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OXIDATION-REDUCTION REACTIONS OF IRON BLEOMYCIN IN THE ABSENCE AND PRESENCE OF DNA

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Using the pulse radiolysis technique, we have demonstrated that bleomycin-Fe(III) is stoichiometrically reduced by CO_2^- to bleomycin-Fe(II) with a rate of $(1.9 \pm 0.2) \times 10^8\ M^{-1}s^{-1}$. In the presence of calf thymus DNA, the reduction proceeds through free bleomycin-Fe(III) and the binding constant of bleomycin-Fe(III) to DNA has been determined to be $(3.8 \pm 0.5) \times 10^4\ M^{-1}$. It has also been demonstrated that in the absence of DNA O_2^- reacts with bleomycin-Fe(III) to yield bleomycin-Fe(II)O_2, which is in rapid equilibrium with molecular oxygen, and decomposes at room temperature with a rate of (700 ± 200) s^{-1}. The resulting product of the decomposition reaction is Fe(III) which is bound to a modified bleomycin molecule. We have demonstrated that during the reaction of bleomycin-Fe(II) with O_2, modification or self-destruction of the drug occurs, while in the presence of DNA no destruction occurs, possibly because the reaction causes degradation of DNA.

KEY WORDS: Bleomycin, Iron, DNA Damage, Radiolysis, Superoxide, CO_2^-

INTRODUCTION

Bleomycin (BLM) is a cytotoxic glycopeptide used for the treatment of cancer.[1]. *In vitro*, efficient cleavage of DNA by BLM requires Fe(II) and molecular oxygen.[2-4] Fe(III) can replace Fe(II), only in the presence of reducing agents.[3] Degradation of DNA by BLM can also proceed in the presence of Fe(III) and H_2O_2.[5,6] Experiments using optical stopped flow spectrophotometry of BLM-Fe(II) and O_2 indicated that an unstable ternary complex was formed quickly and yielded species which closely resembles BLM-Fe(III) in its spectrum.[7] This intermediate reacted fast to yield "activated BLM" and BLM-Fe(III). A one electron reduction of BLM-Fe(II)O_2 by BLM-Fe(II) has been proposed as the mechanism for this step.[5] "Activated BLM" is the species kinetically competent to cleave DNA.[6] In the absence of DNA it has been demonstrated that "activated BLM" causes destruction of the drug itself.[6,8]

In the present study, we have employed pulse and γ radiolysis techniques to investigate the one electron reduction of BLM-Fe(III) by CO_2^- and O_2^- in the absence and presence of DNA.

MATERIAL AND METHODS

Materials

Blenoxane (bleomycin sulfate) was a gift from Bristol Laboratories. It was dissolved

in water and standardized optically using $\varepsilon_{292} = 1.45 \times 10^4\,M^{-1}cm^{-1}$.[9] BLM-Fe(III) was prepared by mixing 10^{-3} M $Fe(NH_4)(SO_4)_2 \cdot 12H_2O$ (Merck), which was prepared in 10^{-2} N H_2SO_4, with a small excess of BLM, followed by neutralization with NaOH. Solutions of BLM-Fe(II), in buffer, were prepared by mixing oxygen-free solutions of BLM, in phosphate buffer at pH 7, with oxygen-free solutions of $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ (Merck) in water. Calf thymus DNA type I, from Sigma, was dissolved in phosphate buffer at pH 7 and standardized optically using $\varepsilon_{260} = 6800\,M^{-1}cm^{-1}$ (per nucleotide). All reagents were of analytical grade and were used as received. Solutions were prepared in distilled water which was further purified by a millipore reagent grade water system. Solutions were purged of oxygen by swirling them for 15 min under a stream of helium.

Methods

Optical absorption spectra were measured on a Bauch & Lomb model Spectronic 2000 spectrophotometer using curvettes with a 1 cm path length.

The pulse radiolysis set-up has been described earlier.[10] We used electron pulses ranging from 0.5–1.5 μs duration, producing 8–17 μM radicals for $G = 6.05$ per pulse. Xe–Hg 150 W or Xenon 200 W lamps were used as the analytical light sources for kinetic measurements. A 4 cm cell with three light passes was used. Analysis of the data was carried out by employing simultaneously an A to D convertor and a Biomation model 8100, so that two different sweep rates could be used with one pulse. Continuous radiolysis studies were carried out with a ^{137}Cs γ-ray source (Radiation Machinery Co. Model M 3813). Dosimetry was done with the Fricke dosimeter using $G(Fe^{3+}) = 15.6$ and $\varepsilon_{302}^{Fe^{3+}} = 2197\,M^{-1}cm^{-1}$.[11] The dose rate of the ^{137}Cs source was 1.25 Krad/min.

