MOLECULAR PHARMACOLOGY, 6, 335-344

Effect of 2, 2′-(9, 10-Anthrylenedimethylene)bis (2-thiopseudourea) on Nucleic Acid Synthesis in Bacteria

PRISCILLA P. SAUNDERS AND GRADY F. SAUNDERS

From the Departments of Pathology and Developmental Therapeutics, The University of Texas M. D. Anderson Hospital and Tumor Institute at Houston, Houston, Texas 77025

(Received February 24, 1970)

SUMMARY

The mechanism of action of 2,2′-(9,10-anthrylenedimethylene)bis (2-thiopseudourea) (ATPU or pseudourea) has been investigated in bacterial systems. Inhibition of the growth of *Bacillus subtilis* by ATPU was reversed by the addition of nucleic acids. The synthesis of DNA and RNA *in vivo* was markedly inhibited by the drug. RNA synthesis *in vitro* catalyzed by purified DNA-dependent RNA polymerase (nucleosidetriphosphate:RNA nucleotidyltransferase, EC 2.7.7.6) of *Bacillus stearothermophilus* was sensitive to ATPU in a concentration-dependent manner. This inhibition was reversed by increasing the concentration of DNA primer. Evidence for a drug-DNA interaction was obtained from viscosity and cesium chloride buoyant density studies, which suggested the possible formation of intercalation complexes between DNA and ATPU.

INTRODUCTION

2, 2′-(9, 10-Anthrylenedimethylene)bis (2-thiopseudourea) (pseudourea) (Fig. 1) has been shown to possess significant growth-inhibitory activity against Leukemia L1210 (1) and is currently undergoing preliminary clinical trials in patients with metastatic cancer.[1] A microbiological assay for the drug has been developed by Pittillo and Woolley (2). The mechanism of action of ATPU, however, is unknown.

This report demonstrates the activity of ATPU[2] toward *Bacillus subtilis* and describes experiments relevant to the mechanism of action of the drug in this organism and in *Bacillus stearothermophilus*. The experiments presented indicate that ATPU is an effective inhibitor of the DNA-primed RNA polymerase of *B. stearothermophilus*.

This work was supported by American Cancer Society Grant T-465, United States Public Health Service Grant CA 06939, and Contract PH 43 66 1156 with the Cancer Chemotherapy National Service Center, National Cancer Institute.

[1] J. K. Luce and E. Frei, III, personal communication.

[2] The abbreviation used is: ATPU, 2,2′-(9,10-anthrylenedimethylene)bis(2-thiopseudourea).

MATERIALS AND METHODS

B. subtilis 168 was grown in a minimal medium described previously (3). Growth was carried out in 250-ml nephelometer flasks (19 × 130 mm side arms) with incubation at 37° in a New Brunswick G-77 water bath shaker. Absorbance measurements were made on a Bausch and Lomb Spectronic 20 colorimeter at 420 mμ.

B. stearothermophilus strain 10 was grown in 24-liter batches in Trypticase medium (4) until the middle of the logarithmic phase of growth. The cells were then chilled with ice, harvested by continuous flow centrifugation, and washed once with 0.05 M Tris-HCl, pH 7.5.

For short-term growth studies, as previ-

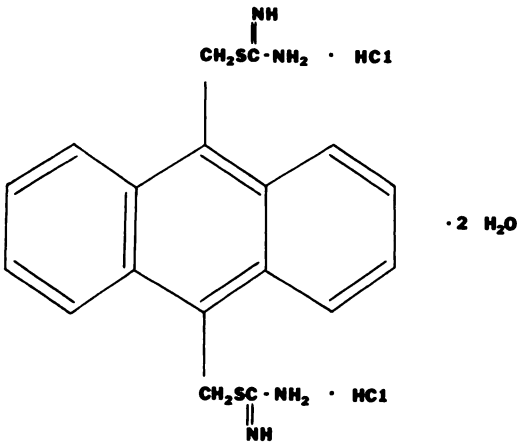

FIG. 1. *Structure of 2,2'-(9,10-anthrylenedimethylene)bis(2-thiopseudourea)*

ously described (5), cells were grown to an absorbance of approximately 0.35 and divided into 10–15-ml portions. The desired additions were made, and incubation was continued for 2–3 hr. Growth was monitored by absorbance readings at appropriate time intervals. Viable cells were determined as described previously (3). The methods used in the colorimetric determinations of the levels of DNA, RNA, and protein in growing cells have been described elsewhere (3).

Standard assay mixtures for RNA polymerase contained 15 mM magnesium acetate, 120 mM ammonium acetate, 0.01 M Tris-HCl (pH 8.3), 4.8 mM 2-mercaptoethanol, 1.6 mM concentration each of GTP, CTP, UTP, and ^{3}H-ATP (5 μCi/μmole), 28 μg/ml of calf thymus DNA, and 0.49–1.0 μg of RNA polymerase (described below) in a final volume of 0.25 ml. The salt concentrations, pH, and incubation temperature used were those described by Remold-O'Donnell and Zillig (6) as optimal for *B. stearothermophilus* RNA polymerase and were verified in our laboratory. Incubation was carried out for 30 min at 45°, and the reaction was terminated by the addition of 2 ml of cold 5% trichloracetic acid. Bovine serum albumin, 100 μg, was added as carrier. After 15 min at 4°, precipitates were collected on Reeve Angel glass fiber filters (2.4-cm diameter, grade 934AH), washed four times with 5-ml portions of cold 5% trichloracetic acid, dried, and counted in a Packard Tri-Carb liquid scintillation spectrometer. The counting solution contained 0.01% 1,4-bis[2-(4-methyl-5-phenyloxazolyl)]benzene and 0.4% 2,5- diphenyloxazole in toluene.

