DNA Cleaving Activity and Cytotoxic Activity of Ferricenium Cations

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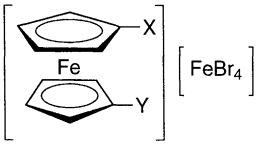
Ferricenium cations showed excellent DNA cleaving activity and cytotoxic activity. Such activities depend on the acyl group introduced into the cyclopentadienyl ring.

There has been considerable interest in cleavage of DNA by protein and small molecules. The antitumor antibiotic bleomycin which recognize DNA with the bithiazole-terminal amine portion and cleave DNA with metal chelation and oxygen activation by the β-aminoalanine-pyrimidine-β-hydroxyhistidine moiety.[1] Dervan *et al.* reported that methidiumpropyl-EDTA-iron(II)[2] and distamycin-EDTA-iron(II)[3] complexes cleave DNA by the activated oxygen. Recently, Komiyama *et al.* reported that hydrolysis of DNA was demonstrated using $Ce^{IV}(NH_4)_2(NO_3)_6$ even in the absence of oxygen.[4] In addition, DNA cleavage by metal complexes such as V(V)[5], Fe(II)[6], Co(III)[7] and Cu(II)[8] have been reported so far.

We have been continuing the screening of the antitumor metal complex[9] and found that ferricenium cations caused excellent inhibition toward cancer cell growth *in vitro*. We also found that ferricenium cations cleaved Form I DNA to small fragments. In order to elucidate the mode of action of ferricenium cations, we report here the cytotoxic activity and DNA cleavage of ferricenium cations. The structures of ferricenium cations used in this study are shown in Scheme 1.



	X	Y
1	H	H
2	$COCH_3$	H
3	$COCH_3$	$COCH_3$
4	COC_2H_5	H

Scheme 1. Ferricenium cations used in this study.

Mono- and diacetyl ferrocene,[10] and monopropyonyl ferrocene[11] were prepared by the Friedel-Crafts acylation with corresponding acyl chloride. Treatment of ferrocene derivatives in bromine at room temperature afforded corresponding ferricenium tetrabromoferrate **1** - **4**. The attempt to obtain dipropyonyl ferricenium tetrabromoferrate was unsuccessful due to the instability of this compound. The room temperature effective magnetic moment (μ_{eff}) of **1** - **4** (6.5 - 6.7 BM) were comparable to that of ferricenium tetrachloroferrate[12] (6.4 BM). The μ_{eff} value of ferricenium picrate[13] (2.4 BM) is close to that of theoretical value of 1.73 BM, suggesting that Fe in ferricenium cation exists as low-spin d^5 Fe(III). On the contrary, Fe in tetrabromoferrate exist as high-spin d^5 Fe(III), since the observed μ_{eff} values of tetraethyl ammonium tetrabromoferrate[14] **5** (6.1 BM) and tetraethyl ammonium tetrachloroferrate[12] (5.9 BM) were in good agreement with those of theoretical value of 5.92 BM. The measured μ_{eff} values of **1** - **4** were smaller than that of the sum of the contribution of ferricenium cation and tetrabromoferrate anion, probably due to a weak inter- or intramolecular antiferromagnetic interaction in the crystal.[12]

Table 1. Cytotoxic activity of ferricenium cations

Ferricenium cation	ID_{50} / μg·ml^{-1}
1	19
2	16
3	8
4	6
5	120

The 50% inhibition dose (ID_{50})[15] of cell growth using the mouse cancer cell line B16 melanoma[16] was measured 3-5 times per each sample, and the average values are summarized in Table 1. The errors in ID_{50} were of the order of 0.5 μg/ml. The ID_{50} values decreased in the order of **1** < **2** < **3** < **4**, suggesting that the cytotoxic activity increased in this order. The introduction of acyl group into the cyclopentadienyl ring enhanced the cytotoxic activity, where the number of acetyl group (**2** and **3**) and the selection of propyonyl group (**4**) depended strongly on the cytotoxic activity. The most excellent cytotoxic activity was observed for **4** (6 μg/ml) which was comparable to that of the excellent therapeutic drug *cis*-dichlorodiammine platinum(II) (2 μg/ml). The control compound, **5** showed 120 μg/ml of ID_{50}, suggesting that excellent cytotoxic activity might be caused by the ferricenium cations rather than the tetrabromoferrate anion.

