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# DNA STRAND SCISSION BY BLEOMYCIN GROUP ANTIBIOTICS[1]

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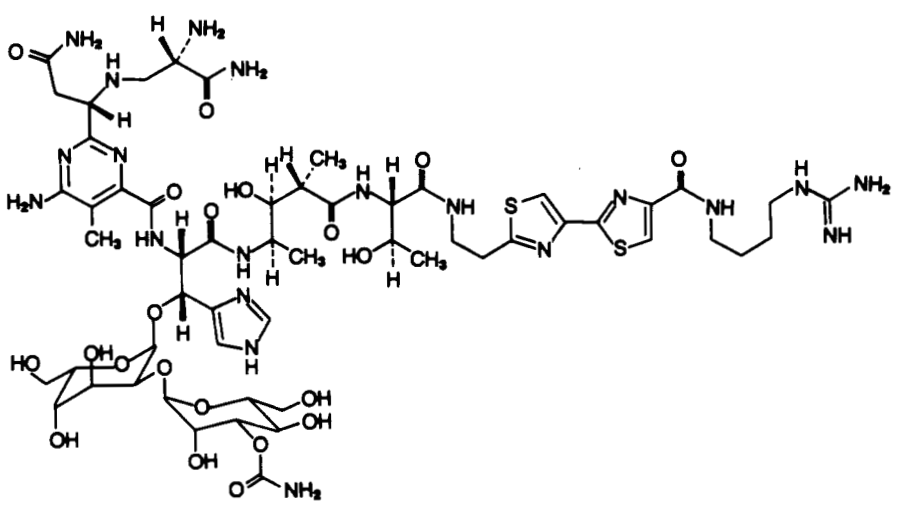
ABSTRACT.—Certain properties of the bleomycin analogs deglycobleomycin $A_2$ and decarbamoylbleomycin $A_2$ have been characterized. In common with bleomycin $A_2$, both deglycobleomycin $A_2$ and decarbamoylbleomycin $A_2$ were found to mediate DNA degradation in the presence of Fe(II)+$O_2$. Both analogs were found to have essentially the same sequence selectivity for DNA strand scission as bleomycin $A_2$ when a 5′-[$^{32}$P]-end-labeled linear duplex DNA derived from SV40 DNA was employed as a substrate. Product analysis for the three analogs was carried out by assay for malondialdehyde (precursors) after digestion of calf thymus DNA, and also by hplc analysis of the digestion products formed from the dodecanucleotide d(CGCTTTAAAGCG). All three Fe(II)·bleomycin $A_2$ analogs produced the same products, albeit not in the same relative amounts.

The bleomycins are a group of antitumor antibiotics used clinically for the treatment of squamous cell carcinomas and malignant lymphomas (1-4). The therapeutic locus of action of the bleomycins is believed to be chromosomal DNA, which is cleaved by bleomycin in a metal ion- and oxygen-dependent reaction (5-9). Blenoxane, the clinically used mixture of bleomycins, consists of several species that differ only at the C-terminus (10,11), i.e., within the putative DNA binding domain (12-14). The major constituents of this mixture, bleomycin $A_2$ and bleomycin $B_2$, have been analyzed with respect to their sequence specificity of DNA strand scission; interestingly, no significant differences were observed for these two congeners (15), although a few different lines of evidence suggest that individual C-substituents can influence the nature of bleomycin-DNA interaction (16). As anticipated for two molecules that employ the same functional groups for metal ligation and oxygen activation (13), the ac-



**bleomycin $B_2$**

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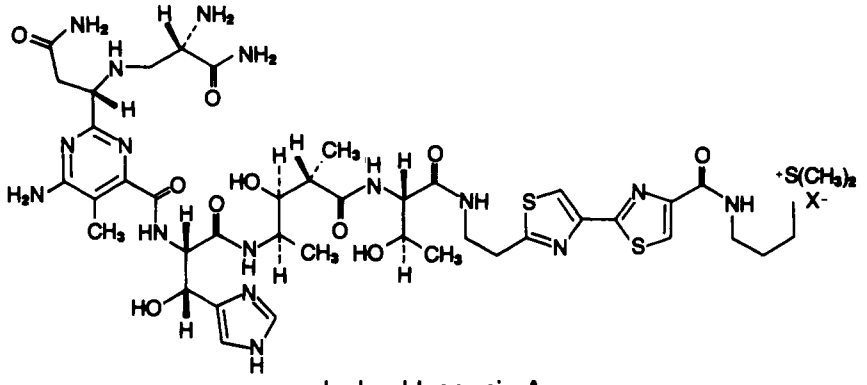


deglycobleomycin $A_2$

tual chemistry employed by bleomycins $A_2$ and $B_2$ for DNA cleavage has also been found to be the same (17).

