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Mode of Action of Streptozotocin

FRITZ REUSSER
The Upjohn Company, Kalamazoo, Michigan 49001

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Streptozotocin induces rapid degradation of deoxyribonucleic acid (DNA) in actively dividing or resting *Bacillus subtilis* cells. Difference spectroscopy showed that the antibiotic interacts specifically with cytosine containing mononucleotides in vitro. This interference occurs only within the very narrow *p*H range of 5 to 5.5 and is reversed immediately upon lowering or increasing the *p*H. No measurable interaction was observed between isolated DNA and streptozotocin. The possibility is discussed that interaction of streptozotocin with cytosine residues in cellular DNA, although possibly taking place at a very low frequency, may constitute the primary step inducing DNA strand breakage.

Streptozotocin is an antibiotic produced by *Streptomyces achromogenes* var. *streptozoticus*. The agent possesses broad-spectrum antibacterial, antitumor, mutagenic, and diabetogenic activities and was recently shown to inhibit primarily deoxyribonucleic acid (DNA) synthesis in bacteria and mammalian cells (1, 2, 5, 16, 19). Streptozotocin is a 2-deoxy-D-glucose derivative of *N*-methyl-*N*-nitroso-urea. The chemical structure is shown in Fig. 1 (6). The antibiotic is most stable at *p*H 4.5 and degrades rapidly in alkaline solutions, forming diazomethane, an alkylating agent.

MATERIALS AND METHODS

B. subtilis strain 23 (obtained from J. Spizizen) was grown in glucose-salt medium as previously described (13). Cellular nucleic acid and protein synthesis were assessed by the incorporation of appropriately labeled precursors for these macromolecular fractions. Specifically, 2 μCi of ^{14}C-L-aspartic acid (specific activity, 217 mCi/mmole; Nuclear-Chicago Corp.) was added per 100 ml of culture medium to follow protein synthesis, 10 μCi of uracil-2-^{14}C (specific activity, 61 mCi/mM; Nuclear-Chicago Corp.) per 100 ml of broth was added to follow ribonucleic acid (RNA) synthesis, and 50 μCi of thymidine-6-^{3}H (specific activity, 22.8 Ci/mmole; Amersham/Searle) was added per 100 ml of broth to follow DNA synthesis. Precursors and streptozotocin were added during the early log phase of the growth cycle. Sample portions were withdrawn from the cultures at appropriate time intervals. The samples were made 5% with cold trichloroacetic acid, the acid-insoluble fractions collected on membrane filters (pore size, 0.45 nm; Millipore Corp.), and counted in 15 ml diotol by conventional techniques.

To assess stability of the cellular DNA, RNA, and protein fractions in *B. subtilis* cells in the presence of streptozotocin, cells were prelabeled for two to three generations at the early log phase by the addition of 20 μCi of ^{3}H-thymidine, 3 μCi of ^{14}C-uracil, or 2.5 μCi of ^{14}C-aspartic acid per 100 ml of culture medium. After the labeling period, cells from 400 ml of culture medium were recovered by centrifugation, washed, and resuspended in 100 ml of fresh culture medium. This suspension was divided into two 50-ml portions, and streptozotocin (150 μg/ml) was added to one. The other served as control. The cultures were reincubated, and sample portions were withdrawn at regular time intervals. The cells were removed by centrifugation, and 0.5-ml portions of clear broth were counted in 15 ml of diotol.

The results presented in this paper show that streptozotocin induces rapid and extensive degradation of cellular DNA in *Bacillus subtilis*. It is concluded that inhibition of DNA synthesis as described previously (16) actually reflects a higher rate of streptozotocin-induced DNA degradation over de novo synthesis.

For the preparation of protoplasts, B. *subtilis* cells were grown in glucose-salt medium as described previously (13). At the time of inoculation, the optical density of the culture broth was 0.30 at 570 nm. After growth had progressed to an optical density of 0.375 (~1.5), 50 μCi of thymidine-6-^{3}H (specific activity, 37.8 Ci/mmole) was added per 100 ml of broth, and the cultures were reincubated until the optical density was approximately 0.450. At this time streptozotocin (75 μg/ml) was added, and the cultures were reincubated as specified below. Protoplasts of *B. subtilis* cells were prepared by the lysozyme-ethylenediaminetetraacetic acid (EDTA) procedure (3, 4). Cells from 300-ml culture portions were washed and suspended in 2 ml of brain heart-sucrose medium (Brain Heart Infusion, Difco, 37 g/liter; sucrose 171 g/liter); 0.2 ml of an aqueous stock solution of lysozyme (1 mg/ml; Calbio-

chem) was added under slow swirling at 32 C. After 5 min, 0.4 ml of a 1% aqueous solution of EDTA was added, and incubation was continued for an additional 30 to 40 min. After this time conversion of the cells to protoplasts was almost complete. The progress of protoplast formation was followed under the microscope. The elongated rod shape of the vegetative cells of *B. subtilis* facilitates these observations.

