0026-895X/89/040428-05$02.00/0

MOLECULAR PHARMACOLOGY, 35:428-432

Transfer RNA Is Cleaved by Activated Bleomycin

RICHARD S. MAGLIOZZO, JACK PEISACH,[1] and MARIA ROSA CIRIOLO

Department of Molecular Pharmacology, Albert Einstein College of Medicine of Yeshiva University, Bronx, New York 10461 (R.S.M., J.P.) and Dipartimento di Biologia, II Università di Roma, 00172 Rome, Italy (M.R.C.)

Received November 4, 1988; Accepted January 4, 1989

SUMMARY

Activated bleomycin is shown for the first time to cleave tRNA in a specific and dose-dependent manner. Adenine and uracil are released in the reaction. Bleomycin and Fe(III)-bleomycin bind to yeast $tRNA^{Phe}$ in analogy with the known behavior of the drug with B-DNA.

The widely used antineoplastic glycopeptide bleomycin can be activated for the degradation of DNA *in vitro* with either Fe(II) and O_2 or Fe(III) and H_2O_2 (1). Activated bleomycin, which is presumably responsible for DNA damage *in vivo*, induces two types of DNA lesions *in vitro*. Under oxygen-limiting conditions, the predominant lesion results from the release of nucleic bases, with strand scission occurring at the base-free sites only upon alkaline hydrolysis (2–6). When additional oxygen is available beyond that required for drug activation, activated bleomycin can directly induce strand breakage (7). This O_2-dependent strand scission involves cleavage of the deoxyribose C3′-C4′ bond, leading to production of base propenals as well as 5′-phosphate and 3′-phosphoglycolate termini (8–10). Although the exact mechanism of these reactions is not known, it has been suggested that all products evolve from a single species generated upon hydrogen atom abstraction from C4′ of the deoxyribose ring (3).

Although bleomycin efficiently cleaves DNA, synthetic deoxyribonucleic acid polymers, and oligonucleotides in a sequence-specific way (5, 11–13), there are a number of reports that the drug does not cleave ribonucleic acid (14, 15). These studies were carried out in the absence of added iron, which was subsequently shown to be required for the activity of the drug in DNA breakage (16). More recently, it was reported that treatment of an RNA-DNA hybrid with activated bleomycin does not cause release of base or base propenals from the RNA strand (17). The origin of the resistance of RNA to damage by bleomycin has not been explained nor has it been demonstrated that the initial C4′ hydrogen abstraction cannot occur in RNA.[2] We, therefore, instituted a search for damage to ribonucleic acid (tRNA) by activated bleomycin and have shown that tRNA is in fact susceptible to breakage, although the small-product profile differs from that found with DNA.

Experimental Procedures

Materials. Bleomycin sulfate (Blenoxane) was a gift of Bristol Laboratories (New York, NY). It was dissolved in distilled deionized water and standardized optically ($E_{291} = 1.7 \times 10^4$ M^{-1} cm^{-1}) (18). Samples prepared with Fe(III) and bleomycin required that the drug and metal ion be mixed in water before the addition of buffer and other components to ensure complexation of the metal and to avoid precipitation of Fe(III). Transfer RNAs were purchased from Boehringer Mannheim (Indianapolis, IN). Calf thymus DNA was purchased from Sigma Chemical Co. (St. Louis, MO). Stock solutions of tRNA and DNA were prepared in 5 mM sodium phosphate buffer, pH 7.0, and their concentrations were determined optically using extinction coefficients, at 260 nm, of 6.8 and 6.6 mM nucleotide^{-1} cm^{-1} for tRNA and DNA, respectively. Solutions of reagent grade ferrous and ferric ammonium sulfate were freshly prepared in distilled deionized water shortly before use. RNase T_1 from *Aspergillus oryzae* was purchased from Boehringer Mannheim. RNase-free RQ1 DNase was purchased from Promega Biotec (Madison, WI). TLC plates (silica gel 60 A) with fluorescent indicator were from Whatman (Clifton, NJ). All electrophoresis reagents were purchased from Bio-Rad (Richmond, CA).

