

The Interaction of Cobalt (III) Mixed-Ligand Coordination Compound Containing Dipyrido[3,2-a:2',3'-c] phenazine With Calf Thymus DNA*

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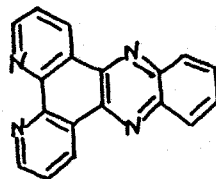
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ABSTRACT The binding of $\text{Co}(\text{phen})_2\text{dppz}^{3+}$ to calf thymus DNA was investigated using absorption spectroscopy, viscosity and electrophoresis measurements, where dppz is dipyrido[3,2-a:2',3'-c]phenazine. The compound shows absorption hypochromicity and the specific viscosity increased upon binding to calf thymus DNA. The complex is also shown to be more efficient photosensitisers for single strand breaks in plasmid DNA.

INTRODUCTION

The interaction of transition metal polypyridyl coordination compounds with DNA and polynucleotide have been extensively studied in the past few years, as their unusual binding properties, combined with their general photoactivity, make them suitable candidate as DNA secondary structure probe, photocleavers and antitumor drugs[1].

Recently, a ruthenium(II) polypyridyl derivative, $\text{Ru}(\text{phen})_2\text{dppz}^{2+}$ was shown to be a remarkable luminescence light switch for DNA[2]. The dppz ligand has an extended aromatic surface area which allows for extensive intercalative stacking in DNA. We have chosen the complex of Co(III), which has not received as much attention as the Ru(II) system but possesses the same interesting characteristics of metallointercalation and DNA cleaving properties. So the mixed-ligand complex $\text{Co}(\text{phen})_2\text{dppz}^{3+}$ has been newly synthesized in our laboratory. In this paper, We investigated the properties of its binding to calf thymus DNA.



dppz

RESULTS AND DISCUSSION

All the measurements were conducted using solutions of the complex in Tris-HCl buffer (pH7. 0) containing 5mmol/L Tris and 50mmol/L NaCl. DNA samples were electrophoresed through 1% agarose gel containing 50mM Tris acetate, 20mM sodium acetate, 18mM sodium chloride, and then stained with ethidium bromide.

Hypochromicity of MLCT Bands. The absorption of MLCT bands of the complex in the presence of calf thymus DNA decreases as compared to that in the absence of DNA. This change is often characteristic of intercalation which involves a strong stacking interaction between an aromatic chromophore and the base pairs of DNA [3]. With increasing amounts of DNA, the hypochromic effect of $\text{Co}(\text{phen})_2\text{dppz}^{3+}$ is more evident. The change of MLCT bands is shown in Figure 1.

Viscosity Study. The viscosity studies provide a strong argument for intercalation[4].

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The specific viscosity contribution (η) due to the DNA in the presence of a binding agent was obtained. The results are summarized in Table 1. The specific viscosity of the DNA sample increases obviously with addition of $\text{Co}(\text{phen})_2\text{dppz}^{3+}$. This is ascribed to the intercalative binding mode of the drug because this could cause the effective length of the DNA to increase [5].

TABLE 1. Effect of the Drug on the Specific Viscosity of DNA

C_{drug} (mmol / L)	0.00	0.02	0.04	0.06	0.08
η	1.98	2.13	2.23	2.29	2.37

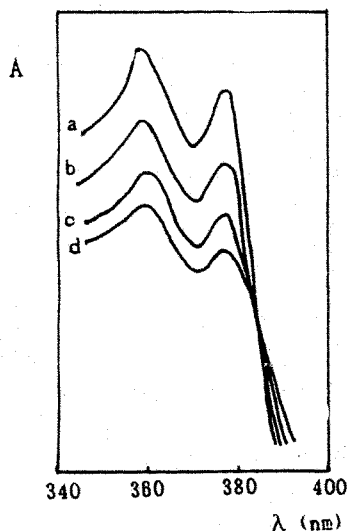


Figure 1. Changes of MLCT Absorption. Complex alone (a), complex + ct DNA (b~d). $R_t = [\text{DNA}]/[\text{Co}] = 0, 0.5, 1, 2$, respectively.

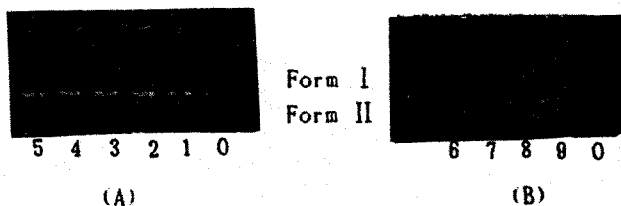


Figure 2. (A) Cleavage of pBR 322 DNA in the presence of $\text{Co}(\text{phen})_2\text{dppz}^{3+}$ and light. DNA alone (lane 0), the concentration of $\text{Co}(\text{phen})_2\text{dppz}^{3+}$ was 2.5, 5, 7.5, 10, 15 μM (lanes 1~5) (B) pBR 322 DNA was incubated with $\text{Co}(\text{phen})_2\text{dppz}^{3+}$ (10 μM) and irradiated for 5, 10, 20, 30 min (lanes 6~9).

Photoactivated Cleavage of pBR322 DNA By $\text{Co}(\text{phen})_2\text{dppz}^{3+}$. Some transition metal polypyridyl coordination compounds can cleave DNA when irradiated at 254nm [6]. The cleavage reaction on plasmid DNA can be monitored by agarose gel electrophoresis. When circular plasmid DNA is subjected to electrophoresis, relatively fast migration will be observed for the intact supercoiled form (Form I). If scission occurs on one strand, the supercoils will relax to generate a slower-moving open circular form (Form II) [6]. Figure 2(A) shows the results of cleaving superhelical pBR322 DNA in the presence of varying concentrations of $\text{Co}(\text{phen})_2\text{dppz}^{3+}$ when irradiated at 254nm for 40 min. Figure 2(B) is the cleavage of pBR322 DNA in the presence of constant concentration of $\text{Co}(\text{phen})_2\text{dppz}^{3+}$ when irradiated for variable times. The results indicate that with increasing concentration of $\text{Co}(\text{phen})_2\text{dppz}^{3+}$ or extended irradiation, Form I of pBR 322 DNA diminishes gradually, whereas the amount of Form II increases. These are the results of single-strand cleavage of pBR322 DNA (nicking).

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