A New Class of Supramolecular, Mixed-Metal DNA-Binding Agents: The Interaction of Ru^{II},Pt^{II} and Os^{II},Pt^{II} Bimetallic Complexes with DNA

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A new type of mixed-metal, supramolecular complex has been designed that incorporates a platinum center to allow binding to DNA. The interaction of two such platinum heterobimetallic complexes of the general formula $[(bpy)_2M(dpb)PtCl_2]Cl_2$ (M = Ru^{II}, Os^{II}; bpy = 2,2′-bipyridine; dpb = 2,3-bis(2-pyridyl)benzoquinoxaline) with DNA is reported herein. The modular design of these systems allows for synthetic variation of individual components within this structural motif. In this case, the remote metal is varied from Ru^{II} to Os^{II}. DNA binding was analyzed using non-denaturing agarose gel electrophoresis. The interaction of these complexes with DNA was studied relative to the known DNA cross-linkers, *cis*-$[Pt(NH_3)Cl_2]$ (cisplatin) and *trans*-$\{[PtCl(NH_3)_2]_2(\mu\text{-}H_2N(CH_2)_6NH_2)\}^{2+}$ (1,1/t,t). Our mixed-metal Ru,Pt and Os,Pt compounds retard the migration of DNA through the gel in both a concentration- and time-dependent manner. Their effect on the migration of DNA is similar to, although much more dramatic than, that observed for either cisplatin or 1,1/t,t. Our evidence suggests a covalent binding of our mixed-metal complexes to DNA through the platinum site. The degree of retardation of DNA migration suggests a large change in DNA conformation is induced by binding of our mixed-metal complexes. This work establishes these inorganic systems as a new class of DNA-binding agents and lays the groundwork for future efforts to enhance binding in an effort to develop novel anticancer drugs through serial design and testing.

Introduction

Discovery and subsequent characterization of the anticancer drug *cis*-$[Pt(NH_3)_2Cl_2]$ (cisplatin) have yielded much information on its activity and have fostered new interest in the field of metallodrugs.[1−5] Today, it is accepted that cisplatin interacts with cellular DNA, resulting in cross-link formation in either an intra- or interstrand fashion. The form believed responsible for activity is a d(GpG) or d(ApG) intrastrand cross-link, which interferes with DNA replication and consequently results in cell death.[4,5] Restrictions in cisplatin's clinical use due to cumulative drug resistance and toxic side effects have prompted an active new area of analog research. Analogs containing the DACH[6,7] (diaminocyclohexane) and the CBDCA[8,9] (cyclobutanecarboxylato) ligand have been developed. Platinum(IV) complexes show potential for oral administration.[1,10]

The development of agents capable of producing activity through new DNA-binding modes has also been explored. Farrell and co-workers have synthesized several complexes having the general formula $[\{cis\text{-}PtCl_2(NH_3)\}_2NH_2(CH_2)_nNH_2]$ (n = 4−6).[11] Studies indicate that these complexes interact with DNA to form interstrand cross-links.[12] Similar studies on the compound $[\{trans\text{-}PtCl(NH_3)_2\}_2\{\mu\text{-}H_2N(CH_2)_6NH_2\}]Cl_2$ (1,1/t,t), show more efficient interstrand cross-linking with unique binding sites.[13]

Recent work has shown that many mono- and bimetallic complexes of the platinum group metals incorporating planar aromatic ligands with extended π-systems can interact with DNA.[14−23] Studies on several polypyridyl compounds of

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ruthenium, osmium, cobalt, nickel, rhodium, and platinum indicate that these complexes bind to DNA, often in an intercalative fashion. Barton and co-workers have demonstrated that $[Ru(bpy)_2(dppz)]^{2+}$, $[Ru(phen)_2(dppz)]^{2+}$, and $[Os(phen)_2(dppz)]^{2+}$ show enhanced photoluminescence in the presence of DNA (bpy = 2,2′-bipyridine; phen = 1,10-phenathroline; dppz = dipyrido[3,2-*a*:2′,3′-*c*]phenazine).[14] They attribute this to the intercalation of the dppz ligand into the DNA double helix. Murphy and co-workers have demonstrated that bimetallic complexes of ruthenium can interact with DNA.[21] Studies on the ruthenium bimetallic complex $\{[(NH_3)_4Ru]_2(dpb)\}^{4+}$ (dpb = 2,3-bis(2-pyridyl)benzoquinoxaline) indicate that the planar dpb bridging ligand intercalates into DNA.[21] McMillin and co-workers have shown that $[Pt(tpy)(OH)]^+$ is a bifunctional DNA-binding agent that competitively binds to DNA in a covalent and intercalative manner (tpy = 2,2′:6′,2″-terpyridine).[22] Mital and co-workers explored the DNA binding of amino acid complexes of (diimine)platinum systems.[23]