Treatment of tRNA with iron bleomycin and electrophoresis of products. All solutions and glassware were autoclaved for 20 min to eliminate ribonuclease activity. Reaction mixtures contained 3 mM tRNA and 0.3 mM bleomycin in 5 mM phosphate buffer, pH 7.0. Reactions at 20° were initiated by the addition of Fe(II) in three aliquots of 0.1 mM over a period of 40 min. After the last addition, the sample was incubated for an additional 20 min. Control reaction mixtures contained 3 mM tRNA plus 0.3 mM bleomycin without iron; 0.3 mM Fe(II); or 0.3 mM EDTA and 0.3 mM Fe(II). Reactions were stopped by dilution of the incubation (1:1, v/v) with electrophoresis sample buffer (7 M urea, 10% sucrose in 25 mM Tris-base, 25 mM boric acid, 0.5 mM EDTA, pH 8.3). Aliquots of 100 μl were applied to a 15% polyacrylamide/0.19% bisacrylamide/7 M urea gel that had been pre-

[1] Recipient of United States Public Health Service Grant GM 40168.

[2] A report of RNA cleavage by a 1,10-phenanthroline-cuprous complex containing an oligonucleotide moiety appeared after submission of this manuscript [C. B. Chen and D. S. Sigman. Sequence-specific scission of RNA by 1,10-phenanthroline-copper linked to deoxyoligonucleotides. *J. Am. Chem. Soc.* 110:6570–6572 (1988)].

ABBREVIATION: TLC, thin layer chromatography.

electrophoresed for at least 3 hr at 200 V (running buffer = 50 mM Tris base, 50 mM boric acid, 1 mM EDTA, pH 8.3). Electrophoreses were carried out at 5° and 150 V until a bromophenol blue marker band reached the bottom of the gel. The gel was then washed and fixed in 1 M acetic acid for 60 min. The gel was stained overnight with 0.2% methylene blue in 0.2 M sodium acetate buffer (pH 4.7) and was destained by multiple washes with water (19).

Identification and isolation of free bases and base propenals. After incubation with activated bleomycin, 2 volumes of ethanol were added to reaction mixtures to precipitate tRNA. The samples were centrifuged, after which the supernatants were evaporated to dryness. The dry material was resuspended in ethyl acetate/isopropanol/water (74:17:9). This step allowed the separation of bleomycin and salts from soluble products, which were then chromatographed on a TLC plate developed in the same solvent. Authentic adenine, guanine, thymine, and cytosine were also chromatographed. DNA was used to provide a product profile because four free bases and four base propenal spots are obtained on TLC after treatment with activated bleomycin. The nucleic bases released from tRNA or DNA were eluted and identified by their UV absorption spectra at alkaline pH. The base propenals released from DNA were identified by the pink color developed on the plate after spraying with thiobarbituric acid followed by heating at 100°. Total base propenal was assayed (20) in aliquots taken from reaction mixtures after incubation with Fe(II)-bleomycin.

Fluorimetric titration of bleomycin with nucleic acids. A Perkin-Elmer fluorescence spectrophotometer (MPF-3L) was used for fluorescence measurements. Bleomycin (20 μM) was titrated with a concentrated solution of DNA or tRNA in 5 mM phosphate buffer, pH 7.0. The emission at 350 nm was monitored upon excitation at 300 nm. A maximum quenching effect was observed at a nucleotide to drug ratio approximately equal to 30:1 for both nucleic acids, and no quenching beyond 50% could be achieved at higher ratios. This quenching factor (0.5) was used in the calculation of a binding equilibrium constant according to a published procedure (21).

EPR spectroscopy. EPR spectra were recorded at 77 K with a Varian E-112 spectrometer equipped with a Varian NMR gaussmeter and a Polytechnic Research and Development Co. frequency meter. Frozen samples, which contained 50% ethylene glycol, were held in a liquid nitrogen cold finger. Precision bore EPR tubes (Wilmad, Buena, NJ) were used when signal intensities needed to be compared from one sample to another. Also, in order to ensure equivalent concentrations of iron in parallel samples, a stock solution of Fe(III)-bleomycin was prepared and divided as required.

Results and Discussion

Our first aim was to compare the interactions known to occur between bleomycin and DNA with those between the drug and tRNA. Experiments were performed using three forms of the drug, ferric bleomycin, activated bleomycin, and apo (metal-free)-bleomycin. The function of bleomycin in DNA cleavage is related to its ability to bind to DNA and to bind a metal ion that can participate in catalysis. It seemed possible that binding to ribonucleic acids differs from DNA binding due to the different helical forms of these polymers. DNA in solution usually assumes the B-form whereas the helical regions of tRNA are in the A-form (17, 22). Differences between the two types of helices lie in the disposition of the sugar rings and their attached bases with respect to the helix axis. There is also an enlarged minor groove in the A-helix form (22).

