

Evidence for C-1' Hydrogen Abstraction from Modified Oligonucleotides by Fe-Bleomycin

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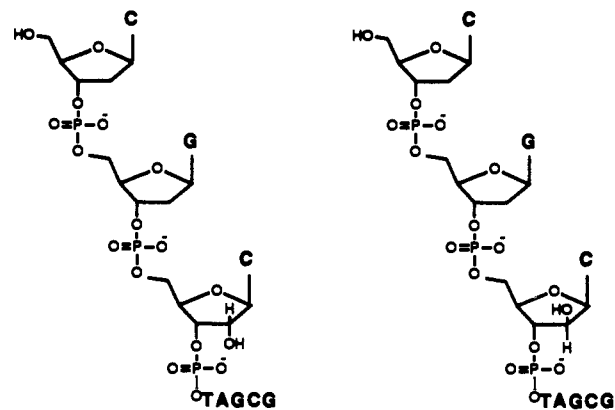
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The bleomycins (BLMs) are antitumor antibiotics employed clinically for the treatment of several malignancies.¹ Fe(II)·BLM can mediate oxidative degradation of DNA² and RNA³ in a sequence selective fashion; it seems likely that damage of one or both of these macromolecules contributes importantly to the observed antitumor effects of the bleomycins.^{2,4}

Oxidative destruction of both DNA^{2,5} and RNA^{3b,d} by activated Fe·BLM, which is believed to be a high-valent metal-oxo complex,² involves abstraction of H from C-4' of (deoxy)ribose. Analogous abstraction of H from C-1' has been postulated for DNA,⁶ and later for the DNA strand of an RNA-DNA heteroduplex.⁷ While reasonable chemically, no evidence has been obtained for DNA degradation by this pathway. Recently, Absalon et al.⁸ have demonstrated convincingly that degradation of the DNA strand of an RNA-DNA heteroduplex formed from two homopolymers does not involve abstraction of C-1' H. Presently, we describe the degradation of two self-complementary oligodeoxynucleotides (Figure 1) containing a single ribo or ara nucleoside at a position susceptible to oxidative degradation by Fe(II)·BLM.^{3b,9,10} In contrast to all results obtained to date with other substrates, these modified octanucleotides were found to afford products that can be envisioned as resulting from abstraction of C-1' H, in addition to those produced from abstraction of C-4' H.

The oligonucleotides d(CGCT₃A₃GCG)⁹ and d(GCG-TAGCG)¹⁰ are efficient, site-selective substrates for Fe·BLM. As anticipated, the products resulted predominantly from ox-

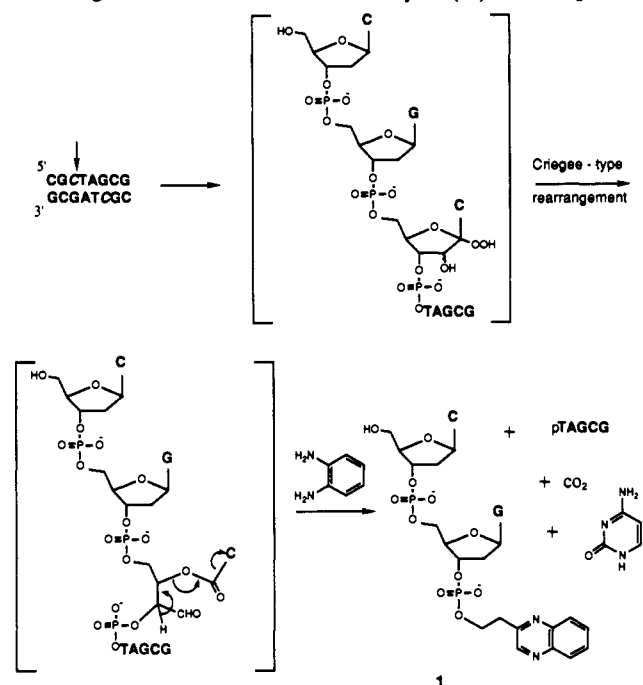


C₃-ribo CGCTAGCG

C₃-ara CGCTAGCG

Figure 1. Structures of two self-complementary oligonucleotides employed as substrates for Fe(II)·BLM A₂.

