Nucleobase Oxidation of DNA by (Terpyridyl)chromium(III) Derivatives

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Keywords: Chromium / DNA cleavage / Photo cleavage / Terpyridyl ligands

The chromium(III) complexes $[Cr(ttpy)_2](ClO_4)_3$ (**1**) and $[Cr(Brphtpy)_2](ClO_4)_3$ (**2**), containing the terpyridyl derivatives ttpy and Brphtpy [ttpy = *p*-tolylterpyridine; Brphtpy = (*p*-bromophenyl)terpyridine] have been synthesized and characterized by ESI-MS, electronic spectroscopy, and cyclic voltammetry. Absorption titration and thermal denaturation studies reveal that both complexes are moderate binders of calf thymus DNA (CT DNA), while viscosity measurements show that they bind with partial intercalation. Binding of the two chromium complexes to DNA and mononucleotides dGMP, dAMP, dCMP, and dTMP decreases the emission intensity of complex **2**. However, the emission intensity of complex **1** is quenched only by DNA and the nucleotides dGMP and dAMP. Excited state potentials of both **1** and **2** have been estimated to be 1.65 and 1.85 V vs. NHE. These results demonstrate that **2** is a stronger photooxidant than **1**, and other (diimine)chromium complexes, and that it can oxidize nucleobases. The photonuclease activity of **1** and **2** has been confirmed by gel electrophoresis.

Introduction

The interaction of various metal ions with DNA has been investigated in great detail to understand the mode of binding of metal ions to DNA. Changes in ligand structure can alter the DNA binding properties of metalloporphyrins, Schiff-base−Mn^{III} complexes, phenanthroline−Cu^{II} complexes, and macrocyclic Ni^{II} complexes.[1−13] We have reported previously the influence of ligand structure on the DNA binding properties of Cr^{III} complexes.[14−18] A main offshoot of these studies has been the development of metal complexes that cleave DNA. Synthetic reagents that cleave DNA are of considerable interest as tools for molecular biologists − such cleavage has led to the development of both sequence-specific nucleases and footprinting agents. The redox properties of various transition metal complexes have been exploited for DNA cleaving. The anticancer drug bleomycin relies on a metal redox center for its DNA-cleaving reaction.[19−20] Polypyridyl complexes of oxoruthenium(IV) are efficient oxidative DNA-cleaving agents.[21] Another class of DNA-cleaving agents are those activated by light. Indeed, light-activated metal complexes have some distinct experimental advantages as they can be added to a protein−DNA complex and remain inert until photoactivation. One, therefore, has control over the timing of these reactions and hence the potential to examine protein−DNA interactions directly with some time resolution. (Polypyridyl)metal complexes of d^6 systems, such as Ru^{II}, Rh^{III}, and Co^{III}, are used extensively as photophysical probe and as photochemical agents because of their room-temperature luminescence.[22−26] Because these complexes are coordinatively saturated, there is no opportunity for reactions other than electron and energy transfer. Thus cleavage of DNA by complicated hydrogen atom or oxo transfer pathways is unlikely, and the binding modes of the complexes can be studied exclusively. Coordinatively saturated phenanthrenequinone diimine (phi) complexes of Rh^{III}, $[Rh(phi)(bpy)_2]^{3+}$, are conformationally selective photocleaving agents.[24−26] Surprisingly, despite its extensive photochemistry, Cr^{III} complexes have found little use as photoprobes for nucleic acids. Even though certain Cr^{III} complexes exhibit nuclease activity, the photochemistry of only some diimine complexes, such as $[Cr(bpy)_3]^{3+}$, $[Cr(phen)_3]^{3+}$, $[Cr(phen)_2(dppz)]^{3+}$, and $[Cr(TMP)_3]^{3+}$, has been investigated in the presence of DNA because of their strong room-temperature luminescence ($^2E_g \rightarrow {}^4A_{2g}$ phosphorescence).[27−29] We report here the synthesis and characterization of the terpyridyl−Cr^{III} complexes **1** and **2** and the photocleavage of DNA in the presence of these complexes.

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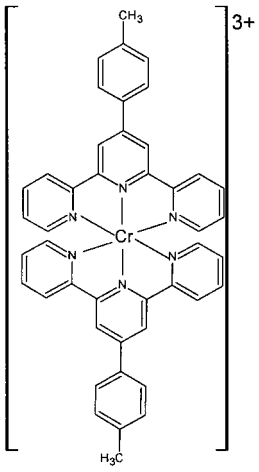
Results and Discussion

Synthesis and Characterization

The chromium(III) complexes **1** and **2** were synthesized by the reaction of $[Cr(H_2O)_6]^{2+}$ and the corresponding ligand under nitrogen followed by air oxidation. Both complexes showed satisfactory elemental analyses and IR spectra. Their authenticity was confirmed by their ESI mass spectra.