Binding studies. A fluorescence study allowed us to demonstrate the binding of apo-bleomycin to yeast $tRNA^{Phe}$. Bleomycin exhibits a fluorescence near 350 nm due to emission from the bithiazole moiety (23). The yield of fluorescence is quenched as a result of the association of the bithiazole ring with DNA, and this effect has been demonstrated in other studies with bleomycin and various nucleic acids (21, 23, 24). Fig. 1 shows the emission spectrum of bleomycin upon titration with DNA or tRNA. In both cases, a nucleotide to drug ratio of 30:1 gives the maximum quenching (50%). This value is dependent on ionic strength and could not be achieved at higher buffer concentration, consistent with a previous study with bleomycin A_2 and DNA (21). The data suggest that apo-bleomycin binds to tRNA as efficiently as it binds to DNA. A control sample with an ATP to drug molar ratio equal to 30:1 gives no quenching.

Fluorescence spectroscopy was also used to determine the equilibrium constant for the binding of bleomycin to tRNA. This method (21) involves titration of a dilute solution of nucleic acid with bleomycin over a broad range of drug concentrations. Data analysis gives the concentration of free and bound bleomycin. A double reciprocal plot allows calculation of the equilibrium constant and the number of binding sites per mole of tRNA (21). The results give a value of 0.86×10^5 M^{-1} for the binding constant and 0.04 for n, which is the number of binding sites per mole of nucleotide. These values are similar to those reported for the binding of bleomycin A_2 to DNA (21). The n value per nucleotide for tRNA extrapolates to 3 bleomycin binding sites/mol. The $tRNA^{Phe}$ structure contains 42 of 76 nucleotides in A-helix domain, although nearly all the bases (71 of 76) participate in stacking interactions (22). The similarity of the fluorescence results for both nucleic acids shows that the binding of the drug is not influenced by the difference in structure between DNA and tRNA. The binding stoichiometry calculated in the fluorescence study with DNA (21) showed that a five-base pair stretch of double helix constitutes a binding unit for one bleomycin molecule. Examination of the tertiary structure of $tRNA^{Phe}$ reveals only three such double helical units available. Therefore, the tRNA result (3 binding sites/mol) is consistent with the binding mode of the drug to DNA. It should be noted that, within the double helical regions of the $tRNA^{Phe}$ molecule (22), there are GpC and GpU(T) sites, which are known to be preferred cleavage sites for bleomycin with DNA (25).

EPR spectroscopy was used to investigate the interaction between Fe(III)-bleomycin and tRNA. The EPR spectrum of Fe(III)-bleomycin can be of the low spin or high spin type, where the spin state depends on buffer type, pH, and the presence or absence of nucleic acids (1). For example, in phosphate buffer, pH 7.0, the iron is high spin and can be converted to low spin by the addition of DNA (1). Even though the physical-chemical origin of this spin state change is not understood, it is useful as an analytical tool to indicate binding of bleomycin to DNA.

Fe(III)-bleomycin with tRNA in 5 mM phosphate buffer, pH 7.0, is nearly all in the low spin form when the nucleotide to drug ratio is 25:1 (Fig. 2). A spectrum of Fe(III)-bleomycin in the presence of a 25-fold molar excess of DNA nucleotide and its spectrum in the absence of nucleic acid are shown for comparison. The spectra illustrate that tRNA is able to induce the same spin state conversion as DNA. However, there is a small increase in the linewidth of the g_{max} ($g = 2.45$) feature with tRNA, compared with DNA. The broadening could reflect structural heterogeneity in the population of bound Fe(III)-bleomycin molecules or may result from differences in nuclear hyperfine interactions in the presence of tRNA. Either phe-