Of considerable interest in the context of the mechanism of DNA strand scission by bleomycin, as well as the design of bleomycins with potentially improved antitumor activity, is the nature of chemical cleavage of DNA by bleomycin congeners having altered metal binding properties. Reported herein are experiments that define aspects of the efficiency, sequence specificity, and chemistry of DNA cleavage mediated by Fe(II) chelates of deglycobleomycin $A_2$ and decarbamoylbleomycin $A_2$.

## RESULTS AND DISCUSSION

Deglycobleomycin is a product of partial hydrolysis of bleomycin (18,19) that has also been prepared synthetically (20,21). As illustrated clearly in Figure 1, when Fe(II)·deglycobleomycin $B_2$ was admixed with SV40 Form I DNA[3] in the presence of

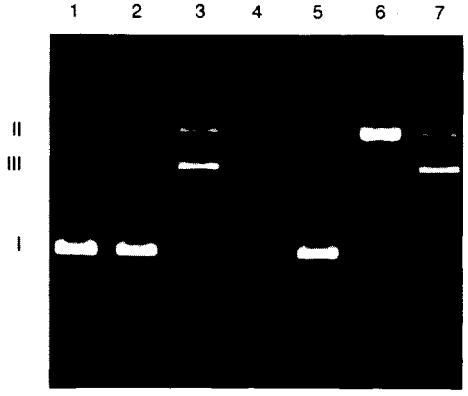



FIGURE 1. Cleavage of SV40 Form I DNA by Fe(II)·bleomycin $B_2$ and Fe(II)·deglycobleomycin $B_2$. Reactions were carried out using 0.1 μM (lane 2), 1.0 μM (lane 3) and 2.5 μM (lane 4) Fe(II)·bleomycin $B_2$, or 0.1 μM (lane 5), 1.0 μM (lane 6) and 2.5 μM (lane 7) Fe(II)·deglycobleomycin $B_2$. A control reaction (lane 1) was run in the absence of any Fe·bleomycin congener.

[3]SV40 Form I DNA denotes a supercoiled, covalently closed circular DNA derived from simian virus 40-infected BSC-1 monkey cells (strain 776). Nicking one strand produces a relaxed circular duplex DNA (Form II DNA), while nicking both strands at proximal sites produces a linear duplex (Form III DNA).

$O_2$, facile conversion to Form II and Form III DNA was noted. Direct comparison with Fe(II)·bleomycin $B_2$ indicated that Fe(II)·deglycobleomycin $B_2$ was about 40%-50% as effective as the parent compound in producing DNA strand breaks (cf. lanes 3 and 7 in Figure 1), a result that was in agreement with observations made for bleomycin $B_2$ and deglycobleomycin $B_2$-mediated degradation of *Escherichia coli* [$^3$H]DNA (19).

In addition to their activation in the presence of $O_2$, both Fe·bleomycin (22) and Cu·bleomycin (22,23) have been shown to effect DNA strand scission following activation with iodosobenzene. Analogous experiments were attempted with deglycobleomycin; as shown in Figure 2, both Fe(III)·deglycobleomycin $B_2$ and Cu(II)·deglycobleomycin $B_2$ relaxed supercoiled SV40 DNA after activation with $C_6H_5IO$. As seen previously for bleomycin, the concentrations of deglycobleomycin required for DNA strand scission were significantly higher when activation was mediated by $C_6H_5IO$ rather than by $O_2$, a finding that held true both for the Fe and Cu complexes (22,23).

