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POLYNUCLEOTIDE RECOGNITION AND STRAND SCISSION BY FE·BLEOMYCIN

BARBARA J. CARTER, KALAKOTA S. REDDY
and SIDNEY M. HECHT*

Departments of Chemistry and Biology,
University of Virginia, Charlottesville,
VA 22901 USA

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Abstract - The structural basis of sequence-selective DNA recognition by Fe·bleomycin (Fe·BLM) has been studied by the use of BLM congeners in which the two functional domains are separated by spacers of variable length. Fe·bleomycin has also been shown to recognize and cleave RNA, which may constitute a new target of therapeutic importance for BLM.

The bleomycins (BLM's) are a group of glycopeptide-derived antitumor antibiotics, some of which are used clinically in the treatment of neoplasms, including malignant lymphomas and squamous cell carcinomas[1,2]. The chemotherapeutic effects of BLM are currently believed to result from the degradation of cellular DNA[3,4] in a sequence-selective reaction that is dependent on oxygen and which requires a metal ion, of which Fe(II) has been the most commonly studied. Bleomycin has been thought to contain two major functional domains: a metal binding region that is also responsible for binding and activation of O_2[5-7], and a C-terminus + bithiazole region that has been thought to be responsible for DNA binding (Fig. 1)[8-11]. The mechanism(s) employed by BLM for recognition of its substrate and the actual mode(s) of binding are not completely understood, although recent studies have provided some insights[3,4,12,13]. Although little definitive evidence exists concerning the orientation of metallobleomycins bound to the DNA helix, the fact that the chemistry of DNA degradation is initiated by abstraction of C-4'H of deoxyribose[14-16] suggests strongly that the metal binding domain is oriented in the minor groove of DNA[17,18].

Bleomycin-mediated DNA cleavage is sequence-selective, exhibiting a preference for oxidative degradation of the pyrimidine nucleotides in a subset of all 5'-GT-3' and 5'-GC-3' sequences, but the structural elements within the BLM molecule that control sequence selectivity have not been established definitively. It has been suggested[19] that the bithiazole region of bleomycin is responsible for the observed sequence selectivity of DNA interaction. Although a plausible molecular mechanism consistent with this suggestion has been provided on the basis of a molecular modeling study,[20] and would be a logical extension of the known role of the bithiazole

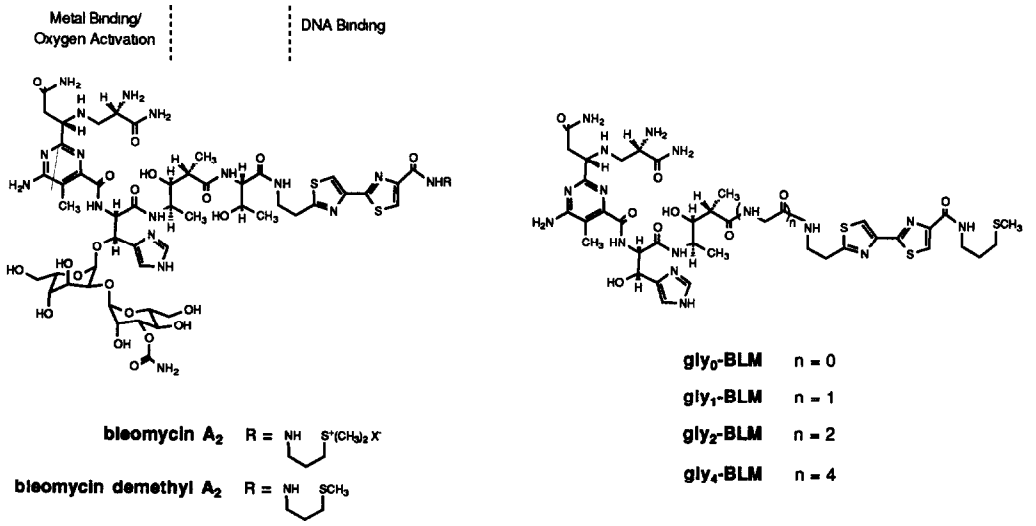


Fig. 1. Structures of bleomycin A_2, bleomycin demethyl A_2 and four bleomycin analogs (gly_X-BLM's) containing variable numbers of glycines connecting the metal binding and bithiazole regions of bleomycin.

domain in DNA binding[8-11], there is presently no definitive evidence that sequence selectivity is determined by the bithiazole domain. In fact, the structurally related phleomycin antibiotics, which differ from the bleomycins only within the bithiazole domain, exhibit the same sequence selectivity of DNA cleavage nonetheless[21].

