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DNA CLEAVAGE BY 7-METHYLBENZOPENTATHIEPIN: A SIMPLE ANALOG OF THE ANTITUMOR ANTIBIOTIC VARACIN

Tonika Chatterji[a] and Kent S. Gates[a,b,*]

Departments of [a]Chemistry and [b]Biochemistry, University of Missouri-Columbia, Columbia, MO 65211, U.S.A.

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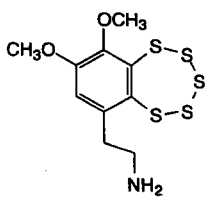
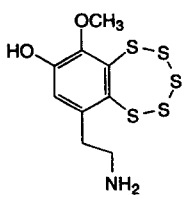
Abstract: The compound 7-methylbenzopentathiepin, a simple analog of the benzopentathiepin antitumor antibiotic varacin, was shown to be a potent thiol-dependent DNA-cleaving agent. Biological experiments previously suggested that DNA cleavage might play a role in the cytotoxicity of varacin; however, this is the first direct evidence that benzopentathiepins can cause DNA strand breaks under physiologically relevant conditions.

A number of biologically active polysulfides have been isolated from natural sources, including the sirodesmins B and C,[1] the leptosins A, B, E, and F,[2] 2-hydroxyethyltrisulfide,[3] allylmethyltrisulfide,[4] lenthionine,[5] varacin,[6] and lissoclinotoxin A.[7,8] In addition, we recently reported that the antitumor antibiotic leinamycin produces polysulfides upon reaction with thiols.[9] Many polysulfides are cytotoxic and display antifungal, antibacterial, or anticancer properties; however, in most cases the mechanism of biological action remains unknown.

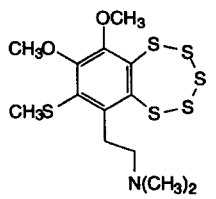
The recent discoveries of the first naturally occurring benzopentathiepin antibiotics, such as varacin,[6] lissoclinotoxin A,[7] and *N,N*-dimethyl-5-(methylthio)varacin,[10] were met with substantial interest because of their interesting structural properties,[8] the unique synthetic challenge they offer,[8,11,12] and their potent biological activities. The polysulfur ring of the pentathiepin antibiotics appears to be crucial for biological activity.[8,10] Varacin displays promising antifungal activity against *Candida albicans* and potent cytotoxicity against a human colon cancer cell line (IC_{90} = 0.05 μg/mL against HCT-116).[6] The selective toxicity of varacin toward a cell line characterized as DNA-repair deficient led to the suggestion that the compound's cytotoxicity may arise through single-strand cleavage of DNA.[6] Inspired by our recent discovery that polysulfides are thiol-dependent DNA-cleaving agents[9] and by the earlier suggestion that single-strand DNA cleavage may underlie the cytotoxicity of varacin, we investigated the DNA-cleaving ability of a simple varacin analog, 7-methylbenzopentathiepin (**1**).[13]



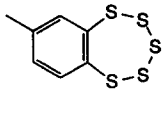
Varacin



Lissoclinotoxin A



N,N-Dimethyl-5-(methylthio)varacin



1

We find that **1** is a potent, thiol-dependent DNA-cleaving agent (Figure 1). In a plasmid-based DNA-cleavage assay, low micromolar concentrations of **1**, in the presence of thiol, efficiently convert supercoiled (form I) DNA to the nicked form (form II). Added thiol is absolutely required for DNA-cleavage by **1** and,

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importantly, the biological thiol glutathione is competent to drive DNA cleavage in this system. The efficiency of thiol-activated DNA cleavage by **1** (5 μM) displays a maxima at approximately 2000 equivalents of thiol (Figure 2). Such thiol dependence is expected in a system where thiol both stimulates the formation of DNA-cleaving reactive intermediates and can also quench those reactive species. A similar trend in the dependence of DNA-cleavage efficiency on thiol concentration is observed for enediyne antibiotics where the formation of thiol-quenchable reactive intermediates is triggered by thiols.[14,15]

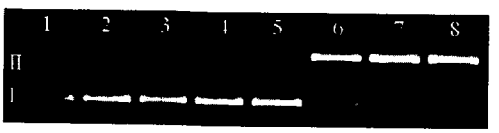


Figure 1. Thiol-Dependent DNA Cleavage by Various Concentrations of **1**. Supercoiled pBR322 DNA (38 μM bp) was incubated for 12 h at 27 °C with various concentrations of **1** and 100 equivalents of 2-mercaptoethanol in sodium phosphate buffer (50 mM, pH 7), followed by agarose gel electrophoretic analysis as previously described.[16] Lane 1, DNA alone; lane 2, **1** (25 μM) no thiol; lane 3, 2.5 mM thiol alone; lane 4, **1** (0.5 μM)+thiol; lane 5, **1** (1 μM)+thiol; lane 6, **1** (5 μM)+thiol; lane 7, **1** (10 μM)+thiol; lane 8, **1** (25 μM)+thiol.

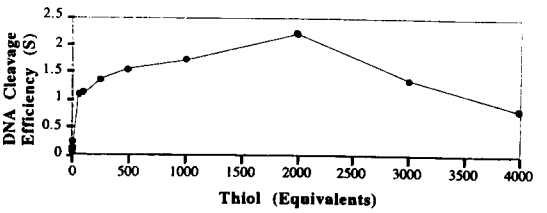
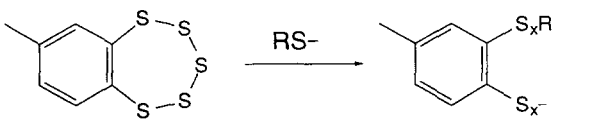


Figure 2. Effect of Thiol Concentration on DNA-Cleavage by **1**. Supercoiled pBR322 DNA (38 μM bp) was incubated for 12 h at 27 °C with **1** (5 μM) and various amounts of 2-mercaptoethanol in sodium phosphate buffer (50 mM, pH 7) followed by agarose gel electrophoretic analysis as previously described.[16] The value S represents the mean number of strand breaks per plasmid molecule obtained using the equation $S = -\ln f_I$, where f_I is the fraction of plasmid present as form I. Values shown represent the average of multiple experiments.