DNA-primed RNA polymerase of *B. stearothermophilus* was prepared essentially by the method of Remold-O'Donnell and Zillig (6), except for the omission of one step, centrifugation through a layered glycerol gradient in low salt buffer, which resulted in considerable losses of enzyme activity. The procedure involved suspending 30 g of cells in 30 ml of standard buffer containing 10 mM Tris-acetate, 22 mM NH_4Cl, 10 mM magnesium acetate, 1 mM 2-mercaptoethanol, and 0.25 mM EDTA, and blending for 15 min with 90 g of glass beads (0.1-mm i.d.). The temperature was maintained below 12° by immersing the container in ice water at 1.5-min intervals. Cell debris was removed by centrifugation at 15,000 rpm for 30 min in a Sorvall RC2-B refrigerated centrifuge. The supernatant fluid was then centrifuged for 20 hr at 34,000 rpm at 4° in a Spinco 50Ti rotor. The supernatant fraction was discarded, and the DNA-enzyme layer was peeled from the ribosomal pellet and dissolved in 20 ml of standard buffer. This solution was then adsorbed on an amount of DEAE-cellulose (equilibrated with standard buffer) equal to the wet weight of starting material. The cellulose was then washed three times with 300-ml portions of a special buffer (50 mM Tris-acetate, 10 mM magnesium acetate, 110 mM NH_4Cl, 0.25 mM EDTA, and 1 mM 2-mercaptoethanol, pH 7.3), packed into a column, and eluted with a linear gradient from 0.11 to 0.70 M NH_4Cl in special buffer (500 ml of each concentration.) The enzyme-containing fractions were combined and brought to 50% $(NH_4)_2SO_4$ saturation. After centrifugation at 15,000 rpm for 10 min, the supernatant fraction was brought to 70% saturation. The precipitate was collected by centrifugation in the same manner, dissolved in 2 ml of special buffer containing 0.5 M NH_4Cl, and dialyzed against 200 ml of the same buffer for 2 hr. One-half the resulting solution was layered onto two 34-ml linear gradients of 55–10% glycerol (w/w) in the special buffer containing 0.5 M NH_4Cl and centrifuged in an SW 27 rotor for 40 hr at 4° in a Spinco model L2-65

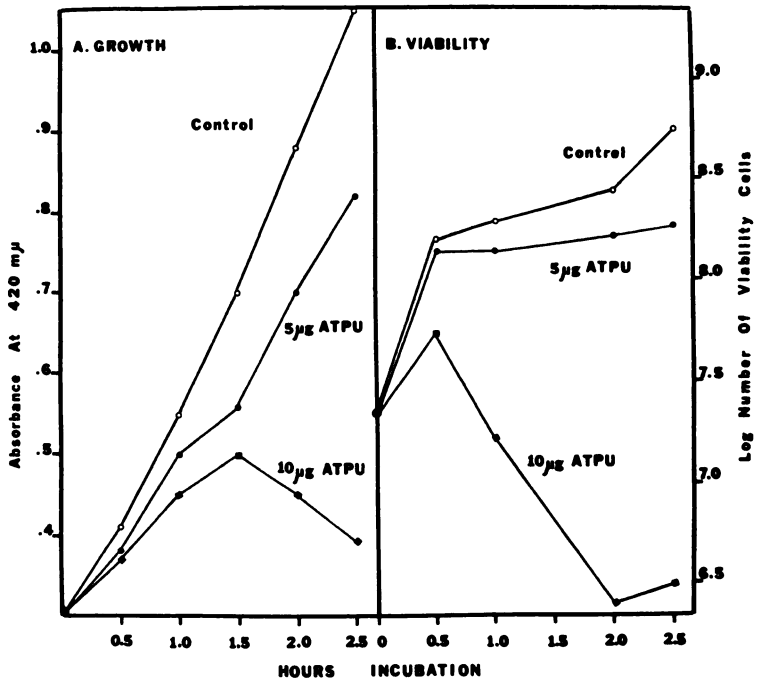

FIG. 2. *Effect of ATPU on growth (A) and viability (B) of B. subtilis*
Concentrations refer to micrograms of ATPU per milliliter. Incubation was carried out, with shaking, at 37°. Growth was measured by absorbance at 420 mμ, and viability was determined by plating appropriate dilutions on a complete medium as described previously (3).

ultracentrifuge. The tubes were pierced, and 1-ml fractions were collected. Enzyme-containing fractions were combined, stored at −20°, and used without further treatment. The enzyme preparation, containing 49 μg of protein per milliliter, catalyzed the incorporation of 3.26 μmoles of ATP per milligram of protein in 30 min under the standard assay conditions with a saturating level of calf thymus DNA template (68 μg/ml).

