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Site-specific cleavage of RNA by Fe(II)·bleomycin

(antitumor antibiotic/oxidative degradation/mechanism of action/human immunodeficiency virus reverse transcriptase mRNA/tRNA precursors)

BARBARA J. CARTER*, ERIK DE VROOM*, ERIC C. LONG*, GIJS A. VAN DER MAREL†, JACQUES H. VAN BOOM†, AND SIDNEY M. HECHT*‡

*Departments of Chemistry and Biology, University of Virginia, Charlottesville, VA 22901; and †Department of Organic Chemistry, University of Leiden, Leiden, The Netherlands

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ABSTRACT **Bleomycin is an antitumor agent whose activity has long been thought to derive from its ability to degrade DNA. Recent findings suggest that cellular RNA may be a therapeutically relevant locus. At micromolar concentrations, Fe(II)·bleomycin readily cleaved a *Bacillus subtilis* $tRNA^{His}$ precursor in a highly selective fashion, but *Escherichia coli* $tRNA^{Tyr}$ precursor was largely unaffected even under more forcing conditions. Other substrates included an RNA transcript encoding a large segment of the reverse transcriptase from human immunodeficiency virus 1. RNA cleavage was oxidative, ≈10-fold more selective than DNA cleavage, and largely unaffected by nonsubstrate RNAs. RNA sequence analysis suggested recognition of RNA tertiary structure, rather than recognition of specific sequences; subsets of nucleotides at the junction of single- and double-stranded regions were especially susceptible to cleavage. The ready accessibility of cellular RNAs to xenobiotic agents, the high selectivity of bleomycin action on RNAs, and the paucity of mechanisms for RNA repair suggest that RNA may be a therapeutically relevant target for bleomycin.**

The bleomycins (BLMs) are antitumor antibiotics believed to function by DNA degradation (1, 2). DNA degradation is sequence-selective, occurring primarily at a subset of all 5′-GT-3′ and 5′-GC-3′ sequences (3, 4). A wealth of information exists concerning the mechanism of BLM-mediated DNA degradation in cell-free (1, 2, 5) and cellular (5, 6) systems.

In contrast, few studies have dealt with RNA as a substrate for BLM. Further, in spite of the compartmentalization of DNA and RNA in nucleated cells, most studies of RNA cleavage have been carried out with DNA present. For example, BLM degraded only the poly(dT) strand of a poly(rA)·poly(dT) hybrid (7, 8). Hori (9) showed that relaxation of simian virus 40 form I DNA was hardly affected by even a large excess of *Escherichia coli* tRNA. Those experiments that employed RNA alone were carried out in the absence of added metal ions (10, 11). Magliozzo *et al.* (12) have reported degradation of yeast $tRNA^{Phe}$ by high concentrations of Fe(II)·BLM; product bands corresponding to <5–10% of the starting tRNA were observed by methylene blue staining of a polyacrylamide gel. Thin layer chromatographic analysis indicated products that comigrated with adenine and uracil.

Although none of the foregoing studies suggested that RNA might constitute an efficient substrate for BLM, ongoing studies in this laboratory of BLM-mediated DNA oligonucleotide degradation have demonstrated that alteration of DNA conformation can significantly change the pattern and extent of cleavage normally obtained with B-form DNA (13, 14). On the chance that the limited RNA cleavage observed (*vide supra*) might actually have resulted from highly efficient cleavage at a few conformationally unique sites, we studied several RNAs as substrates. Presently, we show that Fe(II)·BLM does mediate RNA degradation and that the process is highly site-selective.

MATERIALS AND METHODS

Materials. Restriction enzymes *Bam*HI and *Sau*96I were purchased from BRL, as was pBR322 DNA. *E. coli* SP6 RNA polymerase, RNasin RNase inhibitor, *Eco*RI, and *Pvu* II were from Promega. Calf thymus DNA was purchased from Sigma. Calf intestinal phosphatase was obtained from Boehringer Mannheim; T4 polynucleotide kinase was from United States Biochemical. *Sca* I was purchased from Stratagene. Nensorb-20 cartridges were purchased from DuPont/New England Nuclear; [γ-^{32}P]ATP (≈7000 Ci/mmol; 1 Ci = 37 GBq) was from ICN. A pSP64 plasmid, encoding *Bacillus subtilis* $tRNA^{His}$ precursor, was obtained from Barbara Vold (SRI International, Menlo Park, CA); plasmid pGem-1/HB3, encoding the *E. coli* $tRNA^{Tyr}$ precursor, was provided by Sidney Altman (Yale University). A pGEM plasmid containing the gene for human immunodeficiency virus 1 (HIV-1) reverse transcriptase under the control of an SP6 promoter was obtained from Christine Debouck (Smith Kline-Beecham Pharmaceuticals). All plasmids were isolated from *E. coli* as described (15); RNA and DNA concentrations are expressed as nucleotide concentrations.

