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

Kaushik Mitra,† Woonkki Kim,‡ J. Scott Daniels,† and Kent S. Gates*†,‡

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

Received April 29, 1997

The natural product leinamycin (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.‡ Subsequent experiments in our lab revealed that simple 1,2-dithiolan-3-one 1-oxides (2–4) are thiol-dependent DNA-cleaving agents.‡ 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

\[
\text{O}_2 + \text{H}_2 \text{O}_2 + \text{M}^{n+} \rightarrow \text{HO}^+ + \text{M}^{(n+1)+} \quad (1)
\]

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,‡ reaction of thiols with 1 (Scheme 1) yields an electron-rich 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).5,6 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 alklylation. We find that 1 (25 \(\mu\)M), in the presence of physiologically relevant 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-degrading enzyme catalase, and by the chelator of adventitious trace metals, diethylenetriaminepentaacetic acid (DTPAP), which is known to inhibit the metal-dependent Fenton reaction10 (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 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 DTPAP, which more completely inhibit thiol-dependent DNA cleavage by 2–4,17 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

\[\text{(14) In fact, we have shown that the various additives used in our study have no effect on DNA cleavage by the hemisulfur mustard, 2-chloroethyl benzyl sulfide.}
\[\text{(15) Hara et al. reported, and we have confirmed, that, in tris-(hydroxymethyl)aminomethane (Tris) buffer, addition of catalase, superoxide dismutase, and radical scavengers has no effect on thiol-activated DNA cleavage by 1 (25 \(\mu\)M). We suggest that this is because Tris buffer is an excellent radical scavenger and, thus, effectively suppresses all oxidative DNA damage. Phosphate buffer, used in the experiments described here, is a relatively poor oxygen radical scavenger.}
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

<table>
<thead>
<tr>
<th>Additive</th>
<th>form I remaining (%)</th>
<th>δ</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA alone</td>
<td>89</td>
<td>0.1</td>
</tr>
<tr>
<td>thiol alone (500 μM)</td>
<td>87</td>
<td>0.1</td>
</tr>
<tr>
<td>100 μM 8a alone</td>
<td>88</td>
<td>0.1</td>
</tr>
<tr>
<td>std. rxn + 100 μM 8a + thiol (500 μM)</td>
<td>33</td>
<td>1.1</td>
</tr>
<tr>
<td>methanol (1 M)</td>
<td>88</td>
<td>0.1</td>
</tr>
<tr>
<td>ethanol (1 M)</td>
<td>81</td>
<td>0.2</td>
</tr>
<tr>
<td>mannitol (100 mM)</td>
<td>89</td>
<td>0.1</td>
</tr>
<tr>
<td>DETAPAC (10 mM)</td>
<td>68</td>
<td>0.4</td>
</tr>
<tr>
<td>DETAPAC (1 mM)</td>
<td>47</td>
<td>0.8</td>
</tr>
<tr>
<td>SOD (100 μg/mL)</td>
<td>9</td>
<td>2.4</td>
</tr>
<tr>
<td>catalase (100 μg/mL)</td>
<td>88</td>
<td>0.1</td>
</tr>
<tr>
<td>denatured catalase (100 μg/mL)</td>
<td>35</td>
<td>1.1</td>
</tr>
</tbody>
</table>

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. S is the mean number of strand breaks per plasmid molecule and is calculated using the equation: δ = − ln (form I DNA).29 Values reflect the average of multiple experiments. Standard errors in these measurements are less than 5%.

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 thiosulfide, 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 hydro polysulfides (9) generated in the reaction of thiol 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 antibiotics32 such as varacin,24 lisoclinitoxin A,25 leptomycin 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 proteins28,29 or through chemical reactions that lead to depletion of cellular thiols (e.g., Scheme 2).

For the oxidative component of DNA cleavage by 2 are consistent with radical-mediated oxidative damage. See the Supporting Information for discussion of this issue.

It is not uncommon for naturally occurring antibiotics to be capable of damaging DNA by multiple chemically distinct mechanisms. For example, the anticancer antibiotic mitomycin C alkylates DNA20 and, in the presence of reducing agents and molecular oxygen, can cause oxidative DNA damage through redox cycling of its quinone moiety.21

Acknowledgment. This work was supported by a grant from the National Institutes of Health (GM51565). We are indebted to Dr. Yutaka Kanda and other researchers at Kyowa Hakko Kogyo (Japan) for providing us with samples of leinamycin, and we thank Professor Michael Harmata (University of Missouri) for the use of some facilities.

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

JA971359Z

For the oxidative component of DNA cleavage by 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 thiosulfide, 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 hydro polysulfides (9) generated in the reaction of thiol 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 antibiotics32 such as varacin,24 lisoclinitoxin A,25 leptomycin 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 proteins28,29 or through chemical reactions that lead to depletion of cellular thiols (e.g., Scheme 2).

Acknowledgment. This work was supported by a grant from the National Institutes of Health (GM51565). We are indebted to Dr. Yutaka Kanda and other researchers at Kyowa Hakko Kogyo (Japan) for providing us with samples of leinamycin, and we thank Professor Michael Harmata (University of Missouri) for the use of some facilities.

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

JA971359Z

(18) The DNA “end products” obtained from the thiol-dependent cleavage of DNA by 2 are consistent with radical-mediated oxidative damage. See the Supporting Information for discussion of this issue.

(19) It is not uncommon for naturally occurring antibiotics to be capable of damaging DNA by multiple chemically distinct mechanisms. For example, the anticancer antibiotic mitomycin C alkylates DNA20 and, in the presence of reducing agents and molecular oxygen, can cause oxidative DNA damage through redox cycling of its quinone moiety.21