The protoplast suspensions (0.2 ml) were lysed directly on sucrose gradients by carefully applying them onto a 0.1-ml layer of 0.5 N NaOH and 1% sodium lauryl sulfate (SDS) layered on top of a 4.6-ml 15 to 30% alkaline sucrose gradient containing 0.01 M NaOH and 0.5% SDS (8, 10). The protoplasts were allowed to lyse for 30 min. The gradients were then centrifuged for 60 min at 30,000 rev/min in the SW-39 head of a Beckman Spinco L2 ultracentrifuge at 20 C. After centrifugation the gradients were processed as previously described (14).

The methods used to assess stability of ^{14}C-uracil labeled ribosomes in the presence of streptozotocin have been described (14).

Synthetic polydeoxyribonucleotides and pure DNA were sedimented on 4.6-ml alkaline 5 to 20% sucrose gradients containing 0.1 N NaOH, 0.9 M NaCl, and 1 mM EDTA (9). The synthetic polydeoxyribonucleotides and DNA solutions were treated with streptozotocin for 10 min at room temperature at *p*H 5.0. The mixtures were then made 0.1 N in respect to NaOH by the addition of 1 N NaOH. The polymers were allowed to denature for 10 min before they were applied on top of the gradients. The gradients were centrifuged for 6.5 hr at 39,000 rev/min at 4 C in the SW-30 head. Poly C was separated in neutral 5 to 20% sucrose gradients containing 0.01 M tris(hydroxymethyl)aminomethane (Tris)-hydrochloride buffer (*p*H 7.4) and 10^{-4} M magnesium acetate. These gradients were centrifuged for 17 hr. The tubes were pierced from the bottom, and two-drop fractions were collected. Each fraction was diluted with 1.5 ml of water, and their optical densities were measured in 1-ml cuvettes in a Beckman DU spectrophotometer.

Difference spectra were read in a Beckman DU spectrophotometer by using the cuvettes of Towne and Rabin (18). All substances were dissolved in 0.01 M KPO_4 buffer (*p*H 5.0). A 1-ml portion of reactant (0.2 μmole/ml) was mixed with a 1-ml portion of streptozotocin (20 μg/ml).

Measurements of the thermal transition (T_m) DNA and polydeoxyribonucleotides were made with a Gilford recording thermospectrophotometer (11).

The reaction mixtures contained per ml: 20 μg of salmon sperm DNA; 0.3 units of dAT at an optical density of 260 nm (OD_{260}); or 0.2 OD_{260} units of dG: dC; 25 μg of streptozotocin; 2 μmoles of Tris-hydrochloride (*p*H 7.2); 2 μmoles of NaCl.

Preparation and enzymatic assays of DNA and RNA polymerases from *Escherichia coli* were as described by Reusser and Bhuyan (12).

Salmon sperm DNA was purchased from Calbiochem; *E. coli* DNA was isolated by the Marmur procedure (7).

Synthetic polydeoxyribonucleotides were purchased from Biopolymers, Inc.

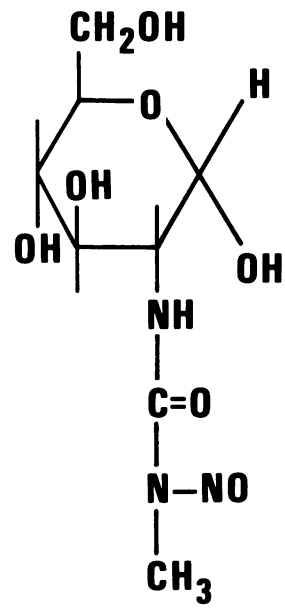

FIG. 1. *Proposed chemical structure of streptozotocin.*

RESULTS

Effect of streptozotocin on protein and nucleic acid synthesis in whole B. subtilis cells. The effect of streptozotocin on protein and nucleic acid synthesis is shown in Fig. 2. It is evident that protein and RNA synthesis remained unaffected in the presence of 50 μg of streptozotocin per ml during the first 40 min after addition of antibiotic. After this time period, only moderate inhibition of these processes was apparent.

Partial but substantial inhibition of ^{3}H-thymidine incorporation, reflecting DNA synthesis, was apparent 20 min after addition of antibiotic. This suggests that streptozotocin interacts with the synthesis or stability of cellular DNA.

Stability of cellular DNA, RNA, and protein fractions in B. subtilis. These cellular fractions were prelabeled with radioactive thymidine, uracil, and aspartic acid, respectively. Leakage of incorporated label from whole cells into the culture medium was then used as a parameter to measure the stability of the cellular nucleic acid and protein fractions.

