Thiol-Mediated DNA Alkylation by the Novel Antitumor Antibiotic Leinamycin

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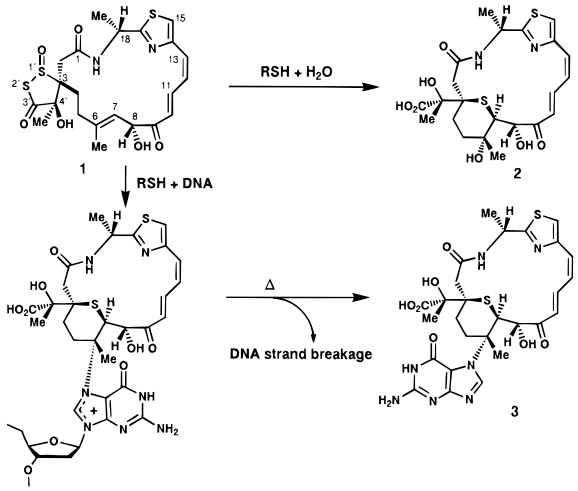
Leinamycin (**1**) was isolated from the culture broth of a *Streptomyces* sp. in 1989,[1−3] and its structure was elucidated by spectroscopic analysis,[1] X-ray crystallography,[4] and chemical synthesis.[5] This antibiotic contains an unusual 1,3-dioxo-1,2-dithiolane moiety, which is connected to the 18-membered lactam through a spiro linkage, and appeared to be a new class of natural product. **1** exhibited significant antitumor activity in some murine tumor models.[2] We previously reported that **1** induces single-strand scission of DNA in the presence of thiol cofactors in vitro. We report here the detailed chemistry of thiol-activation and DNA-cleavage induced by **1**.

Addition of 1.5 equiv of 2-mercaptoethanol (2-ME) to a solution of **1** in MeOH/10 mM phosphate buffer (pH 7) (1/9, v/v) resulted in a rapid conversion to a major product with several minor products. Other thiols including ethanethiol, dithiothreitol, cysteine, and glutathione afforded approximately equal amounts of **2**. We isolated **2** from the reaction mixture with reverse-phase HPLC (70%, yield), but the full characterization of **2** failed due to its instability in DMSO. Treatment of **2** with K_2CO_3 and iodomethane afforded a stable methyl ester **2a** (68%, yield). Characterization of **2a** by 1D and 2D-NMR experiments established an unexpected structure, in which the 1,3-dioxo-1,2-dithiolane moiety and the 6,7-olefin were missing and a new 3,7-sulfide linkage and 6-hydroxyl group were observed. Treatment of **1** with 2-ME in a MeOH-rich solvent, MeOH/0.5 M phosphate buffer (pH 7) (99/1, v/v), afforded the methanol adduct **2b**[6] as the main product.

After reaction of **1** with calf thymus DNA in the presence of 2-ME (drug/DNAbp/2ME = 1/20/1.5), the DNA was purified by ethanol precipitation. The DNA showed a UV spectrum characteristic of the complex with a chromophore. Although it was stable at 4 °C, gradual release of the chromophore from the DNA was observed at 37 °C. The rate and efficiency of the release increased with further an increase in the temperature. On a preparative scale, we isolated the released chromophore **3** from the leinamycin-treated calf thymus DNA (75% yield from **1**). In the ^{13}C NMR spectrum of **3** all of the resonances were comparable to those of **2**, and five additional resonances were found, suggesting the addition of a purine residue to **2**. In the ^1H-NMR spectrum, all of the resonances were also comparable to those of **2**. One additional nonexchangeable resonance at 7.77 ppm (1H) was found. The only nucleobase that gives one nonexchangeable resonance is guanine. Observation of the NOE between the guanine H-8 and the 6-CH_3 is consistent with alkylation of the C-6 carbon by the N-7 of guanine. These spectroscopic data revealed that **3** is a leinamycin-N7 guanine adduct. This was supported by the molecular formula that was established as the sodium salt $C_{27}H_{31}O_7N_7S_2Na$: HRFABMS

Scheme 1[a]



[a] See ref 15.

