Interaction between the Peroxide Adduct of Binuclear Iron(III) Complex with (HPTP) Anion and the Sugar Moiety of Nucleosides

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Z. Naturforsch. **54 b,** 1272–1276 (1999); received February 15, 1999

DNA Cleavage, Peroxide Adduct of Binuclear Iron(III), Interaction with Sugar moiety

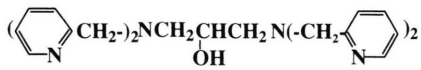
We have in this study obtained the experimental evidence to suggest that the peroxide adduct of binuclear iron(III) complex with H(HPTP) directly reacts with the sugar moiety of the DNA chain, to cleave it; H(HPTP) denotes N,N,N',N'-tetrakis(2-pyridylmethyl)-1,3-diamino-2-propanol.

Introduction

Bleomycins(BLM) are a group of antitumor antibiotics, first isolated by Umezawa in 1966 [1]. These compounds are used clinically in the treatment for head and neck cancer, testicular cancer, and squamous carcinomas [2]; their cytotocity is thought to be related to their ability to bind and tdegrade double-stranded DNA [3]. Extensive efforts by numerous investigators in the past decade have provided much information about the products during BLM-mediated degradation of DNA and the chemistry of their formations [4, 5]. Incubation of BLM with Fe^{2+} and O_2 yields a mixture of ferric-BLM and "activated BLM", a species recently shown to contain BLM-ferric-peroxide by electrospray mass spectroscopy [6]. However, the discussion how the metal-peroxide adduct cleaves DNA is scarce [7], and also there's no evidence to support that the peroxide adduct reacts directly with DNA [8]. In this study we will show clear evidence that the peroxide adduct of a binuclear iron(III) reacts directly with DNA to degrade it.

Experimental Section

Materials: DNA(calf-thymus, pBR322, ϕx174) was obtained from Wako Chemicals (Osaka). In this study, the binuclear iron(III) complex with μ-alkoxo bridge, $Fe_2(HPTP)(OH)(NO_3)_2(ClO_4)_2$ (**1**) and $Fe_2(HPTP)Cl_4ClO_4$ (**2**) were used, where H(HPTP) represents N,N,N',N'-tetrakis(2-pyridylmethyl)-1,3-diamino-2-propanol [9].



H(HPTP)

Preparation of the metal compounds: The ligand, H(HPTP), used in this study was obtained according to the literature method [9]. The methanol solution of H(HPTP) was mixed with a methanolic solution of ferric chloride hexahydrate, to give a yellow powder.

$Fe_2(HPTP)Cl_4FeCl_4$

Calcd C 35.84 H 3.23 N 9.29%,
Found C 35.81 H 3.11 N 9.23%.

When the yellow powder (1.0 g) was recrystallized from a methanol/acetonitrile (v/v = 1/1) solution containing $NaClO_4$ monohydrate (1.0 g), orange prisms were obtained.

$Fe_2(HPTP)Cl_4ClO_4$

Calcd C 40.21 H 3.62 N 10.42%,
Found C 40.41 H 3.85 N 10.99%.

Crystal structure determination of compound **2** · *CH_3OH:* An orange yellow prism having approximately dimensions of 0.25 × 0.25 × 0.35 mm was mounted on a glass fiber. All measurements were made on a Rigaku AFC5S diffractometer with graphite monochromated MoKα radiation and a 12 KW rotating anode generator at Saga University. Cell constants and an orientation matrix for data collection, obtained from a least-squares refinement using the setting angles of 25 carefully centered reflections in the range $10.00 < 2\theta < 22.04°$, corresponded to a monoclinic cell dimension with a = 12.882(6), b = 15.376(2), c = 18.488(3) Å, β = 106.19(2)°, V = 3517(2) Å^3, space group P21/c, Z = 4, F. W. = 838.56.

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The calculated density is 1.584 g cm^{-3}. The structure was solved by direct methods. The non-hydrogen atoms were refined anisotropically. The final cycle of full-matrix least-squares refinement was based on 3350 observed reflections ($I > 3.00\sigma(I)$), and 424 variable parameters and converged with unweighted and weighted agreement factors of $R = \Sigma\,||F_o| - |F_c||/\Sigma\,|F_o| = 0.053$, $R_\omega = [(\Sigma\,\omega(|F_o| - |F_c|)^2/\Sigma\,\omega\,|F_o|^2)]^{1/2} = 0.057$.

Neutral atom scattering factors were taken from Cromer and Weber [10]. Anomalous dispersion effects were included in F_{calc}. The values for $\Delta F'$ and $\Delta F''$ were those of Cromer [11]. All calculations were performed using the TEXSAN Crystallographic software package of Molecular Structure Corporation [12].

