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DNA-Binding and Physical Studies of Pt(4′-NR_2-trpy)CN^+ Systems (trpy = 2,2′:6′,2″-Terpyridine)

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This paper focuses on DNA-binding interactions exhibited by Pt(dma-T)CN^+, where dma-T denotes 4′-dimethylamino-2,2′:6′,2″-terpyridine, and includes complementary studies of the corresponding pyrr-T complex, where pyrr-T denotes 4′-(*N*-pyrrolidinyl)-2,2′:6′,2″-terpyridine. The chromophores are useful for understanding the interesting and rather intricate DNA-binding interactions exhibited by these and related systems. One reason is that the terpyridine ligands employed provide intense visible absorption and enhanced photoluminescence signals. Incorporating cyanide as a coligand further aids analysis by suppressing covalent binding. Physical methods utilized include X-ray crystallography for structures of the individual inorganic complexes. Viscometry as well as spectral studies of the absorbance, emission, and circular dichroism (CD) yield information about interactions with a variety of DNA hosts. Although there is no sign of covalent binding under the conditions used, most hosts exhibit two phases of uptake. Under conditions of high loading (low base-pair-to-platinum ratios), the dma-T complex preferentially binds externally and aggregates on the surface of the host, except for the comparatively rigid host $[poly(dG\text{-}dC)]_2$. Characteristic signs of the aggregated form include a bisignate CD signal in the charge-transfer region of the spectrum and strongly bathochromically shifted emission. When excess DNA is present, however, the complex shifts to intercalative binding, preferentially next to G≡C base pairs if available. Once the complex internalizes into DNA it becomes virtually immune to quenching by O_2 or solvent, and the emission lifetime extends to 11 μs when $[poly(dI\text{-}dC)]_2$ is the host. On the other hand, the host itself becomes a potent quenching agent when G≡C base pairs are present because of the reducing strength of guanine residues.

Introduction

A rich chemistry involving platinum and DNA is emerging because cisplatin has proved to be such a therapeutically useful anticancer drug.[1,2] Lippard and co-workers established a different kind of binding agent when they demonstrated that platinum complexes of the 2,2′:6′,2″-terpyridine (trpy) ligand are capable of intercalating into B-form DNA.[3,4] Subsequently a great deal of work involving platinum(II) terpyridine complexes has appeared including studies dealing with DNA-binding interactions and cytotoxicity.[3−10] Luminescence studies can be informative as shown by investigations of the DNA binding of the Pt(trpy)$(OH)^+$ complex.[6] Most likely because of solvent-induced exciplex quenching, the free hydroxide complex is nonemissive in aqueous solution, but emission appears when the complex intercalates amidst adenine-thymine base pairs. Aside from being base

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dependent, the emission signal is time dependent as well and slowly vanishes as the complex shifts to the thermodynamically preferred, covalent-binding motif.[6]

Further luminescence studies are of interest because of the promise platinum(II) complexes have for sensing applications.[11−14] The Pt(trpy)CN$^+$ system would be intriguing in that regard because the presence of a poor leaving group like cyanide might disfavor covalent binding. Unfortunately, the complex is an intrinsically weak emitter.[15] However, the Pt(dma-T)CN$^+$ complex, where dma-T denotes 4′-dimethylamino-2,2′:6′,2″-terpyridine, offers promise as it is capable of exhibiting relatively long-lived emission, a relatively high luminescence quantum yield, and comparatively strong absorbance in the visible region.[15] The amine substituent is responsible for the enhanced spectral properties and provides for an emissive triplet excited-state with a heavy admixture of intraligand (IL) as well as metal-to-ligand (ML) charge-transfer, that is, ^{3}ILCT/^{3}MLCT character.[15] Results obtained in the present study include crystal structures containing the dma-T complex as well as the Pt(pyrr-T)CN$^+$ analogue, where pyrr-T denotes 4′-(*N*-pyrrolidinyl)-2,2′:6′,2″-terpyridine. Viscometry as well as absorbance, emission, and circular dichroic (CD) spectral data provide insight into DNA-binding interactions that occur with a number of different hosts. In the presence of excess DNA, the hydrophobic platinum complexes bind as monomers, but stoichiometric amounts of DNA promote aggregation, depending on the rigidity of the host.

