Origin of Malondialdehyde from DNA Degraded by Fe(II)·Bleomycin

(Received for publication, February 19, 1980, and in revised form, May 5, 1980)

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Ferrous bleomycin is known to break DNA efficiently in vitro in the presence of O₂ giving rise to oligonucleotides, bases, and compounds resembling malondialdehyde in their chromogenic reaction with 2-thiobarbituric acid. Chromatography of radio-labeled DNA reaction mixtures resolves three kinds of malondialdehyde-like products, related by sequential conversions. The first chromogenic product is linked to DNA, and its formation does not entail the release of a base. It decomposes readily to the second product, a compound containing the base and deoxyribose carbons 1'–3'. Hydrolysis of either product yields the third, which is indistinguishable from authentic malondialdehyde. These findings suggest that the oxygen-dependent cleavage of DNA by Fe(II)·bleomycin can begin with the rupture of the deoxyribose 3'–4'-carbon bond. The initiation of these events is concurrent with the initiation of another mode of DNA degradation, involving the release of free base alone, in a yield similar to that of chromogen.

The degradation of DNA by bleomycin, which is believed to underlie the antitumor activity of this antibiotic (1), follows the formation of a bleomycin complex with both Fe(II) and molecular oxygen (2). The mechanism of the ensuing reaction with DNA is obscure, and none yet proposed can account for all the DNA degradation products so far detected. These reaction products include free nucleic bases (3–6) and baselike species containing the base label of their nucleoside precursors (7). The residual oligonucleotides bear few, if any, terminal 3'-phosphate groups, but 5' termini are predominantly phosphorylated (8). Material resembling malondialdehyde, which is derived from lipid oxidation, has long provided food chemists with a sensitive assay of rancidity (12) by means of its chromogenic reaction with 2-thiobarbituric acid, yielding an intensely colored adduct (ε₅₃₂ = 1.6 x 10⁵ (13)) (Fig. 1). The colored adduct characteristic of malondialdehyde is also formed from products of DNA degraded by ionizing radiation (9, 10, 14, 15), or by aerobic Fe(II) solutions (16), as well as by bleomycin. The possibility that the chromogen from DNA might not be malondialdehyde, but a precursor of malondialdehyde, was appreciated by Kapp and Smith (10), who found that the chromogen precipitated with x-irradiated DNA, while authentic malondialdehyde did not.

In an attempt to determine the chemistry of DNA breakage by ferrous bleomycin, we tried to isolate physically the malondialdehyde reportedly produced. Our recovery of malondialdehyde in distillates of bleomycin-treated DNA reaction mixtures was so inferior to that from model mixtures containing authentic malondialdehyde that we re-examined our reaction mixtures by chromatographic fractionation. Our analysis indicates that in bleomycin reaction mixtures, the chromogen previously considered to be malondialdehyde is not malondialdehyde, but rather consists of two intermediates, each containing the deoxyribose carbons 1'–3' derived from an initial drug-induced cleavage of the 3'–4' carbon bond of the sugar. These intermediates can react with 2-thiobarbituric acid to give an adduct identical with the one produced by malondialdehyde, or they can undergo acid or base hydrolysis to produce malondialdehyde.

EXPERIMENTAL PROCEDURES

Preparation, Assay, and Derivatization of Malondialdehyde—Malondialdehyde was generated (17) from malondialdehyde bis(dimethyl acetal) (Aldrich) by treating a 5 mM solution with 0.1 N HCl at 50°C for 1 h in a stoppered flask. The solution was then cooled and diluted 5-fold with water. Stock solutions were stored at 10°C for up to 2 months with no loss, based on colorimetric assay with 2-thiobarbituric acid (18), and samples eluted simply from Sephadex G-10 columns (Fig. 2).

We assayed malondialdehyde by heating a sample to 92°C with excess 2-thiobarbituric acid at pH 2 to 3 for 20 min, cooling, and measuring A₅₃₂. Samples (≤0.3 ml) were made up to 0.8 ml with a solution containing 45 mM 2-thiobarbituric acid and 1 mM EDTA. Assay mixtures containing 0.05 to 8.0 nmol of malondialdehyde obeyed Beer's law. The published value of ε₅₃₂ = 1.6 x 10⁵ M⁻¹ cm⁻¹ (13) agrees with our assay standardization, for which we assumed that malondialdehyde was obtained quantitatively from the bis(dimethyl acetal).