RNA Substrates Prepared by *in Vitro* Transcription. The transcription reactions were carried out in 20 μl (total volume) of 40 mM Tris·HCl (pH 7.7) containing 6 mM $MgCl_2$, 2 mM spermidine, 10 mM NaCl, 10 mM dithiothreitol, 500 μM ATP, 500 μM GTP, 500 μM UTP, 500 μM CTP, 0.8 μl (30 units) of RNasin, 1 μg of linearized DNA template, and 15 units of SP6 RNA polymerase. The reactions were incubated at 37°C for 1 hr and the transcripts were purified by Nensorb column chromatography (16). The purified transcripts were dephosphorylated (calf intestinal phosphatase) and then 5′-^{32}P-end labeled (T4 polynucleotide kinase and [γ-^{32}P]ATP), as described (16).

Cleavage of RNA Transcripts by Fe(II)·BLM. Reaction mixtures (5 μl, total volume) contained 12 pmol (≈2 × 10^4 cpm) of [5′-^{32}P]$tRNA^{His}$ precursor, 5 mM NaH_2PO_4 (pH 7.0), and 3 or 30 μM BLM A_2. The reactions were initiated by the addition of Fe(II) (freshly prepared solutions) in three equal portions (0, 20, and 40 min) to a final concentration equimolar with BLM A_2. After 1 hr at 22°C, the reaction mixtures were analyzed by PAGE (20% gels).

For the experiments containing RNA and DNA, a 5′-^{32}P-end-labeled 149-base-pair DNA restriction fragment was prepared from *Bam*HI-linearized pBR322 DNA. The DNA was 5′-end-labeled, digested with *Sau*96I, and purified by

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Abbreviations: BLM, bleomycin; HIV, human immunodeficiency virus.

‡To whom reprint requests should be addressed.

PAGE. Reaction mixtures (5 μl) contained 12 pmol (≈2 × 10^4 cpm) of 5′-^{32}P-end-labeled $tRNA^{His}$ precursor, and 3 mM unfractionated *E. coli* tRNA or 3–5 pmol (≈2 × 10^4 cpm) of 5′-^{32}P-end-labeled DNA restriction fragment, and 3 mM sonicated calf thymus DNA, 5 mM NaH_2PO_4 (pH 7.0), and 3, 30, or 300 μM Fe(II)·BLM A_2. The reactions were initiated by the addition of Fe(II) (at 0, 20, and 40 min) to a final concentration equimolar with BLM A_2. The reactions were incubated at 22°C for 1 hr and then analyzed by PAGE (20% gels, 2200 V, 6 hr).

The experiment shown in Fig. 4 was carried out using a 231-nucleotide RNA transcript. Reaction mixtures (5 μl) contained 18 pmol (≈4 × 10^4 cpm) of the radiolabeled RNA, 5 mM NaH_2PO_4 (pH 7.0), and 25 μM Fe(II)·BLM A_2. The reaction was initiated by simultaneous addition of freshly prepared solutions of BLM A_2 and Fe(II). After 10 min at 22°C, the reactions were quenched and analyzed by PAGE (20% gel).

Degradation of Synthetic Octanucleotides. Reaction mixtures (50 μl) contained oligonucleotide (1.0 mM, final concentration), 50 mM sodium cacodylate (pH 7.2), and 0.2 mM Fe(II)·BLM A_2. The reactions were incubated at 0°C for 1 hr and then analyzed by C_{18} reverse-phase HPLC (17).