The goal of this research was to develop a new type of supramolecular structural motif that is modular in design and produces complexes capable of binding DNA. Our approach is to bind a *cis*-$Pt^{II}Cl_2$ moiety to ruthenium and osmium light absorbers using a dpb bridging ligand. These complexes are potentially bifunctional, capable of both intercalative and covalent binding to DNA. Our systems are also modular in design, allowing synthetic coordination chemistry to be used to vary individual components within this structural design. Moreover, these systems incorporate light absorbers that could be used for the photoactivation of these complexes. These remote metals also impart a cationic charge to the complexes that provide the needed water solubility to the $Pt^{II}(dpb)Cl_2$ unit. Herein we explore the change of the nature of the remote metal center.

Our mixed-metal bimetallic complexes are of the general form $[(bpy)_2M(dpb)PtCl_2]Cl_2$ ($M = Ru^{II}, Os^{II}$).[24] Within each molecule, two structural motifs are present, a planar dpb bridging ligand and a platinum unit that has the cis-chloride structure of cisplatin. This paper reports the first evidence for interaction of these mixed-metal complexes with DNA. Agarose gel electrophoresis was used to examine the binding of these complexes to linearized double-stranded plasmid DNA. Cisplatin and 1,1/t,t were used as standards in this assay.

Experimental Section

Materials. Reagent grade chemicals for the preparation of the chloride salts of the heterobimetallic complexes were obtained from Fisher. Cisplatin was obtained from Aldrich. The compound 1,1/t,t was a gift from N. Farrell, Virginia Commonwealth University.[13] Bacteriophage lambda DNA was obtained from Pharmacia. The plasmid, pBluescript KS+, was obtained from Stratagene, and all materials used in amplification and purification were purchased from Fisher. Electrophoresis-grade low EEO agarose, tris(hydroxymethyl)-aminomethane (Tris), boric acid, and ethidium bromide were also obtained from Fisher. EcoRI and HindIII restriction endonucleases were purchased from Promega.

Preparation of Chloride Salts of $[(bpy)_2Ru(dpb)PtCl_2](PF_6)_2$ and $[(bpy)_2Os(dpb)PtCl_2](PF_6)_2$. The heterobimetallic complexes $[(bpy)_2Ru(dpb)PtCl_2](PF_6)_2$ and $[(bpy)_2Os(dpb)PtCl_2](PF_6)_2$ were prepared as described previously.[24] The chloride salts of these compounds, needed to provide water solubility, were prepared via anion exchange chromatography using Dowex-2 anion exchange resin and 1:1 H_2O/acetonitrile as the mobile phase. Resulting solutions were reduced in volume, dissolved in methanol, and flash-precipitated in diethyl ether. The resulting solids were then collected by vacuum filtration and washed with acetonitrile and diethyl ether. All compounds used in this study were dissolved in deionized water and stored at 4 °C in the dark.

Preparation and Purification of Plasmid DNA. The plasmid, pBluescript, was amplified and purified from *Escherichia coli* strain JM109 according to established protocols.[25] Plasmids were isolated using an alkaline lysis procedure, purified in a cesium chloride gradient, and then extensively dialyzed against TE (10 mM Tris (tris(hydroxymethyl)aminomethane), 1 mM EDTA, pH 7.5). Following concentration by ethanol precipitation, the DNA was stored in TE at 4 °C. Plasmid DNA was linearized by overnight incubation at 37 °C with EcoRI endonuclease. Typically, 200 μg of plasmid DNA was combined with EcoRI (3 μL, 240 U) and 20 μL of 10X buffer in a total volume of 200 μL. Protein was removed by extracting with phenol/0.1% hydroxyquinoline (equilibriated with TE pH 8) and 24:1 chloroform/isoamyl alcohol. The DNA was then precipitated with NaCl and ethanol, resuspended in deionized water, and stored at 4 °C.