Recent studies indicate that the metal binding domain of BLM plays an active role in DNA binding, and may participate in determining the sites of cleavage as well[12,13]. For example, a series of BLM congeners differing within the metal binding region, but having identical C-terminus + bithiazole regions, was found to exhibit different sequence selectivity of DNA cleavage[22], and different strand selectivity at a high efficiency DNA cleavage site[23]. Certain bleomycins have also been shown to unwind supercoiled plasmid DNA's effectively, but only if the metal binding domain is bound to Cu(II)[12].

To determine which structural domain in BLM is primarily responsible for determining the sequence selectivity of DNA cleavage, we designed a series of BLM analogs in which the two known functional domains (i.e., the metal binding and C-terminal DNA binding regions, see Figure 1) were separated by relatively rigid spacers of variable, but known lengths. Remarkably, for this series of analogs, the DNA degradation studies indicate that the metal binding region is the primary determinant of sequence selectivity.

Bleomycin has been shown to be capable of mediating the degradation of chromosomal DNA[24,25], which may logically be thought to produce the observed inhibition of cell growth and cell death, as well as the antitumor

effects of bleomycin[1,2]. However, definitive evidence for this is lacking and certain observations, such as the extraordinary concentrations of BLM required to kill cultured mammalian cells[24,25], suggest the involvement of other biochemical loci.

RNA represents another logical cellular target for bleomycin. Although previous studies have generally concluded that RNA is not a substrate for BLM, virtually all of these were carried out in the presence of both RNA and DNA. Recently, Magliozzo et al[26] reported the treatment of yeast $tRNA^{Phe}$ with high concentrations of Fe(II)·BLM; limited amounts of degradation were observed. To explore whether the limited RNA cleavage observed might actually have resulted from highly efficient and selective cleavage at a small number of sites, we have studied several RNA's as substrates for Fe(II)·BLM. Presently, we report that treatment of a $tRNA^{His}$ precursor with bleomycin resulted in highly efficient cleavage at one major site, to the near exclusion of all other sites.

RESULTS AND DISCUSSION

Sequence selectivity of DNA cleavage. Although there is considerable evidence indicating that bleomycin binds to DNA within the minor groove[17,18,20,27], a number of physicochemical experiments suggest that BLM may also be a (partial) DNA intercalating agent[4,9,28]. For example, by the use of 2D agarose gel electrophoresis it has recently been shown that certain Cu(II)·BLM's can unwind supercoiled plasmid DNA's[12]. The results are consistent with (partial) intercalation of the bithiazole moiety, but clearly also reflect a strong dependence on the ionic nature of the BLM congeners studied.

Not surprisingly, the DNA substrate itself can play a large role in the sites at which BLM interacts with DNA. It has been shown, for example, that platination of DNA substrates can cause major alterations in the sequence specificity of BLM-mediated cleavage, including the generation of new sites and the suppression of existing ones[29,30]. Also, the methylation of DNA at selected cytidines and adenosines by *Hha* I and *Hpa* II, or *Taq* I, respectively, resulted in diminished cleavage in proximity to the methylated sites[27]. Presumably, the methylation of selected cytidines and adenosines induced subtle conformational changes in the DNA substrate that were recognized by BLM. These results clearly indicated that site selectivity of bleomycin cleavage can be affected by alterations in the structure of the DNA substrate.

In view of the accumulating evidence that both functional domains of the bleomycin molecule (Fig. 1) participate in DNA binding (*vide supra*), it seemed reasonable to modify the structure of bleomycin systematically in an effort to define those structural elements primarily responsible for controlling sequence-specific DNA cleavage. Our strategy involved the synthesis of a series of BLM analogs each containing the same metal binding region and C-terminus + bithiazole region, but having these regions separated spatially by increasing (and known) distances. If the bithiazole region proved to be the primary determinant of sequence specificity, then each of the synthetic analogs should bind to DNA with this region at a common site; the metal binding region

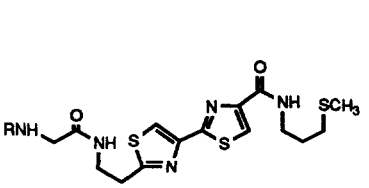
(which actually effects DNA cleavage[3,4,7]) would alter the observed site of cleavage as the distance between the two functional domains was varied. Alternatively, if the metal binding domain were primarily responsible for determining the site of DNA binding, then all of the analogs should bind and cleave DNA at the same site.

