[CANCER RESEARCH 36, 1664-1668, May 1976]

Effect of Bleomycin on Deoxynucleotide-polymerizing Enzymes from Human Cells[1]

R. DiCioccio and B. I. Sahai Srivastava[2]

Department of Experimental Therapeutics and Grace Cancer Drug Center, Roswell Park Memorial Institute, New York State Department of Health, Buffalo, New York 14263

SUMMARY

DNA polymerases α and β from Molt-4 cells are inhibited by bleomycin, whereas DNA polymerase γ assayed with poly(A)·$(dT)_{12-18}$ as the template primer or terminal deoxynucleotidyl transferase assayed with activated DNA, poly(dA), $(dG)_{12-18}$, or $(dA)_{12-18}$ as the initiator are not inhibited by this antibiotic. Inhibition by bleomycin increased the K_m for template DNA but not that for dTTP. Increasing amounts of bleomycin did not affect the V_{max} for DNA polymerase α or β when the amount of template DNA was varied but it reduced the V_{max} for these enzymes when dTTP was varied. Moreover, the addition of extra template reversed the bleomycin inhibition but the addition of extra enzyme did not. Although dithiothreitol was required for bleomycin inhibition of DNA polymerase activity, bleomycin preincubated with dithiothreitol (or β-mercaptoethanol) at pH 6.5 to 9.0 lost its inhibitory activity. This was not the case when DNA was also included in the preincubation mixture. The results obtained in this study indicate that bleomycin inhibits DNA polymerases α and β by a thiol reagent-dependent interaction with the template. Thus, the antitumor activity of bleomycin may be greatly influenced by the concentration of sulfhydryl compounds and their proximity to DNA in the target cells.

INTRODUCTION

Bleomycin, a glycopeptide antibiotic isolated from *Streptomyces verticillus* (23), inhibits both bacterial and mammalian cell growth (5, 6, 9, 19, 22), and possesses antitumor activity against certain tumors such as squamous cell carcinoma (24). Although this drug has been reported to inhibit DNA synthesis (9, 11, 19, 27) and cause strand scission of DNA (1, 4, 10, 12, 13, 18, 20, 21) both *in vitro* and *in vivo*, its effect on the activity of DNA polymerases has been contradictory. For example, bleomycin has been reported to inhibit (9, 11, 27), stimulate (26), or have no effect (11) on DNA polymerase activity *in vitro*. To clarify this situation, we have examined the effect of bleomycin on the activities of TDT[3] and DNA polymerases α, β, and γ from human cells.

MATERIALS AND METHODS

Cells. Molt-4 cells are of acute lymphoblastic leukemia origin (16). Their culturing and harvesting has been described (16).

Enzyme Extraction. About 10 g of Molt-4 cells were homogenized with 50 ml of Buffer A (25 mM Tris-sulfate, pH 8.3; 1 mM $MgSO_4$; 6 mM NaCl; 4 mM dithiothreitol; and 0.1 mM EDTA) and the homogenate was used for the preparation of the 37,000 × *g* supernatant (soluble fraction) and purified chromatin (17). The soluble fraction was used for the purification of DNA polymerases α and γ as described below.

The chromatin was extracted for 4 hr with 100 ml of 1 M NaCl dissolved in Buffer B (0.01 M Tris-HCl, pH 8.0, containing 0.01 M β-mercaptoethanol) and the extract was dialyzed against 600 ml of Buffer B (16). The DNA-histone precipitate formed was removed by centrifugation, and the proteins in the supernatant were precipitated by 70% saturation with ammonium sulfate. The precipitate was dissolved in 2 ml of Buffer C (Buffer A containing 0.1 M NaCl and 10% glycerol) and dialyzed for 4 to 5 hr against Buffer C. This chromatin fraction was used for the purification of TDT and DNA polymerases β and γ.