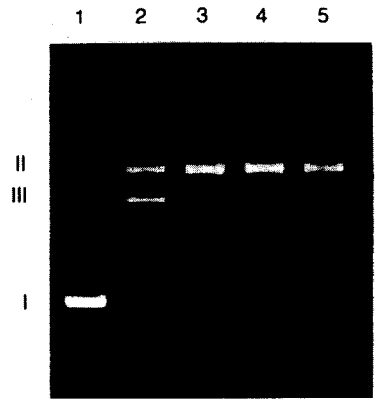



FIGURE 2. Cleavage of SV40 Form I DNA by $C_6H_5IO$-activated deglycobleomycin $B_2$. Reactions contained 1 μM Fe(II)·deglycobleomycin $B_2$ +$O_2$ (lane 2), 100 μM (lane 3) or 50 μM (lane 4) Fe(III)·deglycobleomycin $A_2$+excess $C_6H_5IO$, or 25 μM Cu(II)·deglycobleomycin $A_2$+excess $C_6H_5IO$ (lane 5). A control reaction (lane 1) lacked any metallobleomycin.

Another measure of DNA cleavage by bleomycins involves quantitation of known reaction products. It has been shown that DNA degradation by Fe·bleomycin involves the production of free bases and base propenals (7-9, 24), the latter of which can be measured colorimetrically as a thiobarbituric acid adduct following decomposition to malondialdehyde and free base (7). As shown in Table 1, in common with Fe(II)·bleomycin $A_2$, Fe(II)·deglycobleomycin $A_2$ and Fe(II)·decarbamoylbleomycin $A_2$ also produced malondialdehyde (precursors) concomitant with cleavage of calf thymus DNA. The amount of malondialdehyde produced by decarbamoylbleomycin $A_2$ was intermediate between that produced by bleomycin $A_2$ and deglycobleomycin $A_2$. While these malondialdehyde data provided a ready indication of DNA cleavage

TABLE 1. Malondialdehyde (Precursor) Production by Bleomycin Analogs

| Compound[a] | Malondialdehyde Produced (nmoles) |
|---|---|
| Fe(II)·bleomycin $A_2$ | 3.23±0.35 (n=3)[b] |
| Fe(II)·deglyco bleomycin $A_2$ | 1.25±0.11 (n=2) |
| Fe(II)·decarbamoyl bleomycin $A_2$ | 1.98±0.23 (n=2) |

[a]Reaction mixtures included 300 μM DNA and 100 μM Fe(II)·bleomycin analog.

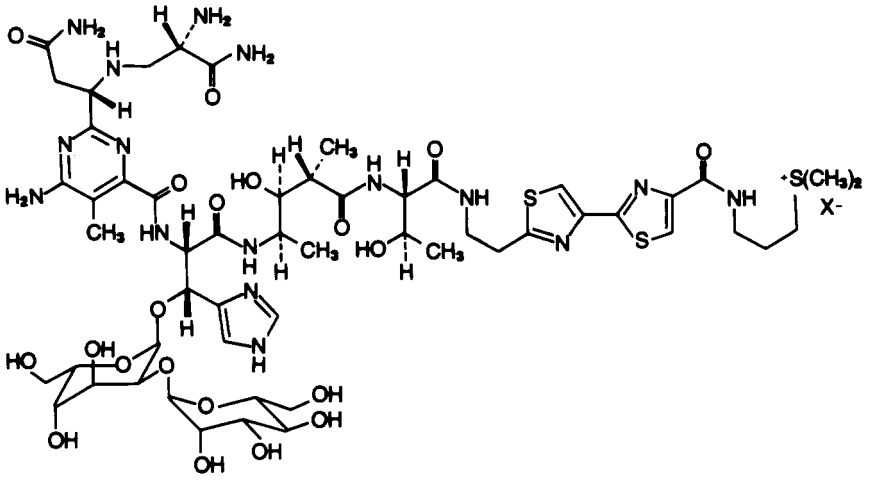
[b]Determinations were made in duplicate; variation in duplicate values was <10%. The number of individual assays=n.

potential by these analogs, and a rough guide to the efficiency of cleavage by individual bleomycin congeners, the known variation (24,25) in the production of individual products according to the specific conditions employed for bleomycin-mediated DNA strand scission precluded the use of this data for direct comparison of DNA cleavage potential by these analogs.

