1,2-Dithiolan-3-one 1-Oxides: A Class of Thiol-Activated DNA-Cleaving Agents That Are Structurally Related to the Natural Product Leinamycin†

Saeid J. Behroozi,‡ Woongki Kim,‡ Jeffrey Dannaldson,‡ and Kent S. Gates*,‡,§

Departments of Chemistry and Biochemistry, University of Missouri—Columbia, Columbia, Missouri 65211

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ABSTRACT: Leinamycin is a recently discovered, thiol-dependent DNA-cleaving natural product. The mechanism of DNA cleavage by leinamycin is unknown. Inspired by this intriguing natural product, we have investigated the DNA-cleaving properties of three 1,2-dithiolan-3-one 1-oxides (**1**−**3**) that are structurally related to the suspected DNA-cleaving "core" of leinamycin. It was found that, similar to leinamycin, these three 1,2-dithiolan-3-one 1-oxides are thiol-dependent DNA-cleaving agents. At the concentrations of **1**−**3** used in these experiments (approximately 100 μM), efficient DNA cleavage is absolutely dependent on added thiol, with optimum cleavage occurring at 5−10 equiv (500 μM−1 mM) of added thiol. 2-Mercaptoethanol, glutathione, dithiothreitol, and thiophenol function with approximately equal efficiency as triggering agents for the cleavage reaction. DNA cleavage by **1**−**3** is not highly pH-dependent. Cleavage of DNA by these sulfur heterocycles is diminished by the removal of molecular oxygen from the reaction medium, by the radical scavengers methanol, ethanol, and mannitol, and by the enzyme catalase. Superoxide dismutase does not suppress DNA cleavage by these compounds. When diethylenetriaminepentaacetic acid is employed in these reactions as a chelator of adventitious trace metal ions, DNA cleavage is efficiently inhibited. The *S*-deoxy analog of **1** does not cleave DNA under conditions where **1** effects efficient thiol-mediated cleavage of DNA. These experiments indicate that, in concert with thiols, 1,2-dithiolan-3-one 1-oxides convert molecular oxygen to DNA-cleaving oxygen radicals. The marked effect of catalase further suggests that molecular oxygen is converted to hydrogen peroxide which ultimately cleaves DNA via a trace metal-dependent Fenton reaction. This work demonstrates that 1,2-dithiolan-3-one 1-oxides represent a general class of thiol-potentiated DNA-cleaving molecules.

DNA-cleaving antibiotics are important due to their potential as anticancer agents (Remers, 1991; Hecht, 1989; Hurley, 1989; Hertzberg & Johnson, 1993). In addition, investigations of novel DNA-cleaving natural products have often been rewarded by the discovery of interesting and unexpected chemical reactions that lead to DNA strand scission [for an example see Lee et al. (1987)]. The antitumor antibiotic leinamycin is reported to cleave DNA (Hara et al., 1990) but does not appear to fall into any of the known classes of DNA-cleaving antibiotics. The chemical mechanism by which leinamycin cleaves DNA is not known but is of interest because leinamycin may be the first in a new class of DNA-cleaving antibiotics.

Leinamycin was isolated from an unnamed strain of *Streptomyces* in 1989 and its structure elucidated by NMR spectroscopy, chemical analysis, X-ray crystallography (Hara et al., 1989; Hirayama & Matsuzawa, 1993), and more recently, chemical synthesis (Kanda & Fukuyama, 1993). Leinamycin exhibits antitumor activity and is also effective against Gram-positive bacteria (Hara et al., 1989). Experiments by its discoverers indicate that this compound probably derives its biological activity through cleavage of DNA and that efficient *in vitro* DNA cleavage by leinamycin is dependent on added thiols (Hara et al., 1990). Other reducing agents such as NADH and ascorbate reportedly do not potentiate DNA cleavage by leinamycin. It was shown that *S*-deoxyleinamycin displays greatly reduced biological activity and does not cleave DNA *in vitro*, under conditions where leinamycin efficiently cleaves DNA (Hara et al., 1990). Thus, the unusual 1,2-dithiolan-3-one 1-oxide heterocycle of leinamycin has been strongly implicated in the cleavage of DNA by the natural product and existing data suggests that nucleophilic attack of thiols on this sulfur-containing ring triggers subsequent DNA-cleavage chemistry. Model studies involving simple 1,2-dithiolan-3-one 1-oxides have shown that this heterocycle does, indeed, react readily with thiols (Behroozi et al., 1995a).

Inspired by the natural product leinamycin, we have investigated DNA cleavage by several variously substituted 1,2-dithiolan-3-one 1-oxides. We find that, similar to leinamycin, the simple 1,2-dithiolan-3-one 1-oxides **1**, **2**, and **3** are thiol-activated DNA-cleaving agents. Our work demonstrates that 1,2-dithiolan-3-one 1-oxides represent a general class of thiol-activated DNA-cleaving agents and supports the notion that the sulfur-containing heterocycle of leinamycin is, in fact, the DNA-cleaving "core" of this natural product (Hara et al., 1990). Our results suggest that, in concert with thiols, 1,2-dithiolan-3-one 1-oxides convert molecular oxygen to DNA-cleaving oxygen radicals.

MATERIALS AND METHODS

Materials. Supercoiled pBR322 plasmid DNA was isolated from *Escherichia coli* and purified by cesium chloride gradient centrifugation (Sambrook et al., 1989) or purchased

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* To whom correspondence should be addressed.

‡ Department of Chemistry.

§ Department of Biochemistry.

