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Articles

DNA Strand Scission by the Novel Antitumor Antibiotic Leinamycin

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ABSTRACT: Leinamycin is a recently discovered antitumor antibiotic with an unusual 1,3-dioxo-1,2-dithiolane structure. It preferentially inhibits the incorporation of [^{3}H]thymidine into the acid-insoluble fraction of *Bacillus subtilis*. In vitro, leinamycin causes single-strand cleavage of supercoiled double-helical pBR322 DNA in the presence of thiol cofactors. Scavengers of oxygen radical did not supress the DNA-cleaving activity. Thiol-activated leinamycin binds calf thymus DNA at 4 °C and thermal treatment of the leinamycin–DNA adduct released a chemically modified leinamycin from the complex. The lack of cytotoxicity and DNA-cleaving activity for *S*-deoxyleinamycin indicates that the 1,3-dioxo-1,2-dithiolane moiety is essential for the activity of leinamycin. Thus, the primary cellular target of leinamycin appears to be DNA. It binds DNA and causes single-strand break at low concentrations, which may account for the potent antitumor activity.

DNA is thought to be the principal target of a number of clinically useful antitumor agents including, mitomycin, bleomycin, adriamycin, and *cis*-platinum. These compounds interfere with DNA through cross-linkage, strand breakage, intercalation, or other kinds of interactions, which result in inhibition of nucleic acid synthesis (Tomasz et al., 1987; Stubbe & Kozarich, 1987; Rowley & Halliwell, 1983; Eastman, 1985). The discovery of these antitumor agents which interact with DNA has provided insight into the mechanistic principle that can be used to mediate DNA recognition and cleavage.

In the course of screening for antitumor antibiotics, we have isolated a new antitumor antibiotic, leinamycin, produced by a *Streptomyces* sp. (Hara et al., 1989a,b). The structure of leinamycin (Figure 1) was revealed by X-ray crystallography and the absolute configuration of the antibiotic was determined by spectroscopic and chemical analysis (Hirayama & Shimizu, 1989; Saitoh et al., 1989). Leinamycin contains an unusual 1,3-dioxo-1,2-dithiolane moiety, which is connected to the 18-membered lactam ring through a spiro linkage, and can be classified as a new group distinct from any known classes of antibiotics or microbial metabolites. Leinamycin exhibited potent antitumor activity against murine experimental tumors leukemia P388 and sarcoma 180. The compound was also active against Gram-positive bacteria (Hara et al., 1989a). The unique structural features as well as the high biological activity of leinamycin prompted us to investigate the mode of action of this antitumor agent. We report here the effect of leinamycin on bacterial DNA synthesis, binding, and single-strand scission of DNA in vitro through the thiol-activated 1,3-dioxo-1,2-dithiolane moiety.

Materials and Methods

Chemicals and Enzymes. Leinamycin was isolated from a cultured broth of *Streptomyces* sp. as reported previously (Hara et al., 1989a,b). Stock solutions were prepared in DMSO and stored at −20 °C. [6-^{3}H]Thymidine (15Ci/mmol), [2-^{14}C]uracil (57.4mCi/mmol), [4,5-^{3}H]leucine (5Ci/mmol), and [γ-^{32}P]ATP (10Ci/mmol) were purchased from New England Nuclear, Boston, MA. All other reagents used were of commercial reagent grade.

Preparation of S-Deoxyleinamycin. The *S*-deoxy derivative was prepared by hydrogenolysis of leinamycin in the presence of 5% Pd–C in methanol. Details of this compound will be discussed elsewhere (Saitoh et al., 1989).

Effect of Leinamycin on the Growth and Macromolecular Synthesis of Bacillus subtilis. B. subtilis was cultured in medium containing 0.2 g of $MgSO_4 \cdot 7H_2O$, 2 g of citric acid (10 g of KH_2PO_4, 3.5 g of $NaNH_4HPO_4 \cdot H_2O$, 5 g of glucose, 1 g of casamino acids, 2 g of yeast extract, 50 mg of tryptophan, and 50 mg of arginine per liter of deionized water (pH 7.0). Various amounts of leinamycin were added at 1.7 h, and

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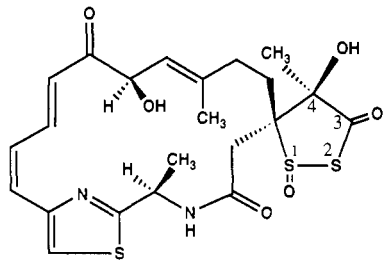

FIGURE 1: Structure of leinamycin.

the growth was automatically recorded with a biophotorecorder (Tokyo Kagaku Sangyo, Tokyo, Japan) at 37 °C.