Comparison of the DNA cleaving activity of **1** - **5** were made in the purpose to explore the mode of action of ferricenium cations. The result of agarose gel electrophoresis[17] is shown in Figure 1. The disappearance of DNA bands in lane 5 suggests that **4** digested DNA to small fragments by the double strand scission. The DNA cleaving activity of **1** - **3** were inferior to that of **4**. Both the acetyl

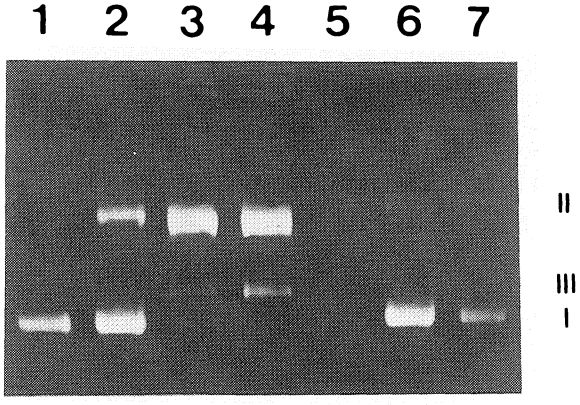


Figure 1. Agarose gel electrophoretic patterns of ethidium bromide-stained φX174 RF I DNA (50 μM in DNA basepair) incubated with ferricenium cations (25 μM) for 30 min. The used ferricenium cations are as follows: lane 2 (**1**), lane 3 (**2**), lane 4 (**3**), lane 5 (**4**), lane 6 (**5**). Lanes 1 and 7 contained intact DNA.

derivatives (**2** and **3**) led a disappearance of Form I DNA and caused a predominant production of Form II over Form III. The ratios of Form II/ Form III were 94/9 and 74/26, respectively, suggesting that both single and double strand scission were occurred and DNA cleaving activity of **3** is superior to that of **2**. In the case of **1**, only the Form II DNA was produced. Thus, the DNA cleaving activity increased in the order of **1** < **2** < **3** < **4**, which is in good agreement with that of ID_{50}. The DNA cleavage was caused by the ferricenium cations rather than tetrabromoferrate anion, since no DNA cleavage was found using tetraethyl ammonium tetrabromoferrate **5**. It is also supported by the fact that the DNA cleaving activity of ferricenium picrate which has no tetrabromoferrate anion is comparable to that of **1**.

In a DNA cleavage system containing Fe(II) species, activated oxygen, especially hydroxyl radical (•OH) acts as the active species for the DNA degradation.[1-3] Thus, DNA cleaving experiment was performed with addition of thiourea which is a famous •OH scavenger. The result is shown in Figure 2. The existence of only the Form I DNA for all of the tested compounds shows complete inhibition of DNA cleavage by the ferricenium cations, suggesting the participation of •OH for the present DNA cleaving system. The production of •OH in an aqueous solution of ferricenium cation was confirmed by the thiobarbituric acid method,[18] where absorption band at 532 nm proved the existence of •OH. In the present system, it is anticipated that Fe(II) species[19] may participate the production of •OH, since it is well known that •OH is produced by the Fenton mechanism in the system containing Fe(II) species. The above anticipation was supported by the experiment adding the reducing agent of dithiothreitol (DTT) to the DNA-ferricenium cation system. The addition of DTT caused enhanced DNA cleaving activity, although addition of DTT alone to the DNA caused no cleavage. This DNA cleaving acceleration may be due to the effective production of Fe(II) species produced by the action of DTT. However, it is still unknown that how is Fe(II) species produced from the ferricenium cation in which Fe exists as Fe(III). Additional work is now in progress.

In conclusion, **4** showed excellent DNA cleaving activity and cytotoxic activity. The present experiments suggest that such activities may be participated by the action of •OH.

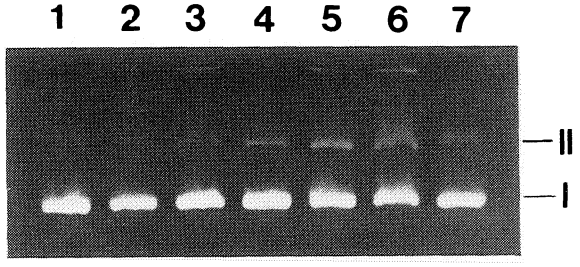


Figure 2. Agarose gel electrophoretic patterns of ethidium bromide-stained φX174 RF I DNA (50 μM in DNA basepair) incubated with ferricenium cations (25 μM) for 30 min in the presence of thiourea (5 μM). The used ferricenium cations are as follows: lane 2 (**1**), lane 3 (**2**), lane 4 (**3**), lane 5 (**4**), lane 6 (**5**). Lanes 1 and 7 contained intact DNA.

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17 A solution of ferricenium cation (25 μM) was immediately added to φX174 RF I DNA (50 μM in DNA basepair) in 100 mM Tris-HCl, pH 8.0. The reaction was allowed to proceed for 30 min at 37 °C, and then DNA was ethanol-precipitated. After resuspension in 100 mM Tris-HCl, pH 8.0, the DNA samples were analyzed by electrophoresis using 1% agarose gel containing 0.5 μg/ml ethidium bromide.

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19 The isolation of the Fe(II) species was unsuccessful. The attempt is now underway.