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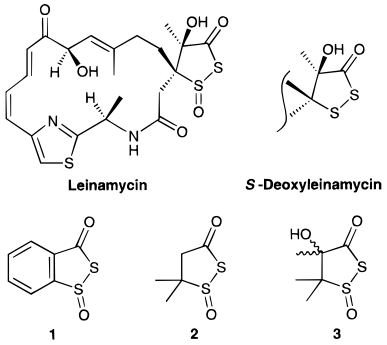

from Boehringer-Mannheim. Buffers, chelators, radical scavengers, and thiols were purchased from Aldrich Chemical Co. or Sigma Chemical Co. and were of the highest purity available. Catalase and superoxide dismutase were purchased from Boehringer-Mannheim. HPLC-grade acetonitrile was purchased from Fisher. Water was distilled, deionized, and glass-redistilled. Seakem ME agarose was from FMC. Ethidium bromide pellets were purchased from Gibco BRL.

*Preparation of 3H-1,2-Benzodithiol-3-one 1-Oxide (**1**).* Compound **1** was prepared by a previously described method (Iyer et al., 1990), purified by column chromatography on silica gel, and recrystallized two times from chloroform−hexane. This compound was subjected to trace metal analysis using inductively coupled plasma atomic spectroscopy (Southern Testing Research Labs, Wilson, NC) and found to contain no detectable traces of chromium, copper, iron, manganese, nickel, titanium, or vanadium. In addition, we have characterized **1** by X-ray crystallography (Behroozi et al., 1995b).

*Preparation of 5,5-Dimethyl-1,2-dithiolan-3-one 1-Oxide (**2**) and 4-Hydroxyl-4,5,5-trimethyl-1,2-dithiolan-3-one 1-Oxide (**3**).* Compounds **2** and **3** were prepared by modifications of synthetic routes described previously (Pattenden & Shuker, 1992). Compound **3** was used as a mixture of diastereomers.

*Preparation of 3H-1,2-Benzodithiol-3-one (S-deoxy-**1**).* *S*-Deoxy-**1** was prepared as described previously (Iyer et al., 1990).

DNA Cleavage Reactions. The 1,2-dithiolan-3-one 1-oxides were used as freshly prepared solutions in acetonitrile and the final reaction mixtures contained 10% acetonitrile by volume. Because dimethyl sulfoxide is an efficient radical scavenger, it is not a suitable cosolvent for these reactions. Acetonitrile is less reactive toward oxygen radicals (Buxton et al., 1988). Thiol solutions were prepared immediately before use. Compounds **1**, **2**, and **3** decompose slowly in aqueous solution; however, their reaction with thiols is rapid; therefore, these DNA-cleaving agents were added either as the final component to a thiol-containing reaction mixture or as the penultimate component, followed immediately by thiol. Thus, in a typical assay (final concentrations 250 μM **1**, 50 mM sodium phosphate, pH 7.0, 1.25 mM 2-mercaptoethanol, and 0.5 μg of pBR322), to a mixture of buffer (2 μL of a 500 mM sodium phosphate, pH 7.0 solution), water (12 μL), and pBR322 DNA (2 μL of a 0.25 μg/mL solution in TE)[1] was added **1** (2 μL of a 2.5 mM solution in acetonitrile) followed immediately by 2-mercaptoethanol (2 μL of a 12.5 mM solution in water). The mixture was agitated on a vortex mixer, spun for 5 s in a tabletop microfuge, and then incubated in the dark for 10−18 h at 37 °C.

Anaerobic DNA-Cleavage Assays. Anaerobic assays were performed as described above, except the reactions were performed in a 200-μL vacuum microhydrolysis tube (Kontes no. 896870). Immediately following addition of the final component, the reaction mixture was subjected to four "freeze−pump−thaw" cycles to effect removal of dissolved gasses. The degassed sample was then sealed and incubated in the dark under vacuum for 10−18 h at 37 °C. We find that benchtop purging of solutions with inert gas is not sufficient to remove dissolved oxygen.[2]

Gel Electrophoresis. Following incubation, 2−3 μL of 50% glycerol loading buffer containing 0.1% bromophenol blue, 100 mM EDTA, 1% SDS, and 50× TAE (Sambrook et al., 1989) was added to the 20-μL reactions and the resulting mixture was loaded immediately onto a 0.9% agarose gel. The gel was electrophoresed at 80 V for approximately 4 h in TAE buffer and then stained in an aqueous ethidium bromide solution (0.2 μg/mL) for 3−6 h. The DNA was then visualized by UV transillumination and the gel was photographed using either Polaroid 665 or 55 film.

Quantitation of DNA Cleavage. The amount of DNA in each band of ethidium-stained gels (generally only forms I and II were present) was quantitated from Polaroid 55 negatives using an Alpha Innotech IS-1000 digital imaging system or a Zeineh SL-TRFF scanning laser densitometer. The values shown are uncorrected for differential ethidium staining of form I, II, and III DNA (Bauer & Vinograd, 1968).

RESULTS

DNA Cleavage by 1,2-Dithiolan-3-one 1-Oxides. We have investigated the DNA-cleaving properties of three 1,2-dithiolan-3-one 1-oxides that vary significantly in their substitution. DNA cleavage was monitored by observing the conversion of supercoiled (form I) plasmid DNA to the circular nicked form (form II) that occurs upon single-strand scission. In some cases, more extensive DNA cleavage leads to the appearance of linearized (form III) plasmid migrating just below the nicked form in the 0.9% agarose gels shown. We find that, in the presence of added thiols, micromolar concentrations of the dithiolanone oxides **1**, **2**, and **3** efficiently carry out single-strand cleavage of plasmid DNA (Figures 1, 2, and 3). At the concentrations used in our studies, **1**−**3** do not cleave DNA efficiently in the absence of added thiol. Although thiols are capable of causing DNA cleavage through reduction of molecular oxygen to superoxide radical (Bode, 1968; Rosenkranz & Rosenkranz, 1971; Misra, 1974; Rowley & Halliwell, 1982; Saez et al., 1982), our controls show that, under the conditions of these experiments, 2-mercaptoethanol alone does not significantly cleave DNA (Figures 1−3, Table 1).

