Tetrahedron Letters,Vol.30,No.51,pp 7213-7216,1989 0040-4039/89 $3.00 + .00
Printed in Great Britain Pergamon Press plc

METALLO·BLEOMYCIN-MEDIATED DEGRADATION OF DEOXYTETRANUCLEOTIDES. UNUSUAL C-4' HYDROXYLATION AT TERMINAL CYTIDINE

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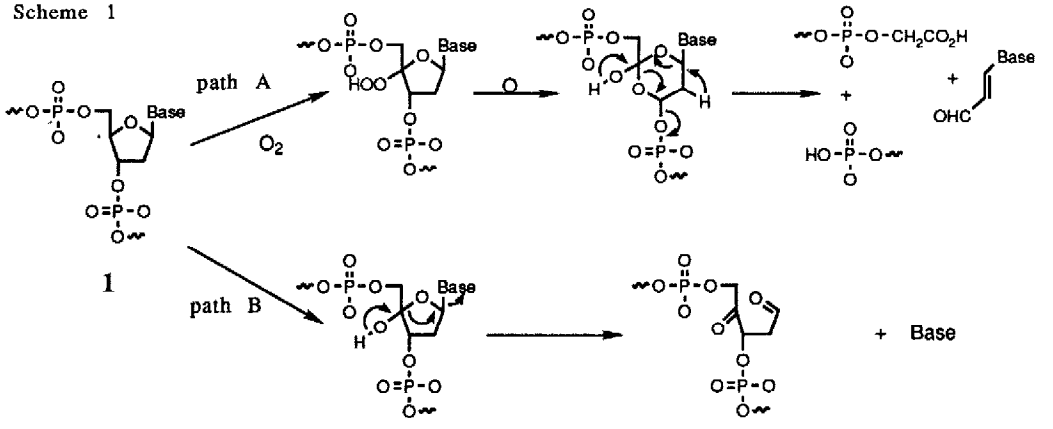
Summary: Degradation of various deoxytetranucleotides by Fe·PEM and Co·PEM was investigated. Tetranucleotides possessing 5'-GC sequence in the middle were found to be cleaved at cytidine3, whereas unusual selective C-4' hydroxylation at terminal cytidine was observed with d(CGAG)-d(CTCG) and d(GCGC)2.

Bleomycins (BLM) are glycopeptide-derived antibiotics capable of effecting DNA strand scission.[1] The products of Fe(II)·BLM·O_2-mediated DNA degradation include base propenals and free bases as well as DNA fragments having 3'-(phosphoro-2"-O-glycolate) termini.[2] Also formed is an alkali-labile site, and its structure was identified as C-4' hydroxylated abasic site by using oligonucleotides as a substrate (Scheme 1).[3] In order to explain all these products, an oxygen-dependent partitioning of intermediate C-4' radical **1** has been proposed.[4] Recently, a dramatic increase of free base formation (path B) relative to propenal production (path A) has been observed in the digestion of DNA-RNA hybrids such as poly(dA)poly(rU), where C-1' hydrogen abstraction by Fe(II)·BLM·O_2 has been proposed.[5] However, the knowledge of the mechanism of metallo·BLM-induced degradation of structurally modulated DNA is scant.[6] In investigating the chemistry of BLM-mediated DNA degradation, methodology using sequence-defined oligonucleotides as a substrate appears to be quite effective.[3,7] Herein described is an unusual observation on the degradation of simple deoxytetranulceotides by metallo·BLM, wherein oxygen-independent path B occurs exclusively at terminal cytidine (C_1) of d(CGAG) in d(CGAG)-d(CTCG) duplex even under oxygen-rich conditions.

Scheme 1



In a series of experiments employing various types of deoxytetranucleotides, tetranucleotides possessing 5'-GC sequence in the middle such as d(CGCG) were found to be effectively cleaved at cytidine3 by Fe·peplomycin (PEM), a derivative of BLM, as well as by Co·PEM.[8] The results of the quantitative analysis including the determination of the cleavage site and the ratio of product partitioning (path A *vs.* path B) are summarized in Table I. The ratio of product partitioning induced by Fe(II)·PEM was fairly constant (entries 1-3). In contrast, an unusual selective C-4' hydroxylation at C_1 of d(CGAG) has been observed in the digestion of d(CGAG)-d(CTCG) duplex (entry 4), where specific cleavage at adenine3 of d(CGAG) is normally expected.[9] A typical reaction mixture containing Fe(II)·PEM (0.3 mM) and d(CGAG)-d(CTCG) (1 mM base concentration[10]) in 50 mM sodium cacodylate (pH 7.0) was incubated for 15 min at 0 °C under aerobic conditions. Direct analysis of the reaction mixture by reverse phase HPLC indicated the formation of free cytosine and an unknown peak eluted at 13.1 min with d(CTCG) remaining unchanged (Figure 1a). For the isolation of the deoxyribose moiety associated with spontaneous cytosine release, immediate $NaBH_4$ reduction was essential due to its chemical instability. When the reaction mixture was reduced with excess $NaBH_4$ at 0 °C, diastereomers of reduced product **2** was obtained (Figure 1b). Enzymatic digestion of **2** with snake venom phosphodiesterase (PDE) followed by alkaline phosphatase (AP) provided two isomers of mononucleotide **3**,[3b,11] together with dA and dG. The structure was further confirmed by independent synthesis of one of the isomers of **2**.[12]