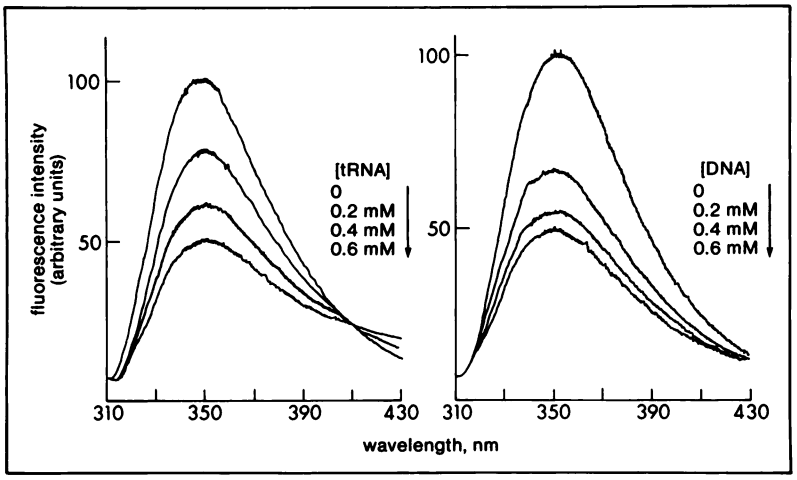

Fig. 1. Titration of bleomycin with tRNA and DNA, monitored by fluorescence quenching. The samples contained 20 μM bleomycin and increasing concentrations of nucleic acids, in 5 mM sodium phosphate buffer, pH 7.0. The excitation wavelength was 300 nm.

