Cleavage of Nucleic Acids by Bleomycin

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Contents

A native New Yorker, Dr. Burger began studying antibiotics after school at the Haskins Laboratories with S. H. Hutner. His predoctoral career included periods at Brandeis, Adelphi, Caltech, Princeton, and Harvard. The Brandeis stays were most productive, yielding publications on insectivorous plants and on mammalian purine metabolism but no Brandeis degree. At Princeton he did nothing publishable but presented a dissertation from Brandeis for the Ph.D. Postdoctoral studies of DNA replication at the Virus Laboratory (Berkeley) preceded an appointment at the Middle East Technical University (Ankara). The likeness above was made en route to Ankara but now the beard is gray. In New York City since 1972, he left the Sloan-Kettering Institute for the Albert Einstein College of Medicine and began bleomycin mechanism studies for which the Leukemia Society of America named him Stohlman Memorial Scholar. At the Public Health Research Institute (a private, nonprofit association of basic biomedical researchers) since 1986, he continues studying bleomycin and other chemotheraputic agents. He is also Volunteer Professor of Chemistry at CCNY (City College of the City University of New York), Visiting Research Professor of Biology at New York University, and Chief Scientist of the Redox Pharmaceutical Corporation of Greenvale, NY. Every other week he performs old sacred motets with the Renaissance Street Singers.

I. Introduction

Bleomycin, unlike most DNA-damaging agents, attacks neither the nucleic bases nor the phosphate linkages, even though products of its attack include both nucleic base derivatives and phosphomonoester termini. These are final products of a deoxyribose oxidation that resembles free radical damage of DNA by ionizing radiation.[1−4] The resolution of bleomycin damage differs from that of radiation damage by producing a narrower spectrum of products. Involvement of iron and reduced oxygen species in bleomycin action suggested that bleomycin might attack DNA via oxygen radicals such as hydroxyl. While radical chemistry almost certainly plays a role in bleomycin mechanisms, including a putative oxygen-centered DNA derivative, bleomycin more closely emulates a micro-oxygenase than a Fenton reaction.

The bleomycins (Figure 1) and their structural congeners are among the best-understood DNA-cleaving antibiotics. The first to be studied was phleomycin, discovered 42 years ago,[5] followed by bleomycin,[6,7] tallysomycin,[8,9] and many others (reviewed by Hecht[10]). Bleomycins are isolated as copper complexes from the culture medium of *Streptomyces verticillis,* but administered to patients in metal-free form, which minimizes irritation at the site of injection. The commercial preparation, called Blenoxane, a mixture of apo-bleomycins, predominantly bleomycins A_2 and B_2, has found wide clinical antitumor use,[11,12] and is being succeeded by congeners such as peplomycin and liblomycin, with milder cytotoxicity than bleomycin.[13,14]

Early evidence that bleomycin cytotoxicity is mediated by its disruption of DNA in vivo, rather than through any effect on protein or RNA synthesis,[15] is consistent with its induction of lysogenic bacteriophage in bleomycin-treated bacteria,[16−19] a process

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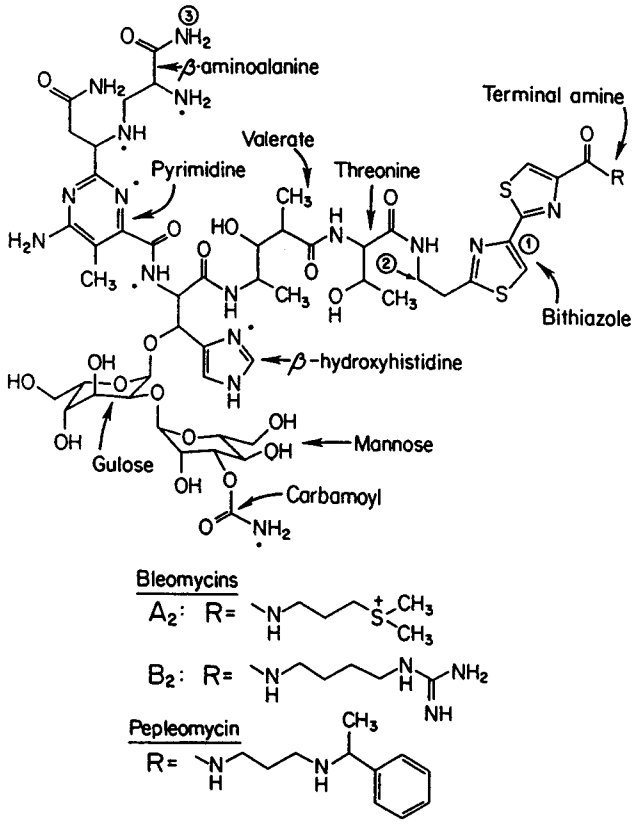


Figure 1. Bleomycin structures. Different bleomycins share the structure shown and vary only in the terminal substituent denoted R. The termini for bleomycins A_1, A_2, and peplomycin are shown. Phleomycin has the same structure as bleomycins except for the lack of conjugation in one of the thiazole moieties indicated (1). Tallysomycin is the same as bleomycin except for a talose 1-glycoside at the position indicated (2) and replacement of the valerate α-methyl by a hydrogen. The metal ligands are indicated by a (•), but sometimes the mannose carbamoyl nitrogen may substitute for that of the β-aminoalanine primary amine, as shown in Figure 5. From ref 24. Copyright 1996 Elsevier Science and reproduced with their kind permission.

triggered by DNA damage. The hypothesis that DNA damage is the primary locus of bleomycin cytotoxicity is further supported by findings that bleomycin-treated cells contain DNA broken with the same site-specificity seen in vitro,[20] that bleomycin survivors are mutagenized,[21−24] and that DNA repair-deficient mutants are especially bleomycin-sensitive.[25,26] There is evidence of collateral damage however, since bleomycin, once thought to atttack DNA exclusively,[27−29] has been shown also to attack RNA,[30,31] cell walls,[32] and small organic molecules in vitro.[33,34] In one study, a bleomycin degradation product rendered incapable of attacking DNA was toxic.[35]

Bleomycin requires oxygen species and metals as cofactors, for reasons that are now understood in considerable detail.[36−38] Analysis of bleomycin reactions began with the discovery[39] that the presence of reducing agents or hydrogen peroxide permits bleomycin to cleave DNA in simple reaction mixtures. Indications that metals[39−42] and oxygen species[40−42] were also required led to the demonstration[43,44] that Fe(II) and O_2 sufficed as bleomycin cofactors for DNA degradation, forming an Fe(II)•bleomycin complex

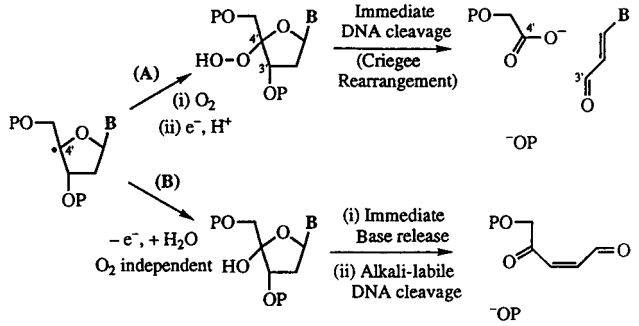


Figure 2. DNA degradation products. There are two sets of products, one of frank strand cleavage (A) and one of oxidative base release (B). The former, including base propenal, are stable and have been isolated directly; the later, which include free nucleic base, induce alkali lability of the injured strand. The proposed product of alkali-induced cleavage by β-elimination of the 3′-phosphate is shown. From ref 4. Copyright 1995 Elsevier Science and reproduced with their kind permission.

that reacted with O_2. Further studies showed that Fe(III)•bleomycin reacted with peroxide[45,46] or superoxide[47−49] to give a drug intermediate that degrades DNA the same way Fe(II)•bleomycin + O_2 does. Thus, bleomycin has several routes to DNA-degrading competence, but the relative importance of these routes in vivo is unclear, as is their dependence on physiological circumstances such as the abundance of oxygen species.

In vitro DNA degradation has two outcomes[50,51] associated with two sets of products.[52] One set of these products includes oligomers terminated by 5′-phosphate[53] and 3′-phosphoglycolate moieties.[54] The remainder of the cleaved nucleoside (which yields the phosphoglycolate) is converted to nucleic base derivatized with deoxyribose carbons 1−3,[52] compounds characterized as base propenal:[54] 3-(pyrimidin-1-yl)-2-propenals and 3-(purin-9-yl)-2-propenals (Figure 2). In the earlier literature[55,56] this is called malondialdehyde, so misidentified because both malondialdehyde and base propenals give the same derivative with 2-thiobarbituric acid,[52] a chromophore ($\epsilon_{532} = 1.6 \times 10^5$) which provides a very sensitive and reproducible assay of bleomycin-induced DNA cleavage. The other set of DNA degradation products[52] includes free nucleic base[57] and an aglycone deoxyribose product that renders its DNA strand alkali labile.[50]

The reactions between bleomycin and DNA are somewhat austere: although every bleomycin intermediate observed manifests interactions with DNA, bleomycin and DNA exchange only electrons, not atoms. The reactions of bleomycin in activating oxygen were often studied separately from the resolution of DNA damage. The major gaps in our understanding occur where the drug activation and DNA degradation pathways meet; where the intermediate species are the shortest-lived and hardest to characterize. These intermediates and their reactions are quite interesting, since they may model inaccessible reactions of other oxidative DNA-cleavers or of the oxygenases and peroxidases that bleomycin seems to emulate.

The reactions enabling iron−bleomycin to attack DNA will be considered first, followed by the events

that complete the degradation of DNA. The intervening rapid reactions of bleomycin and DNA, being the most speculative, are then considered. Reactions of bleomycin with metals other than iron and targets other than DNA are addressed last.

II. Activation of Bleomycin

The dissection of drug activation and DNA degradation pathways proceeded from the observation of bleomycin activity in vitro.[39] The first reaction pathway of Fe−bleomycin activation to be elucidated was that of Fe(II)·bleomycin with O_2. It approximates a single-turnover reaction, since its Fe(III)·bleomycin product does not react with O_2 to initiate subsequent cycles. The reaction of Fe(II)·bleomycin + O_2 attacking DNA was initially thought to be rapid.[43,44] However, when kinetic measurements were based on halting the reaction by acidification rather than using only EDTA, which prevents but does not halt the ongoing reaction, irreversible steps in DNA degradation were observed to approach completion gradually. Indeed, the reaction was slow, displaying $t_{1/2} = 8$ s (at room temperature)[58] or 2 min (at 6 °C).[45] The slowness of the DNA cleaving reaction is visually evident: when Fe(II) is added to a (colorless) solution of bleomycin and DNA even at room temperature, an intense yellowing is followed several seconds later by loss of viscosity, signaling separate changes in the drug and its target.

Since the effects on DNA were relatively slow, it was possible to focus attention on the formation kinetics of oxygenated Fe−bleomycin intermediates and their reactions. These proved to be analogous to those of heme-containing oxygenases and peroxidases.[59] However, the drug and enzyme intermediates differ in their relative stability,[60,61] with the earlier oxygenated bleomycin species being more stable than their enzymatic counterparts, while the later (inferred) bleomycin species seem far less stable than such well-characterized counterparts as peroxidase compounds I and II. The relative stability of the early oxy−bleomycin species makes them amenable to observation, which indicated the one that was kinetically competent to initiate DNA degradation. This species was named activated bleomycin.[45] Considerable effort has been devoted to characterizing its structure and reactions and speculating on what might be the proximate DNA-attacking intermediate.

A. Fe(II) and O_2 as Activation Cofactors

The first step in the reactions leading to activated bleomycin was monitored by stopped-flow spectroscopy.[62] Two kinetic events are observed optically, following addition of O_2 to anaerobic Fe(II)·bleomycin. The rate of the first shows a first-order dependence on Fe(II)·bleomycin and O_2, with $k = 6.1$ M^{-1} s^{-1}.[62] Its product accumulates transiently, but it could be trapped by rapid freezing for EPR spectroscopy. This first intermediate is EPR-silent, and so it was inferred to be an even-spin O_2−Fe(II)·bleomycin complex. At Fe(II)·bleomycin concentrations > 4 μM in the absence of DNA the intermediate was consumed in an apparent first-order reaction with $k = 0.1$ s^{-1}.[62] Thus, under atmospheric oxygen (0.4 mM) the formation reaction is about 30 times as rapid as the consumption. This second reaction is strongly inhibited by DNA when [DNA(nucleotide)]:[bleomycin] > 40,[63] which effectively traps the first oxygenated intermediate in a DNA complex. When this intermediate was submitted to Mössbauer spectroscopy in a high magnetic field, it proved to be diamagnetic. Its unexpectedly large quadrupole splitting, $\Delta E_Q = 2.9$, indicated that the iron valence state was Fe(III). Therefore, the favored resonance form of the ternary complex, O_2·Fe(II)·bleomycin ↔ $^{\bullet}O_2^-$·Fe(III)·bleomycin, is the ferric superoxide complex.[64] Nevertheless, the intermediate is still usually called oxy-ferrous-bleomycin. The reaction of this species was deceptively simple when monitored optically: it spontaneously forms a species that was optically indistinguishable from Fe(III)·bleomycin,[62] the final product of Fe(II)·bleomycin + O_2. This simple optical transition concealed important intermediate steps.

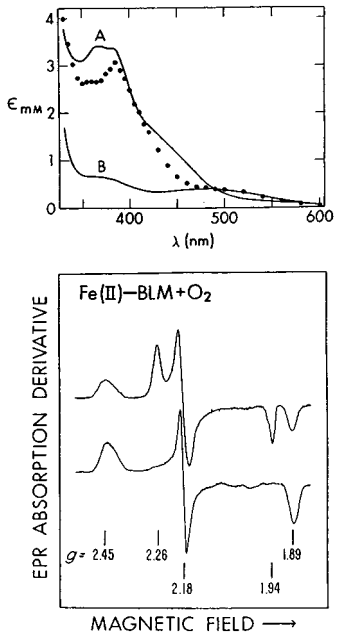


Figure 3. Optical and EPR spectra of iron−bleomycins. The optical species (upper panel) are ferric bleomycin (denoted A; indistinguishable from activated bleomycin), ferrous bleomycin (denoted B), and oxy-ferrous bleomycin (experimental points). The EPR species (lower panel) are a mixture of activated bleomycin and low-spin ferric bleomycin (above), and its decay product, ferric bleomycin, alone (below). In phosphate buffer with DNA absent, ferric bleomycin is a high-spin, $g = 4.3$, species (not shown). From ref 62. Copyright 1979 the American Society for Biochemistry & Molecular Biology.