[1] Abbreviations: TE, 10 mM Tris-HCl, pH 8.0/1 mM EDTA; TAE, 40 mM Tris-acetate, pH 8.0/1 mM EDTA; Tris, tris(hydroxymethyl)-aminomethane; SDS, sodium dodecyl sulfate; MES, 4-morpholineethanesulfonic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; DETAPAC, diethylenetriaminepentaacetic acid; SOD, superoxide dismutase; EDTA, ethylenediaminetetraacetic acid.

[2] J. Scott Daniels and Kent S. Gates, unpublished data.

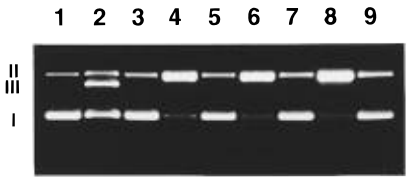

FIGURE 1: Thiol-dependent cleavage of supercoiled pBR322 DNA by **1**, **2**, and **3**. Reactions were incubated at 37 °C for 18 h in 50 mM sodium phosphate, pH 7.0, containing 10% acetonitrile and 0.5 μg of supercoiled pBR322 DNA. Lane 1, pBR322 DNA alone; lane 2, a mixture of supercoiled, nicked, and linearized pBR322 DNA; lane 3, 100 μM **1**; lane 4, 100 μM **1** + 500 μM 2-mercaptoethanol; lane 5, 100 μM **2**; lane 6, 100 μM **2** + 500 μM 2-mercaptoethanol; lane 7, 100 μM **3**; lane 8, 100 μM **3** + 500 μM 2-mercaptoethanol; lane 9, 500 μM 2-mercaptoethanol alone.

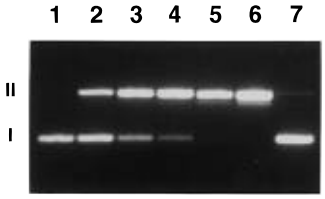

FIGURE 2: DNA cleavage by various concentrations of **1**. Reactions were incubated at 37 °C for 10 h in 50 mM sodium phosphate, pH 7.0, containing 10% acetonitrile and 0.5 μg of supercoiled pBR322 DNA. Lane 1, pBR322 DNA alone; lane 2, 50 μM **1** + 250 μM 2-mercaptoethanol; lane 3, 100 μM **1** + 500 μM 2-mercaptoethanol; lane 4, 250 μM **1** + 1.25 mM 2-mercaptoethanol; lane 5, 500 μM **1** + 2.5 mM 2-mercaptoethanol; lane 6, 1 mM **1** + 5 mM 2-mercaptoethanol; lane 7, 5 mM 2-mercaptoethanol alone. (Each cleavage reaction contains 5 equiv of thiol, based on moles of **1**.)

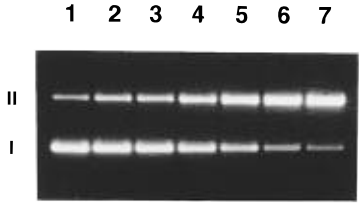

FIGURE 3: DNA cleavage by various concentrations of **2**. Reactions were incubated at 37 °C for 18 h in 50 mM sodium phosphate, pH 7.0 containing 10% acetonitrile and 0.5 μg of supercoiled pBR322 DNA. Lane 1, pBR322 DNA alone; lane 2, 100 μM **2** + no thiol; lane 3, 10 μM **2** + 50 μM 2-mercaptoethanol; lane 4, 25 μM **2** + 125 μM 2-mercaptoethanol; lane 5, 50 μM **2** + 250 μM 2-mercaptoethanol; lane 6, 75 μM **2** + 375 μM 2-mercaptoethanol; lane 7, 100 μM **2** + 500 μM 2-mercaptoethanol. (Each cleavage lane contains 5 equiv of thiol, based on moles of **2**.)

DNA cleavage by **1** increases with increasing amounts of added 2-mercaptoethanol up to approximately 10 equiv (based on moles of dithiolanone oxide) (Figure 4). Increasing the thiol concentration beyond 10 equiv leads to diminished DNA cleavage relative to lower thiol concentrations. Thiol titration of the alicyclic compound **2** reveals a similar trend, with the exception that, whereas DNA cleavage by **1** becomes efficient only upon addition of 2 or more equiv of thiol, cleavage by **2** is relatively efficient even at 1 equivalent of added 2-mercaptoethanol (Figure 5). The behavior of compound **3** is analogous to **2** (data not shown).

The thiol 2-mercaptoethanol is not unique in its ability to potentiate DNA cleavage by **1**, **2**, and **3**. Figure 6 compares the ability of four thiols, 2-mercaptoethanol, glutathione, dithiothreitol, and thiophenol, to trigger DNA cleavage by **1**. Within this series, we find that thiol structure has little effect on the potentiation of DNA cleavage by **1**. Compounds **2** and **3** display analogous behavior.

The pH of the reaction medium does not have a large effect on DNA cleavage by **1**−**3** (data not shown); however, the identity of the buffer has a significant effect on the efficiency

Table 1: Effect of Various Conditions and Additives on Cleavage of Plasmid DNA by **1**[a]

reaction	% form I	% form II
DNA alone	95	5
standard rxn: 100 μM **1** + thiol (5 equiv)[b]	24	76
no thiol	84	16
MES buffer (pH 6.0, 50 mM)	49	51
HEPES buffer (pH 6.8, 50 mM)	80	20
degassed	81	19
S-deoxy-**1** (100 μM)	95	5
added reagent		
methanol (1 M)	80	20
methanol (200 mM)	64	36
ethanol (1 M)	85	15
ethanol (200 mM)	72	28
mannitol (100 mM)	70	30
DEAPAC (10 mM)	87	13
DETAPAC (1 mM)	71	29
EDTA (1 mM)	37	63
SOD (100 μg/mL)	8	92
catalase (100 μg/mL)	82	18
denatured catalase (100 μg/mL)	18	82

[a] Reactions and densitometry performed as described in the Materials and Methods section. Values reflect the average of multiple experiments. Standard errors in these measurements are less than 5%. [b] **1** (100 μM) and 2-mercaptoethanol (500 μM) were incubated with 0.5 mg of supercoiled pBR322 DNA in 50 mM sodium phosphate, pH 7.0, and 10% acetonitrile for 10 h at 37 °C.