Relatively little thymidine leakage was observed in the control culture during an incubation period of 3 to 4 hr (Fig. 3). In the presence of streptozotocin, extensive thymidine leakage from the cells became apparent immediately after addition of the antibiotic. The rate of leakage remained remarkably constant during the whole

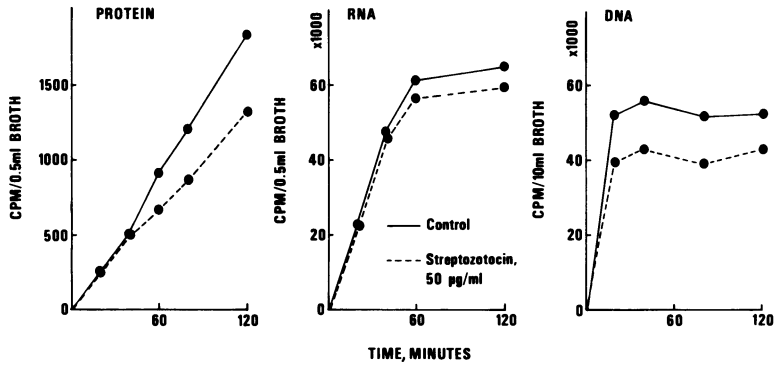

FIG. 2. *Effect of streptozotocin on protein and nucleic acid synthesis in B. subtilis. Two microcuries of ^{14}C-aspartic acid, 10 µCi of ^{14}C-uracil, or 50 µCi of ^{3}H-thymidine was added per 100 ml of broth to follow the progress of protein, RNA, or DNA synthesis, respectively. Precursors and streptozotocin were added during the early log phase of the growth cycle. Sample portions were withdrawn from the cultures at appropriate time intervals. The samples were made 5% with cold trichloroacetic acid; the acid-insoluble fractions collected on membrane filters (Millipore Corp.; pore size, 0.45 nm) and counted in 15 ml of diotol by conventional techniques.*

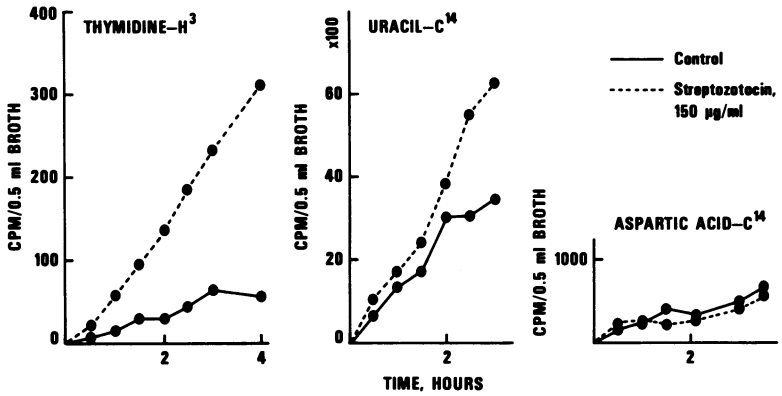

FIG. 3. *Effect of streptozotocin on stability of prelabeled nucleic acid or protein fractions in B. subtilis. Cells were prelabeled for two to three generations at the early log phase with either 20 µCi of ^{3}H-thymidine, 3 µCi of ^{14}C-uracil, or 2.5 µCi of ^{14}C-aspartic acid per 100 ml of culture medium. After the labeling period, cells from four flasks were recovered by centrifugation, washed, and resuspended in 100 ml of fresh culture medium. This suspension was divided into equal portions, and streptozotocin (150 µg/mg) was added to one. The cultures were reincubated, and sample portions were withdrawn at regular time intervals. The cells were removed by centrifugation, and 0.5-ml portions of clear broth were counted in 15 ml of diotol.*

experiment and amounted to an approximately sixfold increase over the control value. Substantial leakage of uracil label into the culture medium was observed in both the control and the streptozotocin-treated cultures. However, for the first 2 hr, loss of uracil label in the streptozotocin-treated culture was only moderately higher than in the control. The leakage rates remained remarkably constant over this time period. After 2 hr, uracil leakage became high in the antibiotic treated culture. Only trace amounts of label were excreted into the culture medium by cells prelabeled with aspartic acid regardless of the presence of streptozotocin. After approximately 1 hr, loss of label was even slightly less in the streptozotocin containing culture than in the control.

Effect of streptozotocin on stability of DNA fraction in dividing or resting B. subtilis cells. In the experiments with dividing cells, cells were prelabeled with ^{3}H-thymidine for two to three generations and then treated with streptozotocin for various periods of time in regular glucose-salts medium. Cellular DNA was isolated from protoplasts. Small portions of protoplast suspension were lysed directly on alkaline sucrose gradients to minimize breakage of DNA during the isolation process.

Exposure of the cells to streptozotocin for 30 min resulted in extensive breakdown of bacterial DNA (Fig. 4). The gradient profiles obtained from streptozotocin-treated cells show no distinctive peak and suggests that the bacterial DNA