Fe(II)·bleomycin $A_2$ has been reported (26,27) to cleave DNA in a sequence-selective fashion; GT and GC are the preferred sequences, and cleavage at these sites involves destruction of the pyrimidine nucleosides. It was of interest to compare the sequence selectivity of DNA cleavage by Fe(II)·bleomycin $A_2$ with those of Fe(II)·deglycobleomycin $A_2$ and Fe(II)·decarbamoylbleomycin $A_2$. This was done using 5′-[$^{32}$P]-end-labeled DNA fragments isolated by restriction endonuclease treatment of SV40 Form I DNA. Shown in Figure 3 is a direct comparison of cleavage of a double-stranded DNA segment 127 nucleotides in length by Fe(II)·bleomycin $A_2$ and Fe(II)·deglycobleomycin $A_2$. As is clear from the figure, while the efficiency of DNA cleavage by Fe(II)·deglycobleomycin $A_2$ was somewhat less than that of Fe(II)·bleomycin $A_2$, the pattern of DNA strand scission was essentially unaltered. Also carried out in the same fashion was a direct comparison of the sequence selectivity of DNA strand scission by

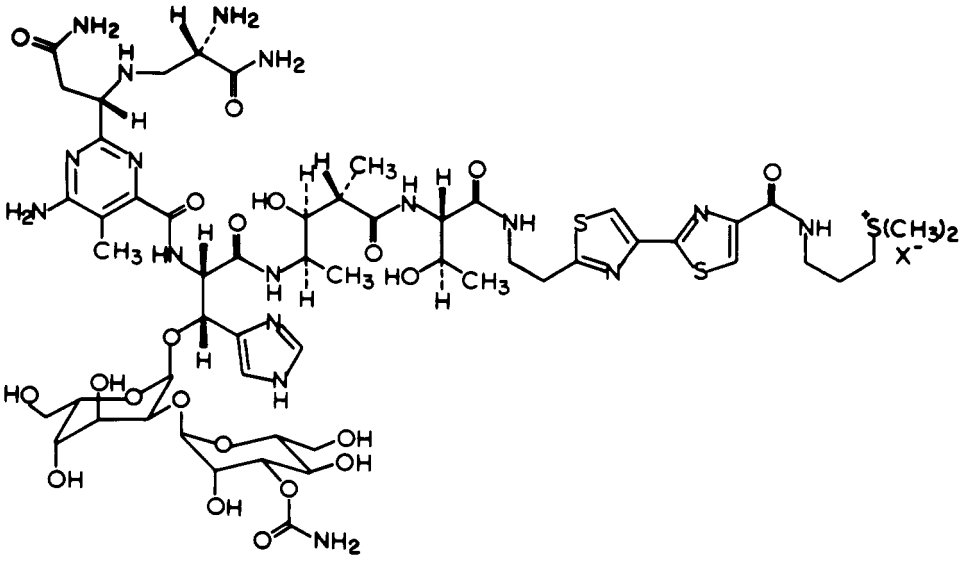

FIGURE 3. Cleavage of a 127 nucleotide long DNA duplex by Fe(II)·bleomycin $A_2$ and Fe(II)·deglycobleomycin $A_2$. Individual reactions contained 5 μM Fe(II)·bleomycin $A_2$ (lanes 4 and 7), 5 μM (lane 5) and 10 μM (lane 6) Fe(II)·deglycobleomycin $A_2$. Control lanes included a Maxam and Gilbert "G-lane" (28) (lane 1), a reaction lacking any Fe·bleomycin (lane 2) and a reaction containing 10 μM $Fe(NH_4)_2(SO_4)_2$ (lane 3).

Fe(II)·bleomycin $A_2$ and Fe(II)·decarbamoylbleomycin $A_2$, both in the presence and absence of dithiothreitol. Again, no significant difference was noted in the pattern of DNA strand scission produced by these two analogs (data not shown).