The results of further experiments indicate that **1**, in conjunction with thiols, mediates the conversion of molecular oxygen to DNA-cleaving oxygen radicals. Using the plasmid-based DNA-cleavage assay, we find that radical scavengers such as ethanol, methanol, dimethyl sulfoxide, and mannitol efficiently inhibit DNA cleavage.[17] The hydrogen peroxide-destroying enzyme catalase also effectively inhibits thiol-dependent strand scission by **1**.[17] Likewise, agents such as diethylenetriaminepentaacetic acid and desferal, which chelate adventitious trace metals in a nonredox-active form,[18] prevent DNA cleavage in this system.[17] DNA cleavage is markedly decreased by the removal of molecular oxygen. Finally, thiol-dependent DNA cleavage by **1** occurs with little sequence specificity, similar to that of a prototypical sequence-independent cleaving system, iron-EDTA (Figure 3).[19] Overall, our evidence suggests that thiol-dependent DNA cleavage by **1** involves conversion of molecular oxygen, probably via superoxide radical, to hydrogen peroxide, followed by a trace-metal-dependent Fenton reaction to generate the well known DNA-cleaving agent hydroxyl radical.[20] The mechanistic results for thiol-dependent DNA cleavage by **1** mirror those that we previously observed for thiol-dependent DNA cleavage by acyclic polysulfides.[9] We speculate that reactive hydropolysulfide anions (e.g., **2**) generated in the reaction of thiols with **1** are key intermediates in the production of DNA-cleaving oxygen radicals (Scheme 1).[9]

Our work provides the first direct evidence that pentathiepins can cleave DNA under physiologically relevant conditions. These findings may pertain to the biological properties of naturally occurring benzopentathiepin antibiotics, as it is well known that the production of reactive oxygen species ($O_2^{\bullet -}$, H_2O_2, $HO^{\bullet}$) by xenobiotics can be cytotoxic.[21,22]



1 2 (X = 2-4)

Scheme 1

Interestingly, all natural benzopentathiepins isolated to date bear an aminoethyl substitutent. This functional group probably exists almost exclusively in the charged (-NHR_2^+) form under physiological conditions and is likely to fundamentally influence the pharmacokinetics (e.g., absorption and transport) of these agents. In the context of DNA as a target, the positively charged amino substituent may confer DNA affinity on the benzopentathiepin antibiotics.[23] It is well known that production of reactive oxygen species by DNA-binding agents affords enhanced DNA cleavage efficiency relative to that by analogous nonbinding compounds.[24,25] We therefore suggest that, due to the (presumed) DNA affinity of the natural pentathiepin antibiotics and their metabolites analogous to **2**, the benzopentathiepin natural products may be somewhat more efficient thiol-activated DNA-cleaving agents than the varacin model compound **1**. In addition, under some conditions the aminoethyl substituent of varacin may catalyze the reaction of thiols with the polysulfur ring of the antibiotic. Although it remains a matter of debate, a similar role has been proposed for the aminoglycosides in the thiol-triggered antibiotics neocarzinostatin and calicheamicin.[26,27] Further investigations of the reaction of thiols with **1** and the mechanism of DNA cleavage by polysulfides are currently underway.

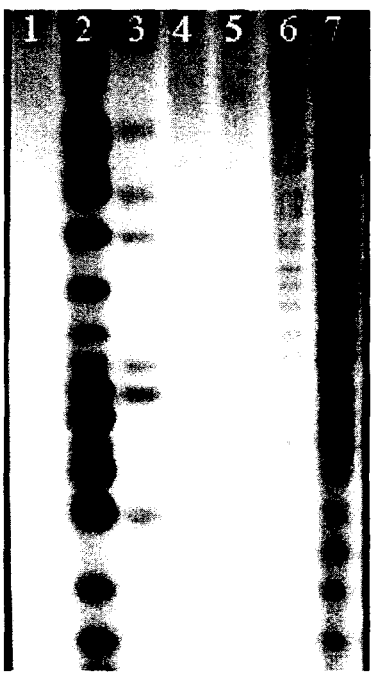


Figure 3. Sequence specificity of thiol-dependent DNA cleavage by **1**. A 5'-^{32}P-labeled restriction fragment (HindIII-BamHI fragment from pBR322, 150,000 cpm) was incubated with **1** (70 μM) and 2-mercaptoethanol (7 mM) in sodium phosphate buffer (50 mM, pH 7.0) for 12 h at 25 °C. The cleavage reaction was then analyzed using 20% polyacrylamide sequencing gel electrophoresis, as previously described.[28] Maxam-Gilbert sequencing reactions[29] and iron-EDTA reaction[28] were performed as previously described. Lane 1, Untreated DNA; lane 2, Maxam-Gilbert A+G reaction; lane 3, Maxam-Gilbert G reaction; lane 4, 2-mercaptoethanol (7 mM) alone; lane 5, **1** (70 μM) alone; lane 6, iron-EDTA control lane; lane 7, **1** (70 μM)+2-mercaptoethanol (7 mM).

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