The course of color development was monitored, when appropriate, with a Cary model 118 recording spectrophotometer thermostatted at 86°C. Preheated cuvettes containing 3 ml of 35 mM 2-thiobarbituric acid and 1 mM EDTA received 0.2-ml samples of authentic malondialdehyde or aerobic DNA reaction mixtures containing Fe(II)·bleomycin. They were then tightly stopped, and A₅₃₂ was measured at 1-min intervals. First order rate constants were calculated by computer, using least squares criteria, as described (16).
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The 2-thiobarbituric acid adduct of malondialdehyde was prepared according to Sinnhuber et al. (18) from the bis(dimethyl acetal) in 1 N HCl. A yield of 84% was achieved, as was observed with crystals obtained spectrophotometrically from DNA. Aqueous solutions were dissolved in H2O for optical spectroscopy, and in perdeuterated dimethyl sulfoxide (98.5% atom % ²H; Aldrich) for ¹H NMR. A similar preparation of crystals was made from material distilled at 80°C. Those few reaction mixtures that froze were discarded. Reaction mixtures (100 pl) contained 18 mM sodium phosphate buffer, pH 6.6, to contain the same amount of LiCl and ethanol present in the supernatants.

Sephadex G-10 columns served to fractionate some products by gel filtration and others by adsorption chromatography (23). Columns (18 x 1 cm diameter) were equilibrated and eluted with 20 mM sodium phosphate buffer, pH 7.0, at 4°C. DNA eluted at 6.0 ml, and H₂O eluted at 11.5 ml, which were taken to indicate void and included volumes, respectively. The reaction mixtures analyzed were incubated at 0°C. Reaction aliquots (0.6 ml) were applied to the column, either at once, after heating to 60°C, or after base hydrolysis with 0.1 N NaOH at 90°C for 10 min followed by neutralization. Columns were eluted at a rate of 4 ml/h with a hydrostatic pressure head of 12 to 14 cm. Thirty-nine 0.66-ml fractions were collected, followed by 3.3-ml fractions. Of each fraction, 0.3 ml was assayed with 2-thiobarbituric acid, and the remainder was mixed with 5.0 ml of TT-21 scintillant (Yokpton) and assayed for radioactivity by scintillation spectrometry. Recovery of radioactivity was always complete (95 to 105%), but recovery of chromogenic activity gradually decreased (from 90%) as the column was re-used, except that the chromogen in hydrolyzed samples was always completely recovered.

Chromatography of radioactive DNA reaction products was carried out on Analtech RPS 0.25-mm plates, developed with ascending 4 mol ethanol, 15 mm sodium phosphate buffer, pH 7, at 6°C for 4.5 h. The solvent front moved 15 cm. Reaction mixtures containing 0.2 mM [³H]thymidine-labeled DNA (30 Ci/mole of nucleotide), 50 μm bleomycin, 50 μm Fe(II), and 18 mm sodium phosphate buffer, pH 7.0, plus 5 μm authentic [³H]thymine (55 Ci/mole) used as an internal standard. They were incubated at 0°C and analyzed either before or after base hydrolysis at 92°C in a sealed glass capillary. Chromatography was started promptly after applying a 1-pl aliquot to the thin layer at 6°C without drying. Fractons (0.5 cm) were scraped from the support plate for scintillation counting in Aquasol (New England Nuclear).

RESULTS

2-Thiobarbituric Acid Reaction Products and Kinetics—When Fe(II) is added to aerobic mixtures of bleomycin and DNA, the ensuing reaction produces material which can react with 2-thiobarbituric acid to form an adduct having the same spectral characteristics as the 2-thiobarbituric acid adduct of 2-thiobarbituric acid and authentic malondialdehyde. One identical with that of the authentic malondialdehyde adduct, as reported previously (5, 8, 24), and the ¹H NMR spectra (Fig. 1) are consistent with the known structure (17). The fluorescence spectra have also been reported to be identical (25).

Although these 2-thiobarbituric acid adducts appear identical, several observations lead us to conclude that they arise from different precursors. The most evident difference between authentic malondialdehyde and the chromogen from DNA is in the kinetics of their reactions with 2-thiobarbituric acid. At 86°C, the adduct from malondialdehyde forms homogeneously with t₁/₂ = 2.2 min, while the reaction with the DNA products is >90% complete in 2 min, the time of our earliest observations. If the Fe(II)-bleomycin/DNA reaction mixtures are exposed to 0.1 N HCl or 0.1 N NaOH at 86°C for 10 min at 92°C before the 2-thiobarbituric acid reaction, their rates of subsequent color development are equal to the rate with authentic malondialdehyde.

