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NICKING OF SUPERCOILED DNA VIA METAL RADICALS GENERATED FROM PHOTOLYSIS OF SPECIES CONTAINING METAL-METAL BONDS

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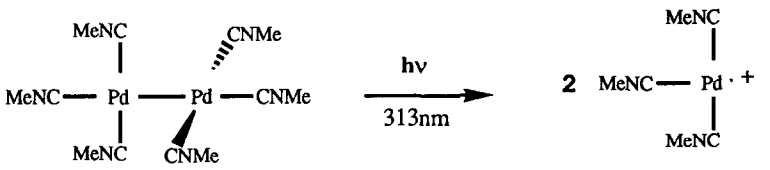
Abstract: The photoactivated metal-dimers, $Pd_2(CH_3NC)_6^{2+}$ and $Pt_2(CH_3NC)_6^{2+}$ are shown to nick supercoiled DNA in a light-dependent reaction.

Recent mechanistic studies on calicheamycin [1], esperamycin [2], neocarzinostatin [3], and related molecules have shown that carbon-centered radical pairs produce single strand nicks and double strand breaks in DNA. Photolysis of metal complexes containing metal-metal bonds produce metal radicals, therefore, it was of interest to investigate the DNA nicking properties of $Pd_2(CH_3NC)_6^{2+}$ and $Pt_2(CH_3NC)_6^{2+}$. These complexes contain an unsupported metal-metal bond, which upon photolysis at ca. 315 nm, generates a pair of the highly reactive Pd(I) or Pt(I) radicals of the form $\cdot M(CH_3NC)^{3+}$ [4] (Figure 1). Previous photochemical studies involving metal complexes and DNA have used coordination complexes such as acridine orange - cisplatin, $Co(DIP)_3$ and $Ru(TMP)_3$ [5, 6, 7]; transition metal dimers had not as yet been explored as possible cleaving reagents towards DNA.

Figure 1:



Photonicking studies were performed on supercoiled pUC8 plasmid. The reaction samples (total volume 50 µl) contained 200 µM $Pd_2(MeNC)_6(PF_6)_2$, or $Pt_2(MeNC)_6(BF_4)_2$, 0.5µg pUC8 plasmid DNA, and 25 mM PO_4 buffer, pH 7.5, and were irradiated at a distance of 10 cm using a 1000W xenon-mercury arc lamp (Oriel) fitted with a 313nm interference filter (Microcoatings). All reactions were degassed with three freeze-pump-thaw cycles prior to irradiation. Irradiation of the reactions was followed by the addition of 25 mM NaCN, 37°C, 15 min. and the DNA was recovered by ethanol precipitation. The added CN^- coordinates to the Pd or Pt which might otherwise remain bound to the DNA. The metal-binding to DNA may interfere with its electrophoretic mobility and with the ability to visualize the DNA with ethidium bromide [8].

As shown in Figure 2, substantial nicking of the supercoiled DNA occurred after 15 min. irradiation in the presence of $Pd_2(CH_3NC)_6^{2+}$. Comparison of lane 4 (standard DNA), lane 3 (DNA irradiated for 15 min. in the absence of Pd-dimer), lane 2 (DNA treated with Pd-dimer without irradiation), and lane 1 (DNA treated with Pd

(DIP) =4,7-diphenyl-1,10-phenanthroline; (TMP) =Tris-3,4,7,8-tetramethylphenanthroline

dimer followed by irradiation), illustrates the photo-induced nicking of supercoiled DNA by $Pd_2(CH_3NC)_6^{2+}$. All of the reactions were treated with NaCN and ethanol precipitated prior to electrophoresis. The choice of buffer used in the photolysis reactions was found to be an important factor in determining the efficiency of nicking by the Pd-dimer. The use of 25 mM Tris, pH 7.5, as the buffer resulted in no conversion of supercoiled DNA to nicked DNA. It would seem that under these conditions, the Tris acts as an efficient metal radical scavenger, preventing their interaction with the DNA. Tris (tris(hydroxymethyl)methylamine) is a potentially reactive primary amine and has been shown to complex metal ions [9]. The quenching of the dimer reactions by Tris is probably a function of one of these side-reactions.

Figure 2: Photo-induced nicking of supercoiled DNA by $Pd_2(MeNC)_6^{2+}$. Lane 1 is DNA + Pd-dimer irradiated at 313 nm and lane 2 is DNA + Pd-dimer without irradiation. Lanes 3 & 4 are DNA irradiated in the absence of Pd-dimer and standard DNA.

Since positively charged metals can bind to DNA, resulting in anomalous electrophoretic mobility, a second method was used to confirm the nicking effects of these metal dimers. The ethidium bromide fluorescence assay has been shown to provide a convenient method for following DNA damage, in the conversion of supercoiled DNA to nicked DNA [10]. The assay is based on the property of ethidium to undergo fluorescence enhancement only when it is intercalated into duplex DNA. The amount of ethidium taken up by native, supercoiled DNA is restricted because of topological constraints. Nicking the DNA relaxes it and removes these constraints. The assay, performed in alkaline pH 12 assay buffer (20 mM K_3PO_4, 0.5 mM EDTA, 0.5 μg/ml ethidium bromide) allows for the distinction of supercoiled, nicked and modified DNAs by denaturing the DNA with heating at 90°C and cooling to room temperature. Under the alkaline condition of the buffer, the denatured nicked form is restricted from renaturing and loses its fluorescence enhancement, while the covalently, closed supercoiled DNA retains its fluorescence enhancement since its strands are topologically prevented from dissociating.

