This student paper was written as an assignment in the graduate course

Free Radicals in Biology and Medicine

(77:222, Spring 2005)

offered by the

Free Radical and Radiation Biology Program
B-180 Med Labs
The University of Iowa
Iowa City, IA 52242-1181
Spring 2005 Term

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Bleomycin – its activation and DNA damage

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March 14, 2005

Abbreviations:

AGM	agmatine
DMS	dimethylsulfonium
GpC	deoxyguanosine-phosphate-deoxycytosine
GpT	deoxyguanosine-phosphate-thymidine

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Abstract

Bleomycin is a group of anti-neoplastic drugs. It is believed to oxidize DNA and induces single and double strand breaks. Bleomycin needs to be activated before damaging DNA. The activation of Bleomycin involves the incorporations of iron and oxygen molecule. The activated Bleomycin abstracts hydrogen atom from the carbon-4 of DNA deoxyribose. This DNA radical reacts with oxygen and subsequent degraded. The strand DNA breaks induced by Bleomycin are sequence-specific. The GpT and GpC are attacked preferentially.

Introduction

Bleomycin is a group of related glycopeptide antibiotics used as anti-neoplastic agents. It was discovered from *Streptomyces verticillus* in the mid 1960s [1, 2]. Its anti-neoplastic effect is believed to produce free radicals, then induce single and double strand DNA breaks [3, 4]. These reactions require Fe(II) and O_2 as cofactors [5]. RNA is also possible target for Bleomycin [6]. The activation of Bleomycin will incorporate ferrous or ferric ion and oxygen molecule. The activated Bleomycin will produce DNA radical and degrade DNA. This review will briefly discuss the proposed mechanism in Bleomycin activation and its DNA damage.

Bleomycin structure

Bleomycin is a complex of related glycopeptides with two major domains; the metal binding domain, DNA binding domain; and one carbohydrate moiety (**Figure 1**) [7].

The DNA binding domain comprised of the bithiazole and positively charged carboxyl-terminal substituent [8]. Modification of this domain will alter the efficiency of DNA cleavage by Bleomycin [9]. Dedon *et al.* suggested that the planar bithiazole group of Bleomycin binds the DNA helix at the minor groove [10, 11]. The positively charged terminal amino group is attracted to the negatively charged phosphate group of DNA [12].

The metal-binding domain binds a metal ion and O_2 and activates Bleomycin [13]. The bound metal ion could be iron or copper [13]. The activation of Bleomycin is essential to attack and damage DNA [3, 14].

The carbohydrate moiety is believed to aid in membrane permeability and selective tumor-cell recognition [7].