Viscosity measurements were made at 20° with a Zimm-Crothers low-shear viscometer (7) (Beckman Instruments) at a shear rate for H_2O of 1.4 sec^{-1}.

ATPU (NSC 56054; mol wt 463.5) and daunomycin (NSC 82151; mol wt 560) were obtained from the Cancer Chemotherapy National Service Center, National Cancer Institute. ATPU was obtained in the form of a solution at 3 mg/ml in propylene glycol. Before use, it was diluted to the desired concentration with deionized water. ^{3}H-ATP and ^{3}H-UTP were purchased from Schwarz BioResearch. Actinomycin D was purchased from Calbiochem.

B. stearothermophilus bacteriophage TP-84 DNA was prepared as previously described (8). *Escherichia coli* DNA was purified by the method of Marmur (9), and human DNA derived from leukocytes of patients with chronic lymphocytic leukemia was purified by the method of Kirby and Cook (10).

RESULTS

Inhibition of B. subtilis by ATPU. The effect of ATPU on growth of *B. subtilis* is shown in Fig. 2A. At a concentration of 5 μg/ml, the drug was slightly inhibitory, as indicated by the decreased growth rate. ATPU at 10 μg/ml caused a dramatic decline in viability (Fig. 2B) after 30 min of incubation, even though the cell mass (as measured by absorbance) continued to increase for another hour of incubation. These growth-inhibitory effects of ATPU are quite reproducible from one experiment to the next,

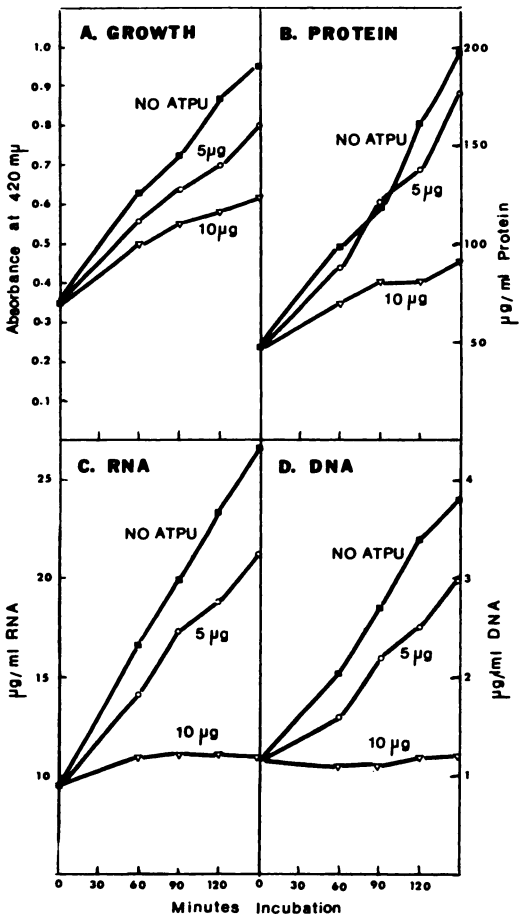

FIG. 3. *Effect of ATPU on levels of protein (B), RNA (C), and DNA (D) in growing B. subtilis*

Minimal medium containing the appropriate additions was inoculated as described in MATERIALS AND METHODS and dispensed into nephelometer flasks (50 ml/flask). Incubation was carried out as described in the text. At the designated intervals, one flask from each set was removed, divided into duplicate 25-ml portions, and placed in ice, and concentrated trichloracetic acid was added to 5%. The samples were allowed to stand overnight at 4° (the recovery was not altered by this step), and the precipitate was harvested by centrifugation and washed twice with cold 5% trichloracetic acid. The pellet was resuspended in 3 ml of 5% trichloracetic acid, heated at 98° for 30 min, cooled in an ice bath for 30 min, and centrifuged. The pellets were dissolved in 0.2 N NaOH by heating in boiling water for 5–10 min and were assayed for protein by the method of Lowry *et al.* (11). The supernatant fractions were assayed for RNA by a modified orcinol reaction (12) with ribose as the standard. DNA was determined by the method of Burton (13), using calf thymus DNA as the standard. Amounts of protein, RNA, and DNA are given in micrograms per milliliter of medium. Numbers on the curves refer to micrograms of ATPU per milliliter of growth medium.

provided that the amount of inoculum is the same. Slight variations in the number of cells present (A_{420}) at the onset of the experiment influence the degree of inhibition observed and the shapes of the growth curves. *S*-Methylthiuronium sulfate, a compound comparable to the side chains of ATPU, was also tested and found to have no inhibitory activity at concentrations up to 50 μg/ml. Propylene glycol alone had no effect on the growth of the cultures.

Effect of ATPU on protein, RNA, and DNA synthesis. When a growing culture of *B. subtilis* was exposed to a slightly inhibitory level of ATPU, 5 μg/ml, little or no effect was observed on macromolecular synthesis (Fig. 3). A higher level of the drug, however, appeared to halt DNA synthesis immediately. RNA accumulation increased gradually during the first 60 min of incubation and then ceased, while protein synthesis continued slowly. Thus, it appears that inhibition of synthesis of DNA and RNA may be considered the primary sites of action of ATPU.

