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Metallo-bleomycin cleaves parallel-stranded DNA similarly to B-DNA

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Abstract

Bleomycin (BLM)-mediated degradation of parallel-stranded (ps) DNA duplex was investigated by HPLC product analysis of the oxidized DNA fragments. It was revealed that Co– and Fe–BLMs effectively degraded a ps DNA decamer at 5′-GC site in a similar manner to that for antiparallel-stranded (aps) B-form DNA. While the strand orientations of ps and aps duplexes are quite different, metallo-BLMs still bind to ps DNA duplex and cause DNA degradation. These results indicate that metallo-BLMs productively interact with the degradable strand of both ps and aps DNA duplexes.

Antitumor antibiotic bleomycins (BLMs) are a group of related glycopeptides isolated from *Streptomyces verticillus.* The currently used peplomycin (PEM) is a less toxic congener of BLM.[1] An active Fe–BLM complex abstracts the C4′-hydrogen of deoxyribose to cause oxidative DNA degradation mainly at the 5′-GC site.[2] The proposed mechanism of oxidative DNA cleavage by Fe–BLM is shown in Scheme 1. There are two major oxidative degradation pathways from C4′ H abstraction (paths A and B). The ratio of these paths is modulated by the O_2 concentration.[3] The Co–BLM-green complex also oxidizes DNA under UV irradiation, but the subsequent degradation reaction is restricted to only path B.[4] Recent studies have shown that Co–BLM and Co–deglycosylated BLM (Co–DegBLM) recognize the amino group and N3-nitrogen of guanine in the minor groove by hydrogen bonds which enable them to bind to the 5′-GC site in duplex DNA. Furthermore, the bithiazole moiety is thought to strengthen the binding affinity by intercalation.[5]

DNA duplexes formed by Watson–Crick base pairs such as B-form, A-form and Z-form duplexes are antiparallel-stranded (aps) duplexes. In contrast, the DNA duplex consisting of reverse Watson–Crick base pairs has been known to form a parallel-stranded (ps) duplex.[6] By incorporating cytosine-isoguanine

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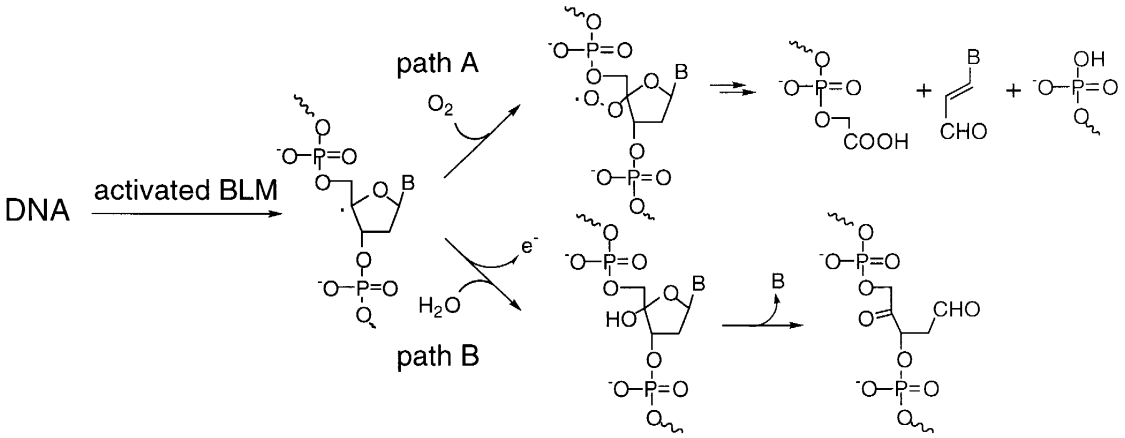

Scheme 1.

(iG) and guanine-5-methylisocytosine (iC) reverse Watson–Crick type base pairs, we demonstrated that a stable ps DNA duplex as well as a DNA/RNA hybrid are formed.[7] Although intercalaters such as ethidium bromide and daunorubicin can bind to the ps duplex,[7b] the molecular recognition and reactivity of the ps duplex by naturally occurring antitumor antibiotics have not been studied. Since metallo-BLMs are known to cleave various types of nucleic acids, such as DNA duplex,[8] DNA–RNA hybrid[9] and RNA which has a tertiary structure,[10] the rationalization of a relationship between reactivity and structure of nucleic acids would provide valuable information for molecular recognition of nucleic acids by BLM. Herein we report an efficient and highly selective cleavage of the ps duplex by Co– and Fe–BLMs in a similar manner to the aps duplex.