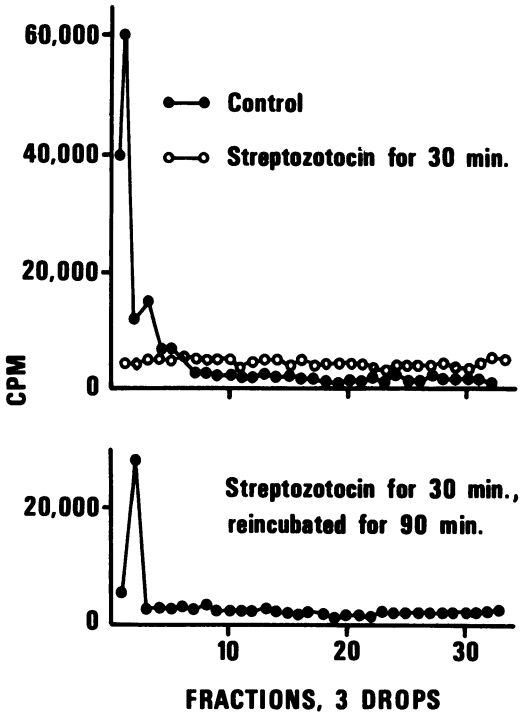

FIG. 4. *Effect of streptozotocin on sedimentation patterns of DNA from B. subtilis. Cells were prelabeled with ^{3}H-thymidine for two to three generations. Streptozotocin was then added (75 μg/ml) and the cultures were reincubated for the times indicated in the figure. Protoplasts were prepared by the lysozyme-EDTA technique. Portions of protoplast suspension (0.2 ml) were directly lysed in 0.1 ml of 0.5 N NaOH containing 1% SDS on top of 4.6 ml of alkaline 15 to 30% sucrose gradients. The gradients were centrifuged at 30,000 rev/min for 60 min at 20 C in a SW-39 rotor. Fractions were collected from the bottom of the tubes. DNA was precipitated with 5% trichloroacetic acid by using tRNA as carrier. The precipitates were collected on membrane filters (Millipore Corp.) and assayed for radioactivity.*

was degraded to fragments of random length and acid-soluble material. The control gradients show that most of the DNA originating from untreated cells sedimented within a narrow region at the bottom of the tube. Cells treated for 30 min with drug were washed twice and then reincubated in fresh medium in the absence of tracer or antibiotic for 90 additional minutes. Under these conditions a small peak was observed at the bottom of the tube which corresponds to material with an identical sedimentation rate to that of control DNA (Fig. 4). This suggests that either some de novo synthesis of DNA from labeled bases or some rejoining of smaller DNA fragments by DNA repair enzymes had taken place during reincubation after removal of the drug.

It was interesting to investigate whether DNA breakdown would be as extensive in resting cells exposed to streptozotocin as in dividing cells. Cells were prelabeled with ^{3}H-thymidine as described above. The cells were then recovered, washed, and suspended in glucose-salts medium lacking all *N*-containing ingredients. DNA from the control resting cells sedimented at the bottom of alkaline sucrose gradients as expected (Fig. 5). DNA from cells treated for 30 min with streptozotocin was degraded extensively. A large portion of the degraded material remained acid precipitable and was located at the top of the gradient. Similar gradient profiles were also obtained from dividing cells treated with streptozotocin for periods shorter than 30 min. This suggests that DNA breakdown induced by streptozotocin also takes place in resting cells, but at a slightly reduced rate. Blockage of oxidative phosphorylation by the addition of kalafungin, an inhibitor of oxidative phosphorylation (15) did not affect the streptozotocin-induced DNA degradation process.

Stability of ribosomes in B. subtilis. Cells were prelabeled with ^{14}C-uracil for two to three generations and were then exposed to streptozotocin (75 μg/ml) for 30 min. Extracts prepared from these cells were separated on neutral sucrose gradients. No extensive degradation of 50 or 30*S* ribosomes nor transfer RNA (4*S* region) was apparent in extracts from streptozotocin-treated cells, although an approximately 20% decrease of label is apparent in all these fractions contained in the extract derived from the streptozotocin-treated cells (Fig. 6). However, we can conclude

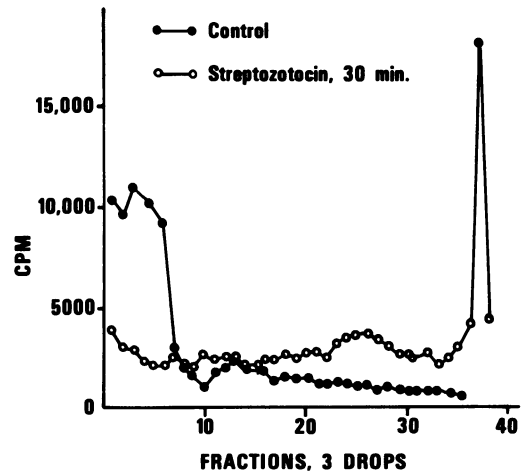

FIG. 5. *Effect of streptozotocin on sedimentation patterns of DNA from resting B. subtilis cells. The gradients were prepared as described in the legend to Fig. 4.*