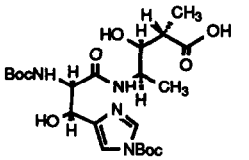
Implementation of the aforementioned strategy was achieved by preparation of four structural analogs of bleomycin. As shown in Fig. 1, these analogs employed (oligo)glycine "spacers" between the two functional domains of BLM in lieu of the threonine moiety normally present. Replacement of the threonine moiety seemed logical, due both to its position between the two domains of interest, and its apparent lack of any essential function. The (oligo)glycine spacer provided a structural element of the same chemical nature as the remainder of the peptide-derived antibiotic; because (oligo)glycines in peptides do not generally support helical structures[31], it was anticipated that this part of the BLM analogs would exist in extended conformations. To facilitate the synthetic work involved, the analogs chosen for preparation were all related structurally to deglycobleomycins[32]; although somewhat less potent than the respective bleomycins as DNA cleaving agents, the aglycones have been shown to degrade DNA with the same sequence selectivity as the bleomycins, and by the use of the same chemical transformations[3,4,23]. The syntheses of the deglycobleomycin analogs containing 0, 1, 2 or 4 glycines in lieu of threonine were carried out in analogy with the synthesis of deglycobleomycin itself[32]; the route employed for gly_2-BLM is outlined in Scheme I.

As shown in the Scheme, bithiazole derivative **1** (accessible in 62% yield by DCC-mediated coupling of *t*Boc-glycine and 2'-(2-aminoethyl)-2,4'-bithiazole-4-(3-thiopropyl)carboxamide)[32] was deblocked (CF_3COOH,$(CH_3)_2S$, 0°C, 80% yield) to afford glycine-linked aminoethylbithiazole **2**. A second glycine moiety was introduced in the same fashion (**3**, 60% yield), and was deblocked with trifluoroacetic acid/dimethylsulfide in 67% yield, affording diglycylaminoethylbithiazole derivative **4** as an amorphous powder. Compounds **4** and **5**[32] were then condensed (DCC,HOBt, DMF, 25°C) to afford intermediate **6**, which was isolated as a colorless glass in 25% yield. This intermediate was deblocked (CF_3COOH,$(CH_3)_2S$, 0°C, 70% yield) and then coupled with pyrimidoblamic acid (**8**) via the agency of DCC/HOBt in dimethylformamide. The crude product (**9**) was isolated in 90% yield following extractive work-up, and was then deblocked with CF_3COOH and dimethylsulfide at 0°C. The product was isolated by extractive work-up, and purified by chromatography on XAD-2 and then on CM-Sephadex C-25. Purified gly_2-BLM (**10**) was isolated as a colorless glass in 30% yield; the structure was confirmed by mass spectrometry (*m/z* 1046 (M+H)+) and ^{1}H-NMR spectroscopy.

As reported previously[13], the four gly_x-BLM were initially assayed for their ability to nick a supercoiled circular DNA in the presence of equimolar concentrations of Fe(II). At 50 μM concentrations, all four of the analogs produced DNA nicks with gly_1-BLM and gly_2-BLM producing breaks to the greatest extent.

The sequence specificity of DNA cleavage by the gly_x-BLM's was studied by using as a substrate a linear DNA duplex that had been 5'-^{32}P end labeled on one strand. Typically, BLM cleaves DNA at many sites in any





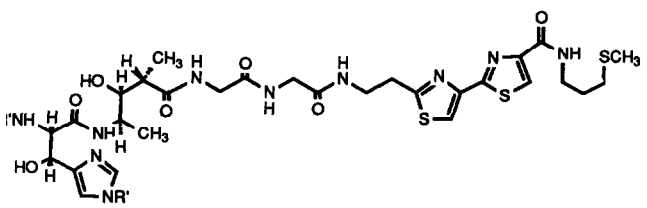
5

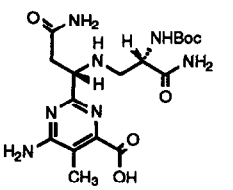
1 R = Boc

2 R = H

3 R = 

4 R = 

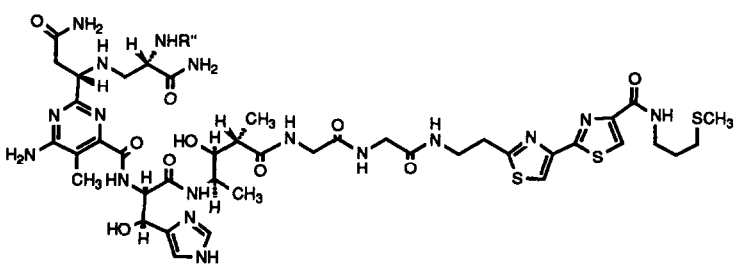




8

6 R' = Boc

7 R' = H



9 R" = Boc

10 R" = H

Scheme I. Synthetic route used for the preparation of gly_2-BLM.