The kinetic spectral event coinciding with DNA attack is not optical but magnetic. In addition to the two optical transitions described above, two magnetic transitions are observed by stop−freeze EPR spectroscopy (Figure 3). The first coincides with the conversion of the second optical species to the third, and consists of the appearance of not one but two EPR species in equal amounts. One is indistinguishable from the final product, Fe(III)·bleomycin (g = 2.45, 2.18, 1.89 or in the presence of phosphate and

no DNA, $g = 4.3$),[46,62,65,66] while the other is more isotropic ($g = 2.26, 2.17, 1.94$) and less stable than Fe(III)·bleomycin. The unstable species converts spontaneously and stoichiometrically to Fe(III)·bleomycin.[45,46] This conversion coincides with the initiation of DNA degradation but not with any evident optical change. These events occur with $t_{1/2} \approx 2$ min at 4 °C, and the rate of EPR conversion (in phosphate buffer) is insensitive to the presence or absence of DNA, suggesting that DNA is not a direct participant in the rate-limiting step of activated bleomycin decay.

The reaction converting oxy-ferrous-bleomycin to activated bleomycin is a disproportionation of oxy-ferrous-bleomycin.[46] This EPR-silent precursor gives rise to equimolar quantities of the odd-spin species: 1 equiv of oxy-ferrous-bleomycin being reduced to activated bleomycin by another equivalent. One Fe(III)-complexed $^{\bullet}O_2^-$ is oxidized to O_2 which is released, being unreactive with Fe(III)·bleomycin, while the other $^{\bullet}O_2^-$ complex is reduced to the level of a ferric peroxide.[67] The first-order kinetics of this reaction (mentioned above) suggests that a bimolecular step, such as dimer formation, is not rate-limiting. The rate-limiting step may be the subsequent redox reaction, but the reactant, an oxy-ferrous-bleomycin dimer, has not yet been observed.

The stabilization of oxy-ferrous-bleomycin by excess DNA has been attributed to a competitive binding of oxy-ferrous-bleomycin monomers by DNA,[63] which would slow their formation of activated bleomycin. In this case oxy-ferrous-bleomycin is gradually converted to Fe(III)·bleomycin without detectable quantities of activated bleomycin accumulating. At marginally inhibitory ratios of DNA:bleomycin, the intermediacy of activated bleomycin is evident, as minor but observable quantities accumulate. Under such inhibitory conditions, the availability of oxy-ferrous-bleomycin might become rate-limiting instead of either the formation or decay of activated bleomycin, causing the rate of DNA attack to become second-order with respect to [bleomycin]. This was seen (unpublished results) at DNA:bleomycin ≥ 20, consistent with the demonstration that the release of O_2 from DNA-stablized oxy-ferrous-bleomycin is a second-order reaction.[68]

The oxygen structure in activated bleomycin remained mysterious for over a decade. Earlier evidence from (a) EPR spectroscopy of complexes containing ^{57}Fe and ^{17}O,[45] (b) Mössbauer spectroscopy,[64] and (c) redox titrations[67] were consistent with it being either a ferric peroxide complex (end-on or Fe-bridged) or a monooxygenated 2e oxidant homologous to peroxidase compound I. Activated bleomycin is

$$\mathrm{Fe(III)O^0} \quad \textit{or} \quad \mathrm{Fe(III)^{-}O\text{-}OH} \quad \textit{or} \quad \mathrm{Fe(III)}\begin{Bmatrix} \mathrm{O} \\ \| \\ \mathrm{O} \end{Bmatrix}$$

represented in papers by different groups as having either a mono- or dioxygenated structure. Proofs of the actual structure emerged from (a) demonstration by electrospray mass spectroscopy that activated bleomycin, when formed from ^{18}O-peroxide, contains both labeled oxygen atoms,[60] and (b) an $^{18}O_2$ kinetic isotope effect on activated bleomycin breakdown of a magnitude consistent only with peroxide O−O cleavage being rate-limiting.[69] Evidence that the peroxide is bound to Fe end-on comes from mass spectroscopic evidence consistent with a hydrogen nucleus accompanying the two peroxide oxygens[60] and from ENDOR (electron−nuclear double resonance) spectroscopy of activated bleomycin formed from $^{17}O_2$, which showed only one Fe-bound O atom.[70] There is no evidence for a short Fe=O bond in activated bleomycin subjected to X-ray absorption spectroscopy (XAS) and extended X-ray absorption fine structure (EXAFS) analysis.[71]

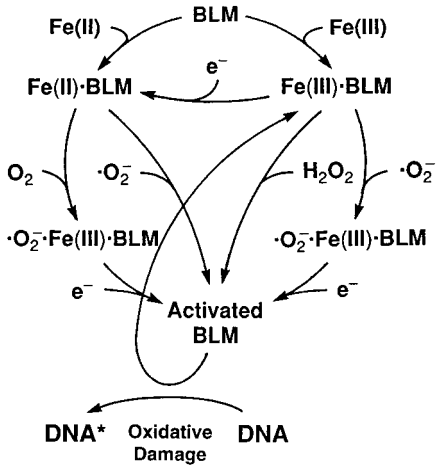


Figure 4. Activated bleomycin formation pathways. Bleomycins react with oxygen species and reductants as shown to form the transient ferric peroxide indicated as activated bleomycin. Details of its conversion to Fe(III)·bleomycin are proposed in Figure 8. From ref 156. Copyright 1996 Kluwer Academic Publishers and reproduced with their kind permission.

In summary, Fe(II)·bleomycin plus O_2 *(*in vitro) yield $^{\bullet}O_2^-$·Fe(III)·bleomycin, a high-spin,[64] EPR-silent[62] Fe(III) species. This undergoes a disproportionation in which the oxidized product is O_2 plus Fe(III)·bleomycin, and the reduced product is activated bleomycin,[46] a peroxide−Fe(III)·bleomycin complex. This breaks down with peroxide cleavage. No further oxygenated intermediates have yet been observed.

B. Other Bleomycin Activation Routes

Several other routes to activated bleomycin formation are available besides that with Fe(II) and O_2 (Figure 4). They have been exploited for resolving the roles of O_2 in bleomycin-induced DNA reactions. DNA cleavage can be obtained when Fe(III) is substituted for Fe(II), provided that additional reducing agents are included.[43] Continuing regeneration of Fe(II)·bleomycin from its O_2 oxidation product, Fe(III)·bleomycin, makes this reaction cyclic.

1. Peroxides

Fe(III)·bleomycin can also react with peroxides, such as H_2O_2 (Figure 4) or ethyl hydroperoxide, to produce a steady-state level of activated bleomycin[45] and form base propenal as a DNA cleavage product. Although peroxide can function as a reductant, its

reaction with Fe(III)·bleomycin appears not to involve reductive recycling to Fe(II)·bleomycin, since activation by peroxides is unaffected by agents that trap Fe(II)·bleomycin, such as ethyl isocyanide,[45] an O_2 analogue that binds Fe(II)·bleomycin and competes with O_2.[58] The direct reaction of H_2O_2 and Fe(III)·bleomycin was confirmed when they were combined in the absence of O_2.[45] The activated bleomycin EPR spectrum formed in such peroxide mixtures[45,46] shows kinetics of decay identical to that of the O_2 product once the cyclic reaction is interrupted, e.g., by addition of DNA at high enough concentrations to inhibit the reaction of peroxide and Fe(III)·bleomycin.[45] As with O_2-derived activated bleomycin, DNA attack by the peroxide product is concurrent with conversion of the activated bleomycin EPR spectrum to that of Fe(III)·bleomycin. Further support for the identity of activated bleomycin formed by these two routes derives from other comparisons, including that of Mössbauer[64] spectra and their DNA degradation products.[72,73]

Paradoxically, anaerobic activated bleomycin solutions can be formed by the O_2 route as well as by the peroxide route, provided that [Fe(II)·bleomycin] $\geq$ 4[O_2] so that all the O_2 is consumed.[72] Such procedures were useful in resolving the additional O_2 requirement for DNA scission[45] and in tracing the origins of the oxygenated DNA products[74] discussed in section III.B.

2. Superoxide

Another dioxygen species, superoxide ($^{\bullet}O_2^-$), can also participate in activated bleomycin formation. Several roles for $^{\bullet}O_2^-$ are possible (Figure 4), so when effects of superoxide consumption by superoxide dismutase (SOD) were noted on the activity of bleomycin,[40,75,76] it was unclear whether the effect was on precursors or products of activated bleomycin. Microsomal enhancement of bleomycin activity is antagonized by SOD,[47,77,78] as is that of soluble superoxide-generating enzymes.[40] Microsomal superoxide was proposed to operate in vivo where the huge excess of DNA to bleomycin[79] may be expected to prevent the activation pathway based on dismutation of oxy-ferrous-bleomycin, as it does in vitro.[63] Superoxide was shown to reverse this inhibition when added to DNA-stabilized oxy-ferrous-bleomycin, reinforcing the proposal that superoxide facilitates bleomycin action in vivo.[48] Superoxide may participate in bleomycin activation in other ways as well (Figure 4). For example, it may react with either Fe(III)- or Fe(II)·bleomycin to respectively form either $^{\bullet}O_2^-$·Fe(III)·bleomycin or activated bleomycin. It may also act as a reductant only, reducing either Fe(III)·bleomycin to Fe(II)·bleomycin or $^{\bullet}O_2^-$·Fe(III)·bleomycin to activated bleomycin. It is difficult to determine which reactions superoxide performs, since it dismutates spontaneously to from H_2O_2 and O_2, which are themselves capable of reacting with Fe−bleomycin species. The reduction of complexed Fe(III) to Fe(II) by superoxide without the product O_2 immediately reacting with the Fe(II) is unlikely to contribute to bleomycin activity. This is because Fe(II)·bleomycin is extremely sensitive to inactivation by H_2O_2, even when furnished together with O_2 (unpublished observations and ref 80), and both form rapidly in aqueous superoxide solutions. The DNA-cleaving reaction of Fe(III)·bleomycin + KO_2[81] may also proceed by formation of $^{\bullet}O_2^-$·Fe(III)·bleomycin and its reduction to activated bleomycin by more superoxide.[48] Superoxide must also be able to react with Fe(II)·bleomycin, since the DNA cleavage obtained with addition of KO_2 solution does not occur when similar concentrations of its products, O_2 + H_2O_2, which are rapidly formed on addition of water, are furnished instead (unpublished observations). The effects of superoxide on bleomycin activity in vivo are even more difficult to interpret due to the complexity of living test systems, e.g., in which superoxide-liberated Fe may enhance Fe-mediated DNA damage independent of bleomycin.[83]

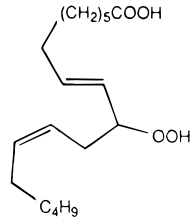
C. Activated Bleomycin Decay

The initial step in conversion of activated bleomycin to Fe(III)·bleomycin does not appear to involve DNA. It occurs in the presence or absence of DNA, which has little, if any, effect on the rate of activated bleomycin decay.[45] An interaction of DNA with activated bleomycin is, however, indicated by an alteration of its $g = 1.94$ EPR feature;[45] interactions with putative short-lived products of activated bleomycin are considered in section IV. Studies of activated bleomycin reactions apart from DNA may provide information about the formation and nature of the bleomycin species that attack both DNA and other target molecules, including bleomycin itself.

1. Oxygen Products

Cleavage of the activated bleomycin peroxide may yield the proximate DNA-attacking intermediate, which might be either hydroxyl radical ($^{\bullet}$OH) or a compound I homologue, [bleomycin·Fe=O]^{V}, depending on whether the cleavage proves to be homolytic or heterolytic. Either species could in principle account for the observed DNA degradation products.[84] The specificity of DNA attack, limited to H4′ (vide infra), suggests that hydroxyl radical, a promiscuous reactant, is unlikely to be involved, since other deoxyribose hydrogens lie unaffected nearby. Regardless of whether the O−O cleavage is homolytic or heterolytic, it is likely that a reoxidation of [bleomycin·Fe=O]^{IV} to [bleomycin·Fe=O]^{V} frequently occurs and that the latter species mediates both DNA cleavage and alkali labilization (see section III.B). The existence of [bleomycin·Fe=O]^{V} strengthens the possibility that it is also the O−O cleavage product.

Attempts were made to determine whether bleomycin O−O cleaves homolytically or heterolytically by assessing the activities expressed by iron−bleomycin presented with a peroxide, 10-hydroperoxy-8,12-octadecadienoic acid, that only cleaves homolytically.[85,86] The two groups carrying out these studies reached opposite conclusions. The mixture of the peroxyacid plus Fe(III)·bleomycin resembled activated bleomycin in cleaving DNA and in oxidizing small, organic molecules, but the DNA cleavage yield was small. Thus, the observed DNA cleavage may



have resulted from a minor reaction not directly involving a homolytic O−O cleavage product.[86] However, 10-hydroperoxy-8,12-octadecadienoic acid is likely to interfere with the close steric approximation of bleomycin and DNA and the putative homolytic O−O cleavage product would probably be short-lived. Therefore, a low yield of DNA cleavage would be expected regardless of whether homolytic O−O cleavage was the normal route to DNA-cleaving competence. A better indicator of whether the homolytically cleaved peroxyacid forms activated bleomycin is probably the spectrum of small molecule reactions mediated by the bleomycin reaction mixture. Examination of the reaction products suggests that the monooxygenated product of activated bleomycin differs from the homolytic peroxide cleavage product,[86] and thus supports the hypothesis of heterolytic O−O cleavage to form an analogue of peroxidase compound I.

