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EVIDENCE FOR HIGH-VALENT IRON-OXO SPECIES ACTIVE IN THE DNA BREAKS MEDIATED BY IRON–BLEOMYCIN

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Abstract—The activity of bleomycin is generally attributed to its ability to cleave DNA through the mediation of transition metal salts, molecular oxygen and electrons. Despite the fact that DNA break products are known with great details, the exact nature of the so-called "activated bleomycin", responsible for the activation of C—H bonds of the deoxyriboses, is still a matter of debate. In the present article, we report evidence that high-valent iron-oxo species might be generated by potassium hydrogen persulfate, $KHSO_5$ (a single oxygen atom donor) and are involved in metallobleomycin-mediated DNA breaks.

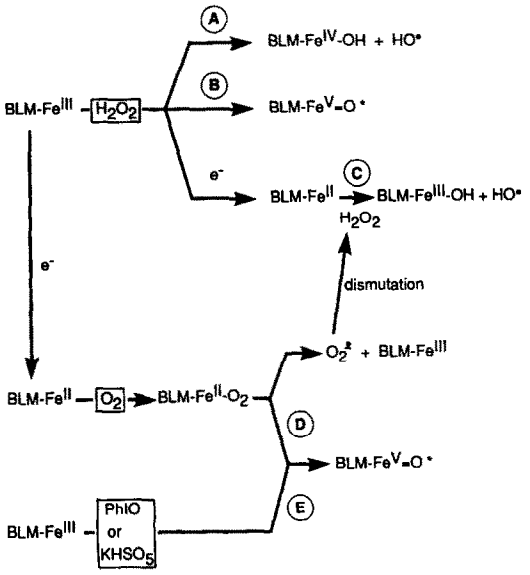
Among all the antitumor agents currently used in cancer therapy, many efforts have been devoted to bleomycin, an antibiotic discovered by Umezawa *et al.* [1]. Actually, bleomycin (BLM)‖ involves a family of glycopeptides which differ by the nature of their bithiazole substituents. The drug used in clinic for treatment of squamous cell carcinomas, Hodgkins disease and other lymphomas [2] is a mixture consisting primarily of BLM A_2 and BLM B_2 (for the detailed structure of BLMs and main review on their mechanism of action, see references [3–5]).

The therapeutic activity of BLM is generally attributed to its DNA binding properties [6, 7] and to its ability to cleave DNA mainly at GpC and GpT sequences [8, 9]. It has been well documented that these DNA breaks are mediated by several redox-active metals (iron, cobalt, copper) which are strongly chelated by nitrogen atoms of the peptide moiety of BLM and are reacting in association with molecular oxygen [3–5]. This unique mechanism of action for an antitumor agent made metallo-bleomycins as the paradigm of a new generation of DNA-damaging transition metal complexes, e.g. methidium propyl–Fe–EDTA [10] or hemin-acridine or -ellipticine [11–14].

The products of DNA strand breaks by metallo-bleomycins are now known with great details: base propenals [15, 16], free bases [17], oligonucleotides having glycolate residue attached at the terminal 3′-phosphate [18] and alkali-labile lesions consisting of fragments with 2,4-dihydroxycyclopentenone at their 3′-phosphate-termini [19].

A key step in DNA lesions is the cleavage of the 4′-carbon-hydrogen bond of deoxyribose rings [16] by the so-called "activated" bleomycin [20–23].

The exact nature of this activated bleomycin is still a matter of debate, but recent articles [4, 21] have pointed out that this form might be relevant to the activated state cytochrome P-450 for which high-valent iron-oxo species have been evidenced using iodosylbenzene as oxygen source [24]. All different possible activation pathways of BLM–Fe^{III} by O_2, H_2O_2 or oxygen donors are summarized in Scheme 1. All the hypotheses on the H_4' abstraction step can be classified in two categories: the Fenton route ($HO^{\cdot}$ as reactive species, routes A and C) or the P-450-like route (with BLM-$Fe^{V}=O$ as hydroxylating



Scheme 1. Different possible activation pathways of BLM–Fe complexes in presence of O_2, H_2O_2 or oxygen donors (PhIO, $KHSO_5$).

* Formal oxidation state illustrating that this highly reactive species is at least at two redox equivalents above BLM–Fe^{III}.