RESULTS AND DISCUSSION

The reduction of BLM-Fe(III) by CO_2^- in the Absence and Presence of DNA

When aqueous formate solutions, saturated with N_2O, are irradiated, all the primary radicals are converted into CO_2^- with a yield of $G(CO_2^-) = 6.05$ molecules/100 eV.[12] In the absence of reactants, CO_2^- dimerizes to oxalate.[13] In γ-radiolysis of BLM-Fe(III), where steady state concentrations of CO_2^- are low, stoichiometric reduction of BLM-Fe(III) takes place:

$$\text{BLM-Fe(III)} + CO_2^- \rightarrow \text{BLM-Fe(II)} + CO_2 \qquad (1)$$

The value of G(BLM-Fe(II)) and the spectrum of BLM-Fe(II), which was obtained through reaction (1), were determined by irradiation of N_2O saturated formate solutions at pH 7 containing BLM-Fe(III) for increasing times. Absorbance changes were followed between 450 and 550 nm, where BLM-Fe(II) absorbs, until all BLM-Fe(III) were reduced and no further changes could be observed. The spectrum of BLM-Fe(II), thus obtained, was similar to that obtained directly by adding Fe(II) to anaerobic solutions of BLM at pH 7 (Figure 1). The G (BLM-Fe(II)) $= 5.4 \pm 0.5$ was lower than that of $G(CO_2^-) = 6.05$ probably because H_2O_2, which is generated during the irradiation with $G_{H_2O_2} = 0.72$,[12] reacts with the metal complexes. Exposing the solutions after the irradiation to air, caused complete oxidation of the complex, but the spectrum, which was obtained was different from the original BLM-Fe(III) (Figure 1). We will define the last species formed as *BLM-Fe(III). The same spec-

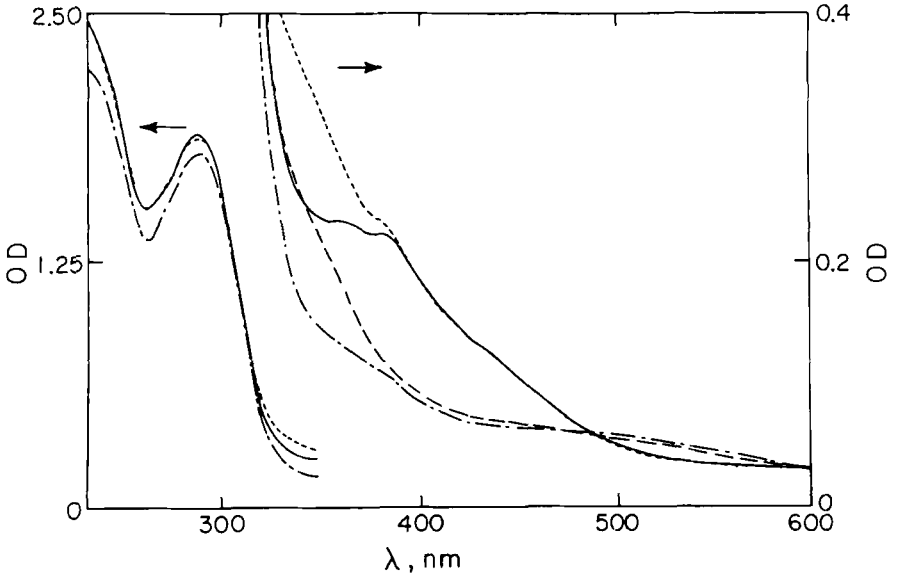


FIGURE 1 The spectra of iron bleomycin complexes. ——— BLM-Fe(III) obtained directly by adding Fe(III) to BLM. —·—· BLM-Fe(II) formed by reducing BLM-Fe(III) by CO_2^-. ------- *BLM-Fe(III) formed by exposing solutions of BLM-Fe(II) to air. – – – – The product of the reduction of *BLM-Fe(III) by CO_2^-. All solutions contained 85 μM BLM-Fe(III),[1] 20 mM formate at pH 7 (2 mM phosphate buffer) and were saturated either with N_2O or air. Irradiations were carried out in a ^{137}Cs γ-ray source which generated 7.56 μM CO_2^-/min in the sample.

trum as that of *BLM-Fe(III) was obtained by adding Fe(II) to air saturated solutions of BLM at pH 7. When N_2O saturated formate solutions of *BLM-Fe(III) were irradiated, CO_2^- reduced *BLM-Fe(III) but the spectrum of the product differed somewhat from that of BLM-Fe(II), (Figure 1). When we irradiated the solutions for short peridos of time as compared to full reduction, and then exposed the irradiated solutions to air, we got spectra which were a mixture of BLM-Fe(III) and *BLM-Fe(III).

It has already been demonstrated by Burger *et al.*[6,8] that drug self-destruction proceeds when BLM-Fe(II) reacts with O_2 in the absence of DNA. From our observations we assume that the spectrum, which we defined as *BLM-Fe(III), is a spectrum of Fe(III) which is bound to a modified BLM, and that modification or self-destruction of BLM occurs during the oxidation reaction of BLM-Fe(II) by O_2.

The kinetics of the reduction of BLM-Fe(III) by CO_2^- was studied by pulse radiolysis. In N_2O saturated formate solutions, under the conditions where [BLM-Fe(III)] > [CO_2^-], we observed a bleaching of the signal at 365 nm as well as at 403 and 435 nm. The kinetics of the reduction reaction followed a biphasic pathway at these wavelengths. A trace of the absorption versus time at 403 nm is shown in the inset of Figure 2. The first phase obeyed first order kinetics and k_{obsd} was linear dependent on [BLM-Fe(III)] (Figure 2). From the slope of the line in Figure 2, we determined $k_{1A} = (1.9 \pm 0.2) \times 10^8\,M^{-1}s^{-1}$. The second phase obeyed first order kinetics and k_{obsd} was independent of [BLM-Fe(III)] and the pulse intensity, $k_{1B} = (45 \pm 10)\,s^{-1}$. We attribute the first phase to the reduction of BLM-Fe(III) by CO_2^- to yield BLM-Fe(II)* or BLM-Fe(II)CO_2 which then follows a change in its configuration or decomposes to BLM-Fe(II), respectively:

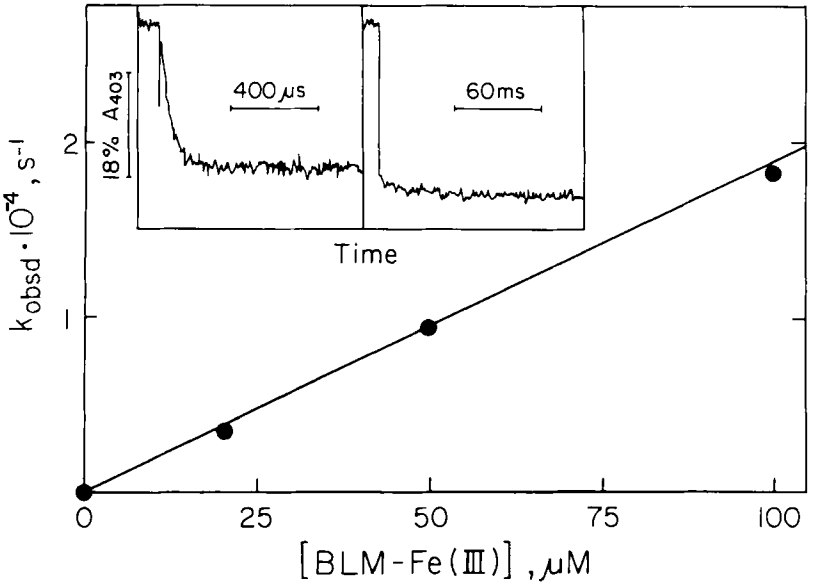


FIGURE 2 The observed rate constant of the first phase of the reduction of BLM-Fe(III) by CO_2^- versus [BLM-Fe(III)]. The inset contains a trace of the absorption of the whole process at 403 nm versus time, where [BLM-Fe(III)] = 100 μM. The pulse duration was 0.5 μsec and the optical pathlength 12.1 cm. All solutions were N_2O saturated and contained 20 mM formate at pH 7 (1 mM phosphate buffer).

$$\text{BLM-Fe(III)} + CO_2^- \rightarrow \text{BLM-Fe(II)}^* + CO_2 \text{ or BLM-Fe(II)}CO_2 \quad (1A)$$

$$\text{BLM-Fe(II)}^* \rightarrow \text{BLM-Fe(II)}$$

or (1B)

$$\text{BLM-Fe(II)}CO_2 \rightarrow \text{BLM-Fe(II)} + CO_2$$

The CO_2 adduct seems to be the more reasonable intermediate as BLM-Fe(III) was not reduced by e_{aq}^-, which is a powerful reducing agent, and also for streometric reasons; and thus electron transfer reaction seems unprobable.

The spectra of BLM-Fe(III) and BLM-Fe(II) in the presence of DNA are very similar to those in the absence of DNA (Figure 3). We assume that in both BLM-Fe(III) and BLM-Fe(III) complexes the metal is hardly affected by DNA. This is in contrast to the cuprous phenanthroline complex, which binds to DNA and its absorption in the presence of DNA at the maximum is reduced to half of the value obtained in the absence of DNA.[14]

While equimolar concentrations of dithionite fully reduced BLM-Fe(III) to BLM-Fe(II) in the presence of DNA, low steady state concentrations of CO_2^- reduced it only partially. We have found that BLM-Fe(II) in the presence of DNA was partially oxidized by CO_2^-. We obtained the same ratio of [Fe(III)]/[Fe(II)] whether BLM-Fe(III) was reduced by CO_2^- or BLM-Fe(II) was oxidized by CO_2^- in the presence of DNA (Figure 3). The ratio [Fe(III)]/[Fe(II)] reached under steady state depended on DNA concentrations. When [DNA] increased the extent of reduction decreased. To explain this dependence on [DNA], we must assume that the reduction process