  DOI: 10.1002/ejic.200300718



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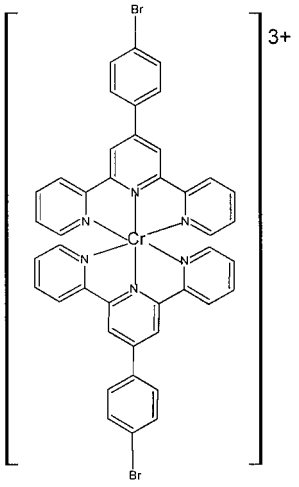

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Table 1. Ground-state absorption spectral details of aqueous solutions of $[Cr(tpy)_2]^{3+}$, $[Cr(ttpy)_2]^{3+}$, and $[Cr(Brphtpy)_2]^{3+}$

$[Cr(tpy)_2]^{3+}$ λ [nm] (log ε)	$[Cr(ttpy)_2]^{3+}$ (**1**) λ [nm] (log ε)	$[Cr(Brphtpy)_2]^{3+}$ (**2**) λ [nm] (log ε)
225 (4.79)	225 (4.38)	225 (4.95)
ca. 239 (4.64)	ca. 239 (4.27)	ca. 239 (4.89)
267 (4.49)	267 (4.13)	267 (4.77)
ca. 286 (4.32)	ca. 286 (4.24)	ca. 286 (4.86)
315 (4.04)	315 (4.01)	315 (4.71)
327 (4.12)	327 (4.05)	327 (4.77)
348 (4.22)	348 (4.09)	348 (4.84)
364 (4.23)	364 (4.12)	364 (4.82)
422 (3.31)	422 (1.3)	422 (2.2)
443 (3.35)	443 (1.12)	443 (2.1)
473 (3.15)	473 (0.87)	473 (1.96)

Like other polypyridyl and terpyridyl complexes of Cr^{III}, complexes **1** and **2** exhibit multiple absorbances between 200 and 800 nm (Table 1), as well as ligand-centered absorbances at 285 and 287 nm, respectively. The visible absorption bands can be attributed to ligand field transitions. The lowest energy ligand field transition for complex **1** appears at 576 nm and the same transition for **2** appears at 564 nm. Other ligand field transitions occur at 481 and 450 nm for complex **1** and at 472 and 442 nm for **2**. Both complexes are electrochemically active and exhibit multiple reduction processes. Their cyclic voltammograms show well-separated reduction waves at −0.19, −0.5, −0.96, −1.913, and −2.196 V (for **1**) and −0.14, −0.45, −0.94, −1.767, and −2.20 V (for **2**) (Figure 1). The first three electrochemical waves are reversible one-electron processes with ΔE_p = 58−65 mV at all scan rates (Table 2). The first two reduction potentials at −0.18 and −0.5 V (**1**) and −0.14 and −0.45 V (**2**) could be due to two reversible one-electron reduction processes arising from Cr^{III}/Cr^{II} and Cr^{II}/Cr^{I} couples, respectively. For $Cr(tpy)_2{}^{3+}$, the Cr^{III}/Cr^{II} and Cr^{II}/Cr^{I} couples are reported to be at −0.28 and −0.69 V vs. SCE, respectively.[30] The less negative reduction potential observed here for these complexes, compared with the parent terpyridyl ligand, could be due to the substituent effect on the terpyridyl ligand. In the present case, on substituting tolyl and bromophenyl group to the terpyridyl ligand, the metal-centered redox potentials shift toward more positive values than for the parent terpyridyl complex of chromium.

DNA Binding Experiments

Absorption Titration of Chromium Complex with Calf Thymus DNA

Interaction of the complexes with DNA was investigated by monitoring the absorption spectra of the complexes in the presence and absence of DNA (Figure 2). The absorption maxima of both chromium(III) complexes are shifted by 3−5 nm in the presence of DNA. A noticeable hypochromicity arises due to interaction of the two chromium complexes with DNA. The intraligand transitions show a decrease in intensity to the maximum of 22% for complex **1** and 19% for complex **2** on addition of an incremental amount of DNA. These spectroscopic changes suggest that there are interactions between the metal complexes and DNA. Such hypochromicity has been attributed to intercalative binding.[31] Notably, the hypochromicities of the peaks are lower than those of classical intercalators such as ethidium bromide and $[Ru(phen)_2(dppz)]^{2+}$ whose percentage hypochromicities are > 25%.[32] The binding constants of the complexes with DNA have been measured from absorption titration as reported earlier.[15] Intrinsic binding constants for complexes **1** and **2** with DNA calculated to be $3.1\pm0.2\times10^3$ and $1.252\pm0.2\times10^4$ M^{-1}, respectively, which are lower than the 10^6-10^7 M^{-1} of classical intercalators,[32] but similar to some of the chromium and ruthenium complexes, which prefer surface binding to DNA.[14−18] These results indicate that the preferred binding mode of complexes **1** and **2** to DNA is surface binding or electrostatic interaction.

Viscosity Measurements

A critical test for a binding model in solution in the absence of crystallographic structural data is hydrodynamic measurement, which is more sensitive to the length change of nucleic acid. Of the hydrodynamic measurements, viscosity measurements provide a tool for the study of metal−DNA interaction since optical studies provide necessary but not sufficient clues to support the binding model. Generally, classical intercalators increase the viscosity of DNA due to the accommodation of the ligand between the base pairs of the helix. In contrast, non-classical intercalators could bend or kink the DNA helix, reduce its effective length and concomitantly its viscosity. Here, the viscosity of CT DNA increased with increasing concentration of **1** and **2** (Figure 3). Such a trend is typical of intercalators. However, since complexes **1** and **2** have octahedral geometry and the ligands are not strictly coplanar one can expect only partial intercalation of these molecules between the DNA bases.