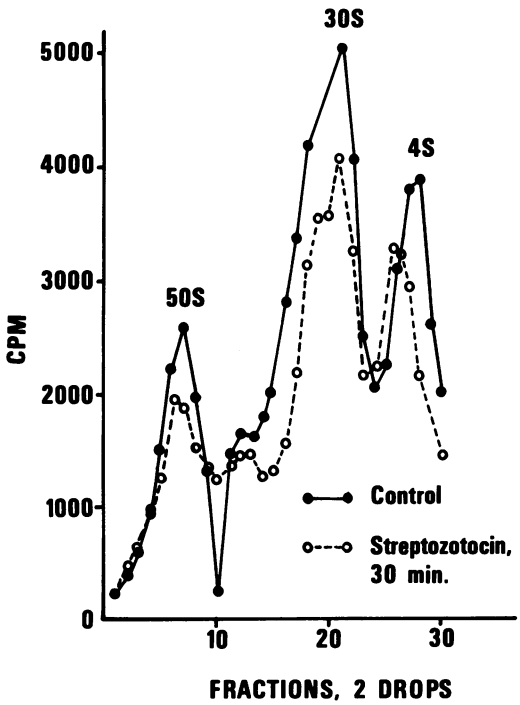

FIG. 6. *Effect of streptozotocin on sedimentation patterns of B. subtilis cell extracts. Cells were prelabeled with ^{14}C-uracil for two to three generations. Streptozotocin (75 μg/ml) was then added, and the cultures were reincubated for 30 min. Cells were disrupted mechanically, and 0.2-ml portions of cell extract were applied to 4.6 ml of 5 to 20% sucrose gradients containing 0.01 M Tris-hydrochloride (pH 7.4) and 10^{-4} M magnesium acetate. The gradients were centrifuged at 38,000 rev/min for 180 min at 4 C. Acid-precipitable material was collected and assayed as described in the legend to Fig. 4.*

that the structural integrity of ribosomes remains largely intact in the presence of streptozotocin.

Stability of synthetic polydeoxyribonucleotides and isolated natural DNA. The synthetic polydeoxyribonucleotides dAT and dG:dC and isolated natural DNA were mixed with streptozotocin and allowed to stand at room temperature for 10 minutes as described above. The solutions were then made alkaline to effect separation of the double stranded polymers. The single stranded products were separated on alkaline sucrose gradients.

Both natural DNA preparations (salmon sperm DNA, *E. coli* DNA) had obviously undergone extensive breakdown due to mechanical shearing during the isolation process and sedimented at a much slower rate than *B. subtilis* DNA from protoplasts (Fig. 7). Streptozotocin did not induce extensive breakdown of either of the two DNA preparations. It is thus evident that isolated DNA remains stable in the presence of streptozotocin as compared to DNA in whole *B. subtilis* cells. Adjustment of the *p*H of the reaction mixtures containing DNA, dAT, or dG:dC and streptozotocin to 5.0 (*see below*) during streptozotocin treatment had no effect on the sedimentation profiles of these polymers. Suzuki et al. (17) reported that bleomycin causes DNA

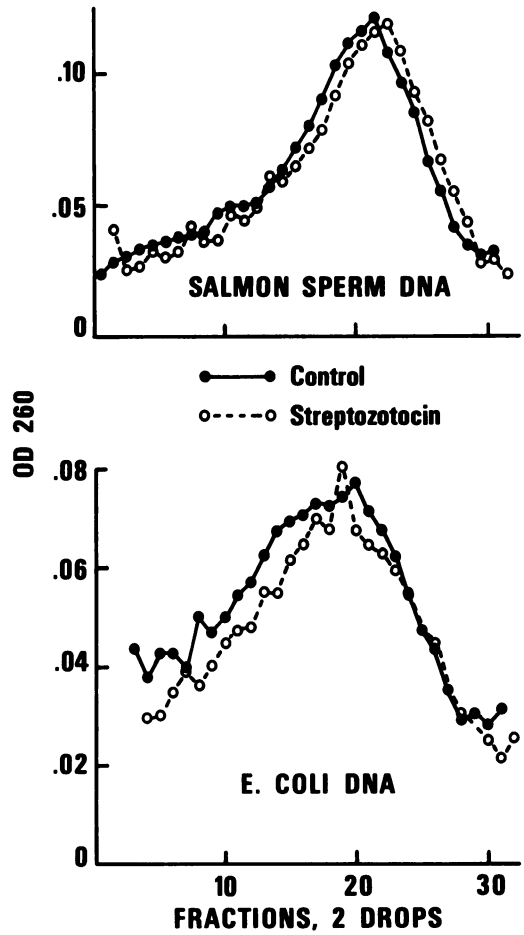

FIG. 7. *Effect of streptozotocin on sedimentation patterns of isolated salmon sperm and E. coli DNA on alkaline sucrose gradients. One hundred micrograms of salmon sperm DNA or 300 μg of E. coli DNA were applied per gradient. Fifty micrograms of streptozotocin was present per gradient where applicable. The gradients had a volume of 4.6 ml of 5 to 20% sucrose containing 0.1 N NaOH and 0.9 M NaCl. The gradients were centrifuged at 39,000 rev/min for 6 hr at 4 C in the SW-39 rotor. Fractions were collected from the bottom of the tubes and processed as described in the text. The salmon sperm DNA gradient containing streptozotocin is offset from the control gradient by one fraction.*

strand breaks in vitro only if the DNA preparation had been pretreated with a reducing agent such as mercaptoethanol. A few crystals of $Na_2S_2O_4$ were added to our DNA-streptozotocin mixtures prepared otherwise as described under Fig. 7. Addition of $Na_2S_2O_4$ had no influence on the sedimentation profile of DNA-streptozotocin mixtures as compared to the control. Thus, streptozotocin did not induce strand breakage of isolated DNA in the presence of the reducing agent $Na_2S_2O_4$.

