Concerning *in Vitro* Mitomycin−DNA Alkylation

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Mitomycin C (**1**) is the prototypical bioreductive alkylating agent.[1] Its clinical effectiveness in the treatment of certain cancers[2] has led to extensive investigations of the mechanism for drug activation[3] and DNA alkylation.[4] These studies provide significant support for the lyer and Szybalski[5] hypothesis, modified by Moore,[6] for the mode of action of **1** (Scheme 1). Although the specific species that reacts with DNA has not been identified, two likely candidates have been proposed: leucoaziridinomitosene **2** and quinone methide **3**. In this paper, we provide evidence of the DNA binding species in *in vitro* mitomycin C transformations.

Earlier studies showed that reductive activation of mitomycin C (**1**) with $Na_2S_2O_4$ in the presence of DNA led to the preferential monoalkylation of guanine units within 5′CG* (G* = modification site) steps[4b,c,7] whereas reductively activated *N*-methylmitomycin A (**6**) selectively alkylates guanine units with little sequence specificity.[8] The differential DNA product profiles observed for **1** and **6** were attributed,[4c] in part, to the facility with which **6** ($E_{1/2} \sim -0.21$ V vs SCE[9a]) underwent reduction compared with **1** ($E_{1/2} = -0.45$ V vs SCE[9b]). We reexamined the sites of DNA modification with **1** and with **6** with the 129-bp restriction fragment from pBR322 plasmid, using the enzymatic reductive system xanthine oxidase (XO)/NADH in place of $Na_2S_2O_4$ (Figure 1). The site of DNA alkylation was determined using the UVRABC assay.[10−12] Our results were in agreement with the earlier findings reported for

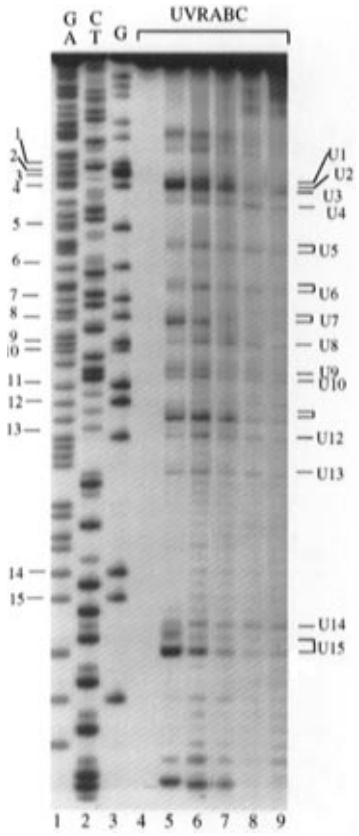
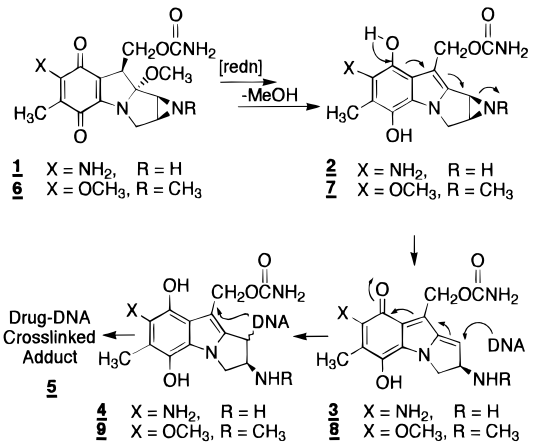


Figure 1. Autoradiogram of UVRABC nuclease cutting of the mitomycin-modified 3′ end ^{32}P-labeled *Bst*NI-*Eco*RI 129-bp fragment of pBR322 plasmid (top strand): lanes 1−3, Maxam−Gilbert sequencing reactions of GA, CT, and G, respectively; lane 4, DNA treated with UVRABC without drug modification (control); lane 5, DNA modified with 1.5 mM **11** after incubation at 37 °C (1 h, pH 7.4); lane 6, DNA modified with 0.09 mM **10** after incubation at 22 °C (1 h, pH 4.0); lane 7, DNA modified with 0.9 mM **1** after reductive activation with XO/NADH at 37 °C (pH 7.4); lane 8, DNA modified with 0.9 mM **6** after reductive activation with XO/NADH at 22 °C (pH 7.4); lane 9, DNA modified with 0.45 mM **11** after reductive activation with $Na_2S_2O_4$ at 22 °C (pH 7.4). The drug modification induced UVRABC nuclease incision bands (U_1−U_{15}) are labeled on the right side of each panel, and the numbers corresponding to the guanine residues (1−15) are listed on the left side of each panel. Approximately the same number of cpm was loaded on each lane.

Scheme 1



$Na_2S_2O_4$.[4c,7,8] Reductively activated **1** (Figure 1, lane 7; Figure 2, panel A) preferentially reacted at 5′CG* sites[4c,7] whereas reductively activated **6** (Figure 1, lane 8; Figure 2, panel B)

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(11) The UVRABC assay is quantitative, and incision proceeds independent of DNA sequence: Li, V.-S.; Choi, D.; Wang, Z.; Jimenez, L.; Tang, M.-s.; Kohn, H. *J. Am. Chem. Soc.*, in press.
(12) The mitomycin concentrations and reaction temperatures were adjusted to permit DNA alkylation to proceed at levels suitable for UVRABC analysis.