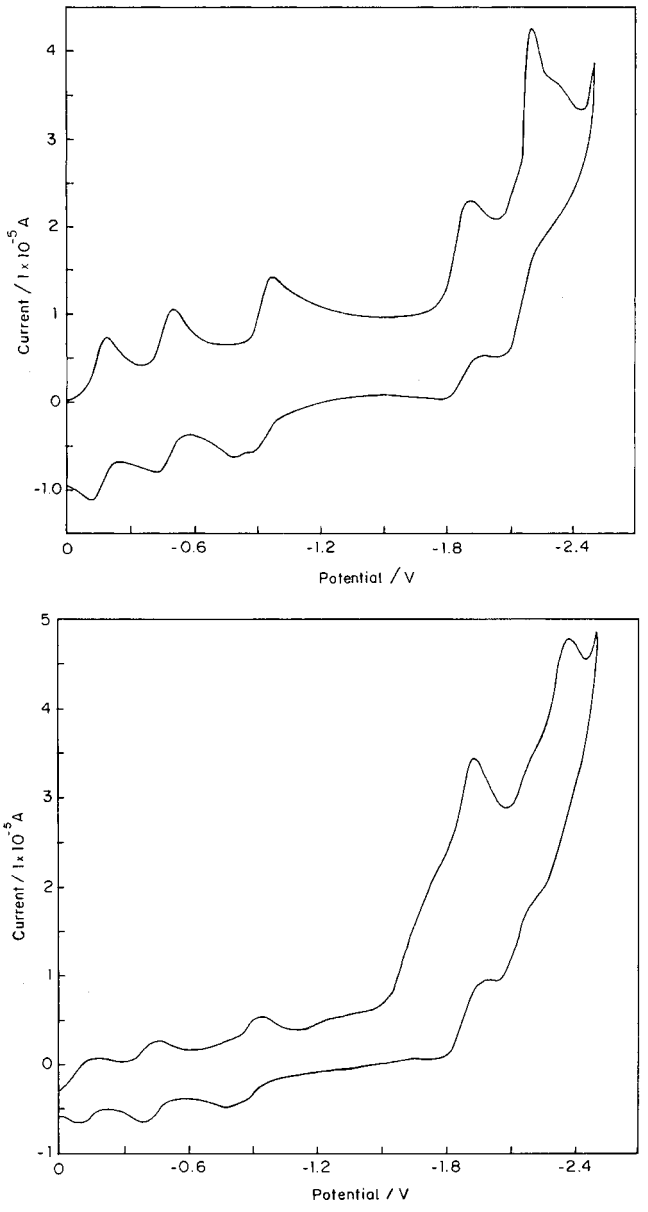

Figure 1. Cyclic voltammogram of the complexes **1** (top) and **2** (bottom) in CH_3CN (0.1 M TBAP as supporting electrolyte, scan rate = 100 mV/s)

Thermal Denaturation Study

The melting of DNA can be used to distinguish between those molecules that bind by intercalation and those that bind externally, i.e. electrostatically. Two parameters can be derived from the thermal denaturation curves of DNA, namely the melting temperature (T_m) at which 50% of the DNA has become single-stranded, and the curve width (σ_T), the temperature range within which 10−90% of the denaturation occurs.[33] DNA melting revealed ΔT_m values of calf thymus DNA in the presence of complexes **1** and **2** of 3±0.5 and 5±0.5 °C, respectively; $\Delta\sigma_T$ values for the duplex in the presence of **1** and **2** were 3±0.5 and 5±0.5 °C, respectively. Generally, one would expect a much larger increase in T_m and σ_T for classical intercalators, e.g. ethidium bromide, which shows an increase in the T_m of DNA of 14 °C and a curve width of 22 °C.[34] The magnitude of the increase in T_m and σ_T for DNA in the presence of **1** and **2** indicates that their interaction with DNA is non-intercalative binding. This is also reflected in the binding constant.

Table 2. Half-wave potentials and peak current ratio of the chromium complexes **1** and **2** compared with the parent compound $[Cr(tpy)_2]^{3+}$

Complex	E_{pc}	E_{pa}	ΔE [mV]	i_{pc}/i_{pa}
$[Cr(tpy)_2]^{3+}$ [a]	−0.177	−0.114	58	1.06
	−0.576	−0.518	58	1.02
	−1.085	−1.022	63	1.03
$[Cr(ttpy)_2]^{3+}$ (**1**)	−0.189	−0.129	62	1.12
	−0.5	−0.443	57	1.14
	−0.968	−0.908	60	1.17
	−1.913	−1.830	83	1.13
	−2.196	−2.158	38	1.99
$[Cr(Brphtpy)_2]^{3+}$ (**2**)	−0.140	−0.084	56	1.1
	−0.451	−0.394	57	1.09
	−0.943	−0.88	63	1.08
	−1.767	−1.705	62	1.59
	−2.198	−2.160	38	1.81

[a] This complex also shows two ligand-centered redox waves with $E_{1/2}$ of −1.99 and −2.37 V.