B. subtilis was cultured as described above. When the cell density reached OD (660 nm) = 0.1, measured with a biophotorecorder, labeled precursors ([^{3}H]thymidine, [^{14}C]uracil, or [^{3}H]leucine) and drug were added to 10 mL of culture. Samples (0.5 mL) taken at indicated times were poured into 2.5 mL of ice-cold 5% trichloroacetic acid and placed for 1 h in an ice bath. They were filtered through HA Millipore filters (0.45 μm) and washed with 15 mL of cold 5% trichloroacetic acid. The filters were dried and radioactivity retained on the filters was determined by liquid scintillation counting.

Antimicrobial Activity and Cytotoxic Activity. The in vitro antimicrobial activity against *B. subtilis* was determined in nutrient agar by a 2-fold serial dilution method. The lowest concentration that inhibited growth of a bacterial strain after 18-h incubation at 37 °C was recorded as the minimum inhibitory concentration (MIC). Cytotoxic activity against HeLa S3 cell was measured and calculated as described previously (Fujimoto & Morimoto, 1983).

Reaction of Leinamycin with pBR322 DNA. The DNA-cleavage activity was determined with purified pBR322 DNA. Typical reaction mixtures included 20 μL of 20 mM Tris-HCl buffer, pH 7.5, 0.3 μg of pBR322 DNA, 0.5 mM 2-mercaptoethanol, leinamycin, and other supplements if necessary. Leinamycin and pBR322 DNA were added into the buffer solutions before adding the various amounts of thiol cofactors. In some studies, different oxygen radical scavengers were added to the buffer solutions before adding leinamycin, pBR322 DNA, and thiol cofactors. To perform studies under unaerobic conditions, nitrogen was bubbled through the assay mixture containing leinamycin and pBR322 DNA in Tris buffer for 5 min. The solution of thiol, which was also nitrogen flushed, was added and the test tubes were then filled with nitrogen and quickly sealed with stoppers. Tris buffer solution was purged with nitrogen for 10 min before use. The reaction mixtures were incubated at 37 °C for 60 min. After the addition of 3.5 μL of 0.02% bromophenol blue and 50% sucrose, 20 μL of the mixture was placed in the well of an agarose slab gel.

Agarose Gel Electrophoresis of pBR322 DNA. Electrophoresis was carried out in 89 mM borate, pH 8.3–2 mM EDTA buffer containing 0.01% SDS at 50 mV for 12 h. Following electrophoresis, gels were stained in an aqueous solution of ethidium bromide (1 μg/mL). DNA bands were visualized by transillumination with ultraviolet light (300 nm) and photographed with Kodak Nos. 24A and 12 filters with Polaroid type 665 positive/negative film. The amount of DNA was quantitated by scanning negatives with a Shimazu scanning densitometer.

Reaction of Leinamycin with Calf Thymus DNA. Leinamycin (0.39 μmol) was dissolved in a small amount of DMSO and was added to 20 mM Tris-HCl buffer solution containing 0.26 mg of calf thymus DNA (Sigma, type I). The nucleic

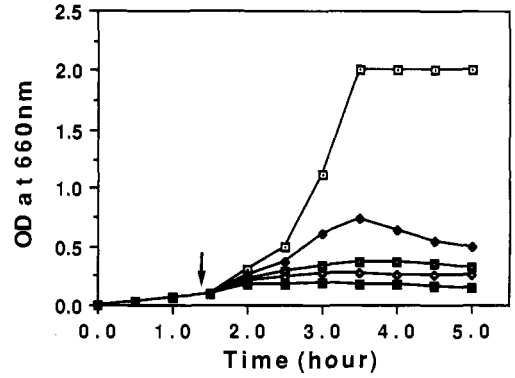

FIGURE 2: Effect of leinamycin on the growth of *B. subtilis*. The exponential culture was incubated with leinamycin at various concentrations: 0 (□), 0.63 (◆), 1.25 (■), 2.5 (◆), and 10 μg/mL (■). Leinamycin was added at the time indicated by the arrow.

acid concentration was equivalent to 0.39 μmol of DNA phosphate, which was calculated by using the average base molar extinction values for calf thymus DNA, 6500 M^{-1} cm (Swenson et al., 1982). Then, 0.78 μmol of 2-mercaptoethanol was added, if necessary, and the reaction mixture was incubated for 24 h at 4 °C with stirring. The reaction was stopped by adding $^1/_{10}$ volumes of 3 M NaOAc, pH 7.5, and 2.5 volumes of cold ethanol. Calf thymus DNA, thus recovered by ethanol precipitation, was subjected to HPLC analysis (YMC AM312 ODS column); the instrument was equipped with the multichannel photodiode array detector (Union Giken, Model MCPD-350).