Preparation of DNA Polymerase α and γ from the Soluble Fraction. The soluble fraction was dialyzed against Buffer D (Buffer A containing 10% glycerol) and chromatographed on DEAE-cellulose (DE-23) and phosphocellulose (P-11) columns as described previously (16). Pooled fractions from the phosphocellulose column containing DNA polymerases α and γ were dialyzed against Buffer E (0.02 M potassium phosphate, pH 7.5, containing 20% glycerol and 1 mM dithiothreitol) and loaded on a column (1 × 5 cm) of hydroxylapatite (Bio Gel-HT) equilibrated with Buffer E (15). After washing with 25 ml of Buffer E, the column was developed with a linear gradient formed with 50 ml of Buffer E and 50 ml of 0.3 M potassium phosphate, pH 7.5, containing 20% glycerol and 1 mM dithiothreitol. The column was subsequently washed with 20 ml of 0.5 M potassium phosphate, pH 7.5, containing 20% glycerol and 1 mM dithiothreitol. DNA polymerase α eluted at 0.12 M and DNA polymerase γ eluted at 0.3 to 0.5 M potassium phosphate.

Preparation of TDT and DNA Polymerases β and γ from the Chromatin Fraction. The dialyzed chromatin fraction was loaded on a column (2 × 40 cm) of Sephadex G-100 equilibrated with Buffer C. The column was developed with Buffer C. The 3 to 4 S DNA polymerase β, which aggregated to 7 S under these conditions (16), was eluted together with DNA polymerase γ near the void volume of the column. The 3 to 4 S TDT did not aggregate and eluted later in the same position as the ovalbumin marker.

Fractions from the Sephadex G-100 column containing TDT were pooled, dialyzed against Buffer F (50 mM Tris-HCl, pH 7.5; 20 mM KCl; 1 mM dithiothreitol; 0.1 mM EDTA; and 20% glycerol) and loaded on a column (1.5 × 10 cm) of

[1] This work was supported in part by Grants CA-13038, CA-16045, and CA-17140 from the National Cancer Institute, USPHS.

[2] To whom requests for reprints should be addressed.

[3] The abbreviation used is: TDT, terminal deoxynucleotidyl transferase.

Received November 17, 1975; accepted February 5, 1976.

DEAE-Sephadex A-25 equilibrated with Buffer F. The column was developed step-wise first with Buffer F and then with Buffer F containing 0.2 M KCl. Under these conditions, TDT is not bound (14), and any contaminating DNA polymerase β and γ, if present, are bound and eluted later with Buffer F containing 0.2 M KCl. Fractions containing TDT were pooled and chromatographed further on a phosphocellulose column as described previously (16).

Fractions from the Sephadex G-100 column containing DNA polymerases β and γ were pooled, dialyzed against Buffer E, and chromatographed on a column of hydroxylapatite as described above. DNA polymerase β was eluted at about 0.16 M and DNA polymerase γ was eluted at 0.3 to 0.5 M potassium phosphate. All final enzyme preparations were dialyzed against Buffer A containing 50% glycerol and stored at −20°. The specific activities of DNA polymerase α, β, γ, and TDT used in this study were 426, 141, 146, and 315 units/mg protein, respectively. One unit of DNA polymerase or TDT activity equals 1 nmole of radioactively labeled nucleotide polymerized per hr at saturating substrate concentration. The amount of protein in the enzyme preparations was estimated by absorbance measurements, using the relationship: $(A_{280} \times 1.54) - (A_{260} \times 0.76)$ = mg/ml of protein (25). The template primer responses of all above enzymes have been published previously (16).