RESULTS AND DISCUSSION

Site-Selective Cleavage of a tRNA Precursor. As shown in Fig. 1 for BLM A_2, treatment of a 5′-^{32}P-end-labeled $tRNA^{His}$ precursor with 3 μM Fe(II)·BLM A_2 or Fe(II)·BLM B_2 effected substantial cleavage of the RNA. A single major site was cleaved in this 118-nucleotide transcript under conditions quite comparable to those utilized for the cleavage of ^{32}P-end-labeled DNA by Fe(II)·BLM (3, 4, 13). Remarkably, 5′-^{32}P-end-labeled $tRNA^{Tyr}$ precursor was largely unaffected under the same conditions, and a generally ^{32}P-labeled $tRNA^{Tyr}$ precursor transcript was not degraded significantly even at much greater ratios of Fe(II)·BLM A_2 to RNA nucleotide (data not shown)! Selective cleavage of $tRNA^{His}$ precursor was also observed in a reaction mixture that contained both $tRNA^{His}$ and $tRNA^{Tyr}$ precursors. Relative to BLM-mediated DNA degradation (1–4), RNA cleavage occurred at comparable drug concentrations but with much greater selectivity.

The selectivity of BLM-mediated RNA cleavage was explored by repeating the degradation of ^{32}P-labeled $tRNA^{His}$ precursor in the presence of a large (>10^3 molar) excess of *E. coli* tRNA. Even in the presence of excess unfractionated tRNA, the $tRNA^{His}$ precursor was cleaved efficiently by 300 μM and detectably by 30 μM Fe(II)·BLM A_2 (Fig. 2, lanes 5 and 6). Thus the ability of unfractionated *E. coli* tRNA to diminish the cleavage of $tRNA^{His}$ precursor was not nearly in proportion to its abundance in the incubation mixture. In comparison, when a 5′-^{32}P-end-labeled 149-base-pair DNA fragment was diluted similarly with calf thymus DNA, little

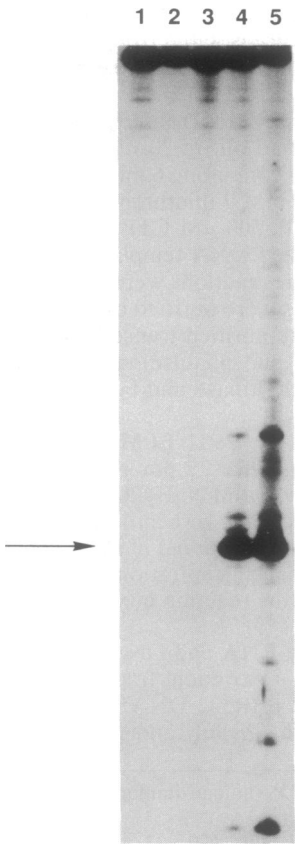

FIG. 1. Fe(II)·BLM-promoted cleavage of *B. subtilis* precursor $tRNA^{His}$. An *in vitro* RNA transcript produced from an *Eco*RI-linearized pSP64 plasmid, and having the sequence shown in Fig. 3, was 5′-^{32}P-end labeled and then treated with Fe(II)·BLM A_2. Lanes: 4 and 5, 3 and 30 μM Fe(II)·BLM A_2, respectively; 1, RNA transcript alone; 2, 300 μM BLM A_2; 3, contained 300 μM Fe(II). Arrow, site of cleavage.