In the absence of streptozotocin, dG:dC formed a broad and shallow peak on the gradient (Fig. 8). This reflects a sizable variation of chain length within the starting material. Addition of streptozotocin did not change the sedimentation pattern. Similarly, the sedimentation patterns obtained with dAT remained unchanged regardless of the presence of streptozotocin.

Poly C was treated with streptozotocin and then sedimented through a neutral sucrose gradient. The sedimentation patterns of the control and the streptozotocin-containing samples were identical.

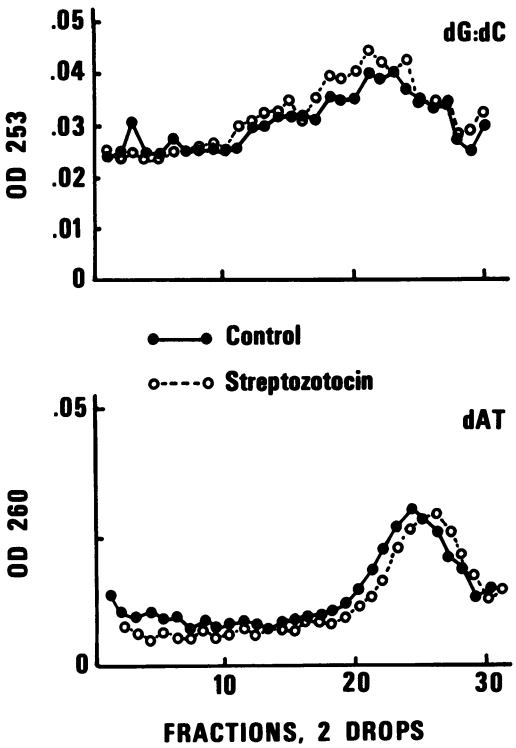

FIG. 8. *Effect of streptozotocin on sedimentation patterns of dG:dC and dAT in alkaline sucrose gradients. One unit of optical density at 260 nm of dG:dC or 0.5 unit of dAT and 50 μg of streptozotocin were applied per gradient. Composition and processing of gradients was as described in the legend to Fig. 6. The dAT gradient containing streptozotocin is offset from the control gradient by one fraction.*

Difference spectroscopy with mononucleotides, nucleosides, and synthetic polymer-streptozotocin mixtures. Difference spectra obtained with deoxycytidine triphosphate (dCTP), deoxycytidine monophosphate (dCMP), cytidine triphosphate (CTP), cytidine monophosphate (CMP), and poly C-streptozotocin mixtures were similar and showed a loss of absorption at approximately 280 nm and a gain at 240 nm (Fig. 9). Mixtures of streptozotocin with DNA (salmon sperm), poly dC, the other usual nucleic acid bases (including cytosine), ribonucleosides and deoxyribonucleosides (including the ones containing cytosine), ribose, deoxyribose, and orthophosphate did not show any changes in the ultraviolet-absorption spectrum. The fact that streptozotocin interacts with cytosine-containing nucleotides but not with the corresponding nucleosides indicates that at least one phosphoric acid ester is essential for interaction with the antibiotic. The interaction of streptozotocin with poly C but not with poly dC or DNA cannot be explained satisfactorily. It is conceivable that some interaction does occur but at such a low frequency that it is not detectable by difference spectroscopy.

The described cytosine-containing nucleotide-

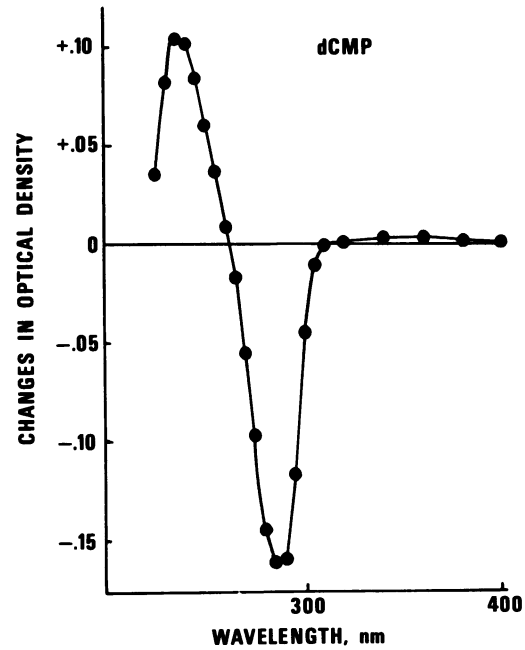

FIG. 9. *Difference spectrum of a streptozotocin-dCMP mixture. Both substances were dissolved in 0.01 M K PO_4 buffer (pH 5.0). A 1-ml portion of dCMP solution containing 0.2 μmoles/ml was mixed with 1 ml of a solution containing 20 μg of streptozotocin per ml.*

streptozotocin interaction is *p*H dependent and occurs only within a very narrow *p*H range. Interaction, as judged by the magnitude of the deflections of the ultraviolet spectra, was optimal at *p*H 5 and somewhat less discrete at *p*H 5.5. No interaction was detected in buffers of *p*H 4.5 and below and *p*H 6.0 and above. The resulting streptozotocin-cytosine association product formed at *p*H 5.0 appears very unstable and dissociated immediately when the *p*H was either lowered below 5.0 or increased to 6.0.