Experimental Section

Materials. Sigma-Aldrich Chemical was the source for silver cyanide, K_2PtCl_4, 1,5-cyclooctadiene (COD), sodium trifluoromethanesulfonate (Na[OTf]), tetrabutylammonium chloride [TBA]Cl, 5′-adenosine monophosphate (AMP), Trizma HCl, pyrrolidine, 4′-Cl-2,2′:6′,2″-terpyridine and Trizma base. The salmon testis (ST) DNA, [poly(dI-dC)]$_2$, [poly(dG-dC)]$_2$, and [poly(dA-dT)]$_2$ came from GE Healthcare. Exciton Inc. supplied the laser dye. The solvents methanol, ethanol, dichloromethane (DCM), and acetonitrile were products from Mallinckrodt.

Methods. An eight-hour reflux of 4′-Cl-2,2′:6′,2″-terpyridine in pyrrolidine resulted in the formation of 4′-(*N*-pyrrolidinyl)-2,2′:6′,2″-terpyridine (hereafter pyrr-T). After cooling and pouring the mixture onto ice, the solid product separated. Following filtration and a water wash, the crude pyrr-T ligand was pure enough for synthesis of the desired platinum complex. ^{1}H NMR in $CDCl_3$: 8.68 ppm (d, 2H), 8.66 ppm (d, 2H), 7.83 ppm (t, 2H), 7.61 ppm (s, 2H), 7.30 ppm (m, 2H), 3.5 ppm (t, 4H), 2.1 ppm (t, 4H). Literature procedures sufficed for the synthesis of 4′-dimethylamino-2,2′:6′,2″-terpyridine (hereafter dma-T),[16] Pt(COD)Cl$_2$[17] as well as the precursor materials [Pt(dma-T)Cl]OTf and [Pt(pyrr-T)Cl]OTf.[18] A published double-exchange method permitted replacement of chloride by cyanide as coligand.[15] The chloride salt, which deposited upon addition of tetrabutylammonium chloride to a solution of the triflate salt in acetone, was the more useful form for studies carried out in aqueous solution. H. Daniel Lee of the Purdue University Microanalysis Laboratory performed all elemental analyses.

[Pt(dma-T)CN]OTf·H$_2$O. Analysis: Calcd for $C_{19}H_{18}F_3N_5O_4PtS$: C, 34.34; H, 2.73; N, 10.54. Found: C, 34.53; H, 2.44; N, 10.38%.

[Pt(pyrr-T)CN]OTf·2H$_2$O. Analysis: Calcd for $C_{21}H_{22}F_3N_5O_5PtS$: C, 35.60; H, 3.13; N, 9.88. Found: C, 35.33; H, 2.84; N, 9.66%.

The platinum concentration was typically 13.4 μM for absorption, luminescence, and CD measurements in methanol or 0.05 M pH 7.8 tris buffer. In serial spectrophotometric titrations, the concentration of platinum remained constant, and the order of reagent addition was platinum stock solution (in methanol), followed by DNA stock solution, and then buffer. The sample history is potentially important as indicated by an attempt to create a solution of [poly(dA-dT)]$_2$ and Pt(dma-T)CN$^+$ at a base-pair-to-platinum ratio of $q = 2$ in two different ways. The experiment involved interrupting a standard titration at a ratio of $q = 4$ and back-titrating to $q = 2$ by the addition of a large aliquot of platinum stock solution. The final solution had half as large a DNA concentration as the original $q = 2$ solution, but both solutions gave the same absorbance-corrected emission signal. However, the apparent molar absorptivity of the complex was about 30% smaller in the final solution, perhaps because microparticle formation reduced the effective platinum concentration. For luminescence measurements, the slit settings were 5 nm, and an appropriate long pass filter was in place to preclude stray excitation light from striking the detector. The excitation wavelength for lifetime measurements was 420 nm. A set of 525 and 555 nm long pass filters allowed isolation of the emission signal. The method used to extract lifetimes has been described.[19]