Nucleotide Sequence Cleavage Experiments. The plasmid pBR322 DNA was initially cut with *Sal*I, and the obtained DNA fragment was incubated with bacterial alkaline phosphatase. The 5′-ends were labeled with ^{32}P by treatment with T_4 polynucleotide kinase and [γ-^{32}P]ATP, and this doubly end-labeled DNA fragment was digested with *Bam*HI. The singly end-labeled 275-bp fragment was isolated by preparative polyacrylamide gel electrophoresis. The reaction samples containing ^{32}P-labeled fragment, leinamycin, and 500 μM DTT were used for the nucleotide sequence analysis. After incubation at 37 °C for 60 min, DNA was recovered as precipitate by the addition of cold ethanol, using 5 μL of tRNA (5 mg/mL) as a carrier nucleic acid. The samples were resuspended in PAGE loading buffer (80% formamide, 10 mM NaOH, 1 mM EDTA, 0.1% xylene cyanol, 0.1% bromophenol blue) and heated in sealed vessels at 90 °C for 1 min. After the samples were cooled in the ice bath, equal amounts of radioactivity were loaded onto 10% polyacrylamide–7 M urea sequencing gel and electrophoresed at 1000 V for 4 h. Autoradiographs were developed with Kodak XRP-1 film.

Results

Effect of Leinamycin on Macromolecular Synthesis. The effect of leinamycin on the growth of *B. subtilis* is shown in Figure 2. Growth was inhibited by 0.03 μg/mL and increasing concentrations showed more extensive effect on growth. The effect of leinamycin on macromolecular synthesis in *B. subtilis* was studied to elucidate the primary action of this drug. The exponentially growing culture of *B. subtilis* was exposed to both drug and radioactive precursors of DNA, RNA, or protein, and radioactivities incorporated into the corresponding fractions were measured at the time indicated. Leinamycin (1 μg/mL) markedly inhibited the incorporation of [^{3}H]thymidine as shown in Figure 3. At concentrations higher than 5 μg/mL, synthesis of RNA and protein was also affected, accompanied by the complete inhibition of DNA synthesis. These results indicate that inhibition of DNA

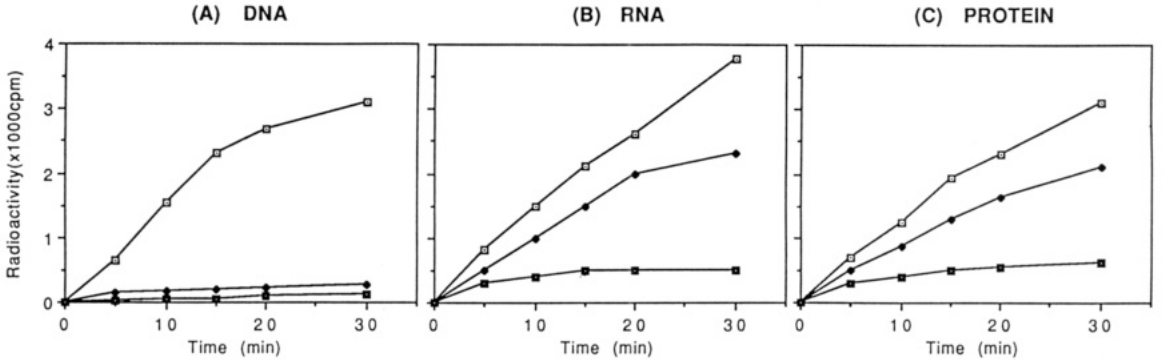

FIGURE 3: Effect of leinamycin on synthesis of macromolecules in *B. subtilis*. The exponential culture of *B. subtilis* was incubated with leinamycin at varying concentrations: (□), 1 (◆), and 5 μg/mL (■) together with [^{3}H]thymidine (A), [^{14}C]uracil (B) or [^{3}H]leucine (C). At the times indicated, aliquots (0.5 mL) were taken for determination of radioactivity as described under Materials and Methods.