Effect of streptozotocin on T_m of DNA. The antibiotic caused a slight decrease in the melting temperature of salmon sperm DNA. The T_m value for the control sample was 64.5 C; in the presence of streptozotocin this value was reduced to 62.0 C. These measurements were complicated by the observation that streptozotocin undergoes rapid degradation at higher temperatures. This resulted in a rapid loss of optical density at 260 nm. The melting curves were corrected for this loss. dAT showed a sharp melting point at 42.0 C regardless of the presence of streptozotocin. The dG:dC preparation on hand could not be induced to melt despite heating of the samples to >90 C.

Effect of streptozotocin on macromolecular biosynthetic processes in cell-free systems. DNA polymerase reactions primed with various synthetic polydeoxyribonucleotides of known base composition remained insensitive to streptozotocin (Table 1). The only exception was found with the dAC:dTG-primed ^{3}H-dCTP incorporation system. Streptozotocin stimulated this reaction by 30 to 40% in the presence of 100 to 200 μg of drug per ml.

Most of the RNA polymerase reactions were moderately but somewhat irregularly inhibited if cytosine was present in the primer and ^{14}C-guanosine triphosphate incorporation was measured (Table 2). Poly dG or dG:dC-primed ^{14}C-CTP incorporation was inhibited to an extent of 10 to 16% by 200 μg of streptozotocin per mg and stimulated to an extent of 10 to 38% by 100 μg of drug per ml. Reactions primed with only ade-

TABLE 1. *Effect of streptozotocin on cell-free E. coli DNA polymerase*[a]

Sample	Primer	Labeled nucleotide	Incorporation (counts per min per sample)	Control (%)
Control	dAT	^{3}H-dATP	24,340	100.0
Streptozotocin				
400 μg/ml	dAT	^{3}H-dATP	23,870	98.1
100 μg/ml	dAT	^{3}H-dATP	22,800	93.6
Control	dAT	^{3}H-TTP	22,000	100.0
Streptozotocin				
400 μg/ml	dAT	^{3}H-TTP	20,170	91.6
100 μg/ml	dAT	^{3}H-TTP	21,440	97.4
Control	dG:dC	^{3}H-dCTP	990	100.0
Streptozotocin				
400 μg/ml	dG:dC	^{3}H-dCTP	950	96.2
100 μg/ml	dG:dC	^{3}H-dCTP	780	78.8
Control	dAC:dTG	^{3}H-dATP	1,180	100.0
Streptozotocin				
400 μg/ml	dAC:dTG	^{3}H-dATP	1,200	101.9
100 μg/ml	dAC:dTG	^{3}H-dATP	1,070	90.8
Control	dAC:dTG	^{3}H-TTP	5,440	100.0
Streptozotocin				
400 μg/ml	dAC:dTG	^{3}H-TTP	5,870	107.9
100 μg/ml	dAC:dTG	^{3}H-TTP	5,760	105.8
Control	dAC:dTG	^{3}H-dCTP	380	100.0
Streptozotocin				
400 μg/ml	dAC:dTG	^{3}H-dCTP	550	141.9
100 μg/ml	dAC:dTG	^{3}H-dCTP	490	127.9

[a] Reaction mixtures contained in a total volume of 0.5 ml: KPO_4 buffer (*p*H 7.2), 35 μmoles; $MgCl_2$, 3.5 μmoles; mercaptoethanol, 0.5 μmole; deoxycytidine triphosphate, deoxyguanosine triphosphate, thymidine triphosphate, and deoxyadenosine triphosphate, 0.05 μmole each; one labeled nucleotide, 0.5 μCi; synthetic polydeoxyribonucleotides, 0.03 to 0.04 units at an optical density of 260 nm; polymerase, 1.2 enzyme units. The assays were carried out at 37 C for 30 min. Further processing of the samples was as described previously (11).

TABLE 2. *Effect of streptozotocin on cell-free E. coli RNA polymerase*[a]

