DNA Binding and Cleaving Activity of Antitumor Metal Complex : Sodium *trans*-dinitrobis(2,4-pentanedionato)cobalt(III)

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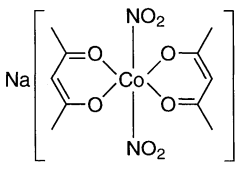
The antitumor metal complex, sodium *trans*-dinitrobis(2,4-pentanedionato)cobalt(III) bound to adenine rich regions of DNA helix and caused single strand scission under the irradiation of light.

There has been considerable interest in site-specific recognition and cleavage of DNA by protein and small molecules since the structure and function of specific region of DNA have become apparent.[1] Compounds of this class have separate sites respectively for interaction with DNA and for chemical reactivity. For example, the antitumor antibiotic bleomycine contains a bithiazole-terminal amine, primarily responsible for DNA binding, and a β-aminoalanine-pyrimidine-β-hydroxyhistidine moiety, for DNA cleaving.[2] Among the metallo-bleomycines, bleomycin-Co(III) complex caused preferential strand scission of DNA at guanine rich sequence in the presence of UV light.[3] In addition, Barton *et al.* reported that tris(diphenyl phenanthroline)cobalt(III) complex caused stereospecific DNA nicking when irradiated at 254 nm.[4] These examples indicate that Co(III) complex with specific base affinity may cleave DNA at a specific base in the presence of light. The choice of a simple and well known complex, sodium *trans*-dinitrobis(2,4-pentanedionato)cobalt(III)[5] **1** which reacts selectively with adenine derivatives among purine and pyrimidine derivatives through coordination of N(7) adenine,[6] would achieve the above point of view.

We have been continuing the screening of the antitumor metal complex[7] and found that **1** caused excellent inhibition toward cancer cell growth *in vitro*. In the 50% inhibition dose (ID_{50})[8] of cell growth using the mouse cancer cell line B16 melanoma,[9] the cytotoxic effect of **1** (6 μg/ml) was comparable to that of the excellent therapeutic drug *cis*-dichlorodiamine platinum(II) (2 μg/ml). We also found the indication that **1** caused DNA cleavage under the room light. In order to elucidate the mode of action of **1**, we report here the DNA binding and cleaving properties of **1** and its *cis*-isomer[10] **2** under the irradiation of light. The structures of Co(III) complexes used in this study are shown below.



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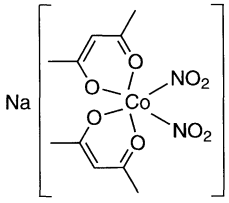

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The effect of light irradiation on cleavage of φX174 RF I DNA was examined under the irradiation of 390 nm light[11] and in the absence of light. The irradiation wavelength of 390 nm was selected since the charge transfer band of **1** was around 400 nm. The most effective irradiation wavelength for DNA cleavage is reported to be that which is likely to excite charge transfer electronic transition.[12] The result of agarose gel electrophoresis[13] is shown in Figure 1. The DNA cleavage was followed by monitoring the conversion of covalently closed circular (form I) φX174 RF I DNA to open circular (form II) and linear duplex (form III) DNAs using the densitometor. In the case of DNA cleavage by **1** (lane 2), production of remarkable amount of form II[14] (95 %) indicates the occurrence of single strand scission under the irradiation of light. On the other hand, slight form II (~1 %) was produced in the absence of light (lane 3). These results suggest the necessity of light for the DNA cleavage by **1**. The charge transfer band of **2** was almost the same as that of **1**. However, **2** hardly caused DNA cleavage even under the irradiation of light (lane 4). It is interesting that difference in *cis* and *trans* geometry effects on the DNA cleavage. Additionally, the emphasis should be paid that the present DNA cleavage was induced by the lower energy light compared to the conventional systems, most of them required UV light.[3,4]

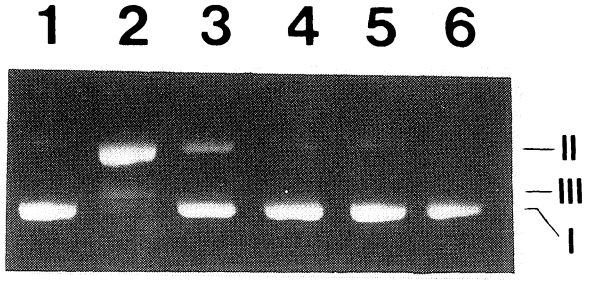

Figure 1. Agarose gel electrophoretic patterns of ethidium bromide-stained φX174 RF I DNA incubated with **1**(lanes 2 and 3) and **2**(lanes 4 and 5). The samples of lanes 2 and 4 were incubated under the irradiation of 390 nm, and those of lanes 3 and 5 were incubated in the absence of light. The concentration of **1** and **2** was 25 μM, and the incubation was performed for 1 h. Lanes 1 and 6 contained intact DNA.

