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Natural products that cleave DNA

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Abstract - The antitumor agent bleomycin is believed to mediate its therapeutic effects at the level of DNA strand scission; this process requires oxygen and an appropriate metal ion and proceeds via oxidative damage to the carbohydrate moiety of DNA. Metallobleomycins participate catalytically in DNA cleavage, apparently via the intermediacy of high valent metal-oxo complexes, which delivers a reactive form of oxygen to a DNA sugar subsequent to binding of the metallobleomycin to the DNA duplex. Also investigated were two structural series of natural products that have been shown for the first time to cleave DNA. One series, exemplified by (-)-epicatechin and procyanidin B_2, was identified initially as constituents of Celastrus pringli Rose. Also studied was a series of 5-alkylresorcinols isolated from Hakea trifurcata. In common with bleomycin, DNA strand scission by both types of agents required a metal ion and O_2. However, for both the flavanoids and 5-alkylresorcinols, DNA cleavage did not require any reductant and was actually diminished in the presence of thiols.

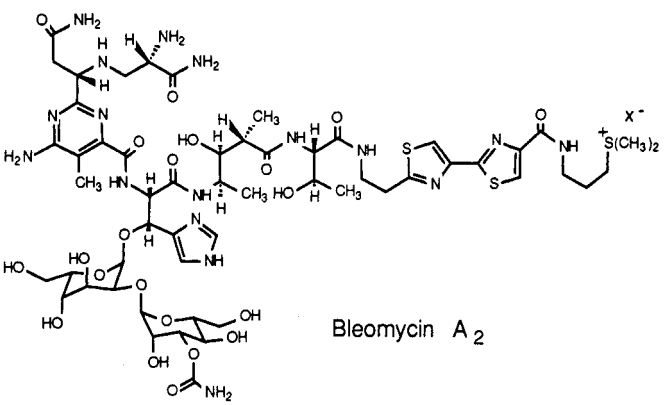
INTRODUCTION

The identification and characterization of molecules that mediate DNA strand scission has led to the development of strategies for determining DNA sequences (ref 1), DNA conformation (ref 2) and the way in which small molecules bind to DNA (ref 3). Of considerable interest in recent years have been species that effect DNA cleavage subsequent to DNA binding, as the combination of these two events has the potential to provide considerable selectivity. Chemical agents that act on DNA in this fashion include both natural products and synthetically derived compounds, as well as previously characterized DNA binders that have been altered structurally to facilitate DNA cleavage (ref 4). New principles for DNA binding (ref 5, 6) and cleavage (ref 7) are of continuing importance and natural product models can contribute importantly in this regard.

The present report describes recent efforts to characterize the mechanism of DNA binding and strand scission by the antitumor agent bleomycin (BLM). Also summarized is the status of a study whose objective is the identification of additional natural products that bind and cleave DNA by novel mechanisms.

BLEOMYCIN-MEDIATED DNA STRAND SCISSION

Figure 1 illustrates the sequence-selective cleavage of a 3'-^{32}P-end labeled DNA restriction fragment by Fe·BLM A_2 in the presence of O_2; as is clear from the figure, strand scission occurred primarily at 5'GT3' and 5'GC3' sequences, and in an overall fashion quite similar for the two metallobleomycins.



Bleomycin A_2

Also investigated was the actual chemistry of DNA strand scission. This was done by the use of self-complementary oligonucleotides such as $^{5'}$d(CGCTTTAAAGCG)$^{3'}$ and $^{5'}$d(CGCTAGCG)$^{3'}$, which have preferred BLM cleavage sites near the 5'-end. It was found (refs 8, 9) that bleomycin produced two types of damage at each site that was modified (Scheme 1). For the octanucleotide depicted in Scheme 1, one lesion produced trans-3-(cytidin-1'-yl)propenal (1), pTAGCG, and a dinucleotide terminating with a 3'-phosphoroglycolate moiety (2). In addition, each site of damage also contained an alkali labile 4'-hydroxyapurinic acid (3), which formed with concomitant release of the heterocylic base. Base treatment of the alkali labile lesion produced strand scission, with rearrangement of the deoxyribase ring to produce diastereomeric 4-hydroxycyclopentenones (4).

