Oligonucleotides Containing a Radical Trap: The Trapping of Deoxyribose C4′ Radical in Bleomycin-Mediated Reactions

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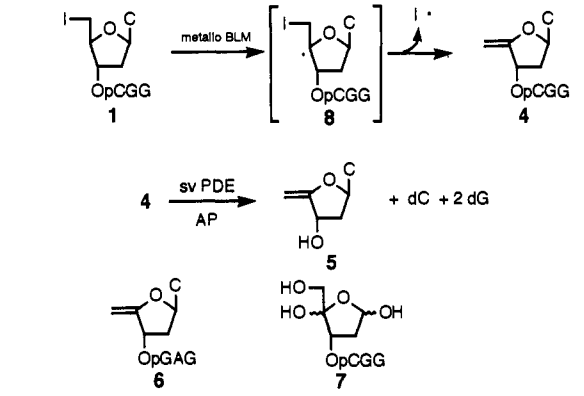
The chemistry of sequence-specific DNA damage induced by antitumor antibiotics via hydrogen abstraction is a topic of intense current interest.[1] While several types of modified oligodeoxynucleotides (ODNs) have been used as a mechanistic probe for drug-induced DNA degradations,[2] ODN designed to trap putative carbon radicals generated on a DNA sugar backbone has not been demonstrated.[3] With the view of developing a new mechanistic probe for drug-induced radical reactions of DNA, we have synthesized a novel type of ODN equipped with a radical trap specific for the deoxyribose C4′ radical, which has long been proposed as an intermediate in metallobleomycin (BLM)-mediated DNA modifications.

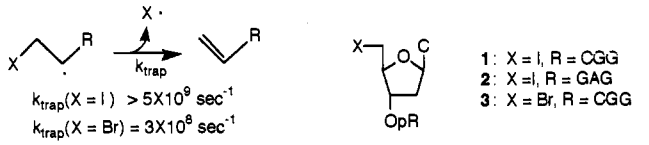
Since we already demonstrated that a selective hydroxylation occurs at terminal cytidine (C_1) of tetranucleotide d($C_1C_2G_3G_4$) by Fe-BLM or Co-BLM,[4] we have attempted to incorporate a radical trap into the C5′ position of the C_1 site. Iodomethyl ($k_{trap} > 5 \times 10^9$ s^{-1}) and bromomethyl ($k_{trap} = 3 \times 10^8$ s^{-1}) groups were used as a radical trap due to the rapid homolysis rates of a carbon–halogen bond β to a radical center.[5] Thus, the radical trap-containing teteramers **1-3** were prepared by automated DNA synthesizer using the β-cyanoethylphosphoramidite of *N*-benzoyl-5′-halo-2′,5′-dideoxycytidine.[6] After deprotection with ammonium hydroxide under mild conditions, oligonucleotides **1-3** were purified by HPLC.

Radical trap-containing tetramer **1** was digested with peplomycin (PEM), a clinically used derivative of BLMs, in the presence of Fe(II) at 0 °C for 15 min under aerobic conditions. Direct

Scheme 1



HPLC analysis of the reaction mixture revealed the formation of a major product. The structure of the product was characterized



as *exo*-methylene-containing tetramer **4** on the basis of enzymatic digestion of the isolated product with snake venom phosphodiesterase (sv PDE) and alkaline phosphatase (AP) providing **5**,[8] dC, and dG in a ratio of 1:1:2 (Scheme 1). *exo*-Methylene-containing oligomer **6** was also produced from **2**, but only in the presence of complimentary strand d(CTCG), showing that the reaction requires a double-stranded structure for DNA, a typical characteristic for the BLM-mediated reaction.[9] These results clearly indicate that C4′ radical **8** when produced via hydrogen abstraction by activated Fe-PEM rapidly eliminates iodo radical to give **4** (Scheme 1). The efficiency for the formation of **4** well corresponded to that observed for the Fe-PEM reaction of d(CCGG), giving **7** under identical conditions (Table 1), implying that the incorporation of radical trap into d(CCGG) does not alter the efficiency of hydrogen abstraction from C4′ of terminal cytidine (C_1). Reaction of **1** with photoactivated Co-PEM also provided **4** as a major product,[10] indicating that the generation of C4′ radical intermediate **8** via hydrogen abstraction is again an initial step of the C4′-hydroxylation induced by photoactivated Co-PEM.[11] The predominant formation of **4** in either Fe-PEM or Co-PEM reaction clearly demonstrated that the trapping rate of C4′ radical **8** by the adjacent iodomethyl group is much faster than that for the subsequent hydroxylation in both cases.

In contrast, the reaction of bromo-substituted ODN **3** with metallo-PEMs under the same conditions gave **4**, but along with it an additional product (Scheme 2). In each case, the formation of a new product was observed with release of cytosine together with radical trapping product **4**. The structure of the new product was assigned as **9** on the basis of $NaBH_4$ reduction and subsequent enzymatic digestion with sv PDE and AP, producing **10** and dG in a ratio of 1:2. The structure of **10** was further confirmed by independent synthesis of one of the diastereomers.[12] These results

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(4) Sugiyama, H.; Tashiro, T.; Dannoue, Y.; Miwa, T.; Matsuura, T.; Saito, I. *Tetrahedron Lett.* **1989**, *30*, 7213. Further investigation in our laboratory indicated that terminal cytidine of d(CCGG)$_2$ is also hydroxylated efficiently by Fe-PEM or photoactivated Co-PEM.

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(10) In a control experiment without Co-PEM, consumption of **1** was never observed under the irradiation conditions.

