

Mechanism of Double-Strand DNA Cleavage Effected by Iron-Bleomycin

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Z. Naturforsch. **53c**, 867–870 (1998); received March 23/May 19, 1998

DNA Cleavage, Activated Bleomycin, Double-Strand Scission, Single-Strand Scission, Iron(III) Compound

We have observed that in the absence of hydrogen peroxide the Fe(III)-bleomycin (BLM) complex exhibits high DNA cleavage efficiency, converting supercoiled Form I DNA(pBR322 or ϕ x174) to Form II (nicked, relaxed circular); the present study may give an important clue to elucidate the fact that iron-bleomycin mediated double-strand DNA cleavage requires at least one molecule of oxygen (O_2) over the amount required to form “activated bleomycin”.

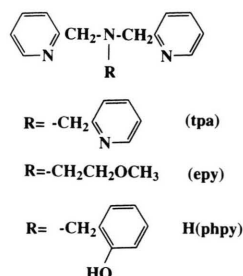
Studies evaluating the extent of single-strand(ss) and double-strand(ds) DNA cleavage mediated by bleomycin (BLM) necessitate the consideration of a mechanism for ds-strand in which a single molecule of BLM is responsible for both strand breaks (Absalon *et al.*, 1995a). Incubation of BLM with Fe^{2+} and O_2 yields a mixture of ferric-BLM and “activated BLM”, a species recently shown by electrospray mass spectroscopy to be BLM-ferric-peroxide which is kinetically competent to cause both ss- and ds-cleavage (Sam *et al.*, 1994). Recently, the requirement in the ds-cleavage process for O_2 , in addition to that needed to form “activated BLM”, has been clearly demonstrated by the absence of ds-cleavage products in reactions performed under anaerobic conditions in which ss-cleavage still occurs (Absalon *et al.*, 1995a). The distance between the two cleavage sites (15–18 Å) in ds-cleavage and the apparent lack of bifunctionality of “activated BLM” thus presents an apparent impasse that has prompted us to investigate a number of proposals regarding the ds-cleavage process mediated by Fe-BLM (Absalon *et al.*, 1995b). In this study we have observed that in the absence of hydrogen peroxide Fe(III)-BLM reacts with DNA(pBR322, ϕ x174, and λ -DNA) in a quite different manner from those by both the monomeric and dimeric model compounds, nicking DNA; the present results may give an important evidence to elucidate the fact

that iron-bleomycin mediated ds-cleavage requires at least one molecule of oxygen (O_2) over the amount required to form “activated BLM”.

Experimental

Materials

Bleomycin A2 was purchased from Nihon Kayaku Co. Supercoiled DNA(pBR322, ϕ x174) were obtained commercially (Wako Chemicals, Osaka). The iron(III) complexes used are $Fe(tpa)Cl_2ClO_4$ (Kojima *et al.*, 1993), $Fe(epy)Cl_2ClO_4$ (Ito *et al.*, 1997), $Fe(phpy)Cl_2$ (Ito *et al.*, 1996), *trans*- $FeCl_2(cyclam)ClO_4$ (Nishida and Tanaka, 1994) and some structures of the ligands are illustrated below (see Scheme 1), where (cyclam) denotes 1,4,8,11-tetraazacyclotetradecane.



Scheme 1.

DNA cleavage

In order to assess the competence of the complexes for DNA strand scission, each complex was incubated with pBR322 Form I DNA under identical reaction conditions (Kobayashi *et al.*, 1996). In a typical run, aqueous solution of the iron(III)

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complex (4 μ l of 0.1–1.0 mM solution), DNA (4 μ l of 0.1 mg/ml solution), Tris(tris(hydroxymethyl)-aminomethane) buffer (3 μ l of 0.1 M solution; pH = 7.8) and hydrogen peroxide (4 μ l of 0.01 mol solution) were mixed and allowed to stand for 1 h at 25 °C. The experiments without hydrogen peroxide were performed as follows; aqueous solution of iron(III) complex (4 μ l of 0.1–2.0 mM solution), DNA (4 μ l of 0.1 mg/ml solution), and Tris buffer (2 μ l of 0.1 mol solution; pH = 7.8) were mixed and allowed to stand for 1 h at 25 °C. The extent of DNA cleavage was assessed by analysis on 0.9% agarose gel containing ethidium (3,8-diamino-5-ethyl-6-phenylphenanthridinium) bromide (Micklos and Freyer, 1990). The bands were photographed with Polaroid 667 film, and the band

intensities were quantified on an ATTO Densitograph Model AE-6920-M/W/V(for Mac).

