Alteration of Macromolecular Synthesis and Membrane Permeability by a *Streptococcus sanguis* Bacteriocin

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Although the elaboration of bacteriocins by streptococci has been described previously (Brock & David, 1963; Kuttner, 1966; Kelstrup & Gibbons, 1969; Overturf & Mortimer, 1970; Kramer & Brandis, 1972), few attempts have been made to purify and characterize these bactericidal factors (Kramer & Brandis, 1972). *Streptococcus sanguis* (strain Challis) produces a streptocin which is lethal for *Streptococcus sanguis* (strain Wicky) and can be purified by ammonium sulphate fractionation and Sephadex G-100 column chromatography. We exposed sensitive strain Wicky cells to Challis streptocin and observed macromolecular synthesis and membrane permeability.

METHODS

The two streptococcal strains used in this study were obtained from the Central Public Health Laboratory, London, via Dr R. Pakula (Toronto). Challis streptocin, STH₁, was prepared by Sephadex G-100 chromatography of Challis culture filtrates and was purified 50-fold relative to supernatant fluid protein. Details of this purification procedure as well as the methods for estimating streptocin titres have been described (Schlegel & Slade, 1973).

Strain Wicky bacteria were grown in Brain Heart Infusion (BHI) medium until the extinction at 550 nm (E_{550}) was 0.30. The culture was then diluted 1:100 with additional BHI medium. Samples (2 ml) were dispensed into sterile test tubes containing [³H]thymidine (12.5 μ Ci/ml, 15 Ci/mmol), [³H]uracil (12.5 μ Ci/ml, 7 Ci/mmol), or [³H]leucine (12.5 μ Ci/ml, 25 Ci/mmol) and incubated at 37 °C for 1.0 h. Purified streptocin, dissolved in 0.15 M-NaCl + 0.2 M-KH₂PO₄ (PBS, pH 7.6), was added to a final multiplicity of 1.9 streptocin 'molecules'/Wicky organism. Control cultures received equivalent volumes of PBS. At 0, 5, 10, 20, 30 and 40 min, Wicky cultures (experimental and control) were immersed in ice water and each filtered through a 0.45 μ m Millipore filter which had been pretreated with 3.0 ml 5% (w/v) bovine serum albumin (BSA). Trapped Wicky bacteria were washed with 50 ml saline and treated with 15 ml cold 10% trichloroacetic acid. The filters were dried and then counted in a Beckman LS-100 scintillation counter. Incorporation of radioactive precursor molecules into trichloroacetic acid-precipitable macromolecules was expressed as pmol ³H-labelled molecules/ml Wicky culture.

RESULTS AND DISCUSSION

Figure 1(a), (b) and (c) shows that Challis streptocin inhibited DNA, RNA and protein synthesis in Wicky organisms. The dramatic inhibition of these three synthetic processes suggested that streptocin might possess a common site of action. According to Nomura's (1967) classical model, the streptocin might mediate alterations of membrane conformation

Fig. 1. Inhibition of macromolecular synthesis by strain Challis bacteriocin (*a*, *b*, and *c*). Strain Wicky organisms were pre-labelled with $12.5 \mu\text{Ci/ml}$ of $[^3\text{H}]$ thymidine (*a*), $[^3\text{H}]$ uracil (*b*), or $[^3\text{H}]$ leucine (*c*). At 0 min, purified Challis streptocin was added to a multiplicity of 1.9 bacteriocin 'molecules'/Wicky organism and estimates of subsequent macromolecular synthesis were obtained by trapping Wicky organisms on Millipore filters and measuring trichloroacetic acid-insoluble radioactivity. ▲, Wicky organisms + streptocin; ●, Wicky organisms + PBS. (*d*) Release of ^{86}Rb from streptocin-treated Wicky organisms. Strain Wicky cultures were pre-incubated with $100 \mu\text{Ci } ^{86}\text{Rb/ml}$ for approximately 3 h. Samples (0.2 ml) were then inoculated into 1.9 ml PBS containing either streptocin (○) or heat-denatured streptocin (●), multiplicity = 1.6. Intracellular ^{86}Rb was determined by filtering 0.2 ml samples of suspension through a Millipore filter and washing with saline.

which could be translated into observable modifications of (i) energy production, (ii) membrane transport and permeability, or (iii) macromolecular synthesis.

A streptocin-induced change in the membrane permeability was therefore sought. In red blood cells (Hingson, Massengill & Mayer, 1969) and bacterial cells (Wendt, 1970), membrane permeability to potassium (K^+) ions can be studied by utilizing the radioactive ion, $^{86}\text{Rb}^+$, since K^+ and Rb^+ transport is competitive (Wendt, 1970).

Wicky bacteria were grown in 3.0 ml TM medium (Schlegel & Slade, 1972) containing $100 \mu\text{Ci } ^{86}\text{Rb/ml}$ to an E_{550} value of 0.20. At this time, intracellular Rb^+ concentrations were more than 100 times the extracellular Rb^+ concentrations. Samples (0.2 ml) of the pre-labelled Wicky bacteria were transferred to a test tube containing 1.9 ml purified streptocin or 1.9 ml streptocin denatured at 65°C for 1.0 h. At intervals, 0.2 ml samples were collected on $0.45 \mu\text{m}$ Millipore filters which had been pretreated with 2.0 ml 5% BSA and 3.0 ml

saline (0.85 % NaCl, w/v). The filters were washed with 30 ml saline, dried, and assayed for ^{86}Rb by counting on the ^{32}P channel of a Beckman LS-100 scintillation counter. (The beta-emitting energies of ^{86}Rb and ^{32}P are 1.8 and 1.7 MeV, respectively.) ^{86}Rb efflux from streptocin-treated Wicky bacteria was more rapid than the ion efflux from control bacteria (Fig. 1*d*). This finding implies that the ability of the Wicky membrane to maintain high intracellular concentrations of ^{86}Rb has been affected. Whether this also reflects a change in the capacity of the membrane to retain other ions (e.g. Mg^{2+} and Ca^{2+}) or small molecules (e.g. ATP, amino acids and sugars) is not known. However, it is possible that permeability changes resulting either in the loss of internal pools of precursor molecules for macromolecular synthesis or in the disturbance of essential intracellular ion concentrations might be responsible for the inhibition of synthesis observed in Fig. 1(*a*), (*b*) and (*c*). Fig. 1 also indicates that the streptocin-treated bacteria apparently do not 'leak' macromolecules, which augments the previous finding (Schlegel & Slade, 1972) that sensitive bacteria are not lysed by the bactericidal factor. Thus, Rb^{+} efflux is probably the result of subtle rather than gross membrane damage or alteration.

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