Enzyme Assays. Except where noted, the assay mixtures in 0.2 ml final volume contained: *(a)* for DNA polymerases α or β, 10 μmoles of Tris-HCl (pH 8.3); 1.2 μmoles of magnesium acetate; 4 μmoles of dithiothreitol; 2 μCi of [^{3}H] dTTP (specific activity, 9 mCi/μmole); 0.16 μmole each of dATP, dCTP, and dGTP; enzyme; and 12 μg of activated (16) calf thymus DNA; *(b)* for DNA polymerase γ, 10 μmoles of Tris-HCl (pH 7.5), 0.1 μmole of $MnCl_2$, 20 μmoles of KCl, 4 μmoles of dithiothreitol, 90 μg of bovine serum albumin, 2 μCi of [^{3}H]dTTP (specific activity 15 mCi/μmole), enzyme, and 8 μg of poly(A)·$(dT)_{10}$; (c) for TDT, 10 μmoles of Tris-HCl (pH 7.5), 0.1 μmole of $MnCl_2$, 20 μmoles of KCl, 4 μmoles of dithiothreitol, 90 μg of bovine serum albumin, 2 μCi of [^{3}H]dGTP (specific activity 9 mCi/μmole), enzyme, and either 8 μg of $(dG)_{12-18}$, $(dA)_{12-18}$, poly(dA), or 12 μg of activated calf thymus DNA. Except where mentioned otherwise, the addition sequence involved template-primer, bleomycin, other components of the assay mixture and, finally, the enzyme. Bleomycin solutions stored less than a week at −20° were used, since longer storage leads to a loss of activity. After 30 min incubation at 37° (30° for DNA polymerase γ), 50 μg of yeast RNA and 1 ml of 20% trichloroacetic acid containing 3% sodium pyrophosphate were added. The precipitates were collected on nitrocellulose membrane filters, washed with 5% trichloroacetic acid, dried, and counted with the use of toluene-based scintillation fluid (16). All reactions were linear with respect to time and enzyme concentration.

RESULTS

The data in Table 1 show that bleomycin is a potent inhibitor of DNA polymerases α and β of Molt-4 cells. The same is also true of DNA polymerase α from phytohemagglutinin-stimulated normal human lymphocytes (data not shown). However, bleomycin did not significantly inhibit DNA polymerase γ or TDT, even when activated DNA was used as the initiator with the latter enzyme.

To determine the mechanism of inhibition of DNA polymerases α and β by bleomycin, kinetic experiments involving variation of substrate (dTTP) or template (activated DNA) concentration were conducted. The data in Charts 1 and 2 show that bleomycin increased the K_m for template DNA but not that for dTTP. Furthermore, the increasing amounts of bleomycin did not affect the V_{max} for DNA polymerase α or β when the amount of template DNA was varied, but it reduced the V_{max} for these enzymes when dTTP was varied in the presence of excess template. Qualitatively similar results were obtained with DNA polymerase α from phytohem-

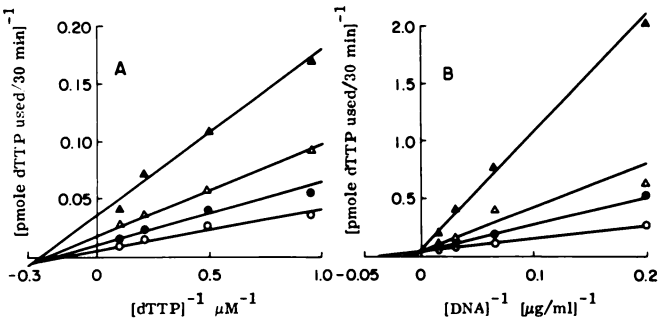

Chart 1. Inhibition of DNA polymerase α by bleomycin. Data plotted according to the method of Lineweaver and Burk (8). The standard DNA polymerase assay mixture containing various concentrations of dTTP (μM) and bleomycin (if added) were used for Chart 1*A*, whereas standard DNA polymerase assay mixture containing various concentrations of activated DNA (μg/ml) and bleomycin (if added) were used for Chart 1*B*. The amount of protein used in the assays was 1.4 μg. Bleomycin concentrations were: ○, 0 μg/ml; ●, 3 μg/ml; △, 5 μg/ml; ▲, 10 μg/ml. K_m for dTTP = 6.3 μM from Chart 1*A* and for activated DNA = 28 μg/ml from Chart 1*B*.

Table 1

Effect of bleomycin on the activity of deoxynucleotide-polymerizing enzymes from Molt-4 cells

The amount of protein used in the assays was 1.4, 2.2, 2.4, and 2.4 μg for DNA polymerase α, β, and γ, and TDT, respectively.