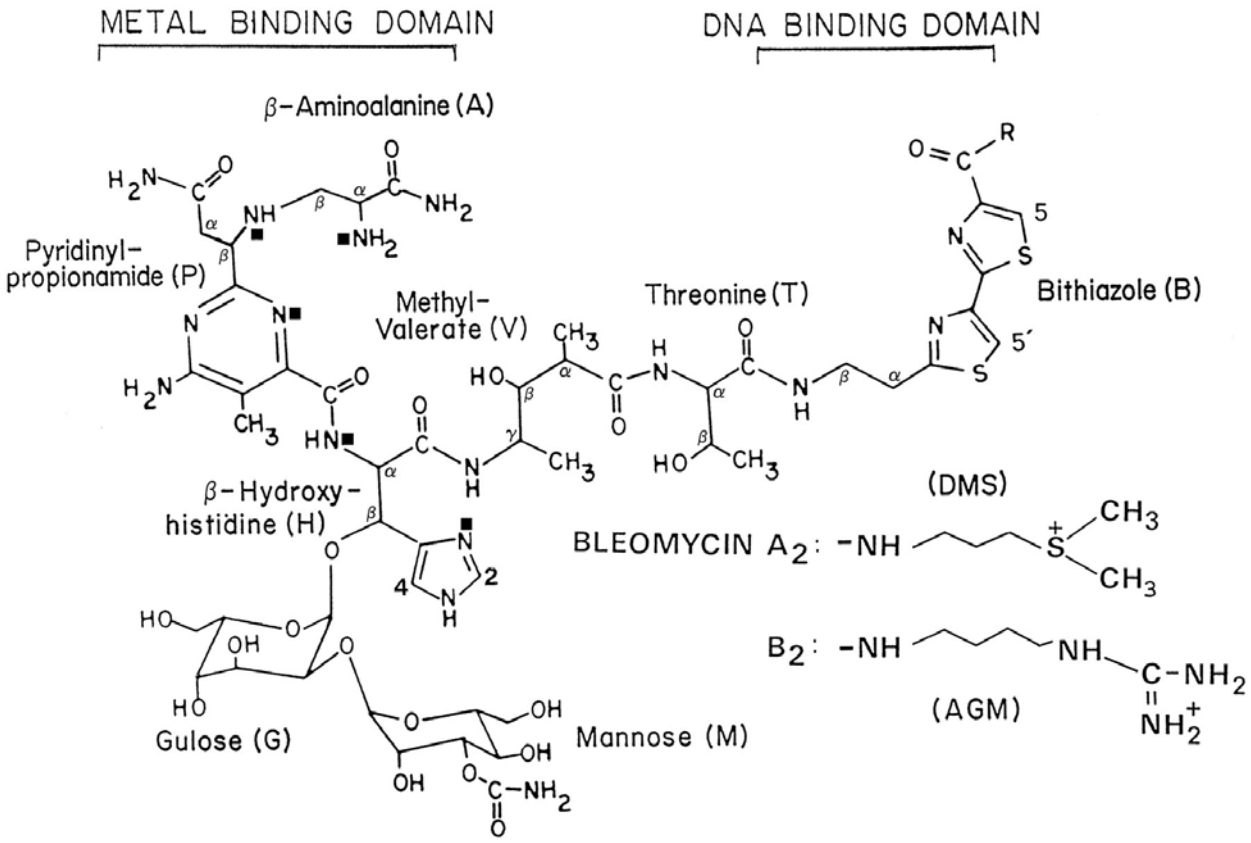

Figure 1: Structure of Bleomycin. Only the deprotonated amide nitrogen contributes to the ligand charge among the metal binding sites (■). It is also assumed that the peroxyl group is protonated. Adapted from [7].

Activation of Bleomycin

Before Bleomycin can damage DNA, it needs to be activated [3, 14]. The activation of Bleomycin involved the incorporation of Fe(II) and O_2 (reaction 1) [3]. Burger et al. reported this reaction is fast and completes in about a second in their experiment. They also observed that DNA does not effectively compete with Bleomycin for Fe(II)[3].

$$Fe(II) + \text{Bleomycin} \rightarrow \text{Fe(II)-Bleomycin} \quad (1)$$

Under atmospheric O_2 tension, Fe(II)-Bleomycin reacts with O_2 and the product, O_2-Fe(II)-Bleomycin is EPR-silent (reaction 2) [3].

$$\text{Fe(II)-Bleomycin} + O_2 \rightarrow O_2\text{-Fe(II)-Bleomycin} \quad (2)$$

The O_2-Fe(II)-Bleomycin reacts with reducing agent and further forms the activated Bleomycin, HO_2^--Fe(III)-Bleomycin (reaction 3)*. In the absence of reducing agent, the Fe(II)-Bleomycin complex can dismutate to form activated Bleomycin and Fe(III)-Bleomycin [15].

$$2\ \text{Fe(II)-Bleomycin} + O_2 + H^+ \rightarrow HO_2^-\text{-Fe(III)-Bleomycin} + \text{Fe(III)-Bleomycin} \quad (3)$$

The activated Bleomycin can also be formed from Fe(III)-Bleomycin with peroxide*[3].

$$\text{Fe(III)} + \text{Bleomycin} \rightarrow \text{Fe(III)-Bleomycin} \quad (4)$$

$$\text{Fe(III)-Bleomycin} + H_2O_2 \rightarrow HO_2^-\text{-Fe(III)-Bleomycin} + H^+ \quad (5)$$

The activated Bleomycin will oxidize its binding DNA and decay to Fe(III)-Bleomycin [3]*.

$$HO_2^-\text{-Fe(III)-Bleomycin} + \text{DNA} \rightarrow \text{Fe(III)-Bleomycin} + \text{oxidized DNA} \quad (6)$$

Mechanism of DNA cleavage by activated Bleomycin

The activated Bleomycin is highly oxidizing. After the binding of activated Bleomycin to

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*Buettner GR. (2005) DNA oxidation. class note in 77:222 Free radical and Radiation Biology. chapter 13; pp 30.

DNA, it abstracts hydrogen atom from the carbon-4 of deoxyribose moiety of pyrimidines. The attacked deoxyribose will become a radical and lead to DNA strand break (**Figure 2**, Pathway A) or abasic DNA strand (**Figure 2**, Pathway B). This carbon-4 radical partitions between two pathways depending on the availability of O_2. Here the oxygen dependent pathway A will be discussed.