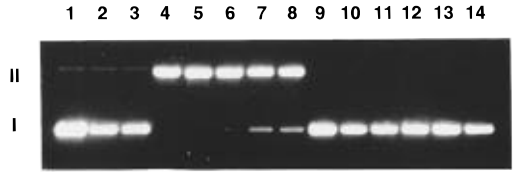

FIGURE 4: DNA cleavage by 500 μM **1**, in the presence of varying amounts of thiol. Reactions were incubated at 37 °C for 10 h in 50 mM sodium phosphate, pH 7.0, containing 10% acetonitrile and 0.5 μg of supercoiled pBR322 DNA. Lane 1, pBR322 DNA alone; lane 2, 500 μM **1** + no thiol; lane 3, 500 μM **1** + 500 μM 2-mercaptoethanol; lane 4, 500 μM **1** + 1 mM 2-mercaptoethanol; lane 5, 500 μM **1** + 2.5 mM 2-mercaptoethanol; lane 6, 500 μM **1** + 5 mM 2-mercaptoethanol; lane 7, 500 μM **1** + 10 mM 2-mercaptoethanol; lane 8, 500 μM **1** + 25 mM 2-mercaptoethanol; lane 9, 500 μM 2-mercaptoethanol; lane 10, 1 mM 2-mercaptoethanol; lane 11, 2.5 mM 2-mercaptoethanol; lane 12, 5 mM 2-mercaptoethanol; lane 13, 10 mM 2-mercaptoethanol; lane 14, 25 mM 2-mercaptoethanol.

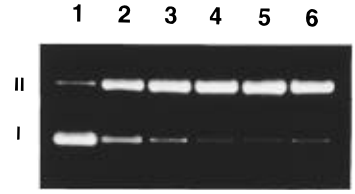

FIGURE 5: DNA cleavage by 100 μM **2**, in the presence of varying amounts of thiol. Reactions were incubated at 37 °C for 18 h in 50 mM sodium phosphate, pH 7.0, containing 10% acetonitrile and 0.5 μg of supercoiled pBR322 DNA. Lane 1, pBR322 DNA alone; lane 2, 100 μM **2** + 100 μM 2-mercaptoethanol; lane 3, 100 μM **2** + 200 μM 2-mercaptoethanol; lane 4, 100 μM **2** + 500 μM 2-mercaptoethanol; lane 5, 100 μM **2** + 1 mM 2-mercaptoethanol; lane 6, 100 μM **2** + 2 mM 2-mercaptoethanol.

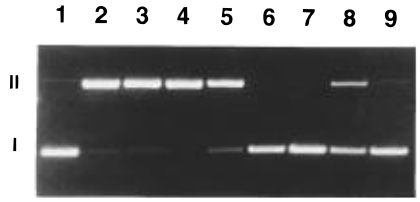

FIGURE 6: DNA cleavage by 250 μM **1** in the presence of four different thiols. Reactions were incubated for 10 h at 37 °C in 50 mM sodium phosphate, pH 7.0, containing 10% acetonitrile and 0.5 μg of supercoiled pBR322 DNA. Lane 1, pBR322 DNA alone; lane 2, 250 μM **1** + 1.25 mM 2-mercaptoethanol; lane 3, 250 μM **1** + 1.25 mM glutathione; lane 4, 250 μM **1** + 1.25 mM dithiothreitol; lane 5, 250 μM **1** + 1.25 mM thiophenol; lane 6, 1.25 mM 2-mercaptoethanol; lane 7, 1.25 mM glutathione; lane 8, 1.25 mM dithiothreitol; lane 9, 1.25 mM thiophenol.

of the DNA cleavage reaction. Strand scission is markedly more efficient in phosphate buffer, as compared to buffers such as HEPES and MES (Table 1). A possible explanation for this effect is offered in the Discussion section.

Investigation of the Mechanism of DNA Cleavage by 1,2-Dithiolan-3-one 1-Oxides. Strand scission can result from reaction of DNA with a variety of species including electrophilic (e.g., alkylating) agents (Lawley & Brookes, 1963), oxygen radicals (Stubbe & Kozarich, 1987; von Sonntag et al., 1981), and carbon-centered radicals (Nicolaou & Dai, 1991; Goldberg, 1991; Myers et al., 1994; Riordan & Wei, 1994). Thus, we undertook experiments designed to identify the nature of the reactive species responsible for thiol-mediated DNA cleavage by 1,2-dithiolan-3-one 1-oxides **1**−**3**. Table 1 presents the results of various mechanistic experiments involving **1**; analogous results were observed for compounds **2** and **3**.

Removal of dissolved atmospheric gases from the samples by "freeze−pump−thaw" degassing of the complete reaction mixture prior to incubation results in substantially diminished DNA cleavage (Table 1). Controls show that reaeration of a degassed mixture allows efficient DNA cleavage to proceed as usual. This important result clearly suggests that molecular oxygen is involved in the cleavage of DNA by **1**−**3**. Oxygen-dependent cleavage of DNA usually indicates involvement of radical species (Pratviel et al., 1995); thus, additional experiments were designed to explore the role of radicals and molecular oxygen in this DNA-cleavage reaction.