Interaction between DNA and binuclear iron(III) complex in the absence or presence of hydrogen peroxide: In a typical run, an aqueous solution of the iron(III) complex (4 μl of 0.1 - 2.0 mmol dm^{-3}), DNA (4 μl of 0.1 mg per ml) and tris buffer (2 μl of 0.1 mol dm^{-3}, pH = 7.8)were mixed and allowed to stand for 1 h at 25 °C. The solution was electrophorated on 0.9 % agarose gel containing ethidium(3,8-diamino-5-ethyl-6-phenylphenanthridinium) bromide [13]. The bands were photographed with Polaroid 667 film. The effect by hydrogen peroxide was evaluated as follows. An aqueous solution of the iron(III) complex (4 μl of 0.05 - 0.10 mmol dm^{-3}), DNA (4 μl of 0.1 mg per ml), tris buffer (2 μl of 0.1 mol dm^{-3}, pH = 7.8), and H_2O_2 (4 μl of 0.05 - 1.0 mmol dm^{-3}) were mixed and allowed to stand for 1 h at 25 °C. The extent of DNA cleavage was assessed by the same procedures as described above.

HPLC containing nucleoside, complex **2**, *and hydrogen peroxide:* The iron(III) complex **2** (1×10^{-5} mole) was dissolved in the solution (20 ml) containing nucleoside (0.3 mM) and the mixture was treated with hydrogen peroxide (10 ml of the 10 mM solution). The reaction mixture was stirred under air at 298 K, and analyzed by HPLC (5C18-AR column, eluted with a 10% methanol solution containing 0.005 mol of ammonium acetate [14] at a flow rate of 0.8 ml/min). The quantity of unreacted substrate was evaluated by calculating the area of the HPLC peak (Fig. 4).

Oxidation of tetrahydrofuran by $Fe_2(HPTP)Cl_4ClO_4$ and H_2O_2: In a typical run, hydrogen peroxide (0.01 mole, acetonitrile solution (10 cm^3) containing 1.13 g of 30% commercial hydrogen peroxide solution) was added dropwise (within 5 minutes) to an acetonitrile solution (30 cm^3) containing the iron(III) complex (0.01 mmole) and tetrahydrofuran (0.01 mole), and the products were determined by GC after the usual work-up. Authentic samples of γ-butyrolactone was obtained commercially (Tokyo Kasei, Co.), and tetrahydrofuran-2-hydroperoxide was obtained according to the published method. A iodometirc method was used to determine the quantity of the hydroperoxide.

Table I. Selected bond distances (Å) and angles(°) of compound $Fe_2(HPTP)Cl_4ClO_4$.

Fe1-Cl1	2.259(2)	Fe1-Cl2	2.291(2)
Fe1-O1	2.037(4)	Fe1-N1	2.198(5)
Fe1-N2	2.196(6)	Fe1-N3	2.170(6)
Fe2-Cl3	2.238(2)	Fe2-Cl4	2.290(2)
Fe2-O1	2.018(4)	Fe2-N4	2.218(6)
Fe2-N5	2.165(6)	Fe2-N6	2.208(6)
Fe1-Fe2	3.720(2)		
Cl1-Fe1-N1	166.1(2)	Cl2-Fe1-N3	170.6(2)
O1-Fe1-N2	153.4(2)	Cl3-Fe2-N4	163.8(2)
Cl4-Fe2-N6	169.8(2)	O1-Fe2-N5	154.9(2)
Fe1-O1-Fe2	133.0(2)	Fe1-O1-C1	115.0(4)
Fe2-O1-C1	112.0(4)		

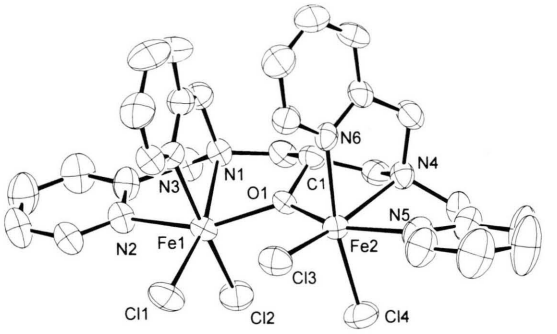
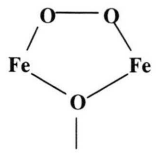


Fig. 1. ORTEP drawing of $Fe_2(HPTP)Cl_4^+$ (50% probability).

Results and Discussion

Crystal structure of $Fe_2(HPTP)Cl_4ClO_4$: Crystal structure of compound **1** was already reported [15]. As expected, the complex **2** was shown to be of a dimeric structure with an alkoxo bridge (see Fig. 1). The selected bond distances and angles are listed in Table I. The blue species which forms on the addition of hydrogen peroxide to the solution of a binucelar iron(III) complex with H(HPTP) [9] should be a peroxide adduct with (μ-η^1:η^1-peroxo)-diiron(III) (see the figure below), which is similar to that reported by Que *et al.* [16].