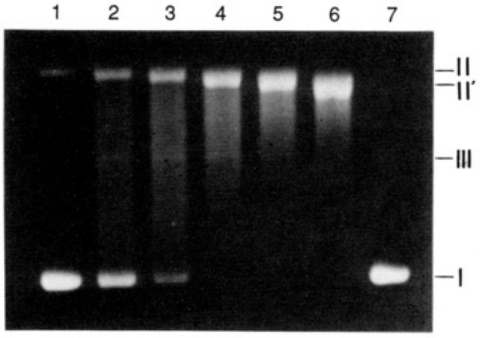

FIGURE 4: Cleavage of supercoiled form I pBR322 DNA to the nicked II form by leinamycin. Lane 1, pBR322 alone, lanes 2–6, pBR322 DNA plus leinamycin at 1.5, 3.1, 6.3, 12.5, and 25 μM in the presence of 0.5 mM DTT, respectively; lane 7, pBR322 DNA plus leinamycin at 25 μM in the absence of DTT. II′ denotes the altered electrophoresis pattern of form II DNA in the presence of 25 μM leinamycin.

synthesis occurs with a dose–response similar to that of growth inhibition, and inhibition of DNA synthesis is a much more sensitive function of drug concentration than growth inhibition. It is therefore concluded that leinamycin preferentially inhibits DNA synthesis, resulting in interference with the growth of susceptible cells.

Single-Strand Scission of DNA by Thiol-Activated Leinamycin. To see whether leinamycin may interact directly with DNA, leading to the damage of DNA concomitant with the inhibition of cellular DNA synthesis, the effect of leinamycin on plasmid pBR322 DNA was analyzed with agarose gel electrophoresis. Figure 4 shows that conversion of form I supercoiled DNA to form II (nicked) DNA is apparent with increasing concentrations of leinamycin in the presence of DTT (0.5 mM). Incubation of thiol with plasmid pBR322 DNA causes any background nicking, as evidenced by Figure 5A. Any strand scission by leinamycin did not occur in the absence of DTT. Figure 4 also shows that leinamycin produced subtle but significant changes in the electrophoreic mobility of form II DNA. This was also observed when the samples were ethanol precipitated to remove unreacted drug. Form III (linear) DNA was not observed even at concentrations as high as 200 μM, indicating that leinamycin causes single-strand breaks but not double-strand breaks in supercoiled plasmid DNA. The same results were observed when phosphate buffer was used instead of Tris-HCl buffer (Figure 5B).

The effect of various reducing agents on the DNA-cleaving activity of leinamycin was examined. When 0.5 mM aliquots of thiol cofactors such as 2-mercaptoethanol, glutathione (reduced form), cysteine, and cysteamine were added to the reaction mixture, conversion to form II DNA occurred to an extent similar to that observed when DTT was used. Whereas, in the presence of the reducing agents (2 mM) $NaBH_4$, NADPH, or ascorbic acid, DNA-cleaving activity was not observed. Thus, the requirement for thiol agents as cofactors is a characteristic of DNA cleavage by leinamycin. The conversion of supercoiled to open circles was dependent on the concentration of thiol cofactors (Figure 5A). In the absence of leinamycin, an increase in the amount of 2-mercaptoethanol did not lead to significant background nicking of pBR322 DNA. On the other hand, the presence of leinamycin and elevated amounts of 2-mercaptoethanol from 1 to 100 μM caused an increase in the amounts of nicked open circular DNA with a concomitant decrease in the amount of form I DNA. Any further strand scission was not seen beyond that