Preparation of Molecular Weight Standards. Molecular weight standards for non-denaturing agarose gel electrophoresis were prepared via digestion of bacteriophage lambda DNA with HindIII endonuclease. Lambda DNA (50 μg, 100 μL of 500 μg/mL stock solution) was combined with HindIII (2 μL, 160U) in 258 μL of H_2O buffered with 10X buffer (40 μL) and incubated for 12 h at 37 °C. Upon completion, 100 μL of 6X type III dye was added and the solution was stored at 4 °C.[26]

Reaction of Metal Complexes with Plasmid DNA. The concentration of the linearized plasmid DNA solution was determined spectrophotometrically.[27] Concentrations of metal solutions were determined using the known extinction coefficients for $[(bpy)_2Ru(dpb)PtCl_2]Cl_2$ ($\epsilon = 9.8 \times 10^4\ M^{-1}\ cm^{-1}$ at 630 nm) and $[(bpy)_2Os(dpb)PtCl_2]Cl_2$ ($\epsilon = 11 \times 10^4\ M^{-1}\ cm^{-1}$ at 638 nm).[24] All reactions contained 1 μg of linearized plasmid DNA and 10 mM sodium phosphate, pH 7 in a total volume of 100 μL. Concentration-dependence studies were performed using DNA base pair (bp) to metal complex (mc) ratios of 5:1 to 100:1 and a 4 h incubation at 37.8 °C. Time course studies were accomplished using a 5:1 bp to mc ratio and 0−2 h incubation at 378 °C. The samples were analyzed by electrophoresis in 300 mL agarose gels (0.8% agarose, 89 mM Tris, 89 mM boric acid, pH 8) at 104 V for 1.5 h, with recirculation of the buffer. Gels were then stained in 0.5 μg/mL ethidium bromide for 30 min and photographed with UV illumination. Polaroid prints were scanned using a MicroTek ScanMaker E_6.

Results

We have previously described the synthesis of the mixed-metal complexes $[(bpy)_2Ru(dpb)PtCl_2]^{2+}$ and $[(bpy)_2Os(dpb)PtCl_2]^{2+}$ shown in Figure 1.[24] The spectroscopic and electrochemical properties of these complexes have also been reported.[24] Both complexes display intense ligand-based $\pi \rightarrow \pi^*$ transitions in the UV and MLCT transitions in the visible region. The lowest lying absorbance is a Ru → dpb CT band at 630 nm for $[(bpy)_2Ru(dpb)PtCl_2]^{2+}$ and an Os → dpb CT band at 638 nm for $[(bpy)_2Os(dpb)PtCl_2]^{2+}$. The electrochemistry of these complexes shows reversible Ru- or Os-based oxidations at 1.61 and 1.05 V vs Ag/AgCl and two reversible dpb-based couples, dpb/dpb^- and dpb^-/dpb^{2-}, that occur prior to bpy reduction. The dpb/dpb^- couples occur at −0.11 and −0.22 V for the Ru- and Os-based complexes, respectively.

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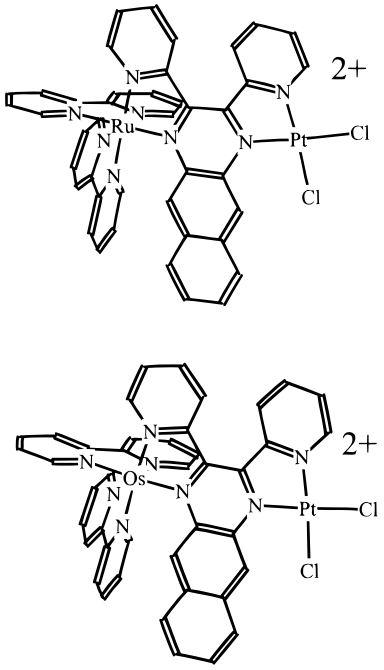

Figure 1. Representations of our supramolecular DNA-binding agents $[(bpy)_2Ru(dpb)PtCl_2]^{2+}$ and $[(bpy)_2Os(dpb)PtCl_2]^{2+}$ (bpy = 2,2′-bipyridine; dpb = 2,3-bis(2-pyridyl)benzoquinoxaline).