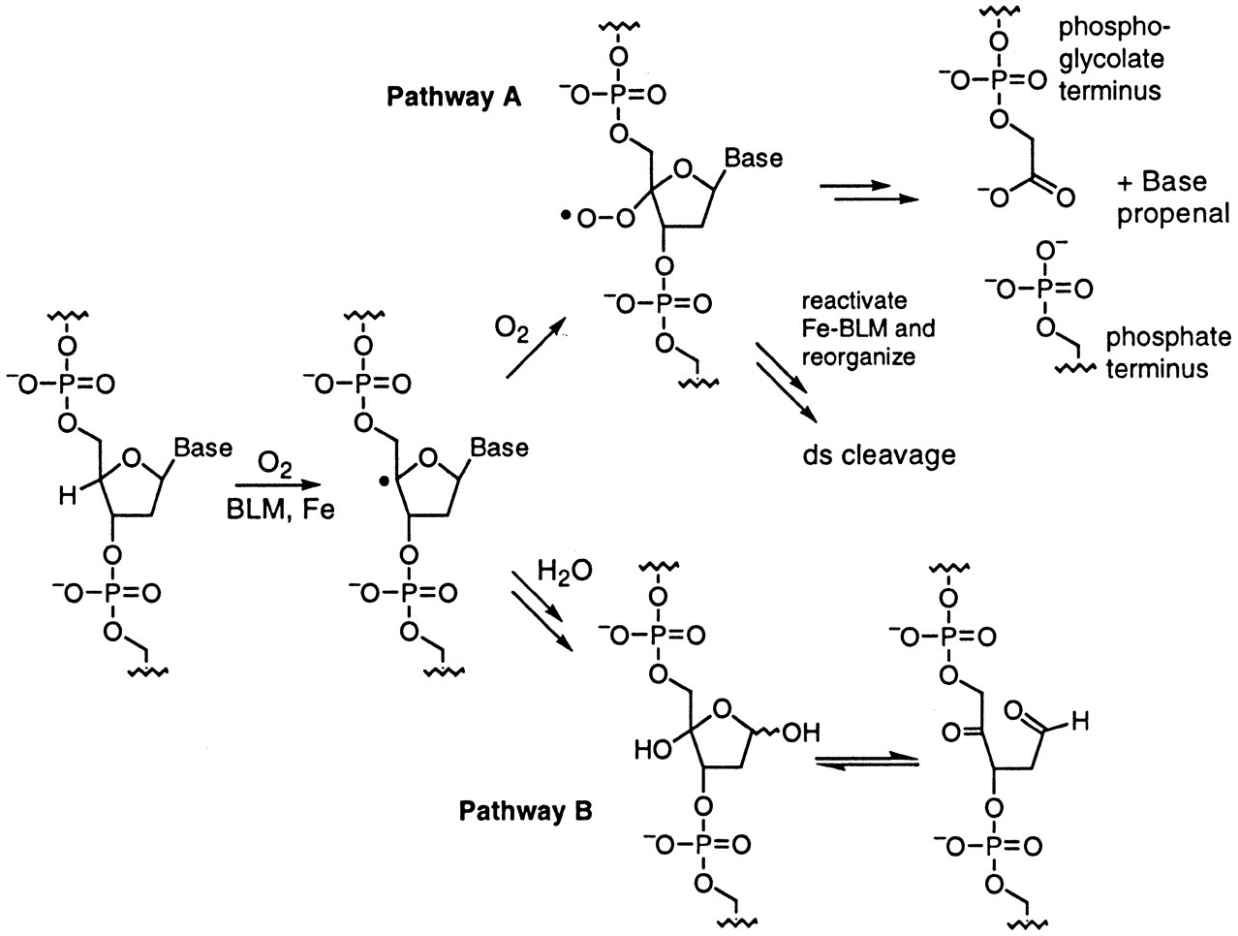

Figure 2. Mechanism of DNA cleavage induced by activated Fe–BLM. Pathway A shows the cleavage mechanism and products in the presence of excess O_2, over and above that required to form activated BLM. Pathway B illustrates the fragmentation in an oxygen limited environment. Adapted from [16].

The oxygen-dependent pathway (**Figure 2**, pathway A) produces a peroxyl radical on the carbon-4 atom of the deoxyribose moiety (**Figure 3**, product 2). This peroxyl radical undergoes further reduction by Fe(II), Fe(II)-Bleomycin or other reducing agents to the hydroperoxy compound (**Figure 3**, product 2). Cleavage of the C3-C4 bond would occur readily under neutral or slightly acidic conditions [17]. Subsequent fragmentation of this reaction yields the base-propenal (**Figure 3**, product 7), free phosphate esters of the 5'- and 3'-terminal groups on DNA strand break (**Figure 3**, product 6), and the phosphate ester of glycolic acid (**Figure 3**, product 8).