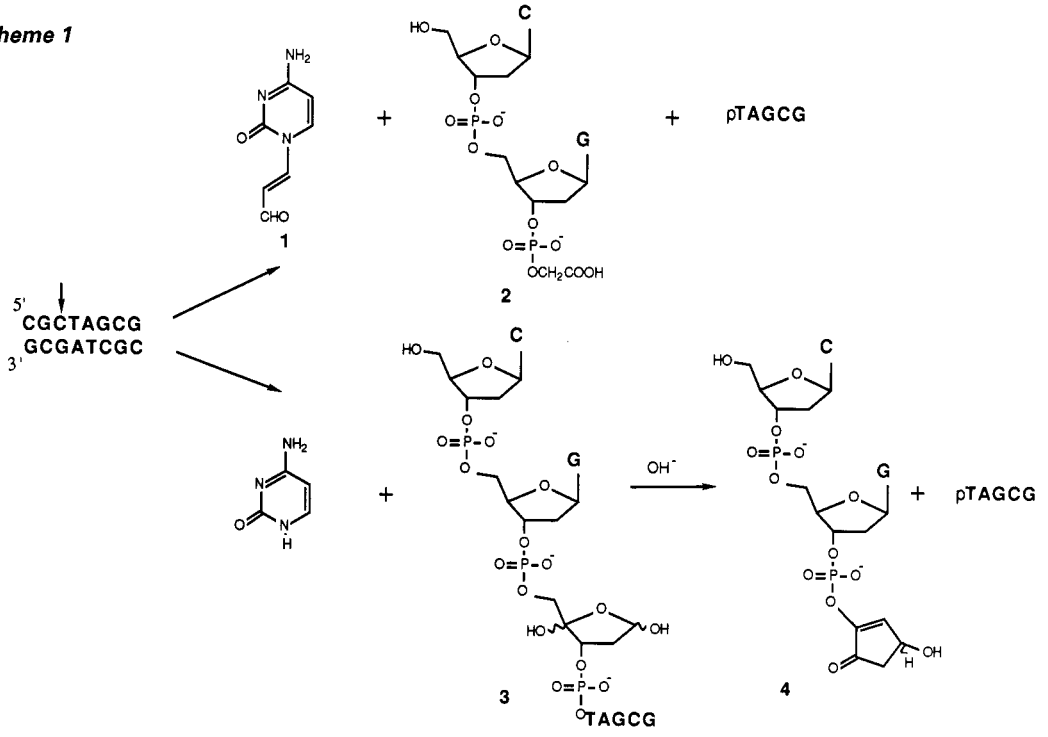
Based on oxygen consumption and other physicochemical data (refs 10, 11), the concentration dependent activation of Fe(II)·BLM in the absence of external reducing agents and the extent of product formation by activated bleomycin in the presence of excess DNA (refs 8, 12), it is believed that activation of Fe(II)·BLM in the presence of O_2 requires an additional electron. This can be provided by an external reductant, or else by the collision and disproportionation of Fe(II)·BLM molecules in the presence of O_2 (refs 9, 10). Cleavage of DNA oligonucleotides by bleomycin gives a pattern quite different than that which obtains with species that generate diffusible oxygen radicals (ref 9). It is suggested that activated Fe·BLM and Cu·BLM exist as high valent metal-oxo complexes capable of oxidizing and oxygenating substrates such as DNA. The chemistry of bleomycin with low molecular weight substrates strongly supports this view (refs 13-15).

IDENTIFICATION OF OTHER NATURAL PRODUCTS THAT CLEAVE DNA

In order to identify additional natural products capable of DNA strand scission, we employed a highly sensitive assay to detect the presence of such compounds in extracts prepared from a number of plants. The assay, illustrated in Fig. 2, involved the use of a negatively supercoiled covalently closed circular DNA (cccDNA) containing several thousand nucleotide base pairs. DNA strand scission at any one of these produced relaxed circles; cleavage on both strands within several base pairs resulted in conversion to linear duplex DNA. Also shown in the figure is the relative mobilities of these three forms of DNA on agarose gels and a "typical" conversion that might be observed with an agent capable of producing both single- and double-strand nicks (e.g., bleomycin).