**decarbamoylbleomycin $A_2$**

Recently we have described the use of a self-complementary dodecanucleotide, d(CGCTTTAAAGCG), as a substrate for Fe(II)·bleomycin-mediated cleavage (25,29). The use of this oligonucleotide as a model for bleomycin-DNA interaction afforded several advantages, including a higher efficiency of bleomycin-mediated cleavage than that obtained with DNA, and the fact that cleavage occurred predominantly at the putative preferred double-strand bleomycin recognition site (Figure 4) over a wide range of experimental conditions. Because the products of bleomycin-mediated cleavage of this dodecanucleotide are of modest size and known structure (25), product quantitation for bleomycin has also been possible. The products of Fe(II)·bleomycin-mediated strand scission of the dodecanucleotide are readily separable by hplc under appropriate conditions (25,29); although a number of products are formed, the number of



**bleomycin $A_2$**

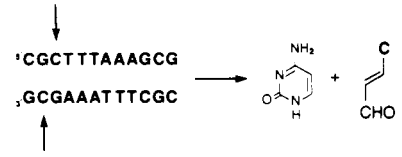



FIGURE 4. Products formed concomitant with Fe(II)·deglycobleomycin $A_2$ degradation of d(CGCTTTAAAGCG) at preferred cleavage sites.

oligonucleotide cleavage events is equal to the sum of all free bases and base propenals (25). Because cleavage of the dodecanucleotide occurs predominantly at GC when Fe(II)·bleomycin is employed, total cleavage events are approximated by the sum of cytosine and cytosine propenal (25).

Because Fe(II)·deglycobleomycin $A_2$ and Fe(II)·bleomycin $A_2$ exhibited sequence specificity of DNA strand scission very similar to that of Fe(II)·bleomycin $A_2$, it seemed reasonable to identify and quantitate the products of these bleomycin congeners by the use of d(CGCTTTAAAGCG) as a substrate. Shown in Figure 5 is the hplc profile corresponding to a reaction mixture in which the dodecanucleotide (83 μM) was treated with 300 μM Fe(II)·decarbamoylbleomycin $A_2$. On the basis of this analysis, which included quantitation in direct comparison with authentic synthetic samples of each product (25,29), it was shown that cleavage of the dodecanucleotide by Fe(II)·decarbamoylbleomycin $A_2$ occurred to the extent of ~89% at the preferred double strand cleavage site, which compared favorably with the value of 76-96% observed for Fe(II)·bleomycin under a range of conditions. The comparable value when Fe(II)·deglycobleomycin $A_2$ was employed at 300 μM concentration was 98%, and the same products of dodecanucleotide cleavage were also observed (data not shown). Thus, the chemistry of DNA strand scission by Fe(II) ·bleomycin $A_2$, Fe(II)·deglycobleomycin $A_2$ and Fe(II)·decarbamoylbleomycin $A_2$ were found to be quite similar, as in regard to sequences cleaved preferentially and products formed, as well as in specificity of cleavage at the preferred site.

Also calculated from the data obtained was the efficiency of dodecanucleotide modification by the three Fe(II)·bleomycin analogs studied here. As shown in Table 2, Fe(II)·deglycobleomycin $A_2$ was about 60% as efficient as Fe(II)·bleomycin $A_2$, in

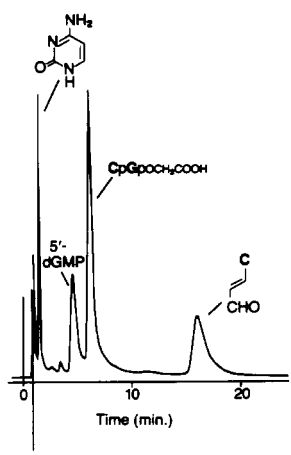



FIGURE 5. Hplc analysis of Fe(II)·decarbamoylbleomycin $A_2$-treated dodecanucleotide.

reasonably good agreement with results obtained for efficiency of relaxation of supercoiled DNA (Figure 1), release of [$^3$H]thymine from radiolabeled DNA (21) and production of malondialdehyde concomitant with DNA degradation. Interestingly, although Fe(II)·decarbamoylbleomycin $A_2$ produced significantly less malondialdehyde from calf thymus DNA under the conditions employed here (Table 1), it was almost as efficient as Fe(II)·bleomycin $A_2$ at modification of d(CGCTTTAAAGCG) (Table 2). Several possibilities may account for this apparent discrepancy; these are under investigation at present.