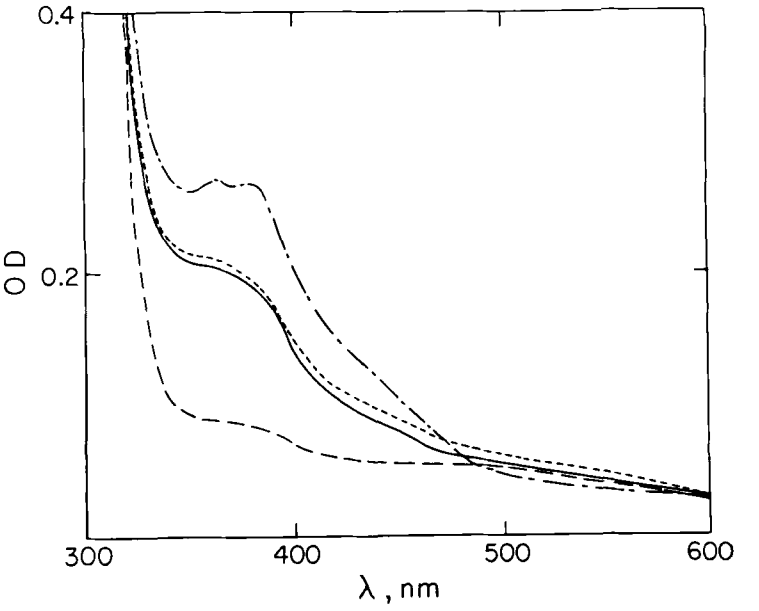


FIGURE 3 The spectra of iron bleomycin complexes in the presence of DNA —·—· BLM-Fe(III) which was prepared directly by adding BLM-Fe(III) to DNA – – – BLM-Fe(II) which was prepared directly by adding oxygen free solutions of BLM-Fe(II) to oxygen-free solutions of DNA ——— Radiolitically reduced BLM-Fe(III) by CO_2^- -------- Radiolitically oxidized BLM-Fe(II) by CO_2^-. All solutions were N_2O saturated and contained 85 μM BLM-Fe(III) or BLM-Fe(II), 420 μM DNA and 20 mM formate at pH 7 (1 mM phosphate buffer). Irradiations were carried out in a ^{137}Cs γ-ray source, which generated 7.56 μM CO_2^-/min.

proceeds through free BLM-Fe(III), while the oxidation process proceeds through the ternary complex of BLM-Fe(II) with DNA.

When solutions of DNA≡BLM-Fe(II), prepared directly or radiolitically in N_2O saturated formate solutions containing DNA≡BLM-Fe(III), were exposed to air, a spectrum similar to that of the original DNA≡BLM-Fe(III) was obtained. When these solutions were resaturated with N_2O and irradiated again, the extent of reduction was higher than that in the case when DNA≡BLM-Fe(III) was prepared directly, indicating that there is more free BLM-Fe(III) in the solution after the exposure to air. From these observations we assume that in the presence of DNA the reaction between BLM-Fe(II) and O_2 causes degradation of DNA, while in the absence of DNA, self-destruction of the drug occurs.

The kinetics of the reduction of BLM-Fe(III) by CO_2^- in the presence of DNA were studied by pulse radiolysis. We assume that in the presence of DNA under the conditions where $[\text{BLM-Fe(III)}]_o > [CO_2^-]_o$, only the reduction of the ferric complexes by CO_2^- proceeded, and competed with the dimerization of CO_2^- to oxalate. The reduction reaction which was followed at 365 nm followed a biphasic pathway as in the absence of DNA. The first phase obeyed first order kinetics and k_{obsd} was linear dependent on [BLM-Fe(III)], and decreased as [DNA] increased. The second phase obeyed first order kinetics and $k_{obsd} = (40 \pm 10)\,s^{-1}$ was independent of [BLM-Fe(III)], [DNA] and the pulse intensity. From these observations we conclude that the

reduction of BLM-Fe(III) by CO_2^- in the presence of DNA proceeds through free BLM-Fe(III), in agreement with our earlier assumption of the extent of reduction of BLM-Fe(III) in the presence of DNA in γ radiolysis, where there are low steady concentrations of CO_2^-.

$$\text{DNA} + \text{BLM-Fe(III)} \rightleftharpoons \text{DNA}\equiv\text{BLM-Fe(III)} \quad (2)$$

$$\text{BLM-Fe(III)} + CO_2^- \rightarrow \text{BLM-Fe(II)}^* + CO_2 \text{ or } \text{BLM-Fe(II)}CO_2 \quad (1A)$$

$$\text{BLM-Fe(II)}^* \rightarrow \text{BLM-Fe(II)}$$

or (1B)

$$\text{BLM-Fe(II)}CO_2 \rightarrow \text{BLM-Fe(II)} + CO_2$$

$$\text{DNA} + \text{BLM-Fe(II)} \rightleftharpoons \text{DNA}\equiv\text{BLM-Fe(II)} \quad (3)$$

We assume that equilibria (2) and (3) are rapid. At high $[DNA]_o$ we obtain for the first phase:

$$k_{obsd} = \frac{k_{1A}[\text{BLM-Fe(III)}]_o}{1 + n_2K_2[DNA]_o} \quad (4)$$

where n_2 is the number of binding sites for BLM-Fe(III) per nucleotide. In Figure 4 $1/k_{obsd}$ is plotted versus $[DNA]_o$. From the slope and intercept of the line we obtained $n_2K_2 = (3.8 \pm 0.5) \times 10^4\,M^{-1}$. It has been demonstrated that BLM binds for every 5 to 6 base pairs in calf thymus DNA.[15] If this is the case also for BLM-Fe(III), then $K_2 = (4.3 \pm 1.0) \times 10^5\,M^{-1}$ which is of the same order of magnitude as that of the binding constant of BLM to DNA, $1.2 \times 10^5\,M^{-1}$.[15]