$(M + Na)^+$ *m/z* 652.1600, calcd 652.1624. **1** did not react with guanosine nucleotide monomer or single stranded DNA, suggesting that the alkylation of DNA could be attributable to the unique interaction of this molecule with double stranded DNA. To determine the specificity of the strand cleavage, we examined the reaction of **1** with a 5′-end-labeled DNA fragment (89bp) obtained from plasmid pBR322. Our preliminary results indicated that **1** induces nonselective strand scission of DNA in the presence of thiol.[3] However, treatment of these cleaved products with hot piperidine resulted in the production of fragments that migrated as guanine-specific cleavage. Recent reports[7−9] have shown that thermal treatment of the DNA that is modified on N7 guanine or N3 adenine produce some products of DNA strand breakage corresponding to fragments that contain the unsaturated aldehyde end and migrate approximately 1.5 bp higher than the fragments possessing a 3′-phosphate terminus produced by the Maxam−Gilbert's chemical reaction. The results from sequencing gel analysis of these products appeared to be attributable to nonspecific cleavage. Subsequent piperidine treatment, which converts the modified sugar termini to 3′-phosphate termini, produces quantitative conversion of these products to faster migrating bands which comigrated with the fragments from Maxam−Gilbert reaction. These results are consistent with the release of a guanine adduct from modified DNA, indicating that the DNA-alkylation is responsible for DNA-cleavage by leinamycin.

In the course of the thiol-mediated conversion of **1** to **2** or DNA-adduct, we found another degradation product **4** that was generated initially and gradually disappeared. Preparation[10] and characterization of **4** and its methyl ester **4a** revealed the formation of a 3,6-sulfide bond and a 7,8-epoxide. The presence of the epoxide ring was supported by $^1J_{C-H}$ couplings of 183 and 182 Hz for the C-7 and C-8 carbons, respectively. Surprisingly in the absence of thiol, **4** gave **2** quantitatively in phosphate buffer (pH 7).[11] We also obtained another degradation product **5**. The yield of **5** was dependent on the concentra-

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(2) Hara, M.; Asano, K.; Kawamoto, I.; Takiguchi, T.; Katumata, S.; Takahashi, K.; Nakano, H. *J. Antibiot.* **1989**, *42*, 1768−1774.
(3) Hara, M.; Saitoh, Y.; Nakano, H. *Biochemistry* **1990**, *29*, 5676−5681.
(4) Hirayama, N.; Matsuzawa, E. S. *Chem. Lett.* **1993**, *11*, 1957−1958.
(5) Kanda, Y.; Fukuyama, T. *J. Am. Chem. Soc.* **1993**, *115*, 8451−8452.
(6) Structure of **2b** was elucidated with spectroscopic analysis of **2c**, the methyl ester derivative of **2b** (MeI, K_2CO_3, 35% from **1**).
(7) Sugiyama, H.; Fujiwara, T.; Ura, A.; Tashiro, T.; Yamamoto, K.; Kawanishi, S.; Saito, I. *Chem. Res. Toxicol.* **1994**, *7*, 673−683.
(8) Sun, D.; Hansen, M.; Clement, J. J.; Hurley, L. H. *Biochemistry* **1993**, *32*, 8068−8074.
(9) Lee, C.; Sun, D.; Kizu, R.; Hurley, L. H. *Chem. Res. Toxicol.* **1991**, *4*, 203−213.
(10) Isolation of **3** from the aqueous reaction mixture resulted in low yields (<10%) because of its lability which leads to **2**. However, treatment of **1** with 2-ME and excess molar triethyl amine in 2-propen-1-ol unexpectedly resulted in the generation of sufficient amounts of **4** (yield: 68%) for spectroscopic characterization.
(11) The details of conversion of **2**, including DNA-cleavage activity in the absence of thiol will be reported elsewhere.

