STREPTONIGRIN-TRANSITION METAL COMPLEXES: BINDING TO DNA AND BIOLOGICAL ACTIVITY

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Summary. Two effects of transition metal divalent cations on the behavior of the antitumor antibiotic streptonigrin are reported: (1) Metal ions such as Mn^{++}, Zn^{++}, and Cd^{++} promote an association of the antibiotic with double-stranded DNA. The metal ion causes a depression and slight red shift of the 365 nm absorption maximum of the antibiotic. Addition of DNA to the zinc-streptonigrin complex causes a further depression and red shift of the absorption maximum, indicating the formation of a DNA-streptonigrin-zinc complex. (2) These metal cations affect the biological activity of streptonigrin, in that they greatly enhance the lethal effect of the antibiotic on Escherichia coli. It is likely that the two effects are related.

Introduction. The antitumor antibiotic streptonigrin causes strand breaks in DNA in vivo (1). Its bactericidal action requires both intracellular reduction of the antibiotic and the presence of oxygen (2). The antibiotic has been shown to generate the superoxide anion $O_2^{\cdot -}$ upon reduction and autoxidation in vitro (3,4), and the superoxide anion has been shown to cause strand breaks in closed circular double stranded DNA (5,6). These observations have led to a proposed mechanism in which the antibiotic generates superoxide during a reduction-oxidation cycle, and this radical beings about single strand breaks (3,6). This mechanism does not involve a direct interaction between streptonigrin and DNA, and indeed no significant interaction has ever been demonstrated. This paper presents evidence that under appropriate conditions streptonigrin does associate physically with DNA and that this interaction is probably involved in its mechanism of action.

Methods and Materials. Escherichia coli strain 15 $Thy^-Arg^-Ura^-$ was grown at 37° with forced aeration in a Tris-buffered minimal medium (2) containing thymine (4µg/ml), L-arginine (20µg/ml), uracil (20µg/ml), and 10^{-5}M $FeCl_3$. A culture in exponential growth at a concentration of 3×10^8 cells/ml was diluted 1:1 into tubes of fresh medium containing streptonigrin and metal ions. Viabilities were determined by serial dilution in 0.15M NaCl and plating on nutrient agar.

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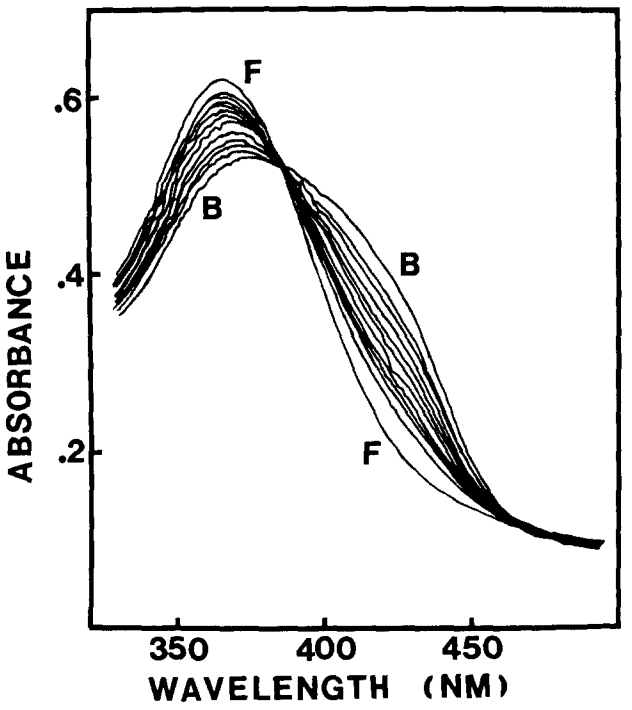

Figure 1. Demonstration of a ternary complex between streptonigrin (4×10^{-5}M) DNA, and Zn^{++} (4×10^{-4}M). The curve marked F is the spectrum of the streptonigrin-Zn^{++} complex. The curve marked B denotes the spectrum of extensively bound antibiotic. Curve B was obtained from a solution containing 1.0 mg/ml DNA. Other curves represent successive decrements of 15% in DNA concentration.

Optical absorbance curves were run on a Cary Model 14 Recording Spectrophotometer. A solution (solution S) of streptonigrin (20µg/ml) and $ZnSO_4$ (4×10^{-4}M) was prepared in dilute saline citrate (0.015M NaCl, 0.0015M trisodium citrate, pH 7.0). To obtain the spectrum of the free streptonigrin-zinc complex, solution S was run against a reference solution containing zinc sulfate (4×10^{-4}M) in dilute saline citrate. The spectrum of extensively bound antibiotic was obtained by running a sample of solution S containing 1.0 mg/ml calf thymus DNA against a reference of the same solution lacking streptonigrin. Solutions containing lower concentrations of DNA were prepared successively by diluting 1.7 ml of a higher concentration with 0.3 ml of solution S. The reference in each case was obtained by dilution of the previous reference with 0.3 ml of $ZnSO_4$ (4×10^{-4}M) in dilute saline citrate.