Luminescence Study

Aqueous solutions of the complexes **1** and **2** emit strongly at room temperature. In aqueous solution, complexes **1** and **2** show strong emission bands ($^2E_g \rightarrow {}^4A_{2g}$) at 773 nm and 707 nm, respectively, on excitation at 340 nm. The emission spectral bands, which represent the 0−0 transition of $^2E_g \rightarrow {}^4A_{2g}$, are only slightly sensitive to the nature of ligand substituents with diimine systems. For example, on substitution of a phenyl group on the bipyridyl ligand, there is a red-shift of 14 nm in the emission spectra of the corresponding chromium complex. $[Cr(tpy)_2]^{3+}$ shows a red-shift of 42 nm in the emission bands compared with $[Cr(bpy)_3]^{3+}$.[35] The $^2E_g \rightarrow {}^4A_{2g}$ band is seen at 728 nm for $[Cr(bpy)_3]^{3+}$ but occurs at 770 nm for $[Cr(tpy)_2]^{3+}$. Interestingly, substituting the bromophenyl group on the terpyridyl ligand results in a drastic blue-shift in the emission band of $[Cr(Brphtpy)_2]^{3+}$, by 63 nm compared with the parent (terpyridyl)chromium complex. However, a *p*-tolylterpyridyl substituent yields a red-shift of 3 nm in the emission maxima compared with the emission maxima of $[Cr(tpy)_2]^{3+}$. Generally, the binding of a luminescent compound to DNA or polynucleotides leads to an increase in the emission and excited state lifetime due to the rigidity of DNA microenvironment and protection from solvent quenching. The influence of DNA on the emission of **1** and **2** has been examined. Interestingly, the emission intensity of the complexes is quenched by the addition of incremental amounts of DNA, in contrast to $[Cr(bzimpy)_2]^+$.[36] Effects of nucleotides dGMP, dAMP, dCMP, and dTMP on the emission intensity of **1** and **2** have also been examined. The emission intensity of complex **1** at 773 nm was quenched by

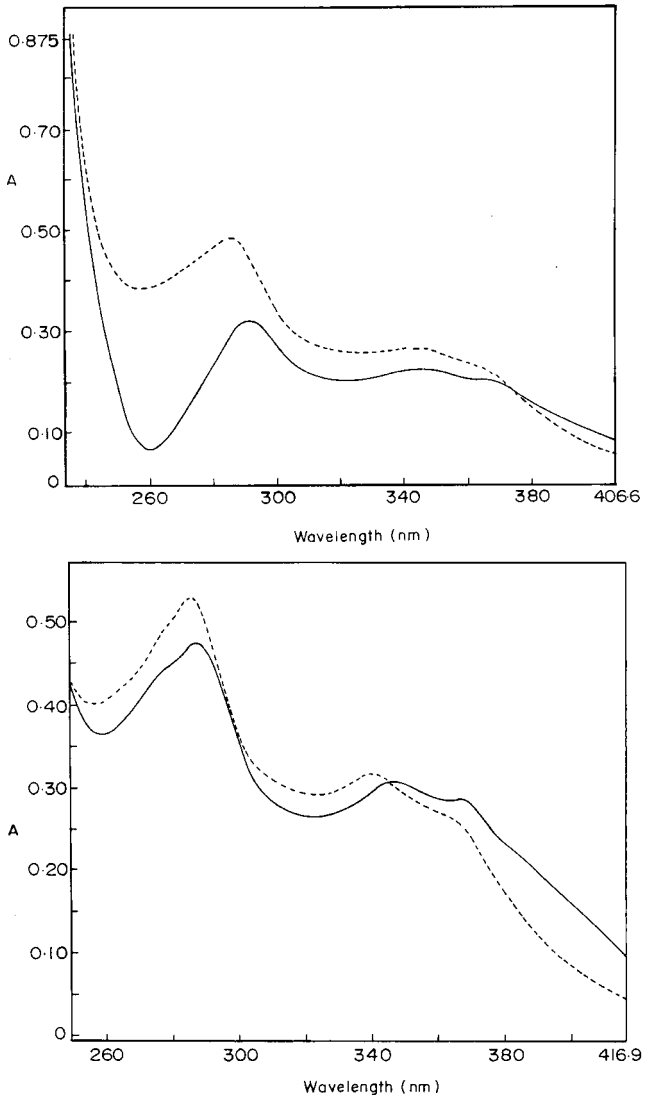

Figure 2. Absorption spectra of **1** (top) and **2** (bottom) in the absence (---) and presence (—) of increasing amounts of CT DNA; [Cr] = 10 μM; [DNA] = 0−220 μM

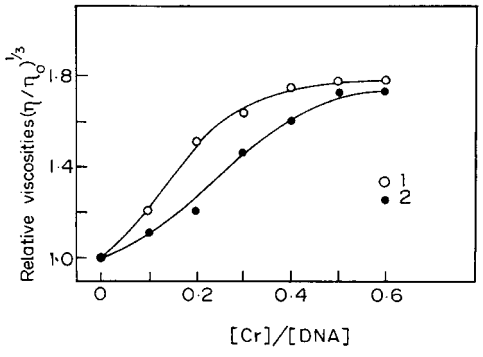

Figure 3. Effect of increasing $[Cr(ttpy)_2]^{3+}$ (**1**) and $[Cr(Brphtpy)_2]^{3+}$ (**2**) on the relative viscosity of DNA