Results and Discussion

As shown in Fig. 1(A), the mononuclear complex, Fe(phpy)Cl₂ is competent to effect DNA strand scission in the presence of hydrogen peroxide, converting the supercoiled Form I DNA-(pBR322) to a mixture of nicked DNA (Form II, relaxed circular) and linear duplex (Form III) (Micklos and Freyer, 1990) (see lane 9). The iron(III) compounds with BLM and (tpa) evidence a much higher DNA cleavage efficiency, producing fragments without supercoiled, relaxed

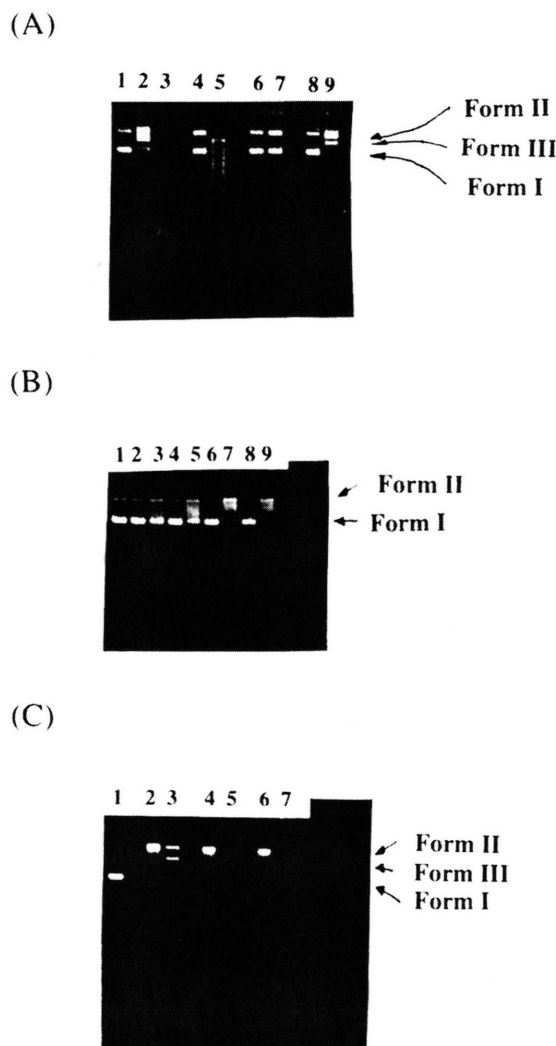


Fig. 1. (A). DNA(pBR322) cleavage by iron(III) complexes (final concentrations of iron(III) and H₂O₂ are 6.67×10^{-3} and 667×10^{-3} mmol, respectively) Lane 1, DNA alone; lane 2, Fe(III)-BLM; lane 3, Fe(III)-BLM + H₂O₂; lane 4, Fe-(tpa); lane 5, Fe-(tpa) + H₂O₂; lane 6, Fe-(epy); lane 7, Fe-(epy) + H₂O₂; lane 8, Fe-(phpy); lane 9, Fe-(phpy) + H₂O₂.

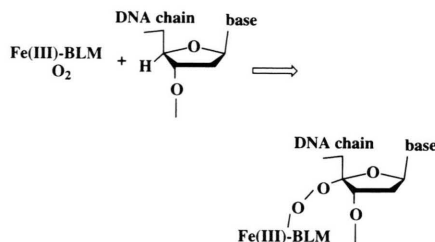
(B). DNA(pBR322) scission by Fe(III)-BLM as a function of concentration of Fe(III)-BLM in the absence of hydrogen peroxide (electrophoresed after 1 h by addition of Fe(III)-BLM. The final concentration of the iron(III) ion in the solution was indicated in parenthesis). Lanes 1, 4, 6, 8; DNA alone; lane 2, Fe(III)-chloride (0.001 mM); lane 3, Fe(III)-BLM (0.0001 mM); lane 5, Fe(III)-BLM (0.0025 mM); lane 7, Fe(III)-BLM (0.005 mM); lane 9, Fe(III)-BLM (0.01 mM).

(C). DNA(pBR322) scission by Fe(III)-BLM as function of time (final concentrations of iron(III) ion and hydrogen peroxide are 0.05 and 0.1 mM, respectively): Lane 1, DNA alone; lane 2, Fe(III)-BLM after 1 h; lane 3, Fe(III)-BLM + H₂O₂ at after 1 h; lane 4, Fe(III)-BLM at after 2 h; lane 5, Fe(III)-BLM + H₂O₂ at after 2 h; lane 6, Fe(III)-BLM at after 3 h; lane 7, Fe(III)-BLM + H₂O₂ at after 3 h.

circular, and linear duplex, as shown in lanes 3 and 5 in Fig. 1(A), indicating that ds-scission occurs in these solutions, since no Form II and Form III DNA was found in the reaction mixture. In the absence of hydrogen peroxide, mononuclear iron(III) compounds used in this study do not effect DNA scission as shown in lanes 4, 6 and 8 under our experimental conditions. However, the remarkable change was observed in the case of Fe(III)-BLM, as depicted in lane 2 in Fig. 1(A) and also in Fig. 1(B), where the effect due to the concentration of the Fe(III)-BLM on DNA cleavage is illustrated.