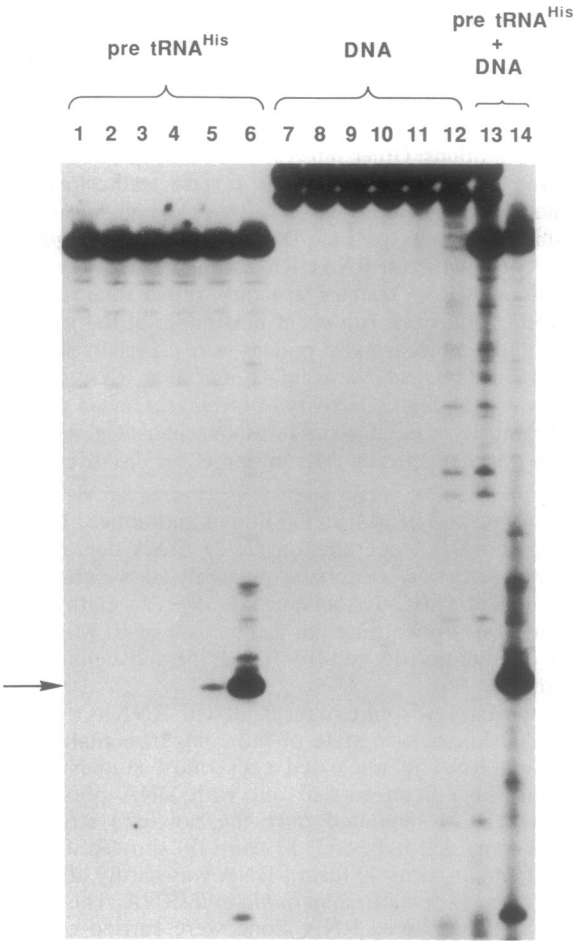

FIG. 2. Comparison of Fe(II)·BLM-mediated cleavage of $tRNA^{His}$ precursor and DNA. Reaction mixtures contained 12 pmol (RNA nucleotide) of 5′-^{32}P-end-labeled $tRNA^{His}$ precursor, 3 mM unfractionated *E. coli* tRNA (lanes 1–6) or 3–5 pmol of 5′-^{32}P-end-labeled DNA restriction fragment, 3 mM sonicated calf thymus DNA (lanes 7–12), 5 mM NaH_2PO_4 (pH 7.0), and 3, 30, or 300 μM Fe(II)·BLM A_2. Lanes 1–6 contained 3 mM unfractionated *E. coli* tRNA and $tRNA^{His}$ precursor as follows. Lanes: 1, $tRNA^{His}$ alone; 2, plus 300 μM BLM A_2; 3, plus 300 μM Fe(II); 4, plus 3 μM Fe(II)·BLM A_2; 5, plus 30 μM Fe(II)·BLM A_2; 6, plus 300 μM Fe(II)·BLM A_2. Lanes 7–12 contained 3 mM sonicated calf thymus DNA and a 149-base-pair DNA restriction fragment as follows. Lanes: 7, DNA alone; 8, plus 300 μM BLM A_2; 9 plus 300 μM Fe(II); 10, plus 3 μM Fe(II)·BLM A_2; 11, plus 30 μM Fe(II)·BLM A_2; 12, plus 300 μM Fe(II)·BLM A_2. Lane 13 contained 12 pmol of radiolabeled $tRNA^{His}$ precursor, ≈4 pmol of radiolabeled DNA restriction fragment, 3 mM calf thymus DNA, and 300 μM Fe(II)·BLM A_2. Lane 14 contained 12 pmol of radiolabeled $tRNA^{His}$ precursor, ≈4 pmol of radiolabeled DNA restriction fragment, and 300 μM Fe(II)·BLM A_2.

cleavage was apparent even by 300 μM Fe(II)·BLM A_2 (Fig. 2, lanes 7–12). The effect of DNA on Fe(II)·BLM-mediated RNA cleavage was studied by incubating equimolar mixtures of the labeled RNA and DNA with Fe(II)·BLM either in the presence (lane 13) or absence (lane 14) of excess DNA. As shown, in the absence of calf thymus DNA, 300 μM Fe(II)·BLM A_2 effected significant cleavage of $tRNA^{His}$ and essentially complete digestion of the labeled DNA substrate. Upon admixture of 3 mM calf thymus DNA, there was very little degradation of either radiolabeled substrate. The effect of DNA on RNA degradation was assessed further by admixture of 10 μM Fe(II)·BLM and $tRNA^{His}$ precursor (270 μM nucleotide concentration) with various amounts of DNA; essentially complete inhibition of RNA degradation was noted when calf thymus DNA or d(CGCTTTAAAGCG) were present at 50 μM (DNA nucleotide) concentration but not at much lower concentrations.

The results suggest that BLM-mediated degradation is generally more efficient for DNA than for RNA but that some RNA sites undergo highly efficient strand scission that is little affected by nonsubstrate RNAs. Accordingly, it seems logical to anticipate that little RNA cleavage would occur in the presence of a large excess of DNA but that populations of RNA molecules relatively isolated from DNA (e.g., in the cytoplasm of nucleated cells) might undergo selective cleavage.

Characterization of Fe·BLM Cleavage Sites in RNAs. The position of $tRNA^{His}$ precursor cleavage by Fe·BLM was established by RNA sequence analysis (18). As shown (Fig. 3), the predominant site of cleavage was found to be uridine-35, located adjacent to guanosine in a single-stranded region at the base of the "acceptor stem." Whereas cleavage at this 5′-GU-3′ is formally analogous to the cleavage of DNA at 5′-GC-3′ and 5′-GT-3′ sequences, the preference for cleavage within a single-stranded region is unprecedented (see, however ref. 19). Further, six other 5′-GU-3′ sequences in the tRNA precursor were cleaved weakly or not at all; the minor sites actually observed (Fig. 3) included all four bases and reflected no obvious sequence selectivity.