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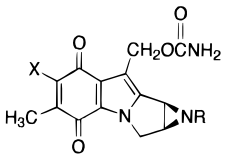
extensively modified 5′TG* and 5′AG* sites and, to a lesser extent, 5′CG* and 5′GG* sites.[8]

We also determined the sites of DNA modification with 7-aminoaziridinomitosene[13] (**10**) and *N*-methyl-7-methoxyaziridinomitosene[3c] (**11**) in the *absence* of reductant. Both **10**[14]



10 X = NH_2, R = H
11 X = OCH_3, R = CH_3

and **11** preferentially reacted at 5′CG* sites (Figure 1, lanes 6,5; Figure 2, panels C,D) and gave a DNA product profile similar to reductively activated **1** (Figure 1, lane 7; Figure 2, panel A).

The stability of **11** in water−dimethyl sulfoxide (9:1) solutions permitted us to examine the DNA profiles of this aziridinomitosene under reductive conditions. $Na_2S_2O_4$ activation of **11** led to low levels of DNA modification, and reaction proceeded with reduced selectivity at the guanine sites distributed throughout the restriction fragment (Figure 1, lane 9).

These findings demonstrated that reductively activated **1** and nonreduced **10** and **11** all alkylate DNA with comparable 5′CG* selectivity. This result indicated that the species responsible for DNA alkylation with reductively activated mitomycin C (**1**) was **10** and that nonreduced **11** behaved similarly to nonreduced **10**. Compound **10** is believed to be generated in mitomycin C reductive processes through a disproportionation reaction between **2** and unreacted **1**.[3d,e,15] Once formed, **10** will rapidly react with nucleophiles.[13b] Our study also showed that both reductively activated **6** and **11** were less selective and less efficient in their reaction with DNA than was nonreduced **11**. This finding indicated that a species other than **11** was responsible for the DNA alkylation with reductively activated **6**. We suspect that this intermediate is either *N*-methyl-7-methoxyleucoaziridinomitosene (**7**) or aziridine ring-opened quinone methide **8**. These species will be formed rapidly in mitomycin A reductive transformations because of the ease with which this quinone system is converted to the hydroquinone.[9] We anticipate that **7** and **8** will be more reactive than **11** and hence show decreased DNA sequence selectivity. Furthermore, **7** and **8** are not expected to alkylate DNA efficiently. We have shown that fully reduced mitomycins react primarily with electrophiles (e.g., protons) rather than nucleophiles.[3a]

These results suggest that *in vitro* and *in vivo* reductive processes that completely convert mitomycin C (**1**) to **2** would produce low levels of DNA modification and that monoalkylation would proceed with little sequence specificity. The mechanism of the **11**−DNA reaction is under investigation.

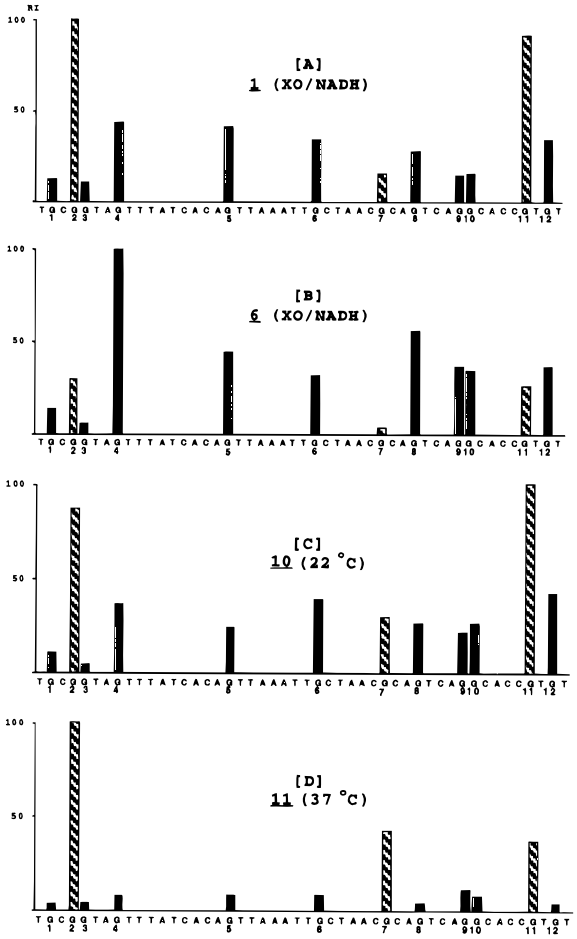


Figure 2. Relative intensities (RI) of UVRABC nuclease incision mitomycin−DNA adducts of 48-base region within 3′ end ^{32}P-labeled *Bst*NI-*Eco*RI 129-bp sequence from pBR322 plasmid (diagonal bars: 5′CG* sites; solid bars: 5′AG*, 5′TG*, 5′GG* sites). Panels A, B, C, and D correspond with Figure 1 lanes 7, 8, 6, and 5, respectively. U_1−U_{12} bands in lanes 5−8 of Figure 1 were scanned by a densitometer. The intensities were normalized for the most intense band (100) within each lane, and the relative intensity of each band is plotted at the corresponding G in the sequence.

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