Oxidative DNA Cleavage by the Antitumor Antibiotic Leinamycin and Simple 1,2-Dithiolan-3-one 1-Oxides: Evidence for Thiol-Dependent Conversion of Molecular Oxygen to DNA-Cleaving Oxygen Radicals Mediated by Polysulfides

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The natural product leinamycin (**1**),[1] thought to derive its antitumor activity through reactions with DNA, is of interest because of its potent biological activity and because it represents a new chemical class of DNA-damaging agents. Early work demonstrated that **1** is a thiol-dependent DNA-cleaving agent and further suggested that the 1,2-dithiolan-3-one 1-oxide heterocycle of the natural product plays an integral role in DNA cleavage.[2] Subsequent experiments in our lab revealed that simple 1,2-dithiolan-3-one 1-oxides (**2**−**4**) are thiol-dependent DNA-cleaving agents.[3] It was found that **2**−**4** mediate oxidative DNA damage through a general mechanism involving the conversion of molecular oxygen to hydrogen peroxide, ultimately yielding the DNA-cleaving agent hydroxyl radical via a trace-metal-dependent Fenton reaction, as shown in the (unbalanced) eq 1.[3]

$$O_2 \rightarrow O_2^{\bullet -} \rightarrow H_2O_2 + M^{n+} \rightarrow HO^{\bullet} + M^{(n+1)+} \qquad (1)$$

Interestingly, experiments carried out concurrently with our studies of leinamycin analogs **2**−**4** showed that the natural product **1**, following reaction with 1 equiv of thiol, alkylates DNA, producing covalent DNA adducts (**5a**) that lead to strand cleavage.[4] Similar to the mechanism proposed for simple 1,2-dithiolan-3-one 1-oxides,[5] reaction of thiols with **1** (Scheme 1) yields an electrophilic oxathiolanone (**6**) that, in a chemical reaction *not* available to the leinamycin analogs **2**−**4**, can be trapped by intramolecular reaction with the C6−C7 alkene of the natural product's 18-membered macrocycle, thus resulting in formation of an episulfonium alkylating species (**7**).[4,6] The episulfonium ion intermediate derived from **1** alkylates DNA at N7 of guanine residues.[4,8]

Our previous studies of oxidative DNA cleavage by simple leinamycin analogs (**2**−**4**) prompted us to investigate whether **1** is capable of mediating oxidative DNA damage *in addition to* the recently reported DNA alkylation. We find that **1** (25 μM), in the presence of physiologically relevant[9] concentrations of thiol (0.5−2.5 mM), cleaves DNA, as measured by the conversion of supercoiled (form I) plasmid DNA to its open circular form (form II) (Figure 1). In order to shed light on the chemical mechanism(s) of thiol-activated DNA cleavage by **1** under these conditions, we performed the reaction in the presence of various additives that are known to have an effect on strand breakage arising from the cascade of reactions involving reduced oxygen species (e.g., eq 1).[10] Under the conditions employed here, thiol-activated DNA cleavage by **1** is partially inhibited by the radical scavengers mannitol and ethanol, by the hydrogen-peroxide-destroying enzyme catalase, and by the chelator of adventitious trace metals, diethylenetriaminepentaacetic acid (DETAPAC), which is known to inhibit the metal-dependent Fenton reaction[10] (Figure 1). Addition of the enzyme superoxide dismutase (SOD) stimulates DNA cleavage. Such an SOD-dependent increase in DNA cleavage has been observed in other systems where superoxide is formed in the presence of thiols.[11−13] This diverse set of reagents is not expected to significantly affect DNA alkylation by a species such as **7**.[14] The effects of these various additives on DNA cleavage by **1** are analogous to those observed previously[3] for **2**−**4** and clearly suggest that the natural product, similar to simple 1,2-dithiolan-3-one 1-oxides, is able to mediate thiol-dependent oxidative DNA damage by a pathway such as that shown in eq 1.[15,16] The fact that thiol-dependent cleavage of DNA by **1** is only partially inhibited by agents such as catalase and DETAPAC, which more completely inhibit thiol-dependent DNA cleavage by **2**−**4**,[3] is consistent with the likelihood that *both* oxidative and alkylative cleavage mechanisms are significant under these conditions.

Further experiments provide evidence that polysulfides (**8**) produced in the reaction of thiols with **1**−**4** may be primarily

Scheme 1

1 → (RSH) → 6 + RSSH (9) → 7 → 5 **a**: R'=DNA, **b**: R'=SSR

2, 3, 4

9 → RSS_xSR (**8 a**: x=1, **b**: x=2, R=CH_2CH_2OH) → (O_2, RSH) → DNA-Cleaving Oxygen Radicals

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(14) In fact, we have shown that the various additives used in our study have no affect on DNA cleavage by the hemisulfur mustard, 2-chloroethyl benzyl sulfide.