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‖ Abbreviations used: BLM, bleomycin; PhIO, iodosylbenzene; $KHSO_5$, potassium hydrogen persulfate or Oxone®.

agent, routes B, D, E). The high-valent iron-oxo species might be generated by three different oxygen sources: molecular oxygen and electrons, hydrogen peroxide and single oxygen atom donors. The latter compounds are the only oxygen source which can unambiguously support the P-450-like route. Iodosylbenzene has been used by Hecht *et al.* to demonstrate this hypothesis in DNA breaks or oxygen transfer reactions to olefins catalyzed by metallobleomycins [25–27]. Recently, we have developed the use of $KHSO_5$ (potassium hydrogen persulfate or Oxone®), *a water-soluble oxygen donor at physiological pH*, in P-450 modeling studies [28, 29]. We report in the present paper our studies on DNA cleavage mediated by BLM-Fe^{III}-$KHSO_5$ supporting the P-450-like mechanism for the hydrogen atom abstraction from deoxyribose rings by metallobleomycins (for a preliminary report, see Ref. 30).

MATERIALS AND METHODS

Materials

Commercial bleomycin was a gift of Roger Bellon (France). This mixture of BLM A_2 (70%) and BLM B_2 (30%) was used without further purification. An average molecular weight of 1550 was taken. $Fe(ClO_4)_3$ (crystalline form) and $KHSO_5$ were obtained from Alfa (Ventron) and Desferoxamine® from Ciba-Geigy. Solutions of $KHSO_5$ were stable at used pH values, only a slow decomposition with molecular oxygen evolution is observed at day scale. Electrophoresis-grade agarose was purchased from Bio Rad Laboratories. The scintillation fluid was Ready-Solv MP from Beckman. The DNA used for EPR studies was DNA of Herring testes, sodium salt type XIV from Sigma. Phage Ø X 174 RFI supercoiled DNA was obtained from Bethesda Research Laboratories and was dialysed with Tris–HCl buffer pH 7, NaCl 10 mM prior to use. λ[^{14}C]DNA was prepared as previously described [30]. 2-[^{14}C]thymidine was obtained from CEA. Concentration of DNA was estimated spectroscopically assuming a millimolar (nucleotide) extinction coefficient, $\varepsilon_{260} = 6.6\ mM^{-1}\ cm^{-1}$. H_2O used for all the solutions was distilled twice.

Methods

Sucrose gradients. The degradation of radioactively labeled λ DNA was assayed by following the migration of the radioactivity of DNA fragments on alkaline sucrose gradients for single-strand breaks and on neutral sucrose gradients for double-strand breaks [31, 32]. Alkaline sucrose gradients consisted of 5–20% sucrose, 0.7 M NaCl, 0.3 M NaOH, 1 mM EDTA and neutral sucrose gradients of 5–20% sucrose, 50 mM Tris–HCl pH 8, 0.9 M NaCl, 10 mM EDTA. All the incubations were of 30 min at 30° under aerobic conditions. $KHSO_5$ (after being neutralized separately by NaOH) is added lastly. The details for each experiments are given in the figure legends. 10 μL of 10 mM Desferoxamine was added to quench the reaction. The whole reaction mixture was layered on sucrose gradients and centrifuged at 45,000 rpm for 2 hr at 20° in a Beckman SW50.1 rotor. Fractions were collected from the bottom to the top of the centrifugation tube and radioactivity counted. In the case of alkaline gradients each fraction was neutralized by addition of HCl 0.6 N before the scintillation fluid was added.

Electrophoresis. The DNA cleavage activity of our system was determined also on phage Ø X 174 DNA by using agarose gel electrophoresis. Incubation of Ø X 174 form I DNA with BLM–Fe^{III} and $KHSO_5$ was performed at 20° for 30 min under partially anaerobic conditions (Atmosbag, nitrogen). BLM and iron were mixed separately. The BLM–iron complex formation was allowed to proceed for 1 min and the resulting solution was added to the DNA solution. Solutions of $KHSO_5$ were neutralized by NaOH up to a pH value of 6 before being added to the reaction. $KHSO_5$ is the last addition. All reactions were stopped by addition of 5 μL of a gel loading solution consisting of sucrose (50%), Na_2EDTA (0.1 M), bromophenol blue (0.05%). The combined solutions were immediately applied to a 0.8% agarose slab gel containing 1 μg/mL ethidium bromide. Electrophoresis was carried out at constant intensity (25 mA) for 16 hr in 89 mM Tris–borate, pH 8.3, 1 μg/mL ethidium bromide. The gel was visualized by UV light (254 nm) and photographed. Typical reaction mixtures (15 μL total volume) were as indicated in the figure legend.