	[^{3}H]TMP (cpm) incorporated			[^{3}H]GMP (cpm) incorporated in following TDT			
Bleomycin (μg/ml)	DNA polymerase α	DNA polymerase β	DNA polymerase γ	Activated DNA initiator	$(dG)_{12-18}$ initiator	$(dA)_{12-18}$ initiator	Poly(dA) initiator
0	80,102 (100)[a]	54,958 (100)	39,422 (100)	56,485 (100)	19,271 (100)	121,331 (100)	6,851 (100)
1	80,959 (101)	44,933 (82)	39,816 (101)				
10	19,222 (24)	11,197 (20)	41,191 (105)	55,366 (98)	18,996 (99)	144,398 (119)	5,620 (82)
100	12,015 (15)	6,595 (12)	29,322 (75)	44,495 (79)	19,724 (102)	91,829 (76)	5,646 (82)

[a] Numbers in parentheses, percentage of control.

agglutin-stimulated normal human lymphocytes (data not shown). These results indicate that bleomycin reduces the affinity of template for enzyme. Consequently, increasing the concentration of template DNA should overcome the inhibition by bleomycin. Table 2 shows that this is indeed the case, as addition of extra template (DNA) 10 min after the start of a reaction overcomes bleomycin inhibition, whereas the addition of extra enzyme does not. In addition, if the amount of enzyme used at the start of the reaction is varied 28-fold, the same percentage inhibition is obtained with a given amount of bleomycin. The above results therefore suggest that bleomycin inhibits DNA polymerases α and β by interaction with the template rather than with the enzyme. To further examine the mechanism of interaction of bleomycin with DNA, the changes in the amount of product recovered with time in the presence of bleomycin were examined. The data in Chart 3 show that the addition of bleomycin 10 min after the start of a reaction resulted in immediate cessation of the reaction. Also, the addition of bleomycin at the beginning of the assay completely inhibited the reaction after a low level of synthesis which was undetectable when bleomycin was added after the reaction started. In either case, however, the amount of newly synthesized DNA did not decrease with time. This indicates that if bleomycin inhibits the DNA polymerase activity by interacting with the template, degradation of DNA to acid-solu-

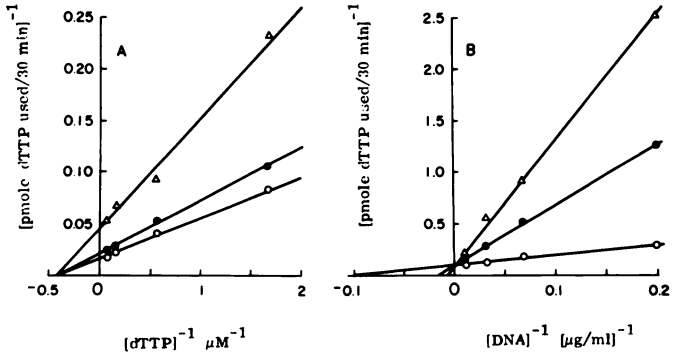

Chart 2. Inhibition of DNA polymerase β by bleomycin. Data plotted according to Lineweaver and Burk (8). Assays were done in the same way as given for DNA polymerase α in Chart 1. The amount of protein used in the assays was 3 μg. Symbols also same as in Chart 1. K_m for dTTP is 2.4 μM from Chart 2A and for activated DNA it is 9.4 μg/ml from Chart 2B.

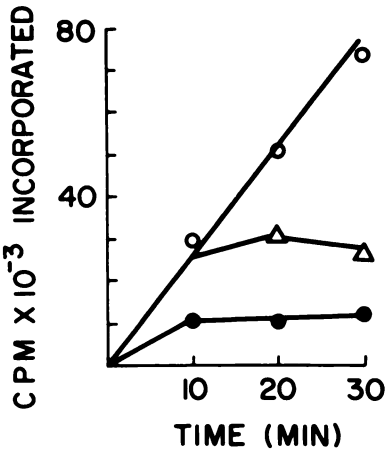

Chart 3. Kinetics of bleomycin inhibition of DNA polymerase α. DNA polymerase activity was assayed as given under "Materials and Methods." The amount of protein used in the assays was 1.4 μg. ○, DNA polymerase activity without bleomycin; ●, bleomycin, 100 μg/ml, present from the start of the reaction; △, bleomycin, 100 μg/ml, added 10 min after the start of the reaction.