Prevention of inhibition of growth by ATPU. The site of drug action is often indicated by determining the compound or class of compounds which will reverse the inhibitory effects of the drug. Several substances (i.e., deoxyguanosine, AMP, ATP, and nucleic acids) were tested in short-term growth experiments with *B. subtilis* for their ability to reverse the inhibitory effects of ATPU. Of these, only nucleic acids were effective. Figure 4 shows that while calf thymus DNA had essentially no effect on growth in the absence of ATPU, it could reverse ATPU inhibition at appropriate concentrations. A relatively high concentration of DNA, 200 μg/ml, was necessary to reverse completely the inhibition of growth caused by 10 μg/ml of ATPU.

When DNA was added to cultures at various times after the introduction of ATPU, as shown in Fig. 5, an apparent reversal of

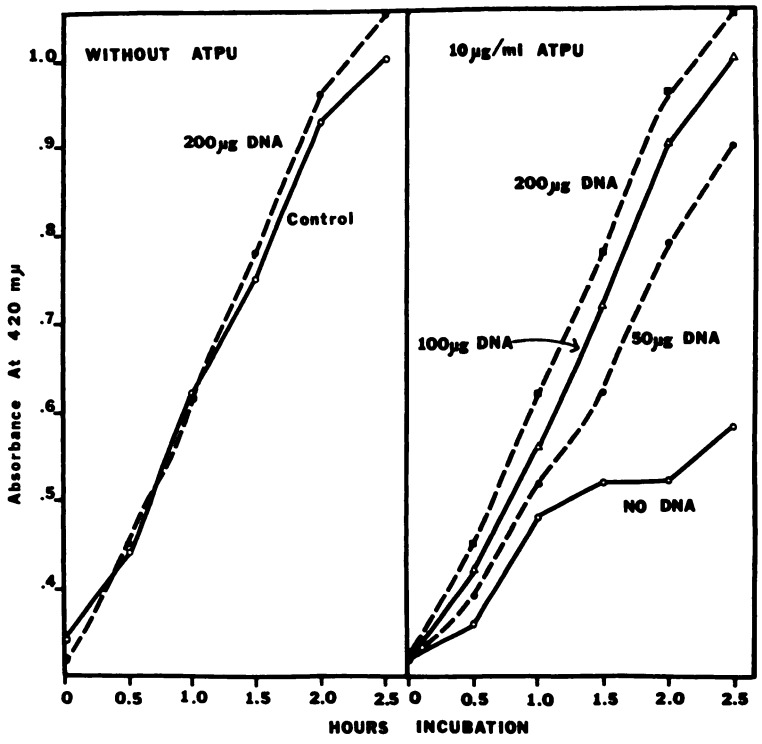

FIG. 4. *Effect of DNA on inhibition of growth of B. subtilis by ATPU*
Concentrations refer to micrograms of calf thymus DNA per milliliter. The experiment was conducted as described for Fig. 2.

inhibition was observed even when the DNA was introduced up to 60 min after ATPU. The abrupt decrease in absorbance (presumably due to cell lysis) following the addition of DNA at 30 to 60 min is difficult to explain without additional experiments; however, since Fig. 2 shows that the absorbance of an ATPU-inhibited culture can increase while the number of viable cells decreases, it is possible that the portion of the population that lysed may reflect those cells that were not viable. The remainder of the population was then able to continue growth without interference by ATPU. The most likely explanation of this reversal of inhibition is that the DNA interacted directly with ATPU, thus preventing its entrance into the cell. DNA has been shown to reverse the growth-inhibitory activities of certain other drugs, such as actinomycin (14), which were later found to exert their effects through the formation of DNA-drug complexes.

Effect of ATPU on RNA polymerase. The results shown in Fig. 3 indicate that the synthesis of both DNA and RNA in whole cells is markedly inhibited by ATPU. If ATPU does interact directly with DNA, as suggested by the reversal experiments, it might be expected to produce such a result by interfering with the enzymatic synthesis of either or both of these polynucleotides. This possibility was tested by measuring the effect of ATPU on cell-free RNA synthesis catalyzed by purified DNA-dependent RNA polymerase. Initial experiments were carried out with purified *E. coli* RNA polymerase; however, the work described here, which was carried out in more detail, employed the RNA polymerase from *B. stearothermophilus*, an obligate thermophile. The *B. stearothermophilus* enzyme is quite similar to the *E. coli* enzyme in its general properties (6) and sensitivity to ATPU, but is considerably more stable. Table 1 shows the comparative inhibitory activities of daunomycin, actinomycin D, and ATPU in this RNA-synthe-