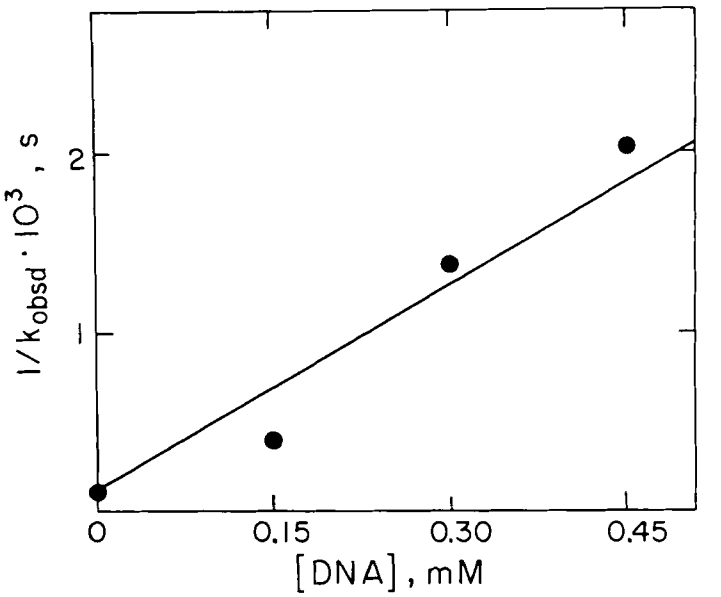


FIGURE 4 The reciprocal of k_{obsd} of the first phase of the reduction of BLM-Fe(III) by CO_2^- versus $[DNA]_o$. All solutions contained 50 μM BLM-Fe(III), 20 mM formate at pH 7 (1 mM phosphate buffer) and were saturated with N_2O. The pulse duration was 0.5 μsec.

The reduction of BLM-Fe(III) by O_2^- in the Absence and Presence of DNA

When aqueous formate solutions, saturated with air, are irradiated, all the primary radicals are converted to O_2^- with a yield of $G(O_2^-) = 6.05$ molecules/100 eV.[12] Under low steady state concentrations of O_2^-, BLM-Fe(III) in the presence of O_2 was fully converted to *BLM-Fe(III) (Figure 1). We assume that O_2^- reduced BLM-Fe(III) and subsequently degradation of the drug occurred, similarly to the case where BLM-Fe(II) or radiolitically reduced BLM-Fe(III) by CO_2^- were exposed to air.

The kinetics of the reduction of BLM-Fe(III) by O_2^- in the presence of oxygen was studied by pulse radiolysis under pseudo first order conditions. A trace of absorption at 385 nm is shown in Figure 5. Two sequential kinetic events could be observed. The rate of the first one, the bleaching of the signal, was found to be first order, and the observed rate constant was independent of $[O_2]$, $[\text{BLM-Fe(III)}]_o$ and the pulse intensity with $k_{obsd} = (1.2 \pm 0.1) \times 10^5\,s^{-1}$. The extent of the bleaching decreased with increasing $[O_2]$. The second process was first order and the observed rate constant was independent of $[\text{BLM-Fe(III)}]_o$, $[O_2]$ and the pulse intensity with $k_{obsd} = (700 \pm 200)\,s^{-1}$.

The rate of the bleaching of the absorption was independent of $[\text{BLM-Fe(III)}]_o$. Therefore we assume that the reaction of BLM-Fe(III) with O_2^- was completed within the pulse. This implies that the rate constant of the reaction must be $\sim 2 \times 10^{10}\,M^{-1}s^{-1}$, which is much faster when compared to other reactions of Fe(III) complexes with O_2^-.[16] This rate is comparable to the rate of O_2^- with BLM (unpublished results). The reaction of BLM-Fe(III) with O_2^-, which was completed within the pulse, cannot yield BLM-Fe(II) as in such a case one would expect to observe at the end of the pulse a decrease in the absorption ($\varepsilon^{385}_{\text{BLM-Fe(III)}} > \varepsilon^{385}_{\text{BLM-Fe(II)}}$). Therefore we assume that O_2^- reacts with BLM-Fe(III) not at the metal site either by forming BLM-Fe(III) or BLM-Fe(III) . . . O_2^-. After the end of the pulse, an intra-

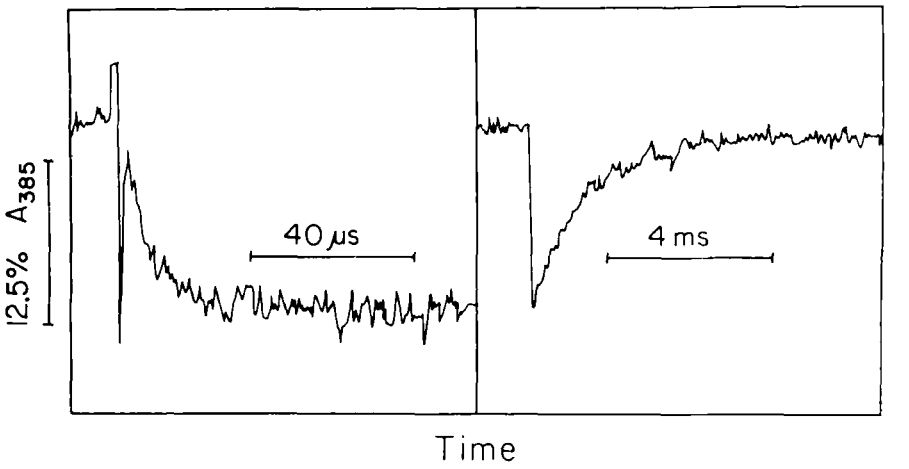


FIGURE 5 A trace of the absorption at 385 nm of the reduction of BLM-Fe(III) by O_2^- in the presence of oxygen. Solutions were air saturated and contained 45 μM BLM-Fe(III), 20 mM formate at pH 7 (1 mM phosphate buffer). The optical path length was 12.1 cm and the pulse duration 1.5 μs. (The sharp negative absorption at the left picture represents the Cerenkov radiation).

