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Structure-activity Relationships Involved in the Site-specific Fragmentation of Linear Duplex DNAs by Talisomycin and Bleomycin Analogs

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Summary. *The fragmentation of Hind III- and Pst I-digested PM2 DNA and of Hind III-digested pBR322 DNA by bleomycin A_2 and B_2 and talisomycins A, B, S_2b, and $S_{10}b$ was investigated. As observed by electrophoresis on agarose gels, the ethidium bromide staining band patterns produced following incubation of the various restriction endonuclease-digested DNAs with the compounds were different for the bleomycin analogs and for the talisomycin analogs. Quantitation of the degree of fragmentation of various segments of linear PM2 DNA by either bleomycin A_2 or talisomycin A indicated that certain segments of the PM2 genome serve as better substrates than other segments for double-strand fragmentation by either of the two antitumor antibiotics. These results show that in this system bleomycin and talisomycin analog treatment of linear PM2 or pBR322 DNA resulted in breakage of DNA, producing different-length DNA fragments, and demonstrate the importance of the two amino acids and the 4-amino-4,6-dideoxy-L-talose sugar, which are located near the bithiazole in talisomycin but absent in the bleomycin structure in conferring a different site-specificity for DNA fragmentation to talisomycin than to bleomycin.*

Introduction

The bleomycins (BLM) are a group of glycopeptide antibiotics, isolated from *Streptomyces verticillus* [31], that have been shown to be effective against a variety of neoplasms [7]. The primary target for BLM cytotoxicity appears to be interaction with cellular DNA. The effects of BLM on isolated DNA have been shown to include liberation of free bases [9], single-strand breakage [23], double-strand breakage [10, 14, 26], site-specific single- and double-strand breakage [8, 14, 15, 20, 30], non-covalent intermolecular crosslinks [16], and the reduction of DNA melting temperature [24]. BLM also produced breakage of DNA in cells grown in tissue culture and the extent of degradation was correlated with the cell cycle-specific cytotoxicity of the drug [4].

Talisomycin (TLM) is a new antitumor antibiotic related structurally to BLM. Structures of the two major components of the antibiotic, TLM A and TLM B, and of two analogs, TLM S_2b and TLM S_{10}b, have been determined. They contain two new amino acids and a unique sugar, 4-amino-4, 6-dideoxy-L-talose, that have not been previously found in the BLM complex [13]. The drug has exhibited significantly greater antibiotic activity against a variety of bacteria and fungi than did BLM [12]. Both TLM A and TLM B had antitumor activity in several experimental animal tumor systems [1, 12]. However, TLM A is less active than BLM A_2 in in vitro DNA-breakage systems [21, 28].

Initial studies have indicated significant differences in the site-specificity of double-strand breakage of PM2 DNA between TLM A and BLM A_2 [20]. The purpose of the present study was further investigation of the different site-specificity of DNA breakage by BLM and TLM with various analogs of the parent compounds and two different species of DNA molecules.

Materials and Methods

Preparation of PM2 DNAs. Covalently closed circular PM2 DNA was isolated as previously described [28]. The closed circular DNA

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was isolated from the lower band in buoyant CsCl[1] gradients containing saturating levels of Eth Br. After removal of Eth Br by extraction with NaCl-saturated isopropyl alcohol, the DNA samples were dialyzed against five changes of 0.015 *M* NaCl and stored at −20° C. Only PM2 DNA of greater than 90% covalently linked superhelical form (form I) was used.

Preparation of pBR322 DNA. Covalently closed circular plasmid pBR322 DNA was grown in and isolated from *E. coli* RR1 according to the procedure described by Clewell et al. [5, 6]. The DNA was dialyzed against five changes of 0.015 *M* NaCl and stored at −20° C.

Restriction Endonuclease Cleavage of PM2 and pBR322 DNAs. Samples of PM2 or pBR322 form I DNA were incubated in 50 m*M* NaCl, 10 m*M* $MgCl_2$, 14 m*M* DTT, 10 m*M* Tris-HCl at pH 7.5 with Hind III restriction endonuclease (Boehringer Mannheim, Biochemica, FRG) at a concentration of 2 units enzyme per μg DNA for 4 h at 37° C. Pst I restriction endonuclease (Boehringer Mannheim, FRG) digestion of PM2 DNA was carried out in solutions containing 50 m*M* NaCl, 10 m*M* $MgCl_2$, 10 m*M* Tris-HCl at pH 7.5 with an enzyme-to-DNA ratio of 1 μ/4μg DNA for 2 h at 37° C.

Restriction Endonuclease Extraction Following Digestion of PM2 DNA. Following digestion of PM2 DNA by Hind III, the enzyme was extracted from the incubation mixture and the PM2 DNA was then precipitated as described by Strong and Hewitt [29]. Following removal of ethanol by nitrogen the precipitated PM2 DNA was resuspended in 0.015 *M* NaCl. The 260/280 absorbance ratio of the DNA mixture was equal to 2.05 following resuspension.