given sequence. Therefore, to facilitate interpretation of the data, we intentionally chose DNA substrates having relatively isolated BLM cleavage sites. Two appropriate DNA fragments, 127 and 242 base pairs (bp) in length, respectively, were obtained by digestion of SV40 Form I DNA with restriction endonucleases *Bcl* I and *Eco* RII. As shown in Fig 2, treatment of the radiolabeled 127-bp duplex with Fe(II)·gly$_1$-BLM or Fe(II)·gly$_2$-BLM

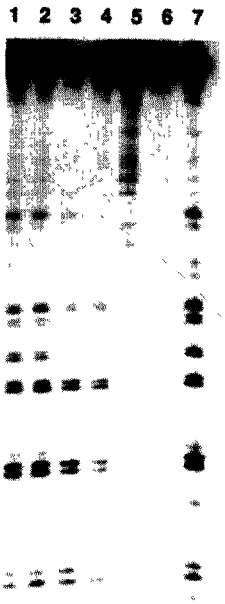


Fig. 2. Polyacrylamide gel analysis of gly$_x$-BLM analog-mediated cleavage of 5'-^{32}P-end labeled SV40 DNA-derived 127-bp restriction fragment. Lanes 1-4, 50 μM Fe(II) + 100 or 200 μM gly$_1$-BLM or gly$_2$-BLM, respectively; lane 5, DNA control: lane 6, 50 μM Fe(II); lane 7, 10 μM Fe(II)·deglyco BLM demethyl A$_2$. All reactions contained ~3 pmol of the DNA substrate and 50 mM Na cacodylate buffer, pH 7.5. This 20% gel resolved both the nucleoside 3'-phosphoroglycolate and 3'-phosphate cleavage products, which appear as doublets on the gel at the prominent cleavage sites.

afforded cleavage at precisely the same positions; the same results were also obtained using deglyco BLM demethyl A$_2$ (lane 7). The 20% sequencing gel used in this experiment permitted resolution of the cleaved products into doublets, indicating that both nucleoside 3'-phosphoroglycolates and nucleoside 3'-phosphates were produced in the cleavage reactions. These are the same products that result from Fe(II)·BLM A$_2$-mediated cleavage of DNA[3,4,33], indicating that the substitution of (oligo)glycine for the threonine normally present in BLM did not change the chemistry that bleomycin utilizes to degrade its DNA substrates.

In another series of experiments, samples of the 5'-^{32}P end labeled 242-bp duplex were incubated with the Fe(II) derivatives of each of the four gly$_x$-BLM analogs, and also with Fe(II)·BLM A$_2$ and Fe(II)·BLM demethyl A$_2$. Once again, each of the Fe(II)·BLM congeners effected DNA strand scission at precisely the same sites[13].

In the context of the strategy that led to the preparation of the gly$_x$-BLM's (*vide supra*), the observation

that all of the analogs cleaved DNA at the same sites is consistent with the interpretation that the metal binding region is dominant in determining the sequence selectivity of Fe·BLM. The observation also indicates that the C-terminus + bithiazole region is not primarily responsible for the sequence selectivity obtained here, although both domains are clearly required to achieve efficient degradation of the DNA duplex. The foregoing interpretation rests on the assumption that the (oligo)glycine moieties in gly_2-BLM and gly_4-BLM were in an extended conformation when these BLM congeners bound to the DNA substrate. The assumption seems reasonable on the basis of what is known about the conformational preferences of oligoglycine peptides[31]; it is also likely that the spacers must assume fairly rigid conformations to permit the metal binding and bithiazole regions to act cooperatively in promoting DNA binding (see, however, ref 34), since neither domain binds strongly to DNA by itself[7-11].

Not excluded by the present study is the possibility that the C-terminus + bithiazole region of BLM can bind selectively to certain sequences in DNA, but that this selectivity is obscured by the dominant behavior of the metal binding region. It seems possible, for example, that a series of gly_x-BLM derivatives structurally related to BLM A_2 (rather than BLM demethyl A_2) might reflect a greater influence of binding preferences actually exhibited by the C-terminus + bithiazole region of BLM.

The finding that a single structural domain of BLM is responsible for metal binding, oxygen activation and sequence selectivity of DNA cleavage has important implications for the design of DNA cleaving agents with enhanced efficiency. To the extent that this process constitutes the basis for the antitumor activity of BLM, the present finding can also potentially guide the design of improved antitumor agents.

