## Thiol-Independent DNA Alkylation by Leinamycin

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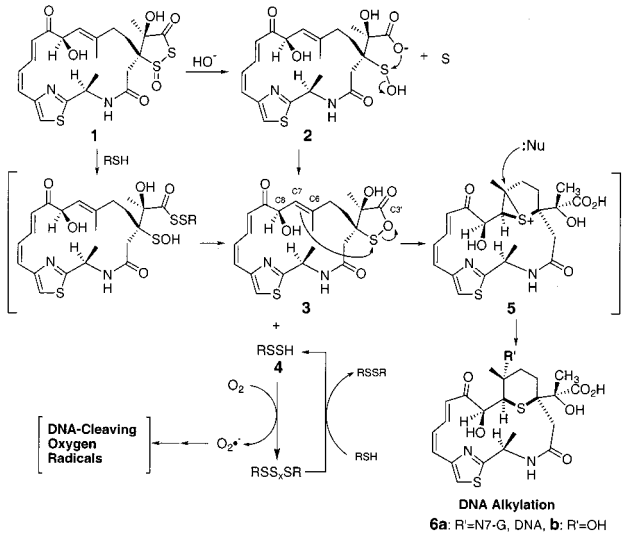
Covalent modification of cellular DNA can have profound biological consequences and, therefore, the mechanisms by which small organic molecules react with DNA are of interest in both medicinal chemistry and toxicology.[1−6] The reactions underlying the biological activity of DNA-damaging agents are diverse and chemically interesting.[1] Some agents contain functional groups that are inherently reactive with DNA, while others require in vivo chemical or enzymatic activation to transform the parent molecule into the reactive intermediate actually responsible for covalent modification of DNA.[1,7] In cases where in vivo activation is required, characterization of the relevant chemical or enzymatic reactions is crucial for understanding the overall mechanism of action. In addition, characterization of novel bioactivation processes offers fundamental new insights regarding the types of chemical reactions by which small organic molecules can efficiently modify biomolecules under physiological conditions.

Leinamycin (**1**) is a structurally unique antitumor agent that is bioactivated by reaction with cellular thiols.[8] Thus far, leinamycin has been characterized exclusively as a thiol-triggered DNA-damaging agent. Thiol attack on leinamycin triggers DNA damage by two unprecedented mechanisms (Scheme 1). Thiol-dependent oxidative DNA damage is triggered by release of a hydrodisulfide species (**4**) that mediates the production of oxygen radicals.[9−13] In addition, leinamycin alkylates DNA through a remarkable rearrangement reaction in which the antibiotic is initially converted by reaction with thiol to the oxathiolanone intermediate **3**.[9,14] The electrophilic sulfur of the oxathiolanone group then reacts with the C6−C7 alkene of the antibiotic's 18-membered macrocycle to yield an episulfonium ion alkylating agent (**5**) that efficiently modifies duplex DNA at the N7-position of guanine residues.[14]

While previous studies have characterized thiol-triggered DNA damage by leinamycin, we report here experiments that reveal a new *thiol-independent* mode of DNA alkylation by this antibiotic.[15,16] Sequencing gel analysis shows that treatment of a 5′-$^{32}$P-labeled, 377 base pair fragment of duplex DNA with leinamycin *in the absence of thiol*, followed by Maxam−Gilbert workup,[17] generates cleavage at guanine residues (Figure 1). The pattern of alkali-labile DNA cleavage resulting from incubation with leinamycin in the absence of thiol is virtually identical to that obtained from thiol-triggered DNA alkylation by the antibiotic (Figure 2, Figures S1 and S2, Supporting Information).[18] Thiol-independent DNA modification by leinamycin occurs more slowly ($t_{1/2} \approx 11$ h, 37 °C) than the analogous thiol-promoted DNA alkylation process ($t_{1/2} \approx 3$ h, 37 °C, 20 equiv of glutathione) (Figures S3 and S4, Supporting Information). The final yield of strand breaks produced by leinamycin in the absence of thiol is approximately 30% of that obtained in the presence of thiols (Figure 2).