Photoexcited Co·PEM also provided **2** exclusively, showing that both Fe(II)·PEM and Co·PEM react preferentially at terminal cytidine1 rather than at the middle GA site.[14] It has been well established that metallo-BLMs cleave DNA preferentially at pyrimidine moieties of 5'-GC and 5'-GT sequences.[8b,9] The guanine 2-amino group of 5'-GC site in the minor groove has been suggest to play a crucial role in GC recognition by BLM.[15] In fact, all tetranucleotides containing 5'-GC sequence in the middle were preferentially cleaved at cytidine3. In contrast, there is no such recognition element in d(CGAG). While it has not yet been clarified that BLM binds to DNA by intercalation or groove binding,[1b,c] our results indicate an alternative possibility that BLM is capable of expressing a different type of binding mode at the blunt end of the duplex.

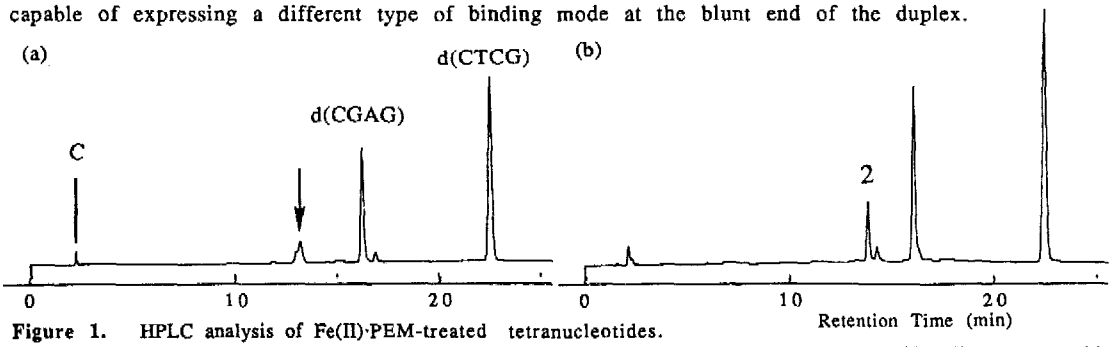


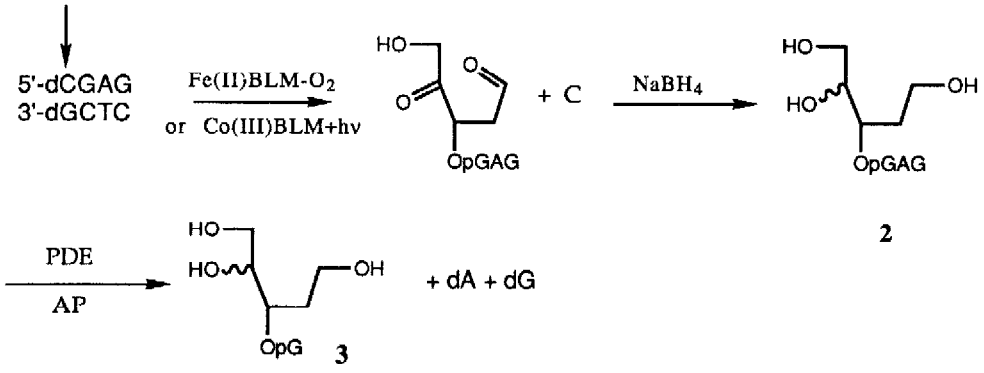
Figure 1. HPLC analysis of Fe(II)·PEM-treated tetranucleotides.
The reaction mixture (total volume 50 μL) contained 1 mM d(CGAG)-d(CTCG) (final nucleotide concentration), 0.3 mM PEM, and 0.3 mM $Fe(NH_4)_2(SO_4)_2$ in 50 mM sodium cacodylate at pH 7.0. The reaction was initiated by addition of Fe(II), and the mixture was incubated at 0 °C for 15 min and then analyzed (a) directly or (b) after $NaBH_4$ reduction by means of HPLC. Analysis was carried out on a Cosmosil $5C_{18}$ column (4.6 x 150 mm); elution was with 0.05 M ammonium formate, 0 - 15 % acetonitrile, linear gradient, 30 min, at a flow rate of 1.5 mL/min.