Radical scavenging agents methanol, ethanol, and mannitol significantly inhibit DNA cleavage by **1**−**3** (Table 1). Methanol and ethanol are known to react rapidly with hydroxyl radical and, although more slowly, with alkoxyl radicals (Buxton et al., 1988; Halliwell & Gutteridge, 1990; Rao et al., 1988). Mannitol is reported to be an efficient scavenger of hydroxyl radical but not alkoxyl radicals (Halliwell & Gutteridge, 1990; Rao, 1988). Thus, inhibition of DNA cleavage by these alcohols is consistent with a notion that oxygen radicals are involved in the cleavage of DNA by **1**−**3**. We cannot rule out, however, that these agents may act by trapping reactive, nonradical intermediates generated in the reaction of **1**−**3** with thiols. The radical scavengers employed in our experiments do not react directly with **1**−**3** at a significant rate in the absence of thiol.

Metal chelators such as desferal and diethylenetriaminepentaacetic acid (DETAPAC) are known to sequester adventitious traces of transition metals in a form that inhibits these metals from catalyzing the conversion of species such as peroxides to DNA-cleaving oxygen radicals such as hydroxyl radical (Halliwell & Gutteridge, 1990; Graf et al., 1984; Williams et al., 1992). Thus, the finding that addition of DETAPAC to our reaction mixtures causes marked inhibition of thiol-dependent DNA cleavage by **1**−**3** provides additional evidence implicating oxygen radicals in these cleavage reactions (Table 1).

Addition of the superoxide-destroying enzyme superoxide dismutase (SOD) (Fridovich, 1972) to our reaction mixtures does not inhibit thiol-mediated DNA cleavage by **1**−**3**. Interestingly, in all cases, DNA cleavage is actually slightly *enhanced* in the presence of SOD (Table 1). On the other hand, addition of the hydrogen peroxide-destroying enzyme catalase nearly completely inhibits the cleavage of DNA by **1**−**3**. Addition of heat-denatured catalase to the reaction mixtures has no effect on the cleavage reactions. The marked effect of catalase on these DNA-cleavage reactions suggests that 1,2-dithiolan-3-one 1-oxides, in combination with thiols, convert molecular oxygen to hydrogen peroxide and that DNA cleavage is ultimately caused by hydroxyl radicals that arise from hydrogen peroxide decomposition via the Fenton reaction (Halliwell & Gutteridge, 1990; Rhaese & Freese, 1968; Pogozelski et al., 1995; Hertzberg & Dervan, 1984; Luo et al., 1994). Possible origins of hydrogen peroxide are considered in the Discussion section of this paper.

We find that, under conditions where **1** causes efficient thiol-mediated DNA cleavage, identical concentrations of the *S*-deoxy analog of **1** do not effect measurable cleavage of DNA (Table 1).

DISCUSSION

The intriguing thiol-dependent DNA-cleaving properties of the natural product leinamycin (Hara et al., 1990) motivated us to examine DNA cleavage by three synthetic 1,2-dithiolan-3-one 1-oxides that are structurally related to the suspected DNA-cleaving "core" of leinamycin. We have investigated the mechanism of DNA cleavage by these compounds and explored the effects of varying dithiolanone oxide structure and substitution.

The compounds that we investigated vary significantly in substitution and include one compound (**3**) that is structurally identical to the dithiolanone oxide heterocycle of the natural product leinamycin. We find that all three of the 1,2-dithiolan-3-one 1-oxides, **1**, **2**, and **3**, are thiol-activated DNA-cleaving agents. The DNA-cleaving properties and cleavage efficiency of these agents are remarkably *unaffected* by the substituents on 1,2-dithiolan-3-one 1-oxide heterocycle. This important result indicates that the 1,2-dithiolan-3-one 1-oxide heterocycle is sufficient to cause thiol-mediated DNA cleavage and that additional substituents on the sulfur-containing heterocycle are not required for activity.

The oxidation state of sulfur in the 1,2-dithiolan-3-one ring system appears important to the DNA-cleaving properties of these compounds, as demonstrated by the fact that the *S*-deoxy analog of **1** does not cleave DNA under conditions where **1** causes efficient thiol-mediated cleavage of DNA. The well-known increased reactivity of thiosulfinates as compared to disulfides (Kice & Rogers, 1974; Oae et al., 1977; Hara et al., 1990) provides a useful analogy to rationalize the differences in activity observed for **1** versus the corresponding *S*-deoxy compound. In addition, chemical model studies have shown that while **1** reacts rapidly with

thiols, *S*-deoxy-**1** is relatively unreactive toward thiols (Behroozi et al., 1995a).

Compounds **1**−**3** react rapidly (within minutes) and completely with thiols in aqueous buffered solution (data not shown), and it is possible that the resulting products, not the intact 1,2-dithiolan-3-one 1-oxide heterocycle, are responsible for the observed DNA cleavage chemistry. We have characterized a portion of the products resulting from the reaction of thiols with 1,2-dithiolan-3-one 1-oxides with thiols (Behroozi et al., 1995a); however, it is not clear whether these products or other, as yet uncharacterized, reaction products are involved in the DNA cleavage reaction. Efforts to completely characterize the products of the reactions between **1**−**3** and thiols in aqueous solution are currently underway in our laboratory.

Under the conditions of our experiments, efficient DNA cleavage by **1**−**3** is absolutely dependent on added thiol. 2-Mercaptoethanol, glutathione, dithiothreitol, and thiophenol function with approximately equal efficiency as cofactors for the cleavage of DNA by **1**−**3**, suggesting that the thiol functional group alone in these molecules is sufficient to trigger the cleavage reactions.