Also instructive is a comparison of the secondary structure of *B. subtilis* $tRNA^{His}$ precursor with that of *E. coli* $tRNA^{Tyr}$ precursor. Although the $tRNA^{Tyr}$ precursor was not a good substrate for cleavage by Fe(II)·BLM, this tRNA precursor contains a 5′-GU-3′ sequence analogous to the one cleaved in $tRNA^{His}$ precursor (Fig. 3). Clearly, the dramatic difference in susceptibilities of these two tRNA precursors to degradation by Fe(II)·BLM argues for selective recognition of the $tRNA^{His}$ precursor at the level of tertiary structure; in fact, an alternate conformation for $tRNA^{Tyr}$ precursor has been suggested (20). This would parallel the alteration of DNA sequence selectivity as DNA conformation was altered (13, 14, 21).

Several other RNA transcripts were tested as potential substrates. Among these was a 5′-^{32}P-end-labeled 231-nucleotide RNA transcript produced from pSP64 plasmid DNA. As shown in Fig. 4, treatment with 25 μM Fe(II)·BLM A_2 afforded cleavage at two major sites. One of these was also at the uridine-(−93) in a 5′-GU-3′ sequence; although RNA folding analyses (22) cannot be considered definitive, the extensive complementarity involved in the present case suggests that this site can be represented reasonably as the last nucleotide within the double-stranded region of a stem–loop structure (Fig. 4). This structure is not unlike the major cleavage site in the $tRNA^{His}$ precursor. The second cleavage occurred at guanosine-122, which is part of a 5′-CG-3′ sequence in which the cytidine is in a single-stranded region and the guanosine is the first of several nucleotides in a double-stranded region. Cleavage of this RNA occurred at Fe(II)·BLM to RNA nucleotide ratios comparable to those required for degradation of $tRNA^{His}$ precursor.

Susceptibility of Viral RNA to Fe·BLM. The present results challenge the assumption that DNA is the biochemical locus at which BLM mediates its therapeutic effects; it seemed logical to study the possible involvement of RNA by the use of an organism in which the genetic material consists of RNA. Accordingly, we have studied the effect of Fe(II)·BLM on the mRNA that encodes HIV-1 reverse transcriptase. A 270-nucleotide 5′-^{32}P-end-labeled RNA encoding the N terminus of HIV reverse transcriptase was obtained by transcription from a (*Sca* I linearized) pGEM plasmid. Treatment with Fe(II)·BLM at a BLM to RNA nucleotide ratio similar to that used for $tRNA^{His}$ precursor effected cleavage at four sites,