EPR. EPR spectra were obtained with a Bruker ER 200-D spectrometer operating in the X-band mode. Reaction aliquots (in EPR quartz tubes) were slowly frozen (30 sec) in liquid nitrogen vapours before EPR examination. No ethylene glycol was added to the reaction aliquots to be frozen. EPR spectra were taken before or at various times after addition of $KHSO_5$ in the reaction mixture. The reactions were carried out at 4°. For details, see figure legends.

RESULTS AND DISCUSSION

The DNA damages by BLM-Fe^{III}-$KHSO_5$ have been studied by three different methods: (i) neutral sucrose gradients in order to detect double strand breaks, (ii) alkaline sucrose gradients for single strand breaks, and (iii) agarose gel electrophoresis with supercoiled Ø X 174 DNA.

Double strand breaks of DNA. Neutral sucrose gradients

[^{14}C]labelled DNA of bacteriophage λ (prepared from $\lambda(C_{I857}Sam_7)$ lysogen of *E. coli*) was used to analyze double strand breaks created by the association of persulfate to iron–bleomycin. Incubation of a buffered solution of λ[^{14}C]DNA with increasing amounts of BLM–Fe^{III}–$KHSO_5$ (the molar ratio of the three components was kept constant: BLM/Fe^{III}/$KHSO_5$ equal to 1/4/10) resulted in an increase of DNA double strand breaks as shown by neutral sucrose density gradients (Fig. 1). Sedimentation patterns of phage DNA showed a decrease in molecular size when increasing the amount of artificial nuclease material.

In control experiments (not shown), when DNA was incubated with only one of the three components (BLM, Fe^{III} or $KHSO_5$, incubation time = 30 min at 30°) or with two of them (BLM + $KHSO_5$ or Fe^{III} + $KHSO_5$), no degradation was observed. The

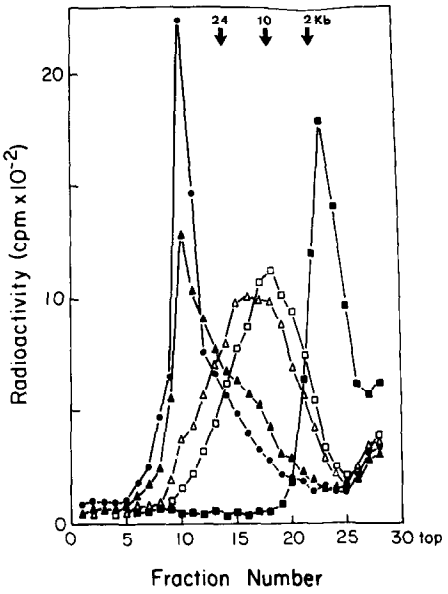

Fig. 1. Neutral sucrose gradient. λ[^{14}C]DNA strand scission by BLM–Fe^{III} and $KHSO_5$. Individual reaction mixture contained in a final volume of 100 μl: 200 ng of λ[^{14}C]DNA (6.25 μM nucleotides), 10 mM Tris–HCl buffer, pH 7.5 and the following concentrations of BLM, Fe^{III} and $KHSO_5$, respectively: (a) (●) 0.062 μM; 0.25 μM; 0.625 μM; (b) (▲) 0.3 μM; 1.25 μM; 3 μM; (c) (△) 0.625 μM; 2.5 μM; 6.25 μM; (d) (□) 1.25 μM; 5 μM; 12.5 μM; (e) (■) 2.5 μM; 10 μM; 25 μM; $KHSO_5$ was the last addition. BLM/bases = (●) 1/100; (▲) 1/20; (△) 1/10; (□) 1/5; (■) 1/2.5.

concentrations used for these controls were those of experiment (e) of Fig. 1. However, for control experiments carried out in the absence of persulfate, i.e. incubation of DNA with BLM + Fe^{III} for all the concentrations indicated in the legend of Fig. 1, some DNA breaks were observed when Fe^{III} concentration was higher than 2.5 μM. However, it should be noted that the number of DNA breaks in control experiments was always below the level of breaks observed in the corresponding assays with the complete set of cleaving agents. This basal level of breaks can be attributed to traces of Fe^{III} complexed by BLM. This point will be discussed in the following paragraph on the single strand breaks.

The data presented in Fig. 1 clearly indicate that Fe–BLM activated by a single oxygen atom donor is able to damage DNA. This generation of "activated bleomycin" without prerequisite reduction of Fe–BLM to a low valent species supports the hypothesis of BLM–Fe^{V} = 0 as being a key intermediate in the promotion of double strand breaks which are expected to be largely irreparable—and consequently lethal—alterations.