Scheme I. Products Formed from Substrate Octanucleotides following Putative Oxidation at C-1' by Fe(II)·BLM A₂



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idative destruction of the sugar moieties in cytidine-3 and cytidine-7; these included 2'-deoxycytidylyl(3'→5')(2'-deoxyguanosine 3'-(phosphoro-2''-O-glycolate)) (CpGpCH₂COOH), which presumably formed by initial abstraction of C-4' H from cytidine.^{2,5,9} The same product was obtained upon treatment of C₃-ribo and C₃-ara CGCTAGCG and constitutes one line of evidence for the nature of the chemical products formed from RNA-containing oligonucleotides by Fe·BLM.^{3b} In the belief that these modified oligonucleotides might possess altered conformations at cytidine-3 conducive to Fe·BLM-mediated abstraction of C-1' H,¹¹ the reaction mixtures were analyzed for products that might result from this initial event.

(11) As noted previously,⁹ opening of the minor groove to give a duplex having more A-form character leads to greater accessibility in the minor groove of C-1' H as compared with C-4' H. In fact, substitution of ara-C for dC is known to destabilize local B-form conformation (Gao, Y.-G.; van der Marel, G. A.; van Boom, J. H.; Wang, A. H.-J. *Biochemistry* **1991**, *30*, 9922); alteration of conformation has been noted specifically for the ara-C-containing octanucleotide studied here (Pieters, J. M. L.; de Vroom, E.; van der Marel, G. A.; van Boom, J. H.; Koning, T. M. G.; Kaptein, R.; Altona, C. *Biochemistry* **1990**, *29*, 788).

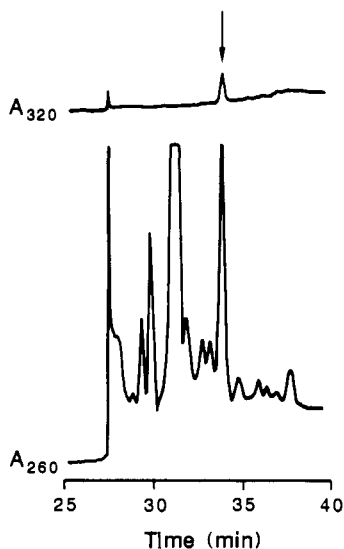


Figure 2. HPLC analysis of Fe(II)-BLM-treated C₃-ara CGCTAGCG, following additional incubation with 1,2-diaminobenzene. Separation was achieved on an Alltech Lichrosorb C₁₈ column (10 μM); elution was with aqueous 0.1 M NH₄OAc containing increasing amounts of CH₃CN (0–20 min, 0.1 M NH₄OAc; 20–22 min, linear gradient, 0 → 6% CH₃CN in 0.1 M NH₄OAc; 22–32 min, 6 → 15% CH₃CN in 0.1 M NH₄OAc; 32–50 min, 15% CH₃CN) at a flow rate of 2 mL/min. The eluate was monitored at 260 and 320 nm; authentic dinucleotide **1** coeluted with the peak at 33.9 min (see arrow). The small A₃₂₀ peak at 27.5 min was an artifact due to the gradient maker.

As outlined in Scheme I, initial formation of a C-1' ribose radical might be expected to lead to the formation of a C-1' hydroperoxynucleotide intermediate, in analogy with transformations noted for the putative C-4' radical.^{2,5,9} As illustrated, Criegee rearrangement of the C-1' hydroperoxide would not lead directly to strand scission; accordingly, 1,2-diaminobenzene was added to effect conversion of the envisioned intermediate to 2'-deoxycytidylyl(3'→5')(2'-deoxyguanosine 3'-(phospho-2''-O-(hydroxyethyl)-2-quinoline) (**1**).