Concentration-Dependent Interaction with DNA. To determine whether these complexes, $[(bpy)_2Ru(dpb)PtCl_2]Cl_2$ and $[(bpy)_2Os(dpb)PtCl_2]Cl_2$, exhibit DNA binding activity, reactions were performed with linearized plasmid DNA in a range of bp to mc ratios. The DNA used is 2958 base pairs in length with a 50.2% GC content. To provide a basis for comparison, incubations of DNA with the known cross-linkers *cis*-$[Pt(NH_3)_2Cl_2]$ and *trans*-$\{[PtCl(NH_3)_2]_2(\mu\text{-}H_2N(CH_2)_6NH_2)\}^{2+}$ (1,1/t,t) were also performed using equivalent bp to mc ratios. Cisplatin is known to form primarily intrastrand cross-links and 1,1/t,t is known to form primarily interstrand cross-links, but both complexes form covalent bonds to DNA via the Pt center. All samples were then analyzed by non-denaturing agarose gel electrophoresis.

Figure 2 shows the results of the concentration-dependent DNA-binding study. Each gel contains eight lanes. Lanes 1 and 8 are molecular weight standards. Lanes 2 and 7 contain the plasmid control with no metal complex added. Lanes 3−6 contain the plasmid DNA incubated for 4 h at 37 °C with varying bp to mc ratios, 5:1, 10:1, 20:1, and 100:1. Results are shown for cisplatin (A), 1,1/t,t (B), $[(bpy)_2Ru(dpb)PtCl_2]Cl_2$ (C), and $[(bpy)_2Os(dpb)PtCl_2]Cl_2$ (D).

As shown in Figure 2A, cisplatin, which forms covalent cross-links with DNA,[1−5] had a marked effect on the migration of linearized plasmid DNA in the gel. In the absence of metal complex, the plasmid control migrated at a rate inversely proportional to the logarithm of its molecular weight relative to the size standard. In contrast, after incubation with cisplatin, lanes 3−6, migration of the DNA was significantly reduced relative to the plasmid control. This effect was most pronounced at the lowest ratio of bp to mc, lane 3, which is the highest relative concentration of the metal complex. This complex also interfered with the staining of the DNA with ethidium bromide. Similar effects were observed for 1,1/t,t (Figure 2B), which interacts with DNA primarily via interstrand cross-links.[13]

The effects of the two mixed-metal complexes $[(bpy)_2Ru(dpb)PtCl_2]Cl_2$ and $[(bpy)_2Os(dpb)PtCl_2]Cl_2$ on DNA migration are shown in Figure 2C,D. Incubation with either of our metal complexes resulted in even more pronounced effects than were

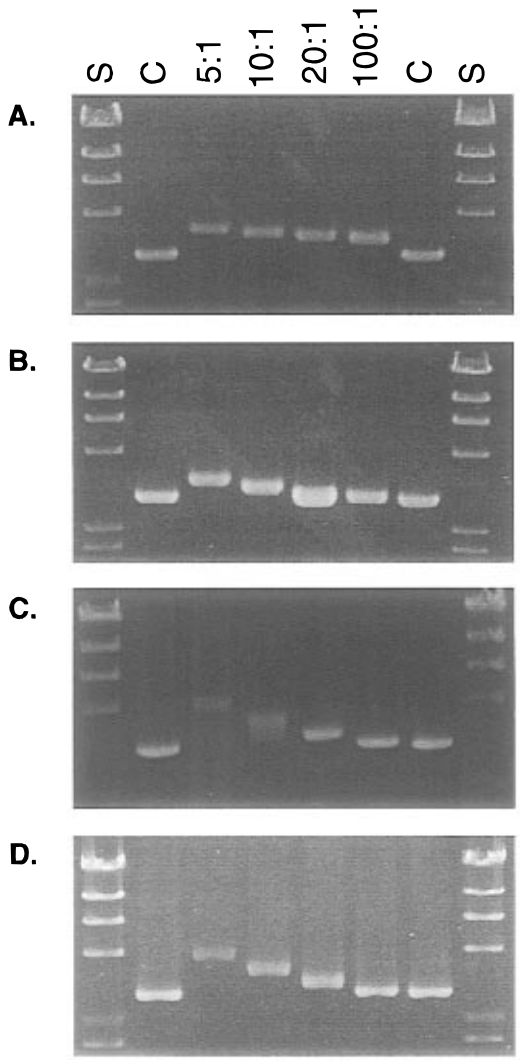

Figure 2. Concentration-dependent interaction of metal complexes with DNA. One microgram of linearized plasmid DNA was incubated with metal complexes at a ratio of 5, 10, 20, or 100 base pairs to 1 metal complex at 37.8 °C for 4 h (5:1, 10:1, 20:1, 100:1). A control sample (C) containing only plasmid DNA was incubated under identical conditions. Samples were analyzed on 0.8% agarose gels which were then stained with 0.5 mg/mL ethidium bromide and photographed under UV illumination. A molecular weight standard (S) was included for reference. Key: (A) *cis*-$[Pt(NH_3)_2Cl_2]$; (B) *trans*-$\{[PtCl(NH_3)_2]_2(\mu\text{-}H_2N(CH_2)_6NH_2)\}^{2+}$; (C) $[(bpy)_2Ru(dpb)PtCl_2]Cl_2$; (D) $[(bpy)_2Os(dpb)PtCl_2]Cl_2$.