However, it is known that BLM induces more single strand breaks than double strand ones [23, 24]. So, we investigated DNA breaks by alkaline sucrose gradient method in order to evidence single strand scissions.

Single strand breaks of DNA. Alkaline sucrose gradients

The single strand breaks have been studied by two different BLM activation routes: (i) using persulfate and BLM–Fe^{III} and (ii) using BLM–Fe^{II}–O_2 in order to compare the biological activation process.

DNA single strand breaks by BLM–Fe^{III}–$KHSO_5$. Incubation of λ[^{14}C]DNA with BLM–Fe^{III}–$KHSO_5$ (the persulfate being the last reagent added before a 30 min incubation time at 30°) resulted in very efficient single strand breaks as illustrated by the migration of DNA fragments on denaturating alkaline sucrose gradients in Fig. 2. As for neutral sucrose gradients, the molar ratio BLM/Fe^{III}/$KHSO_5$ was kept constant at 1/4/10 and the three reported experiments correspond to a BLM/base ratio of 1/10, 1/5 and 1/2.5, respectively.

The radioactivity profile is shifted to the top of the alkaline sucrose gradient when the BLM/base ratio increases. For the same BLM/base ratio of 1/10 (conditions (a) in Fig. 2 and conditions (c) in Fig. 1), the maximum profile is observed for fractions No. 16–18 for double strand breaks and fractions No. 21–23 for single strand breaks, i.e. for smaller DNA fragments, indicating thus that single strand breaks are more frequent than double strand breaks, as previously observed with molecular oxygen as oxygen source for the oxidative DNA cleavage [33, 34].

As observed for the neutral sucrose gradients, the control experiments carried out in the absence of persulfate, some DNA breaks were observed even at low Fe^{III} concentrations. In order to elucidate this rather unexpected result, we have investigated this aspect of control experiments having in mind that

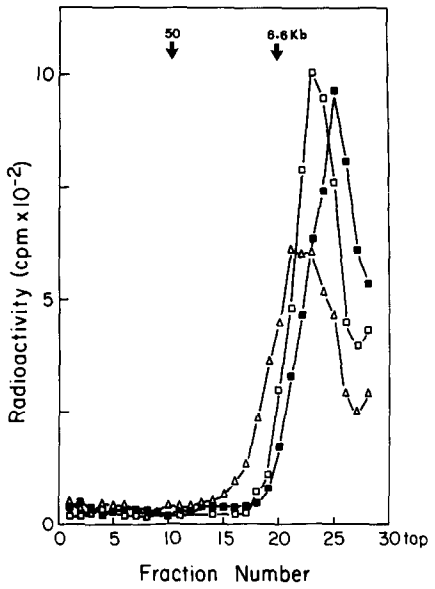

Fig. 2. Alkaline sucrose gradient. λ[^{14}C]DNA single-strand breaks mediated by BLM–Fe^{III} and $KHSO_5$. Individual reaction mixture contained in a final volume of 200 μl: 400 ng of λ[^{14}C]DNA (6.25 μM nucleotides), 15 mM Tris–HCl buffer, pH 7.5 and the following concentration of BLM, Fe^{III} and $KHSO_5$, respectively: (a) (△) 0.625 μM; 2.5 μM; 6.25 μM; (b) (□) 1.25 μM; 5 μM; 12.5 μM; (c) (■) 2.5 μM; 10 μM; 25 μM; BLM/bases = (△) 1/10; (□) 1/5; (■) 1/2.5.

these breaks might be attributed to traces of BLM–Fe^{II} complex (as current hypothesis on the origin of Fe^{II} in BLM–Fe^{III} experiments, traces of reducing agent in the incubation medium might be considered).

DNA single strand breaks by BLM–Fe^{III} *and* BLM–Fe^{II} *complexes.* Figure 3A shows typical profiles of alkaline sucrose gradients when $\lambda[^{14}C]$DNA is treated by increased concentration of BLM–Fe^{III} complex in air (without persulfate) for 30 min (the BLM/Fe^{III} ratio is kept constant at 1/1.5). For the same BLM/base ratio, first of all, it must be noted that levels of DNA breaks in Fig. 3A are always lower than those described in Fig. 2 where persulfate was present.

However, similar patterns for DNA single strand breaks are observed when Fe^{III} is replaced by Fe^{II} at 1% level of the iron concentration used in the experiments of Fig. 3A. These data are reported in Fig. 3B. The single strand breaks increased when the BLM–Fe^{II} complex/base ratio increased. In these experimental conditions, the breaks can be attributed to the known activation of molecular oxygen by BLM–Fe^{II} ([19], see also reference [35] for possible biological reduction pathways of BLM–Fe^{III} to BLM–Fe^{II}).