The results in Fig. 1(A) and (B) clearly indicate that Fe(III)-BLM does effect DNA cleavage, converting Form I DNA to Form II or Form III even in the absence of hydrogen peroxide; this behavior is quite different from those observed for the mononuclear compounds as illustrated in Fig. 1(A), and the novel electrophoresis patterns observed for Fe(III)-BLM were not found for the corresponding Ni(II)- or Zn(II)-BLM compounds. This may be consistent with the fact reported by Steighner and Povirk (Steighner and Povirk, 1990); they observed the release of bases from the reaction mixture of Fe(III)-BLM and DNA (calf-thymus). The proceeding of the DNA cleavage by Fe(III)-BLM complex without hydrogen peroxide is dependent on the concentration of the complex (see Fig. 1(B)), but not on the reaction time (see lanes 2, 4 and 6 in Fig. 1(C)). It should be noted that the results in Fig. 1(C) indicates that the DNA cleavage by Fe(III)-BLM in the absence of hydrogen peroxide is due to single-strand scission, not to ds-scission. This is clearly indicating that the active species in DNA strand scission by Fe(III)-BLM in the absence of hydrogen peroxide is different from that of an "activated BLM", because the latter species gives ds-scission in the presence of oxygen molecule, and this should exclude the possibility that an iron(II) species existing in solution as an impurity may play a role in DNA strand scission observed in the solution without hydrogen peroxide. These facts demonstrate that there are at least two species to induce strand scission, and this may correspond to the two pathways in DNA strand scission reported by McGall *et al.* (1992). In pathway A proposed by McGall *et al.* the oxidative

degradation of the sugar ring occurs *via* formation of C-4' hydroperoxide, and the degraded DNA chain cannot capture the iron(III)-BLM furthermore, and break of the DNA strand by Fe(III)-BLM stops at this point. This leads to ss-scission. Thus it seems likely that the ss-break by Fe(III)-BLM and oxygen molecule proceeds *via* formation of C-4' hydroperoxide (see Scheme 2). Similar



Scheme 2.

acyl-hydroperoxide formation was confirmed in the Fe(III)-(cyclam)/O₂/linolenic acid system (Nishida and Tanaka, 1994) and above discussion may be consistent with the fact that *trans*-FeCl₂(cyclam)⁺ can degrade pBR322 DNA in the absence of hydrogen peroxide, but its activity is lower than that of Fe(III)-BLM (data not shown). As DNA degradation induced by Fe(III)-BLM and oxygen molecule observed in this study was not detected for the corresponding deglyco-derivative under the same experimental conditions (Hamamichi *et al.*, 1992), it seems reasonable to assume that the presence of sugar moiety in BLM should play an important role in formation of the C-4' hydroperoxide adduct.

Above discussion may give an important information to elucidate the fact that Fe(III)-BLM mediated ds-cleavage requires at least one molecule of oxygen over the amount required to form "activated BLM"; that is, at first an "activated BLM" gives a ss-break (Absalon *et al.*, 1995a; Wu *et al.*, 1985) through C-4' hydroxide formation (in pathway-B) (McGall *et al.*, 1992) and after this ss-break the resulted Fe(III)-BLM, which is linked continuously with the cleaved DNA strand, and oxygen molecule effect cleavage of DNA at another strand, which should be near to the place where the first ss-break occurred, leading to the ds-break (see Fig. 2).

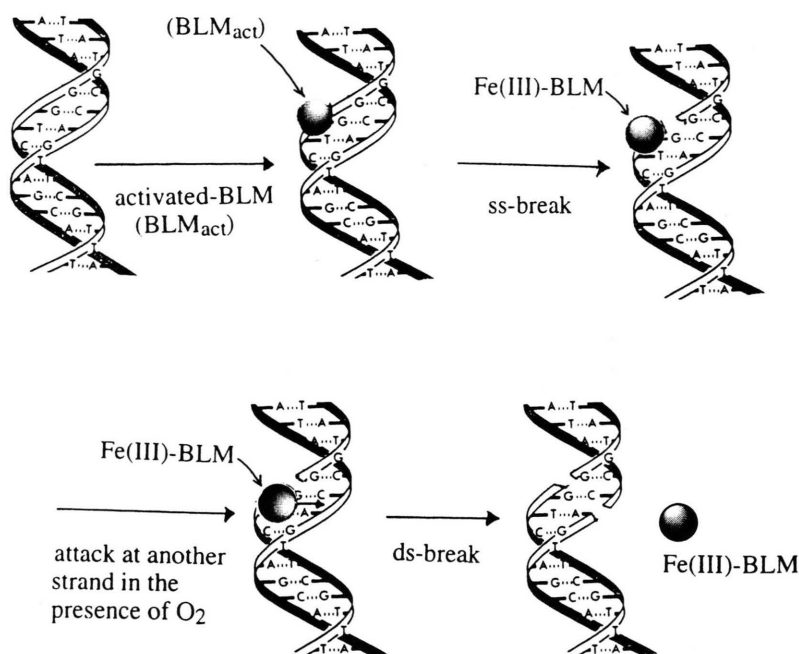


Fig. 2. Assumed scheme for ds-break of DNA by activated-BLM in the presence of oxygen.

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