For the purpose to assume the binding site of **1** and **2** to DNA, *Dra* I-digesting experiment[15] was performed. The result of the agarose gel electrophoresis is shown in Figure 2. Restriction enzyme *Dra* I recognizes sequence specific loci (AAA↓TTT) and digests φX174 RF I DNA in two DNA fragments, A and B (lane 4). The *Dra* I-digesting experiment for **1** (lane 2) showed considerable amount of form II (87%) and small quantity of form I (9%). No *Dra* I-digesting fragments were detected. This result suggests that binding of **1** to the adenine bases in the recognition sites of *Dra* I (AAA↓TTT) inhibits the digesting activity of *Dra* I. The present inhibition of *Dra* I-digesting activity was not caused by the inactivation of *Dra* I by **1**, since the ethanol-precipitation operation performed before the *Dra* I-digesting experiment removed the unreacted **1**. Moreover, this inhibition is explained by the fact that both sites of the Co(III) binding of N(7) adenine and the recognition site of *Dra* I for DNA are located in the major

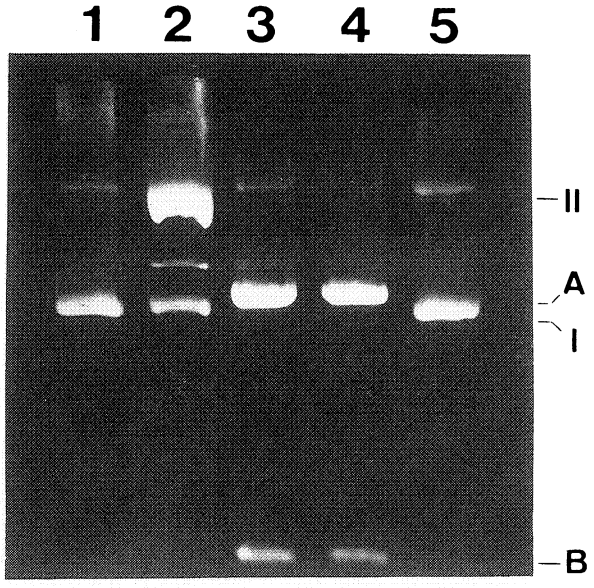

Figure 2. Agarose gel electrophoretic patterns of ethidium bromide-stained ϕX174 RF I DNA incubated with 25 μM of **1** (lane 2) or **2** (lane 3) and treated with *Dra I*. The samples of lanes 2 and 3 were incubated under the irradiation of 390 nm for 1 h, and those of lanes 2-4 were digested with *Dra* I. Lanes 1 and 5 contained intact DNA.

groove of DNA.[7] On the contrary, no inhibitory effect of **2** on *Dra* I-digesting activity (lane 3) indicates that **2** may not bind to adenine moiety in DNA. The lack of binding and cleaving ability of **2** for DNA probably relates with the low cytotoxic effect of **2** (ID_{50} >15 μg/ml).

The reaction of **1** with excess amine (B) in a moderate condition gives mixed-ligand complex of the type $[Co(acac)_2(NO_2)B]$,[5b] since $[Co(acac)_2(NO_2)(H_2O)]$ formed by aquation of **1** readily leads to exchange of ligand with amine.[10] Among the purine and pyrimidine derivatives, **1** forms stable mixed-ligand complex with only adenine derivatives through N(7) coordination. Specificity of **1** requires a nitrogen donor for coordination to cobalt as well as a strategically placed hydrogen bond donor, since the X-ray structure of the stable complex with deoxyadenosine showed a bifurcated hydrogen bond between exocyclic amine group at C(6) and two of the equatorial 2,4-pentanedionato oxygen atoms,[6] suggesting that hydrogen bonding is critical to effective binding.

Once the **1** binds to adenine base of DNA, further substitution of adenine base is impossible under the present reaction condition since the substitution of the remaining nitro group of $[Co(acac)_2(NO_2)B]$ with amine is difficult unless the reaction is performed under a severe condition.[16] Thus, the binding mode of **1** to DNA may be expected to be monofunctional adduct formation with no cross-linking rather than bifunctional adduct formation by intrastrand or interstrand cross-linking. As for **2**, formation of mixed-ligand complex with amine is difficult even under a severe reaction condition.[16] This fact supports that **2** does not bind to DNA at all.

In conclusion, coordination of *trans* isomer **1** to the adenine rich regions of DNA helix caused single strand scission under the irradiation of light, while the *cis* isomer **2** did not.

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References and Notes

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11 Light irradiation was carried out using a 500 W tungsten lamp narrowed to 390 ± 20 nm with C-50S and C-39B color filter glass (Toshiba Co. Ltd.). The distance between the lamp and sample was 30 cm, and the incident intensity at sample was 70 W/cm^2 measured by 6x10^{-3} M potassium tris(oxalato)iron(III).
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13 The preliminary study of dependence of irradiation time on cleavage of DNA indicated that disappearance of form I DNA and production of form II DNA was observed after one hour. Thus, irradiation was performed for one hour. The detailed experimental procedure is as follows. A solution of Co(III) complex (25 μM) was immediately added to ϕX174 RF I DNA (50 μM in DNA base pair) in 100 mM Tris-HCl, pH 8.0. The 20 μl sample in glass tube was then irradiated by a 500 W lamp. The reaction was allowed to proceed for 1 h 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.
14 The mobility of this band increased slightly compared to that of the reference DNA sample (lanes 1 and 6). This slight increased mobility of form II DNA is unexpected because coordination of cobalt to DNA would be expected to retard the migration in the agarose gel. It may be caused by the structure change of DNA such as shortening the length of the DNA. The same phenomenone was also observed in Figure 2 (lane 2).
15 To the DNA sample treated with Co(III) complex under the irradiation of light and then ethanol-precipitated, restriction enzyme *Dra* I was added in 3-fold excess. The mixture was incubated at 37 °C for 1 h, and then analyzed by electrophoresis using 1% agarose gel.
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