These single strand breaks are largely increased when the concentration of Fe^{II} increased (see Fig. 3C). In this latter case, the concentration of Fe^{II} was twice that of experiments of Fig. 3B, i.e. 2% of the quantity of Fe^{III} used for data of Fig. 3A.

The results confirm the great activity of BLM–Fe^{II}–O_2 as cleaving agent and suggest at least one hypothesis for the origin of the basal level of breaks observed in the control experiments with BLM–Fe^{III}. It is unlikely that trace amounts of Fe^{II} are present in the assays when $KHSO_5$ is added as oxygen atom donor. In this case Fe^{II} traces are quickly oxidized by the persulfate salt. Thus the profiles of alkaline sucrose gradients of the controls without $KHSO_5$ and assay experiments cannot be directly compared. The only way to avoid this hypothesized side reaction (BLM–Fe^{II}–O_2) in the control experiments would be to carry out these controls under totally anaerobic conditions (i.e. in any inert gas containing less than 1 ppm of oxygen).

Strand scission of Ø X 174 DNA by BLM–Fe^{III}*–*$KHSO_5$

Strand scissions have also been investigated on supercoiled Ø X 174 RFI bacteriophage DNA (Form I) at BLM/base pair ratios from 1/4 to 2.6/1.

The early stage DNA breaks on Ø X 174 DNA are revealed by form II (nicked circular form resulting from form I by one single strand break) and form III (full-length linear duplex resulting from form I by a double strand break or from form II by a single strand break, nearby on the strand opposite to a previous nick). The incubation of Ø X 174 DNA with BLM–Fe^{III}–$KHSO_5$ results in the conversion of form I to forms II and III while the intensities of bands corresponding to II and III are dependent