molecular electron transfer may proceed to form either BLM-Fe(II), or BLM-Fe(II)O_2, respectively. According to the first mechanism we would expect a bigger bleaching than that obtained as $\varepsilon_{\text{BLM-Fe(III)}} > \varepsilon_{\text{BLM-Fe(II)}}$ at 385 nm, while according to the other mechanism, the small bleaching results from the fact that $\varepsilon_{\text{BLM-Fe(III)}} \sim \varepsilon_{\text{BLM-Fe(II)O}_2}$ at 385 nm.[7] The small bleaching in the first mechanism and the dependence of the extent of bleaching in both mechanisms inversely on $[O_2]$ may be explained by assuming that in the first mechanism BLM-Fe(II) or that in the other mechanism BLM-Fe(II)O_2 are in rapid equilibrium with oxygen:

Mechanism I

$$\text{BLM-Fe(III)} + O_2^- \rightarrow \text{B}\bar{\text{L}}\text{M-Fe(III)} + O_2 \quad (5)$$

$$\text{B}\bar{\text{L}}\text{M-Fe(III)} \rightarrow \text{BLM-Fe(II)} \quad (6)$$

$$\text{BLM-Fe(II)} + O_2 \rightleftharpoons \text{BLM-Fe(II)}O_2 \quad (7)$$

Mechanism II

$$\text{BLM-Fe(III)} + O_2^- \rightarrow \text{BLM-Fe(III)} \ldots O_2^- \quad (5a)$$

$$\text{BLM-Fe(III)} \ldots O_2^- \rightarrow \text{BLM-Fe(II)}O_2 \quad (6a)$$

$$\text{BLM-Fe(II)}O_2 \rightleftharpoons \text{BLM-Fe(II)} + O_2 \quad (7a)$$

We assume that reactions (6) or (6a) are the rate determining steps as otherwise the rate of the bleaching would depend on $[O_2]$. As the extent of the bleaching was inversely depended on $[O_2]$ and $\varepsilon^{385}_{\text{BLM-Fe(III)}} \sim \varepsilon^{385}_{\text{BLM-Fe(II)O}_2} > \varepsilon^{385}_{\text{BLM-Fe(II)}}$,[7] we conclude that (7) or (7a) are in rapid equilibria. According to those mechanisms, the first step observed (Figure 5) is reaction (6) or (6a) with $k = (1.2 \pm 0.1) \times 10^5\ s^{-1}$.

Mechanisms I and II are kinetically and spectrally indistinguishable. Mechanism II has already been proposed by Sugiura *et al.*[17] and seems to be more reasonable. However, as we have found that O_2^- reacts very fast with BLM alone with cmparable rate to that with BLM-Fe(III), we feel that both alternatives should be considered.

We assume that the second process that we observed is the decomposition of BLM-Fe(II)O_2:

$$\text{BLM-Fe(II)}O_2 \rightarrow \text{*BLM-Fe(III)} + \text{products} \quad (8)$$

We have already shown through γ-radiolysis experiments that the resulting product of the irradiation of air saturated formate solutions containing BLM-Fe(III) was *BLM-Fe(III). Therefore we assume that BLM-Fe(II)O_2 decomposes to yield *BLM-Fe(III), which at 385 nm has a similar absorption to that of BLM-Fe(III) and therefore we expect that the signal will return roughly to its initial absorption. The observed rate constant of the last process is given by equation (9) for both mechanisms:

$$k_{\text{obsd}} = k_8/(1 + 1/K_7[O_2]) \quad (9)$$

where $K_7 = [\text{BLM-Fe(II)}O_2]/[\text{BLM-Fe(II)}][O_2]$ and $K_{7a} = 1/K_7$.

Since we have found that k_{obsd} of the last process was independent of $[O_2]$, we conclude that $K_7[O_2] > 1$ and equilibrium (7) is shifted to the right and hence $k_8 = (700 \pm 200)\ s^{-1}$.

Burger *et al.*[7], using stopped flow technique, have studied the kinetics of the reaction of BLM-Fe(II) with O_2 at 2°C. They observed three sequential kinetic events, which were attributed to : a) the formation of BLM-Fe(II)O_2 with a rate of

$6.1 \times 10^3\ M^{-1}s^{-1}$; b) the decay of BLM-Fe(II)O_2 to BLM-Fe(III) with a rate of $0.11\ s^{-1}$, and c) the loss of iron from BLM-Fe(III) to form complex ferric oxides and hydroxides. They observed in the third process, a rise in the absorption from about 430 nm to a very large absorbance near 360 nm as compared to the spectrum of BLM-Fe(III). They attributed these changes to light scattering by insoluble ferric oxides/hydroxides formed by loss of iron from BLM-Fe(III). If this is true, one should expect that this loss of iron would occur in the case where BLM-Fe(III) is formed directly and not only through the rection of BLM-Fe(II) with O_2. We suspect that the changes that were observed were due to self-destruction of the drug, which causes a change in the absorption of Fe(III) which is bound to a modified BLM molecule.