(15) Hara *et al.* reported,[2] and we have confirmed, that, in tris(hydroxymethyl)aminomethane (Tris) buffer, addition of catalase, superoxide dismutase, and radical scavengers has no affect on thiol-activated DNA cleavage by **1** (25 μM). We suggest that this is because Tris buffer is an excellent radical scavenger[16] and, thus, effectively suppresses all oxidative DNA damage. Phosphate buffer, used in the experiments described here, is a relatively poor oxygen radical scavenger.[16]

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Figure 1. Thiol-dependent cleavage of DNA by leinamycin (**1**) and the effect of various additives. Assays were performed in 50 mM sodium phosphate buffer, pH 7.0, 10% acetonitrile, containing 38 μM bp of pBR322 DNA. The number in parentheses following the description of each lane indicates the mean number of strand breaks per plasmid molecule.[29] Lane 1: pBR322 DNA alone (0.1). Lane 2: 2.5 mM 2-mercaptoethanol alone (0.2). Lane 3: 25 μM **1** alone (0.2). Lane 4: 25 μM **1** + 2.5 mM of 2-mercaptoethanol (standard cleavage reaction) (0.8). Lanes 5−10, standard reaction with additives: lane 5, with 200 mM ethanol (0.4); lane 6, with 100 mM mannitol (0.5); lane 7, with 100 μg/mL catalase (0.5); lane 8, with 100 μg/mL denatured catalase (1.0); lane 9, with 10 mM DETAPAC (0.6); lane 10, with 100 μg/mL superoxide dismutase (3.9).

Table 1. Cleavage of Plasmid DNA by **8a** and the Effect of Various Additives[a]

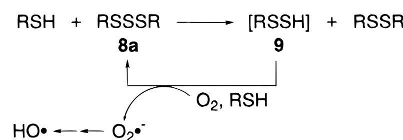
reaction	form I remaining (%)	S^b
DNA alone	89	0.1
thiol alone (500 μM)	87	0.1
100 μM **8a** alone	88	0.1
std. rxn: 100 μM **8a** + thiol (500 μM)	33	1.1
std. rxn + additive		
methanol (1 M)	88	0.1
methanol (200 mM)	81	0.2
ethanol (1 M)	89	0.1
ethanol (200 mM)	87	0.1
mannitol (100 mM)	79	0.2
mannitol (50 mM)	59	0.5
DETAPAC (10 mM)	68	0.4
DETAPAC (1 mM)	47	0.8
SOD (100 μg/mL)	9	2.4
catalase (100 μg/mL)	88	0.1
denatured catalase (100 μg/mL)	35	1.1

[a] In a typical assay, **8a** (100 μM) and 2-mercaptoethanol (500 μM) were incubated with supercoiled pBR322 DNA (38 μM bp) in 50 mM sodium phosphate (pH 7.0) containing 10% acetonitrile, for 7−8 h at 24 °C. The reaction was analyzed by agarose gel electrophoresis, and the amount of DNA cleavage quantitated by digital imaging, as described in the Supporting Information. [b] S is the mean number of strand breaks per plasmid molecule and is calculated using the equation: $S = -\ln$ (% form I DNA).[29] Values reflect the average of multiple experiments. Standard errors in these measurements are less than 5%.

responsible for the thiol-dependent oxidative cleavage of DNA by these sulfur heterocycles. Polysulfides, formed by decomposition of hydrodisulfides (**9**) released during the initial reaction of thiols with the 1,2-dithiolan-3-one 1-oxide heterocycle (see Scheme 1), were previously identified as major products resulting from the reaction of **2** and **3** with thiols.[5] We have examined DNA-cleaving ability of polysulfides **8a** and **8b** isolated from the reaction of **2** with 2-mercaptoethanol and also prepared by independent synthesis,[17] and we find that these polysulfides, in conjunction with excess thiol, efficiently cleave plasmid DNA (data for **8a** shown in Table 1). Importantly, cleavage efficiency and the results of mechanistic experiments for these polysulfides (Table 1) mirror those reported previously[3] for thiol-dependent DNA cleavage by 1,2-dithiolan-3-one 1-oxides (**2−4**) as well as those described above for the apparent oxidative component of DNA cleavage by **1** and point to a mechanism where polysulfides, in concert with thiols, mediate the reduction of molecular oxygen to DNA-cleaving oxygen radicals.[18]

Following the precedent described above for simple 1,2-dithiolan-3-one 1-oxides (**2−4**), polysulfides may be responsible

Scheme 2



for the oxidative component of DNA cleavage reported here for the natural product **1**. Isolation of **5b** previously provided evidence for the formation of a hydrodisulfide (**9**) in the reaction of **1** with thiols.[4] We find that treatment of **1** (300 μM) with thiol (1.5 equiv) in sodium phosphate buffer (50 mM, pH 7.0) at room temperature affords the corresponding trisulfide, as identified by comparison of HPLC retention times and NMR spectra with that of authentic polysulfides.[17]

Our results suggest that **1**, in conjunction with thiols, can damage DNA by both oxidative and alkylative mechanisms.[19−21] The efficiency of oxidative vs alkylative DNA damage by **1** is likely to depend upon the conditions under which the initial reaction of the antibiotic with thiol occurs. We speculate that the thiol-dependent formation of oxygen radicals mediated by polysulfides hinges upon the unusual reactivity of intermediates such as hydropolysulfides (**9**) generated in the reaction of thiols with polysulfides (Scheme 2).[22]

The finding that polysulfides are thiol-dependent DNA-damaging agents may be relevant not only to the action of **1** but to the biological activity of polysulfide-containing natural product antibiotics[23] such as varacin,[24] lissoclinotoxin A,[25] leptosin A, B, E, and F,[26] and sirodesmin B and C.[27] Finally, in addition to their oxygen-radical-producing ability, polysulfides may derive biological activity through reactions with thiol groups on proteins[28,29] or through chemical reactions that lead to depletion of cellular thiols (e.g., Scheme 2).

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Supporting Information Available: Complete experimental details (18 pages). See any current masthead page for ordering and Internet access instructions.

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