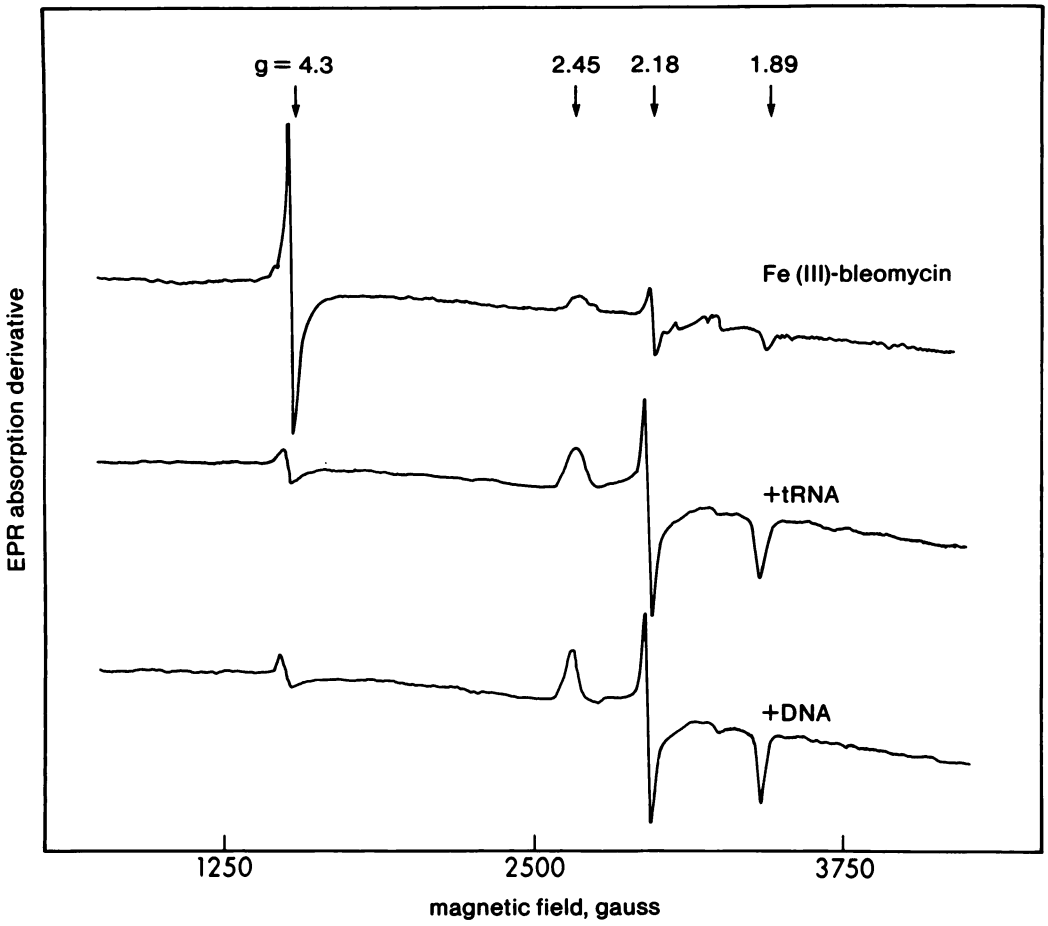

Fig. 2. EPR spectra of Fe(III)-bleomycin in the presence and absence of nucleic acid. The signal at $g = 4.3$ indicates high spin Fe(III)-bleomycin. The signal with $g = 2.45$, 2.18, and 1.89 indicates low spin Fe(III)-bleomycin. Solutions were 50% in ethylene glycol and contained 0.1 mM Fe(III)-bleomycin and 2.5 mM tRNA or DNA in 2.5 mM sodium phosphate buffer, pH 7.0. EPR conditions: microwave frequency, 9.09 GHz; microwave power, 7.0 mW; modulation amplitude, 20.0 gauss; $T = 77$ K.

nomenon could indicate small changes in the ligand environment of the Fe(III) ion induced by tRNA.

The results of other experiments suggest that Fe(III)-bleomycin, as well as activated bleomycin (see below), binds to tRNA. EPR analysis showed that a large fraction of Fe(III)-bleomycin was found associated with the tRNA pellet collected from a mixture of the drug and tRNA after ethanol precipitation of the nucleic acid. A similar behavior was observed with Fe(III)-bleomycin and DNA. No Fe(III)-bleomycin was precipitated in a similar experiment without nucleic acid.

Drug protection by tRNA. Activated bleomycin in the absence of nucleic acid decays to ferric bleomycin, with concomitant damage to a fraction of the drug (1, 26). This self-inactivation is prevented to some extent by the addition of DNA, presumably as a result of drug binding and the protection of the "catalyst," i.e., activated bleomycin, by a substrate that it can attack. A protection of the drug was also found in an experiment with tRNA. The production of base propenal from a given amount of activated bleomycin in the presence of DNA allows for the quantitation of drug available in a sample because the product stoichiometry per Fe(II) supplied is well established (1). In the case in which the activated drug is protected by the nucleic acid, more product would be expected upon the final addition of Fe(II) and DNA to a sample that had been through several activation cycles.

We determined the concentration of bleomycin that remains available for activation after the cycling of the drug with several additions of Fe(II), in the presence and absence of tRNA and DNA. Table 1 shows the yield of base propenal from incubations with and without tRNA, in which the bleomycin had been cycled by activation with three additions of Fe(II). No base propenal is found in the incubation with tRNA and activated bleomycin before the addition of DNA and the fourth aliquot of Fe(II) (lower limit of detection corresponds to approximately 0.1 μM base propenal). The drug sample cycled in the presence of tRNA gave nearly twice the product upon final activation with Fe(II) in the presence of 1 mM DNA. A similar experiment in which the drug was cycled in the presence of DNA showed release of an equal amount of product upon final assay. These results show that the activated drug is protected equally well by DNA and tRNA.[3] Furthermore, an aliquot of the sample containing tRNA, after cycling with Fe(II) but before addition of DNA, exhibits an Fe(III)-bleomycin EPR signal intensity approximately twice that of the sample cycled in the absence of nucleic acid. The EPR analysis, therefore, is in agreement with the assay for yield of base propenal. It may be concluded that the self-inactivation process involves damage to Fe(111)-binding ligands that are protected by tRNA.

The demonstration that bleomycin binds to tRNA, and more importantly that the activated drug binds, implies that the potential exists for activated drug to damage ribonucleic acid.

tRNA cleavage. Several types of tRNA were treated with bleomycin activated *in situ*. Fig. 3 shows a representative electrophoretic pattern of the products after treatment of $tRNA^{phe}$. Several new bands can be observed in the sample treated with activated bleomycin. A similar breakage was observed with other tRNA species $tRNA^{tyr}$, total tRNA from yeast, and $tRNA^{phe}$ or $tRNA^{tyr}$ from *Escherichia coli*), although the patterns are slightly different for each tRNA. The breakage was not observed upon treatment with bleomycin without Fe(II). Treatment with Fe(II) without drug achieves some degradation, which is enhanced by EDTA, but the electrophoretic patterns in these cases are different. Also, the band pattern is different from one obtained upon alkaline hydrolysis of tRNA (data not shown). The extent of breakage was dependent on the concentration of Fe(II)-bleomycin, although all focused patterns show that a large proportion of the tRNA is focused near the same position as the starting material. In fact, the persistence of ethanol-precipitable tRNA in the sample cycled with activated bleomycin indicates that extensive digestion of the polymer did not occur. (The yield of fragments in the sample shown in Fig. 3, *lane 6* represents less than 5–10% breakage of the starting tRNA.) The breakage was not inhibited by 2 μM superoxide dismutase (not shown).