Electrophoretic Analysis of Restriction Endonuclease-Digested DNA Following Incubation with BLM and TLM Analogs. Aliquots containing 8 μg PM2 or pBR322 DNA-restriction endonuclease reactions were incubated in solutions containing BLM A_2, BLM B_2, TLM A, TLM S_2b, or TLM S_{10}b (Bristol Laboratories, Syracuse, NY) at drug concentrations of 0.1−10 μ*M*, 10 m*M* Tris, 20 m*M* NaCl, and 40 m*M* DTT at pH 7.5 in a final volume of 50 μl for selected periods of time at 37° C. In reactions containing $FeCl_2$, solutions of $FeCl_2$ were freshly made immediately before use and aliquots added to the final reaction mixture. The reactions were stopped by the addition of an equal volume of a solution containing 25 m*M* EDTA, 70% glycerol, and 0.05% bromphenol blue at pH 7.5 and the mixture was stored at −20° C. Equal volumes containing 4 μg (Hind III-digested PM2 or pBR322) or 2 μg (Pst I-digested PM2) DNA in the final incubation mixture were electrophoretically separated on 1% agarose gels under conditions previously described [28]. Slab gels were stained with Eth Br dissolved in electrophoresis buffer for a minimum of 2 h and were then photographed on an ultraviolet light plate (Ultra-Violet Products, Inc., San Gabriel, Mass, USA). The negative films of the gels were scanned with an RFT Scanning Densitometer Model 2955 (Transsidyne General Corporation, Ann Arbor, Mich, USA) and the relative position of the Eth Br-staining DNA fragments determined.

Quantitation of Double-strand Breakage on Restriction Endonuclease Fragments of DNA by BLM A_2 and TLM^A. Following densitometric scanning of the negative pictures of the gels as described above, the areas under the peaks, resulting from the measurement of intensity of the Eth Br fluorescence in areas in the gel corresponding to Hind III PM2 DNA fragment A, Hind III pBR322 DNA digestion product, or Pst I PM2 DNA digestion product following incubation with BLM A_2 or TLM A were measured. The extent of double-strand breakage due to the drug was calculated as the percentage of a particular DNA-restriction endonuclease fragment remaining following incubation with either drug at concentrations from 1−5 μ*M* for selected time periods as compared with control (incubation mixture minus drug).

1 *Abbreviations used:* BLM, bleomycin; TLM, talisomycin; CsCl, cesium chloride; Eth Br, ethidium bromide; EDTA, ethylene diamine tetraacetic acid; DTT, dithiothreitol

Results

Structures of BLM-related Compounds

Figure 1 shows the basic structure and the structural modification of the BLM analogs studied in this report. These compounds include BLM A_2, BLM B_2, TLM B, TLM S_2b, and TLM S_{10}b. The detailed structural differences of these related compounds will be described, along with the apparent significance of these differences in determining the site-specificity of the molecule in causing double-strand breaks on isolated linear DNA.

Double-Strand Breakage of Hind III-Digested PM2 DNA by BLM-related Compounds

When PM2 DNA was digested by Hind III restriction endonuclease, six of the seven linear duplex fragments which are limit products of the reaction [2] were resolved by agarose gel electrophoresis and visualized by fluorescence after staining with Eth Br (Fig. 2). The fragments ranged in molecular weight from 3.53×10^6 (fragment A) to 1.88×10^5 (fragment F). The mixture of Hind III PM2 fragments was subsequently treated with the BLM-related compounds mentioned above and the reaction products electrophoresed in agarose gels as described in *Materials and Methods*. As shown in Fig. 2, similar DNA-banding patterns resulted following treatment of the Hind III PM2 fragments with either BLM A_2 or B_2. The resulting subfragments are detectable between the A and B and B and C Hind III PM2 fragments. The densitometric scans at high gain indicate at least six peaks between A and B fragments (Fig. 3, labeled Bl thru 6). Two DNA bands were detectable between the B and C fragments following incubation with either analog, as shown by the densitometric scan of this region of the gel following BLM A_2 incubation (Fig. 4, labeled B-7 and 8). The similarities of the Eth Br staining pattern for both BLM A_2- and BLM B_2-cleaved DNA fragments indicate that substitutions in the molecule at the C-terminal amine of either 3-aminopropyl-dimethyl-sulfonium (BLM A_2) or agmatine (BLM B_2)