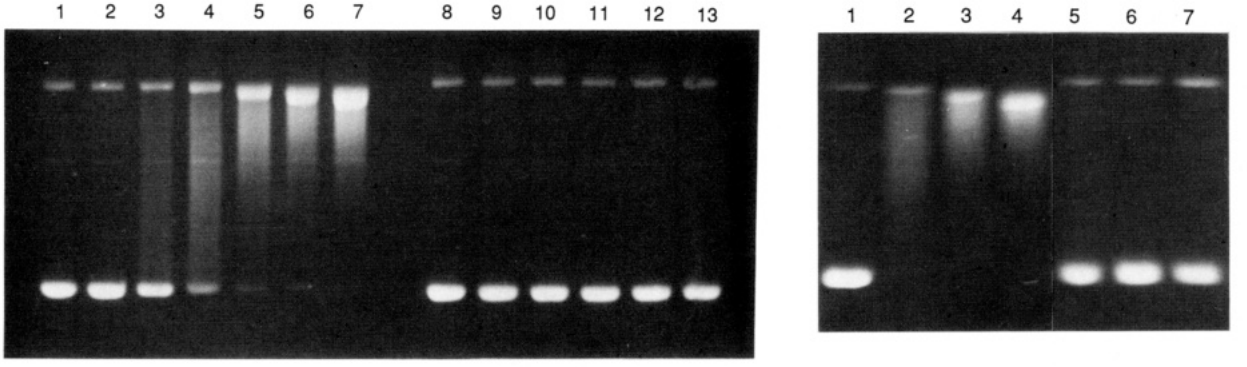

FIGURE 5: (A, left) DNA strand scission by leinamycin in the presence of various amounts of 2-mercaptoethanol. Lane 1, pBR322 DNA alone; lanes 2–7, pBR322 plus 25 μM leinamycin in the presence of 2-mercaptoethanol at 0, 1, 5, 20, 100, and 500 μM, respectively; lanes 8–13, pBR322 DNA plus 2-mercaptoethanol at 0, 1, 5, 20, 100, and 500 μM, respectively. (B, right) DNA strand scission by leinamycin in the presence of 2-mercaptoethanol and at various pH (50 mM phosphate buffer was used). Lane 1, pBR322 DNA alone; lanes 2–4, pBR322 DNA plus 25 μM leinamycin in the presence of 500 μM 2-mercaptoethanol at pH 4, 5, and 6, respectively; lanes 5–7, pBR322 DNA in the presence of 500 μM 2-mercaptoethanol at pH 4, 5, and 6, respectively.

Table I: Effect of Radical Scavengers on Leinamycin-Mediated DNA Strand Scission[a]

agent	scavengers	% distribution form I	form III	form II
		84.7	0.6	14.5
leinamycin (25 μM)	SOD (μg/mL)	8.9	0	91.9
	12.5	0.4	0	99.6
	50	0.2	0	99.8
	β-carotene (mM)			
	0.5	0.2	0	99.8
	1	0.3	0	99.7
	D-mannitol (mM)			
	50	0.2	0	99.8
	100	0.3	0	99.7

[a] Assays were carried out as described under Materials and Methods. The incubation mixture contains 0.3 μg of pBR322 DNA, 500 μM 2-mercaptoethanol, 25 μM leinamycin with various amounts of oxygen radical scavengers in 20 mM Tris-HCl buffer, pH 7.5.

observed in 1-h incubations. An approximately equal molar amount of thiol is necessary for the DNA-cleaving activity of leinamycin. Leinamycin (100 μM) preincubated with 400 μM of 2-mercaptoethanol for 30 min at 37 °C did not cause any DNA strand scission. The presence of EDTA did not supress the DNA-cleaving activity, confirming that the observed effect is not due to the presence of adventitious metal ions (data not shown). All of the above results indicate that leinamycin is activated only by agents containing a mercapto group and the transiently activated form of leinamycin has the potential to cause single-strand scission of DNA.

Figure 5B shows the effect of pH on the DNA strand scission in the presence of thiol. The changes in electrophoretic pattern observed at various pHs are quite similar to those observed for varying amounts of leinamycin or stoichiometric titration of 2-mercaptoethanol (compare lanes 2–4 in Figure 5B with lanes 3–6 in Figure 4 or lanes 4–7 in Figure 5A). These observations indicate that DNA-cleaving activity slightly increased with increasing pH from 4 to 6. Since thiol have pK_a's of ca. 8, one would not expect to see a pH-dependent DNA-cleaving behavior caused by thiols. We would then have the implication that the pH-dependent profile of strand scission in the presence of thiol is associated with the change in the intrinsic reactivity of leinamycin toward DNA and/or thiol. It will be well to keep in mind, however, that those arguments are indirect and not completely free from ambiguity.

Some antitumor agents has been reported to cleave DNA by generating a oxygen radical species (Lown et al., 1977; Lown & Weir, 1978; Lown et al., 1982; Tanida et al., 1982). In order to examine whether DNA strand scission by leinamycin is due to the production of oxygen-dependent free radicals, DNA cleavage was investigated with the addition of several oxygen radical scavengers (Table I). Scavengers such as superoxide dismutase, β-carotene, and D-mannitol did not supress the DNA-cleaving activity of leinamycin. Exclusion of oxygen from the reaction mixture did not inhibit strand scission. These results suggest that diffusible oxygen radical species are not involved in the strand scission.