Another difference, which is similarly nullified upon treatment of reaction products with acid or base, is seen in the stability of chromogenic activity. The DNA-derived chromogen is lost from reaction mixtures at 6°C with t₁/₂ = 70 h, while the chromogenic activity of authentic malondialdehyde added to unproductive control incubations is stable, like our 1 mm malondialdehyde stock solutions.
A minor but consistent difference is also seen in the effect of ethanol on the yield of adduct. Addition of 1 to 5 mM ethanol to the 2-thiobarbituric acid assay mixture has no effect on color development with malondialdehyde, but enhances the yield from the DNA products by 8%.

These results are consistent with the hypothesis that reaction mixtures contain a product that may be converted to malondialdehyde by hydrolysis. The product is less stable, and reacts faster than does malondialdehyde in forming the 2-thiobarbituric acid adduct.

Fractionation of 2-Thiobarbituric Acid Chromogens—Malondialdehyde does not co-purify with DNA in cold ethanol, but when bleomycin-treated DNA is ethanol-purified, as described in the legend to Table I, as much as 88% of its chromogenic product is recoverable in the precipitate. Such completeness of precipitation is lost if reactions are performed at higher temperatures, for longer times, or contain a smaller ratio of DNA to bleomycin.

A more detailed analysis of the DNA degradation products was obtained by fractionating reaction mixtures on a Sephadex G-10 column (Fig. 2). Four chromogenic fractions were resolved, each present in an amount depending on the conditions of the reaction and of postreaction treatment.

When a 0°C reaction mixture is applied directly to the column (Fig. 2b), most of the chromogenic material elutes in two fractions: the first with the void volume (Peak 1), and the second subsequent to the included volume near the position of the pyrimidine bases (Peak 3). A minor amount of chromogen elutes at about four times the included volume (Peak 4) near the position of the purine bases.

A rechromatography experiment suggests that the chromogen appearing in the region between peaks 1 and 3 (Fig. 2, b, e, and h) is due to the release of chromogen from DNA during chromatography. When the central Peak 1 fractions of a reaction mixture were applied within 1 h of collection to an identical column, the chromogen material eluted mainly as Peak 1, but 15% of it eluted as Peak 3.

When DNA cleavage reactions are run at room temperature or warmed after their completion, the transfer of chromogen from Peak 1 to Peaks 3 and 4 is enhanced. It is almost complete in mixtures heated to 50°C (at pH 7) for 10 min (Fig. 2c). Chromogen material eluting like authentic malondialdehyde is now manifest in a minor shoulder preceding Peak 3; no trace of color is obtained between the vestige of Peak 1 and this shoulder.

When reaction mixtures are treated with 0.1 mM NaOH or HCl at 92°C for 10 min, then chilled, neutralized, and chromatographed (Fig. 2d), all chromogenic material elutes like malondialdehyde, in Peak 2. The rate of color development in the 2-thiobarbituric acid assay of these fractions is also characteristic of authentic malondialdehyde and is unlike that of the chromogens eluting elsewhere (Peaks 1, 3, and 4).

Fate of Thymine Radioactivity—The origin of the materials eluting in Peaks 2, 3, and 4 was investigated by fractionating reaction products of radioactive DNA. A reaction run at -5°C, stopped at <75% completion, and ethanol-purified released 0.13 eq of ethanol-soluble [6-3H]thymine radioactivity/mol of 2-thiobarbituric acid chromogen formed (Table I). This amount of radioactivity is one-third of that expected if chromogen production were contingent on base release, since thymine normally accounts for about half of the base released by bleomycin from typical DNA’s (5-7, 15). At slightly higher temperatures (6°C; Fig. 2b, d, and h), 0.43 eq of [6-3H]thymine are released per mol of chromogen formed. Other experiments (below) indicate that the deoxyribose-base linkage also remains intact in material cleaved from DNA by bleomycin reactions analyzed at 6°C.