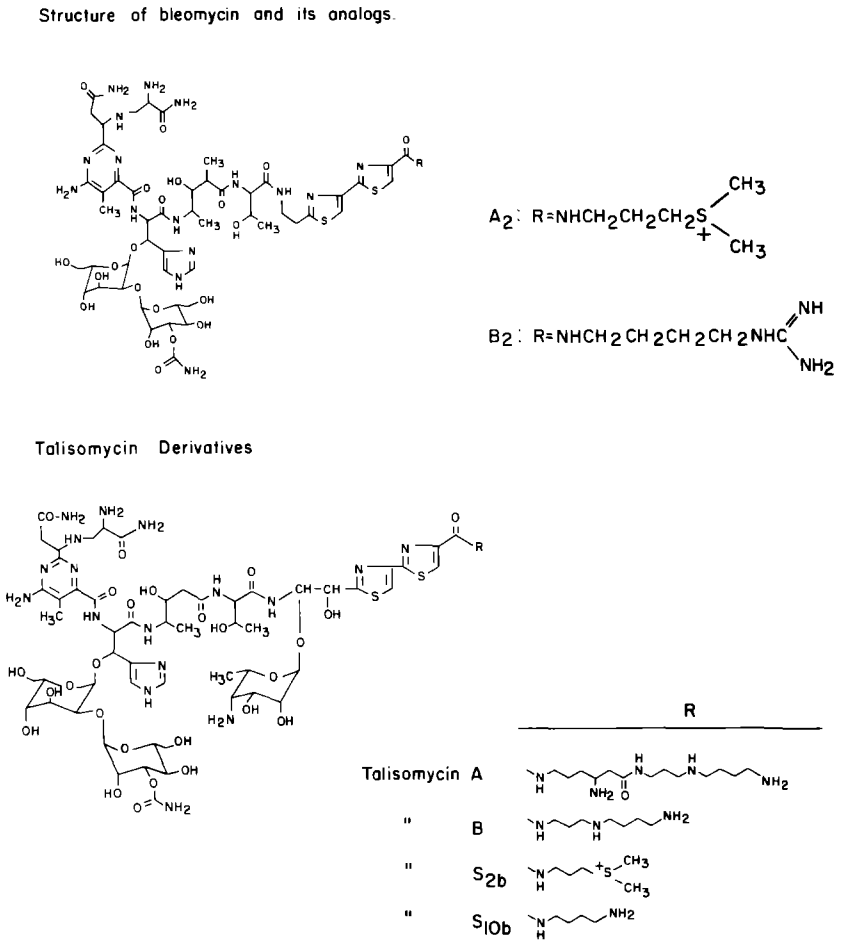

Fig. 1. Structure of tested bleomycin-related drug compounds

resulted in similar site-specific subfragmentation of hind III-digested PM2 DNA.

The Eth Br staining patterns resulting from incubation of the Hind III PM2 DNA mixture with TLM A, B, S_2b, or $S_{10}b$ were similar (Fig. 2). However, the staining pattern of the DNA fragments treated with this group of TLM analogs was distinct from that resulting from BLM A_2 or B_2 incubation. The densitometric scans of the region between the A and B Hind III PM2 fragments resulting from incubations with the TLM showed at least six peaks, with the third peak resolving into a doublet (Fig. 3, labeled T1 thru 7). Unlike that found with BLM A_2 and BLM B_2, no peaks are detectable between the B and C Hind III PM2 DNA fragments following treatment with the TLMs. This is illustrated in the densitometric scans comparing the BLM A_2 and TLM A reaction products between the B and C Hind III PM2 DNA fragments (Fig. 4).

The previous results indicate that the sizes of the detectable DNA subfragments produced following incubation with TLMs A, B, S_2b, or $S_{10}b$ differed from those resulting from BLM A_2 or B_2. The observed differences were produced with different batches of PM2 DNA, and in multiple experiments. As with the BLMs, changes in the C-terminal amine of the TLMs molecule did not change its apparent site-specific subfragmentation of Hind III digested PM2 DNA.

With higher concentrations of and/or longer incubation times with the BLM and TLM analogs, increased Eth Br staining was produced in the gels in the areas between the subfragments, indicating extensive non-specific double-strand breakage (data not shown). The non-specific DNA breakage by BLM and TLM is evidenced in Fig. 4, as the scans corresponding to lanes containing BLM A_2- and TLM A-produced DNA subfragments show a progressive decrease in background fluorescence, with a return to baseline preceding Hind III PM2 fragment C. Under conditions of relatively high drug concentration (20 μg/ml), which resulted in increased background fluorescence due to non-specific breakage, the discrete subfragments were still observed and the