To investigate BLM-mediated DNA degradation we used two pairs of 10-mers, 5′-ACTTGCCTGA (ODN I) and 5′-TiGAAiCiGiGAiCT (ODN II), which form a ps duplex, and ODN I and 5′-TCAGGCAAGT (ODN III), which form an aps duplex. Fig. 1a shows the HPLC profile of the reaction mixture of the 50 μM of Co–PEM-treated ps duplex (ODN I–II) after UV irradiation. A new peak at 32 min was identified as C4′-hydroxylated abasic site-containing decamer **1** generated by C4′H abstraction of C_6 of ODN I through path B.[11] The control reaction of the aps duplex (ODN I–III) with Co–PEM also produced **1** (Fig. 1(b)). Since degradation of the aps duplex with 50 μM Co–PEM caused a side reaction at T_{10} of the ODN III, 10 μM of Co–PEM was used for the aps duplex. It appears that cleavage of the ps duplex is less efficient based on the efficiency of the reaction, which is the amount of oxidation products devided by that of Co–PEM. Degradation sites and yields of cleavage are summarized in Fig. 1. In contrast, Co–PEM showed almost no reactivity toward the single-stranded DNA, ODN I (data not shown). These results clearly indicate that the ps duplex is degraded by photoactivated Co–BLM in a similar manner to the aps duplex.

Fe–BLM was also found to cleave ps DNA. Fig. 2a shows the HPLC profile of the degradation products of the ps duplex (ODN I–II) by Fe–BLM. In addition to the formation of **1**, two major products were formed, subsequently revealed as ACTG-glycolate **2** and d(CTGA) **3** by enzymatic digestion and ESMS, respectively.[3d,13] These compounds were produced by oxidative damage on C_6 of the 5′-GC site through path A (Scheme 1). Similar to the degradation by Co–PEM, Fe–PEM cleaved ODN I–III at C_6 of ODN I (Fig. 2b).[14] Analogous GC selective degradation was observed for degradation of the ps DNA duplex, 5′-ACGCATGG (ODN IV)/5′-TiGiCiGTAiCiC (ODN V) by Co– and Fe–BLMs.

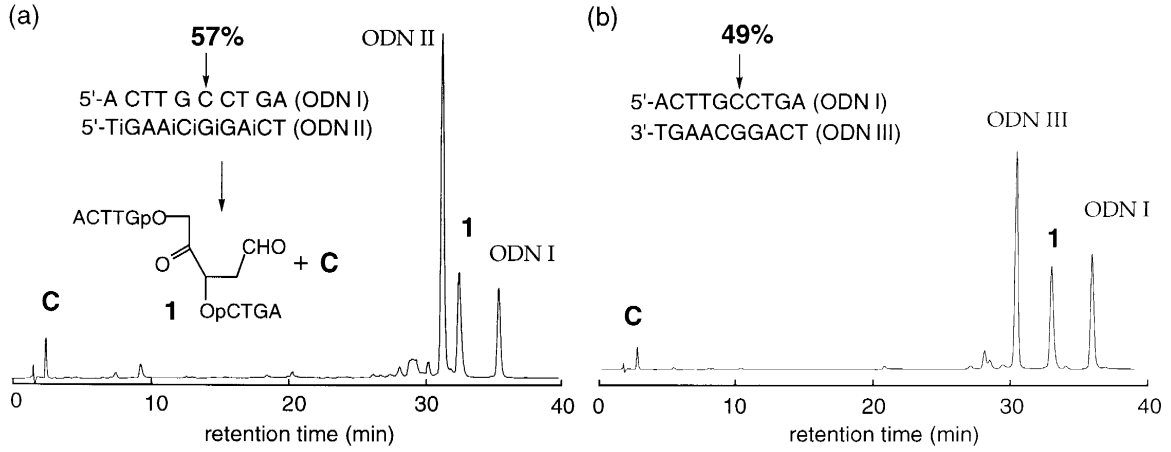

Fig. 1. HPLC profiles of degradation reactions by Co–PEM. (a) ps 5′-ACTTGCCTGA (ODN I)/5′-TiGAAiCiGiGAiCT (ODN II); (b) aps ODN I/5′-TCAGGCAAGT (ODN III). The reaction mixtures (50 μL) contained 10 μM DNA duplex or single strand, Co–PEM-green complex[12] (50 μM for ps and 10 μM for aps), and 50 mM sodium cacodylate buffer (pH 7.0). UV (365 nm) light was irradiated for 2 h at 0°C under aerobic conditions by a transilluminator and the reaction mixture was directly analyzed by HPLC on a Cosmosil 5C18 AR II column (4.6×150 mm) at 254 nm; 0.05 M ammonium formate (pH 7.0) containing 0–10% acetonitrile was eluted with a linear gradient, over 40 min, at a flow rate of 1.0 mL/min, at 40°C. Arrows represent location and yield of cleavage