Another type of experiment suggests how activated bleomycin decay produces a species capable of oxidizing its bound peroxide. Activated bleomycin, a two-electron oxidant,[67] may yield the final product, Fe(III)·bleomycin, by oxidizing either DNA or some other reductant, but when activated bleomycin decays spontaneously in the absence of reductants, it must react with an oxygenated bleomycin species. Then the stoichiometry of complete oxygen reduction by iron−bleomycin is the expected four Fe(II) per O_2, and only half the oxygen bound as peroxide in activated bleomycin is reduced.[46] Activated bleomycin releases no $^{\bullet}O_2^-$ and minor (≤0.1 equiv) quantities of H_2O_2.[62] The only product of $^{17}O_2$ detected, using NMR and $^{17}O_2$-depleted water, was $H_2{}^{17}O$ and, as expected, only half of the activated bleomycin oxygen was converted to water in the absence of reductants.[87] This stoichiometry is consistent with the destruction of bound peroxide by disproportionation to yield $H_2O + {}^1/_2O_2$, thereby regenerating bound O_2. When DNA or other reductants are present, the net O_2 consumption is greater.[46,87] Activated bleomycin disproportionation is not a bimolecular reaction of activated bleomycin, since activated bleomycin decay displays first-order kinetics and since the rate-limiting reaction is an O−O rupture.[69] To determine whether a monooxygenated intermediate such as [bleomycin·Fe═O]^V participates in a bimolecular reaction like

$$2[\text{bleomycin}\cdot\text{Fe}{=}\text{O}]^{V} \rightarrow 2\text{bleomycin}\cdot\text{Fe}^{III} + O_2 \quad (1)$$

activated bleomycin was prepared from a mixture of $^{16}O_2$ and $^{18}O_2$, and the reaction products were submitted to mass spectroscopy to detect ^{16}O-^{18}O. Surprisingly, only the two original O_2 species were found, and no recombinant O_2. Once separated, activated bleomycin oxygen atoms must never recombine (unpublished result, R. M. Burger, B. Luz, and M. Bender). To explain this result, a catalase-type reaction is necessary:

$$[\text{bleomycin}\cdot\text{Fe}^{-}\text{O}{-}\text{OH}]^{V} + [\text{bleomycin}\cdot\text{Fe}{=}\text{O}]^{V} \rightarrow 2\text{bleomycin}\cdot\text{Fe}^{III} + O_2 + OH^- \quad (2)$$

with the activated bleomycin peroxide serving as the reductant. The apparent first-order kinetics of activated bleomycin decay with DNA absent, which are identical to those of DNA attack, are consistent with activated bleomycin peroxide cleavage being the rate-limiting step leading to reaction 2.

2. Bleomycin Suicide

When activated bleomycin decays in the absence of DNA, an irreversible change in bleomycin occurs. It undergoes conversion to one or more Fe(III) complexes unable to attack DNA when aerobic Fe(II) is subsequently added.[58] This conversion is probably responsible for the inactivation of bleomycin seen in reaction mixtures containing ascorbate as a reductant.[88,89] The reaction is called activated bleomycin suicide because activated bleomycin is kinetically competent in this reaction.[45] The presence of DNA can prevent bleomycin suicide, and its protective capacity disappears just as activated bleomycin decays. Suicide chemistry is complex.[90] Several products have been distinguished,[60] and it appears that modification of the bithiazole moiety is involved.[91] The iron ligation properties evident in EPR spectra are unchanged, and the inactivated drug can still react with Fe(II) and O_2 to give a species with the EPR spectrum of activated bleomycin.[91] This is consistent with structural studies placing the peroxide adjacent to the bithiazole except in the presence of DNA.[92,93] Little is known mechanistically, but O_2 is not required.[72] Since the anaerobic suicide products are unexamined, it is possible that oxygen may be involved in alternative suicide reactions. The suicide products, when characterized, should be consistent with the reactivity of whatever active bleomycin species is proposed to initiate DNA degradation, and thus provide an additional means to verify its structure.

Other reactions destroying bleomycin activity are not mediated by activated bleomycin. These include the reaction of Fe(II)·bleomycin + H_2O_2,[80] which may involve hydroxyl radical formation, and the reaction with iodosylbenzene,[94] which proved to be metal-independent and is attributed to hypervalent iodine.[95]

III. DNA Degradation

The binding of bleomycin to the minor groove of duplex DNA was inferred from observations of interference by known minor groove binders[96,97] and inability of major groove obstructions to affect cleavage.[97,98] Moreover, the primary DNA target, H4′, is accessible only from the minor groove. Although bleomycin fragments[99] and model compounds[100,101] have provided structures by X-ray crystallography, no complete bleomycin has been crystallized with or

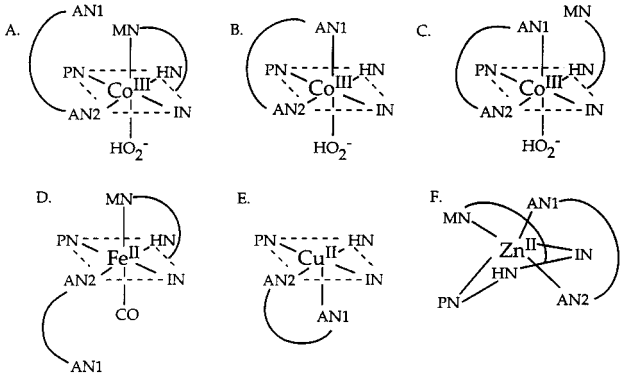


Figure 5. Ligation of metal ions by bleomycin, peplomycin, and P-3A, a truncated bleomycin biosynthetic intermediate. The indicated ligands are: β-aminoalanine primary amine (AN1), β-aminoalanine secondary amine (AN2), pyrimidine N5 (PN), β-hydrohistidine amide (HN), imidazole N1 (IN), and mannose carbamoyl nitrogen (MN). The structures depicted are from (A) Co(III)·peplomycin,[115] (B) Co(III)·deglycopeplomycin,[115] (C) Co(III)·bleomycin A_2[92,114] (D) Cu(II)·P-3A,[99] (E) CO·Fe(II)·bleomycin,[108] (F) Zn(II)·bleomycin.[106] Figure kindly provided by J. Caceres-Cortes and A. H.-J. Wang.

without an oligonucleotide. Direct evidence of drug conformation and the influence on it of DNA and specific nucleotide sequences is emerging rapidly from multinuclear NMR studies of free and oligonucleotide-complexed bleomycin and metallobleomycins. Recent NMR studies confirm that the bleomycin bithiazole moiety, which is planar and aromatic, binds to the nucleic bases in a mode that is partially intercalative.[93,102−104] Positively charged moieties distal to the bithiazole penetrate the major grove and may provide ionic interactions with DNA phosphates. Interference with high-resolution NMR by paramagnetic Fe led to examination of apo-blemycin[105,106] of the low-spin bleomycin·Fe(II)·CO complex,[107,108] and of non-iron metallobleomycins containing such diamagnetic nuclei as Zn(II)[103,106,109−112] or Co(III)[92,93,104,113−115] to model iron−bleomycin species. However, doubts persist about the equivalence of ligation geometries to different metal ions[112,113,115] (Figures 5 and 6), and even about the correct assignment of ligands to the same metals by different laboratory groups. Most important are the differences in axial ligands of the Co and Fe complexes shown in Figure 5. The ligand chiralities reported for HOOCo(III)·bleomycin[92,114] are identical only to those in deglycosylated HOOCo(III)·peplomycin[115] and not to those in intact HOOCo(III)·peplomycin[115]—which are equivalent to those in COFe(II)·bleomycin.[108] The change in axial ligation seen to result from deglycoslation[115] is accompanied by a change in cleavage site specificity,[116] which also changes upon removal of the mannose carbamoyl ligand moiety (Table 1[117]). A misjudgment of how the metal is bound in the drug−DNA complex could confound efforts to deduce the specificity-determining interactions between drug and target.

Activated bleomycin, being short-lived, is unsuitable for liquid-phase NMR, but some structural information such as metal−phosphate distance, deducible from the NMR spectra of other complexes, is subject to verification by ENDOR spectroscopy of

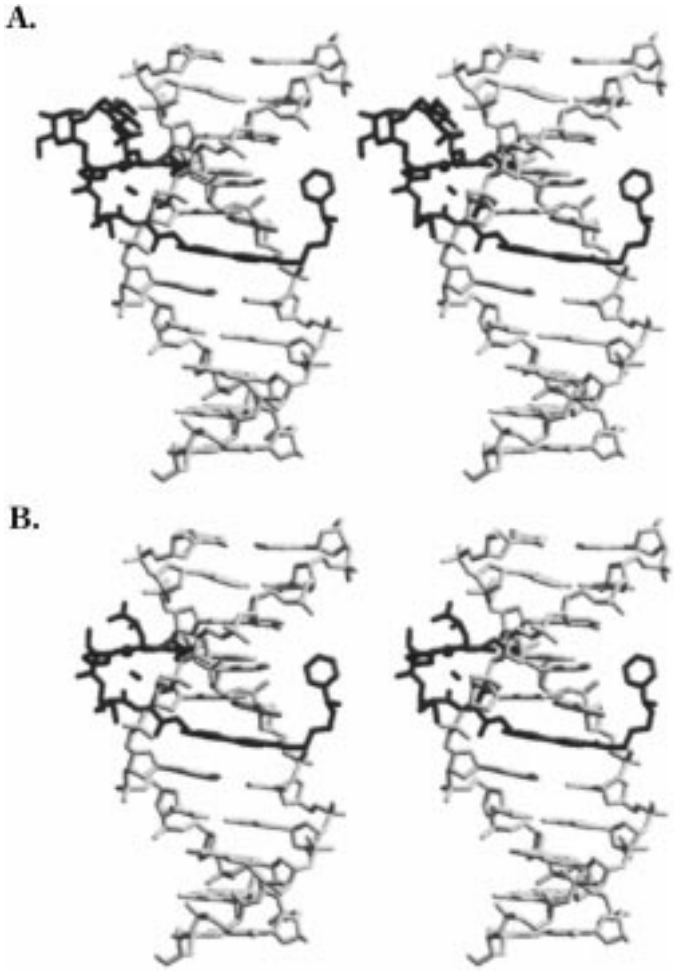


Figure 6. Stereoscopic views of bleomycin−DNA interactions. The structures of d(ATCGTACGAT)$_2$ complexed with HOOCo(III)·peplomycin or with HOOCo(III)·deglycopeplomycin were determined using 2D NMR. The respective ligand conformations are A and B as shown schematically in Figure 5. Figure kindly provided by J. Caceres-Cortes and A. H.-J. Wang.

frozen samples.[70,118] The best evidence that the stable bleomycin·Co(III)−OOH "green complex" is a good model for activated bleomycin conformation derives from comparisons of NMR-derived structures of green complex and Fe(II)·bleomycin. Proton resonances shifted by the paramagnetic Fe(II) have been assigned to putative iron ligand moieties. Their distances from Fe, calculated from relaxation times, T_1,[119] correlate closely with their distances from Co.[114] Complete NMR assignments for several complexes have recently been published by several laboratories along with detailed structures obtained by computerized atomic modeling and energy minimization. The complete NMR assignments are available within, or as supporting material to, the referenced papers for: bleomycin A_2 ^1H[105] and ^{13}C,[106] Zn(II)·bleomycin complexed with d(CGCTAGCG)$_2$,[110] Co(III)·bleomycin and peroxyCo(III)·bleomycin;[92] peroxyCo(III)·bleomycin[114] and its complex with d(CCAGGCTGG)$_2$;[113] bleomycin, Zn(II)·bleomycin, tallysomycin and Zn(II)·tallysomycin,[112] and Co(III)·pepleomin.[93,115] In some cases atomic coordinates are also available,[93] on the basis of intra- and intermolecular nuclear Overhauser effect measurements that have become in-

Table 1. Relative DNA Cleavage Efficiency of Fe(II)·Bleomycin Congener (Reprinted from Ref 117. Copyright 1988 American Chemical Society)[a]

	Fe(II)−bleomycin derivative			
dinucleotide (5′-XY-3)	BLM A_2	epi-BLM A_2	decarbamoyl-BLM A_2	bleomycinic acid
AA	20	0	0	0
AC	20	0	0	0
AG	0	0	0	0
AT	60	20	20	0
CA	0	0	0	0
CC	0	0	0	0
CC	0	0	0	0
CG	0	0	0	0
CT	40	60	0	40
GA	80	60	60	60
GC	100	80	80	20
GG	60	80	40	20
GT	100	100	100	80
TA	40	20	20	20
TC	60	20	40	20
TG	0	60	0	0
TT	40	0	40	0
PuPu	40	35	25	20
PuPy	70	35	40	25
PyPu	25	35	5	5
PyPy	40	20	15	5

[a] The congener structures are as shown in Figure 1 except that (a) epi-bleomycin A_2 differs stereochemically at the propionamide C-1 (between pyrimidine and β-aminoalanine residues), (b) decarbamoyl-bleomycin A_2 lacks the mannose carbamoyl function, and (c) bleomycinic acid has no terminal amine, so R = OH.

creasingly numerous since 1994.

NMR has confirmed and clarified earlier structural and conformational inferences, such as the minor groove contacts of bleomycin with DNA and the details of bithiazole intercalation. It has verified the approximation of the target 4′H to complexed peroxide, and the approximation of the bithiazole to the peroxide−ligand site in the absence of DNA.[92,93,113] The detailed conformational information includes indications about which bleomycin moieties recognize the target-specifying nucleic bases and how they do it.[113] Evidence that the metal-binding domain confers most of bleomycin's sequence specificity[120,121] may now be understood in terms of a H-bonding network that includes two bonds between the bleomycin metal-binding pyrimidine and the target-specifying guanine, which thereby form a three-base structure.[113,122] Other fine-structural information has provided suggestions about mechanism, such as the locations of three exchangeable hydrogens providing H-bonds between the peroxide and the bleomycin threonine and methylvalerate moieties,[113] a role consistent with a multiple solvent H isotope effect on peroxide cleavage (unpublished data).

In addition to serving as structural models, some nonferrous bleomycins show activities more or less similar to that of iron−bleomycins, and these are described in section IV.B.

A. Specificity

The specificity of DNA attack has two related aspects: nucleotide sequence specificity and mechanistic specificity. For DNA the latter appears quite narrow: bleomycin attacks at the deoxyribose 4′-H only.[123] For activities against non-DNA targets, such as RNA, the degradation mechanisms are not so well understood.[38] The nucleotide sequence specificity may be simply stated, but bleomycin shows a range of target preference. Duplex DNA is preferred,[27,124,125] and the earliest sequence work indicated preferential cleavage at pyrimidine nucleotides that are 3′ substituents of guanosines (i.e., GpC and GpT, designated GpY).[126−128] The purine 2-amino group is crucial in target site recognition.[129,130] Sites other than GpC and GpT are also cleaved, and the range of cleavage at the 16 possible dinucleotides was determined for four bleomycins.[117] However, it is expected that the distribution of cleavages will also depend on reaction conditions such as the relative proportions of DNA and bleomycin. For example, low ratios of bleomycin:DNA have revealed "hot spots" of cleavage selectivity, and so DNA from bacteriophage PM2 can sustain up to 20% of its total bleomycin cleavages at five specific positions among 10^4 nucleotides.[131] Nucleotides adjacent to the GpY sequences also have an influence on cleavage susceptibility,[36,132−134] but the stringency of specificity, except for the case of PM2, does not approach that of restriction nucleases.