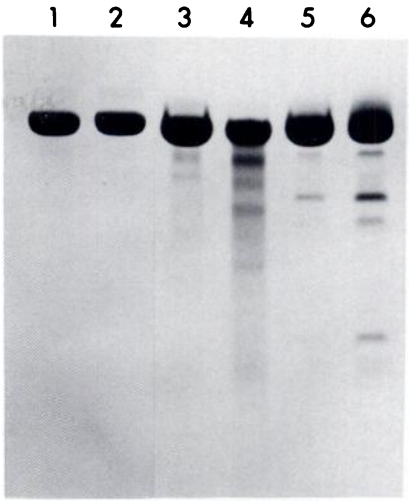

Fig. 3. Polyacrylamide gel electrophoresis of $tRNA^{Phe}$ treated with 0.1 mM or 0.3 mM activated bleomycin (*lanes 5* and *6*, respectively). *Lane 1*, untreated $tRNA^{Phe}$; *lane 2*, treatment with apo-bleomycin; *lane 3*, treatment with Fe(II); *lane 4*, treatment with Fe(II)-EDTA; see Experimental Procedures for details.

We demonstrated that the bands in the focused patterns corresponded to tRNA fragments and not to breakage products from DNA contamination in the following way: 1) the bands do not appear in the pattern of a sample treated with RNase T1 after incubation of tRNA with Fe(II)-bleomycin and 2) the pattern is identical for a sample of tRNA treated with Fe(II)-bleomycin after pretreatment with DNase.

As stated above, the breakage of tRNA by activated bleomycin was not accompanied by release of base propenal, as occurs with DNA. The nucleic bases are other products that can be released from DNA in reactions with activated drug. Therefore, we used TLC to search for purines and pyrimidines in the products from tRNA. A typical digestion of DNA gave a pattern of four nucleic bases and four base propenals, the latter reacting with thiobarbituric acid. Chromatography of the products obtained from tRNA showed two spots, which were identified spectrophotometrically (27) as adenine (R_F = 0.39) and uracil (R_F = 0.64) after elution from the silica in 0.02 M NaOH.

The presence of adenine and uracil demonstrated for the first time that activated bleomycin can initiate free base release from ribonucleic acid, with no concomitant release of base propenals.[4] A recent report (17) suggests that abstraction of the 1′-hydrogen from deoxyribose can occur and could lead to release of free base without formation of base propenal. Furthermore, it was suggested that attack at the 1′-position would be favored in the A form of nucleic acid helix. The helical

TABLE 1

Yield of base propenal from DNA breakage after cycling of bleomycin with Fe(II)

Reaction mixtures (in 20 mM sodium phosphate, pH 7.0) contained 2 mM nucleic acid, 0.1 mM bleomycin, and 0.24 mM Fe(II) added in three 0.08 mM portions. Base propenal was assayed in an aliquot removed 20 min after addition of 1.0 mM DNA and a fourth portion of Fe(II). No base propenal was detected in the tRNA sample before addition of DNA and the fourth portion of Fe(II). Numbers in parentheses are number of experiments.

Reaction Mixture	Base Propenal
	μM
Bleomycin, Fe(II)	3.37 ± 0.06 (4)
tRNA, bleomycin, Fe(II)	6.84 ± 0.039 (4)
DNA, bleomycin, Fe(II)	6.47 ± 0.11 (4)

[3] The results also contain a clue concerning the nature of the self-inactivation reaction. The activated drug may be degraded in an intramolecular oxidation, which is prevented when it is bound to DNA where the oxidation of deoxyribose occurs instead, or the auto-oxidation may be intermolecular, with attack by activated bleomycin on a second drug molecule. The rate of this intermolecular reaction should be slowed when the drug is bound to nucleic acid. Equal protection occurs with tRNA and DNA, although the yield of substrate oxidation seems to be far less in the tRNA case. This suggests that the auto-oxidation is an intermolecular reaction.