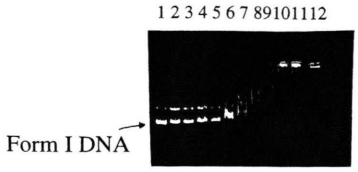
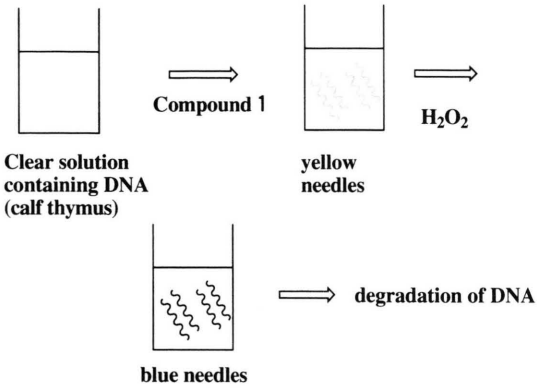


Fig. 2. Supercoiled ds-DNA(pBR322) was incubated aerobically with iron complex **1** for 1 h at 298 K. The concentrations of the iron(III) complex solution were varied as indicated: Lane 1, DNA alone; lane 2, 0.1 mmol solution lane 3, 0.2 mmol. lane 4, 0.3 mmol. lane 5, 0.4 mmol; lane 6; 0.5 mmol; lane 7, 0.6 mmol; lane 8, 0.7 mmol; lane 9, 0.8 mmol; lane 10, 0.9 mmol; lane 11, 1.0 mmol; lane 12, 2.0 mmol.



Scheme 1.

Interaction between blue peroxide adduct of iron(III) complex with H(HPTP) and DNA: When we added the yellow solution (10 ml) of **1** (or **2**) (in water, 0.01 M) to a clear aqueous solution (50 ml) containing DNA (calf-thymus, 1mg/1ml solution), yellow fibrous needles deposited (see Scheme 1). This may be due to a), a strong binding affinity of the iron(III) complex with a positive charge to the phosphate backbone of DNA, [17] and b), the negative charge on the phosphate group is diminished, causing the material to be insoluble in water. This is supported by the electrophoresis, done on pBR322 DNA; as shown in lanes 10-12 of Fig. 2, DNA does not move by the electronic gradient in the presence of the compound **1**. On the addition of the hydrogen peroxide solution (10 ml, 0.1 M aqueous solution) to the solution containing calf-thymus DNA and **1**, the yellow needles turned to blue, even though they are yet insoluble in water. This means that the blue peroxide adduct of the binuclear iron(III) complex [9, 16] is attached to the DNA chain (see Scheme 1).

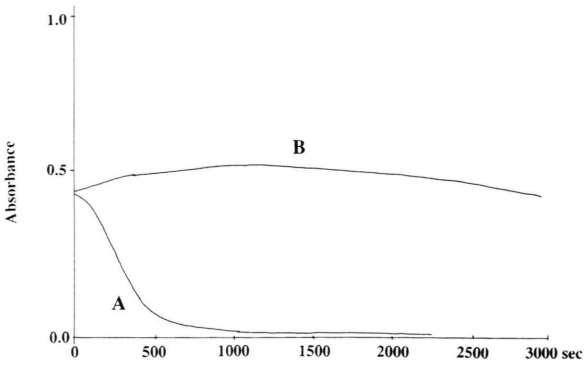


Fig. 3. Time course of absorbance at 560 nm of the blue species at 295 K in aqueous solution. A) without 2'-deoxyguanosine and B) in the presence of 2'-deoxyguanosine(8-equivalent). Equi-volumes of iron(III) complex solution (0.150 mg / 25 ml) and ydrogen peroxide (0.567 g (commercial 30% solution) / 50 ml) were mixed.

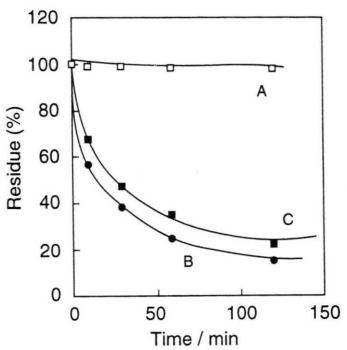


Fig. 4. Residual nucleosides in the solution containing $Fe_2(HPTP)Cl_4ClO_4$ and hydrogen peroxide (aqueous solution, 295 K). A: adenine; B: adenosine; C: cyclic(3',5')-AMP.