dGMP and dAMP, but not affected by dCMP and dTMP (Figure 4, top). Conversely, the emission intensity complex **2** was quenched by all four mononucleotides (Figure 4, bottom). Stern−Volmer plots for steady-state emission intensity quenching are expected to be a straight line for dynamic quenching. Figure 4 (top and bottom), show plots with upward curvature at higher nucleotide concentrations. Such behavior indicates an additional deactivation pathway involving static quenching, attributed in this case to nucleotide-bound chromium(III) complex. To probe the mechanism for the quenching of the complexes **1** and **2** by DNA and mononucleotides, the excited state potentials were calculated using Equation (1), where $E°(Cr^{3+}/Cr^{2+})$ is the ground-state reduction potential and $E_{0-0}(Cr^{3+}/Cr^{2+})$ is the potential calculated from the emission energy. The excited state potentials for complexes **1** and **2** were calculated as +1.65 V and +1.85 V vs. NHE, respectively. The excited state potential of complex **1** is higher than that of dGMP (1.29 V) and dAMP (1.4 V) but similar to those of dCMP (1.53 V) and dTMP (1.63 V).[37] However, for complex **2**, $E°(*Cr^{3+}/Cr^{2+})$ is much higher than that of all the four nucleotides. Hence, the quenching process clearly could be due to electron transfer from a nucleobase to an excited state complex. Such luminescent quenching has been reported for various ruthenium compounds as well as chromium complexes containing diimine systems. However, in the present investigation, chromium(III) complexes having a triimine system, such as the (*p*-bromophenyl)terpyridyl derivative, exhibited a much higher excited-state oxidizing power than complex **1** and also diimine−Cr^{III} systems such as $[Cr(bpy)_3]^{3+}$, $[Cr(phen)_3]^{3+}$, and $[Cr(TMP)_3]^{3+}$. Notably, coordination of triimine ligands like ttpy and Brphtpy gives chromium complexes with a much higher excited state potential than that observed for the diimine−Cr^{III} complexes such as $[Cr(bpy)_3]^{3+}$, $[Cr(phen)_3]^{3+}$, and $[Cr(TMP)_3]^{3+}$, whose excited state potentials are in the range of 1.2−1.5 V.

$$E°(*Cr^{3+}/Cr^{2+}) = E°(Cr^{3+}/Cr^{2+}) - E_{0-0}(Cr^{3+}/Cr^{2+}) \quad (1)$$

Photonuclease Activity

One possible consequence of electron transfer from a nucleobase to a molecule in the ground state or excited state is a single strand break of DNA. This process is generally demonstrated by gel electrophoresis of plasmid DNA, because the conversion of the supercoiled, closed circular form of plasmid DNA into an open circular form, induced by a single strand nick, is easily monitored. To support the electron transfer from the nucleobase to the excited state of a chromium(III) complex, we have carried out gel electrophoresis of plasmid DNA after irradiating it in the presence of the complexes **1** and **2**. On excitation of complexes **1** and **2** at 340 nm in the presence of supercoiled plasmid DNA, a single strand break was observed (lanes 4 and 5 of Figure 5). DNA cleavage was not observed for the complex with DNA without irradiation (lanes 2 and 3). A possible role of singlet oxygen has also been ruled out in the present case because the strand break was not inhibited by the addition of a singlet oxygen scavenger such as sodium azide (lanes 6 and 7); furthermore, reactions carried out in D_2O

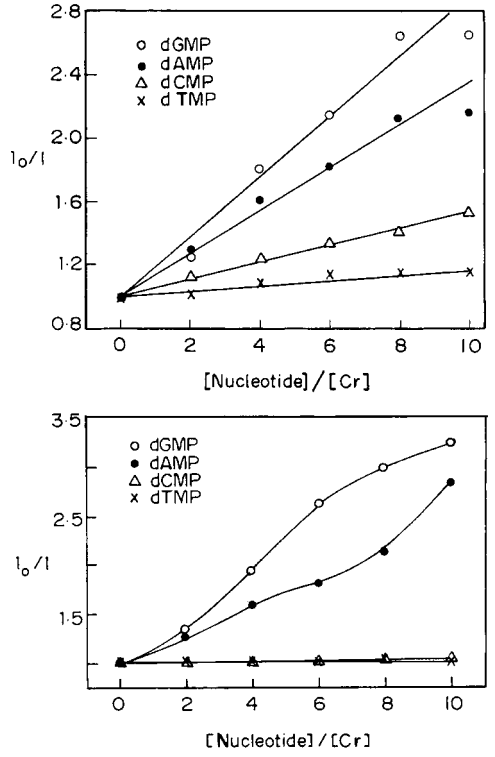

Figure 4. Relative emission intensity of the complexes **1** (bottom) and **2** (top) in the presence of mononucleotides; [Cr]= 10 μM; [nucleotides] = 20−100 μM

showed no enhancement in cleavage reaction (not shown). From the above observations, it is assumed that formation of a radical cation of guanine by electron transfer to the excited state of the complex plays a key role in this damage event since the guanine base is the most oxidizable base of all the four bases. Similar photonuclease activity has also been reported for photooxidizing agents such as $[Ru(TAP)_3]^{2+}$, $[Ru(bzimpy)_2]^{2+}$, $[Ru(bpz)_3]^{2+}$, and $[Ru(bpz)_2(DPPZ)]^{2+}$.[38−41]

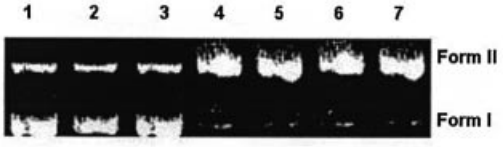

Figure 5. Photoinduced cleavage of pBR 322 DNA by complex **1** and **2** (20 μM) following 340 nm photolysis for 20 min at 25 °C (0.8% agarose gel); form I: supercoiled plasmid; form II: nicked plasmid; lane 1: control DNA; lane 2: DNA + **1**; lane 3: DNA + **2**; lane 4: DNA + **1** + *hv*; lane 5: DNA + **2** + *hv*; lane 6: DNA + **1** + NaN_3 (2 mM) + *hv*; lane 7: DNA + **2** + NaN_3 (2 mM) + *hv*

Conclusions

The cations $[Cr(ttpy)_2]^{3+}$ and $[Cr(Brphtpy)_2]^{3+}$ exhibit very high excited-state oxidizing power and bring about electron transfer from DNA bases to the excited state of the complex. These complexes also bring about cleavage of plasmid DNA upon photoirradiation. Such photonuclease activity by metal complexes can be exploited to develop footprinting agents in molecular biology.