Sample	Primer	Labeled nucleotide	Incorporation (counts per min per sample)	Control (%)
Control	Poly dC, 0.04 unit	^{14}C-GTP	837	100.0
Streptozotocin				
200 μg/ml	Poly dC, 0.04 unit	^{14}C-GTP	620	74.4
100 μg/ml	Poly dC, 0.04 unit	^{14}C-GTP	520	63.1
Control	dG:dC, 0.04 unit	^{14}C-GTP	290	100.0
Streptozotocin				
200 μg/ml	dG:dC, 0.04 unit	^{14}C-GTP	290	100.0
100 μg/ml	dG:dC, 0.04 unit	^{14}C-GTP	280	96.2
Control	dI:dC, 0.03 unit	^{14}C-GTP	370	100.0
Streptozotocin				
200 μg/ml	dI:dC, 0.03 unit	^{14}C-GTP	320	85.6
100 μg/ml	dI:dC, 0.03 unit	^{14}C-GTP	290	77.1
Control	Poly C, 20 μg	^{14}C-GTP	890	100.1
Streptozotocin				
200 μg/ml	Poly C, 20 μg	^{14}C-GTP	590	66.6
100 μg/ml	Poly C, 20 μg	^{14}C-GTP	670	75.4
Control	Poly dG, 0.03 unit	^{14}C-CTP	230	100.0
Streptozotocin				
200 μg/ml	Poly dG, 0.03 unit	^{14}C-CTP	200	88.7
100 μg/ml	Poly dG, 0.03 unit	^{14}C-CTP	320	138.1
Control	dG:dC, 0.04 unit	^{14}C-CTP	330	100.0
Streptozotocin				
200 μg/ml	dG:dC, 0.04 unit	^{14}C-CTP	280	83.9
100 μg/ml	dG:dC, 0.04 unit	^{14}C-CTP	370	110.1
Control	dAT, 0.05 unit	^{14}C-UTP	3,960	100.0
Streptozotocin				
200 μg/ml	dAT, 0.05 unit	^{14}C-UTP	4,120	104.1
100 μg/ml	dAT, 0.05 unit	^{14}C-UTP	3,840	97.0
Control	Poly A, 20 μg	^{14}C-UTP	1,100	100.0
Streptozotocin				
200 μg/ml	Poly A, 20 μg	^{14}C-UTP	1,100	100.0
100 μg/ml	Poly A, 20 μg	^{14}C-UTP	980	88.9
Control	dAT, 0.05 unit	^{14}C-ATP	1,480	100.0
Streptozotocin				
200 μg/ml	dAT, 0.05 unit	^{14}C-ATP	1,620	109.3
100 μg/ml	dAT, 0.05 unit	^{14}C-ATP	1,550	104.6
Control	Poly U, 20 μg	^{14}C-ATP	370	100.0
Streptozotocin				
200 μg/ml	Poly U, 20 μg	^{14}C-ATP	380	103.2
100 μg/ml	Poly U, 20 μg	^{14}C-ATP	370	100.0

[a] Assay mixtures contained in a total volume of 0.25 ml: Tris-hydrochloride buffer (*p*H 7.9), 5 μmoles; $MgCl_2$, 1 μmole; mercaptoethanol, 3 μmoles; $MnCl_2$, 0.25 μmole; guanosine triphosphate, uridine triphosphate, cytidine triphosphate, and adenosine triphosphate, 0.1 μmole each; one labeled nucleotide containing 0.045 μCi; and polymerase, 35 μg. Reactions were run at room temperature for 15 min, tubes were then chilled in ice, and 3 ml of cold 3.5% perchloric acid containing 50 mg of Celite per 100 ml was added. The acid-insoluble product was collected on filters. The discs were then dried and assayed for radioactivity

nine-, thymine-, or uracil-containing primers remained insensitive to streptozotocin.

Poly U-directed ^{14}C-phenylalanine and poly C-directed ^{14}C-proline incorporation in cell-free amino acid incorporation systems prepared as previously described were not affected by streptozotocin (11). This is of particular interest since difference spectroscopy has shown that poly C can interact with streptozotocin.

DISCUSSION

Streptozotocin causes rapid and extensive degradation of cellular DNA in *B. subtilis* cells. In contrast, no significant breakdown of isolated DNA and synthetic polydeoxyribonucleotides by streptozotocin was observed. Although there is no obvious explanation for this discrepancy, the DNA degradation process in cells might be accelerated by exonucleases after a limited number of single- and double-strand breaks have been induced by streptozotocin.

Streptozotocin also preferentially inhibits DNA synthesis as compared to RNA or protein synthesis in *B. subtilis* cells. The inhibition of DNA synthesis is only partial and is probably a reflection of a higher rate of streptozotocin-induced DNA degradation as compared to de novo synthesis.

Difference spectroscopy has shown that the antibiotic interacts exclusively with cytosine-containing mononucleotides and some polynucleotides but not with isolated DNA or cytosine-containing nucleosides. This interaction is *p*H dependent and reversible, and takes place within a *p*H range of 5.0 to 5.5 The cell-free macromolecular biosynthetic systems used are carried out in neutral or slightly alkaline buffer systems where little or no interaction with cytosine residues takes place. This would explain the moderate or absent interference of the antibiotic with nucleic acid and peptide synthesis in cell-free systems.

It is difficult to determine whether the described interaction of streptozotocin with cytosine-containing mononucleotides has any relationship with the observed rapid degradation of the cellular DNA fraction. It is conceivable that the *p*H within the cellular DNA fraction either at certain positions of the strands, the whole strand or during a certain physiological state might be sufficiently low to permit interaction to occur between the antibiotic and cytosine residues in DNA. This interaction in turn might result in strand breakage. No difference in the sedimentation profiles was observed if the DNA or polydeoxyribonucleotide-streptozotocin mixtures were incubated at *p*H 7.2 instead of *p*H 5.0 before analysis on alkaline sucrose gradients.

The cellular RNA fraction remains fairly stable in the presence of streptozotocin. If RNA-streptozotocin interaction occurs, the resulting modification does not appreciably impair stability or function of cellular RNA. This can be inferred from the observation that ribosomes remain stable and functional in the presence of streptozotocin and poly C remains an effective messenger for ^{14}C-proline incorporation in a cell-free amino acid incorporation system.

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