When bleomycin makes closely spaced lesion in opposite strands, which constitute 10−20% of all lesions[50,135] the GpY specificity is strong in one strand but absent in the other.[136] This was taken to indicate that nucleotide specificity constrained the first lesion only and that the second, nearby, lesion was a consequence of the first, rather than of a coincidence of independent site-specific events.[135−137] A mechanism for bleomycin reactivation proposed to explain this phenomenon[135] appears in section IV, the central reactions.

B. Mechanism

Analysis of the DNA degradation mechanism was facilitated by the discovery that there were two kinds of DNA damage inflicted by bleomycin:[50,51] release of free nucleic bases and of aldehydic base derivatives (of deoxyribose carbons 1−3). The two are on separate reaction pathways, since bleomycin-treated DNA that had released most of its base propenal still retained the majority of bases to be released as free base.[52] Liberation of base derivatives containing C3′, a DNA backbone atom, necessitates strand scission, whereas base release does not.

Further details of the DNA degradation chemistry were inferred by identifying which of the deoxyribose hydrogens are released when free base or this plus base propenal are produced. Bleomycin treatment released similar quantities of ^3H_2O from [1′,2′-^{3}H]-DNA under mild conditions and from [5′-^{3}H]DNA upon base hydrolysis.[52] The origin of the former ^3H_2O was clarified when the aldehydic base derivative was characterized as base propenal,[54] which lacks one 2′-hydrogen. The ^3H_2O from [5′-^{3}H]DNA is associated only with free base release,[138] suggesting (correctly) that the abasic product is a 4-ketodeoxyribose product. Another finding that facilitated resolving the two DNA degradation pathways was

that activated bleomycin could be formed and consumed anaerobically.[45,72] This made it possible to verify a proposal of Johnson and Haim (cited in ref 56) that endogenous O_2 was additionally required to form DNA cleavage products. In the absence of O_2 no base propenal forms; only the pathway of base release is operative.[72]

The remaining deoxyribose fragmentation product, the complement of base propenal, is a 3′-phosphoglycolate DNA terminus;[54] this and base propenal are found in equimolar quantities. These products represent the only mode of bleomycin-mediated DNA cleavage, for the number of cleavages (ascertained by DNA oligomer size analysis) does not exceed the amount of base propenal formed.[139]

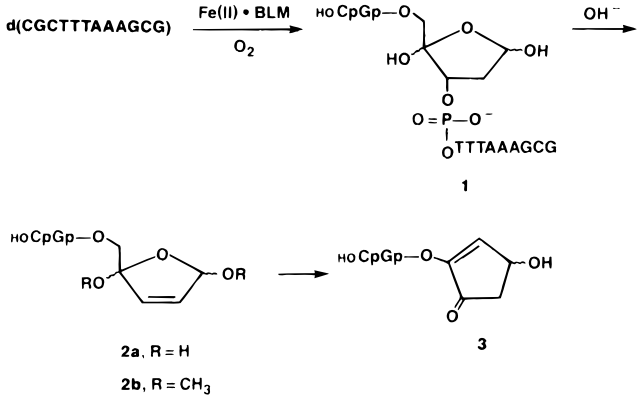
The early steps in DNA degradation were inferred mainly from the use of specifically tritiated poly dA·dU deoxyriboses in the laboratories of J. Stubbe and J. Kozarich.[138] The idea that 4′-H abstraction[140] initiates DNA degradation by bleomycin was proposed by Johnson and Haim,[56] but awaited verification until [4′-^{3}H]DNA was prepared and tested. The 4′-^{3}H appeared in ^3H_2O, whereas the 1′-^{3}H did not, and although some of the 3′-^{3}H appeared in water, none was lost from the base propenal. Loss of 3′-H is therefore related to free base release only. These findings are consistent with both base propenal release and free base release being consequences of a 4′-H abstraction followed by a partitioning of the resulting 4′-radical by reaction with either O_2 or •OH, respectively. An enrichment of released 4′-^{3}H over base propenal plus free base was seen, an isotope effect indicating that this abstraction is a rate-limiting reaction.[138] This isotopic selection remained in the range of 7.2−12.5 as the ratio of free base to base propenal production was manipulated over a 300-fold range by adjusting $[O_2]$,[141] indicating that the 4′-H abstraction was indeed rate-limiting for both processes. The application of DNA-sequencing electrophoresis methodologies to 5′-^{32}P end-labeled DNA containing 4′-deuterated thymidine permitted measurements of this isotope effect at individual target sites, which were found to differ by up to 60%.[142] Identical site-specific isotope effects are seen on both cleavage and alkali-labilization outcomes.[142]

The subsequent DNA degradation mechanisms responsible for DNA cleavage and for free base release will be considered separately before addressing relationships between these two events. The partition of the initial lesion is not as simple as expected, for while base propenal production increases with oxygen concentration, free base release can remain constant, independent of oxygen, rather than declining, provided that an excess of DNA is available.[72] This implies a commitment to a certain level of free base release prior to any encounter with O_2 and is addressed in section IV.

1. Oxygen-Independent DNA Degradation

Free base release and alkali labilization of the DNA strand are facile consequences of a putative 4′-hydroxylation.[138] The putative hemiketal product was expected to eliminate the nucleic base while rearranging to yield a 4-ketodeoxyribose product susceptible to alkali attack at H2′ and β-elimination of the 3′-phosphate ester.[143] This 4-ketodeoxyribose product was difficult to characterize because of its instability.[144] The levulinic acid identified in an acid hydrolysate of bleomycin-treated DNA[54] is presumably a final product of this intermediate. Experimental support for the putative 4-ketodeoxyribose intermediate was provided by the observations that H3′[138] and H5′ were labilized in the base-releasing reaction, labilities suppressed by $NaBH_4$ reduction of the putative carbonyls.[143]

Scheme 1. Products of Oxygen-Independent Bleomycin Attack (Reprinted from Ref 144. Copyright 1985 American Chemical Society)[a]

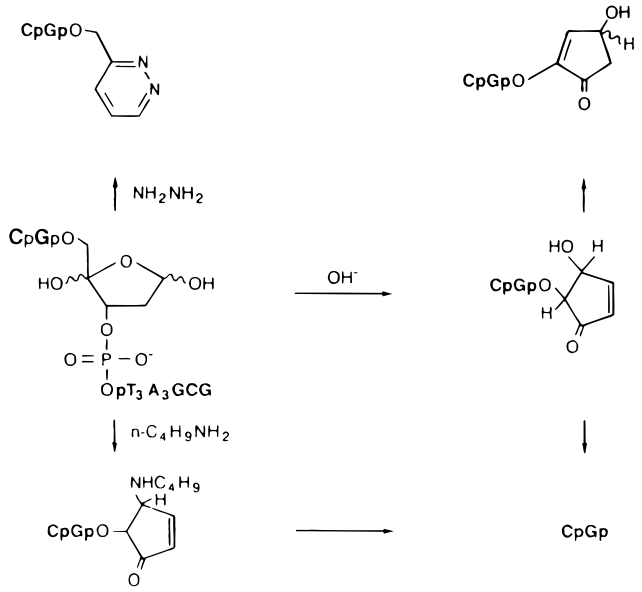


[a] Synthetic **2a**, which is unstable, was prepared from **2b** by hydrolysis.

Further structural evidence for a 4-ketodeoxyribose as a degradation product was obtained by synthesis. Sugiyama et al.[144,145] prepared a d(CpGp) dinucleotide having as a 3′-phospho substituent a congener of the expected 4-keto aldehyde, only lacking its 3′-phosphate extension (**2a**, Scheme 1). The degradation and derivatization products of this synthetic congener were then compared to those of an oligonucleotide target, $d(CGCT_3A_3GCG)_2$, chosen because cytosine-3 is a major site of bleomycin damage.[53,146] When it sustains alkali-labilizing damage, it yields a modified C_3 analogous to the synthetic congener (but preserving its 3′-phosphate oligonucleotide linkage). As expected, the synthetic material, **2a**, was unstable, with $t_{1/2} \approx 1$ h in 0.1 N HCl at 37 °C.[145] Its stable products, however, were suitable for characterization by NMR and HPLC and for comparison with those of the bleomycin-treated oligonucleotide products. After treatments with either alkali, hydrazine, or *n*-butylamine, the dinucleotide bleomycin products proved identical with those of the synthetic dinucleotide derivative (Scheme 2). This was taken as evidence for the structural identity of the reactants. However, products other than **3** are also found in HPLC analyses of the bleomycin-treated oligonucleotide,[145] which left some ambiguity in the identification of a 4-ketodeoxyribose as a unique intermediate in this degradation pathway.

The stoichiometric relationship between free base release and formation of its putative deoxyribose product, the unstable 4-keto aldehyde, was established (within 10%) by promptly derivatizing it with labeled hydrogens from $NaBH_4$ to form deoxypentitols.[73] For this purpose the target $d(CGCGCG)_2$ was

Scheme 2. Derivatives of the Oxygen-Independent Attack Product, (Reprinted from Ref 145. Copyright 1988 American Chemical Society)



used, and the techniques thereby validated were subsequently applied to confirm results with large copolymers. The reduced product of attack on the hexanucleotide dimer (predominantly at dC-5) was stable and could be subjected to dissection with nucleases and phosphatases, preparatory to mass spectroscopy of deuterated products and chromatography of tritiated products. When the trimethylsilyl derivatives of the deuterated DNA product were compared by gas chromatography/mass spectrometry to those of 2-deoxy-D-erytherotol, positions 4 and 1 were both found to be monodeuterated, as expected for an initial 4-ketodeoxyribose. Loss of stereospecificity at C4, detected by chromatographic resolution of the tritiated epimers, supported the intermediacy of a 4-ketone. When the yields of deoxypentitol-containing products were compared to free base detected, ratios approached 0.9, supporting 4′-keto aldehyde as the predominant (and possibly only) sugar product of base release.[73] Thus, the oxidized sugar product of free base release is consistent with the hypothesis that hydroxylation of deoxyribose C-4 initiates the process.

The base-releasing precursor, a putative 4′-hydroxylated nucleotide intermediate even less stable than the 4′-keto aldehyde, has not been detected or trapped as a derivative. However, the sluggish release of free nucleic base from DNA at low temperature[52] suggests that this hydroxylated intermediate may have a lifetime sufficient to permit its detection, (although a slow release could be due to an artifactual adherance to the DNA by intercalation, as was seen with base propenals).[147]

Assuming that deoxyribose C4 becomes hydroxylated, how is the oxygen inserted, and what is its origin? Even in anaerobic reactions oxygen might derive from either activated bleomycin or from the solvent. One plausible conjecture[138] was that bleomycin acts like cytochrome P-450,[148] hydroxylating with an O_2 atom. However, this seems unlikely: the 4′-oxygen (stabilized by $NaBH_4$ reduction) originates not in activated bleomycin (prepared with $^{18}O_2$) but with water, and control experiments rule out solvent exchange with the ketone prior to trapping.[84] This exclusion of bleomycin as a vehicle of oxygen insertion was made with the reservation that water oxygen exchanging into activated bleomycin might then be inserted to give this labeling pattern. However, this is unlikely because preparations of activated bleomycin made with ^{17}O are stable enough to produce a characteristically broadened EPR signal even in the presence of $H_2{}^{16}O$[45] and vice versa.[149] Thus the inserted oxygen probably comes directly from water.[84] To attack water, a more active species than a C-4′-centered radical seemed necessary, and a C-4′ carbocation was postulated.[84] Since activated bleomycin is a 2e oxidant, it could conceivably produce such a lesion. While this proposal appears to conflict with the evidence that both pathways of DNA degradation begin with a 4′-H abstraction to yield a 4′-C radical as the initial lesion, the 4′-C radical might sometimes undergo further oxidation to a carbocation,[84,150] accounting for divergence of the pathways soon after the initial lesion. The sparse evidence for this attractive possibility will be considered below with the elusive, "central" reactions of the bleomycin/DNA pathways.

2. Oxygen-Dependent DNA Degradation

Frank strand cleavage is the more obvious form of bleomycin-induced DNA degradation and, like the base-releasing lesion, it begins with 4′-H abstraction. DNA scission depends on the presence of O_2 in addition to activated bleomycin. The second O_2 must be available at the time of activated bleomycin decay.[72] The two oxygen requirements were distinguished by their distinct kinetic loci: oxygen uptake for bleomycin activation is about a 1000-fold more rapid[62] than the rate of activated bleomycin decay. Thus by furnishing isotopically labeled O_2 at different times, it was possible to distinguish their different fates. The alternative procedure of using Fe(III)·bleomycin with isotopically labeled H_2O_2 and O_2 was also used. As with the oxygen-independent lesion, no oxygen from bleomycin (<3.5%)[74] is found in the DNA product, supporting the idea that both pathways begin with a common event. The second, non-bleomycin O_2 provides one O atom of the DNA degradation product, nucleotide-3′-phosphoglycolate.[74] The base propenal oxygen derives exclusively from water.[151] This ruled out early proposals in which a 4′-hydroperoxide adduct rearranged to insert its two oxygens at C3′ and C4′. Details of the reaction pathway yielding the observed cleavage products from the putative peroxide adduct[56] have emerged from experimental efforts to verify mechanistic hypotheses.[151]

Initially, bleomycin-induced DNA cleavage was thought to be a rapid reaction, and suitable reaction mechanisms were proposed[36,56,152,153] in which the events of DNA scission were largely concerted. Subsequent kinetic measurements indicated that DNA cleavage was not the final step in the DNA cleavage pathway, so these proposals were modified to accom-

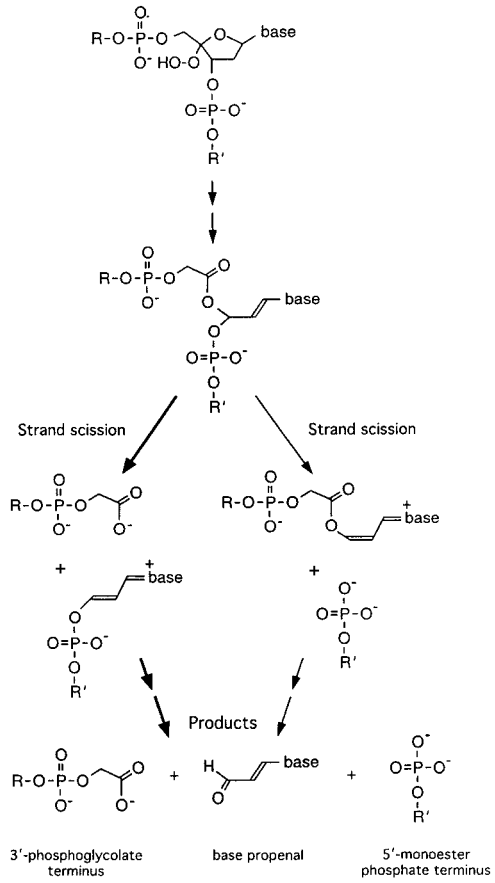


Figure 7. Proposed intermediates in base propenal formation,[151] redrawn.[147] Only the pathway shown with bold arrows (left) is consistent with the kinetics of phosphomonoester formation monitored by NMR. Copyright 1994 the American Society for Biochemistry & Molecular Biology.

modate the emerging details of kinetically distinct events.[151] The final DNA cleavage products require the breakage of three carbon bonds[52,54,154] shown in Figure 7, but not all need be broken to accomplish strand cleavage. Thus, DNA was seen to release base propenal significantly after cleavage.[155] Since the DNA cleaving reactions of Fe(II)·bleomycin + O_2 approximate a single-turnover system (vide infra) with constituent steps taking seconds (at room temperature) or minutes (near freezing), these events could be observed sequentially even though intermediates were difficult to trap. Real-time methods of monitoring, such as spectroscopies, were especially useful in determining the sequence or simultaneity of the reactions.[156] These events are (1) activated bleomycin decay, (2) 2′-H release, (3) DNA strand cleavage, and (4) base propenal formation.