Although the life-time of the blue peroxide adduct is very short in water in the presence of excess of hydrogen peroxide (within 10 minutes, see trace A in Fig. 3) [9], the blue-needles deposited on the DNA chain are not degraded for about 1 h. We also found that the presence of nucleosides, such as 2'-deoxyguanosine or adenosine in the solution increases the life-time of the blue species, verified by measuring the time course of the absorbance at 560 nm of the blue peroxide adduct (see Fig. 3). The fact that $Fe_2(HPTP)Cl_4^+$ in the presence of hydrogen peroxide system can decompose adenosine and also cyclic-AMP (adenosine monophosphate), whereas this mixture cannot degrade the adenine molecule under the same experimental conditions

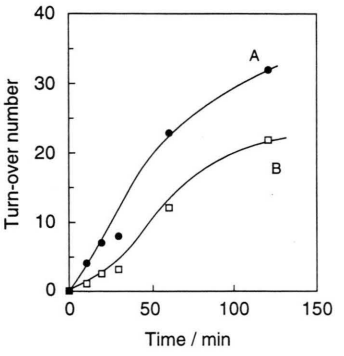
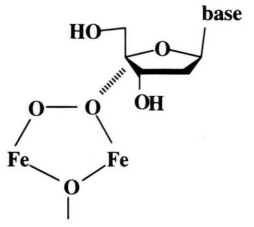


Fig. 5. Time course of turn-over numbers (= [mole of product]/[mole of iron(III) complex]) of products from tetrahydrofuran. A: tetrahydrofuran-2-hydroperoxide; B: γ-butyrolactone.

(see Fig. 4) supports that the peroxide adduct of the binuclear iron(III) complex directly interacts with the sugar moiety of the nucleosides. One possible scheme is illustrated below, which is based on the results reported by Stubbe *et al.* for the interaction between oligomers and the hydroperoxide adduct of Co(III)-BLM, [8]. This interaction may induce the lengthening of the life-time of the blue species (Fig. 3), since it prevents the approach of another hydrogen peroxide molecule to the peroxide adduct. In the figure below, it seems quite likely that electronic interaction [18] occurs between the terminal oxygen atom of the peroxide ion and carbon atom of C4'-position (IIII denotes the presence of electronic interaction; here the peroxide adduct acts as an electrophile [18]):



This may be consistent with the fact the $Fe_2(HPTP)Cl_4^+$ complex shows high ability to generate γ-butyrolactone and 2-hydroperoxide-tetrahydrofuran in the reaction with THF and hydrogen peroxide (see Fig. 5).

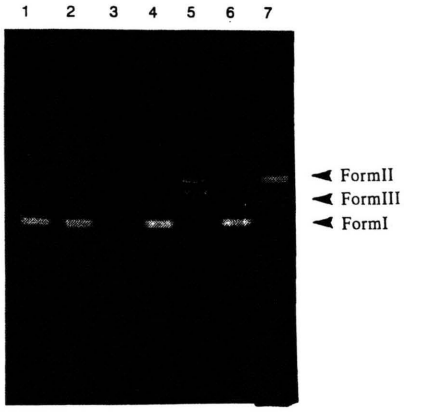
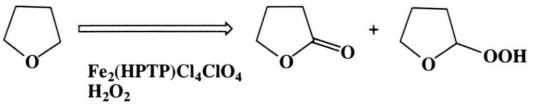


Fig. 6. Electrophoresis containing DNA(pBR322), iron(III) complex 2 and hydrogen peroxide. lane 1, DNA alone; lane 2, DNA and Fe-(HPTP) (0.1 mmol solution); lane 3, DNA, Fe-(HPTP) (0.1 mmol solution) and H_2O_2 (0.5 mmol); lane 4, DNA and Fe-(HPTP) (0.1 mmol solution); lane 5, DNA, Fe-(HPTP) (0.1 mmol solution) and H_2O_2 (0.1 mmol); lane 6, DNA and Fe-(HPTP) (0.05 mmol solution); lane 7, DNA, Fe-(HPTP) (0.05 mmol solution) and H_2O_2 (0.1 mmol).



The blue-needles attached to the calf-thymus DNA slowly disappeared within three hours at room temperature. This process correposnds to the degradation of DNA, which is consistent with the results in the lanes 3, 5, and 7 in Fig. 6. In the presence of excess hydrogen peroxide (5 equivalents), complete degradation of DNA occurred (see lane 3 in Fig. 6). At present, the detailed mechanism of DNA cleavage is unknown [19], but it is clear that the peroxide adduct of $Fe_2(HPTP)Cl_4ClO_4$ directly interacts with the sugar moiety of DNA chain, leading to the cleavage of DNA.

Acknowledgements

This work was supported by the Grant-in-Aid for Scientific Research on Priority Areas of Metal-assembled Complexes No. 10149205 from the Ministry of Education, Science, Sports and Culture.

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