Table 2

Effect of enzyme or template addition 10 min after the start of the reaction or variation in the amount of initial enzyme on bleomycin inhibition

A 0.2-ml final volume of the reaction mixture and an incubation period of 30 min were used. The amount of protein used in the assays was 0.4 and 1.6 μg for DNA polymerase α and 0.6 and 2.4 μg for DNA polymerase β in Experiment 1 and 0.06, 0.3, and 1.7 μg for DNA polymerase α and 0.6 and 3.0 μg for DNA polymerase β in Experiment 2.

Reaction conditions	DNA polymerase α		DNA polymerase β	
	cpm incorporated	% of control	cpm incorporated	% of control
Experiment 1				
A. 1 μl initial enzyme plus 12 μg DNA	31,239	100[a]	14,484	100[a]
B. A + 1 μg bleomycin	11,135	36	6,228	43
C. A + 4 μl enzyme added 10 min after the start of the reaction	87,419	100[a]	59,613	100[a]
D. C + 1 μg bleomycin	37,567	43	28,852	48
E. A + 24 μg DNA added 10 min after the start of the reaction	29,432	100[a]	14,178	100[a]
F. E + 1 μg bleomycin	24,466	83	11,244	79
Experiment 2				
A. 1 μl enzyme and 12 μg DNA	4,466	100[a]	13,682	100[a]
B. A + 1 μg bleomycin	1,682	38	3,564	26
C. 5 μl enzyme and 12 μg DNA	21,412	100[a]	71,265	100[a]
D. C + 1 μg bleomycin	8,264	38	21,131	30
E. 28 μl enzyme and 12 μg DNA	106,631	100[a]		
F. E + 1 μg bleomycin	47,688	45		

[a] Control.

ble fragments does not occur concurrently.

During the course of this work it was noticed that bleomycin that was preincubated (15 min at 37°) with either DNA polymerase α or β and assay mixture (without DNA) lost its inhibitory activity against these enzymes. However, if bleomycin was preincubated with the assay mixture containing DNA either in the presence or in the absence of the enzyme, inhibition was observed (Table 3). In addition, bleomycin preincubated with the enzyme alone was inhibitory. This suggested that one or more components of the assay mixture was responsible for the inactivation of bleomycin in the absence of DNA. Therefore, components of the assay mixture individually and in combination were preincubated with bleomycin. Only a combination of Tris-HCl buffer and dithiothreitol or of magnesium acetate and dithiothreitol inactivated bleomycin (data not shown). β-Mercaptoethanol could substitute for dithiothreitol in the inactivation of bleomycin. Unbuffered dithiothreitol (pH 4.6) did not inactivate bleomycin. Moreover, an adequate amount of dithiothreitol (2 to 20 mM) was required for bleomycin to interact with DNA and inhibit the DNA polymerase reaction (data not shown) (27). Since Tris-HCl buffer and magnesium acetate both have a pH of about 8.0, it was considered that both the alkaline pH and dithiothreitol were probably required to inactivate bleomycin. Indeed, preincubation of bleomycin with either $MgCl_2$ (pH 5.7) or magnesium acetate (adjusted to pH 4.6) in the presence of dithiothreitol did not inactivate the drug, but preincubation with phosphate buffer, pH 8.3, or glycine-NaOH buffer, pH 8.3, in the presence of dithiothreitol caused inactivation. The data in Chart 4 show the bleomycin in the absence of DNA is inactivated by dithiothreitol in the pH range 6.5 to 9.0. Since bleomycin is not inactivated by dithiothreitol at any pH as long as DNA is also present, the reaction among bleomycin, dithiothreitol, and DNA must be very rapid and the product formed should be resistant to inactivation, as is apparent by inhibition of DNA polymerase α or β by bleomycin in assay mixture buffered at pH 8.3 and containing dithiothreitol.

Table 3

Inhibition of DNA polymerase α by bleomycin preincubated with various components of the reaction system

After preincubation (15 min at 37°) the missing components required for DNA polymerase activity were added and the complete system was incubated for 30 min at 37°. The amount of protein used in the assays was 1.4 μg. The control without bleomycin gave 99,987 cpm. DNA polymerase β yielded similar results.