Activated bleomycin decay and its coincidence with the second O_2 requirement have been noted above. The next detected event, release of 2′-*pro*-*R*-^{3}H into solvent, which was well-characterized stoichiometrically and sterically,[138,154] proved to be more rapid than base propenal formation or DNA cleavage. This was established by removing freshly attacked [1′,2′-methyl-^{3}H]thymine-containing DNA from the reaction mixture by rapid ethanol precipitation, and assaying distillable ^3H_2O in the supernatant, thereby demonstrating the rapid ($t_{1/2}$ = 1.8 min) release[155] of 2′-H, the only labilizable labeled hydrogen in this experiment.[138] The rapidity of this reaction is also reflected in a ^{1}H NMR study.[147] NMR spectra of oligonucleotide degradation in progress include two signals in the aromatic region which may belong to deoxyribose hydrogens 1′ and 2′ after the 2′-H release. They appeared fully formed by 4 min at 4 °C when first observed, and were progressively replaced by the 2,3-olefinic proton resonances of base propenal,[54] expected consequences of early 2′-dehydrogenation. This relatively rapid release of 2′-H was rigorously verified[151] using as bleomycin targets stereospecifically tritiated poly(dA[2′-^{3}H]dU). Both base propenals and ^3H_2O were assayed in both supernatant and precipitate fractions for both isomers, confirming the rapid release of the 2′-*pro*-*R*-H. This, together with the release of base propenal occurring after strand scission (vide infra), led to a mechanistic proposal[151] distinguished by a new precursor of cleavage shown in Figure 7, where the currently favored form of this proposal is indicated by bold arrows.

Earlier measurements of strand scission kinetics,[155] which demonstrated first-order DNA cleavage kinetics ($t_{1/2}$ = 2.5−5 min) at bleomycin concentrations differing by 10^3, were confirmed by an optical real-time assay. Cleavage of d(CAAGCTTG)$_2$ was monitored by the consequent denaturation hyperchromicity, showing $t_{1/2}$ = 4.1 ± 0.5 min,[147] significantly slower than activated bleomycin decay ($t_{1/2} \approx$ 2 min[45]).

The same oligonucleotide, d(CAAGCTTG)$_2$, was used to monitor formation of base propenal by real-time NMR spectrometry of the aldehyde proton. This proton appears with $t_{1/2}$ = 6.7 ± 0.3 min, coinciding with 5′-phosphomonoester formation determined by real-time ^{31}P NMR: $t_{1/2}$ = 7.4 ± 0.8 min.[147] Because base propenal formation lags strand scission, the originally proposed alternative cleavage events of Figure 7 are experimentally distinguishable. The concurrent formation of base propenal and 5′-phosphomonoester indicates that the release of base propenal from the oligonucleotide-5′-phosphate is the final event of DNA cleavage. A distinctive feature of the present mechanistic proposal which remains to be documented is the persistence of a C3−C4 oxygen bridge for the interval between 2′-H release and strand cleavage.

The latest proposal for a DNA cleavage mechanism consistent with present data[151] concludes as shown in Figure 7. A putative 4′-hydroperoxide is formed by O_2 addition to the 4′-DNA radical, followed by reduction of the resultant peroxyl radical to a peroxide. The latter undergoes a Criegee rearrangment, possibly with iron catalysis, and forms a 4′-carbocation with an oxygen inserted between C3′ and C4′. This intermediate is thought to eliminate the 2′-*pro*-*R*-proton and the C1′−O bond to form a glycosidic N-cationic intermediate that is a mixed diester of base propenal with glycolic acid and the 3′-phosphate. Elimination of either oxygen would accomplish strand scission; subsequent reaction of the base propenal monoester cation with water would form a hemiacetal expected to decompose quickly to base propenal and the other acid. The glycolate proved to be the better leaving group, always released before the phos-

phate.[147] A similar pathway in which the peroxide intermediate is replaced by a 4′-alkoxyl cation intermediate[157] is described in section IV.

IV. The Central Reactions

Rapid reactions convert activated bleomycin to Fe(III)·bleomycin and commence the degradation of DNA, if present. These events will be called the central reactions. They include reactions responsible for initiating two distinct resolution pathways from a single initial DNA lesion. The central reactions bridge the more experimentally accessible portions of pathways that account for activated bleomycin formation and for the resolution of DNA damage. The central reactions are thus at the crux of what remains unknown about bleomycin reaction pathways.

The ability of activated bleomycin reaction mixtures to perform either one- or two-electron oxidations[67] is consistent with the idea that partitioning of the DNA degradation pathways is a consequence of whether the initial bleomycin-mediated oxidation removes one or two electrons from the deoxyribose C4′.[84,135,150,158] Activated bleomycin itself need not be the species that directly attacks DNA. In the base-releasing, alkali-labilizing pathway of DNA degradation, the two-electron oxidation product, a C4′ cation, is thought to react with water to form a 4′-hydroxylated deoxyribose intermediate.[84] It is unclear whether the oxidation occurs directly or by a further one-electron oxidation of a deoxyribose 4′-radical. The latter possibility is consistent with observations of DNA product partitioning discussed below. The bleomycin degradation product of an aristeromycin-containing oligonucleotide, a 1′,5′-desaturated ring, is also consistent with the proposed formation of a C4′-carbocation from a C4′-radical.[158]

In the DNA-cleaving pathway, the putative one-electron oxidation product of hydrogen atom abstraction is a C4′ radical that forms an adduct with endogenous O_2.[36,56,152,153] This peroxyl radical then reacts to yield DNA cleavage, putatively after reduction to a peroxide.[56] This peroxide adduct has been formed as a product of a model C4′-DNA radical generated by photolysis of a phenylselenide precursor, and observed by matrix-assisted laser desorption/ionization mass spectroscopy (MALDI-MS).[159] If O_2 is not made available promptly in the bleomycin reaction, only base release occurs.[72] This contingency of bleomycin cleavage on prompt availability of O_2 is not observed with photolytically formed 4′-DNA radicals, which can cleave the 3′-phosphate anaerobically.[160,161] Bleomycin appears to limit the ability of a 4′-radical to migrate elsewhere. That a 4′-DNA radical actually forms in bleomycin reactions was verified by experiments employing a radical-trapping analogue of the target oligonucleotide d(CCGG).[150] It seems difficult to maintain that the reaction with O_2 of the DNA radical is the product-partitioning step, however, because the quantity of free base released is not diminished when base propenal formation is enhanced.[72] Conversely, the fate of the C4′ radical in the absence of O_2 was difficult to understand, since O_2 removal does not enhance free base release and since no other product, including a restored 4′-H,[151] has been identified. A solution for this dilemma is emerging from an unexpected quarter: a model for the formation of closely spaced double lesions.

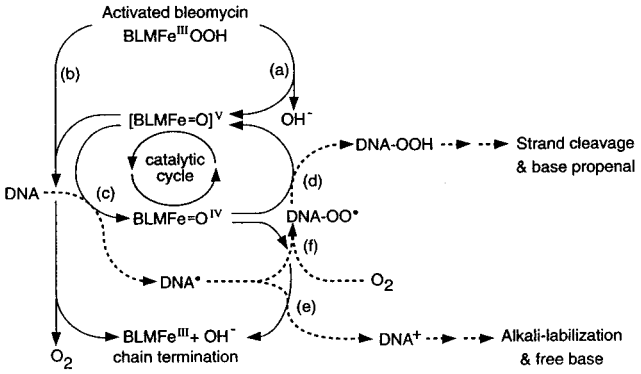


Figure 8. Putative "central reaction" pathways of activated bleomycin and DNA. The rate-limiting reaction of activated bleomycin (BLM·Fe^{III}OOH$^-$) is O−O cleavage (a), which is thought to be heterolytic. The intermediate product is [BLM·Fe=O]^V, which is analogous to peroxidase compound I. This [BLM·Fe=O]^V can participate in two reactions. In one (b), it oxidizes activated bleomycin to yield 2BLM·Fe^{III} + O_2 + OH$^-$ in a catalase-type reaction that results in chain termination. In the other (c), [BLM·Fe=O]^V reacts with DNA, producing a DNA-C4′ radical and BLM·Fe=O^{IV}, which is analogous to peroxidase compound II. The DNA radical and BLM·Fe=O^{IV} can react in two ways. One (d) regenerates [BLM·Fe=O]^V, while the second (e) produces BLM·Fe^{III}, which reacts with neither O_2 nor DNA and thus results in chain termination. The chain-propagating oxidation of BLM·Fe=O^{IV} to [BLM·Fe=O]^V (d) depends on O_2 addition (f) to the DNA radical to form a DNA peroxyl radical, which is then reduced (d) to the peroxide by BLM·Fe=O^{IV}. The DNA peroxide resolves to DNA cleavage with base propenal formation. The chain-terminating reduction (e) of BLM·Fe=O^{IV} to BLM·Fe^{III} + OH$^-$ by the DNA radical is proposed to yield a DNA-C4′ cation independent of O_2. This DNA carbocation reacts with water and releases free nucleic base as it forms a 4-ketodeoxyribose 3,5-diphosphate derivative, which thereby renders the DNA strand alkali labile.

An explanation of the partition stoichiometry paradox and lack of anaerobic phosphate cleavage emerges from the idea that bleomycin and DNA tend to react a second time soon after forming the initial lesion. The reaction, proposed[135−137] to account for a high incidence[50] of closely spaced cleavages in opposing strands, is depicted in Figure 8. It also accounts for the reduction of the 4′-DNA peroxyl radical to the peroxide[56] shown in Figures 7 and 8 as an intermediate in DNA cleavage. The required reductant is thought to be an analogue of peroxidase compound II, produced from the proximate active bleomycin species when it makes the 4′-H abstraction by a one-electron oxidation and thus is reduced by one electron. By returning an electron to the DNA peroxyl radical,[135] the compound II analogue would thereby produce a DNA hydroperoxide and revert to its two-electron oxidant status.[135] Such recycling constitutes an oxygen-driven chain reaction in which O_2 would execute one-electron oxidations of both the DNA and bleomycin products immediately after their formation.[156] The unstable proximate active species, once regenerated, is likely to react again before it can travel far,[135] which would account for the closely space double-strand lesions that constitute 10−20%

of cleavage events.[50] The plausibility of this proposal for double-lesion formation is reinforced by the finding that double lesions may include one alkali-labile DNA lesion, but they never include a pair of them:[162] an oxygen-dependent DNA degradation event is required for this phenomenon.[135]

The same mechanism may account for another, less obvious, double lesion. The size distribution of same-strand cleavage events includes an anomalous >2-fold enhancement of same-strand double cleavages spaced 3 nucleotides apart, even at low levels of total cleavage.[139] Alkali-labile lesions formed aerobically also contribute to a similar distribution anomaly. However, when anaerobic activated bleomycin degrades DNA, forming only alkali-labile lesions,[139] the size distribution anomaly disappears. Thus, single-strand lesion pairs, like opposite-strand pairs, must include at least one frank, oxygen-dependent cleavage. Closely spaced lesions in either the opposing or the same DNA strands would reflect the action of the newly reformed proximate attacking species.

The putative regeneration of [bleomycin·Fe=O]^{V} from a bleomycin·Fe=O^{IV} product by •O−O−C4′− DNA in a chain reaction (Figure 8) is a welcome proposal because it argues for the existence of a bleomycin intermediate that is an obvious source of the electron required to reduce a peroxyl radical to the putative 4′-deoxyribose peroxide shown in Figure 8. Competing with O_2 for the C4′ radical, the bleomycin·Fe=O^{IV} might instead act as an oxidant and convert the radical to a 4′-carbocation. This would initiate free base release and yield the unreactive Fe(III)·bleomycin, a chain-terminating reaction denoted **e** in Figure 8. If free base is thus a product of the major chain-termination reaction, free base yield would be independent of chain length, and therefore [O_2]-insensitive. The other mode of chain termination, which would result in bleomycin suicide, is the decay of activated bleomycin while dissociated from DNA. This is probably minor, since the presence of DNA is demonstrably protective.[58] One confirmed prediction of the Figure 8 model is that the yield of free base should be independent of base propenal yield.[72] Another prediction is stoichiometric: for each equivalent of attacking bleomycin, one free base should appear, provided that adequate DNA is available. This prediction differs from the current stoichiometric estimates.[45] Thus, the observed stoichiometries of base propenal formation to free base release and of base propenal formation to activated bleomycin gave an estimate of only 0.5 equiv base release per activated bleomycin. To better test this chain-termination hypothesis, the ratio of free base to activated bleomycin should be reexamined, particularly under a range of conditions known to alter the yield of base propenal, such as [O_2].