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(12) One of the diastereoisomers of **10** was synthesized by six steps from 2-deoxy-D-ribose. ^{1}H NMR and FABMS data for **10** are consistent with the assigned structure.

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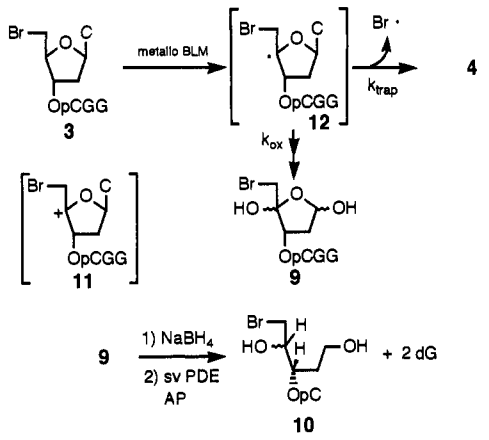
Table 1. Quantitative Analysis of the Products Formed in Metallo-PEM-Mediated Degradation of Radical Trap-Containing Tetranucleotides **1** and **3**[a]

metallo-BLM	ODN	consumed ODN(μM)	*C* (μM)	**4** (μM)	**9** (μM)	**7** (μM)	k_{ox}/k_{trap}[b]	$k_{ox}(s^{-1})$[c]
Fe-PEM	**1**	28	1.0	18.7			0	
Fe-PEM	**3**	43	11.5	13.5	9.2		0.68	2.0×10^8
Fe-PEM	d(CCGG)	23	15.5			15.1		
Co-PEM	**1**	52	5.0	24.7			0	
Co-PEM	**3**	63	34.7	10.2	33.0		3.24	9.7×10^8
Co-PEM	d(CCGG)	49	42.4			45.6		

[a] Degradation by Fe-PEM was as follows. The reaction mixture (total volume, 20 μL) contained tetranucleotide (1 mM, final base concentration) and PEM (0.1 mM) in 50 mM sodium cacodylate at pH 7.0. The reaction was initiated by addition of freshly prepared aqueous $Fe^{+2}(NH_4)_2(SO_4)_2$ solution (0.1 mM), and the mixture was incubated at 0 °C for 15 min under aerobic conditions. Degradation by Co-PEM was as follows. The reaction mixture (total volume, 50 μL) contained tetranucleotide (1 mM, final base concentration), 50 mM sodium cacodylate (pH 7.0), and 300 μM green Co-PEM. Irradiation was performed with a transilluminator (TL 33, 365 nm) for 2 h at 0 °C from a distance of 10 cm. The reaction mixture (10 μL) was analyzed by HPLC on a Cosmosil $5C_{18}$ column (4.6 × 150 mm), detected at 254 nm; elution was with 0.05 M ammonium formate, 0–10% acetonitrile, linear gradient, 20 min, at a flow rate of 1.5 mL/min. [b] The value was obtained from the ratio **4**/**9**. [c] Apparent rate constant for the oxidation of radical **12** leading to **9** on the basis of reported homolysis rate (3×10^8 s^{-1})[5] of the C–Br bond β to a radical center.

Scheme 2



imply that the C4′-hydroxylation giving **9** and the radical trapping reaction occur competitively at the C_1 site in the degradation of bromo-substituted ODN **3**. Although a carbocation intermediate such as **11** has been proposed as an intermediate in the Fe-BLM-mediated reaction on the basis of the incorporation of solvent $H_2{}^{18}O$ into the C4′ position of the product,[13] the exact mechanism of the hydroxylation giving **9** in the present Fe-PEM reaction is still not clear. However, it seems very reasonable to assume that C4′-hydroxylation product **9** arises from carbocation **11** produced by further oxidation of the C4′ radical **12** in the Fe-PEM reaction,[2e,14] whereas C4′-hydroxylation by Co-PEM does not seem to involve such a carbocation intermediate. Table 1 shows all the degradation products obtained from ODNs **1** and **3** and the calculated apparent rate constants (k_{ox}) for the oxidation of radical **12** leading to **9** on the basis of the product ratio (**4** vs **9**). The results obtained in the present study indicate (i) that C4′ radical intermediate such as **8** or **12** is actually produced at C_1 site in the Fe- and Co-PEM reactions and (ii) that the rate of subsequent hydroxylation leading to **9** in the Co-PEM reaction is about 5 times faster than that for the corresponding Fe-PEM reaction. The apparent rate constant (2×10^8 s^{-1}) for the oxidation of C4′ radical **12** to **11** in the Fe-PEM reaction was much smaller than that for the oxygen rebound by cytochrome P-450 (ca. 2×10^{10} s^{-1}),[15] suggesting a mechanistic difference in the two systems.

In summary, we have demonstrated for the first time the intermediacy of the deoxyribose C4′ radical in metallo-BLM reactions by using ODN equipped with a radical trap. The present study indicates that radical trap-containing ODN can be used as a powerful mechanistic probe for the oxidative DNA modifications by a number of other DNA-cleaving molecules. Obviouly, radical trap-containing ODNs described here are limited to the reaction at terminal residues. Further work on the development of a new radical trap for internal site of ODN is in progress.

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Supplementary Material Available: Structure of peplomycin, HPLC profiles of metallo-BLM-treated tetranucleotides **1** and **3**, and spectral data (^1H NMR, FABMS) of **5** and **10** (4 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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(14) Alternatively, O_2 trapping of C4′ radical **12** would also be able to produce **9**. However, the ratio of **4** to **9** was not changed appreciably whether the reaction was conducted under either O_2-limiting conditions or at 1 atm of O_2 pressure, suggesting that **9** is not derived from an O_2-dependent process.

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