observed for cisplatin or 1,1/t,t. Both migration of DNA through the gel and ethidium bromide staining were affected in a strongly concentration-dependent manner. Again, increasing effects were observed with decreasing ratios of bp to mc.

Time-Dependent Interaction with DNA. It has previously been shown that the interaction of cisplatin and 1,1/t,t with DNA occurs very rapidly. For example, significant binding of cisplatin to supercoiled plasmid DNA at a 20:1 bp to mc ratio occurs within 5 h of incubation at 37.8 °C.[28] Although similar time courses have not been reported for 1,1/t,t, several assays using 1−3 h incubation times have been described.[13] To assess the relative time dependence of the DNA-binding activities of the four complexes, incubations were performed at a 5:1 bp to mc ratio for 0 min, 15 min, 30 min, 1 h, and 2 h. The 0 min times indicate no incubation of the metal complex with the DNA, but the DNA is exposed to the metal complex for ca. 15

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min at room temperature prior to applying a voltage to the gel. All samples were again examined by agarose gel electrophoresis. Each gel contains eight lanes. Lanes 1 and 8 contain the molecular weight standards. Lanes 2 and 7 contain the plasmid DNA without the addition of the metal complex. Lanes 3−6 contain the plasmid DNA after incubation at 37 °C for 0, 0.25, 0.5, 1, and 2 h. Figure 3 shows the results for five different metal complexes, cisplatin (A), 1,1/t,t (B), $[(bpy)_2Ru(dpb)PtCl_2]Cl_2$ (C), $[(bpy)_2Os(dpb)PtCl_2]Cl_2$ (D), and $[(bpy)_2Ru(dpb)]Cl_2$ (E).

In this study, different results were obtained for the mixed-metal complexes, $[(bpy)_2Ru(dpb)PtCl_2]Cl_2$ and $[(bpy)_2Os(dpb)PtCl_2]Cl_2$, and the two standards. Parts A and B of Figure 3 show that, even at short incubation times, cisplatin and 1,1/t,t had a significant effect on the migration of plasmid DNA through the gel. This effect increased slightly with increasing incubation times over the 2 h time course. Some effect on ethidium bromide staining was also observed for 1,1/t,t. The two mixed-metal complexes, $[(bpy)_2Ru(dpb)PtCl_2]Cl_2$ and $[(bpy)_2Os(dpb)PtCl_2]Cl_2$, also appeared to react rapidly with plasmid DNA, as shown in Figure 3C,D. However, a greater overall effect on DNA migration was observed that increased over the time period shown. This effect was accompanied by a significant decrease in ethidium bromide staining intensity.

To confirm that the interaction observed in these studies was due to the presence of the Pt center of the bimetallic complexes and not simply to the monometallic ruthenium or osmium component, a time course study was performed using $[(bpy)_2Ru(dpb)]^{2+}$. As shown in Figure 3E, the monometallic $[(bpy)_2Ru(dpb)]^{2+}$ had no effect on DNA migration in the gel. This result demonstrates that the Pt component of the bimetallic complexes is essential for the interaction with DNA responsible for the change in its migration in the gel electrophoresis experiment.

Discussion

From these gel electrophoresis studies, it has been clearly shown that our mixed-metal complexes, $[(bpy)_2Ru(dpb)PtCl_2]Cl_2$ and $[(bpy)_2Os(dpb)PtCl_2]Cl_2$, interact with linearized plasmid DNA and form the basis of a new class of metal-containing DNA-binding agents. Significant retardation of DNA band migration was seen for both $[(bpy)_2Ru(dpb)PtCl_2]Cl_2$ and $[(bpy)_2Os(dpb)PtCl_2]Cl_2$ relative to the control which contains no metal complex. Retardation trends appear to be equivalent for both compounds, and this effect appears to be both concentration and time dependent. Both of our complexes lead to a more pronounced retardation of DNA migration through the gel than the known DNA cross-linkers, cisplatin and 1,1/t,t. In addition, it appears that the complexes interfere with staining of DNA with the intercalative dye, ethidium bromide, as we observe a decrease in the intensity of ethidium bromide fluorescence at higher concentrations of metal complex and at longer incubation times. This effect is also observed for 1,1/t,t and, to a lesser degree, cisplatin.