The chain reaction hypothesis also predicts that the proportion of closely spaced lesions will be higher under conditions known to enhance the yield of base propenal. Higher base propenal yield was conspicuous in incubation mixtures containing $^{2}H_2O$.[58] Mixing experiments with solutions of DNA and of activated bleomycin prepared in $^{2}H_2O$ and H_2O indicate that the $^{2}H_2O$ kinetic locus occurs as a part of the central reactions rather than during drug activation or DNA cleavage resolution (unpublished data). The predicted increase in the incidence of closely spaced lesions in $^{2}H_2O$ should be verified.

An attractive variant to this chain proposal involves a recently proposed alternative to the formation of DNA hydroperoxide.[157] Like other early intermediates in DNA degradation which have not been observed, the 4′-hydroperoxide was thought to be short-lived. This is difficult to maintain because a model of the intermediate has been prepared and appears to be stable enough for observation.[153,163] In another experiment a hydroperoxide was produced in an oligonucleotide with a photolytic, radical-generating 4′ substituent.[159,160] In this experiment light treatment for 100 min was used to accumulate the peroxide for MALDI-MS characterization. This discrepancy between actual and assumed hydroperoxide stabilities is avoided in a proposed mechanism which gives the same cleavage outcome without the peroxide intermediate.[157] Were the newly formed DNA peroxyl radical to interact with the bleomycin·Fe=O^{IV} and displace the O as OH^{-}, it would form a transient complex of bleomycin·Fe(III)OODNA. Heterolysis of the O−O bond could then regenerate [bleomycin·Fe=O]^{V} and release a 4′-DNA alkoxyl cation which would undergo ring enlargement by a C3′−C4′ oxygen insertion and proceed to base propenal formation as previously proposed.[151] The only alteration to the prior proposals[135,151] would be substitution of the bleomycin−peroxide−DNA and alkoxy cation intermediates for the hydroperoxide, and a change in the origin of the oxygen atom in the regenerated [bleomycin·Fe=O]^{V}.

V. Other Bleomycin Reactions

Bleomycin reactivity is not limited to DNA damage by iron−bleomycin, as mentioned above. The actions of activated bleomycin on small molecules have been likened to those of cytochrome P-450, but Padbury and Sligar[33] argued for a specificity closer to that of chloroperoxidase. When single-oxygen donors such as iodosylbenzene are combined with bleomycin, a wider range of activities is observed,[34] but it is not clear that they involve bleomycin species resembling or derived from activated bleomycin.

A. RNA Cleavage

The mechanism of RNA cleavage is not as well understood as that of DNA cleavage. Bleomycin was not thought to attack RNA until 1989, when it was shown that high concentrations cleave transfer RNA at several sites, releasing uracil and adenine.[30] Relatively fewer sites in RNA than in DNA sustain cleavage, and not all RNA cleavage sites have the sequence GpY. Some tRNAs are not cleaved at all. The secondary structures of RNA play an important role, and regions of transition between single- and double-stranded structures of tRNAs and r-RNA are especially prone to cleavage. This relaxation of sequence specificity, together with failures to detect cleavage in heteroduplex ribo/deoxyribonucleotide polymers,[28,29] led to proposals that irregular second-

ary structures characterized RNA sites susceptible to bleomycin.[38] Now that some cleavage has been found in RNA:DNA hybrids,[164] more subtle rules are sought for predicting site specificity. Comparisons were therefore made of cleavage in DNA and RNA targets of similar structure such as precursor tRNA and its single-strand DNA counterpart,[165] and in targets with alternative structures, such as 5S ribosomal RNA, which assumes very different secondary structures alone and in DNA heteroduplex.[164] To distinguish between fine and gross structural determinants of differences in cleavage of DNA and RNA, cleavage of *B. subtilis* $tRNA^{His}$ precursor was compared to that of its analogous tDNA. Both targets showed the same principal cleavage site: U_{35} (or T_{35}) in a GUG between two duplex regions, called a stem−stem transition. This was surprising, as DNA cleavage canonically requires a duplex configuration. However, there were still differences in cleavage of similarly configured DNA and RNA: the tRNA cleavage at its unique site is less efficient than that of DNA, even though the tDNA is significantly cleaved at 10 additional sites.[165] In another experiment, when 10S ribosomal RNA was heteroduplexed with its complementary DNA, the RNA became much more bleomycin-sensitive than the rRNA alone, being cleaved at several sites, none of them a GpY.[164] An effect of the RNA on the DNA strand was also seen: the heteroduplex DNA was cleaved at GpY sites, but at fewer of them than when homoduplexed. Sequences predisposing certain DNA sites to resist cleavage when heteroduplexed have been identified,[134,166] but a coherent theory has yet to be verified.

The chemistry of RNA cleavage must differ from that of DNA, since the 2′-*pro-R*-H, which is released early in DNA cleavage, is replaced in ribose by OH. The substitution of OH for H in either 2′ position does not prevent cleavage: in the chimeric oligonucleotides, $[d(CG)rCd(TAGCG)]_2$ containing one ribose at its C_3 target site, and $[d(CG)araCd(TAGCG)]_2$, containing arabinose, where the 2′-H and -OH in C_3 are reversed, both *ribo*C_3 and *ara*C_3 are cleaved,[31] but cleavage at arabinose is three times that at the ribose.[167] Perhaps the stereospecificity of 2′-H loss seen with DNA may be relaxed with RNA. Cleavage at the chimeric *ribo*C is inhibited; its oligomer is cleaved predominantly (87%) at dC_7, yielding about 1 equiv of cytosine propenal. With the arabinose chimera, cleavage occurs equally at dC_7 and *ara*C_3 and equimolar cytosine propenal appears, which was not expected.[38] The difference in cleavage between ribose and arabinose indicates that stereospecificity at C2′ is important, but there is no sign that a 2′-hydroxy base propenal ever formed, as would be expected if the ribose 2′-H were removed as from deoxyribose. No base propenal was found in the initial RNA cleavage study.[30] Tracing the fates of ribose carbons 1−3 would buttress the mechanistic proposals so far expounded.[38,167] These proposals include the abstraction of H4′ and insertion of a hydroperoxide there, as with DNA. The principal evidence for this is an oligonucleotide product that comigrates with the authentic 5′-phosphoglycolate produced by degradation of an analogous oligodeoxynucleotide. The 4′-H kinetic isotope effects that accompany DNA degradation have not been reported for RNA. A requirement of O_2 for RNA degradation by Fe(II)·bleomycin has been established[167] but the only direct evidence for or against a second O_2, as required for DNA cleavage, is the formation of phosphoglycolate as a product. A proposal has been made that bleomycin can attack RNA by abstracting H1′,[30] and a derivatve predicted of such an event and sequelae was produced in bleomycin−RNA reaction mixtures.[168] This interpretation of the derivatization experiment could be strengthened by tracing the fates of C1′ and H1′ and, if possible, by demonstrating a H1′ isotope effect.

Finally, there is evidence that yet another bleomycin reaction with RNA is possible that must have an entirely different mechanism: in the absence of metals bleomycin can promote a hydrolytic cleavage of tRNA, evidenced byproducts comigrating electrophoretically with those of RNA hydrolysis.[169] Bleomycin is a molecule of many talents.

B. Nonferrous Metal Cofactors

Although iron appears to be the most effective bleomycin cofactor, other metals bind strongly to bleomycin, and some, including Cu, Co, Mn, Ru, and V, can facilitate DNA cleavage by mechanisms less well understood than that of iron.[170] Nonferrous metals were not initially considered possible bleomycin cofactors, since they inhibited bleomycin DNA cleaving activity.[44,171] For bleomycin to show activity with nonferrous metal ions, more is required than the presence of O_2 as a cofactor. Thus, Co(III)·bleomycin[172] and Ru(II)·bleomycin[173] require light to nick DNA. The Co complexes reportedly produce 3′-phosphoglycolate termini in the presense or absence of O_2, but no base propenal is produced.[174] Thus cobalt−bleomycins must initiate reactions differing from those of iron−bleomycins. Among the DNA products free nucleic base is found, and 4′-hydroxylated sites were identified, suggesting that a C4′ carbocation is produced by the cobalt reaction. Of several Co(III)·bleomycin complexes examined, the active ones were the formato and hydroperoxo ternary complexes.[174]

The activation reactions of Co(II)·bleomycin parallel those of Fe(II)·bleomycin activation:[176,177]

$$2\text{Co(II)}\cdot\text{bleomycin} + 2O_2 \rightarrow\rightarrow \text{HOOCo(III)}\cdot\text{bleomycin} + \text{Co(III)}\cdot\text{bleomycin} + O_2 \quad (3)$$

However, there are differences: the two Co(III)·bleomycin species are differently colored, Co(III)·bleomycin does not react with peroxide,[177] and HOO-Co(III)bleomycin is stable for months.[114] This stability plus its extremely strong binding to DNA ($K_d \approx 10^{-7}$ M[114,174]) has made HOOCo(III)·bleomycin the choice for modeling activated bleomycin (HOOFe(III)·bleomycin) and its structural interactions with DNA.[113]

Like Co, Ru(II) requires light but, unlike the Co(III) complex, it requires O_2 to nick DNA.[173] Light also potentiates DNA cleavage by Fe(III)·bleomycin, and the proposed reduction to Fe(II),[81] which would

permit the familiar reactions with O_2 and DNA, was verified by formation of the chromophore with added ethyl isocyanide in the absence of O_2, and of DNA cleavage products in its presence.[178] Mn(II) and VO-(IV) complexes plus H_2O_2 also cleave DNA, but the reactions are not well-characterized. The DNA sequence specificity of the VO(IV) complex differs somewhat from that of iron, its cleavage efficiency is 50-fold lower, and the products have not been identified.[179] In one study the Mn(II) complex yields free base and base propenal from DNA when H_2O_2 is used, but at 1−3% the yield of similar Fe(III) mixtures.[180] In another study, DNA cleavage occurred with Mn(II)·bleomycin lacking H_2O_2, but it required O_2 and produced no base propenal.[181] In every case where nucleotide sequence specificity has been examined, cleavage is favored at the GpY sites as with iron−bleomycin,[182] and the product oligonucleotide electrophoretic mobilities appear characteristic of 5′-phosphoglycolate where controls were run permitting direct comparison with products of Fe−bleomycin and of Maxam−Gilbert digests.[183]

Cu(I)·bleomycin may also cleave DNA notwithstanding reports that Cu ions are inactive or inhibitory,[44] do not produce base propenal or much free base,[184] and evidence that the activity in Cu-containing reaction mixtures may be due to adventitious Fe.[185] Conditions were found under which additions of Cu(II) augmented the cleavage of DNA seen in the presence of metal-free bleomycin and the reductant dithiothreitol, which was significant.[186] In another experiment Cu(II)- and Fe(II)·bleomycin were shown to produce similar yields of cytosine and/or (unspecified) cytosine propenal, provided the metal ions were combined with bleomycin and dithiothreitol for 5 min prior to DNA addition. Under these circumstances the reductively recycled iron−bleomycin would be largely inactivated in the suicide reaction. Cu(II)·bleomycin differs from Fe(III)·bleomycin in requiring > 10 min preincubation with the reductant before showing significant activity. These studies[186] make it clear that copper can enhance cleavage of DNA in bleomycin reaction mixtures, but they do not indicate the relevant mechanism(s), which may be complex and different from that with iron alone.

VI. Pharmacological Considerations

In considering how bleomycin cleaves nucleic acids, little attention was given to what makes bleomycins useful drugs or what limits their usefulness. Bleomycin is selectively toxic to tumor cells not because their DNA is different but because they take up bleomycin more readily than normal cells do. When complexed to NMR-visible, or radioactive metal ions, bleomycin may be used as a tumor-imaging agent or for radiotherapy.[187−189] Like all antitumor drugs, bleomycin is toxic to normal cells too. Skin and lung are especially sensitive tissues, but their apparent accessibility to oxygen may be less important than their relative paucity of bleomycin hydrolase,[190] a detoxifying enzyme that removes the β-aminoalanine carbamoylamine (indicated (3) in Figure 1). Bleomycin finds some use in wart removal;[191] the limiting side effect of its antitumor use is a fatal lung fibrosis.[192] Bleomycin does not enter cells readily, and providing it to cultured cells by electroporation enhances its potency 1000-fold.[193] Electroporation of tumors in situ has shown promise in the clinic.[194] Tumor cells may become resistant to bleomycin by destroying it,[195] excluding it,[196] or repairing DNA more actively.[197] Skirting the dangers of toxicity and resistance may be the most practical challenge in enhancing bleomycin therapy. Predicting these limitations in individual patients is important because susceptibility varies greatly among individuals, and the onset of irreversible lung fibrosis is delayed. Progress has been made both toward predictive testing and discovery of less toxic drug congeners. For reviews on these medicinal considerations and on how bleomycin interacts with the vast complications of cell physiology, such as oxidant-protection systems,[198] the reader should seek elsewhere.[11,12,199,200]

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VIII. References

(1) von Sonntag, C. *The Chemical Basis of Radiation Biology;* Taylor & Francis: Philadelphia, 1987.
(2) Henner, W. D.; Rodriguez, L. O.; Hecht, S. M.; Haseltine, W. A. *J. Biol. Chem.* **1983**, *258*, 711.
(3) Janicek, M. F.; Haseltine, W. A.; Henner, W. D. *Nucleic Acids Res.* **1985**, *13*, 9011.
(4) Breen, A. P.; Murphy, J. A. *Free Radical Biol. Med.* **1995**, *18*, 1033.
(5) Maeda, K.; Kosaka, H.; Yagishita, K.; Umezawa, H. *J. Antibiot. (Ser. A)* **1956**, *9*, 82.
(6) Umezawa, H.; Maeda, K.; Takeuchi, T.; Okami, Y. *J. Antibiot. (Tokyo) Ser. A* **1966**, *19*, 200.
(7) Umezawa, H.; Suhara, Y.; Takita, T.; Maeda, K. *J. Antibiot. (Tokyo) Ser. A* **1966**, *19*, 210.
(8) Kawaguchi, H.; Tsukuira, H.; Tomita, K.; Konishi, M.; Saito, K.; Kobaru, S.; Numata, K.; Fujisawa, K.; Miyaski, T.; Hatori, M.; Koshiyama, H. *J. Antibiot. (Tokyo)* **1977**, *30*, 779.
(9) Konishi, M.; Saito, K.; Numata, K.; Tsuno, T.; Asama, K.; Tsukuira, H.; Naito, T.; Kawaguchi, H. *J. Antibiot. (Tokyo)* **1977**, *30*, 789.
(10) Hecht, S. M. In *Cancer Chemotherapeutic Agents;* Foye, W. O., Ed.; American Chemical Society: Washington, DC, 1995; p 369.
(11) Sikic, B. I.; Rosencweig, M.; Carter, S. K. *Bleomycin Chemotherapy*; Academic Press: Orlando, 1985.
(12) Lazo, J. S.; Chabner, B. A. In *Cancer Chemotherapy and Biotherapy: Principals and Practice*, 2nd ed.; Chabner, B. A.; Longo, D. L., Eds.; Lippincott-Raven: Philadelphia, PA, 1996; p 379.
(13) Takita, T.; Ogino, T. *Biomed. Pharmacother.* **1987**, *41*, 219.
(14) Takahashi, K.; Ekimoto, H.; Minamide, S.; Nishikawa, K.; Kuramochi, H.; Motegi, A.; Nakatani, T.; Takeuchi, T.; Umezawa, H. *Cancer Treat. Rev.* **1987**, *14*, 169.
(15) Suzuki, H.; Nagai, K.; Yamaki, H.; Tanaka, N.; Umezawa, H. *J. Antibiot. (Tokyo)* **1968**, *21*, 379.
(16) Haidle, C. W.; Weiss, K. K.; Mace, M. L., Jr. *Biochem. Biophys. Res. Commun.* **1972**, *48*, 1179.
(17) Gudas, L. J.; Pardee, A. B. *Proc. Natl. Acad. Sci. U.S.A.* **1975**, *72*, 2330.
(18) Gudas, L. J.; Pardee, A. B. *J. Mol. Biol.* **1976**, *101*, 459.
(19) Baluch, J.; Chase, J W.; Sussman, R. *J. Bacteriol.* **1980**, *144*, 489.
(20) Murray, V.; Martin, R. F. *J. Biol. Chem.* **1985**, *260*, 10389.
(21) Moore, C. W. *Mutat. Res.* **1978**, *58*, 41.
(22) Umezawa, K.; Haresaku, M.; Muramatsu, M.; Matsushima, T. *Biomed. Pharmacother.* **1987**, *41*, 214.