Intact DNA labeled with [6-3H]- or [methyl-14C]thymidine elutes from Sephadex G-10 columns entirely in Peak 1. After incubation with Fe(II)-bleomycin at 0°C and chromatography at 6°C (Fig. 2h), about 10% of the label is eluted subsequent to Peak 1, just like the chromogens described above. Heating to 50°C (Fig. 2i) doubles the amount of label transferred from Peak 1 to Peak 3, while reducing the amount eluting between them. Hydrolysis with base (Fig. 2j) or acid as above has little further effect except to transfer a trace of the 6-3H label to the elution position of H2O. Thus, neither [6-3H]-nor [methyl-14C]thymidine base labels co-chromatograph with malondialdehyde (Peak 2) or the purine-like chromogen (Peak 4) after any treatment described. The participation of thymine in the
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Fig. 2. Sephadex G-10 column fractionation of Fe(II)-bleomycin-degraded DNA products. The DNA cleavage reactions to be fractionated were incubated at 0°C, and the reaction mixtures were applied to the column directly or after the indicated treatments. Columns were run at 5°C and pH 7.0, as described under "Experimental Procedures." a, the elution of the indicated reference compounds (arbitrary ordinate); b to d, the elution of chromogenic incubation products; e to g, the elution of radioactivity from DNA with [U-14C]thymidine; h to j, the elution of radioactivity from DNA with [6-3H]thymidine. The ordinate indicates the radioactivity per fraction and the A_{252} developed in assaying a 0.3-ml aliquot with 2-thiobarbituric acid. The arrows indicate the elution position of malondialdehyde (MDA). The peaks are numbered for reference to the text. Reference compounds tested but not shown here are: formate, thymidine, and cytosine, which elute with peaks centered at 10, 13, and 15.5 ml, respectively.

chromogenic Peak 3 product is not demonstrable using Sephadex G-10 columns, since thymine itself elutes in Peak 3.

Thin layer chromatography of Fe(II)-bleomycin/DNA reaction mixtures reveals that much of the thymine label first released from DNA partitions not as free thymine, but as a separable species that is susceptible to hydrolysis, and then yields a product with the mobility of the free base. When a digest of [6-3H]thymidine-labeled DNA is fractionated by reversed-phase thin layer chromatography (Fig. 3), most of the radioactivity remains associated with oligonucleotides near the origin, but the remainder is found in two mobile fractions. One (R_f = 0.73) co-migrates with an authentic [14C]thymine internal marker; the other is less mobile (R_f = 0.56). Unlike thymine (R_f = 0.75; not shown), this less mobile fraction is susceptible to hydrolysis in 0.1 N NaOH at 92°C for 10 min. When a reaction aliquot is hydrolyzed before chromatography, the fraction having R_f = 0.56 is absent, but the radioactivity found to co-migrate with thymine is enhanced by the amount otherwise found in the missing fraction. The ratio of these two products is variable and depends on the separation system used. We obtained results similar to those of Povirk et al. (7) when we used a cellulose thin layer, but found that the recovery of the non-thymine (R_f = 0.56) product is much enhanced with reversed-phase chromatography.

Fate of Deoxyribose Radioactivity—On Sephadex G-10 fractionation, all the radioactivity in digests of [5'-3H]thymidine-labeled DNA eluted with the oligonucleotide fraction, as in Fig. 2, Peak 1, unless the completed reaction had been hydrolyzed prior to fractionation. The radioactive product then released (5%) eluted as [3H2O] and probably derives from tritium exchange with the solvent. No radioactive formate was detected in any of our reaction mixtures.

When DNA containing [U-14C]thymidine is incubated with bleomycin but not hydrolyzed (Fig. 2, e and f), the distribution of label in the Sephadex G-10 effluent is qualitatively like that of thymine-labeled DNA (Fig. 2, h to j). When the reaction mixture was first hydrolyzed, radioactivity was also found in the Peak 2 eluant (Fig. 2g), otherwise, no new peaks or shoulders in the distribution of radioactive products are discerned. The distribution of radioactivity in Peak 2 eluants...
(Fig. 2g) appears identical with that of the chromogen (Fig. 2d).

The release of deoxyribose fragments was studied quantitatively using DNA doubly labeled with [U-14C]thymidine and [6-3H]thymidine. Fe(II)-bleomycin digests of this DNA which were otherwise untreated (0°C), warmed to 50°C, or base-hydrolyzed were fractionated on Sephadex G-10 columns, and the ratios of isotopes released permitted calculation of the fraction of thymidine carbons appearing in Peak 2 and 3 eluants. Thus, for example, if only the thymine base moiety were released in a particular reaction, the fraction of [6-3H]thymine label released would be twice that of the [U-14C]thymidine label released, since only half of the thymidine carbon atoms are in the base. Such calculations are interpreted cautiously: they express averages that reflect a possible mixture of products.