The fact that cleavage of DNA by these molecules is significantly diminished by freeze−pump−thaw degassing of the reaction mixtures strongly suggests that molecular oxygen is involved in the cleavage reaction. There are several ways that organic molecules can utilize molecular oxygen to cleave nucleic acids. For example, a number of organic reducing agents are known to convert molecular oxygen to superoxide radical (Williams et al., 1994; Weiner, 1994; Gaudiano & Koch, 1991; Cone et al., 1976; Misra & Fridovich, 1972; Misra, 1974). Spontaneous dismutation of superoxide radical produces hydrogen peroxide, which subsequently undergoes trace metal-catalyzed breakdown to the known DNA-cleaving agent hydroxyl radical (Haber-Weiss cycle and Fenton reaction) (Lesko et al., 1980). The resulting hydroxyl radicals cleave DNA primarily by abstracting hydrogen atoms from the deoxyribose backbone (von Sonntag et al., 1981; Stubbe & Kozarich, 1987; Pratviel et al., 1995). Molecular oxygen may also combine with organic radicals to yield reactive peroxyl radicals or hydroperoxides (March, 1992). Peroxyl radicals might directly abstract hydrogen atoms from the DNA backbone (Marnett, 1987; Bregant et al., 1994; Barvian & Greenberg, 1995; Deeble & von Sonntag, 1986) and hydroperoxides, in a reaction analogous to the Fenton reaction, decompose in the presence of adventitious transition metal ions to yield peroxyl and alkoxyl radicals (Davies & Slater, 1987; Black, 1978) that could potentially abstract hydrogen atoms from the deoxyribose backbone. The results of our degassing experiments, implicating the involvement of molecular oxygen, prompted us to pursue experiments designed to further define the role of molecular oxygen and oxygen radicals in the DNA cleavage reactions of **1**−**3**.

Several lines of evidence indicate that DNA-cleaving oxygen radicals derived from molecular oxygen are generated in the reaction of dithiolanone oxides **1**−**3** with thiols. Oxygen radical scavengers ethanol, methanol, and mannitol significantly inhibit the cleavage reactions of **1**−**3**. Decreased DNA cleavage by **1**−**3** at high thiol concentrations (Figures 4 and 5) may be attributed to the fact that thiols can also act as radical scavengers (Asmus, 1990; Huston et al., 1992; Buxton et al., 1988). A similar trend has been observed previously for neocarzinostatin, another thiol-activated DNA-cleaving agent that generates radical intermediates (Kappen & Goldberg, 1978). In addition, the observed buffer dependence of this DNA-cleavage reaction (cleavage in HEPES and MES < phosphate) is consistent with the known abilities of these buffers to react with radicals (Buxton et al., 1988; Pogozelski et al., 1995).

The extremely effective inhibition of these thiol-dependent DNA cleavage reactions by catalase suggests that molecular oxygen may ultimately be converted to hydrogen peroxide in our reactions and that DNA cleavage results from hydroxyl radicals that are produced by hydrogen peroxide decomposition (Fenton reaction). Hydrogen peroxide may be formed directly or from spontaneous dismutation of superoxide radical. The dependence of DNA cleavage on adventitious trace metal ions, as demonstrated by the ability of the metal chelator DETAPAC to inhibit DNA strand scission, is consistent with trace metal-dependent, Fenton reaction-mediated production of hydroxyl radicals from hydrogen peroxide. It must be noted, however, that catalase is known to decompose some organic hydroperoxides (Schomburg et al., 1994); thus, it is not possible to rule out involvement of such species at this time.

As noted above, hydrogen peroxide may be formed either directly or by spontaneous dismutation of superoxide radical. Involvement of superoxide radical in other DNA cleavage reactions has been indicated by diminished DNA cleavage upon addition of the superoxide-decomposing enzyme SOD to reaction mixtures (Williams et al., 1992). We find that SOD does not inhibit DNA cleavage by the dithiolanone oxides in our study. When considered carefully, however, this finding *cannot* be taken as evidence that superoxide has no role in thiol-mediated DNA cleavage by **1**−**3**. Superoxide dismutase efficiently catalyzes the conversion of two molecules of superoxide radical to one molecule of molecular oxygen and one molecule of hydrogen peroxide (Fridovich, 1972; Walsh, 1979). The product of the superoxide dismutase reaction, hydrogen peroxide, as discussed above, can cause DNA cleavage through Fenton reaction-mediated production of hydroxyl radicals. In cases where superoxide-mediated DNA cleavage is inhibited by superoxide dismutase (Williams et al., 1992), the inhibition probably results from the fact that although SOD catalyzes the formation of hydrogen peroxide, the enzyme removes superoxide, which may serve as a necessary reducing agent to drive the Fenton chemistry (Halliwell & Gutteridge, 1990). In our system, the fact that superoxide dismutase does not inhibit DNA cleavage cannot rule out involvement of superoxide radical due to the possibility that enzymatically produced hydrogen peroxide could lead to DNA cleavage through a Fenton reaction in which *thiol* serves as the reducing agent. The slight increase in DNA cleavage that we observe upon addition of SOD (Table 1) may, in fact, suggest the involvement of superoxide radical and provide further support for the important role of hydrogen peroxide; however, the presence of superoxide radical in these reactions has not yet been unambiguously shown.

When considered together, our experiments suggest that, in concert with thiols, 1,2-dithiolan-3-one 1-oxides lead to the conversion of molecular oxygen to DNA-cleaving oxygen radicals. The marked effect of catalase on these reactions suggests that hydrogen peroxide is involved and that DNA cleavage may ultimately result from hydroxyl radicals

produced via the Fenton reaction. Our results are not consistent with thiol-dependent production of alkylating agents, exceptionally nucleophilic species, or carbon-centered radicals as principal intermediates in the cleavage of DNA by 1,2-dithiolan-3-one 1-oxides such as **1**−**3**.

Finally, it is perhaps useful to compare and contrast our findings to those reported previously for the natural product leinamycin (Hara et al., 1990). In making comparisons between the compounds in question, it must be remembered that significant differences in the observed DNA-cleaving properties of **1**−**3** versus leinamycin could result either from differences in the DNA-binding properties of these molecules or from differences in the chemical mechanism of DNA cleavage. Accordingly, at this time it is not clear whether the somewhat higher efficiency of DNA cleavage by leinamycin (3−25 μM concentrations completely nick 0.3 μg of supercoiled pBR322 DNA) versus our synthetic analogs (100 μM concentrations of **1**−**3** completely nick 0.5 μg of pBR322 DNA) is a result of differences in DNA affinity or DNA cleavage chemistry.