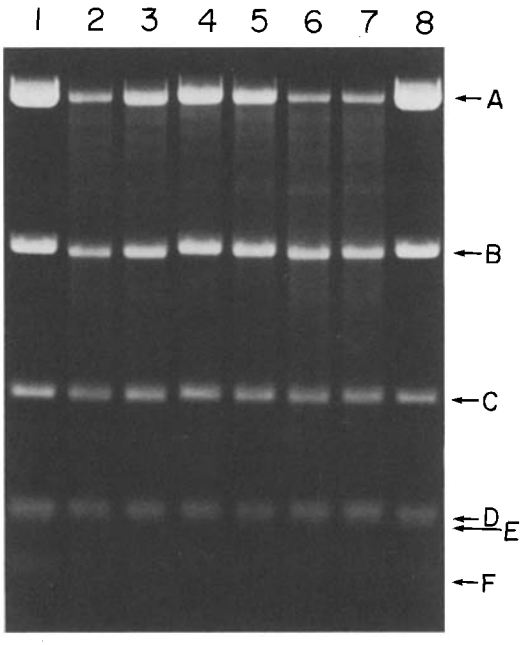

Fig. 2. BLM and TLM analog fragmentation at discrete sites on PM2 DNA. PM2 DNA was digested with the Hind III-restriction endonuclease, followed by BLM and TLM treatment at a drug concentration of 5 μg/ml for 30 min to give fragments of discrete sizes which were resolved by agarose gel electrophoresis. Lanes 1–8 correspond to Hind III- PM2 mixtures incubated with 1, no drug; 2, BLM A_2; 3, BLM B_2; 4, TLM A; 5, TLM B; 6, TLM S_2b; 7, TLM $S_{10}b$; 8, no drug. Hind III fragments of PM2 DNA are indicated in order of decreasing sizes, A–F by *arrows* at *right*

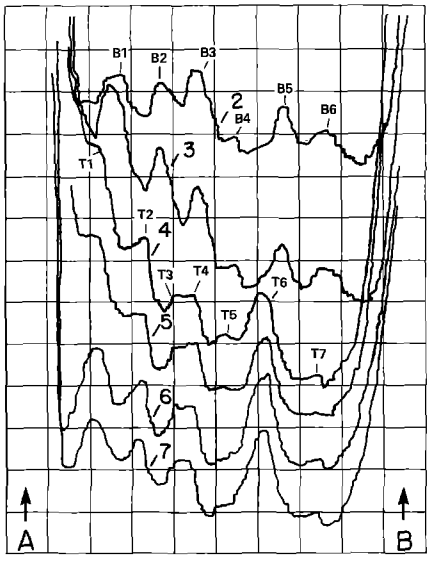

Fig. 3. Densitometric scans of agarose gels containing products of BLM and TLM analog treated Hind III-PM-2 DNA. Scans labeled 2–7 correspond to the respective lanes in Fig. 1. Scans correspond to the area between A and B fragments of Hind III-digested PM-2 DNA and are normalized to the point of origin (gel wells). All scans were produced with identical scan settings

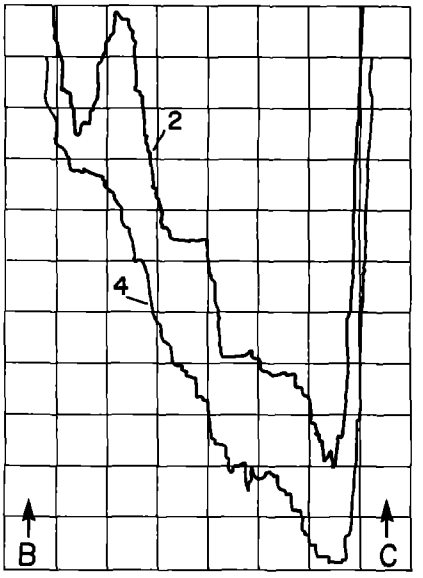

Fig. 4. Densitometric scans of agarose gels containing products of BLM A_2- and TLM A-treated Hind III-PM-2 DNA between Hind III-PM-2 DNA fragments B and C. Scans labeled 2 and 4 correspond, respectively, to lanes 2 and 4 in Fig. 1. Scans are normalized to the points of origin and were produced with identical scan settings

relative positions and patterns of the BLM- or TLM-induced specific cleavage products were not changed.

BLM A_2 and TLM A were also incubated with Hind III PM2 fragments after removal of the restriction endonuclease from the digestion mixture. The subfragments produced with BLM A_2 or TLM A following enzyme extraction were similar to those produced in the presence of the enzyme, as shown by the Eth Br staining pattern in agarose gels following electrophoresis (not shown). Therefore the presence or absence of the restriction endonuclease during treatment did not alter the DNA-banding pattern induced by incubation with either BLM A_2 or TLM.

Quantitation of Double-strand Breakage of Hind III PM2 DNA Restriction Fragment A by BLM A and TLM A

The extent of double-strand degradation of the Hind III PM2 A fragment induced by BLM A_2 and TLM A was determined as described in *Materials and Methods*. As illustrated in Fig. 5, incubation with BLM A_2 produced more double-strand degradation of the A fragment than did TLM A under identical conditions of drug concentration and incubation duration.