A comparison of the cleavage activities of the $Pd_2(CH_3NC)_6^{2+}$ and $Pt_2(CH_3NC)_6^{2+}$ towards supercoiled DNA using the ethidium bromide fluorescence enhancement assay is shown in Figure 3. The increase in the ethidium bromide before-heat fluorescence, along with the decrease in after-heat fluorescence is further evidence that the dimers nick supercoiled DNA.

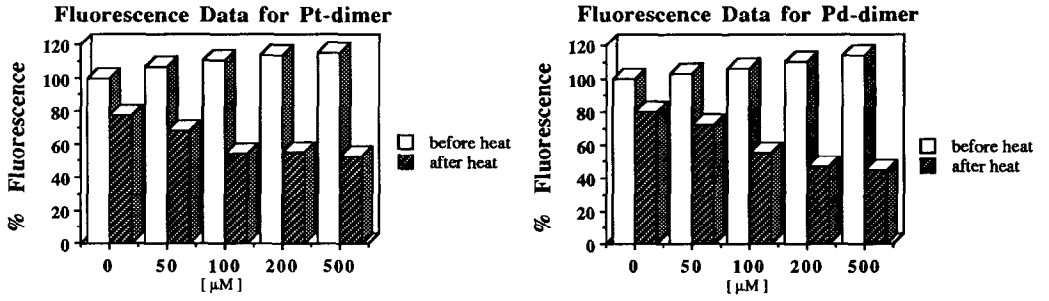


Figure 3: Nicking activity of Pd- and Pt-dimers towards supercoiled DNA analyzed by ethidium bromide fluorescence assay. Metal-dimer reactions were irradiated at 313 nm for 15 min. and treated with NaCN before addition of assay buffer. Fluorescence emission of ethidium bromide at 600 nm (excitation 525 nm).

The DNA nicking ability of $Pd_2(MeCN)_6^{2+}$ was inhibited by the addition of deoxyribose as shown in Figure 4. At 200 μM, the sugar prevented the nicking of supercoiled DNA. This concentration corresponds to ca. 10^3 molar excess of sugar molecules relative to plasmid DNA. Electrostatic interactions between the doubly positive-charged metal dimer may account for the need of such an excess neutral sugar to compete with the plasmid DNA as a source of reactive sites for the transition metal-based radicals.

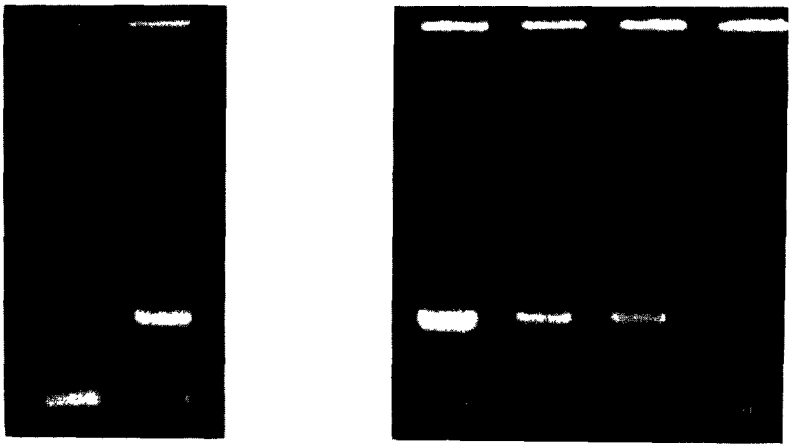


Figure 4: Inhibition of Pd-dimer nicking of supercoiled DNA by deoxyribose. Reactions are 200 μM Pd-dimer with added deoxyribose: 0 μM (lane 2), 20μM (lane 3), 50 μM (lane 4), 100μM (lane 5), and 200μM (lane 6).

Though our studies, we have demonstrated that the complexes $Pd_2(MeNC)_6(PF_6)_2$ and $Pt_2(MeNC)_6(BF_4)_2$ nick supercoiled DNA by a photo-induced reaction. The detailed mechanisms of interaction of the metal-radicals with DNA is currently unknown, although covalent attachment of the metal to the DNA is evident through inhibition of ethidium bromide binding subsequent to treatment of the DNA-metal complex with NaCN [11]. The inhibition of cleavage by deoxyribose may suggest that strand scission is initiated by hydrogen abstraction. Further studies, including GC-MS analysis of the degradation products, are underway to elucidate the mechanism of action of the metal radical on DNA and to ascertain whether the dimers exhibit any sequence selectivity.

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