These effects may be explained by considering the characteristics of DNA migration through agarose gels. The electrophoretic mobility of DNA is determined by many factors including molecular weight, molecular shape, charge, and gel voltage. Alteration of any of these factors may affect the migration of DNA through the gel; i.e., a shorter DNA molecule will move farther and faster than a longer one, as will a more compact or more negatively-charged DNA molecule. The retardation observed in our gels is due to binding of the metal complexes to the DNA. Such binding would alter the molecular weight of the DNA molecule considerably. Since our complexes have higher molecular weights than either cisplatin or 1,1/t,t, this could explain the fact that our complexes have a more dramatic effect on the retardation of the DNA through the gel. However, the magnitude of the retardation of the DNA through the gel cannot be explained by the molecular weight increase alone. The overall negative charge of DNA, due to the phosphate groups in the sugar−phosphate backbone, would

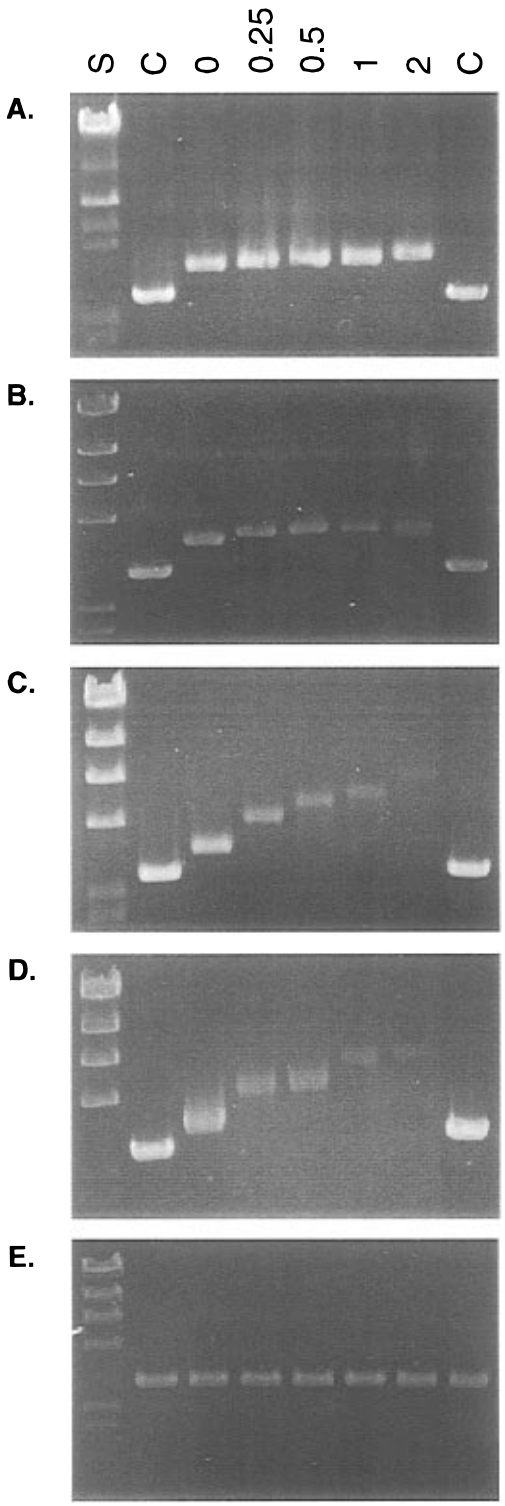

Figure 3. Time course of the interaction of metal complexes with DNA. One microgram of linearized plasmid DNA was incubated with metal complexes at a ratio of 5 base pairs to 1 metal complex at 378 °C for 0, 0.25, 0.5, 1, or 2 h. A control sample (C) containing only plasmid DNA was incubated for 4 h. Samples were analyzed on 0.8% agarose gels which were then stained with 0.5 mg/mL ethidium bromide and photographed under UV illumination. A molecular weight standard (S) was included for reference. Key: (A) *cis*-$[Pt(NH_3)_2Cl_2]$; (B) *trans*-$\{[PtCl(NH_3)_2]_2(\mu\text{-}H_2N(CH_2)_6NH_2)\}^{2+}$; (C) $[(bpy)_2Ru(dpb)PtCl_2]Cl_2$; (D) $[(bpy)_2Os(dpb)PtCl_2]Cl_2$; (E) $[(bpy)_2Ru(dpb)]Cl_2$.