The 1,2-dithiolan-3-one 1-oxides that we have investigated, like leinamycin, are thiol-activated DNA-cleaving agents. Therefore, in general, our work supports the notion (Hara et al., 1990) that the 1,2-dithiolan-3-one 1-oxide heterocycle is intimately involved in DNA cleavage by leinamycin. However, our conclusion that molecular oxygen-derived radicals are involved in DNA cleavage by 1,2-dithiolan-3-one 1-oxides stands in direct contrast with that reached by the discoverers of leinamycin, who concluded that radicals were not involved in DNA cleavage by the natural product (Hara et al., 1990). The discoverers of leinamycin suggested that thiol-dependent formation of alkylating species may be responsible for DNA strand scission. In light of our findings, we are able to suggest a reinterpretation of results presented by Hara and co-workers regarding the mechanism of leinamycin. In an experiment designed to probe the involvement of trace metal ions in DNA cleavage by leinamycin, Hara et al. observed no effect resulting from addition of the metal chelator EDTA to the reactions. However, EDTA is much less effective at sequestering trace metals in a manner that prevents conversion of hydrogen peroxide to hydroxyl radical as compared to DETAPAC, the chelator used in our experiments (Leskso et al., 1980; Graf et al., 1984; Williams et al., 1992). Indeed, we find that moderate concentrations of EDTA have little inhibitory effect on DNA cleavage by **1** (Table 1), while an identical concentration of DETAPAC significantly inhibits cleavage. It was also reported that degassing the leinamycin cleavage reactions had no effect on DNA cleavage; however, we find that complete removal of dissolved gases from aqueous solutions is difficult, and in our hands, purging aqueous solutions with inert gas, the method utilized by Hara et al., is not sufficient to remove dissolved oxygen, rather, freeze-pump−thaw degassing is required. Thus, traces of oxygen remaining in solution may account for the DNA cleavage observed in the degassing experiments performed with leinamycin. Though Hara and co-workers report that the radical scavengers mannitol (100 mM) and β-carotene (1 mM) have no effect on the DNA-cleavage reaction of leinamycin, it is possible that radical species generated by a DNA-bound 1,2-dithiolan-3-one 1-oxide are not susceptible to reaction with scavenging agents. Although it has not been shown that leinamycin binds to DNA, this is a common feature of DNA-cleaving natural products and thus is not unexpected. Finally, the effect of catalase, a key experiment in our study, was not investigated in the case of DNA cleavage by leinamycin. This experiment might provide useful information regarding the mechanism of DNA cleavage by the natural product. Thus, while it is possible that leinamycin cleaves DNA by a mechanism that is distinct from the 1,2-dithiolan-3-one 1-oxides described in this paper, we feel that existing evidence indicates it is equally possible that the natural product and our synthetic agents share a common mode of DNA cleavage involving oxygen radicals.

CONCLUSION

1,2-Dithiolan-3-one 1-oxides represent an intriguing class of molecules that, in combination with thiols, utilize molecular oxygen for the cleavage of DNA. Our studies indicate that DNA cleavage is due to molecular oxygen-derived oxygen radicals and further suggest that molecular oxygen may ultimately be converted to hydrogen peroxide, which cleaves DNA via the Fenton reaction. Efficient thiol-activated DNA cleavage by **1**, **2**, and **3** may be the result of a thiol-driven redox cycle, similar to the well-known redox cycling of quinones (Weiner, 1994). Alternatively, slow production of hydrogen peroxide or superoxide radical may result in particularly efficient conversion of these species to hydroxyl radical. Such a theory has been put forward to explain certain aspects of DNA cleavage by the antitumor agent quinocarcin (Williams et al., 1992). The chemical mechanism of thiol-mediated DNA cleavage by 1,2-dithiolan-3-one 1-oxides is the subject of ongoing investigations in our laboratory.

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REFERENCES

Asmus, K.-D. (1990) *Methods Enzymol. 186*, 168−180.
Barvian, M. R., & Greenberg, M. M. (1995) *J. Am. Chem. Soc. 117*, 8291−8292.
Bauer, W., & Vinograd, J. (1968) *J. Mol. Biol. 33*, 141−171.
Behroozi, S. J., Kim, W., & Gates, K. S. (1995a) *J. Org. Chem. 60*, 3964−3966.
Behroozi, S. J., Barnes, C., & Gates, K. S. (1995b) *J. Chem. Crystallog.* (in press).
Black, J. F. (1978) *J. Am. Chem. Soc. 100*, 527−535.
Bode, V. C. (1967) *J. Mol. Biol. 26*, 125−129.
Bregant, T. M., Groppe, J., & Little, R. D. (1994) *J. Am. Chem. Soc. 116*, 3635−3636.
Buxton, G. V., Greenstock, C. L., Helman, W. P., & Ross, A. B. (1988) *J. Phys. Chem. Ref. Data 17*, 513−886.
Cone, R., Hasan, S. K., Lown, J. W., & Morgan, A. R. (1976) *Can. J. Biochem. 54*, 219−223.
Davies, M. J., & Slater, T. F. (1987) *Biochem. J. 245*, 167−173.
Deeble, D. J., & von Sonntag, C. (1986) *Int. J. Radiat. Biol. 49*, 927−936.
Fridovich, I. (1972) *Acc. Chem. Res. 5*, 321−326.
Gaudiano, G., & Koch, T. H. (1991) *Chem. Res. Toxicol. 4*, 2−16.
Goldberg, I. H. (1991) *Acc. Chem. Res. 24*, 191−198.
Graf, E., Mahoney, J. R., Bryant, R. G., & Eaton, J. W. (1984) *J. Biol. Chem. 259*, 3620−3624.
Halliwell, B., & Gutteridge, J. M. C. (1990) *Methods Enzymol. 186*, 1−85.
Hara, M., Asano, K., Kawamoto, I., Takiguchi, T., Katsumata, S., Takahashi, K., & Nakano, H. (1989) *J. Antibiot. 42*, 1768−1774.
Hara, M., Saitoh, Y., & Nakano, H. (1990) *Biochemistry 29*, 5676−5681.
Hecht, S. M. (1989) *Pure Appl. Chem. 61*, 577−80.
Hertzberg, R. P., & Dervan, P. B. (1984) *Biochemistry 23*, 3934−3945.