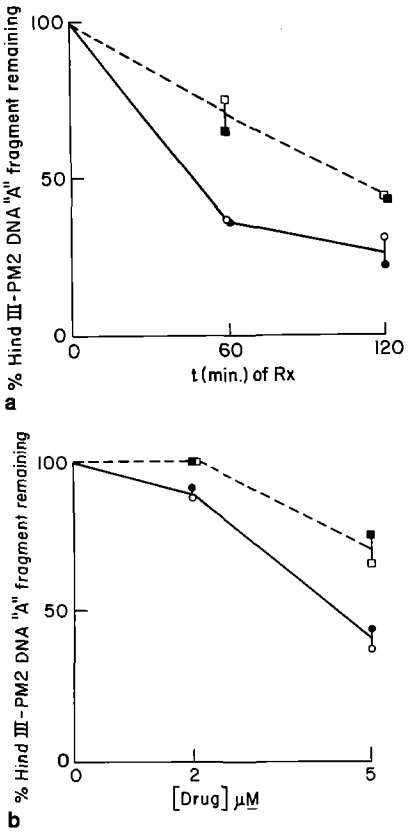

Fig. 5a and b. Comparison of degradation of Hind III-PM2 A fragment following incubation with BLM A_2 and TLM A (as described in *Materials and Methods*). *Closed* and *open symbols* represent results of two separate experiments, as in Figs 7 and 10. **a** Percent Hind III-A fragment remaining following incubation with μM BLM A_2 (○———●) or TLM A (□———■) versus time of incubation. **b** Percent Hind III-a fragment remaining following 60 min incubation with BLM A_2 (○– – –●) or TLM A (□———■) versus drug concentration. *Closed* and *open symbols* represent data obtained in two separate experiments

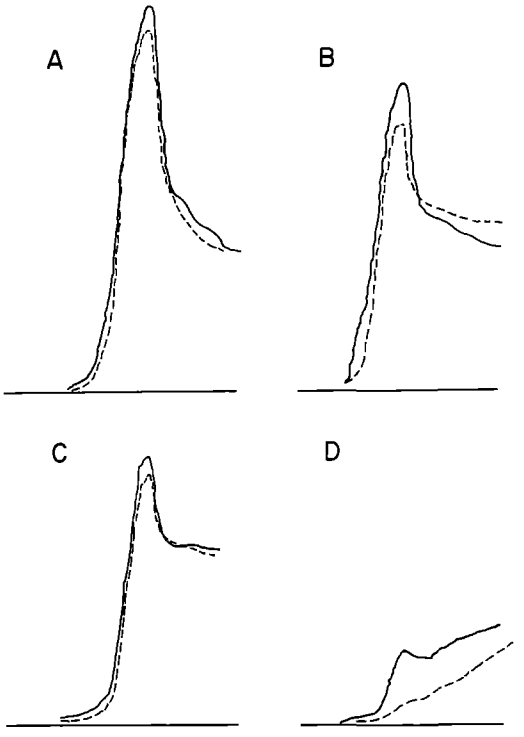

Fig. 6A–D. Densitometric scans of area in agarose gels corresponding to Pst I-digested PM2 DNA following incubation with increasing concentrations and time of incubation with BLM A_2 (———) and TLM A (– – –). **A** 2 μ*M* for 30 min; **B** 2 μ*M* for 60 min; **C** 5 μ*M* for 30 min; **D** 5 μ*M* for 60 min

Quantitation of Double-strand Breakage of Pst I-Digested PM2 DNA by BLM A_2 and TLM A

PM2 DNA was incubated in the presence of the endonuclease Pst I as described in *Materials and Methods*. Agarose gel electrophoresis of the reaction products demonstrated an Eth Br staining band with the same electrophoretic mobility as whole linear PM2 DNA (mol. wt. 6.3×10^6), confirming the existence of only one double-strand breakage site on the DNA for Pst I [2]. Subsequent incubation of the Pst I-digested PM2 DNA with BLM A_2 followed by agarose gel electrophoresis resulted in a different Eth Br staining pattern than that which resulted from incubation with TLM A (not shown). Quantitation of the extent of double-strand breakage of Pst I-digested PM2 DNA BLM A_2 or TLM A was determined for various drug concentrations and incubation times (Fig. 6). Unlike the results obtained for the extent of degradation of the Hind III PM2 DNA fragment A by BLM A_2 and TLM A, the extent of degradation of Pst I PM2 DNA fragment was consistently found to be at least equal and probably slightly greater following incubation with TLM A than after incubation with BLM A_2 under similar conditions of drug concentration and incubation time (Fig. 7).