Beer's law plots yielded molar absorptivities for the two platinum complexes in methanol: 10,500 M^{-1} cm^{-1} for Pt(dma-T)CN$^+$ and 10,200 M^{-1} cm^{-1} for Pt(pyrr-T)CN$^+$, both at 402 nm. Beer's law also provided information about DNA concentrations in solution. The following is a list of the molar absorptivities used for DNA determinations (all in units of base pairs): 13,200 M^{-1} cm^{-1} for ST,[20] 13,600 M^{-1} cm^{-1} for [poly(dA-dT)]$_2$,[21] 16,800 M^{-1} cm^{-1} for [poly(dG-dC)]$_2$,[22] and 13,800 M^{-1} cm^{-1} for [poly(dI-dC)]$_2$.[23] On a nucleotide basis, the literature value for 5′-AMP is 15,400 M^{-1} cm^{-1}.[24] The standard reduced viscosity, defined in eq 1, revealed the effect ligand binding had on the flow properties of DNA in solution.

$$\eta/\eta_0 = (t - t_0)/(t' - t_0) \quad (1)$$

In eq 1, t is the flow time with platinum complex and DNA present, t_0 is the flow time of buffer alone, and t' is the flow time of the DNA control solution in the viscometer.[25] For the viscosity

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Table 1. Crystal Data and Structure Refinement

[Pt(pyrr-T)CN]OTf	[Pt(dma-T)CN]OTf
$C_{21}H_{18}F_3N_5O_3PtS$	$C_{19}H_{16}F_3N_5O_3PtS$
$a = 7.3286(9)$ Å	$a = 18.6654(14)$ Å
$b = 16.2604(19)$ Å	$b = 14.3779(11)$ Å
$c = 19.315(2)$ Å	$c = 15.5628(6)$ Å
$\alpha = 83.37(5)°$	$\beta = 102.95(4)°$
$\beta = 88.92(5)°$	
$\gamma = 85.21(8)°$	
$V = 2278.2(5)$ Å^3	$V = 4070.3(5)$ Å^3
formula weight = 672.56	formula weight = 646.52
space group $P\bar{1}$ (No. 2)	space group $C2/c$ (No. 15)
$T = 150.$ K	$T = 150.$ K
$\lambda = 0.71073$ Å	$\lambda = 0.71073$ Å
$\rho_{calc} = 1.961$ g cm^{-3}	$\rho_{calc} = 2.110$ g cm^{-3}
$\mu = 6.367$ mm^{-1}	$\mu = 7.123$ mm^{-1}
transmission coeff = 0.823−0.853	transmission coeff = 0.465−0.491
$R(F_o)^a = 0.099$	$R(F_o)^a = 0.044$
$R_w(F_o^2)^b = 0.248$	$R_w(F_o^2)^b = 0.099$

^{a} $R = \sum||F_o| - |F_c||/\sum|F_o|$ for $F_o^2 > 2\sigma(F_o^2)$. ^{b} $R_w = [\sum w(|F_o^2| - |F_c^2|)^2/\sum w|F_o^2|^2]^{1/2}$.

measurements the temperature was 28 °C, and the DNA base pair concentration was 700 μM. See the literature for the protocol.[26]

Crystal Structures. A Nonius KappaCCD area-detector diffractometer collected scattering data secured with graphite-monochromatized Mo Kα radiation. After initial integration and data scaling by the DENZO and SCALEPACK packages,[27] the SHELXS-97 program yielded structure solutions via direct methods.[28] Subsequent Fourier-difference map analyses yielded the positions of the non-hydrogen atoms. The SHELXL-97 package also performed refinements via full-matrix least-squares analyses of F^2 with anisotropic displacement parameters for all non-hydrogen atoms. The refinement included hydrogen atoms, but in calculated positions. Table 1 summarizes the crystal data and provides more details about data collection and refinement. In the case of the Pt(dma-T)CN$^+$ structure, adjustment of the residual electron density by means of the SQUEEZE option[29] in PLATON was necessary because of the disorder of the triflate counterion. The geometrical agreement between the platinum complexes in the two structures, vide infra, confirms the validity of the approach.

Instrumentation. The absorption spectrophotometer was a Varian Cary 100 Bio instrument, while the spectrofluorimeter was a Varian Cary Eclipse and the circular dichroism spectropolarimeter was a Jasco J-815. Mass spectra were obtained on a Waters Micromass Z_Q mass spectrometer. A description of the nitrogen-pumped dye laser and associated equipment used for lifetime determinations appears in the literature.[30] The viscometer was a Cannon-Fenske model 25.