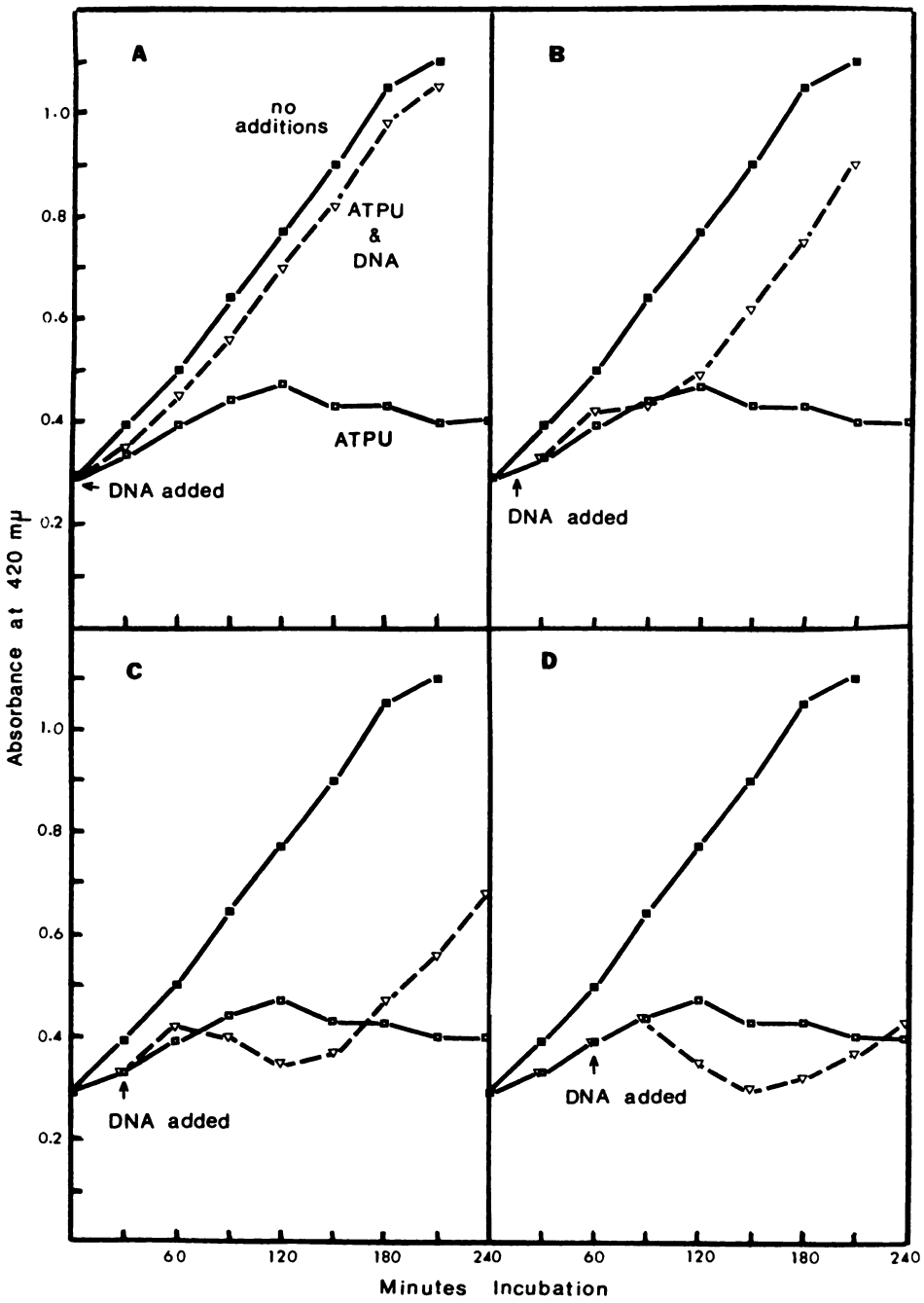

FIG. 5. *Effect of time of addition of DNA on its reversal of inhibition by ATPU*
Inhibition was effected with 7 μg/ml of ATPU. Growth was measured as described for Fig. 2, with the addition of 100 μg/ml of calf thymus DNA simultaneously with ATPU (A), or 15 min (B), 30 min (C), or 60 min (D) after ATPU was added.

TABLE 1

Inhibition of B. stearothermophilus RNA polymerase by ATPU

Incubation mixtures contained 7 μg of calf thymus DNA and 0.98 μg of RNA polymerase in the standard assay described in MATERIALS AND METHODS. Incubation was carried out for 30 min at 45°.

DNA primer	Additions	Drug concentration	^{3}H-ATP incorporated	Inhibition
		μM	mμmoles	%
+	—		2.46	0
−	—		0.16	
+	ATPU	2.72	1.42	42.3
−	ATPU	2.72	0.19	
+	ATPU	13.6	0.78	68.3
−	ATPU	13.6	0.16	
+	ATPU	40.8	0.28	93.5
−	ATPU	40.8	0.13	
+	Daunomycin	4.0	1.11	54.9
−	Daunomycin	4.0	0.10	
+	Daunomycin	10.0	0.40	83.7
−	Daunomycin	10.0	0.16	
+	Daunomycin	20.0	0.23	90.6
−	Daunomycin	20.0	0.14	
+	Actinomycin D	0.32	1.09	55.7
−	Actinomycin D	0.32	0.14	
+	Actinomycin D	3.20	0.57	76.8
−	Actinomycin D	3.20	0.14	
+	Actinomycin D	16.00	0.25	89.8
−	Actinomycin D	16.00	0.15	

sizing system. Actinomycin D is obviously the most inhibitory of the three compounds, giving 76% inhibition at a level of 3.2 mM. The ability of ATPU to inhibit RNA polymerase was somewhat less than that of daunomycin, but inhibition was nevertheless relatively efficient and depended on drug concentration. In subsequent experiments it was found that the DNA concentration employed in Table 1 was below saturation; however, all the reaction mixtures containing DNA were identical in this respect.