Experimental Section

Chemicals: 2-Acetylpyridine and *p*-tolualdehyde were obtained from Sigma Aldrich chemicals and used as received. *p*-Bromobenzaldehyde was purchased from Lancaster chemicals, and used as received. Calf thymus DNA, Hepes, Tris, and mononucleotides were obtained from SRL chemicals, Mumbai. The stock solution of DNA was prepared by stirring the sample, dissolving in Hepes buffer (10 mM, pH = 7.0), at 4 °C. The solution was then dialyzed exhaustively against buffer for 48 h and filtered using a membrane filter obtained from Sartorius (0.45 μm). Filtered DNA solution in the buffer gave a UV absorbance ratio A_{260}/A_{280} of about 1.9, indicating that the DNA was sufficiently free from protein.[42] The concentration of DNA was determined by using the molar absorption coefficient of 6600 $M^{-1}\cdot cm^{-1}$ at 260 nm.[43] The stock solution of the Cr^{III} complex was prepared in water and its concentration estimated according to a reported procedure.[44] All solvents used were of analytical grade, received from Ranbaxy, Mumbai, and were used without further purification. All experiments were carried out in Hepes buffer at pH = 7.0 in Milli Q water.

Methods: UV/Vis spectra of the chromium(III) complexes and DNA binding studies were recorded with a Perkin−Elmer Lambda 35 double beam spectrophotometer at 25 °C. Elemental analyses were performed using a Heraeus-CHN-Rapid Analyzer at RSIC, IIT, Madras. Emission spectra were recorded with a Hitachi 650-40 spectrofluorimeter. Electrospray ionisation (ESI) mass spectra of the complexes were recorded with a Hewlett Packard 1100 mass spectrometer equipped with an electron spray source. Infrared spectra of the complex were recorded with a Perkin−Elmer FT-IR spectrometer. Cyclic voltammetry experiments were performed with an EG and G PAR 173 potentiostat/Galvanostat analyzer. Tetrabutylammonium perchlorate (TBAP) was used as supporting electrolyte. A standard three-electrode system was used, consisting of a glassy carbon as working electrode, platinum electrode as auxiliary electrode and a saturated calomel solution as reference electrode (SCE).

Synthesis of $[Cr(ttpy)_2](ClO_4)_3$ (1): The ligand ttpy was prepared according to a literature procedure.[45] Subsequently, ttpy (0.646 g, 2 mmol), dissolved in methanol (10 mL), was taken and purged with nitrogen for 20 min. To this a deaerated solution of the ligand, a $[Cr(H_2O)_6]^{2+}$ solution (1 mmol), was added under nitrogen. The resulting brown solution was then bubbled with air for 3 h. The resultant orange-yellow compound was filtered off and recrystallized from a mixture of acetone/water (2:1). The recrystallized product was then filtered off, washed with diethyl ether, and dried under vacuum. ESI-MS: m/z = 696 $[M - 2H^+ - 3ClO_4]^+$. $C_{44}H_{34}Cl_3CrN_6O_{12}$ (997.12): calcd. C 53.00, H 3.44, N 8.43, Cr 5.21; found C 52.85, H 3.32, N 8.38, Cr 5.11.

Synthesis of $[Cr(Brphtpy)_2](ClO_4)_3$ (2): The ligand Brphtpy was prepared according to a literature procedure.[46] The chromium(III) complex was synthesized according to the above procedure, using Brphtpy in place of ttpy. ESI-MS: m/z = 826 $[M - 2H^+ - 3ClO_4]^+$. $C_{42}H_{28}Br_2Cl_3CrN_6O_{12}$ (1126.86): calcd. C 44.70, H 2.50, N 7.46; Cr, 4.61; found C 44.75, H 2.41, N 7.38; Cr, 4.43.

Caution! Although we had no difficulty during working, perchlorate salts are potentially explosive! Utmost care should be exercised in handling perchlorate salts.

DNA Binding Studies: Electronic spectra of Cr^{III} complexes were monitored in the presence and absence of DNA. The binding constants for the interaction of Cr^{III} complexes with DNA were obtained from absorption titration data. A fixed amount of the complex (10 μM) was titrated with increasing amounts of DNA, over the range 20−220 μM. The binding constant was then determined using Equation (2), where ε_A, ε_F, and ε_B correspond to A_{obsd}/[Cr], the molar absorption coefficient for the free chromium complex and the molar absorption coefficient for the chromium complex in the fully bound form, respectively. A plot of [DNA]/($\varepsilon_A - \varepsilon_F$) vs. [DNA], gives K_b as the ratio of the slope to intercept.

$$[DNA]/(\varepsilon_A - \varepsilon_F) = [DNA]/(\varepsilon_B - \varepsilon_F) + 1/K_b(\varepsilon_B - \varepsilon_F) \quad (2)$$