[4] Free bases were also released from tRNA treated with Fe(II)-EDTA, in a reaction that was completely inhibited by mannitol (10 mM), a reagent known to scavenge hydroxyl radicals (28). Mannitol did not affect base release from tRNA by activated bleomycin.

regions of tRNA are known to be in the A form (22) and the release of adenine and uracil is, therefore, consistent with 1′-hydrogen abstraction. From these preliminary studies, it is not yet possible to correlate the free base release with the chain breakage demonstrated by electrophoresis. Nevertheless, the observation of four new bands as the principal degradation fragments suggests that there are at least three cutting sites preferred by the drug, which agrees nicely with the number of double helical regions that provide potential binding domains (29). Also, the appearance of uracil and adenine is consistent with the expected cleavage site preference of the drug for GT(U) loci in DNA and with the fact that thymine and adenine are the bases released most readily from DNA in reactions with activated bleomycin.

Conclusions. We have demonstrated for the first time that iron bleomycin can bind to tRNA and can induce breakage of this ribonucleic acid. The failure of other investigations to demonstrate this phenomenon could be ascribed to several factors, among which are 1) the omission of iron from reaction mixtures in some studies; 2) a choice of nucleic acid substrate that binds the drug poorly; or 3) an inadequate sensitivity in the product analyses. The finding that bleomycin attacks tRNA implies that, in the cell, the drug, which has always been considered cytotoxic due to its DNA-damaging potential, could damage a cytoplasmic molecule before its access to nuclear DNA.

Acknowledgments

The authors thank Dr. LaDonne Schulman for helpful discussions.