The results of these experiments are summarized in Table II. When a 0°C reaction mixture is applied directly to the column, the material eluting after Peak 1 contains 8 (of 10) thymidine 14C carbons for every 6-3H]thymine equivalent released. The 5'-H (and, presumably, the 5'-carbon) was seen to remain associated with the oligonucleotide fraction (Peak 1), and no thymidine was detected.

When reactions run at 0°C are heated to 50°C before column chromatography, our calculations indicate that 92% of the increase in released 14C radioactivity derives from thymine and 8% derives from deoxyribose products. The number of thymidine carbons found per eq of [6-3H]thymidine, either in Peak 3 or in all fractions subsequent to Peak 1, is now 6. This would result if, at 50°C, for every new fragment containing 8 thymidine carbons, four fragments now appeared, containing only 5 carbons. Although the release of thymine label more than doubles on heating to 50°C, no significant increase in total chromogen is seen.

When the same reaction products are hydrolyzed before column chromatography, little additional radioactivity is released, but the products are altered so that only Peak 2 is chromogenic. It now includes some deoxyribose radioactivity that would otherwise have appeared in Peak 3 (Fig. 2f and g). The overall 13C:H fractional release ratio remains 0.6, but when Peak 3 alone is considered, the ratio is 0.5, indicating that Peak 3 now contains only thymine. The deoxyribose carbons now elute as malondialdehyde in Peak 2.

A parallel experiment was done using DNA labeled with [1',2',methyl-3H] and [methyl-14C]thymidine (Fig. 4). DNA cleaved at 0°C released equal fractions of both labels, which is consistent with a continuing association of base with deoxyribose carbons 1'-3'. However, in this experiment, not all the released 3H appears in Peak 3: about 20% of the released 3H elutes as 18O. An exchange of 2'-3H with solvent could result from enolization of a 3'-aldehyde, which is a possible structure for the base-sugar fragment. As expected, heating the reaction mixture to 50°C at pH 7 releases additional radioactivity, with 14C predominating, but no additional 3H2O appears. The 3H2O detected exceeds by 5-fold that found in similarly treated controls containing undigested DNA. Base hydrolysis releases 9% of DNA tritium as 3H2O after incubation with bleomycin, but releases very little from untreated DNA. These experiments are refractory to more complete interpretation, since the quantitative distribution of tritium in this thymidine is not precisely known.

The release of 50°C of chromogen from DNA (Fig. 2c) without the release of equivalent thymidine deoxyribose label (Table II) requires comment. It appears that the chromogen formed at 0°C but released from DNA at 50°C must derive mainly from nucleosides other than thymidine. Conversely, the chromogen released at 6°C might be expected to derive mainly from thymidine, and the ratio of chromogen recovered (Fig. 2b) to the thymine and deoxyribose radioactivity released (Fig. 2, e and h) indicates that this is so. Thus, it appears that release from DNA of different nucleoside degradation products is differentially affected by incubation temperature.

**DISCUSSION**

The first observed effects of bleomycin on DNA were a reduction in melting temperature and sedimentation velocity, reflecting DNA polymer cleavage in vitro and in vivo (26). Müller *et al.* (4) observed the formation of aldehyde groups and proposed this to be a probable consequence of the liberation of free thymine. They titrated 0.58 aldehyde eq per thymine released. The aldehydic species was characterized by Kuo and Haidle (8) as malondialdehyde-like in forming the characteristic 2-thiobarbituric acid adduct. They noted the similarity of products of DNA damaged by x-rays and by

**TABLE II**

*Release of base and deoxyribose moieties from DNA*

The double label incubations of Fig. 2, e to j, were analyzed by comparing the fraction of [U-14C]thymidine label released to the fraction of [6-3H]thymidine label released. A ratio of 1 signifies the stoichiometric release of base label and nucleoside label. Details are given under "Experimental Procedures."

| Treatment | Thymidine label | Label eluting after | 14C/1H eluting after |
|-----------|----------------|---------------------|---------------------|
| 0°C, pH 7  | U-14C          | 10 ml               | 14 ml               |
|           | 6-3H           | 9.6                 | 6.6                 |
|           |                | 0.77                | 0.80                |
| 50°C, pH 7 | U-14C          | 13.4                | 12.1                |
|           | 6-3H           | 22.1                | 20.1                |
|           |                | 0.61                | 0.60                |
| 92°C, pH 12.3 | U-14C | 14.8                | 11.2                |
|           | 6-3H           | 23.8                | 22.5                |
|           |                | 0.62                | 0.50                |
bleomycin. Free bases, 5'-phosphate termini, and a malondialdehyde-like chromogen were formed. Haidle et al. (3) and subsequent workers (5-7) observed liberation of all four bases. Haidle et al. (3) proposed that the bleomycin acted primarily by removing bases, as an alkylating agent. Such lesions would then render the DNA polymer unstable. Closer examination of the products of the drug-treated DNA by Povirk et al. (7, 27) revealed further complications: the DNA contained alkali-labile sites in addition to breaks, and the base-like products included species that were distinguishable from authentic free base. The alkali-labile sites were attributed to base-free deoxyribose residues (27).