**Scheme 1**




The similarities between thiol-independent and thiol-triggered DNA damage by leinamycin (Figure 2) suggested to us that a common alkylating intermediate (i.e., **5**) might be involved in these two processes. The 1,2-dithiolan-3-one 1-oxide moiety is unstable in aqueous solution (for example, $t_{1/2} = 39$ min for 5,5-dimethyl-1,2-dithiolan-3-one 1-oxide at pH 7, Figure S6, Supporting Information) and we suspected that attack of water on this heterocycle in leinamycin may initiate a cascade of chemical reactions leading to the formation of the episulfonium ion **5**. Consistent with this hypothesis HPLC and LC/MS analysis reveals that leinamycin undergoes conversion to the characteristic, episulfonium ion-derived hydrolysis product **6b** upon incubation in aqueous buffer. In accord with the yields of thiol-independent DNA damage reported above, the yield of **6b** obtained from leinamycin in the absence of thiol is approximately 20% of that produced by treatment of the antibiotic with thiol (1.5 equiv of 2-mercaptoethanol). The pseudo-first-order disappearance of leinamycin (and simultaneous appearance of the rearranged product **6b**) in aqueous buffer occurs with a rate constant of $4.3 \pm 0.5 \times 10^{-2}$ h$^{-1}$ (in 200 mM Hepes, pH 7, 24 °C, Figure

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(15) Asai et al. have reported on DNA alkylation by a metabolite isolated from the reaction of thiol with leinamycin.[16] Alkylation by this thiol-derived metabolite does not require additional thiol and, thus, was described as "thiol-independent"; however, the chemistry described by Asai and co-workers is distinct from the alkylation chemistry reported here, in which there is absolutely no thiol involvement.
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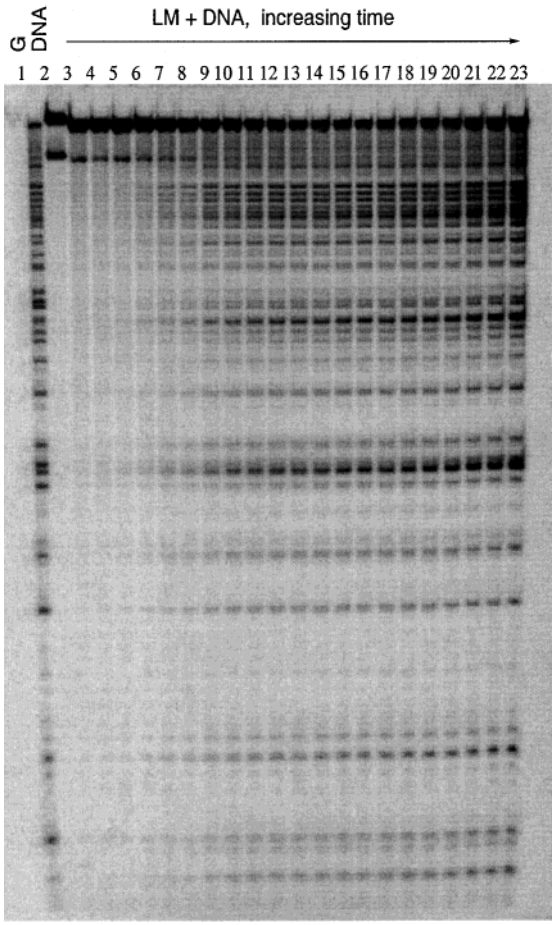


**Figure 1.** Thiol-independent DNA alkylation by leinamycin (LM). A 5′-$^{32}$P-labeled 377 bp restriction fragment was incubated at 37 °C with leinamycin (50 $\mu$M) for varying amounts of time in Hepes buffer (10 mM, pH 7.0) containing $NaClO_4$ (50 mM) and herring sperm DNA (250 $\mu$M bp), followed by Maxam−Gilbert workup, denaturing 11% polyacrylamide gel electrophoresis, and phosphorimager analysis, as described in the Supporting Information. Lane 1: Maxam−Gilbert G reaction; lane 2: DNA alone; lanes 3−23: leinamycin + DNA incubated for 0, 1, 2, 4, 6, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, 48, 52, 56, 60, 64, and 70 h, respectively.

S5, Supporting Information) and is subject to general base catalysis. On the other hand, the pseudo-first-order rate constants for the reaction of 0.5, 0.75, and 1 mM glutathione with leinamycin (10 $\mu$M in 50 mM Hepes, pH 7, 24 °C) are $15 \pm 2$ $h^{-1}$, $26.3 \pm 0.7$ $h^{-1}$, and $40.7 \pm 0.4$ $h^{-1}$, respectively. A plot of these pseudo-first-order reaction rates versus thiol concentration yields a second-order rate constant of $10.4 \pm 0.6$ $M^{-1}s^{-1}$ for the reaction of leinamycin with glutathione (Figures S7 and S8, Supporting Information).