The incorporation of ^{3}H-UTP was also inhibited by ATPU, as shown in Table 2. This suggests that ATPU does indeed interfere with over-all RNA synthesis rather than specifically inhibiting ATP incorporation. In several experiments, the addition of ATPU,

TABLE 2

Inhibition of RNA polymerase by ATPU as measured by ^{3}H-UTP incorporation

Incubation mixtures contained 17 μg of calf thymus DNA and 0.49 μg of RNA polymerase in the standard assay described in MATERIALS AND METHODS. Incubation was carried out for 30 min at 45°. The labeled nucleotide was ^{3}H-UTP (1 μCi/μmole) instead of ^{3}H-ATP.

Incubation mixture	^{3}H-UTP incorporated	
	Without ATPU	With 40.8 μM ATPU
	mμmoles	
Complete	2.4	0.29
Complete; omit DNA	0.03	0.02

TABLE 3

Effect of nucleotide concentrations on inhibition of RNA polymerase by ATPU

Incubation mixtures contained the designated concentrations of nucleotides, 17 μg of DNA, and 0.49 μg of RNA polymerase in the standard assay. Incubation was carried out for 30 min at 45°. Each value is the average of duplicate analyses.

Incubation mixture	^{3}H-ATP incorporated		Inhibition by ATPU
	Without ATPU	With 40.8 μM ATPU	
	mμmoles		%
Complete (1.6 mM each ATP, GTP, CTP, UTP)	1.66	0.39	76.5
Complete (1.6 mM each ATP, GTP, CTP, UTP); omit DNA	0.11	0.09	
Complete (3.2 mM each ATP, GTP, CTP, UTP)	1.70	0.43	74.7
Complete (3.2 mM each ATP, GTP, CTP, UTP); omit DNA	0.15	0.18	

at concentrations somewhat higher than those used here, resulted in slight precipitation of nucleotides. This complication was avoided by using low concentrations of the drug.

In order to rule out the possibility of ATPU-nucleotide complex formation as the mechanism of inhibition, the experiment described in Table 3 was carried out. Doubling the nucleotide concentration of the incubation mixture did not significantly influence

TABLE 4

Effect of DNA concentration on inhibition of RNA polymerase by ATPU

Incubation mixtures contained various levels of calf thymus DNA and 0.49 μg of RNA polymerase in the standard assay. Incubation was carried out for 30 min at 45°. Each value is the average of duplicate analyses.

DNA content in standard incubation mixture	^{3}H-ATP incorporated		Inhibition with ATPU
	Without ATPU	With 40.8 μM ATPU	
μg	mμmoles		%
0	0.06	0.04	
17	1.29	0.46	64.4
34	1.18	0.81	31.4
51	1.18	1.04	11.9
68	1.16	1.25	0

TABLE 5

Poly A synthesis by B. stearothermophilus RNA polymerase, and effect of ATPU

Incubation mixtures contained various levels of calf thymus DNA and 0.49 μg of RNA polymerase in the standard assay. Incubation was carried out for 30 min at 45°. Each value is the average of duplicate analyses.

Incubation mixture	^{3}H-ATP incorporated				
		With ATPU			
	Without ATPU	27.2 μM ATPU	Inhibition	54.5 μM ATPU	Inhibition
	mμ-moles	mμ-mole	%	mμ-mole	%
Complete	1.84	0.98	46.8	0.52	71.8
Complete; omit DNA	0.08				
Complete; omit CTP, GTP, UTP	0.99	0.60	39.4	0.26	73.8
Complete; omit CTP, GTP, UTP, DNA	0.18				
Complete; omit CTP, GTP	0.41				
Complete; omit GTP, UTP	0.32				

the degree of inhibition produced by a constant level of ATPU. Conversely, 64% inhibition of RNA polymerase by ATPU could be reversed completely by increasing the concentration of the DNA primer 4-fold (Table 4).