If we compare our results to those obtained by Burger *et al.*[7], our results indicate that the whole process of the reduction of BLM-Fe(III) by O_2^- in the presence of O_2 was completed within 10 ms, which is within a time interval much shorter than that obtained by Burger *et al.*[7] at 2°C. One possible explanation for the very different behaviour between the two systems may result from different species. We assume that BLM-Fe(II) is the same whether it is prepared directly as done by Burger *et al.*[7], or whether it is prepared by reducing BLM-Fe(III) by O_2^- as we did. If this is not the case, and different species are formed, their kinetic behaviour may differ. In such a case it would imply that different reducing agents may yield different species. (We have found that reduction of BLM-Fe(III) by dithionite or by CO_2^- did not yield exactly the same spectrum in the UV, possibly due to some changes in the drug in the case of dithionite (results not shown)). This possibility may be of great interest in the biological or chemotherapeutic action of the drug.

In the presence of DNA, under low steady state concentrations of O_2^-, we did not observe any spectral changes in BLM-Fe(III). We were not able to follow the kinetics of the reaction of O_2^- with BLM-Fe(III) in the presence of DNA, since the absorption changes were too small for detection.

The reactions with peroxide

It has already been demonstrated that catalase does not inhibit the degradation of DNA induced by BLM-Fe(II) and O_2 or BLM-Fe(III), a reducing agent and O_2.[18] On the contrary, it enhances the degradation reaction.[19–20] Therefore it is assumed that H_2O_2 is not a cofactor in the degradation reaction.

We did not investigate in detail the reactions of the various complexes with H_2O_2 as we have found that all the iron BLM complexes in the presence and absence of DNA react with H_2O_2 yielding different species, some of which we were not able to identify.

However, we can summarize our findings as follows: BLM-Fe(III) rects slowly with H_2O_2 yielding in the absence of DNA the same products obtained when BLM-Fe(II) was oxidized by oxygen (Figure 1). The kinetics of this reaction was first order with respect to both reactants. The rate of the reaction, but not the reaction products, depended on whether the solutions were air or helium saturated or whether formate was present in the solutions. In the presence of DNA the reaction was much slower as compared to the case where DNA was absent, and the spectrum of the reaction products was different from that of BLM-Fe(II) or BLM-Fe(III) in the presence of DNA.

It has already been demonstrated that degradation of DNA proceeds either by BLM-Fe(II) and O_2 or BLM-Fe(III) and H_2O_2.[6] In the absence of DNA, our results demonstrate that degradation of the drug occurs during this reaction of BLM-Fe(III)

with H_2O_2 in agreement with previous studies.[6,8] In the presence of DNA, although degradation of DNA takes place, the spectrum of the reaction products did not resemble those obtained when DNA≡BLM-Fe(II) reacted with O_2.

Both BLM-Fe(II) and DNA≡BLM-Fe(II) reacted rapidly with H_2O_2 yielding spectra which were different from those of BLM-Fe(III), *BLM-Fe(III) or DNA≡BLM-Fe(III).

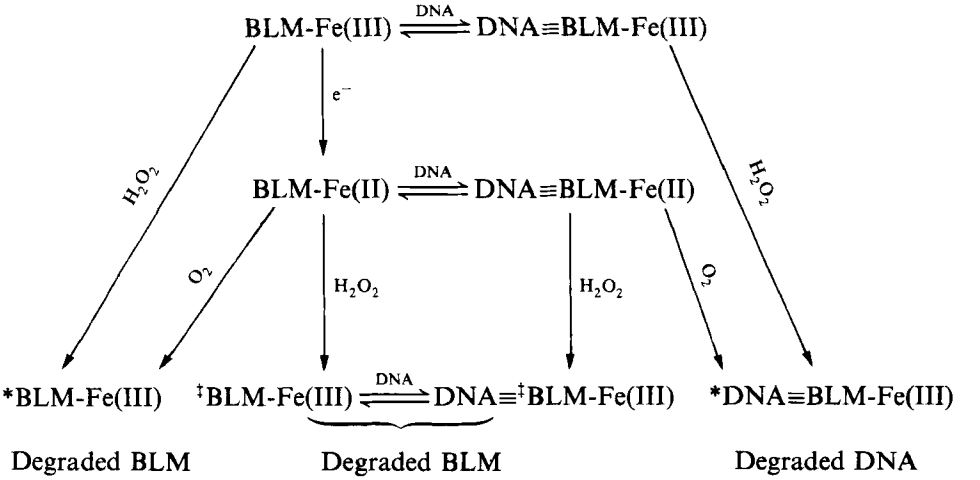
It is possible that the oxidation of BLM-Fe(II) by H_2O_2, which is produced possibly during the oxidation of BLM-Fe(II) by O_2 in the absence and presence of DNA, causes degradation of the drug and therefore catalase, by destroying H_2O_2, enhances the degradation reaction of DNA.

The correlation between the Iron-BLM and Copper-BLM Systems

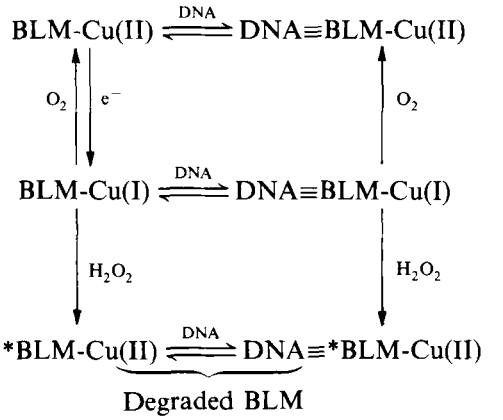
Both BLM-Fe(III) and BLM-Cu(II) are stiochiometrically reduced by CO_2^- with rates of $(1.9 \pm 0.2) \times 10^8\ M^{-1}s^{-1}$ and of $(6.7 \pm 0.5) \times 10^8\ M^{-1}s^{-1}$,[21] respectively. In the presence of DNA, the reduction process in both cases slows down and proceeds through the free complex. We have determined the binding constants of BLM-Fe(III) and BLM-Cu(II) to calf thymus DNA to be $(3.8 \pm 0.5) \times 10^4\ M^{-1}$ and $(1.5 \pm 0.1) \times 10^3\ M^{-1}$,[21] respectively. While CO_2^- reduces stiochimetrically BLM-Cu(II) in the absence and presence of DNA, BLM-Fe(III) in the prsence of DNA is only partially reduced because the ternary complex of BLM-Fe(II) with DNA is oxidized by CO_2^-.