RNA strand scission by Fe(II)·BLM. While few studies have dealt specifically with RNA as a substrate for BLM degradation, there are several that have been carried out in the presence of both RNA and DNA. For example, Haidle and Bearden[35] found that only the poly(dT) strand of a poly(rA)·poly(dT) hybrid was a substrate for bleomycin; the same conclusion was reached recently by Krishnamoorthy et al. following a detailed product analysis of the degradation of poly(rA)·poly(dT) and poly(dA)·poly(U) duplexes with Fe(II)·BLM[36]. Hori[37] demonstrated that *E. coli* transfer RNA had little effect on the BLM B_2-mediated relaxation of SV40 Form I DNA, even when a large excess of the RNA was present. Those few studies that did use RNA as the sole substrate were performed in the absence of exogenous metals[38,39].

On the basis of a recent report by Magliozzo et al.[26], which demonstrated limited degradation of yeast $tRNA^{Phe}$ by very high concentrations of Fe(II)·BLM, we chose to study a number of RNA's as possible substrates for Fe(II)·BLM. Each of the RNA's was obtained by RNA polymerase-catalyzed transcription from an appropriate DNA template. Interestingly, Fe(II)·BLM was shown to mediate strand scission of RNA substrates in a highly selective and efficient manner, cleaving some RNA substrates at 2.5 μM concentration, but not cleaving others even at 300 μM concentration[40]. One RNA that proved to be an excellent substrate for BLM was *B. subtilis* $tRNA^{His}$ precursor. This RNA substrate was cleaved by as little as 3 μM Fe(II)·BLM A_2 and

equally well by Fe(II)·BLM B_2, but not by Fe(III)·BLM A_2[40,41]. Remarkably, cleavage occurred only at one major site, to the near exclusion of all others.

The major BLM cleavage site on the $tRNA^{His}$ substrate was identified using RNA enzymatic sequencing[40,41]. Fig. 3 shows Fe(II)·BLM cleavage of a 5'-^{32}P-end labeled $tRNA^{His}$ precursor by 300 μM

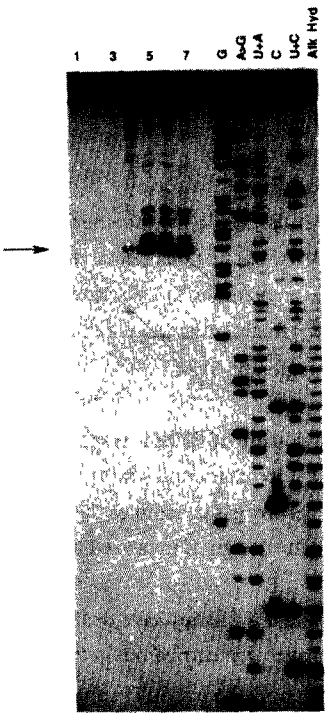


Fig. 3. Temperature-dependent cleavage of 5'-^{32}P-end labeled *B. subtilis* $tRNA^{His}$ precursor. The reactions were carried out at the indicated temperatures for 1 hr in the presence of 3 mM unlabeled carrier tRNA. Lane 1, $tRNA^{His}$ precursor alone; lane 2, 300 μM BLM; lane 3, 300 μM Fe(II); lanes 4-7, 300 μM Fe(II)·BLM at 0, 22, 37 and 55°C, respectively; lanes 8-13, RNA sequencing reactions.

Fe(II)·BLM at temperatures varying from 0° to 55°C (lanes 4-7). The enzymatic sequencing reactions are in lanes 8-13. As is clear from the figure, there was no change in the position of the major cleavage site at any of the temperatures studied, although very little actual strand scission was observed at 0°C (lane 4).

Figure 4 illustrates the primary sequence and a plausible secondary structure for *Bacillus subtilis* $tRNA^{His}$ precursor. The major and minor sites of Fe(II)·BLM-mediated strand scission are indicated by the arrow and asterisks, respectively. The major cleavage site was at uridine$_{35}$, which is part of a 5'-GU-3' sequence at the junction between a double- and single-stranded region of the RNA. The preference for cleavage at a 5'-GU-3' site

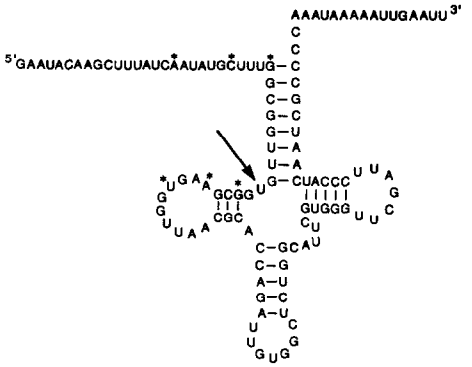


Fig. 4. Cloverleaf representation of *B. subtilis* $tRNA^{His}$ precursor. The major site of Fe(II)·BLM-mediated cleavage is denoted by the arrow; the minor sites by asterisks.

bears formal analogy to the preferential cleavage of DNA at 5'-GT-3' and 5'-GC-3' sequences, although it may be noted that six other 5'-GU-3' sequences in the $tRNA^{His}$ precursor were cleaved weakly at best. More interesting was the fact that cleavage occurred at a nucleotide believed to be in a single-stranded region of the RNA. There is as yet no example of cleavage of single-stranded DNA by BLM, although it has been reported that BLM cleaved DNA with enhanced efficiency at sites adjacent to bulges[42], and in a segment of a DNA duplex in which the interstrand H-bonding was believed to have been distorted by platination of a proximate guanine nucleotide[30].