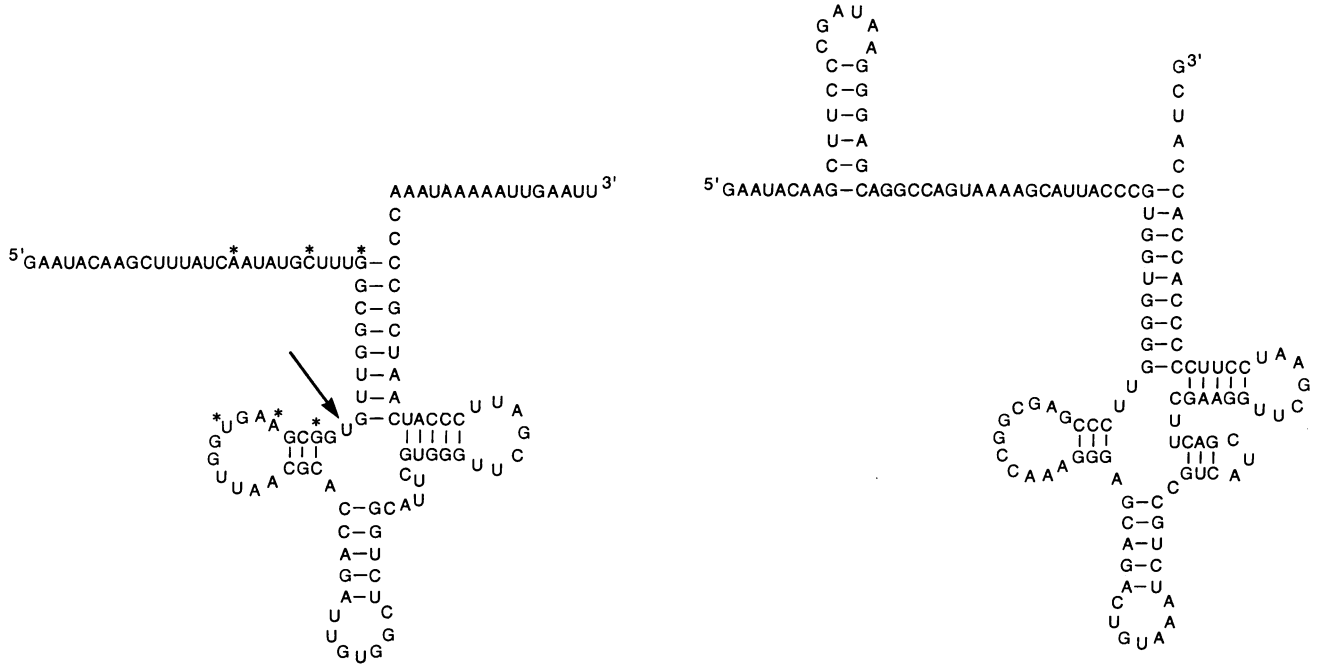

FIG. 3. Structures of *B. subtilis* $tRNA^{His}$ precursor (*Left*) and *E. coli* $tRNA^{Tyr}$ precursor (*Right*). The arrow denotes the major site of Fe·BLM-mediated cleavage of the $tRNA^{His}$ precursor; the asterisks denote the minor sites.

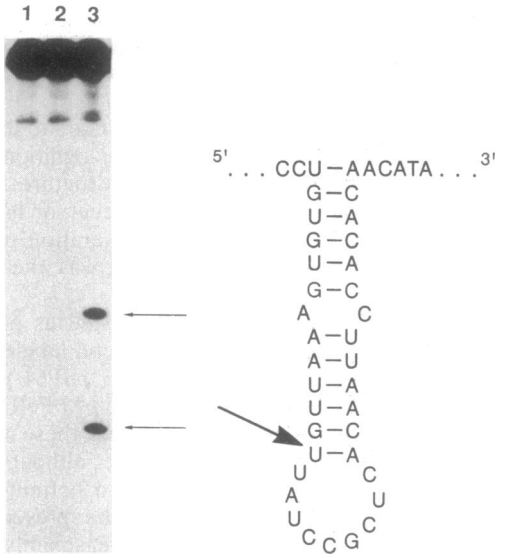

FIG. 4. Fe(II)·BLM-promoted cleavage of an *E. coli* RNA transcript. (*Left*) A 231-nucleotide RNA, obtained by *in vitro* transcription from a *Pvu* II-linearized pSP64 DNA plasmid, was 5'-^{32}P-end-labeled and then treated with Fe(II)·BLM A_2. Lanes: 1, RNA alone; 2, 25 μM Fe(II); 3, 25 μM Fe(II)·BLM A_2. (*Right*) A possible secondary structure for one BLM cleavage site. The arrow indicates the cleavage site, which occurs at uridine-93 of the RNA transcript.

two of which were strong cleavage sites (data not shown). The observation that this RNA undergoes facile Fe(II)·BLM-mediated cleavage is of substantial importance as it provides a vehicle for studying the effects of BLM on an organism whose viability depends critically on maintenance of RNA structure and function. It should be noted that this particular RNA target could be especially favorable, since any HIV reverse transcriptase actually produced in spite of the presence of BLM would elaborate a (DNA) product also potentially susceptible to BLM.

Recognition of Polynucleotide Conformation by Fe·BLM. The data from several RNA substrates suggest that RNA cleavage by Fe(II)·BLM is at least 10-fold more selective than DNA cleavage. Studies of DNA cleavage have demonstrated that Fe(II)·BLM produces lesions with the greatest efficiency at a subset of all 5'-GC-3' and 5'-GT-3' sites, although it has become increasingly clear that BLM is also responsive to DNA conformational alterations (13, 14, 19, 21, 23). Recognition of B-form DNA by Fe(II)·BLM primarily involves recognition of two adjacent bases and is optimal for 2 of the 10 possible distinguishable duplex sequences for a 2-base binding site (24). For RNA, the present data do not indicate a preferred 2-base recognition sequence, although the cleavage of 5'-GU-3' sequences having appropriate conformations is clearly favorable, and many RNA nicks occur at or in proximity to guanosine residues. It seems unlikely that recognition of RNA by Fe(II)·BLM involves a recognition sequence of different length than for DNA.