	% inhibition of DNA polymerase α activity
No preincubation (complete system containing bleomycin, 5 μg/ml)	65
Bleomycin (5 μg/ml) preincubated with:	
Assay mixture containing DNA, no enzyme	61
Assay mixture without DNA, plus enzyme	8
Assay mixture without DNA and enzyme	0
Enzyme	52
DNA	59
H_2O	55

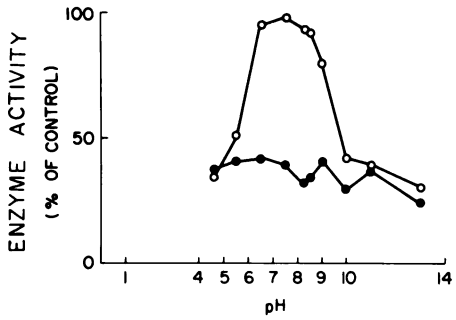

Chart 4. Effect of bleomycin, preincubated at different pH, on DNA polymerase activity. The control without bleomycin, gave 84,397 cpm. *Open circles:* bleomycin (5 μg/ml) was preincubated with various buffers (0.01 M) and dithiothreitol (0.02 M) for 15 min 37°. Activated DNA (60 μg/ml); Tris-HCl, pH 8.3 (50 μM); magnesium acetate (6 mM); dATP, dCTP, and dGTP (each 0.8 mM); [^{3}H]dTTP of specific activity 9 mCi/μmole (10 μCi/ml); and DNA polymerase α (1.4 μg protein) were then added in a final volume of 0.2 ml and the incubation was continued for 30 min at 37°. Trichloroacetic acid-precipitable radioactivity was collected and counted as described in "Materials and Methods." *Closed circles:* same as above except that activated DNA was pipetted as the first constituent of the preincubation mixture. Buffers used for preincubation were: Sodium acetate-acetic acid for pH 4.6, 5.5, and 6.5; Tris-HCl for pH 7.5, 8.3 and 9.0; glycine-NaOH for pH 10 and 11; and NaOH for pH 13. Preincubation of bleomycin with any one of the above buffers in the absence of dithiothreitol did not inactivate bleomycin and resulted in inhibition similar to that shown by the *closed circles*, provided that DNA was added to the assay mixture before dithiothreitol.

DISCUSSION

Three lines of evidence indicate that bleomycin inhibits DNA polymerases α and β by interacting with the DNA template and thus impairing its use for replication. First, bleomycin increases the apparent K_m of template DNA, implying a diminished affinity of template for enzyme. Second, the addition of extra template overcomes bleomycin inhibition but extra enzyme does not. Third, preincubation of bleomycin with either dithiothreitol or β-mercaptoethanol at pH 6.5 to 9.0 prevents the inhibition by bleomycin, but inclusion of template in the preincubation does not. The latter observation is especially interesting since thiol compounds have been found to enhance bleomycin inhibition of DNA polymerase from Molt-4 cells (data not shown) and *Escherichia coli* (27) as well as the degradation of DNA *in vitro* (1, 10, 13, 18, 20). Consequently, it may be that dithiothreitol and bleomycin at pH 6.5 to 9.0 react to form an active intermediate which can interact with DNA and make it inactive as a template. If DNA is not present, the active intermediate is converted into an inactive form. Alternatively, the inactivation of bleomycin as a result of preincubation with dithiothreitol at pH 6.5 to 9.0 and the action of bleomycin when preincubated with DNA and dithiothreitol at pH 6.5 to 9.0 may be different but competing reactions. If the latter occurs faster than the former, inhibition of DNA polymerase activity will be observed in the presence of bleomycin, DNA, and dithiothreitol at pH 6.5 to 9.0.

Bleomycin was found in this study to increase the K_m for template DNA without affecting the V_{max} for DNA polymerase α and β when DNA concentration was varied (Charts 1*B* and 2*B*). However, Yamazaki *et al.* (27) found that bleomycin had no effect on the K_m of template DNA but reduced the V_{max} for *E. coli* DNA polymerase when DNA concentration was varied. Müller *et al.* (11) obtained similar results for the DNA-directed DNA polymerase activity of Rauscher murine leukemia virus DNA polymerase. The reason for these dis-

crepancies is not clear. However, if bleomycin inhibits DNA polymerase activity by rendering the template inactive, increasing the template concentration should overcome the inhibition, as was actually found in this study (Table 2). This result is consistent with an inhibitor that increases the K_m for template without affecting the V_{max} for the enzyme but not with an inhibitor that reduces the V_{max} without affecting the K_m.