Following treatment of C₃-ara CGCTAGCG with Fe(II)-BLM A₂ in the presence of 1,2-diaminobenzene,¹² the reaction mixture was analyzed by reversed-phase HPLC (Figure 2). An authentic, synthetic sample of dinucleotide **1**¹³ was found to have an absorption maximum at 320 nm (ϵ 5900) in addition to the

(12) Reaction mixtures (50-μL total volume) contained 250 μM octanucleotide (2 mM nucleotide concentration), 0.4 mM BLM A₂, and 0.8 mM Fe^{II}(NH₄)₂(SO₄)₂ in 100 mM sodium cacodylate, pH 7.0. Reactions were initiated by Fe²⁺ addition and incubated (O₂ bubbling) at 0 °C for 30 min. Following treatment with 25 μL of 1:1 EtOH-H₂O containing 0.5 M 1,2-diaminobenzene, the combined solution was heated at 55 °C for 30 min. The cooled solution was extracted with ether and concentrated prior to HPLC analysis.

characteristic absorption at shorter wavelength, so the HPLC eluate was monitored at both 260 and 320 nm. As shown in Figure 2, a product absorbing at 260 and 320 nm eluted at 33.9 min; this material had the same UV spectrum as authentic **1**. Repetition of the experiment using [¹⁴C]-1,2-diaminobenzene (14.7 Ci/mol) afforded a peak containing radioactive material coincident with the A₃₂₀ peak.¹⁴ The same results were obtained when C₃-ribo CGCTAGCG was treated with Fe(II)-BLM A₂ and then with 1,2-diaminobenzene, although significantly less **1** was obtained.

Quantification of products formed from d(CGCTAGCG), C₃-ara CGCTAGCG, and C₃-ribo CGCTAGCG was carried out by HPLC analysis using authentic synthetic standards having known response factors.¹⁴ Treatment of 250 μM oligonucleotide with 400 μM Fe(II)-BLM A₂ gave comparable amounts of products (114–120 μM) in each case, although the ratio of products derived from cytidine-3 and cytidine-7 varied substantially.¹⁵ For C₃-ara CGCTAGCG, dinucleotide **1** constituted 58% of the products derived from degradation of cytidine-3; the comparable figure for C₃-ribo CGCTAGCG was ~10%.

The detection of dinucleotide **1** in the foregoing experiments provides strong suggestive evidence for the degradation of C₃-ribo and C₃-ara CGCTAGCG via a pathway involving initial abstraction of C-1' H from the susceptible sugar moiety of each oligonucleotide.¹⁷ Thus, in common with other DNA-cleaving agents whose mode of DNA degradation can change according to the substrate employed,¹⁸ Fe-BLM would seem to be capable of abstracting either C-4' H or C-1' H from appropriate substrate oligonucleotides.

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(13) The synthesis of dinucleotide **1** was carried out in analogy with the synthesis of CpGpCH₂COOH (Murugesan, N.; Xu, C.; Ehrenfeld, G. M.; Sugiyama, H.; Kilkuskie, R. E.; Rodriguez, L. O.; Chang, L.-H.; Hecht, S. M. *Biochemistry* **1985**, *24*, 5735); 2-(2-hydroxyethyl)quinoline was prepared as described (Elina, A. S.; Tsyrl'nikova, L. G. *Zh. Obshch. Khim.* **1964**, *34*, 2077). Characterization of dinucleotide **1** included analysis of its UV, ¹H-NMR, and FAB mass spectra.

(14) Dinucleotide **1** was also detected (30.2-min elution time) on an Alltech Econosphere C₁₈ column (3 μM); elution was with 0.1 M NH₄OAc, pH 6.8; flow rate 1.4 mL/min. This column was employed for quantification of key products.⁹

(15) The ratio of cleavage at the two GC sites has been shown to change as the structure of the BLM congener^{9a} or oligonucleotide substrate¹⁶ was varied.

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(17) Although less likely, we cannot presently exclude the possibility that dinucleotide **1** arose from an oxidative process involving C-2' of the sugar moiety of cytidine-3.

(18) See, e.g.: (a) Goldberg, I. H. *Acc. Chem. Res.* **1991**, *24*, 191. (b) Kappen, L. S.; Goldberg, I. H. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 6706.