Interaction of Leinamycin with Calf Thymus DNA. As described in the former section, the changes in the electrophoretic mobility of leinamycin-induced form II DNA is thought to be due to the association of drug with nicked DNA. To explore this observation further, we examined the interaction of leinamycin with calf thymus DNA. The DNA–drug complex, which was formed in the presence of 2-mercaptoethanol, was recovered by ethanol precipitation. HPLC analysis of thus recovered DNA gave rise to an adduct with a UV absorption maximum at 370 nm in addition to the

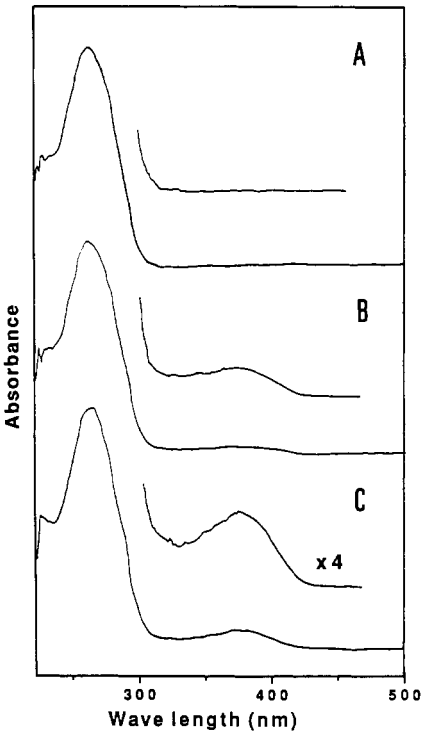

FIGURE 6: UV absorption spectra of leinamycin–calf thymus DNA complex at several temperatures. Leinamycin was reacted with calf thymus DNA as described under Materials and Methods. DNA was recovered by ethanol precipitation and subjected to HPLC analysis. UV spectra: Analyzed after 2 h of incubation at (A) 55 or (B) 37 °C, respectively; (C) analyzed immediately after ethanol precipitation.

expected DNA absorption at 256 nm, as shown in Figure 6. It is to be noted that the absorption maximum of leinamycin at 310 nm (Hara et al., 1989) shifted to 370 nm upon interaction with DNA. When the DNA–leinamycin complex was incubated at 37 °C, peak absorption at 370 nm decreased and disappeared at 55 °C. In the absence of 2-mercaptoethanol, on the other hand, calf thymus DNA treated with leinamycin did not exhibit any absorption maxima besides the DNA absorption at 259 nm (data not shown). These results allow us to suggest that thiol-activated leinamycin binds to DNA and the resulting DNA–leinamycin complex is thermolabile.

Nucleotide Sequence Cleavage of DNA by Thiol-Activated Leinamycin. To determine the specificity of the strand cleavage, we examined the reaction of leinamycin with 5′-end-labeled *Sal*I–*Bam*HI restriction fragment obtained from plasmid pBR322 DNA. DNA fragments treated with leinamycin were heated at 90 °C for 1 min to remove the DNA-bound antibiotic. The results of sequencing studies at several drug concentrations are shown in Figure 7. At the more dilute leinamycin concentration, a number of bands were observed. They became more prominent with increasing concentrations of leinamycin. Although the somewhat preferred cleavage sites were observed, there is little sequence specificity among them. These results indicate the randum DNA cleavage mode of leinamycin.

Essential Role of the 1,3-Dioxo-1,2-dithiolane Structure for the DNA-Cleaving Activity of Leinamycin. With regard to the thiol-mediated activation of leinamycin, we predicted that the 1,3-dioxo-1,2-dithiolane moiety might be the possible target of thiol cofactors. In order to gain further insight into this point, the effect of *S*-deoxyleinamycin on plasmid DNA was studied. Despite the close structural similarity between leinamycin and its *S*-deoxy derivative, the latter compound was found to be inactive; cytotoxicity on cultured cells and antimicrobial activity against Gram-positive bacteria were less