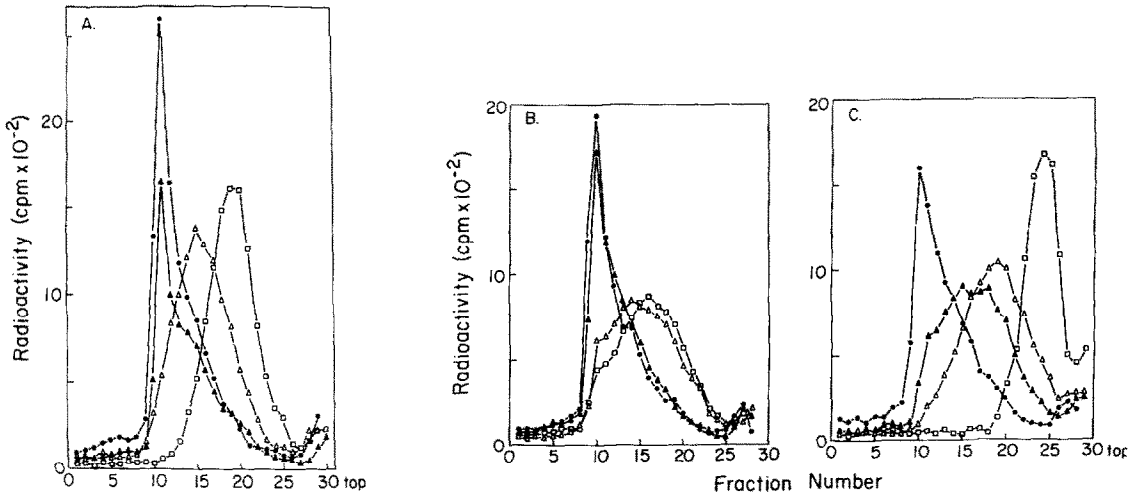

Fig. 3. Alkaline sucrose gradients. $\lambda[^{14}C]$DNA single-strand breaks mediated by trace amounts of BLM–Fe^{II} in the presence of molecular oxygen. (A) Individual reaction mixture (80 μM total volume) contained 312 ng of $\lambda[^{14}C]$DNA (12 μM nucleotides), 25 mM NaCl (pH 6) and the following concentration of BLM and Fe^{III}, respectively: (●) 0.09 μM; 0.135 μM; (▲) 0.18 μM; 0.27 μM; (△) 0.9 μM; 1.35 μM; (□) 1.8 μM; 2.7 μM. BLM/bases = (●) 1/133; (▲) 1/66; (△) 1/13; (□) 1/6.6. (B) Experimental conditions same as described in A excepted that Fe^{III} is replaced by Fe^{II} (0.015 equivalents of Fe^{II} per BLM: 1% of total Fe^{III} usually added as in A). The concentrations of BLM and Fe^{II} are respectively: (●) 0.09 μM; 1.35 10^{-3} μM; (▲) 0.18 μM; 2.75 10^{-3} μM; (△) 0.9 μM; 0.0135 μM; (□) 1.8 μM; 0.0275 μM. BLM/bases = (●) 1/133; (▲) 1/66; (△) 1/13; (□) 1/7. (C) Experimental conditions same as in A but with 2% of total Fe^{III} of A experiments added in the reaction media. 156 ng of $\lambda[^{14}C]$DNA are incubated in NaCl 25 mM (pH 6) (45 μl total volume) with various concentrations of BLM and Fe^{II}. The concentrations of BLM and Fe^{II} are respectively: (●) 0.08 μM; 2.44 10^{-3} μM; (▲) 0.16 μM; 4.88 10^{-3} μM; (△) 0.8 μM; 0.025 μM; (□) 1.6 μM; 0.05 μM. BLM/bases = (●) 1/137; (▲) 1/69; (△) 1/14; (□) 1/7.

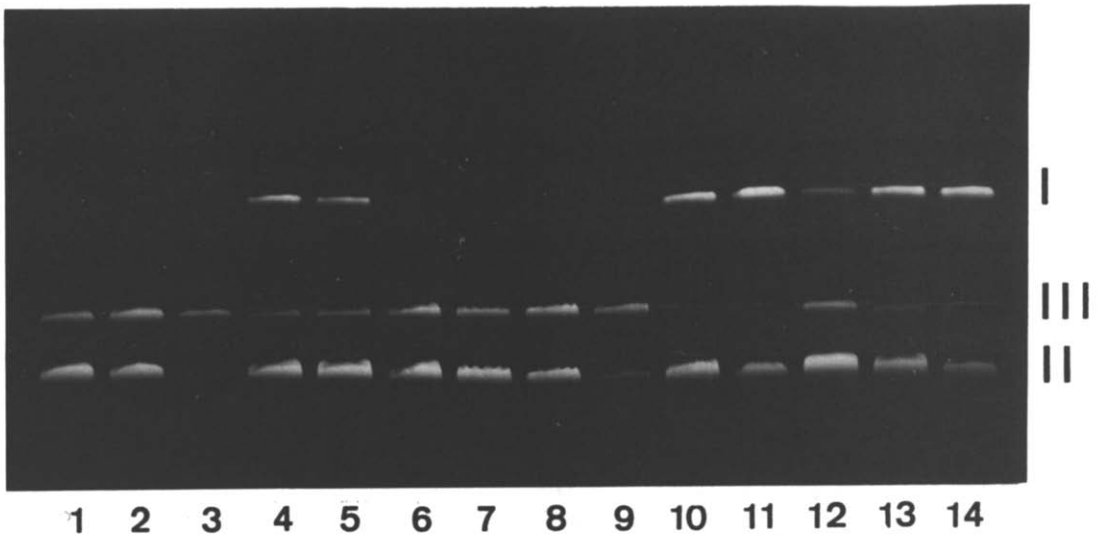

Fig. 4. Agarose gel electrophoretic pattern of Ø X 174 DNA stained with ethidium bromide. Cleavage of Ø X 174 phage by BLM–Fe^{III} and $KHSO_5$. 250 ng of Ø X 174 DNA (38 μM nucleotides) in 20 mM Tris–HCl buffer pH 7.8, 3 mM NaCl are incubated with the following concentrations of BLM–Fe^{III} complex and $KHSO_5$, respectively: lane 1: 5 μM, 50 μM; lane 2: 10 μM, 100 μM; lane 3: 50 μM, 500 μM; lane 4: 5 μM, none; lane 5: 10 μM, none; lane 6: 50 μM, none; lane 7: 5 μM, 500 μM; lane 8: 10 μM, 500 μM; lane 9: 50 μM, 2500 μM; lane 10: 500 μM $KHSO_5$; lane 11: 50 μM Fe^{III}, 500 μM $KHSO_5$; lane 12: 50 μM BLM, 500 μM $KHSO_5$; lane 13: 50 μM BLM and lane 14: untreated DNA.