(23) Povirk, L. F.; Goldberg, I. H. *Biochimie* **1987**, *69*, 815.
(24) Povirk, L. F. *Mutat. Res.* **1996**, *355*, 71.
(25) Onishi, T.; Shimada, K.; Takagi, Y. *Biochim. Biophys. Acta* **1973**, *312*, 248.
(26) Levin, J. D.; Demple, B. *Nucleic Acids Res.* **1996**, *24*, 885.
(27) Müller, W. E. G.; Yamazaki, Z.; Breter, H.-J.; Zahn, R. K. *Eur. J. Biochem.* **1972**, *31*, 518.
(28) Haidle, C. W.; Bearden, J., Jr. *Biochem. Biophys. Res. Commun.* **1975**, *65*, 815.
(29) Krishnamoorthy, C. R.; Vanderwall, D. E.; Kozarich, J. W.; Stubbe, J. *J. Am. Chem. Soc.* **1988**, *110*, 2008.
(30) Magliozzo, R. S.; Peisach, J.; Ciriolo, M. R. *Mol. Pharmacol.* **1989**, *35*, 428.
(31) Carter, B. J.; de Vroom, E.; van der Marel, G. A.; van Boom, J. H.; Hecht, S. M. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 9373.
(32) Lim, S. T.; Jue, C. K.; Moore, C. W.; Lipke, P. N. *J. Bacteriol.* **1995**, *177*, 3534.
(33) Padbury, G.; Sligar, S. G. *J. Biol. Chem.* **1985**, *260*, 7820.
(34) Murugesan, N.; Hecht, S. M. *J. Am. Chem. Soc.* **1985**, *107*, 493.
(35) Berry, D. E.; Kilkuskie, R. E.; Hecht, S. M. *Biochemistry* **1985**, *24*, 3214.
(36) Stubbe, J.; Kozarich, J. W. *Chem. Rev.* **1987**, *87*, 1107.
(37) Dedon, P. C.; Goldberg, I. H. *Chem. Res. Toxicol.* **1992**, *5*, 311.
(38) Hecht, S. M. *Bioconjugate Chem.* **1994**, *5*, 513.
(39) Nagai, N.; Yamaki, H.; Suzuki, H.; Tanaka, N.; Umezawa, H. *Biochim. Biophys. Acta* **1969**, *179*, 165.
(40) Ishida, R.; Takahashi, T. *Biochem. Biophys. Res. Commun.* **1975**, *66*, 1432.
(41) Onishi, T.; Iwata, H.; Takagi, Y. *J. Biochem. (Tokyo)* **1975**, *77*, 745.
(42) Sleigh, M. J. *Nucleic Acids Res.* **1976**, *3*, 891.
(43) Sausville, E. A.; Peisach, J.; Horwitz, S. B. *Biochem. Biophys. Res. Commun.* **1976**, *73*, 814.
(44) Sausville, E. A.; Peisach, J.; Horwitz, S. B. *Biochemistry* **1978**, *17*, 2740.
(45) Burger, R. M.; Peisach, J.; Horwitz, S. B. *J. Biol. Chem.* **1981**, *256*, 11636.
(46) Kuramochi, H.; Takahashi, K.; Takita, T.; Umezawa, H. *J. Antibiot. (Tokyo)* **1981**, *34*, 578.
(47) Bickers, D. R.; Dixit, R.; Mukhtar, H. *Biochim. Biophys. Acta* **1984**, *781*, 265.
(48) Ciriolo, M. R.; Magliozzo, R. S.; Peisach, J. *J. Biol. Chem.* **1987**, *262*, 6290.
(49) Ciriolo, M. R.; Peisach, J.; Magliozzo, R. S. *J. Biol. Chem.* **1989**, *264*, 1443.
(50) Povirk, L. F.; Wübker, W.; Köhnlein, W.; Hutchinson, F. *Nucleic Acids Res.* **1977**, *4*, 3573.
(51) Povirk, L. F.; Köhnlein, W.; Hutchinson, F. *Biochim. Biophys. Acta* **1978**, *521*, 126.
(52) Burger, R. M.; Berkowitz, A. R.; Peisach, J.; Horwitz, S. B. *J. Biol. Chem.* **1980**, *255*, 11832.
(53) Sugiyama, H.; Kilkuskie, R. E.; Hecht, S. M.; van der Marel, G. A.; van Boom, J. H. *J. Am. Chem. Soc.* **1985**, *107*, 7765.
(54) Giloni, L.; Takeshita, M.; Johnson, F.; Iden, C.; Grollman, A. P. *J. Biol. Chem.* **1981**, *256*, 8608.
(55) Kuo, M. T.; Haidle, C. W. *Biochim. Biophys. Acta* **1974**, *335*, 109.
(56) Takeshita, M.; Grollman, A. P. In *Bleomycin: Chemical, Biochemical, and Biological Aspects*; Hecht, S. M., Ed.; Springer-Verlag: New York, 1979; p 207.
(57) Haidle, C. W.; Weiss, K. K.; Kuo, M. T. *Mol. Pharmacol.* **1972**, *8*, 531.
(58) Burger, R. M.; Peisach, J.; Blumberg, W. E.; Horwitz, S. B. *J. Biol. Chem.* **1979**, *254*, 10906.
(59) Dawson, J. W. *Science* **1988**, *240*, 433.
(60) Sam, J. W.; Tang, X.-J.; Peisach, J. *J. Am. Chem. Soc.* **1994**, *116*, 5250.
(61) Loeb, K. E.; Zaleski, J. M.; Westre, T. E.; Guajardo, R. J.; Mascharak, P. K.; Hedman, B.; Hodgson, K. O.; Solomon, E. I. J. *Am. Chem. Soc.* **1995**, *117*, 4545.
(62) Burger, R. M.; Horwitz, S. B.; Peisach, J.; Wittenberg, J. B. *J. Biol. Chem.* **1979**, *254*, 12299.
(63) Albertini, J. P.; Garnier-Suillerot, A.; Tosi, L. *Biochem. Biophys. Res. Commun.* **1982**, *104*, 557.
(64) Burger, R. M.; Kent, T. A.; Horwitz, S. B.; Münck, E.; Peisach, J. *J. Biol. Chem.* **1983**, *258*, 1559.
(65) Sugiura, Y.; Kikuchi, T. *J. Antibiot. (Tokyo)* **1978**, *31*, 1310.
(66) Dabrowiak, J. C.; Greenaway, F. T.; Santillo, F. S.; Crooke, S. T. *Biochem. Biophys. Res. Commun.* **1979**, *91*, 721.
(67) Burger, R. M.; Blanchard, J. S.; Horwitz, S. B.; Peisach, J. *J. Biol. Chem.* **1985**, *260*, 15406.
(68) Fulmer, P.; Petering, D. H. *Biochemistry* **1994**, *33*, 5319.
(69) Burger, R. M.; Tian, G.; Drlica, K. *J. Am. Chem. Soc.* **1995**, *117*, 1167.
(70) Veselov, A.; Sun, H.; Sienkiewicz, A.; Taylor, H.; Burger, R. M.; Scholes, C. P. *J. Am. Chem. Soc.* **1995**, *117*, 7508.
(71) Westre, T. E.; Loeb, K. E.; Zaleski, J. M.; Hedman, B.; Hodgson, K. O.; Solomon, E. I. *J. Am. Chem. Soc.* **1995**, *117*, 1309.
(72) Burger, R. M.; Peisach, J.; Horwitz, S. B. *J. Biol. Chem.* **1982**, *257*, 3372.
(73) Rabow, L. E.; Stubbe, J.; Kozarich, J. W. *J. Am. Chem. Soc.* **1990**, *112*, 3196.
(74) McGall, G. H.; Rabow, L. E.; Stubbe, J.; Kozarich, J. W. *J. Am. Chem. Soc.* **1987**, *109*, 2836.
(75) Sausville, E. A.; Stein, R. W.; Peisach, J.; Horwitz, S. B. *Biochemistry* **1978**, *17*, 2746.
(76) Lown, J. W. In *Bleomycin: Chemical, Biochemical, and Biological Aspects;* Hecht, S. M., Ed.; Springer-Verlag: New York, 1979; p 184.
(77) Yamanaka, N.; Kato, T.; Nishida, K.; Ota, K. *Cancer Res.* **1978**, *38*, 3900.
(78) Trush, M. A. *Chem.-Biol. Interact.* **1983**, *45*, 65.
(79) Roy, S. N.; Horwitz, S. B. *Cancer Res.* **1984**, *44*, 1541.
(80) Gutteridge, J. M. C.; Beard, A. P. C.; Quinlan, G. J. *Biochem. Int.* **1985**, *10*, 441.
(81) Sugiura, Y.; Suzuki, T.; Kuwahara, J.; Tanaka, H. *Biochem. Biophys. Res. Commun.* **1982**, *105*, 1511.
(82) Valentine, J. S.; Curtis, A. B. *J. Am. Chem. Soc.* **1975**, *97*, 224.
(83) Keyer, K.; Imlay, J. A. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 13635.
(84) Rabow, L. E.; McGall, G. H.; Stubbe, J.; Kozarich, J. W. *J. Am. Chem. Soc.* **1990**, *112*, 3203.
(85) Padbury, G.; Sligar, S. G.; Labeque, R.; Marnett, L. J. *Biochemistry* **1988**, *27*, 7846.
(86) Natrajan, A.; Hecht, S. M.; van der Marel, G. A.; van Boom, J. H. *J. Am. Chem. Soc.* **1990**, *112*, 4532.
(87) Barr, J. R.; van Atta, R. B.; Natrajan, A.; Hecht, S. M.; van der Marel, G. A.; van Boom, J. H. *J. Am. Chem. Soc.* **1990**, *112*, 4058.
(88) Onishi, T.; Iwata, H.; Takagi, Y. *Biochim. Biophys. Acta* **1975**, *378*, 439.
(89) Buettner, G. R.; Moseley, P. L. *Biochemistry* **1992**, *31*, 9784.
(90) Cheng, C.-C.; Groll, J. G.; Neyhart, G. A.; Welch, T. W.; Singh, P.; Thorp, H. H. *J. Am. Chem. Soc.* **1995**, *117*, 2970.
(91) Nakamura, M.; Peisach, J. *J. Antibiot. (Tokyo*) **1988**, *41*, 638.
(92) Xu, R. X.; Nettesheim, D.; Otvos, J. D.; Petering, D. H. *Biochemistry* **1994**, *33*, 907.
(93) Caceres-Cortes, J.; Sugiyama, H.; Ikudome, K.; Saito, I.; Wang, A. H.-J. *Biochemistry* **1997**, *36*, 9995.
(94) Murugesan, N.; Ehrenfeld, G. M.; Hecht, S. M. *J. Biol. Chem.* **1982**, *257*, 8600.
(95) Sam, J. W.; Tang, X.-J.; Magliozzo, R. S.; Peisach, J. *J. Am. Chem. Soc.* **1995**, *117*, 1012.
(96) Sugiura, Y.; Suzuki, T. *J. Biol. Chem.* **1982**, *257*, 10544.
(97) Suzuki, T.; Kuwahara, J.; Sugiura, Y. *Biochem. Biophys. Res. Commun.* **1983**, *117*, 916.
(98) Hertzberg, R. P.; Caranfa, M. J.; Hecht, S. J. *Biochemistry* **1988**, *27*, 3164.
(99) Iitaka, Y.; Nakamura, H.; Nakatani, T.; Muraoka, Y.; Fujii, A.; Takita, T.; Umezawa, H. *J. Antibiot. (Tokyo)* **1978**, *31*, 1070.
(100) Brown, S. J.; Mascharak, P. K.; Stephan, D. W. *J. Am. Chem. Soc.* **1988**, *110*, 1996.
(101) Farinas, E.; Tan, J. D.; Baidya, N.; Mascharak, P. K. *J. Am. Chem. Soc.* **1993**, *115*, 2996.
(102) Povirk, L. F.; Hogan, M.; Dattagupta, N. *Biochemistry* **1979**, *18*, 96.
(103) Manderville, R. A.; Ellena, J. F.; Hecht, S. M. *J. Am. Chem. Soc.* **1994**, *116*, 10851.
(104) Wu, W.; Vanderwall, D. E.; Stubbe, J.; Kozarich, J. W.; Turner, C. J. *J. Am. Chem. Soc.* **1994**, *116*, 10843.
(105) Haasnoot, C. A. G.; Pandit, U. K.; Kruk, C.; Hilbers, C. W. *J. Biomol. Struct. Dyn.* **1984**, *2*, 449.
(106) Akkerman, M. A. J.; Haasnoot, C. A. G.; Pandit, U. K.; Hilbers, C. W. *Magn. Reson. Chem.* **1988**, *26*, 793.
(107) Oppenheimer, N. J.; Rodriguez, L. O.; Hecht, S. M. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 5616.
(108) Akkerman, M. A. J.; Neijman, E. J. W. F.; Wijmenga, S. S.; Hilbers, C. W.; Bermel, W. *J. Am. Chem. Soc.* **1990**, *112*, 7462.
(109) Akkerman, M. A. J.; Haasnoot, C. A. G.; Hilbers, C. W. *Eur. J. Biochem.* **1988**, *173*, 21.
(110) Manderville, R. A.; Ellena, J. F.; Hecht, S. M. *J. Am. Chem. Soc.* **1995**, *117*, 7891.
(111) Otvos, J. D.; Antholine, W. E.; Wehrli, S.; Petering, D. H. *Biochemistry* **1996**, *35*, 1458.
(112) Calafat, A. M.; Won, H.; Marzilli, L. G. *J. Am. Chem. Soc.* **1997**, *119*, 9, 3656.
(113) Wu, W.; Vanderwall, D. E.; Turner, C. J.; Kozarich, J. W.; Stubbe, J. *J. Am. Chem. Soc.* **1996**, *118*, 1281.
(114) Wu, W.; Vanderwall, D. E.; Lui, S. M.; Tang, X.-J.; Turner, C. J.; Kozarich, J. W.; Stubbe, J. *J. Am. Chem. Soc.* **1996**, *118*, 126.
(115) Caceres-Cortes, J.; Sugiyama, H.; Ikudome, K.; Saito, I.; Wang, A. H.-J. *Eur. J. Biochem.* **1997**, *244*, 818.
(116) 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.
(117) Shipley, J. B.; Hecht, S. M. *Chem. Res. Toxicol.* **1988**, *1*, 25.
(118) Veselov, A.; Burger, R. M.; Scholes, C. P. *J. Am. Chem. Soc.* **1998**, *120*, 1030.
(119) Lehmann, T. E.; Ming, L.-J.; Rosen, M. E.; Que, L., Jr. *Biochemsitry* **1997**, *36*, 2807.