The possibility that the breaks occurring without alkali treatment give rise to a derivatized base species is suggested by the discovery (14) among the products of irradiated deoxyribonucleotides, of compounds comprising base and deoxyribose fragments, as well as the malondialdehyde-like chromogen. Products originating in DNA degradation and in lipid oxidation resemble malondialdehyde in their product with thiobarbituric acid but appear by other criteria to be different from, though possibly precursors of, malondialdehyde (10, 28).

Scheme 1 summarizes our interpretation of the reactions initiated by bleomycin. The early products of DNA degradation, X and Y, are relatively stable at low temperatures and are macromolecular. They must contain lesions, however, which predispose them to two modes of disintegration. Even at low temperatures, Y slowly releases a compound containing the carbon atoms of the nucleic base and 3 of the 5 deoxyribose carbons. This compound is relatively stable in its chromogenic properties, and a hydrolysis procedure is necessary to cleave the deoxyribose fragment, as malondialdehyde, from the nucleic base moiety. (At room temperature and pH 7, the stoichiometry of chromogen formed per DNA cleavage is about 1.) Following cleavage of the deoxyribose 3'-4' bond, carbons 5' and, probably, 4' remain associated with the degraded oligomer. A mechanism for such a cleavage has been proposed (29).

The other modes of disintegration take effect when reaction mixtures are warmed, releasing free thymine and, presumably, other bases. This lesion yields no malondialdehyde-like chromogen, but the removal of nucleic base renders the residual phosphodeoxyribose oligomer susceptible to cleavage in moderate alkali. Thus, under appropriate conditions, DNA incubated with oxygenated ferrous bleomycin may release malondialdehyde and free nucleic bases, a compound comprising the nucleic base and three deoxyribose carbon atoms, a mixture of the latter two, or nothing. The hypothesis that DNA cleavage results as a consequence of free base release is only partly true, since the released compound combining base and deoxyribose carbons 1'-3' preserves the glycosidic bond. The hypothesis that the free base detected is a breakdown product of this compound is, likewise, only partly true, since base is also released independently.

The events preceding the cleavage reactions were elucidated by Sausville et al. (30, 31), who demonstrated the necessary participation of both Fe(II) and O₂ in the bleomycin-catalyzed reaction. They appreciated the radiomimetic aspects of bleomycin activity and proposed that such free radicals as -OH and -O₂⁻ might be formed as a consequence of Fe(II)-bleomycin oxidation, and that these might attack DNA. Indeed, the detection of free radicals using spin traps in aerobic Fe(II)-bleomycin mixtures (32, 33) has been interpreted as confirming the proposal that ·OH or ·O₂⁻ accumulate and damage DNA in a way analogous to that resulting from irradiation or from treatment with aerobic Fe(II) solutions (34-36). Although this proposal is attractive, there is no compulsion to assume that radicals formed from O₂⁻-Fe(II)-bleomycin autoxidation are the species responsible for the specific DNA cleavage outlined in this paper. The oxygenated complex itself (2) may be the active species or may give rise to one that is not necessarily a free radical.

Oxidation reactions are well known for the heterogeneity of their pathways and products, so the observed release of base, both with and without the chromogenic deoxyribose fragment, does not in itself require that bleomycin inflict upon DNA more than one kind of primary lesion. However, it seems likely that the early intermediates in Scheme 1, X and Y, are

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different, since the prior formation of one final product does not seem to prejudice the yield of the other.

Acknowledgments—We are grateful for the advice and help of Drs. C. Fred Brewer, Felicia A. Gaskin, Pradip Bandyopadhyay, James A. Wechsler, and Robert A. Scalfani. We thank Drs. S. T. Crooke and W. T. Bradner of Bristol Laboratories for supplying bleomycin sulfate.

Addendum—After submission of this paper, an abstract appeared which reported the characterization, by mass spectrometry, of a derivative of adenine plus a 3-carbon deoxyribose fragment obtained from bleomycin-degraded DNA (37).

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