Fractionation of Celastrus pringli Rose yielded epicatechin (5) and procyanidin B_2 (6) (ref 4), both of which mediated relaxation of supercoiled cccDNA at micromolar concentrations in the presence of Cu^{2+} and O_2. As shown in Fig. 3, the extent of DNA cleavage at fixed [Cu^{2+}] increased in proportion to the concentration of ligand present. The addition of reducing agents such as alkyl thiols did not enhance the extent of DNA cleavage by 5 and 6, suggesting

Scheme 1



Degradation of 5'-d(CGCTAGCG) by bleomycin.

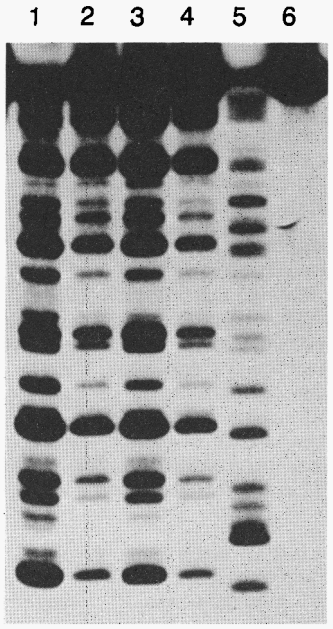


Fig. 1. Site selective cleavage of a ^{32}P-end labeled DNA by BLM A_2 in the presence of Fe, and Cu + dithrothreitol. Reaction mixtures contained: lane 1, 10 μM BLM + 20 μM Fe(II); lane 2, 5 μM BLM + 10 μM Fe(II); lane 3, 10 μM BLM + 20 μM Cu(II) + 1 mM DTT; lane 4, 5 μM BLM + 10 μM Cu(II) + 1 mM DTT; lane 5, G-specific reaction; lane 6, DNA alone.

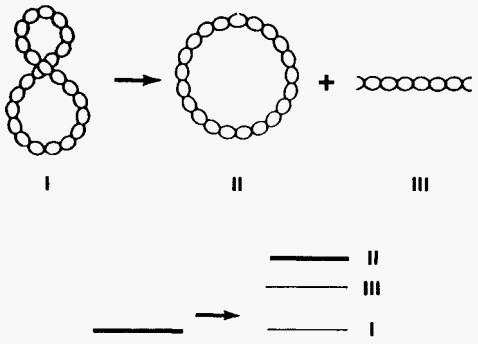


Fig. 2. Conversion of Form I DNA to Form II (nicked circular) DNA and Form III (linear duplex) DNA

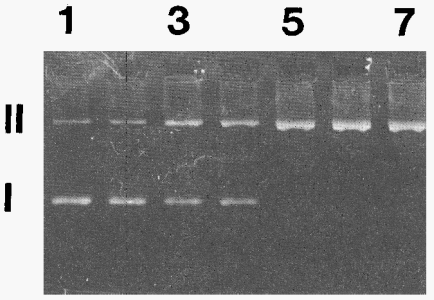
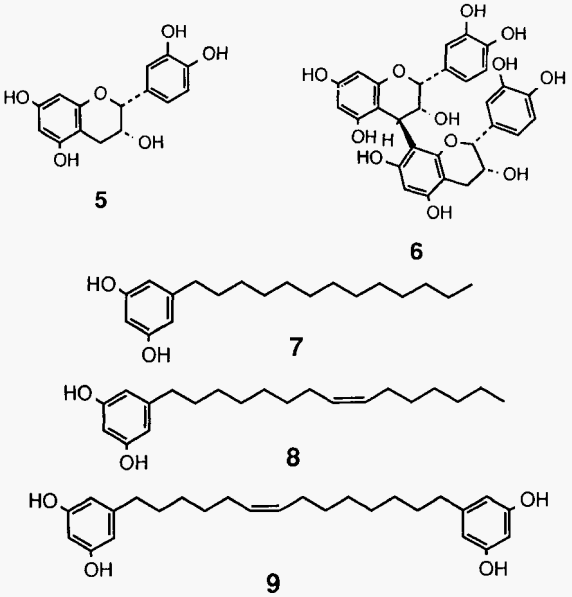


Fig. 3. Relaxation of ΦX174 Form I DNA by procyanidin B_2 (6) in the presence of Cu(II). Lane 1, DNA + 10 μM Cu(II); lanes 2-7, 10 μM Cu(II) + 1, 5, 10, 25, 50 and 100 μM procyanidin B_2, respectively.