on the concentration of BLM–Fe^{III}–$KHSO_5$ (Fig. 4, lanes 1–3 and 7–9). Control experiments showed that neither $KHSO_5$ alone (lane 10), nor Fe^{III}–$KHSO_5$ (lane 11), nor BLM alone (lane 13) are able to modify largely the relative population of forms I and II of the native DNA (lane 14). A slight cleaving activity is observed for BLM–Fe^{III} controls (lanes 4–6) and BLM–$KHSO_5$ (lane 12) which can be attributed in the first case to traces of Fe^{II} due to contamination of Fe^{III} or very partial reduction of Fe^{III} salts during incubation (see above, paragraph 2.2 on the very high activity of BLM–Fe^{II}–O_2) and in the second case to traces of metallic salts in BLM samples or chemicals. It has to be remembered that the association constant of BLM for Fe^{II} is extremely high ($K_a = 3.7 \times 10^8\ M^{-1}$) [36]. However, the only complete disappearance of form I is obtained with the complete system BLM–Fe^{III}–$KHSO_5$ (except in lane 6 where the cleaving system is highly concentrated). In addition, lanes 1–3 and 7–9 showed that form I vanished in favour of form II and that form III grew mainly from form II, confirming that single strand breaks are predominant compared to double strand breaks. Increasing the concentration of $KHSO_5$, lanes 7–9, did not increase the cleaving efficiency of the system. We had previously reported [30] that below 50 equivalents of $KHSO_5$ per BLM–Fe^{III} molecule, increasing amounts of $KHSO_5$ in the reaction mixture enhanced DNA scission but above this ratio the oxidation properties of $KHSO_5$ may induce a degradation of the system instead of promoting its catalytic activity.

EPR studies on the activated form of BLM–Fe generated by $KHSO_5$

EPR studies have been performed in order to elucidate the structure of activated BLM resulting from O_2 interaction with BLM–Fe^{II} or peroxides with BLM–Fe^{III} [22, 23, 37, 38]. Three different BLM–Fe complexes have been well characterized by EPR so far: (i) activated BLM (three signals at g = 2.26, 2.17 and 1.94), (ii) high spin BLM–Fe^{III} (g = 4.3), and (iii) low-spin BLM–Fe^{III} (g = 2.45, 2.18 and 1.89) [22]. We report in Figs 5A–D the EPR spectra of BLM–Fe complexes before and after addition of persulfate in the presence or absence of DNA and using two different buffers (phosphate or Tris–HCl).

In phosphate buffer at pH 8, in the presence of DNA and before addition of $KHSO_5$, the two BLM–Fe^{III} complexes (high-spin and low-spin) are detected (Fig. 5A(a)). The high-spin BLM–Fe^{III} complex has a phosphate anion as sixth ligand, as proposed by Dabrowiak *et al.* [39]. After addition of persulfate, a new sharp signal is observed at g = 2.0005. Since sulfate or hydroxyl radicals are usually not directly observed by EPR [40, 41] and also because the same resonance peak is observed in the absence of DNA (Fig. 5B(b)), this signal might be attributed to an organic radical centred on the BLM itself. The same phenomenon is observed in Tris–HCl buffer. Before addition of persulfate, the low-spin BLM–Fe^{III} is the main species in solution (Fig. 5C(a)) and a new signal at g = 2.0005 is superimposed to the starting BLM–Fe^{III} complex after $KHSO_5$ addition (Fig. 5C(b)). In the absence of DNA, the same signal is also observed but the intensity of the low-spin BLM–Fe^{III} is reduced and a signal at g = 4.3 appears (a five-coordinate high-spin BLM–Fe^{III} complex after partial degradation of BLM?). These facts confirm that the activated BLM is rapidly degraded in the absence of DNA. The direct oxidation of BLM itself by