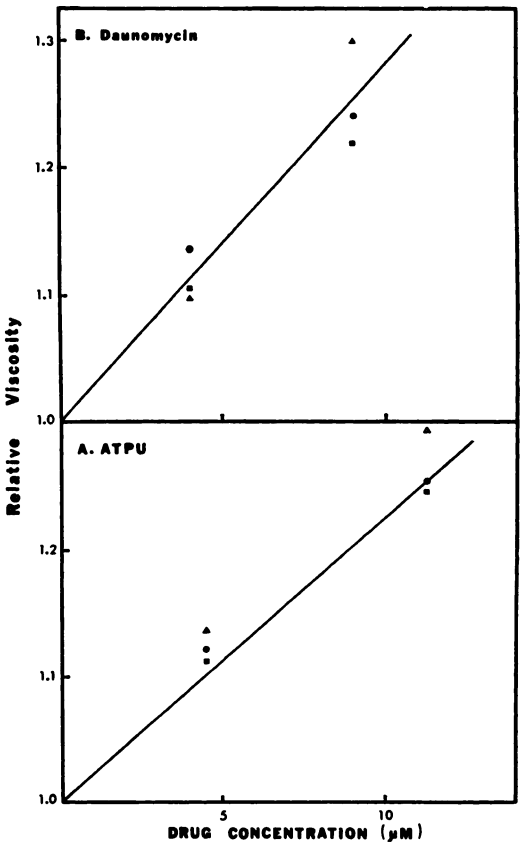

FIG. 6. *Effect of ATPU (A) and daunomycin (B) on viscosity of DNA*

□, TP-84 bacteriophage DNA; ○, *E. coli* DNA; △, human DNA. The DNA concentration was 10 μg/ml in a solvent containing 10^{-3} M Na_2HPO_4 and 10^{-4} M EDTA, pH 7.6. Relative viscosity = (viscosity of DNA + drug)/(viscosity of DNA).

Table 5 describes the synthesis of polyriboadenylic acid (poly A) by *B. stearothermophilus* RNA polymerase in the absence of CTP, GTP, and UTP. Poly A synthesis was dependent on the addition of DNA primer and was partially decreased by the addition of CTP or UTP. ATPU appeared to inhibit poly A synthesis to approximately the same extent as this compound inhibited RNA synthesis in the complete incubation mixture.

Effect of ATPU on viscosity and buoyant density of DNA. Direct evidence for the interaction of ATPU with DNA was obtained

TABLE 6.

Effect of added ATPU on buoyant density of DNA in a CsCl density gradient

Increasing amounts of ATPU were added to 4 ml of ^{32}P-labeled phage (*B. subtilis* phage SP02) DNA in a solvent containing 10^{-3} M Na_2HPO_4 and 10^{-4} M EDTA, pH 7.6. Solid CsCl was added to give an initial buoyant density of 1.690 g/ml. Samples were centrifuged for 24 hr in the SW 50.1 rotor at 45,000 rpm and 18° in a Spinco L2-65B ultracentrifuge. Fractions (0.15 ml) were collected directly into counting vials, and radioactivity was monitored by Cerenkov counting (16). The buoyant density was computed from refractive index measurements. Radioactive phage SP02 DNA was kindly provided by Mr. Bruce Dishman and had a ρ of 1.704 and a G + C content of 43%. Each tube contained 2.5 μg of DNA (2.5×10^7 cpm of ^{32}P).

ATPU added	Buoyant density shift
μg	g/ml
0	0
30	0.006
120	0.010
180	0.016
240	0.020

from viscosity studies. The addition of ATPU to DNA solutions (10 μg/ml) results in a marked increase in viscosity (Fig. 6). Measurements were limited to low ATPU concentrations, since considerable variation in viscosity was observed at 10 μg/ml (22 μM) ATPU (sometimes completely stopping the viscometer rotor). The compositional difference in the DNAs studied was not great, and the effect of the nucleotide base composition of the DNA on the viscosity of drug-DNA complexes did not appear to be profound, since *E. coli* DNA (50% G + C) was only slightly more responsive than human chronic lymphocytic leukemia leucocyte DNA (40% G + C) or bacteriophage TP-84 DNA (42% G + C). The enhanced viscosity of drug-DNA complexes satisfies one of the major requirements for intercalative drugs (15). Further evidence for intercalation of ATPU is shown by the decrease in buoyant density of DNA in a CsCl density gradient (Table 6). Since these measurements were made in a high salt (7.7 M CsCl) medium, the decreased viscosity is presumptive evidence for tight binding, i.e., intercalation of the drug with DNA. Satisfaction of other requirements for the intercalation of DNA by drugs, such as reduced sedimentation coefficient, spectral shifts, and increased melting temperature, are complicated by the extremely high absorption of ATPU at 260 mμ. The changes in viscosity and buoyant density strongly support the thesis that ATPU could, indeed, exert its effect through the formation of DNA-ATPU complexes.

DISCUSSION

The essence of this report can be summarized as follows: (a) ATPU is an effective inhibitor of the growth of *B. subtilis*; (b) it interferes with the accumulation of protein, DNA, and RNA *in vivo*; (c) it can inhibit the activity of *B. stearothermophilus* RNA polymerase *in vitro*, presumably as the result of a DNA-ATPU interaction, as shown by viscometry; and (d) viscosity and CsCl buoyant density studies suggest that ATPU may form intercalative complexes with DNA similar to those obtained with daunomycin, ethidium bromide, and nogalamycin (8, 15, 17).

The presence of an anthracene nucleus in ATPU may facilitate binding with nucleic acids by intercalation similar to that found with actinomycin, daunomycin, ethidium, and related antibiotics. Presumably this occurs by insertion of the planar anthracene nucleus between adjacent base pairs in the DNA helix (14). Conventional methods of detecting such interactions depend primarily upon ultraviolet absorption measurements (i.e., changes in the drug absorption spectrum, DNA melting profile, DNA buoyant density in CsCl solution, and sedimentation coefficient). ATPU, however, has a much higher molar extinction coefficient than DNA at the same wavelength (5 μg/ml of ATPU gives A_{260} 2.0), thus making DNA absorbance studies ineffective for this purpose. It was possible, however, to detect changes in DNA buoyant density in CsCl by using isotopically labeled DNA. DNA viscosity changes in the presence of ATPU have also provided a simple means of detecting an interaction, although this method is not adequate for a detailed characterization of the complex formed.