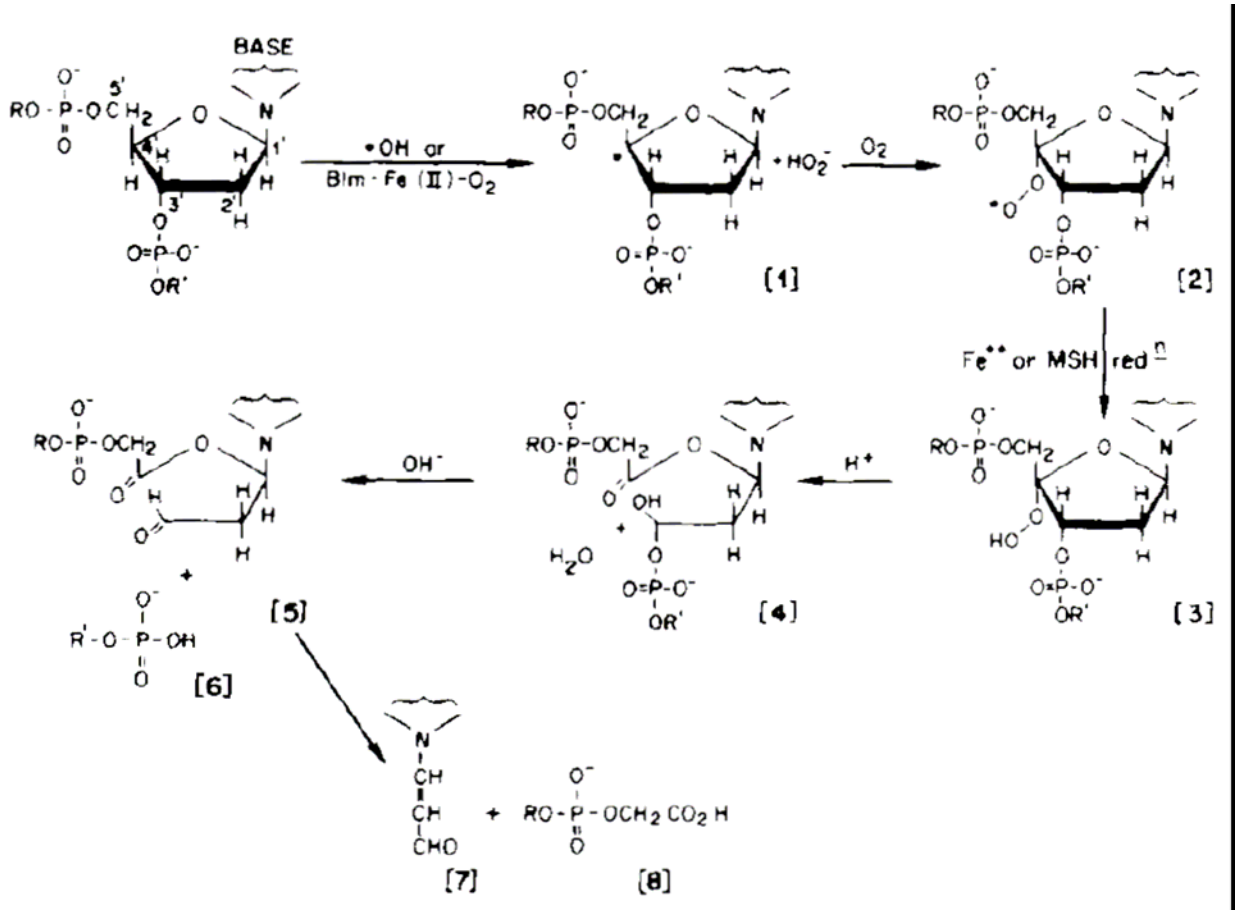

Figure 3. Proposed reaction mechanism for the cleavage of deoxyribose by Bleomycin. Adapted from [18]

Both the single and double strand DNA strand breaks induced by activated Bleomycin are sequence-specific [11, 19-21]. Activated Bleomycin prefers to abstract 4'-hydrogen atom from the deoxyribose moiety of pyrimidines located 3' to a deoxyguanosine unit [22, 23]. Therefore, the DNA sequences of 5'-GpT-3' and 5'-GpC-3' are attacked by Bleomycin preferentially [24, 25]. Double strand breaks occurs less frequently by the damage of activated Bleomycin [25, 26].

Summary

Bleomycin is used as antineoplastic drugs in clinic. It is observed that the activated Bleomycin produces DNA strand breaks. The activation of Bleomycin requires metal ions and oxygen molecules. The activated Bleomycin abstracts hydrogen from carbon-4 of DNA deoxy-ribose. The DNA radical then attacked by oxygen and peroxyl DNA radical was formed. The peroxyl DNA radical then subsequently degraded into base-propenal, free phosphate, and phosphate ester of glycolic acid. Finally DNA strand cleavage was done. The strand DNA breaks induced by Bleomycin are sequence-specific. The GpT and GpC are attacked preferentially.

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