Table I. Sequence Dependence of Peplomycin-Mediated Oxidation of Various Tetranucleotides

	Nucleotide	PEM·Fe(II)-O_2[a] % scission[c] (position)	path A:path B[c]	total product[d] (μM)	PEM·Co-green[b] % scission[c] (position)	total product[d] (μM)
1	5'-d(CGCG)	85 (C_3)	64:36	99	90 (C_3)	80
2	5'-d(GGCC)	100 (C_3)	64:36	89	98 (C_3)	59
3	5'-d(CGCC) 3'-d(GCGG)	90 (C3) 100 (C3)	74:26 68:32	103	96 (C3) 100 (C3)	55
4	5'-d(CGAG) 3'-d(GCTC)	100 (C_1) 0	0:100	32	100 (C_1) 0	17
5	5'-d(GCGC)	90 (C_4)	0:100	52	83 (C_4)	47

[a]Reaction conditions are shown in Figure 1. [b]The reaction mixture (total volume 50 μL) contained 1 mM deoxytetranucleotides, 50 mM sodium cacodylate (pH 7.0), and 300 μM green Co·PEM.[8] After irradiation (transilluminator TL-33, 10 cm distance) for 1 h at 0°C, analysis of the degradation products was effected by reverse phase HPLC. [c]The position of the cleavage and the ratio of product partitioning (path A *vs.* path B) at major cleavage site were determined by analyzing the products shown in Scheme 1 by means of HPLC in comparison with authentic samples[2b] prepared independently in each case. [d]Total product is equal to the sum of all free bases and base propenals.

Scheme 2



Of special interest is that Fe(II)·PEM-induced oxidation at cytidine1 proceeded exclusively via path B (entry 4). Oxygen-dependent products via path A such as d(pGAG) or cytosine propenal have never been observed even under oxygen atmosphere in this reaction. Surprisingly, degradation of self-complimentary d(GCGC) also proceeded exclusively via path B at terminal cytidine C_4 (entry 5).[16] These results are not easily explained by the oxygen-dependent C-4' radical partitioning mechanism shown in Scheme 1. Such selective C-4' hydroxylation at terminal residue of certain tetramers described here seems to be very important for the investigation of the interaction mode of BLM with DNA.[17,19]

Reference and Notes

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9) BLM A_2 is known to preferentially cleave DNA at GC, GT, and GA sites. See, Takeshita, M.; Grollman, A. In "Bleomycin: Chemical, Biochemical and Biological Aspects"; Hecht, S. M. Ed., Springer-Verlag, New York, **1979**; p 207.
10) Concentrations of all tetranucleotides were determined by HPLC after complete digestion with snake venom phosphodiesterase and alkaline phosphatase.
11) Formation of two diastereomers of **3** by $NaBH_4$ reduction strongly suggests that initial hydroxylation occurs at C-4' of deoxyribose moiety.[3b]
12) Phosphoramidite of 1-O-methyl-5-dimethoxytrityl-2-deoxy-D-ribose was prepared by the reported procedure,[13] and subjected to automatic DNA synthesizer. By usual deprotection procedure, d(GAG) possessing 1-O-methyl-2-deoxy-D-ribose moiety at 5'-termini was obtained. Upon standing at room temperature in 0.1 N HCl for 1 h and following $NaBH_4$ reduction, one isomer of **2** was obtained.
13) Nielsen, J.; Taagaard, M.; Marugg, J. E.; van Boom, J. H.; Dahl, O. *Nucleic Acids Res.* **1986**, 19, 7391.
14) Degradation products resulting from the reaction at adenine4 have never been observed by HPLC.
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16) Alkali-labile product formed at cytidine4 of d(GCGC) will be reported elsewhere. Neither d(GCGp) having glycolic acid at 3'-phosphate termini nor cytosine propenal was detected.
17) Although detail mechanism of O_2 independent free base release is not clear, binding of BLM to the opened blunt end of the duplex might alter the distance between active site of Fe(II)·BLM·O_2 and C-4' hydrogen to result in an exclusive terminal cytosine release. A similar mode of unusual binding of actinomycin D to non GC site has been reported recently.[18]
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19) This work was supported by Grant-in-Aid for Priority Research from Ministry of Education and the Naito Foundation. We are grateful to Nippon Kayaku Co., Ltd. for providing PEM.

(Received in Japan 17 October 1989)