Results

Crystal Structure of [Pt(dma-T)CN]OTf. Figure 1 provides a thermal-ellipsoid view of the complex complete with the atom-numbering scheme and a list of important bond angles and bond distances. The distances and angles are similar to those of other platinum terpyridine complexes.[31−35]

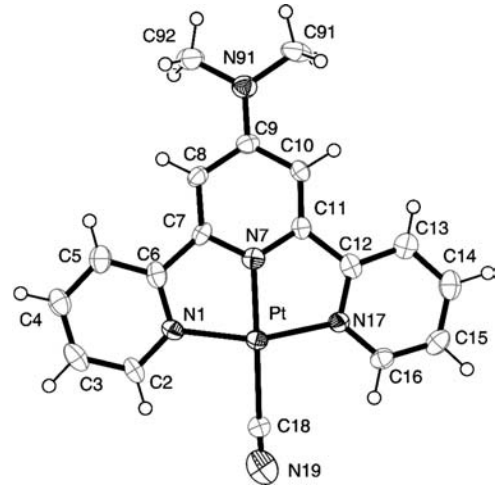

Figure 1. Molecular structure of Pt(dma-T)CN$^+$ with thermal ellipsoids set at 50%. Selected bond lengths (Å) and angles (deg) of the planar complex: N1−Pt 2.024(6), N7−Pt 1.947(6), N17−Pt 2.009(6), C18−Pt 2.086(8), C18−N19 0.978(10); N1−Pt−N7 80.7(3), N7−Pt−N17 80.2(3), N17−Pt−C18 100.7(3), C18−Pt−N1 98.4(3).

As expected, the Pt−C≡N angle of 176.3(9)° is nearly linear in accordance with the value of 176.1(8)° previously observed within the related Pt{4′-phenyl-(2,2′:6′,2″-terpyridine)}CN$^+$ complex.[36] The C91−N91−C92 plane of the dimethylamino group makes a dihedral angle of 5.9° with the best plane through the carbon and nitrogen atoms of the trpy ligand.

That compares with a corresponding dihedral angle of 3.7° in a manganese complex of the dma-T ligand[37] and a related torsion angle of 3.0° in a structure reported for the free ligand.[38] In the dma-T structure reported herein, the platinum complexes stack along the *c* direction as dimers; see Figure 2 for a view down the *b* axis. The Pt···Pt distance of 3.48 Å within each pair is indicative of at best a weak metal−metal interaction because the maximum separation compatible with appreciable d(z^2)-d(z^2) overlap is reportedly 3.5 Å.[39] The Pt(dma-T)CN$^+$ structure is unusual in that the C18−Pt−Pt−C18 torsion angle is 79.9°. Ordinarily, platinum terpyridines pack in a head-to-tail fashion, in which case the C18−Pt−Pt−C18 torsion angle would be 180°, to avoid unnecessary electrostatic repulsions.[40] The two faces of the dimer are essentially

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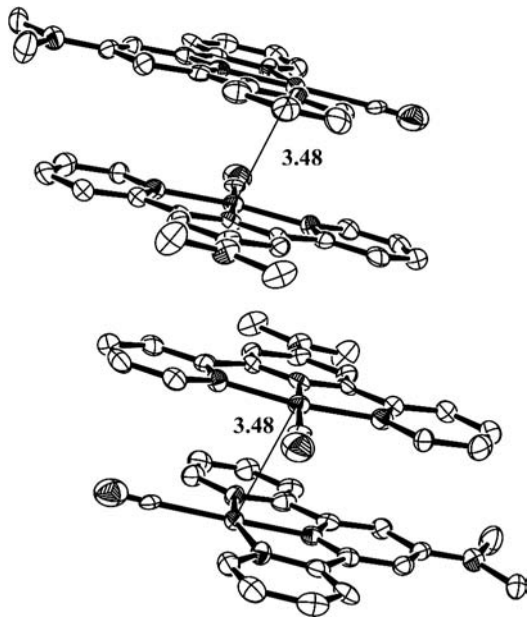

Figure 2. View of Pt(dma-T)CN$^+$ stacking along the *c* axis viewed down the *b* axis of the adopted unit cell.