If bleomycin inhibits DNA polymerase α and β by interaction with the template, then why were DNA polymerase γ and TDT not inhibited by bleomycin? As for DNA poly merase γ, the template-primer used in the assay, poly(A)·$(dT)_{12-18}$, is probably resistant to bleomycin action since bleomycin reacts with neither polyadenylic acid *in vitro* (3) nor with short oligodeoxyribonucleotides (7). Recently, it was reported that high concentrations of bleomycin (1.5 mg/ml) degrade the poly(dT) portion of a poly(A)·poly(dT) hybrid synthesized with viral reverse transcriptase (2). Much lower concentrations of bleomycin were used here, and inhibition of DNA polymerase was observed at bleomycin concentrations that did not degrade DNA into acid-soluble fragments (Chart 3). The lack of inhibition of DNA polymerase γ by bleomycin is not due to a low K_m for template, since the concentration of poly(A)·$(dT)_{12-18}$ (8 μg/ml) used here is about twice the K_m. As for TDT, which does not require a template, the alteration of DNA by bleomycin must not significantly interfere with its function as an initiator. Consequently, when DNA is used in an assay, bleomycin can be useful in distinguishing TDT from DNA polymerases α and β.

Because bleomycin is an anticancer drug, the finding in this study that its DNA polymerase inhibitory activity can be inactivated (Table 3; Chart 4) is important. The effectiveness of bleomycin as an antitumor agent could depend upon the concentration of sulfhydryl compounds and their proximity to DNA in the target cells.

