markedly reduced, indicating the degradation of RNA. This effect was independent of protein synthesis in the treated cells. Similar effects on macromolecule synthesis and RNA degradation have been shown to be associated with the lethal activity of two staphylococcal bacteriocins (12, 29). The phage type 71 staphylococcus bacteriocin was shown to cause the breakdown of both newly formed and previously formed RNA in susceptible organisms and it was speculated that this may occur via the activation of an endogenous nucleolytic system (12).

The bactericidal activity of streptocin A in liquid media, including its effect on RNA degradation, was shown to be significantly less than that of preparations of a staphylococcal bacteriocin having an identical inhibitory titer against the indicator strain in lawn culture assays. Several other investigators have similarly noted that the action of boticins (30), pesticins (31), or colicins (32) may also be substantially greater against susceptible organisms grown on agar medium than when grown in broth. In any such studies it is important to distinguish carefully between the determination of general inhibitory activity (including bacteriostatic effects) in lawn culture assays and the measurement of specific bactericidal activity from viable counts of survivors after exposure to the bacteriocin.

Streptocin A appears to have several properties in common with the phage type 71 staphylococcus bacteriocin, in addition to the aforementioned similarity in biochemical effects on a susceptible bacterium. Production of both bacteriocins seems excellent in tryptic soy broth and moreover, both are noninducible, heat-stable, extracellular products of the producer strains and have broadly similar spectrums of activity against other organisms (5, 7). Significant differences are also apparent though. The staphylococcin appears to be stable in alkali and is nondialyzable (19). Furthermore, production of the staphylococcal bacteriocin is considerably greater in shaken tryptic soy broth cultures, while no streptocin A is recovered under these conditions. Interesting differences in the activity spectra are also apparent (5, 7). Streptocin A (but not the staphylococcin) has activity against Group B streptococci, but only the staphylococcin inhibits strains of *S. aureus*, *Bacillus megaterium*, *Listeria monocytogenes*, *Corynebacterium diphtheriae*, *Diplococcus pneumoniae*, and Group D streptococci. Moreover, the immunity of the streptocin A producer strain to the homologous bacteriocin seems less complete than that of the staphylococcin producer to its bacteriocin. Although the staphylococcin has been shown to be antigenic (33)

our preliminary experiments (unpublished data) have indicated a failure of streptocin A to elicit the production of neutralizing antibodies, possibly due in part to its low molecular weight. In some of these properties streptocin A seems more closely to resemble the staphylococcal bacteriocin described by Barrow (34) which was slowly dialyzable, sensitive to alkaline pH but extremely heat resistant when acidic. Lachowicz and Walczak (35) have also described a staphylococcin having an active component of molecular weight 9,700, similar in size to streptocin A. All other characterized bacteriocins have been substantially larger, making streptocin A perhaps the smallest of the known bacteriocins.

Our present studies are being directed towards the investigation of a possible role for streptocin A in the pathogenesis of rheumatic fever. It has been suggested (36) that the attachment to and damage of heart cells by streptocin A may lead to the development of carditis in some individuals infected with bacteriocinogenic Group A streptococci. More definitive studies of the toxicity of streptocin A for mammalian cells have necessarily been dependent upon the development of effective procedures for the production and purification of the bacteriocin such as those described in the present investigation.

SUMMARY

A bacteriocin, streptocin A, was isolated from the supernatant fluid of tryptic soy broth cultures of Group A streptococcus strain FF-22. Evidence was obtained which supports the view that the failure to recover active streptocin A after growth of the producer strain in certain fluid media is due to the inactivation of the bacteriocin by concomitantly synthesized streptococcal proteinase.

The bacteriocin was purified 139-fold and the active product appeared to be of uniform size, having a molecular weight of approximately 8,000.

Streptocin A was bactericidal, but not lytic, for a susceptible Group A streptococcus and the lethal effect was markedly temperature dependent. The bacteriocin inhibited the synthesis of DNA, RNA, and protein, and also prevented the uptake and incorporation of glucose by the sensitive cells.

Degradation of RNA occurred, but appeared to be less than that produced by a staphylococcal bacteriocin. This effect may be due to differences in the killing potency of the two bacteriocins in preparations having similar inhibitory activity when measured by lawn culture assays.

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