(120) Carter, B. J.; Murty, V. S.; Reddy, K. S.; Wang, S.-N.; Hecht, S. M. *J. Biol. Chem.* **1990**, *265*, 4193.
(121) Carter, B. J.; Reddy, K. S.; Hecht, S. M. *Tetrahedron* **1991**, *47*, 2463.
(122) Boger, D. L.; Ramsey, T. M.; Cai, H.; Hoehn, S. T.; Kozarich, J. W.; Stubbe, J. *J. Am. Chem. Soc.* **1998**, *120*, 53.
(123) Absalon, M. J.; Kozarich, J. W.; Stubbe, J. *Nucleic Acids Res.* **1992**, *20*, 4179.
(124) Kross, J.; Henner, W. D.; Hecht, S. M.; Haseltine, W. A. *Biochemistry* **1982**, *21*, 4310.
(125) Ueda, K.; Kobayashi, S.; Sakai, H.; Komao, T. *J. Biol. Chem.* **1985**, *260*, 5801.
(126) Takeshita, M.; Grollman, A. P.; Ohtsubo, E.; Ohtsubo, H. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 5983.
(127) D'Andrea, A. D.; Haseltine, W. A. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 3608.
(128) Takeshita, M.; Kappen, L. S.; Grollman, A. P.; Eisenberg, M.; Goldberg, I. H. *Biochemistry* **1981**, *20*, 7599.
(129) Kuwahara, J.; Sugiura, Y. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 2459.
(130) Bailly, C.; Waring, M. J. *J. Am. Chem. Soc.* **1995**, *117*, 7311.
(131) Lloyd, R. S.; Haidle, C. W.; Robberson, D. L.; Dodson, M. L., Jr. *Current Microbiol.* **1978**, *1*, 45.
(132) Povirk, L. F. *Mutat. Res.* **1987**, *180*, 1.
(133) Murray, V.; Tan, L.; Matthews, J.; Martin, R. F. *J. Biol. Chem.* **1988**, *263*, 12854.
(134) Bansal, M.; Lee, J. S.; Stubbe, J.; Kozarich, J. W. *Nucleic Acids Res.* **1997**, *25*, 1836.
(135) Absalon, M. J.; Wu, W.; Kozarich, J. W.; Stubbe, J. *Biochemistry* **1995**, *34*, 2076.
(136) Povirk, L. F.; Han, Y.-H.; Steighner, R. J. *Biochemistry* **1989**, *28*, 5808.
(137) Steighner, R. J.; Povirk, L. F. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 8350.
(138) Wu, J. C.; Kozarich, J. W.; Stubbe, J. *J. Biol. Chem.* **1983**, *258*, 4694.
(139) Burger, R. M.; Peisach, J.; Horwitz, S. B. *J. Biol. Chem.* **1982**, *257*, 8612.
(140) Pogozelski, W. K.; Tullius, T. D. *Chem. Rev.* **1998**, *98*, 1089.
(141) Wu, J. C.; Kozarich, J. W.; Stubbe, J. *Biochemistry* **1985**, *24*, 7562.
(142) Kozarich, J. W.; Worth, L., Jr.; Frank, B. L.; Christner, D. F.; Vanderwall, D. E.; Stubbe, J. *Science* **1989**, *245*, 1396.
(143) Wu, J. C.; Stubbe, J.; Kozarich, J. W. *Biochemistry* **1985**, *24*, 7569.
(144) Sugiyama, H.; Xu, C.; Murugesan, N.; Hecht, S. M. *J. Am. Chem. Soc.* **1985**, *107*, 4104.
(145) Sugiyama, H.; Xu, C.; Murugesan, N.; Hecht, S. M.; van der Marel, G. A.; van Boom, J. H. *Biochemistry* **1988**, *27*, 5.
(146) Murugesan, N.; Xu, C.; Ehrenfeld, G. M.; Sugiyama, H.; Kilkuskie, R. E.; Rodriguez, L. O.; Chang, L.-H.; Hecht, S. M. *Biochemistry* **1985**, *24*, 4, 5735.
(147) Burger, R. M.; Drlica, K.; Birdsall, B. *J. Biol. Chem.* **1994**, *269*, 25978.
(148) Groves, J. T.; McClusky, G. A. *J. Am. Chem. Soc.* **1976**, *98*, 859.
(149) Sam, J. W.; Peisach, J. *Biochemistry* **1993**, *32*, 1488.
(150) Sugiyama, H.; Ohmori, K.; Saito, I. *J. Am. Chem. Soc.* **1994**, *116*, 10326.
(151) McGall, G. H.; Rabow, L. E.; Ashley, R. G.; Wu, S. H.; Kozarich, J. W.; Stubbe, J. *J. Am. Chem. Soc.* **1992**, *114*, 4958.
(152) Grollman, A. P.; Takeshita, M.; Pillai, K. M. R.; Johnson, F. *Cancer Res.* **1985**, *45*, 1127.
(153) Saito, I.; Morii, T.; Matsuura, T. *J. Org. Chem.* **1987**, *52*, 1008.
(154) Ajmera, S.; Wu, J. C.; Worth, L., Jr.; Rabow, L. E.; Stubbe, J.; Kozarich, J. W. *Biochemistry* **1986**, *25*, 6586.
(155) Burger, R. M.; Projan, S. J.; Horwitz, S. B.; Peisach, J. *J. Biol. Chem.* **1986**, *261*, 15955.
(156) Burger, R. M.; Drlica, K. In *DNA and RNA Cleavers and Chemotherapy of Cancer and Viral Disease;* Meunier, B., Ed.; Kluwer: Dordrecht, Netherlands, 1996; p 91.
(157) Rashid, R.; Langfinger, D.; Wagner, R.; Schuchmann, H.-P.; von Sonntag, C. Manuscript in preparation.
(158) Sugiyama, H.; Sera, T.; Dannoue, Y.; Marumoto, R.; Saito, I. *J. Am. Chem. Soc.* **1991**, *113*, 2290.
(159) Giese, B.; Beyrich-Graf, X.; Erdman, P.; Giraud, L.; Imwinkelried, P.; Müller, S. N.; Schwitter, U. *J. Am. Chem. Soc.* **1995**, *117*, 6146.
(160) Giese, B.; Beyrich-Graf, X.; Erdman, P.; Petretta, M.; Schwitter, U. *Chem. Biol.* **1995**, *2*, 367.
(161) Gugger, A.; Batra, R.; Razadek, P.; Rist, G.; Giese, B. *J. Am. Chem. Soc.* **1997**, *119*, 8740.
(162) Povirk, L. F.; Houlgrave, C. W. *Biochemistry* **1988**, *27*, 3850.
(163) Saito, I.; Morii, T.; Matsuura, T. *Nucleic Acids Res.* **1983**, *Symposium Series No. 12*, 95.
(164) Morgan, M. A.; Hecht, S. M. *Biochemistry* **1994**, *33*, 10286.
(165) Holmes, C. E.; Hecht, S. M. *J. Biol. Chem.* **1993**, *268*, 25909.
(166) Bansal, M.; Stubbe, J.; Kozarich, J. W. *Nucleic Acids Res.* **1997**, *25*, 186.
(167) Holmes, C. E.; Duff, R. J.; van der Marel, G. A.; van Boom, J. H.; Hecht, S. M. *Bioorg. Med. Chem.* **1997**, *5*, 1235.
(168) Duff, R. J.; de Vroom, E.; Geluk, A.; Hecht, S. M.; van der Marel, G. A.; van Boom, J. H. *J. Am. Chem. Soc.* **1993**, *115*, 3350.
(169) Keck, M. V.; Hecht, S. M. *Biochemistry* **1995**, *34*, 12029.
(170) Petering, D. H.; Byrnes, R. W.; Antholione, W. E. *Chem. Biol. Interact.* **1990**, *73*, 133.
(171) Suzuki, H.; Nagai, K.; Akutsu, E.; Yamaki, H.; Tanaka, N. *J. Antibiot. (Tokyo)* **1970**, *23*, 473.
(172) Chang, C.-H.; Meares, C. F. *Biochemistry* **1982**, *21*, 6332.
(173) Subramanian, R.; Meares, C. F. *Biochem. Biophys. Res. Commun.* **1985**, *133*, 1145.
(174) Chang, C.-H.; Meares, C. F. *Biochemistry* **1984**, *23*, 2268.
(175) Sugiyama, H.; Kawabata, H.; Fujiwara, T.; Dannoue, Y.; Saito, I. *J. Am. Chem. Soc.* **1990**, *112*, 5252.
(176) Xu, R. X.; Antholine, W. E.; Petering, D. H. *J. Biol. Chem.* **1992**, *267*, 950.
(177) Xu, R. X.; Antholine, W. E.; Petering, D. H. *J. Biol. Chem.* **1992**, *267*, 944.
(178) Burger, R. M.; Peisach, *J. Inorg. Chim. Acta, Bioinorg. Sect.* **1983**, *79*, 304, V24.
(179) Kuwahara, J.; Suzuki, T.; Sugiura, Y. *Biochem. Biophys. Res. Commun.* **1985**, *129*, 368.
(180) Burger, R. M.; Freedman, J. H.; Horwitz, S. B.; Peisach, *J. Inorg. Chem.* **1984**, *23*, 2215.
(181) Ehrenfeld, G. M.; Murugesan, N.; Hecht, S. M. *Inorg. Chem.* **1984**, *23*, 1496.
(182) Suzuki, T.; Kuwahara, J.; Goto, M.; Sugiura, Y. *Biochim. Biophys. Acta* **1985**, *824*, 330.
(183) Maxam, A. M.; Gilbert, W. *Methods Enzymol.* **1980**, *65*, 499.
(184) Ehrenfeld, G. M.; Rodriguez, L. O.; Hecht, S. M.; Chang, C.; Basus, V. J.; Oppenheimer, N. J. *Biochemistry* **1985**, *24*, 81.
(185) Suzuki, T.; Kuwahara, J.; Sugiura, Y. *Biochemistry* **1985**, *24*, 4719.
(186) Ehrenfeld, G. M.; Shipley, J. B.; Heimbrook, D. C.; Sugiyama, H.; Long, E. C.; Van Boom, J. H.; Van der Marel, G. A.; Oppenheimer, N. J.; Hecht, S. M. *Biochemistry* **1987**, *26*, 931.
(187) Grove, R. B.; Reba, R. C.; Eckelman, W. C.; Goodyear, M. *J. Nucl. Med.* **1974**, *15*, 386.
(188) Kono, A.; Matsushima, Y.; Kojima, M.; Maeda, T. *Chem. Phar. Bull.* **1977**, *25*, 1725.
(189) Jekunen, A. P.; Kaireme, K. J.; Ramsay, H. A.; Kajanti, M. *J. Clin. Nucl. Med.* **1996**, *21*, 129.
(190) Lazo, J. S.; Humphreys, C. J. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 3064.
(191) Munn, S. E.; Higgins, E.; Marshall, M.; Clement, M. *Br. J. Dermatol.* **1996**, *135*, 969.
(192) Thrall, R. S.; Scalise, P. J. *Lung Biol. Health Dis.* **1995**, *80*, 213.
(193) Poddevin, B.; Orlowski, S.; Belehradek, J., Jr.; Mir, L. M. *Biochem. Pharmacol.* **1991**, *42*, S67.
(194) Domenge, C.; Orlowski, S.; Luboinski, B.; De Baere, T.; Schwab, G.; Belehradek, J., Jr.; Mir, L. M. *Cancer* **1996**, *77*, 956.
(195) Sebti, S. M.; Lazo, J. S. *Pharmacol. Ther.* **1988**, *38*, 321.
(196) Lazo, J. S.; Schisselbauer, J. C.; Meandzija, B.; Kennedy, K. A. *Biochem. Pharmacol.* **1989**, *38*, 2207.
(197) Zuckerman, J. E.; Raffin, T. A.; Brown, J. M.; Newman, R. A.; Etiz, B. B.; Sikic, B. I. *Cancer Res.* **1986**, *46*, 1748.
(198) Demple, B.; Ambile-Cuevas, C. F. *Cell* **1991**, *67*, 837.
(199) Povirk, L. F.; Austin, M. J. F. *Mutation Res.* **1991**, *257*, 127.
(200) Huang, L.; Xie, Y.; Lown, J. W. *Exp. Opin. Ther. Patents* **1996**, *6*, 893.

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