A number of other *in vitro* RNA transcripts were investigated as substrates for degradation by Fe(II)·BLM. Although an *E. coli* $tRNA^{Tyr}$ precursor ostensibly related in structure to the $tRNA^{His}$ precursor was largely refractory to Fe·BLM-mediated cleavage, certain other RNA's were degraded efficiently by Fe(II)·BLM. These included a 231-nucleotide (nt) RNA transcribed from a pSP64 plasmid. Fe(II)·BLM readily cleaved this RNA substrate at three major sites. Comparison of the RNA sequencing data with a folding analysis of the RNA structure[43] indicated that one of the cleavage sites was G_{75}, part of a 5'-GU-3' sequence located in a double-strand region adjacent to a loop structure. The second site occurred at U_{93} which was the uridine of a 5'-GU-3' sequence located at the start of a single-stranded region (*vida supra*). The third BLM cleavage site in this 231-nucleotide RNA substrate occurred at G_{122}, which appears to be part of a 5'-CG-3' sequence in which the cytidine is in a single-stranded region and the guanosine is located at the beginning of a double-stranded region. It may be noted that in the case of DNA, CG sequences are not routinely observed to be good sites for BLM degradation[22].

Also investigated was a 270-nt RNA transcript corresponding to the 5'-terminal portion of the messenger RNA encoding HIV reverse transcriptase. This RNA was cleaved at four sites by Fe(II)·BLM, two of which were strong cleavage sites.

For RNA, the characterized cleavage sites all can be represented as junctions between single- and double-stranded regions of the RNA. So far, the available data concerning the recognition of RNA substrates by Fe·BLM do not indicate the recognition of any specific two-base sequence (which would parallel the recognition of 5'-GT-3' and 5'-GC-3' sequences in DNA), and it seems unlikely that Fe(II)·BLM recognition of RNA involves a sequence of different length than for DNA. Considering the apparent lack of RNA sequence recognition by BLM, and the highly selective RNA cleavage by Fe(II)·BLM, it is logical to think that the primary determinant of the BLM cleavage sites in RNA substrates is RNA conformation. In fact, there have been occasional reports of DNA sites that undergo exceptionally efficient Fe·BLM cleavage[44,45], which could reflect local DNA conformations particularly susceptible to Fe(II)·BLM-mediated cleavage. The identification and structural characterization of such sites in RNA and DNA would establish Fe(II)·BLM as a very useful probe of polynucleotide conformation, and possible tertiary structures.

These results challenge the assumption, which is presently generally accepted as valid, that DNA is the biochemical locus at which BLM mediates its therapeutic effects. RNA would seem to constitute another possible therapeutic target for BLM, especially when one considers the remarkable selectivity for degradation of certain structural elements within RNA, even in the presence of large excesses of other (nonsubstrate) RNA's. In comparison with DNA as a therapeutic target for BLM, RNA has a few obvious advantages, including its greater accessibility (i.e., due to its localization in the cytoplasm of nucleated cells), and the paucity of cellular mechanisms for repair of damaged RNA. If this thesis proves to be correct, it might extend the range of diseases amenable to treatment with BLM to include those that involve pathogens whose template molecules are RNA.