Thermal denaturation studies were conducted in a Perkin−Elmer Lambda 35 spectrophotometer equipped with thermostatted cell holder. The temperature was controlled by a Peltier system (±0.1 °C). The absorbance at 260 nm was monitored for DNA in the absence and the presence of the complexes. Viscometric experiments were carried out using an Ostwald-type viscometer (2 mL capacity), thermostated in a water bath maintained at 25±1 °C. Flow rates of buffer (10 mM), DNA (150 μM), and DNA in the presence of Cr^{III} complex at various concentrations (15−90 μM) were measured with a manually operated timer at least three times to agree within 1 s. The viscosity was calculated according to $\eta = (t - t_0)/t_0$, where t_0 is the flow time for the buffer and t is the observed flow time for DNA in the presence and absence of the complex. Plots of $(\eta/\eta_0)^{1/3}$ vs. R (R = [complex]/[DNA]) were constructed from the viscosity measurements.[47] Steady-state emission spectra of complexes **1** and **2** in the presence of DNA were recorded. The concentration of complexes was fixed (10 μM) while that of DNA was varied from 20 to 100 μM. The Cr^{III} complexes were excited at 340 nm and the emission intensity of the complexes **1** and **2** monitored at 773 and 707 nm, respectively. Time-resolved fluorescence for the complexes was determined using a picosecond-laser-excited TCSPC spectrometer. The excitation source was a tunable Ti-Sapphire laser (Tsunami, Spectrophysics, USA) with a pulse width of < 2 ps and repetition rate of 82 MHz. Samples were excited at 340 nm and the emission monitored using an MCP-PMT (Hamamatsu-C 4878) detector. The decay traces were deconvoluted using a non-linear least-squares analysis with IBH software.

Gel electrophoresis of plasmid DNA (pBR322 DNA) was carried out with a reaction mixture (25 μL) containing plasmid DNA (4 μL of 200 μg·mL^{-1}), a solution of complex **1** and **2** (5 μL of 0.1 mM) and remaining buffer. NaN_3 solution (5 μL) was then added to one of the samples. Metal/DNA solutions were preincubated for 1 h in the dark and the samples were kept in a spectrofluorimeter sample chamber. The sample was then irradiated at 340±5 nm for 20 min and subsequently analyzed by 0.8% agarose gel electrophoresis (Tris/boric acid/EDTA buffer, pH = 8.0) at 50 V for 4 h. The gel was stained with 0.5 μg·mL^{-1} ethidium bromide and then illuminated under a UV lamp and photographed with Polaroid film using a Kodak DC-40 digital camera.

Acknowledgments

V. G. V. thanks CSIR for a research fellowship. The authors thank the National Centre for Ultrafast Processes for providing the facilities to carry out time-resolved fluorescence experiments.