For RNA, the characterized lesions all reflect damage at what can be represented as junctions between single- and double-stranded regions. Given the lack of any obvious recognition sequence for BLM in RNA and the extraordinary selectivity of RNA cleavage, it seems highly likely that RNA conformation is the primary determinant of the position(s) of RNA degradation.

Fe(II)·BLM-mediated DNA cleavage has long been known to proceed with high efficiency at a few sites that are a subset of those utilized under more forcing conditions (e.g., refs. 23 and 25). It may be that these sites reflect microheterogeneity of DNA structure that produces sites highly susceptible to cleavage by Fe(II)·BLM. Clearly, if tertiary interactions in RNA and DNA do constitute the source of highly selective cleavage, the structural characterization of such sites would permit the subsequent use of BLM as a tool for analysis of polynucleotide tertiary structure.

Chemistry of RNA Nucleotide Cleavage by Fe·BLM. The foregoing assignment of the position of BLM-mediated cleavage rests on the assumption that the chemistry of RNA strand scission by Fe(II)·BLM is mechanistically analogous to that of DNA; accordingly, this has been studied directly in several ways.

BLM-mediated DNA degradation is an oxidative process that requires oxygen and any one of several redox-active metals ions; it is greatly facilitated by reducing agents. The products of DNA degradation include free bases and base propenals (1, 2). We found that the degradation of $tRNA^{His}$ precursor also proceeded readily in the presence of Fe(II)·BLM but not in the presence of Fe(III)·BLM and was enhanced substantially by ascorbate. Although RNA degradation in a fashion analogous to that of DNA would not be expected to produce base propenals, due to differences in the sugar moieties of RNA and DNA, Magliozzo *et al.* (12) reported that adenine and uracil were produced from tRNA. To confirm that the $tRNA^{His}$ precursor was degraded oxidatively at uridine-35, we prepared a $tRNA^{His}$ precursor transcript containing [^{3}H]uridines labeled within the pyrimidine moiety. Treatment with Fe(II)·BLM produced cleavage at the site believed to be uridine-35; free [^{3}H]uracil, identified by HPLC analysis, was obtained in amounts consistent with the extent of degradation produced at position 35 (data not shown).

Products have been characterized after the degradation of DNA (26, 27) and of short DNA oligonucleotides having limited numbers of sites degraded efficiently by Fe·BLM (17). The latter substrates have proven particularly useful because degradation can occur near the end of a DNA duplex, affording relatively small products amenable to precise chemical analysis. As shown in Fig. 5, for example, d(CGCTAG CG) undergoes strand scission at cytidine-3, resulting in the formation of three products: (*i*) a dinucleotide (Structure **1**) having a 3'-(phosphoro-2''-*O*-glycolate) terminus, (*ii*) cytosine propenal (Structure **2**), and (*iii*) a pentanucleotide (Structure **3**) containing a 5' phosphate. Also formed (data not shown) is a second set of products consisting of free cytosine and an alkali-labile lesion; these are believed to derive from an initially formed C-4' hydroxynucleotide intermediate (28, 29).

Because none of the strong RNA cleavage sites characterized thus far is close to the end of an RNA substrate, none could be used for the type of product analysis illustrated above for DNA. Therefore, to determine whether Fe·BLM could degrade an RNA nucleotide oxidatively, we employed

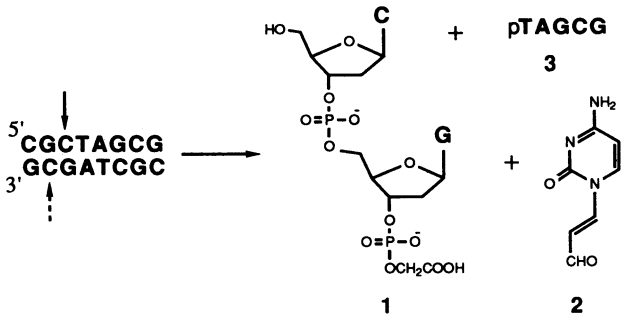

FIG. 5. Strand-scission products formed from deoxycytidine-3 of the self-complementary oligonucleotide d(CGCTAGCG) upon treatment with Fe(II)·BLM plus O_2. The broken arrow indicates the position of another major cleavage site, which afforded the same type of oxidative products (17).