TABLE 2. Dodecanucleotide Cleavage by Bleomycin Analogs

| Compound[a] | Cytosine + Cytosine Propenal[b] | Dodecanucleotide Events[b,c] |
|---|---|---|
| Fe(II)·bleomycin $A_2$ . . . . . | 62 | 80 |
| Fe(II)·deglyco bleomycin $A_2$ . . | 52 | 53 |
| Fe(II)·decarbamoyl bleomycin $A_2$ . | 60 | 67 |

[a]Reaction mixtures included 83 μM d(CGCTTTAAAGCG) and 300 μM Fe(II)·bleomycin analog.

[b]Concentration formed (μM).

[c]Equal to the sum of all bases and base propenals formed (25).

## EXPERIMENTAL

MATERIALS.—SV40 Form I DNA, restriction enzymes *Bcl* I and *Eco* RII, and T4 polynucleotide kinase were obtained from Bethesda Research Laboratories. Calf intestine alkaline phosphatase was purchased from Boehringer-Mannheim. γ-[$^{32}$P]ATP was obtained from ICN Radiochemicals; calf thymus DNA was from Sigma Chemicals.

Bleomycin $A_2$ and $B_2$ were obtained by fractionation of blenoxane (12), the latter of which was obtained from Bristol Laboratories. Deglycobleomycin $A_2$ and $B_2$ were obtained by partial hydrolysis of the respective bleomycins (18,19), or by synthesis (21). Decarbamoylbleomycin $A_2$ was obtained by partial hydrolysis of bleomycin $A_2$ by a modification of the published procedure (30); the structure of the product was verified by total synthesis.

RELAXATION OF FORM I DNA.—To reaction mixtures (80 μl total volume) containing 50 mM $Na^+$ cacodylate, pH 7.0, and 500 ng of SV40 Form I DNA was added the appropriate Fe(II)·bleomycin $B_2$ analog to the final concentration given in the legend to Figure 1. The reactions were maintained at 25° for 30 min, then stopped by the addition of 1 μl of 200 mM EDTA. Five μl of 0.15% bromophenol blue - 75% glycerol was added to each reaction mixture, and each was analyzed on a 1.2% agarose gel containing 1 μg/ml of ethidium bromide. Horizontal gel electrophoresis was carried out at 80 volts for 7 h in 40 mM Tris buffer, pH 7.8, containing 5 mM NaOAc and 1 mM EDTA.

The experiment employing iodosobenzene for Fe(III) and Cu(II)·deglycobleomycin $B_2$ activation was carried out similarly, in $Na^+$ cacodylate buffer that had been purged with argon. The individual reactions employed the analogs at the concentrations indicated in the legend to Figure 2. Iodosobenzene (twentyfold excess relative to deglycobleomycin) was added to each reaction mixture over a period of 15 min. The reactions were then run for 30 min at 25° and analyzed by agarose gel electrophoresis as indicated above.

MALONDIALDEHYDE (PRECURSOR) PRODUCTION BY BLEOMYCIN ANALOGS.—Reaction mixtures (0.2 ml total volume) containing 300 μM calf thymus DNA, 100 μM $Fe(NH_4)_2(SO_4)_2$ and 100 μM bleomycin $A_2$ analog in 20 mM $Na^+$ cacodylate, pH 7.0, were incubated at 25° for 15 min. A solution (0.8 ml) containing 42 mM thiobarbituric acid and 1 mM EDTA was added to the reaction mixture and the combined solution was heated at 90° for 30 min. The amount of malondialdehyde formed was estimated from the measured $A_{532}$ value [$\epsilon = 1.6 \times 10^5\ M^{-1}\ cm^{-1}$ (31)].

SEQUENCE SPECIFICITY OF Fe(II)·DEGLYCO BLEOMYCIN $A_2$.—Double-stranded SV40 DNA was digested with 40 units of restriction enzyme *Bcl* I in a reaction mixture (100 μl total volume) containing 50 mM Tris-HCl, pH 8.0, 5 mM $MgCl_2$, 50 mM NaCl and 1 mM dithiothreitol. SV40 DNA was added to a final nucleotide concentration of 1.2 mM and bovine serum albumin was included at a final concentration of 200 μg/ml. The reaction mixture was incubated at 37° for 1 h, then treated with 100 μl of NaOAc and the DNA precipitated with EtOH.