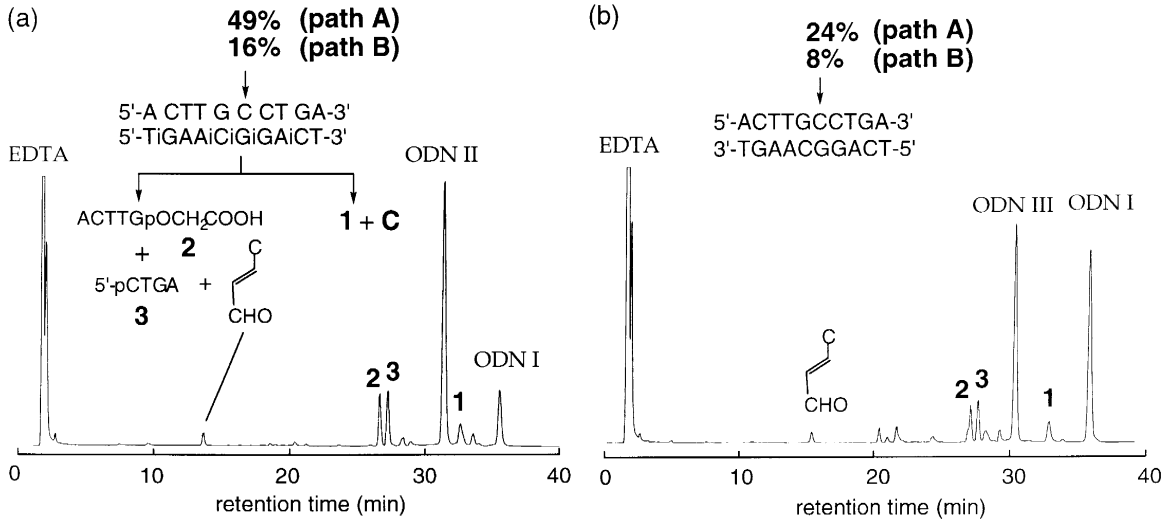

Fig. 2. HPLC profiles of degradation reactions by Fe–PEM. (a) ps ODN I/ODN II; (b) aps ODN I/ODN III. The reaction mixtures (50 μL) contained 10 μM DNA duplex or single strand, Fe–PEM (40 μM for ps and 20 μM for aps), 0.02 U/μL alkaline phosphatase, and 50 mM sodium cacodylate buffer (pH 7.0). The reactions were started by the addition of a $FeSO_4$ solution and left for 1 h at 0°C under aerobic conditions. The reaction mixture was quenched by the addition of 1 μL of 500 mM EDTA and directly analyzed by HPLC as described in the legend of Fig. 1. Arrow represents location and yield of cleavage. Respective selectivity for paths A and B is also indicated

The present study demonstrated that both Co– and Fe–BLMs degraded the ps DNA duplex in the same manner as observed for antiparallel DNA with almost the same selectivity, implying that metallo-BLM strongly interacts with only one strand that is cleaved and further suggests that BLM binds to the ps duplex by similar interactions as those seen in the B-form DNA duplex, i.e. hydrogen bonds enabling the recognition of G in the minor groove and the intercalation[5a,b] of the bithiazole moiety. The present results also suggest that DNA local structures that are deviated from typical B-form DNA can also be a target of BLM.

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11. Enzymatic digestions of **1** afforded dA, dC, dG, and dT in ratio of ca. 2:2:1:3. Electrospray mass spectrum (ESMS) (negative); **1**: calcd: 2907.9; found: 2907.5; **1** (hydrate): calcd: 2926.0; found: 2925.8.
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13. Enzymatic digestion of **2** provided dGpOCH$_2$COOH, dC, dG, dT, and dA in ratios of ca. 0.8:1:0.2: 2:1. Enzymatic digestion **3** afforded dC, dG, dT, and dA in ratios of ca. 1:1:1:1. ESMS (negative) **2**: calcd: 1616.1; found: 1615.8.
14. Analogous to the degradation of aps duplex by Co–PEM, degradation of aps duplex with 50 μM Fe–PEM caused side reactions at G_5 and C_6 of the ODN III.