On the basis of precedents from closely related model systems,[19−23] we believe that thiol-independent DNA alkylation by leinamycin is initiated by attack of water (or hydroxide) on the C3′-carbonyl of the antibiotic to yield the sulfenic acid intermediate **2** (Scheme 1).[24] Subsequent intramolecular attack of the carboxylate on the neighboring sulfenic acid group in **2** is envisioned to afford the oxathiolanone **3**, which leads to DNA alkylation through the episulfonium ion **5**. Our rate data allows us to estimate that, under typical cellular conditions (~1 mM thiol, pH 7.5),[25] leinamycin is ~800 times more likely to react via the thiol-promoted pathway as opposed to the thiol-independent

(19) For production of a sulfenic acid intermediate in a closely related reaction, see: Mitra, K.; Barnes, C. L.; Gates, K. S. *J. Chem. Crystallogr.* **1999**, *29*, 1133−1136.

(20) For production of and subsequent intramolecular nucleophilic attack on a sulfenic acid in a related reaction, see: Kim, W.; Dannaldson, J.; Gates, K. S. *Tetrahedron Lett.* **1996**, *37*, 5337−5340.

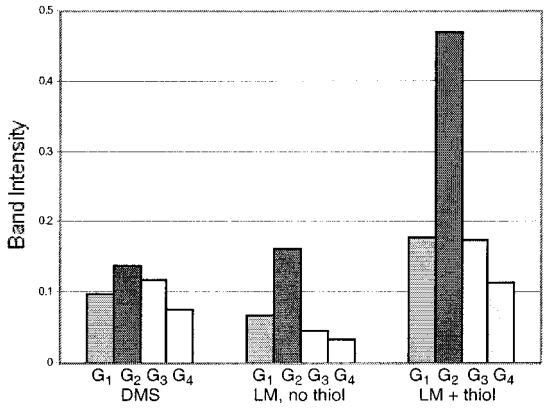


**Figure 2.** Sequence specificity and relative efficiency of thiol-dependent and thiol-independent DNA alkylation by leinamycin (LM). The height of each bar represents the amount of cleavage at each guanine in the oligonucleotide duplex 5′-$^{32}$P-ATA ATT ATT AAA T**G$_4$**A **G$_3$**TA **G$_2$G$_1$**A TAA ATA TAA-3′. Leinamycin (50 $\mu$M) was incubated either in the absence or presence of thiol (2 equiv of $\beta$-mercaptoethanol) with the labeled DNA duplex at 37 °C for 72 h in Hepes buffer (50 mM, pH 7.0) containing herring sperm carrier DNA (200 $\mu$M bp), followed by Maxam−Gilbert workup, denaturing 20% polyacrylamide gel electrophoresis, and phosphorimager analysis as described in the Supporting Information. The sequence preferences for alkylation of this oligonucleotide duplex by dimethyl sulfate (DMS) are shown for comparison.

pathway. In serum, where thiol concentrations are considerably lower (~5 $\mu$M),[26] the two pathways are expected to be competitive. While it is currently unclear whether hydrolytic activation in serum plays an important role in the cytotoxicity of leinamycin, the results presented here indicate that this possibility deserves consideration.

In conclusion, while reactions with water often lead to the destruction of electrophilic antitumor agents,[1−3] it appears that hydrolysis of leinamycin does not lead exclusively to nonproductive decomposition of the antibiotic. In fact, our experiments reveal a new, chemically interesting bioactivation pathway in which hydrolysis of the unstable 1,2-dithiolan-3-one 1-oxide heterocycle unmasks leinamycin's latent alkylating abilities.

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**Supporting Information Available:** Complete procedures and data for leinamycin−DNA reactions, gel electrophoresis, and kinetic measurements (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(21) The decreased efficiency of DNA alkylation and generation of **6b** in the thiol-independent as opposed to the thiol-triggered activation processes may be due to decomposition[22] of the intermediate sulfenic acid **2**. Sulfenic acids can decompose under some conditions to produce thiols;[22] however, addition of high concentrations of thiol-trapping agents such as *N*-ethylmaleimide has no effect on either the rate of leinamycin decomposition or the production of **6b** in our experiments. Thus, it is deemed unlikely that thiols derived from the decomposition of **2** are responsible for the observed activation of leinamycin in the absence of added thiol. In addition, intramolecular participation of leinamycin's amide group[23] in thiol-independent activation of the antibiotic is possible, but appears not to be *required* based upon observations of analogous chemistry in model systems (Chatterji and Gates, unpublished data).

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