Hertzberg, R. P., & Johnson, R. K. (1993) *Annu. Rep. Med. Chem. 28*, 167−176.
Hirayama, N., & Matsuzawa, E. S. (1993) *Chem. Lett. 11*, 1957−1958.
Hurley, L. H. (1989) *J. Med. Chem. 32*, 2027−2033.
Huston, P., Espenson, J. H., & Bakac, A. (1992) *J. Am. Chem. Soc. 114*, 9510−9516.
Iyer, R. P., Phillips, L. R., Egan, W., Regan, J. B., & Beaucage, S. L. (1990) *J. Org. Chem. 55*, 4693−4699.
Kanda, Y., & Fukuyama, T. (1993) *J. Am. Chem. Soc. 115*, 8451−8452.
Kappen, L. S., & Goldberg, I. H. (1978) *Nucleic Acids Res. 5*, 2959−2967.
Kice, J. L., & Rogers, T. E. (1974) *J. Am. Chem. Soc. 96*, 8015−8019.
Lawley, P. D., & Brookes, P. (1963) *Biochem. J. 89*, 127−138.
Lee, M. D., Dunne, T. S., Chang, C. C., Ellestad, G. A., Siegel, M. M., Morton, G. O., McGahren, W. J., & Borders, D. B. (1987) *J. Am. Chem. Soc. 109*, 3466−3468.
Lesko, S. A., Lorentzen, R. J., & Ts'o, P. O. P. (1980) *Biochemistry 19*, 3023−3028.
Luo, Y., Han, Z., Chin, S. M., & Linn, S. (1994) *Proc. Natl. Acad. Sci. U.S.A. 91*, 12438−12442.
March, J. (1992) *Advanced Organic Chemistry: Reactions, Mechanisms, and Structure*, p 705, John Wiley & Sons, New York.
Marnett, L. J. (1987) *Carcinogenesis 8*, 1365−1373.
Myers, A. G., Cohen, S. B., & Kwon, B.-M. (1994) *J. Am. Chem. Soc. 116*, 1670−1682.
Misra, H. P. (1974) *J. Biol. Chem. 249*, 2151−2155.
Misra, H. P., & Fridovich, I. (1972) *J. Biol. Chem. 247*, 188−192.
Nicolaou, K. C., & Dai, W.-M. (1991) *Angew. Chem., Int. Ed. Engl. 30*, 1387−1416.
Oae, S., Takata, T., & Kim, Y.-H. (1977) *Tetrahedron Lett. 48*, 4219−4222.
Pattenden, G., & Shuker, A. J. (1992) *J. Chem. Soc., Perkin Trans. 1*, 1215−1221.
Pogozelski, W. K., McNeese, T. J., & Tullius, T. D. (1995) *J. Am. Chem. Soc. 117*, 6428−6433.
Pratviel, G., Bernadou, J., & Meunier, B. (1995) *Angew. Chem., Int. Ed. Engl. 34*, 746−769.
Rao, P. S., Luber, J. M., Milinowicz, J., Lalezari, P., & Mueller, H. S. (1988) *Biochem. Biophys. Res. Commun. 150*, 39−44.
Remers, W. A. (1991) in *Textbook of Organic, Medicinal and Pharmaceutical Chemistry* (Delgado, J. W., & Remers, W. A., Eds.) pp 313−358, Lippincott, Philadelphia, PA.
Rhaese, H.-J., & Freese, E. (1968) *Biochim. Biophys. Acta 155*, 476−490.
Riordan, C. G., & Wei, P. (1994) *J. Am. Chem. Soc. 116*, 2189−2190.
Rosenkranz, H. S., & Rosenkranz, S. (1971) *Arch. Biochem. Biophys. 146*, 483−487.
Rowley, D. A., & Halliwell, B. (1982) *FEBS Lett. 138*, 33−36.
Saez, G., Thornalley, P. J., Hill, H. A. O., Hems, R., & Bannister, J. V. (1982) *Biochim. Biophys. Acta 719*, 24−31.
Sambrook, J., Fritsch, E. F., & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* Vol. 3, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
Schomburg, D., Salzmann, M., & Stephan, D. (1994) *Enzyme Handbook*, Vol. 7, Class 1.5−1.12: Oxidoreductases, p 1.11.1.6, Springer-Verlag, Berlin.
Stubbe, J., & Kozarich, J. W. (1987) *Chem. Rev. 87*, 1107−1136.
von Sonntag, C., Hagen, U., Schon-Bopp, A., & Schulte-Frohlinde, D. (1981) *Adv. Radiat. Biol. 9*, 109−142.
Walsh, C. T. (1979) *Enzymatic Reaction Mechanisms*, p 443, W. H. Freeman and Co., New York.
Weiner, L. M. (1994) *Methods Enzymol. 233*, 92−105.
Williams, R. M., Glinka, T., Flanagan, M. E., Gallegos, R., Coffman, H., & Pei, D. (1992) *J. Am. Chem. Soc. 114*, 733−740.
Williams, R. M., Flanagan, M. E., & Tippie, T. N. (1994) *Biochemistry 33*, 4086−4092.

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