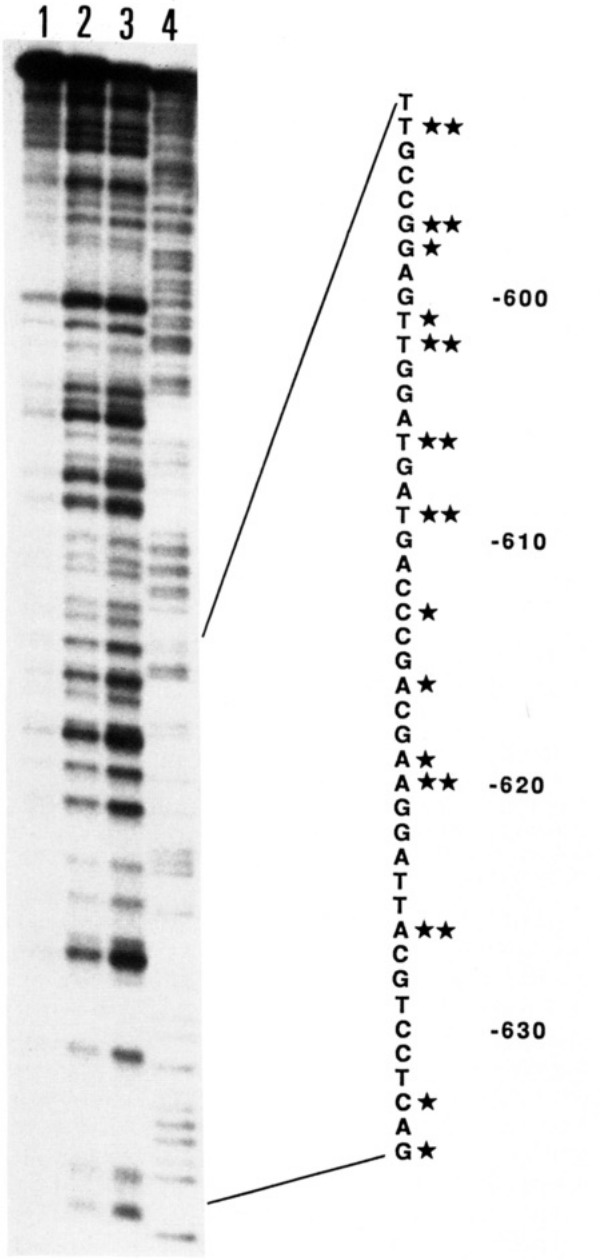

FIGURE 7: Autoradiogram of 10% polyacrylamide–7 M urea gel of a 5′-end-labeled 275-bp *Sal*I–*Bam*HI fragment from pBR322 DNA. The reaction mixture contained the restriction fragment, 500 μM DTT, 20 mM Tris-HCl buffer, and the following additives: lanes 1–3, 6.3, 25, and 100 μM leinamycin, respectively. The samples were incubated at 37 °C for 60 min. Lane 4 shows the Maxam–Gilbert's chemical reactions for C. Asterisks indicate the cleavage site and the relative intensity of the band on the autoradiogram.

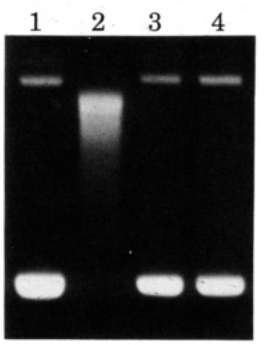

FIGURE 8: Agarose gel electrophoresis of pBR322 DNA treated with *S*-deoxyleinamycin. Lane 1, pBR322 DNA alone; lane 2, pBR322 DNA plus 25 μM leinamycin and 0.5 mM DTT; lanes 3 and 4, pBR322 DNA plus *S*-deoxyleinamycin at 25 and 100 μM, respectively, in the presence of 0.5 mM DTT.

than 1% that of leinamycin, i.e. IC_{50} against HeLa S3 cells was 2.1 μg/mL and minimum inhibitory concentration against *B. subtilis* was more than 100 μg/mL for *S*-deoxyleinamycin. In contrast, the values for leinamycin were 0.014 and 0.08 μg/mL, respectively. Consistent with this weak biological activity, incubation of *S*-deoxyleinamycin with DNA did not cause any cleavage in the presence of thiol cofactor (Figure 8). This observation indicates that the presence of the sulfoxide group in dithiolane moiety is necessary for the DNA-cleaving activity of leinamycin and demonstrates the critical nature of the 1,3-dioxo-1,2-dithiolane moiety of leinamycin.