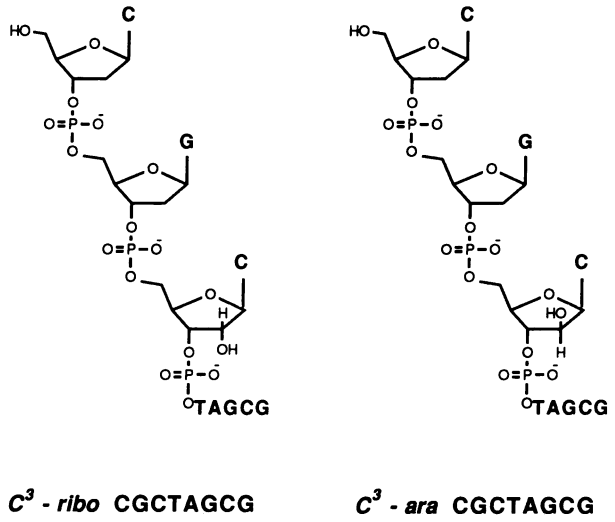

FIG. 6. Two self-complementary oligonucleotides structurally altered at deoxycytidine-3 that act as substrates for Fe·BLM.

a synthetic self-complementary deoxyoctanucleotide containing a single ribonucleotide constituent at the site at which Fe·BLM-mediated degradation was anticipated. The octanucleotide C^3-ribo 5′-CGCTAGCG-3′ (Fig. 6) was treated with Fe(II)·BLM A_2 under aerobic conditions and the products were analyzed by HPLC (17). Because BLM-mediated DNA strand scission has been shown to involve specific abstraction of the C-2′ α hydrogen from deoxyribose at the site of the lesion (28), a functionality that is absent in C^3-ribo 5′-CGCTAGCG-3′, we also prepared C^3-ara 5′-CGCTAGCG-3′ and studied its degradation by Fe(II)·BLM in comparison with that of d(CGCTAGCG). Each oligonucleotide was a substrate for cleavage by aerobically activated Fe(II)·BLM; each afforded substantial quantities of $CpGpCH_2COOH$ (Fig. 5, Structure 1), unequivocally demonstrating the oxidative degradation of nucleotide-3 regardless of the orientation of the C-2′ hydrogen. Also formed from each oligonucleotide was cytosine, consistent with the observation of free base formation from tRNA (12).

Implications. Although BLM is generally thought to exert its antitumor effects by DNA damage, a few observations suggest that DNA may not constitute the only relevant locus. These include the extraordinary levels of BLM-mediated chromatin damage required to render cultured mammalian cells nonviable (5) and the lack of correlation between growth inhibition and DNA damage for individual BLM congeners (6).

Given the exceptional selectivity for destruction of certain RNAs, even in the presence of much larger amounts of nonsubstrate RNAs, it seems possible that RNA is a therapeutic target for BLM. This thesis has the obvious virtues that (*i*) RNA is readily accessible within the cytoplasm, thus obviating the problem of delivery to the cell nucleus, (*ii*) while bound to proteins in many cases, cytoplasmic RNAs may nonetheless be more accessible than DNA, and (*iii*) the selectivity of RNA cleavage may well be sufficient to account for the observed therapeutic selectivity of BLM. In addition, although the cellular capacity for repair of double-strand DNA breaks is not clear, single-strand DNA damage can be repaired readily (5, 6, 30, 31). In contrast, BLM-mediated damage to (m)RNA would be irreversible, possibly leading to an insufficiency of critical proteins whose turnover is fast relative to mRNA production (32). The selective destruction of the mRNA for a protein required for progression of cells into mitosis (33, 34) could also account for the observed growth arrest of BLM-treated cells in late G_2 phase (5, 6, 30, 31). Alternatively, the inhibitory effects of BLM could be expressed by highly selective destruction of an RNA critical for cellular function (e.g., a rRNA or specific tRNA).

The foregoing thesis should be amenable to experimental inquiry, e.g., by monitoring the deletion of specific proteins after BLM treatment of cells. The present findings also suggest other possible uses for BLM, such as in the treatment of pathogens that utilize RNA as their template molecules.

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