References

1. Burger, R. M., J. Peisach, and S. B. Horwitz. Activated bleomycin: a transient complex of drug, iron and oxygen that degrades DNA. *J. Biol. Chem.* **256**:11636–11644 (1981).
2. Burger, R. M., A. R. Berkowitz, J. Peisach, and S. B. Horwitz. Origin of malondialdehyde from DNA degraded by Fe(II)-bleomycin. *J. Biol. Chem.* **255**:11832–11838 (1980).
3. Wu, J. C., J. W. Kozarich, and J. Stubbe. Mechanism of bleomycin: evidence for a rate-determining 4′-hydrogen abstraction from poly(dA-dU) associated with the formation of both free base and propenal. *Biochemistry* **24**:7562–7568 (1985).
4. Wu, J. C., J. Stubbe, and J. W. Kozarich. Mechanism of bleomycin: evidence for 4′-ketone formation in poly(dA-dU) associated exclusively with free base release. *Biochemistry* **24**:7569–7573 (1985).
5. Sugiyama, H., C. Xu, N. Murugesan, and S. M. Hecht. Structure of the alkali-labile product formed during iron(II)-bleomycin mediated DNA strand scission. *J. Am. Chem. Soc.* **107**:4104–4105 (1985).
6. Rabow, L., J. Stubbe, J. W. Kozarich, and J. A. Gerlt. Identification of the alkaline-labile product accompanying cytosine release during bleomycin-mediated degradation of d(CGCGCG). *J. Am. Chem. Soc.* **108**:7130–7131 (1986).
7. Burger, R. M., J. Peisach, and S. B. Horwitz. Stoichiometry of DNA strand scission and aldehyde formation by bleomycin. *J. Biol. Chem.* **257**:8612–8614 (1982).
8. Giloni, L., M. Takeshita, F. Johnson, C. Iden, and A. P. Grollman. Bleomycin-induced strand-scission of DNA. *J. Biol. Chem.* **256**:8608–8615 (1981).
9. Uesugi, S., T. Shida, M. Ikehara, Y. Kobayashi, and Y. Kyogoku. Identification of oligonucleotide fragments produced in a strand scission reaction of the d(C-G-C-G-C-G) duplex by bleomycin. *Nucleic Acids Res.* **12**:1581–1592 (1984).
10. Murugesan, N., G. Xu, G. M. Ehrenfeld, H. Sugiyama, R. E. Kilkuskie, L. O. Rodriguez, L. H. Chang, and S. M. Hecht. Analysis of products formed during bleomycin-mediated DNA degradation. *Biochemistry* **24**:5735–5744 (1985).
11. Takeshita, M., A. P. Grollman, E. Ohtsubo, and H. Ohtsubo. Interaction of bleomycin with DNA. *Proc. Natl. Acad. Sci. USA* **75**:5983–5987 (1978).
12. D'Andrea, A. D., and W. A. Haseltine. Sequence specific cleavage of DNA by the antitumor antibiotics neocarzinostatin and bleomycin. *Proc. Natl. Acad. Sci. USA* **75**:3608–3612 (1978).
13. Mirabelli, C. K., A. Ting, C. H. Huang, S. Mong, and S. T. Crooke. Bleomycin and talisomycin sequence-specific strand scission of DNA: a mechanism of double-strand cleavage. *Cancer Res.* **42**:2779–2785 (1982).
14. Haidle, C. W., M. T. Kuo, and K. K. Weiss. Nucleic acid specificity of bleomycin. *Biochem. Pharmacol.* **21**:3308–3312 (1972).
15. Suzuki, H., K. Nagai, E. Akutsu, H. Yamaki, N. Tanaka, and H. Umezawa. On the mechanism of action of bleomycin-strand scission of DNA caused by bleomycin and its binding to DNA *in vitro*. *J. Antibiot.* **23**:473–480 (1970).
16. Sausville, E. A., J. Peisach, and S. B. Horwitz. Properties and products of the degradation of DNA by bleomycin and Fe(II). *Biochemistry* **17**:2740–2754 (1978).
17. Krishnamoorthy, C. R., D. E. Vanderwall, J. W. Kozarich, and J. Stubbe. Degradation of DNA-RNA hybrids by bleomycin: evidence for DNA strand specificity and for possible structural modulation of chemical mechanism. *J. Am. Chem. Soc.* **110**:2008–2009 (1988).
18. Burger, R. M., S. B. Horwitz, and J. Peisach. Stimulation of Fe(II) bleomycin activity by phosphate-containing compounds. *Biochemistry* **24**:3623–3629 (1985).
19. Grierson, D. in *Gel Electrophoresis of Nucleic Acids*, (D. Rickwood and B. D. Hames, eds.). IRL Press, Oxford, England, 1–38 (1982).
20. Haidle, C. W., K. K. Weiss, and M. T. Kuo. Release of free bases from deoxyribonucleic acid after reaction with bleomycin. *Mol. Pharmacol.* **8**:531–537 (1972).
21. Chien, M., A. P. Grollman, and S. B. Horwitz. Bleomycin-DNA interactions: fluorescence and proton magnetic resonance studies. *Biochemistry* **16**:3641–3647 (1977).
22. Saenger, W. in *Principles of Nucleic Acid Structure* (C. Cantor, ed.). Springer-Verlag, New York, 220–300 (1985).
23. Kasai, H., H. Naganawa, T. Takita, and H. Umezawa. Chemistry of bleomycin. XXII. Interaction of bleomycin with nucleic acids, preferential binding to guanine base and electrostatic effect of the terminal amine. *J. Antibiot.* **31**:1316–1320 (1978).
24. Huang, C.-H., L. Galvan, and S. T. Crooke. Interactions of bleomycin analogues with deoxyribonucleic acid and metal ions studied by fluorescence quenching. *Biochemistry* **19**:1761–1767 (1980).
25. Stubbe, J., and J. W. Kozarich. Mechanisms of bleomycin-induced DNA degradation. *Chem. Rev.* **87**:1107–1136 (1987).
26. Nakamura, M., and J. Peisach. Self-inactivation of Fe(II)-bleomycin. *J. Antibiot.* **41**:638–647 (1988).
27. H. A. Sober, ed. *CRC Handbook of Biochemistry.* Chemical Rubber Co. Cleveland, OH (1968).
28. Halliwell, B., and J. M. C. Gutteridge. Formation of a thiobarbituric-acid-reactive substance from deoxyribose in the presence of iron salts. *FEBS Lett.* **128**:347–352 (1981).
29. Saenger, W. in *Principles of Nucleic Acid Structure* (C. Cantor, ed.). Springer-Verlag, New York, 331–349 (1985).

Send reprint requests to: Dr. Richard S. Magliozzo, Department of Molecular Pharmacology, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461.