be reduced by binding of our cationic metal complexes. Since our complexes, as well as 1,1/t,t, will become 4+ cations upon labilization of the two cis chloride ligands if they substitute neutral water ligands, they will possess a higher positive charge than cisplatin, which will become a 2+ cation upon labilization of the chlorides. This decrease in the overall negative charge of the DNA could also lead to the observed increase in the retardation of DNA migration. If this were the major factor controlling the migration of the DNA through the gel, one would expect our mixed-metal complexes to produce results similar to those for 1,1/t,t which should be about twice those observed for cisplatin. This is clearly not the case, as cisplatin and 1,1/t,t show similar effects and our mixed-metal complexes exhibit much more dramatic retardation. Another important consideration is that binding of these metal complexes to DNA will induce local or global changes in DNA conformation, resulting in shape disturbances and hence altered migration through the agarose matrix. Marzilli et al. have demonstrated that platinum-based anticancer drugs such as cisplatin and Pt(ethylenediamine)Cl_2 induce an unusual hairpinlike structure in DNA.[29] These structural changes would be expected to be different for our larger complexes relative to cisplatin and 1,1/t,t and could be a major contributor to the observed differences in DNA migration in the gel.

Our data clearly show that both $[(bpy)_2Ru(dpb)PtCl_2]Cl_2$ and $[(bpy)_2Os(dpb)PtCl_2]Cl_2$ bind to DNA. This establishes these complexes as a new class of metal-based DNA-binding agents. The complexes have two possible modes of binding to DNA: intercalation of the dpb ligand and covalent binding through the platinum metal site. From the study that included the $[(bpy)_2Ru(dpb)]Cl_2$ species, it is clear that dpb intercalation is not responsible for the observed change in migration of DNA exposed to our $[(bpy)_2Ru(dpb)PtCl_2]Cl_2$ and $[(bpy)_2Os(dpb)PtCl_2]Cl_2$ complexes. This indicates that the platinum site is needed for the observed trends and strongly suggests that our complexes form covalent bonds to DNA through this site. This is also consistent with the observed similarity in the effect of exposure to our mixed-metal Ru,Pt and Os,Pt complexes on the DNA migration relative to the two known DNA cross-linkers, cisplatin and 1,1/t,t.

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Conclusion

The $[(bpy)_2Ru(dpb)PtCl_2]Cl_2$ and $[(bpy)_2Os(dpb)PtCl_2]Cl_2$ complexes interact with DNA in a concentration- and time-dependent fashion. Non-denaturing agarose gel electrophoresis of linearized plasmid DNA provides a simple preliminary assay for probing the covalent binding of our complexes to DNA and comparing them to the known DNA cross-linkers, cisplatin and 1,1/t,t. Our simultaneous analysis of the $[(bpy)_2Ru(dpb)]Cl_2$ system allowed us to rule out a purely intercalative mode of interaction for our mixed-metal complexes $[(bpy)_2Ru(dpb)PtCl_2]Cl_2$ and $[(bpy)_2Os(dpb)PtCl_2]Cl_2$. This strongly suggests that these complexes form covalent bonds to DNA through the platinum metal site. Comparison of the extent of retardation of DNA migration by our complexes relative to cisplatin and 1,1/t,t suggests that our systems cause a more dramatic change in DNA conformation upon binding of the metal complexes. These mixed-metal complexes form an entirely new class of metal-based systems capable of binding DNA. They are modular in design, which will allow for component modification to probe the effect of the terminal ligands (bpy), terminal metal center (Ru or Os), bridging ligand (dpb), and active Pt site on the DNA-binding characteristics of these types of systems. The presence of the remote Os or Ru metal center allows for variation of this center to provide an inorganic method to tune the reactivity at the active platinum site. More extended structures can also be developed by coupling of other components to this remote metal site. Further work aimed at elucidating the molecular basis of the interaction of these mixed-metal complexes with DNA and developing the second generation of mixed-metal complexes is currently in progress.

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