The polynucleotide resulting from the initial restriction enzyme digest was dephosphorylated with calf intestine alkaline phosphatase (50 µl of Tris-HCl, pH 9.0, containing 1 mM $MgCl_2$, 0.1 mM $ZnCl_2$, 1 mM spermidine and 60 units of phosphatase) at 37° over a period of 1 h. After denaturation of the phosphatase (10% aqueous sodium dodecyl sulfate, 65°, 15 min) and extraction with phenol, the oligonucleotides were isolated by EtOH precipitation. The dephosphorylated oligonucleotides were 5′-[$^{32}$P]-end labeled using T4 polynucleotide kinase and [$\gamma$-$^{32}$P]ATP, essentially as described (28).

The 5′-[$^{32}$P]-end-labeled polynucleotide was digested with the restriction endonuclease *Eco* RII; the conditions were essentially the same as those employed for the first digest, except that the total reaction volume was 50 µl and the incubation was carried out for 90 min. The reaction mixture was then treated with 15 µl of a 90 mM Tris-borate solution, pH 8.3, 50% in glycerol and 1% in bromophenol blue. The combined solution was applied to an 8% polyacrylamide gel; electrophoresis at 300 volts for 1.5 h effected separation of the $^{32}$P-labeled DNA fragments of interest, which were 127 and 242 nucleotides in length. Following isolation from the gel, the 127-mer was employed as a substrate for deglycobleomycin $A_2$.

The reactions (30 µl total volume) were run in 20 mM $Na^+$ cacodylate, pH 7.4, containing 40 mM NaCl and DNA at a final (nucleotide) concentration of 15 µM. Where indicated, 1 mM dithiothreitol was also present. The reactions were initiated by the addition of the appropriate Fe(II)·bleomycin $A_2$ analog to the final concentration indicated in the legend to Figure 3. Incubations were carried out at 25° for 2 min, after which the reaction mixtures were treated with 5 µl of 300 mM NaOAc, then with 120 µl of cold absolute EtOH to effect DNA precipitation. The isolated DNA oligonucleotides were dissolved in 6 µl of 90 mM Tris-borate, pH 8.3, containing 50% formamide. The oligonucleotides were denatured by heating (90°, 2 min) and then chilled on ice for 3 min prior to loading on a 20% polyacrylamide gel. The gel was run at 1500 volts for 5.5 h and analyzed by autoradiography.

DODECANUCLEOTIDE DEGRADATION BY Fe(II)·BLEOMYCIN ANALOGS.—Reaction mixtures (50 µl total volume) contained 1 mM d(CGCTTTAAAGCG) and 300 µM bleomycin analog in 50 mM $Na^+$ cacodylate, pH 7.0. Reactions were initiated by the addition of freshly prepared $Fe(NH_4)_2(SO_4)_2$ and incubated at 0° for 15 min. Then, 20-µl aliquots were analyzed promptly by hplc on a Rainin Microsorb $C_{18}$ column (3µ); elution was with 0.1 M ammonium formate at a rate of 1.5 ml/min. Uv detection was at 254 nm; quantitation of individual products was carried out by comparison with authentic samples of each.