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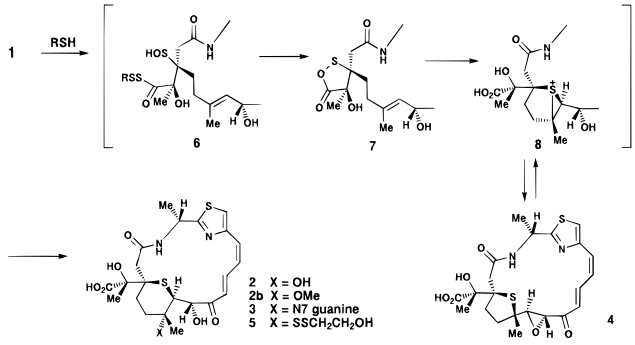
Scheme 2. Proposed Mechanism for Thiol-Activation of Leinamycin



tion of **1** in the reaction mixture. Higher concentrations of **1** (3 mM in 20% CH_3CN/pH 7 phosphate buffer) resulted in the higher efficiency of production of **5** (**2**:**4**:**5** = ca. 3:1:2, HPLC analysis). Characterization of **5** revealed that **5** is an analog of **2** in which the hydroxyl group at C-6 was substituted with 2-hydroxyethyldisulfide. The isolation of **5** implies the generation of 2-hydroxyethylhydrodisulfide by the reaction of **1** with 2-ME.

A plausible mechanism that explains the formation of these products is summarized in Scheme 2. The oxathiolanone moiety[12] could be produced after the addition of thiol at the S-2′ sulfur atom in the 1,3-dioxo-1,2-dithiolane moiety of **1**, giving a hydrodisulfide.[13] Further, we propose the intramolecular addition of the oxathiolane moiety to the 6,7-olefin in the 18-membered lactam moiety to give a possible episulfonium intermediate **8**. The conversion of epoxide **4** to **2** in the absence of thiol implies that **8** could be readily regenerated from **4** and subsequently converted to the final inactive product **2**, that is, the conversion of **8** to **4** would be reversible. Two pathways are considered to explain these reactions. **8** would undergo intermolecular nucleophilic attack to generate the stable products **2**, **2b**, **3**, **5** respectively, while **8** would undergo intramolecular nucleophilic attack of the 8-hydroxyl group at the C-7 carbon to generate **4** saving its potency for DNA-alkylation.

The results indicate that the nucleophilic attack of thiol at the S-2′ sulfur atom of the 1,3-dioxo-1,2-dithiolane moiety triggers the conversion of **1** to an electrophilic molecule, presumably episulfonium **8**, which is responsible for DNA-alkylation and subsequent DNA-cleavage by leinamycin.[14]

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Supporting Information Available: Full details of the preparation of compounds with tables listing complete ^1H- and ^{13}C-NMR chemical shift assignments, figures showing long-range $^1H-^{13}C$ correlations and NOE interactions, and sequencing gel analysis for specificity of the strand cleavage (11 pages). See any current masthead page for ordering and Internet access instructions.

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(12) Regarding previously proposed oxathiolane intermediates: Stanwinski, J.; Thlein, M. *J. Org. Chem.* **1991**, *56*, 5169−5175.

(13) Behroozi, S. J.; Kim, W.; Gates, K. S. *J. Org. Chem.* **1995**, *60*, 3964−3966. Gates et al. also showed some evidence which indicate the generation of an electrophilic oxathiolanone intermediate from thiol treated 1,3-dioxo-1,2-dithiolane heterocyclic compounds.

(14) Behroozi, S. J.; Kim, W.; Dannaldson, J.; Gates, K. S. *Biochemistry* **1996**, *35*, 1768−1774. Gates et al. proposed that the mechanism of DNA-cleavage by dithiolane oxides is thiol-mediated generation of oxygen radicals from the results of their experimental system employing the simple dithiolane oxide compounds.

(15) NOE experiments revealed the relative stereochemistry at C-6 and C-7 of **2c**, **3**, and **4a**.