Double-strand Breakage of Hind III-Digested pBR322 DNA by BLM A_2 and TLM A

Incubation of pBR322 DNA with Hind III caused one double-strand break in the pBR322 DNA molecule and resulted in a linear molecule with a mol. wt. of

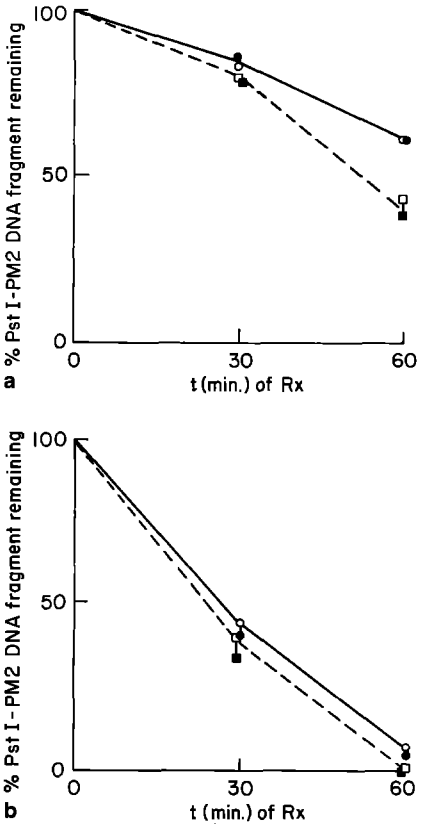

Fig. 7a and b. Comparison of degradation of Pst I-digested PM2 DNA fragment following incubation with BLM A_2 and TLM A (as described in *Materials and Methods*). **a** Percent Pst I-digested PM2 DNA fragment remaining following incubation with 2 μM BLM A_2 (○---●) or TLM A (□———■) versus time of incubation. **b** Percent Pst I-digested PM2 DNA fragment remaining following incubation with 5 μM BLM A_2 (○---●) or TLM A (□———■) versus time of incubation

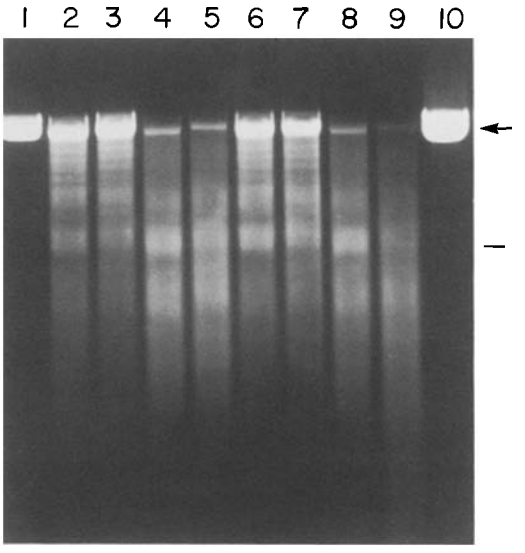

Fig. 8. BLM A_2 and TLM A fragmentation at discrete sites on pBR322 DNA. pBR322 DNA was digested with the Hind III restriction endonuclease followed by BLM A_2 and TLM A treatment to give fragments of discrete sizes which were resolved by agarose gel electrophoresis. Treatment at increasing drug concentration and incubation duration: lane 1, no drug; 2, 1 μM BLM A_2 for 60 min; 3, 1 μM TLM A for 60 min; 4, 2 μM BLM A_2 for 60 min; 5, 2 μM TLM A for 60 min; 6, 1 μM BLM A_2 for 120 min; 7, 1 μM TLM A for 120 min; 8, 2 μM BLM A_2 for 120 min; 9, 2 μM TLM A for 120 min; 10, no drug. Hind III digestion product of pBR322 DNA is indicated by an *arrow* on *right*

2.6×10^6 daltons [3]. When Hind III-digested pBR322 DNA was subsequently incubated with BLM A_2 a series of DNA fragments of discrete sizes was produced. Incubation of Hind III-pBR322 DNA with TLM A produced a series of discretely sized DNA fragments which differed from those produced by BLM A_2 as determined by agarose gel electrophoresis (Fig. 8). As with the subfragmentation of Hind III-digested PM2 DNA by BLM and TLM, the relative positions and patterns of the drug-induced specific cleavage did not change at high drug concentrations or longer incubation time. Conditions which produced increased background fluorescence due to increased non-specific double-strand breakage still resulted in the different banding patterns (Fig. 8). In Fig. 9 the densitometric scans of the agarose gels following electrophoresis of product induced by BLM A_2 and TLM A within a mol. wt. range of 2.6×10^6 to 5×10^5 are compared. As illustrated in Fig. 9, the