EXPERIMENTAL

Cleavage of 5'-^{32}P-end labeled DNA by Fe(II)·gly$_X$-BLM's. The DNA substrate was prepared by treatment of SV40 Form I DNA with restriction endonucleases *Bcl* I and *Eco* RII. The 127-bp DNA duplex was isolated by preparative polyacrylamide gel electrophoresis, and then 5'-end labeled via the agency of polynucleotide kinase + [γ-^{32}P]ATP.[13] DNA cleavage reactions (20 μL total volume) contained 4 x 10^4 cpm (~3 pmol) of the radiolabeled DNA duplex in 50 mM Na cacodylate buffer, pH 7.5, 50 μM Fe(II)$(NH_4)_2(SO_4)_2$, and 100 or 200 μM gly$_X$-BLM analog as indicated. A control reaction contained 10 μM Fe(II)·deglyco BLM demethyl A_2. Reactions were initiated by simultaneous addition of Fe(II) and BLM analog, and incubated at 25°C for 45 min (gly$_X$-BLM's) or 0°C for 5 min (deglyco BLM demethyl A_2). The reactions were quenched by the addition of 5 μL of 50 mM NaOAc, pH 5.5, containing 10 mM $MgCl_2$, and then precipitated by successive additions of 4 μg of calf thymus DNA, 2 μL of 3M NaOAc, pH 5.5, and 250 μL of cold ethanol. The precipitated DNA was recovered by centrifugation, redissolved in 4 μL of loading buffer (80% formamide containing 50 mM Tris-borate, pH 8.3, 1 mM EDTA, 0.1% xylene cyanol and 0.1% bromophenol blue) and analyzed by electrophoresis (2000 V, 2 hr, 50 mM Tris-borate, pH 8.3, containing 1 mM EDTA) on a 10% polyacrylamide gel. The gel was visualized by autoradiography.

Cleavage of 5'-^{32}P end labeled tRNA^{His} precursor by Fe(II)·BLM. Reaction mixtures (5 μL total volume) contained 5 mM NaH_2PO_4, pH 7.0, 2x10^4 cpm (2.5 μM RNA nucleotide concentration) of 5'-^{32}P end labeled tRNA^{His} precursor and 300 μM Fe(II)·BLM A_2. The reactions were maintained at 0, 22, 37 or 55°C for 1 hr, and then quenched by the addition of 3 μL of loading buffer and analyzed by electrophoresis (2000 V, 2 hr) on a 20% polyacrylamide gel. The gel was visualized by autoradiography.

REFERENCES

1. Umezawa, H. in *Bleomycin: Chemical, Biochemical, and Biological Aspects* (Hecht, S. M., Ed.) **1979**, p 24 ff, Springer-Verlag, New York.
2. Umezawa, H. in *Medicinal Chemistry Series: Anticancer Agents Based on Natural Product Models* (Cassady, J. M., & Douros, J. D., Eds.) **1980**, p 148 ff, Academic, New York.
3. Hecht, S. M. *Acc. Chem. Res.* **1986**, *19*, 383-391.
4. Stubbe, J.; Kozarich, J. W. *Chem. Rev.* **1987**, *87*, 1107-1136.
5. Sausville, E. A.; Stein, R. W.; Peisach, J.; Horwitz, S. B. *Biochemistry* **1978**, *31*, 1073-1077.
6. Takita, T.; Muraoka, Y.; Nakatani, T.; Fujii, A.; Iitaka, Y.; Umezawa, H. *J. Antibiot.* **1978**, *31*, 1073-1077.
7. Kilkuskie, R. E.; Suguna, H.; Yellin, B.; Murugesan, N.; Hecht, S. M. *J. Am. Chem. Soc.* **1985**, *107*, 260-261.
8. Chien, M.; Grollman, A. P.; Horwitz, S. B. *Biochemistry* **1977**, *16*, 3641-3646.
9. Povirk, L. F.; Hogan, M.; Dattagupta, N. *Biochemistry* **1979**, *18*, 96-101.
10. Kross, J.; Henner, W. D.; Haseltine, W. A.; Rodriguez, L.; Levin, M. D.; Hecht, S. M. *Biochemistry* **1982**, *21*, 3711-3721.
11. Fisher, L. M.; Kuroda, R.; Sakai, T. T. *Biochemistry* **1985**, *24*, 3199-3207.
12. Levy, M. J.; Hecht, S. M. *Biochemistry* **1988**, *27*, 2647-2650.
13. Carter, B. J.; Murty, V. S.; Reddy, K. S.; Wang, S.-N.; Hecht, S. M. *J. Biol. Chem.* **1990**, *265*, 4193-4196.
14. Takeshita, M.; Grollman, A. P. in *Bleomycin: Chemical, Biochemical and Biological Aspects* (Hecht, S. M., Ed.), **1979**, p 207ff, Springer-Verlag, New York.
15. Burger, R. M.; Peisach, J.; Horwitz, S. B. *J. Biol. Chem.* **1982**, *257*, 3372-3375.
16. Wu, J. C.; Kozarich, J. W.; Stubbe, J. *J. Biol. Chem.* **1983**, *258*, 4694-4697.
17. Kozarich, J. W.; Worth, L., Jr.; Frank, B. L.; Christner, D. F.; Vanderwall, D. E.; Stubbe, J. *Science* **1989**, *245*, 1396-1399.
18. Long, E. C.; Hecht, S. M.; van der Marel, G. A.; van Boom, J. H. *J. Am. Chem. Soc.* **1990**, *112*, 5272-5276.
19. Sugiura, Y.; Suzuki, T. *J. Biol. Chem.* **1982**, *257*, 10544-10546.