Although one cannot generalize regarding

the biological effects of drugs which interact with nucleic acids, it may be informative to compare the structural and inhibitory properties of certain well-studied antibiotics with those of ATPU. The ability of ATPU to interfere with both DNA and RNA synthesis parallels that of ethidium bromide (18) and proflavine (19, 20). The latter two compounds have been shown to form complexes by intercalation between adjacent base pairs of the DNA helix (21, 22).

The chromophores of actinomycin and daunomycin are intercalated between the base pairs of DNA; however, they preferentially inhibit RNA synthesis rather than DNA synthesis (21–23). Controversy over the effects of actinomycin on the hydrodynamic properties of DNA (14, 23) has led to the finding that the molecular weight of the DNA used can influence the results obtained (24). Low molecular weight DNA–actinomycin complexes behave as true intercalated DNA (increased viscosity and decreased sedimentation coefficient), while the addition of actinomycin to larger DNA molecules (mol wt 10^8) results in a decrease in viscosity. Müller and Crothers (24) attributed the behavior of the high molecular weight DNA complexes to side effects due to the peptide rings.

It is of interest that an excellent correlation exists between the ratios of concentrations required to produce a given degree of RNA polymerase inhibition for the various drugs and the ratios of the doses found to be safe, but to produce some degree of toxicity in man. For example, the tolerated dose of a 5-day course of ATPU is 750 mg/m^2 of body surface; for daunomycin, 150 mg/m^2; and for actinomycin D, 1.5 mg/m^2.[3] This general ratio also obtains in toxicity studies in rodents. The ratio corresponds closely to the concentrations required to inhibit RNA polymerase *in vitro*. This is also true for the drug concentrations required to produce inhibition of *B. subtilis* cell growth (15). These correlations suggest that inhibition of DNA-dependent RNA biosynthesis may be the primary mechanism of action; that is, the one responsible for the growth-inhibitory activity of these compounds.

[3] E. Frei, III, personal communication.

ACKNOWLEDGMENTS

The authors are indebted to Mrs. V. Liu, Miss G. Schultz, and Miss E. Fortin for excellent technical assistance. We thank Dr. E. Frei, III, for discussions on the clinical aspects of ATPU.

REFERENCES

1. S. K. Carter, *Cancer Chemother. Rep.* **1**, 153 (1968).
2. R. F. Pittillo and C. Woolley, *Appl. Microbiol.* **18**, 519 (1969).
3. P. P. Saunders, G. A. Schultz and G. F. Saunders, *J. Bacteriol.* **96**, 560 (1968).
4. G. F. Saunders and L. L. Campbell, *J. Bacteriol.* **91**, 340 (1966).
5. P. P. Saunders and G. A. Schultz, *Biochem. Pharmacol.* In press.
6. E. Remold-O'Donnell and W. Zillig, *Eur. J. Biochem.* **7**, 318 (1969).
7. B. H. Zimm and D. M. Crothers, *Proc. Nat. Acad. Sci. U. S. A.* **48**, 905 (1962).
8. G. F. Saunders, W. N. Reese and P. P. Saunders, *Biochim Biophys. Acta* **190**, 406 (1969).
9. J. Marmur, *J. Mol. Biol.* **3**, 208 (1961).
10. K. S. Kirby and E. A. Cook, *Biochem. J.* **104**, 254 (1967).
11. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. Biol. Chem.* **193**, 265 (1951).
12. G. Ashwell, *Methods Enzymol.* **3**, 73 (1957).
13. K. Burton, *Biochem. J.* **62**, 315 (1956).
14. W. Kersten, H. Kersten and H. M. Rauen, *Nature* **187**, 60 (1960).
15. W. Kersten, H. Kersten and W. Szybalski, *Biochemistry* **5**, 236 (1966).
16. T. Clausen, *Anal. Biochem.* **22**, 70 (1968).
17. D. C. Ward, E. Reich and I. H. Goldberg, *Science* **149**, 1259 (1965).
18. M. J. Waring, *Biochim. Biophys. Acta* **87**, 358 (1964).
19. I. H. Goldberg and M. Rabinowitz, *Science* **136**, 315 (1962).
20. J. Hurwitz, J. J. Furth, M. Malamy and M. Alexander, *Proc. Nat. Acad. Sci. U. S. A.* **48**, 1222 (1962).
21. L. S. Lerman, *J. Cell. Comp. Physiol.* **64**, Suppl. 1, 1 (1964).
22. M. J. Waring, *J. Mol. Biol.* **13**, 269 (1965).
23. M. Gellert, C. E. Smith, D. Neville and G. Felsenfeld, *J. Mol. Biol.* **11**, 445 (1965).
24. W. Müller and D. M. Crothers, *J. Mol. Biol.* **35**, 251 (1968).