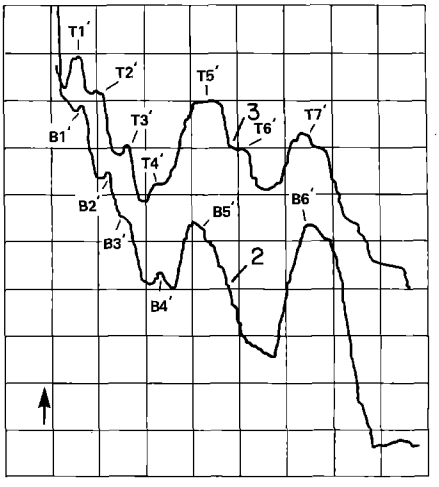

Fig. 9. Densitometric scans of agarose gel containing products of BLM A_2- and TLM A-treated Hind III-pBR322 DNA. Scans labeled 2 and 3 correspond to lanes 2 and 3, respectively in Fig. 8. Scans correspond to area between Hind III-pBR322 fragment to point on gel indicated by *line* at *right* in Fig. 8b. Scans are normalized to the points of origin and were produced with identical scan settings

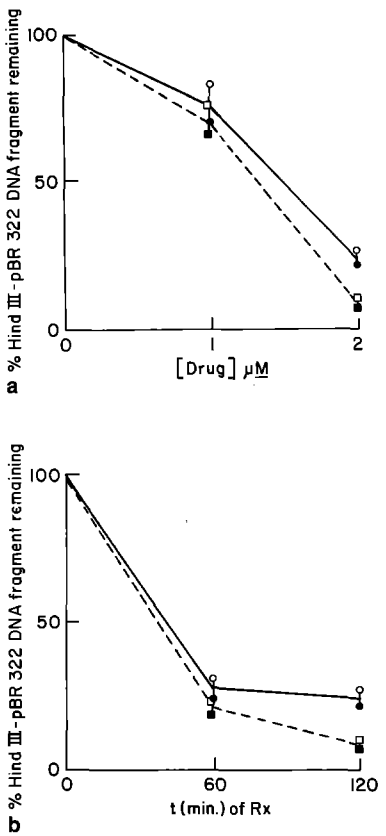

Fig. 10a and b. Comparison of degradation of Hind III-pBR322 DNA fragment following incubation with BLM A_2 and TLM A (as described in *Materials and Methods*). **a** Percent Hind III-pBR322 fragment remaining following 120 min incubation with BLM A_2 (○——●) and TLM A (□——■) versus drug concentration. **b** Percent Hind III pBR322 DNA fragment remaining following incubation with 2 μ*M* BLM A_2 (○——●) or TLM A (□——■) versus time of incubation

scan profiles for the BLM A_2- and TLM A-treated Hind III pBR322 DNA differed from one another, with some of the BLM A_2- and TLM A-produced subfragments having similar electrophoretic mobilities (T3′ and B3′, T4′ and B4′ and T7′ and B6′) and others having unique electrophoretic mobilities (T1′, T2′, T5′, T6′, B1′, B2′, B5′). These distinct patterns were reproduced in multiple experiments.

Quantitation of Double-strand Breakage of Hind III-Digested pBR322 DNA by BLM A_2 or TLM A

As illustrated in Fig. 10, Hind III endonuclease-digested pBR322 DNA (whole-linear pBR322 DNA) was slightly more susceptible to double-strand breakage by incubation with TLM A than with BLM A_2. This slightly greater susceptibility to double-strand breakage by TLM A than by BLM A_2 was consistently seen at all drug concentrations (1–5 μ*M*) and incubation durations (30–120 min) used in these experiments.

It has been reported that ferrous ions (Fe^{2+}) enhanced the DNA-breakage activity of BLM [18, 27, 30], and TLM [12, 28]. D'Andrea and Haseltine [8] reported that in the presence of exogenous ferrous ions the sequence-specificity for single-strand cleavage of DNA by BLM was broadened to include TT, AT, and TA sequences as well as the GC and GT sequences, which were the two sequences preferentially recognized in the absence of exogenous ferrous ions.

To investigate the effects of ferrous ions on breakage of pBR322 DNA by TLM A and BLM A_2, Hind III-digested pBR322 DNA was incubated with either of the two drugs in the absence and presence of added $FeCl_2$ (data not shown). At a concentration of 0.3 μ*M* TLM A or BLM A_2, the double-strand fragmentation of whole linear pBR322 DNA was increased by 50% and 20%, respectively, in the presence of 0.2 μ*M* $FeCl_3$, as compared with reactions in the absence of exogenous ferrous ions. As evidenced by the Eth Br banding patterns in agarose gels, the presence of the exogenous $FeCl_2$ did not alter the site-specificities for breakage of pBR322 DNA by either BLM A_2 or TLM A, as observed in the absence of added $FeCl_2$.

Discussion

The effects of the TLMs on DNA appear to be similar to those of the BLMs in that both groups of agents produce strand breaks in purified isolated DNA and intracellular DNA [28], and BLM- or TLM-induced double-strand breakage may be a significant factor in the mechanism of action of the compounds. Cytological studies with cultured mouse fibroblasts showed that fragmentation of metaphase chromosomes occurred when cells were incubated with high concentrations of BLM [25]. Other studies suggested that cytotoxicity correlates with the degree of DNA degradation induced by BLM [4, 11, 22]. Therefore, differences in the mechanisms, rates, and site-specificities of double-strand DNA breakage between TLM and BLM may be involved in the greater activity of TLM against a variety of bacteria and fungi and the different levels of antitumor activities of the two drugs in experimental tumor systems [12].