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## LITERATURE CITED

1. H. Umezawa, in: *Bleomycin: Current Status and New Developments*. Ed. by S.K. Carter, S.T. Crooke, and H. Umezawa. New York, Academic Press, 1978, pp. 15ff.
2. S.M. Hecht, in: *Bleomycin: Chemical, Biochemical and Biological Aspects*. Ed. by S.M. Hecht. New York, Springer-Verlag, 1979, pp. 1ff.
3. H. Umezawa, *Lloydia*, **40**, 67 (1977).
4. S.T. Crooke, in: *Bleomycin: Current Status and New Developments*. Ed. by S.K. Carter, S.T. Crooke, and H. Umezawa. New York, Academic Press, 1978, pp. 9-14.
5. E.A. Sausville, J. Peisach, and S.B. Horwitz, *Biochemistry*, **17**, 2740 (1978).
6. E.A. Sausville, R.W. Stein, J. Peisach, and S.B. Horwitz, *Biochemistry*, **17**, 2746 (1978).
7. R.M. Burger, A.E. Berkowitz, J. Peisach, and S.B. Horwitz, *J. Biol. Chem.*, **255**, 11832 (1980).
8. L. Giloni, M. Takeshita, F. Johnson, C. Iden, and A.P. Grollman, *J. Biol. Chem.*, **256**, 8608 (1981).
9. J.C. Wu, J.W. Kozarich, and J. Stubbe, *J. Biol. Chem.*, **258**, 4694 (1983).
10. H. Umezawa, K. Maeda, T. Takeuchi, and Y. Okami, *J. Antibiot.* (Tokyo) Ser A, **19**, 200 (1966).
11. H. Umezawa, Y. Suhara, T. Takita, and K. Maeda, *J. Antibiot.* (Tokyo) Ser. A, **19**, 210 (1966).
12. M. Chien, A.P. Grollman, and S.B. Horwitz, *Biochemistry*, **16**, 3641 (1977).
13. R.E. Kilkuskie, H. Suguna, B. Yellin, N. Murugesan, and S.M. Hecht, *J. Am. Chem. Soc.*, **107**, 260 (1985).
14. J. Kross, W.D. Henner, W.A. Haseltine, L. Rodriguez, M.D. Levin, and S.M. Hecht, *Biochemistry*, **21**, 3711 (1982).
15. J. Kross, W.D. Henner, S.M. Hecht, and W.A. Haseltine, *Biochemistry*, **21**, 4310 (1982).
16. C.H. Huang, L. Galvan, and S.T. Crooke, *Biochemistry*, **19**, 1761 (1980).
17. H. Sugiyama, R.E. Kilkuskie, and S.M. Hecht, unpublished observations.
18. Y. Muraoka, M. Suzuki, A. Fujii, Y. Umezawa, H. Naganawa, T. Takita, and H. Umezawa, *J. Antibiot.* (Tokyo), **34**, 353 (1981).

19. N.J. Oppenheimer, C. Chang, L.H. Chang, G. Ehrenfeld, L.O. Rodriguez, and S.M. Hecht, *J. Biol. Chem.*, **257**, 1606 (1982).
20. S. Saito, Y. Umezawa, H. Morishima, T. Takita, H. Umezawa, M. Narita, M. Otsuka, S. Kobayashi, and M. Ohno, *Tetrahedron Lett.*, **23**, 529 (1982).
21. Y. Aoyagi, H. Suguna, N. Murugesan, G.M. Ehrenfeld, L.H. Chang, T. Ohgi, M.S. Shekhani, M.P. Kirkup, and S.M. Hecht, *J. Am. Chem. Soc.*, **104**, 5237 (1982).
22. G.M. Ehrenfeld, L.O. Rodriguez, S.M. Hecht, C. Chang, V.J. Basus, and N.J. Oppenheimer, *Biochemistry*, **24**, 81 (1985).
23. N. Murugesan, G.M. Ehrenfeld, and S.M. Hecht, *J. Biol. Chem.*, **257**, 8600 (1982).
24. R.M. Burger, J. Peisach, and S.B. Horwitz, *J. Biol. Chem.*, **257**, 8612 (1982).
25. H. Sugiyama, R.E. Kilkuskie, S.M. Hecht, G. van der Marel, and J.H. van Boom, *J. Am. Chem. Soc.*, (in press).
26. A.D. D'Andrea and W.A. Haseltine, *Proc. Natl. Acad. Sci. U.S.A.*, **75**, 3608 (1978).
27. M. Takeshita, A.P. Grollman, E. Ohtsubo, and H. Ohtsubo, *Proc. Natl. Acad. Sci. U.S.A.*, **75**, 5983 (1978).
28. A.M. Maxam and W. Gilbert, *Methods Enzymol.*, **65**, 499 (1980).
29. N. Murugesan, C. Xu, G.M. Ehrenfeld, H. Sugiyama, R.E. Kilkuskie, L.O. Rodriguez, L.H. Chang, and S.M. Hecht, *Biochemistry*, (in press).
30. H. Naganawa, Y. Muraoka, T. Takita, and H. Umezawa, *J. Antibiot.* (Tokyo), **30**, 388 (1977).
31. V.S. Waravdekar and L.D. Saslaw, *J. Biol. Chem.*, **234**, 1945 (1959).