Discussion

In the present study, leinamycin preferentially inhibited DNA synthesis in *B. subtilis* and exhibited single-strand scission of DNA in vitro. Thiol cofactors were required for this DNA-cleaving activity. Reducing agents without mercapto groups failed to activate leinamycin to cause strand breakage. These results allow us to suggest that the thiol-mediated activation of leinamycin might result from nucleophilic attack by thiol rather than reductive activation. The lack of cytotoxicity and DNA-cleaving activity of *S*-deoxyleinamycin demonstrates the essential role of the 1,3-dioxo-1,2-dithiolane moiety. These results are consistent with the chemical reactivity of the 1,3-dioxo-1,2-dithiolane moiety (i.e. mixed anhydride of thiocarboxylic acid and thiosulfinic acid), which is reported to be more reactive than 3-oxo-1,2-dithiolanes (i.e. dithioester) (Hortmann et al., 1978). We therefore suggest that thiol nucleophilic activation at the 1,3-dioxo-1,2-dithiolane region leads to DNA strand cleavage. Preliminary results indicate that incubation of leinamycin with an equivalent molar amount of 2-mercaptoethanol affords a component with the same UV absorption but that is inactive toward Gram-positive bacteria (Hara et al., unpublished observation). Isolation and structure determination of this thiol-treated product are underway and are expected to provide information on the mode of action of leinamycin.

Recently, thiol-mediated DNA strand scission has also been reported for the calicheamicin/esperamicin antitumor antibiotics (Zein et al., 1988, 1989a,b). These compounds induce strand scission via a proposed mechanism that involves benzene biradical species produced by endiyne-1,4-benzenediyl rearrangement in the molecule (Zein et al., 1988; Magnus & Carter, 1988; Nicolau et al., 1988). There is no structural moiety in leinamycin that is thought to be responsible for generating a biradical species. Therefore, the mechanism of DNA cleavage by leinamycin is clearly different from that proposed for calicheamicin/esperamicin.

It is noteworthy that leinamycin produces subtle but significant changes in the electrophoretic mobility of form II DNA. The nicked form II DNA, which was ethanol precipitated and thereby presumably free from the noncovalently associated drug with DNA, also exhibited the same change in electrophoretic pattern. Inspection of the UV absorption spectrum for calf thymus DNA treated with leinamycin at 4 °C demonstrates that leinamycin binds to DNA in a thiol-dependent manner. The binding of thiol-activated leinamycin to DNA is supposed to accompany certain chemical changes of the leinamycin molecule, as manifested as the change in absorption maximum of drug upon association of DNA. Incubation of the leinamycin–DNA complex at 37 or 55 °C results in a decrease of UV absorption due to the DNA-bound leinamycin molecule. From the above results and discussion, we suggest the following mechanism, which accounts for

thiol-mediated DNA strand scission of leinamycin. Leinamycin, which is activated by nucleophilic attack of thiol on the 1,3-dioxo-1,2-dithiolane, binds DNA through a covalent bonding. The resulting leinamycin–DNA complex is thermolabile, so that thermal treatment of the leinamycin-modified DNA results in the release of a chemically modified leinamycin covalently attached to certain DNA constituents. Subsequently, it leads to strand cleavage of the DNA. Even at the physiologically relevant temperature of 37 °C, the release of a chemically modified leinamycin occurs to some extent. This might explain our results of agarose electrophoresis of pBR322 plasmid DNA treated with leinamycin at 37 °C.

Two types of single-strand cleavage, in which free-radical scavengers have no effect, are known (Lown, 1983). One is due to base alkylation, depurination, or depyrimidination and cleavage of the AP site (Hurley et al., 1984; Lown & McLaughlin, 1979a,b). The other is due to phosphate residue alkylation and subsequent hydrolysis of the phosphotriesters (Ludlum, 1967, 1969; Lawley, 1973; Schooter et al., 1974). The present observation of a random cutting of DNA restriction fragment by leinamycin might be due to the alkylation of phosphate residue and subsequent DNA cleavage. Alternatively, leinamycin alkylates more than one kind of the base residue so that random cutting was observed. In order to descriminate among single-strand scission resulting from the above two known mechanisms or another unknown mechanism, we attempted to isolate chemically modified leinamycins that were released from the leinamycin–calf thymus DNA complex by thermal treatment. However, such compounds are labile and we do not succeed in obtaining them at the present stage. Further efforts are continuing.

In summary, leinamycin appears to mediate its biological activity through DNA strand breakage. The single-strand scission by leinamycin is dependent on thiol activation of the 1,3-dioxo-1,2-dithiolane structure, binding of thiol-activated leinamycin to DNA, and release of chemically modified leinamycin covalently attached to DNA. A combination of the 1,3-dioxo-1,2-dithiolane DNA-cleaving moiety with a DNA recognition element might provide a novel approach for the design of new cancer chemotherapeutic agents.

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