In a previous study from this laboratory it was demonstrated that the site-specific fragmentation of Hind III-digested PM2 DNA by TLM A was different from that produced by BLM A_2 in that the detectable

TLM A-induced subfragments were of a different number and size than those produced by BLM A_2 [20]. These results were interpreted as indicating different site-specificities for DNA fragmentation by the two antitumor antibiotics. In the present study it has been demonstrated that different site-specific fragmentation of DNA by the TLMs as compared to the BLMs is also observed with incubation of the drugs with Hind III-digested pBR322 DNA. Therefore, the relative different site-specificities for fragmentation of DNA by the BLMs and TLMs is not a phenomenon restricted to PM2 DNA.

The apparent differences in site-specific fragmentation of linear DNA by TLM and BLM indicate the importance of the structural differences between these structurally related molecules in conferring their different site-specificities. The results of this study, with the use of BLM and TLM analogs, suggest that changes in the terminal amine moiety of BLM do not change the site-specificity for fragmentation of DNA. This is demonstrated by the similar pattern of subfragments in agarose gels following electrophoresis of Hind III-PM2 DNA incubated with either BLM A_2 or B_2 (Fig. 2 and 3). Similarly, structural and charge differences conferred by the terminal amine moieties of the various analogs did not change TLMs site-specificity for fragmentation of Hind III digested PM2 DNA, as shown by the similar migration patterns of TLM A, B, S_2b, and S_{10}b produced subfragments in agarose gels (Figs. 2 and 3). TLM S_2b and BLM A_2 contain an equivalent terminal amine moiety. However, their site-specificities of fragmentation appear to differ from one another. Thus these results demonstrate the importance of the two amino acids and the 4 amino-4,6-dideoxy-L-talose sugar, which are located near the bithiazole group in talisomycin but absent in the bleomycin structure, in conferring a different site-specificity for DNA fragmentation to TLM as compared to BLM.

As demonstrated by the different degrees of degradation of different fragments of Hind III-digested PM2 DNA by BLM A_2 and TLM A, it appears that certain segments of the PM2 genome serve as better substrates than other segments for double-strand fragmentation by either BLM A_2 or TLM A (Figs. 5–7). This differential susceptibility of the different segments to double-strand fragmentation by the two drugs may be the result of an unequal distribution of specific sites for BLM- or TLM-induced fragmentation. The Hind III-PM2 DNA restriction fragmentation A may contain more sites for BLM A_2 site-specific fragmentation than TLM A sites. However, the entire linear PM2 genome may contain slightly more sites for TLM A site-specific fragmentation than BLM A_2 sites. The results from the quantitation of double-strand degradation of Hind III-digested pBR322 DNA suggest that the pBR322 genome may contain more TLM A double-strand fragmentation sites than BLM A_2 sites. The results from the quantitation of double-strand degradation of Hind III-digested pBR322 DNA suggest that the pBR322 genome may contain more TLM A double-strand fragmentation sites than BLM A_2 sites (Fig. 10). Using pBR322 DNA restriction fragments labeled with ^{32}P at one 5′ end, we have recently determined that DNA fragments which are more susceptible to degradation by TLM A than BLM A_2 contain more TLM A-specific fragmentation sites than BLM A_2 fragmentation sites (results not shown).

The experiments shown in this paper, in which linear PM2 DNA was used as substrate, indicate that at similar concentrations TLM A is more active that BLM A_2 in inducing double-strand breakage of whole linear PM2 DNA. When covalently closed circular superhelical PM2 DNA was used as a substrate in an agarose gel electrophoresis assay to measure the single- and double-strand breakage, BLM A_2 was found to be 25 times more active than TLM A in terms of total DNA breakage. However, at concentrations which resulted in a similar level of reduction of superhelical PM2 DNA, TLM A induced more double-strand breaks than BLM A_2 [21]. Therefore, the structural and/or conformational differences between BLM A_2 and TLM A appear to be important in conferring their respective site-specificity of fragmentation of DNA and different abilities to produce either single- or double-strand breaks in DNA.

The results presented in this study in combination with data concerning comparisons of BLM and TLM with respect to production of double- and single-strand breakage of isolated DNA [21] and cell cycle-specificities [19] may begin to offer molecular and cytological explanations for their different effects when compared in terms of antibacterial and antitumor activity. With this information it may be possible to design new BLM analogs which cleave at specific sites in cellular DNA, resulting in relatively more selective cytotoxic activities. These analogs may also have potential use as molecular biological tools in areas such as DNA sequencing.

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