REFERENCES

1. Haidle, C. W. Fragmentation of Deoxyribonucleic Acid by Bleomycin. Mol. Pharmacol., *7:* 645–652, 1971.
2. Haidle, C. W., and Bearden, J. Effect of Bleomycin on an RNA-DNA Hybrid. Biochem. Biophys. Res. Commun., *65:* 815–821, 1975.
3. Haidle, C. W., Kuo, M. T., and Weiss, K. K. Nucleic Acid-Specificity of Bleomycin. Biochem. Pharmacol., *21:* 3308–3312, 1972.
4. Haidle, C. W., Weiss, K. K., and Thien Kuo, M. Release of Freebases from Deoxyribonucleic Acid after Reaction with Bleomycin. Mol. Pharmacol., *8:* 531–537, 1972.
5. Hazez-Delatte, J., and Feremans, W. Etude des Aberrations Chromosomiques Provoquées par la Bleomycine sur les Lymphocytes Humains *in vitro*. Buell. Cancer, *62:* 29–36, 1975.
6. Ichikawa, T., Matsuda, A., Yamamoto, K., Tsubosaki, M., Kaihara, T., Sakamoto, and Umezawa, H. Biological Studies on Bleomycin A. J. Antibiotics Tokyo Ser. A, *20:* 149–155, 1967.
7. Kuo, M. T., Haidle, C. W., and Inners, L. D. Characterization of Bleomycin Resistant DNA. Biophys. J., *13:* 1296–1306, 1973.
8. Lineweaver, H., and Burk, D. The Determination of Enzyme Dissociation Constants. J. Am. Chem. Soc., *56:* 658–666, 1934.
9. Müller, W. E. G., Totsuku, A., Nusser, I., Zahn, R. K. and Umezawa, H. Bleomycin Inhibition of DNA Synthesis in Isolated Enzyme Systems and in Intact Cell Systems. Biochem. Pharmacol., *24:* 911–915, 1975.
10. Müller, W. E. G., Yamazaki, Z., Breter, H. J., and Zahn, R. K. Action of Bleomycin on DNA and RNA. European J. Biochem., *31:* 518–525, 1972.
11. Müller, W. E. G., Yamazaki, Z., and Zahn, R. K. Bleomycin, A Selective Inhibitor of DNA-Dependent DNA Polymerase from Oncogenic RNA Viruses. Biochem. Biophys. Res. Commun., *46:* 1667–1673, 1972.
12. Nagai, K., Suzuki, H., Tanaka, N., and Umezawa, H. Decrease of Melting Temperature and Single Strand Scission of DNA by Bleomycin in the Presence of Hydrogen Peroxide. J. Antibiotics Tokyo Ser. A, *22:* 624–628, 1969.
13. Nagai, K., Yamaki, H., Suzuki, H., Tanaka, N., and Umezawa, H. The Combined Effects of Bleomycin and Sulfhydryl Compounds on the Thermal Denaturation of DNA. Biochim. Biophys. Acta, *179:* 165–171, 1969.
14. Sarin, P. S., and Gallo, R. C. Terminal Deoxynucleotidyl Transferase in Chronic Myelogenous Leukemia. J. Biol. Chem., *249:* 8051–8053, 1974.
15. Spadari, S., and Weissbach, A. HeLa Cell R-Deoxyribonucleic Acid Polymerases. J. Biol. Chem., *249:* 5809–5815, 1974.
16. Srivastava, B. I. S. Deoxynucleotide-Polymerizing Enzymes in Normal and Malignant Human Cells. Cancer Res., *34:* 1015–1026, 1974.
17. Srivastava, B. I. S., and Minowada, J. Ribonuclease-sensitive Endogenous DNA Polymerase Activity and DNA-directed DNA Polymerase in Human Tissue Culture Lines. Cancer Res., *22:* 2481–2486, 1972.
18. Suzuki, H., Nagai, K., Akutsu, E., Yamaki, H., Tanaka, N., and Umezawa, H. Strand Scission of DNA Caused by Bleomycin and Its Binding to DNA *In Vitro*. J. Antibiotics Tokyo, Ser. A, *23:* 473–480, 1970.
19. Suzuki, H., Nagai, K., Yamaki, H., Tanaka, N., and Umezawa, H. Mechanism of Action of Bleomycin. Studies with the Growing Cultures of Bacterial and Tumor Cells. J. Antibiotics Tokyo Ser. A, *21:* 379–386, 1968.
20. Suzuki, H., Nagai, K., Yamaki, H., Tanaka, N., and Umezawa, H. On the Mechanism of Action of Bleomycin: Scission of DNA Strands *In Vitro* and *In Vivo*. J. Antibiotics Tokyo, Ser. A, *22:* 446–448, 1969.
21. Umezawa, H., Azakura, H., Oda, K., and Hori, S. The Effect of Bleomycin on SV_{40} DNA: Characteristics of Bleomycin Action which Produce a Single Scission in a Super Helical Form of SV_{40} DNA. J. Antibiotics Tokyo, Ser., A, *26:* 521–527, 1973.
22. Umezawa, H., Ishizuka, M., Kimura, K., Iwanga, J., and Takeuchi, T. Biological Studies on Individual Bleomycins. J. Antibiotics Tokyo Ser. A, *21:* 592–602, 1968.
23. Umezawa, H., Maeda, K., Takeuchi, T., and Okami, Y. New Antibiotics Bleomycin A and B. J. Antibiotics Tokyo Ser. A, *19:* 200–209, 1966.
24. Umezawa, H., Takeuchi, T., Hori, S., Sawa, T., Ishizaka, M., Ishikawa, T., and Komai, T. Studies on the Mechanism of Antitumor Effects of Bleomycin on Squamous Cell Carcinoma. J. Antibiotics Tokyo, Ser. A, *25:* 409–420, 1972.
25. Warburg, O., and Christian, W. Isolierung und Kristallization des Garungsferments Enolase. Biochem. Z., *310:* 384–421, 1941.
26. Yamaki, H., Suzuki, H., Nagai, K., Tanaka, N., and Umezawa, H. Effects of Bleomycin A_2 on Deoxyribonuclease, DNA Polymerase and Ligase Reactions. J. Antibiotics Tokyo, Ser. A, *24:* 178–184, 1971.
27. Yamazaki, Z., Muller, W. E. G., and Zahn, R. Action of Bleomycin on Programmed Synthesis. Influence on Enzymatic DNA, RNA, and Protein Synthesis. Biochim. Biophys. Acta, *308:* 412–421, 1973.