[1] D. J. Gravert, J. H. Griffin, *Inorg. Chem.* **1996**, *35*, 4837−4847.
[2] C. J. Burrows, J. G. Muller, G. T. Poulter, S. E. Rokita, *Acta Chem. Scand.* **1996**, *50*, 337−344.
[3] R. F. Pasternack, P. Garrity, B. Ehrlich, C. B. Davis, E. J. Gibbs, G. Orloff, A. Giartosio, C. Turano, *Nucleic Acids Res.* **1986**, *14*, 5919−5931.
[4] R. F. Pasternack, E. J. Gibbs, J. J. Villafranca, *Biochemistry* **1983**, *22*, 2406−2414.
[5] C. J. Burrows and S. E. Rokita, in: *Metal Ions in Biological Systems* (Eds.: A. Sigel and H. Sigel), Marcel Dekker, New York, **1996**, vol. 33, p. 537−560.
[6] J. G. Muller, R. P. Hickerson, R. J. Perez, C. J. Burrows, *J. Am. Chem. Soc.* **1997**, *119*, 1501−1506.
[7] J. G. Muller, P. Zheng, S. E. Rokita, C. J. Burrows, *J. Am. Chem. Soc.* **1996**, *118*, 2320−2325.
[8] C. J. Burrows, S. E. Rokita, *Acc. Chem. Res.* **1994**, *27*, 295−301.
[9] X. Chen, C. J. Burrows, S. E. Rokita, *J. Am. Chem. Soc.* **1992**, *114*, 322−325.
[10] J. G. Muller, X. Chen, A. C. Dadiz, S. E. Rokita, C. J. Burrows, *J. Am. Chem. Soc.* **1992**, *114*, 6407−6411.
[11] J. G. Muller, X. Chen, A. C. Dadiz, S. E. Rokita, C. J. Burrows, *Pure Appl. Chem.* **1993**, *65*, 545−550.
[12] L. E. Marshall, D. R. Graham, K. A. Reich, D. S. Sigman, *Biochemistry* **1981**, *20*, 244−250.
[13] J. M. Veal, K. Merchant, R. L. Rill, *Biochemistry* **1991**, *30*, 1132−1140.
[14] R. Vijayalakshmi, M. Kanthimathi, V. Subramanian, B. U. Nair, *Biochem. Biophys. Res. Commun.* **2000**, *271*, 731−734.
[15] R. Vijayalakshmi, M. Kanthimathi, V. Subramanian, B. U. Nair, *Biochim. Biophys. Acta* **2000**, *1475*, 157−162.
[16] R. Vijayalakshmi, A. Dhathathreyan, V. Subramanian, B. U. Nair, T. Ramasami, *Langmuir* **1999**, *15*, 2898−2900.
[17] R. Vijayalakshmi, V. Subramanian, B. U. Nair, *J. Biomol. Struct. Dyn.* **2002**, *19*, 1063−1069.
[18] V. G. Vaidyanathan, R. Vijayalakshmi, V. Subramanian, B. U. Nair, *Bull. Chem. Soc. Jpn.* **2002**, *75*, 1143−1149.
[19] R. M. Burger, J. Peisach, S. B. Horwitz, *J. Biol. Chem.* **1981**, *256*, 11636−11644.
[20] H. Kuromochi, K. Takahashi, T. Takita, H. Umezawa, *J. Antibiot. (Tokyo)* **1981**, *34*, 576−582.
[21] N. Grover, H. H. Thorp, *J. Am. Chem. Soc.* **1991**, *113*, 7030−7031.
[22] J. M. Kelly, M. J. Murphy, D. J. McConnell, C. OhUigin, *Nucleic Acids Res.* **1985**, *13*, 167−184.
[23] L. A. Lipscomb, F. X. Zhou, S. R. Presnell, R. J. Woo, M. E. Peek, R. R. Plaskon, L. D. Williams, *Biochemistry* **1996**, *35*, 2818−2823.
[24] D. B. Hall, R. E. Holmin, J. K. Barton, *Nature* **1996**, *382*, 73−75.
[25] J. K. Barton, A. T. Danishefsky, J. M. Goldberg, *J. Am. Chem. Soc.* **1984**, *106*, 2172−2176.
[26] J. K. Barton, A. L. Raphel, *J. Am. Chem. Soc.* **1984**, *106*, 2466−2468.
[27] R. T. Watson, N. Desai, J. Wildsmith, J. F. Wheeler, N. A. P. Kane-Maguire, *Inorg. Chem.* **1999**, *38*, 2683−2687.
[28] K. D. Barker, B. R. Benoit, J. A. Bordelon, R. J. Davis, A. S. Delmas, O. V. Mytykh, J. T. Petty, J. F. Wheeler, N. A. P. Kane-Maguire, *Inorg. Chem. Acta* **2001**, *322*, 74−78.
[29] N. A. P. Kane-Maguire, J. F. Wheeler, *Coord. Chem. Rev.* **2001**, *211*, 145−162.
[30] R. P. Bonomo, S. Musumeci, E. Rizzarelli, S. Samartano, *Gazz. Chim. Ital.* **1974**, *104*, 1067−1074.
[31] X.-H. Zou, B.-H. Ye, H. Li, Q.-L. Zhang, H. Chao, J.-G. Liu, L.-N. Ji, X.-Y. Li, *J. Biol. Inorg. Chem.* **2001**, *6*, 143−150.
[32] A. E. Friedmann, J. C. Chambron, J. P. Sauvage, N. J. Turro, J. K. Barton, *J. Am. Chem. Soc.* **1990**, *112*, 4960−4962.

[33] J. M. Kelly, A. B. Tossi, D. J. McConnell, C. OhUigin, *Nucleic Acids Res.* **1985**, *13*, 6017−6034.
[34] G. A. Neyhart, N. Grover, S. R. Smith, W. A. Kalsbeck, T. A. Fairley, M. Cory, H. H. Thorp, *J. Am. Chem. Soc.* **1993**, *115*, 4423−4428.
[35] N. Serpone, M. A. Jamieson, M. S. Henry, M. Z. Hoffman, F. Bolletta, M. Maestri, *J. Am. Chem. Soc.* **1979**, *101*, 2907−2916.
[36] V. G. Vaidyanathan, B. U. Nair, *Eur. J. Inorg. Chem.* **2003**, 3633−3638.
[37] C. J. Burrows, J. G. Muller, *Chem. Rev.* **1998**, *98*, 1109−1152.
[38] J. M. Kelly, D. J. McConnell, C. OhUigin, A. B. Tossi, A. Kirsch-De Mesmaeker, A. Masschelein, J. Nasielski, *J. Chem. Soc., Chem. Commun.* **1987**, *24*, 1821−1823.
[39] J. P. Lecomte, A. Kirsch-De Mesmaeker, J. M. Kelly, A. B. Tossi, H. Gorner, *Photochem. Photobiol.* **1992**, *55*, 681−689.
[40] A. Kirsch-De Mesmaeker, G. Orellama, J. K. Barton, N. J. Turro, *Photochem. Photobiol.* **1990**, *52*, 461−472.
[41] V. G. Vaidyanathan, B. U. Nair, *J. Inorg. Biochem.* **2002**, *91*, 405−412.
[42] J. Marmur, *J. Mol. Biol.* **1961**, *3*, 208−218.
[43] R. Reichmann, S. A. Rice, C. A. Thomas, P. Doty, *J. Am. Chem. Soc.* **1954**, *76*, 3047−3053.
[44] K. Lingane, R. Peesok, *Anal. Chem.* **1948**, *20*, 425−428.
[45] J. P. Collin, S. Guillerez, J. P. Sauvage, F. Barigelletti, L. D. Cola, L. Flamigni, V. Balzani, *Inorg. Chem.* **1991**, *30*, 4230−4238.
[46] W. Spahni, G. Calzaferri, *Helv. Chim. Acta* **1984**, *67*, 450−454.
[47] G. Cohen, H. Eisenberg, *Biopolymers* **1969**, *8*, 45−55.

Received October 10, 2003
Early View Article
Published Online March 23, 2004