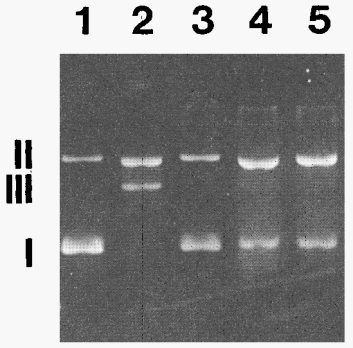


Fig. 4. Cleavage of ΦX174 Form I DNA by a CH_2Cl_2 extract prepared from Hakea trifurcata. Lane 1, DNA alone; lane 2, 10 μM Fe(II) + 0.1% H_2O_2; lane 3, 10 μM Cu(II); lane 4, 20 μg of extract + 10 μM Cu(II); lane 5, 10 μg of extract + 10 μM Cu(II).

that the mechanism of DNA cleavage by these agents differs fundamentally from that of metallobleomycins such as Fe·BLM and Cu·BLM. In experiments that employed ^{32}P-end labeled DNA as a substrate for Cu·epicatechin (i.e., analogous to Fig. 1), cleavage was found to occur at every nucleotide position. In the aggregate these data are consistent with a mechanism involving binding of Cu^{2+} by the catechol moieties in epicatechin and procyanidin B_2; reduction of the bound Cu^{2+} with concomitant ligand oxidation would produce an intermediate capable of reduction of O_2. The oxygen radicals so produced should be capable of mediating the observed DNA strand scission. That 5 and 6 actually do mediate DNA degradation subsequent to DNA binding is suggested by a few different lines of evidence, including

the inability of structurally related flavanoids to mediate DNA cleavage to nearly the same extent, and the alteration of the circular dichroism spectrum of DNA and the ultraviolet spectrum of epicatechin upon admixture of the two.

Assay of a CH_2Cl_2 extract prepared from a dried specimen of Hakea trifurcata indicated the presence of some species capable of mediating DNA strand scission in the presence of Cu^{2+} and O_2 (Fig. 4). When the extract containing this activity was employed at high concentrations, a marked shift in the mobility of Forms I and II DNA on agarose gels was also observed, suggestive of the presence of one or more compounds that bound DNA strongly.

Bioassay-guided fractionation of this extract afforded three compounds (7 - 9) capable of mediating cccDNA relaxation in the presence of Cu^{2+} and O_2. As with compounds 5 and 6, DNA cleavage was not potentiated by added reducing agent, and cleavage of ^{32}P-end labeled DNA was found to produce cleavage at each nucleotide position. Interestingly, it was found that incubation of 7 - 9 under conditions (e.g., high pH in the presence of Cu^{2+}) known to result in oxygenation of the aromatic nucleus (refs 16, 17) dramatically enhanced the potency of these agents as DNA cleaving agents; approximately the same order of potency was observed for the 5-alkyl-1,3,4-trihydroxybenzene derivatives containing structurally comparable 5-alkyl substituents. We suggest that the chemical mechanism of DNA cleavage by these agents involves initial oxygenation of the aromatic nucleus, producing species whose behavior parallels that of epicatechin and procyanidin B_2 in utilizing ligated Cu^{2+} to effect reductive activation of O_2.

As regards the mechanism of DNA binding by 7 - 9, it is interesting to note that synthetic 5-alkylresorcinols and 5-alkyl-1,2,4-trihydroxybenzene derivatives exhibited DNA cleavage efficiencies in rough proportion to the lengths of the individual substituents. It may be possible that the mechanism of association of such agents with DNA is primarily hydrophobic in nature, i.e. that upon dissolution in an aqueous medium containing DNA, compounds 7 - 9 simply associate with the least polar component of that medium (i.e., with the interior of the DNA duplex).

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