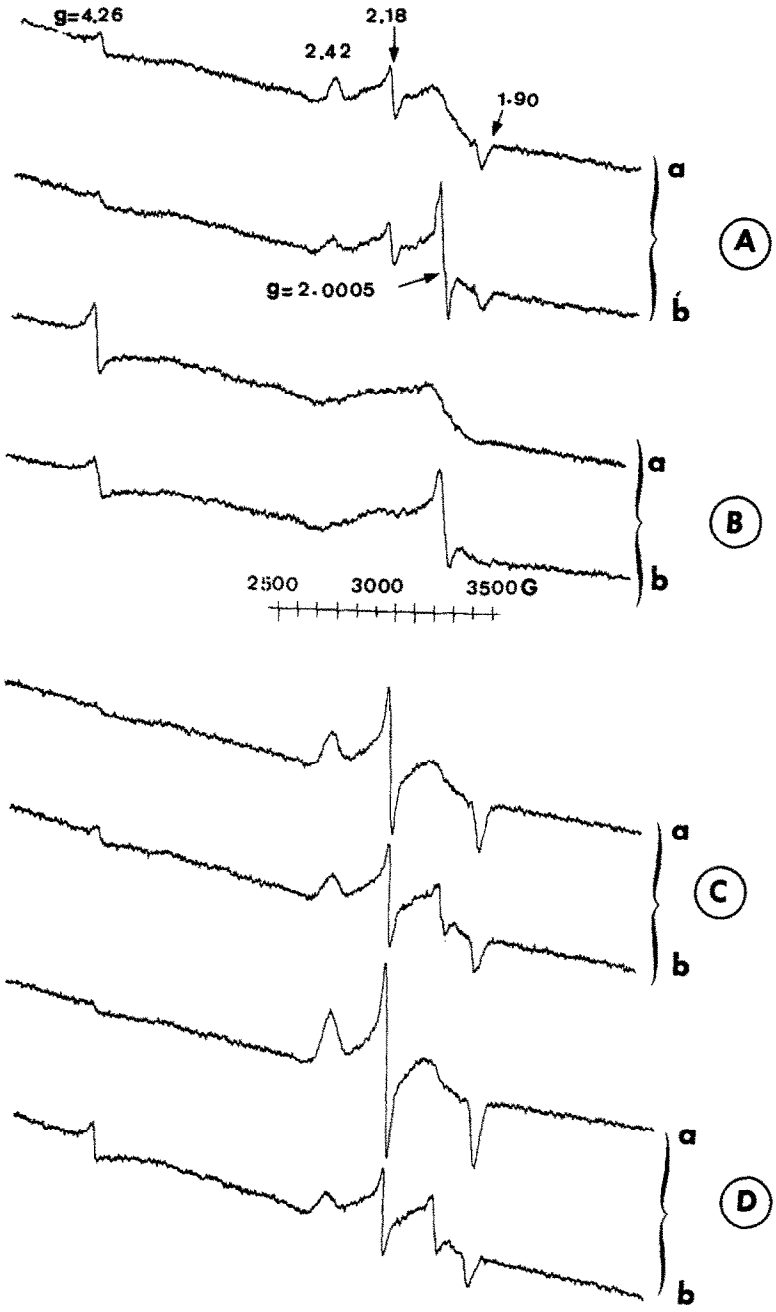

Fig. 5. EPR spectra of BLM–iron complexes before (a) and less than 1 min after (b) the addition of $KHSO_5$ in various conditions. 0.5 mM $KHSO_5$ (neutralized by NaOH) is added to aerobic solutions containing 0.25 mM BLM, 0.2 mM Fe^{III} and: (A) with 1.2 mM DNA (nucleotide content) in 20 mM phosphate buffer pH 7.8; (B) without DNA. 20 mM phosphate buffer pH 7.8; (C) with 1.2 mM DNA in 20 mM Tris–HCl buffer pH 7.8; (D) without DNA. 20 mM Tris–HCl buffer pH 7.8.

persulfate is much slower than the degradation of BLM–Fe after activation by $KHSO_5$ (data not shown).

We do not observe an EPR signal for a possible BLM–Fe^{V} = O active species in the present case. This is not unexpected since even with more robust ligands than BLM (namely porphyrins) it has not been possible to have a direct observation by UV-visible or EPR of a high-valent metal-oxo generated by $KHSO_5$ [42]. Such reactive species able to cleave C—H bonds from very inert substrates are probably too reactive to allow a detection by ordinary spectroscopic methods when the time scale in the sample preparation is in minutes.

CONCLUSIONS

The degradation of nucleic acids by an oxidative process catalyzed by BLM–Fe is now well documented. However, the exact nature of "activated iron–bleomycin" is still a matter of debate. Among the different hypotheses, the possible cleavage of DNA by a high-valent iron-oxo species has recently emerged in the literature. This "cytochrome P-450 like" activity of the BLM–Fe complex is supported by the use of potassium monopersulfate as single oxygen atom donor, soluble at physiological pH. With this peroxidic agent, known to generate metal-oxo entities in metalloporphyrin-catalyzed oxygenation, we have shown in the present article that BLM–Fe^{III} activated by $KHSO_5$ is able to give single-strand and double-strand breaks on DNA, as illustrated by two different methods (sucrose gradients and agarose gel electrophoresis).

By EPR spectroscopy, it has been possible to evidence an organic radical which might be centred on the BLM itself. Such an intermediate has to be only considered as a transient species in the inactivation procedure of BLM during the degradation of nucleic acids.

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