Calf thymus DNA (highly polymerized) was purchased from Calbiochem. Streptonigrin was obtained from Charles Pfizer & Co., Inc.

Results. Spectrophotometric evidence for formation of a number of streptonigrin-metal-DNA complexes has been obtained. The data for Zn^{++} are presented in Fig. 1. Aqueous streptonigrin has an absorption maximum at

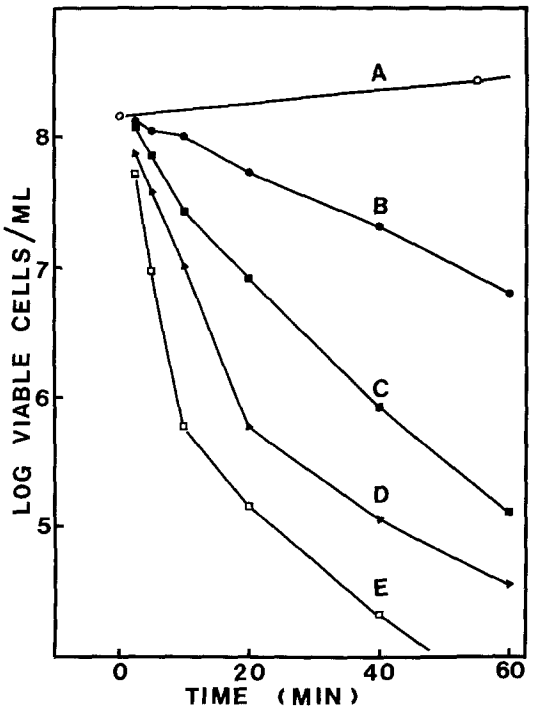

Figure 2. Enhancement of the bactericidal action of streptonigrin by Mn^{++}, Zn^{++}, and Cd^{++}. A: uninhibited; B: 3µg/ml streptonigrin; C: 3µg/ml streptonigrin and 10^{-4}M $MnCl_2$; D: 3µg/ml streptonigrin and 10^{-5}M $ZnCl_2$; E: 3µg/ml streptonigrin and 10^{-5}M $CdCl_2$. Inhibitors were added at 0 min.

365 nm (not shown). Addition of Zn^{++} causes a slight depression and red shift of this maximum, indicating complex formation (curve F). Addition of 1.0 mg/ml of DNA causes a further suppression and red shift of the maximum (curve B). Intermediate concentrations of DNA reveal the existence of a fairly crisp isosbestic point at 385 nm.

Other transition metal divalent cations show similar effects, including Mn^{++}, Cd^{++} and Cu^{++}; however, the alkaline earth cations Mg^{++} and Ca^{++} are ineffective. The phenomena have been confirmed in other buffers.

The effectiveness of Cd^{++}, Zn^{++} and Mn^{++} in stimulating the bactericidal action of streptonigrin is displayed in Fig. 2. In the absence of streptonigrin Mn^{++} and Zn^{++} did not affect the increase in viability during the

experiment, while Cd^{++} was bacteriostatic. Other cations that show less marked synergism include Cu^{++}, Co^{++} and Fe^{++}. Other bacterial strains, such as E. coli B/r, show similar patterns, but effectiveness varies from strain to strain.

Discussion. The complex between streptonigrin and metal ion may involve the two ring nitrogens of the antibiotic. In the conformation of streptonigrin determined by Chiu and Lipscomb (7) a 180° rotation of the B and C rings brings the quinoline and pyridine nitrogens into a bipyridyl-like configuration. If the metal ion is indeed complexed with these nitrogens, then the negatively charged carboxyl group of the C ring would be brought into a position near the Zn^{++} and could help stabilize the conformation. Such a role for the carboxyl might explain the large loss of antibiotic potency reported when this carboxyl is converted to its methyl ester (8,9).

The metal ion could promote formation of the ternary complex (streptonigrin metal ion-DNA) in two ways: (1) by changing the net charge on the streptonigrin molecule from -1 to +1, thereby changing the ionic interaction between streptonigrin and the DNA phosphates from repulsive to attractive; (2) by holding the B and C rings in a coplanar configuration.

The ternary complex may be intercalative in nature, but this is uncertain, since a spectral red shift can arise in other ways (10). It has been reported that Cu^{++} does not promote an intercalative DNA-streptonigrin complex (6). Nevertheless, spectral evidence for a streptonigrin-Cu^{++}-DNA complex is unambiguous (unpublished results). A possible role for intercalative interaction in some of these complexes remains to be investigated.

Enhancement of the antibacterial potency of streptogrin by transition metal divalent cations suggests that these cations play an important role in the action of the antibiotic. One may imagine that a complex between a metal ion and a reduced form of streptonigrin associates with the DNA. Ensuing autoxidation of the antibiotic in close vicinity to the chromosome would be much more effective in causing lethal damage than autoxidation, with generation of superoxide, in a less sensitive part of the cytoplasm.

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