Both BLM-Cu(I) and BLM-Fe(II) react rapidly with oxygen and H_2O_2. In the copper system, the oxidation of BLM-Cu(I) by oxygen does not cause degradation of the drug,[21] while in the iron system, degradation of the drug occurs. In the presence of DNA, in both systems the oxidation by O_2 does not cause degradation of the drug, but causes degradation of DNA only in the iron system.

The reaction of BLM-Cu(I) with H_2O_2 in the absence and presence of DNA causes degradation of the drug.[21] This may be the reason why BLM-Cu(II) in the presence of reducing agens and O_2 or H_2O_2 does not cleave DNA, while BLM-Fe(II) in the presence of O_2 does. The various reaction schemes of copper and iron bleomycin are given below:



SCHEME 1 Reaction mechanism for degradation of either BLM or DNA by iron-blemoycin.



SCHEME 2 Reaction mechanism for degradation of BLM by copper-bleomycin.

CONCLUSIONS

We have demonstated that BLM-Fe(III) which binds to DNA, is reduced by CO_2^- through free BLM-Fe(III). We suggest that when a metal complex binds to DNA, its reduction proceeds through the free and not the ternary complex as in the case of copper-phenanthroline,[14] copper-bipyridine[14] and copper-BLM.[21]

We have shown that degradation of the drug occurs in the iron system in the absence of DNA, while in the presence of DNA, cleavage of DNA takes place.

The kinetics of the reaction of BLM-Fe(III) with O_2^- in the absenbce of DNA differs from that obtained by Burger *et al.*[7] It may be that different species are formed when BLM-Fe(III) is reduced by different reducing agents. This point may be relevant in biological systems where different reducing agents are present.

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References

1. Blum, R.H., Carter, S.K. and Agre, K. *Cancer*, **31**, 903 (1973).
2. Ishida, R. and Takahashi, T. *Biochem. Biophys. Res. Commun.*, **66**, 1432 (1975).
3. Sausville, E.A., Peisach, J. and Horwitz, S.H. *Biochem. Biophys. Res. Commun.*, **73**, 814 (1976).
4. Sausville, E.A., Peisach, J. and Horwitz, S.B. *Biochemistry*, **17**, 2740 (1978).
5. Kuramochi, H., Takahashi, K., Takita, T. and Umezawa, H. *J. Antibiot. (Tokyo)*, **34** , 576 (1981).
6. Burger, R.M., Peisach, J. and Horwitz, S.B. *J. Biol. Chem.* **256**, 11636 (1981).
7. Burger, R.M., Horwitz, S.B., Peisach, J. and Wittenberg, J.B. *J. Biol. Chem.*, **254**, 12299 (1979).
8. Burger, R.M., Peisach, J. and Horwitz, S.B. *J. Biol. Chem.*, **257**, 3372 (1982).
9. Dabrowiak, J.C., Greenaway, F.T., Longo, W.E., Can Husen, M. and Crooke, S.T. *Biochem. Biophys. Acta*, **517** 517 (1978).
10. Cohen, H. and Meyerstein, D. *Inorg. Chem.*, **13**, 2434 (1974).

11. Schested, K. in: *Manual in Radiation Chemistry,* ed. N.W. Holn and R.J. Berry, Marcel Dekker Inc., New York, (1970) p. 313.
12. Bielski, B.H.J. and Allen, A.O. *Int. J. Radiat. Phys. Chem.,* **1**, 153 (1969).
13. Ross, A.B. and Neta, P. in: *Rate Constants for Reactions of Aliphatic Carbon-Centered Radicals in Aqueous Solutions,* 1982, NSRDS-NBS, 70.
14. Goldstein, S. and Czapski G. *J. Am. Chem. Soc.,* **108** 2244 (1986).
15. Chien, M., Grollman, A.P. and Horwitz, S.B. *Biochemistry* **16**, 3641 (1977).
16. Bielski, B.H.J., Cabelli, D.E., Arudi, R.P. and Ross, A.B. *J. Phys. Chem., Ref. Data,* **14**, 1041 (1985).
17. Sugiura, Y., Suzuki, T., Kuwahara, J. and Tanaka, H. *Biochem. Biophys. Res. Commun.,* **105**, 1511 (1982).
18. Sausville, E.A., Stein, R.W., Peisach, J. and Horwitz, S.B. *Biochemistry,* **17**, 2746 (1978).
19. Gutteridge, J.M.C., Beard, A.P.C. and Quinlan, G.J. *Biochem. Inter.,* **10**, 441 (1985).
20. Aronovich, Y., and Godinger, D. unpublished results.
21. Goldstein, S. and Czapski, G. submitted for publication.

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