20. Kuwahara, J.; Sugiura, Y. *Proc. Natl. Acad. Sci. USA.* **1988**, *85*, 2459-2463.

21. Kross, J.; Henner, W. D.; Hecht, S. M.; Haseltine, W. A. *Biochemistry* **1982**, *21*, 4310-4318.

22. Shipley, J. B.; Hecht, S. M. *Chem. Res. Toxicol.* **1988**, *1*, 25-27.

23. Sugiyama, H.; Kilkuskie, R. E.; Chang, L.-H.; Ma, L.-T.; Hecht, S. M.; van der Marel, G. A.; van Boom, J. H. *J. Am. Chem. Soc.* **1986**, *108*, 3852-3854.

24. Berry, D. E.; Kilkuskie, R. E.; Hecht, S. M. *Biochemistry* **1985**, *24*, 3214-3219.

25. Berry, D. E.; Chang, L.-H.; Hecht, S. M. *Biochemistry* **1985**, *24*, 3207-3214.

26. Magliozzo, R. S.; Peisach, J.; Ciriolo, M. R. *Mol. Pharmacol.* **1989**, *24*, 428-432.

27. Hertzberg, R. P.; Caranfa, M. J.; Hecht, S. M. *Biochemistry* **1988**, *27*, 3164-3174.

28. Povirk, L. F.; Hogan, M.; Dattagupta, N.; Buechner, M. *Biochemistry* **1981**, *20*, 665-670.

29. Mascharak, P. K.; Sugiura, Y.; Kuwahara, J.; Suzuki, T.; Lippard, S. J. *Proc. Natl. Acad. Sci. USA* **1983**, *80*, 6795-6798.

30. Gold, B.; Dange, V.; Moore, M. A.; Eastman, A.; van der Marel, G. A.; van Boom, J. H.; Hecht, S. M. *J. Am. Chem. Soc.* **1988**, *110*, 2347-2349.

31. Fraser, R. D. B.; MacRae, T. P.*Conformation in Fibrous Proteins and Related Synthetic Polypeptides* **1973**, p 285-286, Academic Press, New York.

32. Aoyagi, Y.; Suguna, H.; Murugesan, N.; Ehrenfeld, G. M.; Chang, L.-H., Ohgi, T.; Shekhani, M. S.; Kirkup, M. P.; Hecht, S. M. *J. Am. Chem. Soc.* **1982** *104*, 5237-5239.

33. Sugiyama, H.; Xu, C.; Murugesan, N.; Hecht, S. M.; van der Marel, G. A.; van Boom, J. H. *Biochemistry* **1988**, *27*, 58-67.

34. Jencks, W. P. *Proc. Natl. Acad. Sci. USA* **1981**, *78*, 4046-4050.

35. Haidle, C. W.; Bearden, J., Jr. *Biochem. Biophys, Res. Commun.* **1975**, *65*, 815-821.

36. Krishnamoorthy, C. R.; Vanderwall, D. E.; Kozarich, J. W.; Stubbe, J. *J. Am. Chem. Soc.* **1988**, *110*, 2008-2009.

37. Hori, M. in *Bleomycin: Chemical, Biochemical and Biological Aspects,* (Hecht, S. M., Ed.) **1979**, p 195 ff, Springer-Verlag, New York.

38. Suzuki, H.; Nagai, K.; Akutsu, E.; Yamaki, H.; Tanaka, N.; Umezawa, H. *J. Antibiot.* **1970**, *23*, 473-480.

39. Haidle, C. W.; Kuo, M. T.; Weiss, K. K. *Biochem. Pharmacol.* **1972**, *21*, 3308-3312.

40. Carter, B. J.; de Vroom, E.; Long, E. C.; van der Marel, G. A.; van Boom, J. H.; Hecht, S. M. *Proc. Natl. Acad. Sci. USA.* **1990**, *87*, 9373-9377.

41. Carter, B. J.; Holmes, C. E.; Van Atta, R. B.; Dange, V.; Hecht, S. M. *Nucleosides & Nucleotides* **1990**, *9*, xxxx

42. Williams, L. D.; Goldberg, I. H. *Biochemistry* **1988**, *27*, 3004-3011.

43. Zuker, M.; Stiegler, P. *Nucleic Acids Res.* **1981**, *9*, 133-148.

44. Mirabelli, C. K.; Huang, C.-H.; Crooke, S. T. *Biochemistry* **1983**, *22*, 300-306.