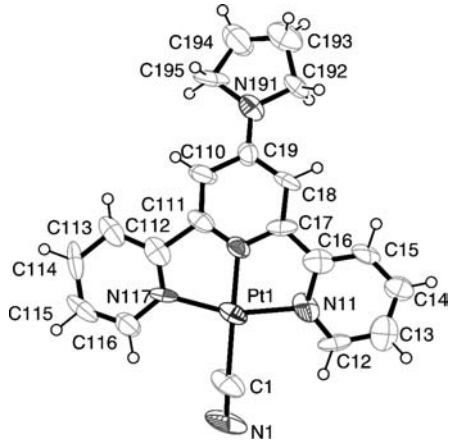

Figure 3. Molecular structure of Pt(pyrr-T)CN$^+$ with thermal ellipsoids set at 50%. Selected bond lengths (Å) and angles (deg): N117−Pt 2.01(3), N111−Pt 1.94(2), N11−Pt 2.05(2), C1−Pt 1.97(4), C1−N1 1.11(4); N117−Pt−N111 80.0(9), N111−Pt−N11 80.3(10), N11−Pt−C1 99.6(12), C1−Pt−N117 100.1(12). The atom numbers pertain to one complex of the asymmetric unit, but the quoted geometric values are averages over both molecules.

parallel because the dihedral angle between the mean planes through the non-hydrogen atoms of the two trpy groups is only 1.3°.

Crystal Structure of [Pt(pyrr-T)CN]OTf. The crystal structure of the pyrr-T complex has two molecules in the asymmetric unit, but the bond distances and angles are the same within accepted confidence limits. To be specific, the thermal-ellipsoid view in Figure 3 depicts the Pt1 complex along with the atom-numbering scheme. However, the reported bond angles and bond distances are the averages over the two structures and are very similar to those found for the dma-T complex. Relative to the best plane through

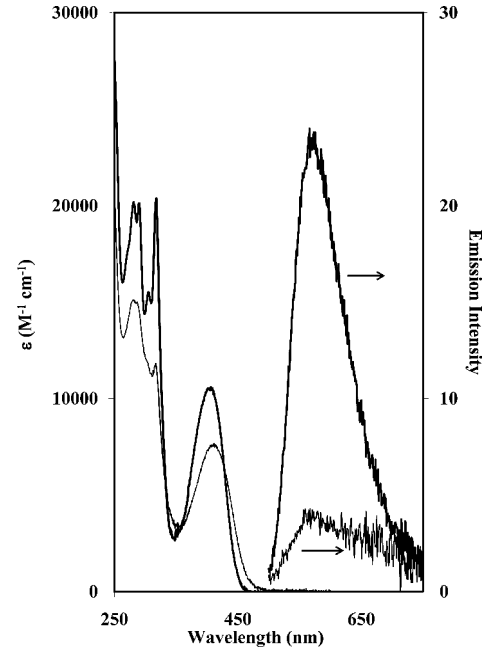

Figure 4. Room-temperature absorption (left) and emission spectra (right) of Pt(dma-T)CN$^+$ in MeOH (thick trace) and 0.05 M pH 7.8 tris buffer (thin trace). For the spectra used to estimate apparent ε values, the input platinum concentration was 11.8 μM as determined from known dilutions of a stock solution.

the trpy moiety attached to Pt1, the plane through the C192−N191−C195 atoms of the pyrrolyl group defines a dihedral angle of 7.01°. The corresponding angle is 7.93° in the structure containing Pt2. The average Pt−C≡N angle is 175(2)°. The platinum complexes stack along the *a* axis of the crystal lattice with the Pt1 and Pt2 systems residing in alternating columns. Within each column, the complexes segregate into offset, head-to-tail dimers, but there are no significant platinum−platinum interactions. The closest Pt1···Pt1 distance is 4.162 Å, and the closest Pt2···Pt2 distance is 4.235 Å. If one draws a plane through the non-hydrogen atoms of the trpy neighbors, the plane-to-plane distances are 3.393 Å (Pt1 dimer) and 3.432 Å (Pt2 dimer).

Solution Spectra. In methanol both platinum complexes exhibit a CT absorption maximum at 402 nm, as well as a series of structured, intraligand absorptions toward shorter wavelengths (Figure 4). Data in Figure 4 also reveal that the apparent molar absorptivity values are much smaller in aqueous solution. The suppression of absorbance is a consequence of aggregation, a well documented effect for platinum terpyridines in aqueous media.[8,31,34]

In MeOH the emission spectrum of Pt(dma-T)CN$^+$ is broad like the CT absorption band (Figure 4). The emission maximum falls at 565 nm, and the lifetime of the emission is 450 ns in aerated methanol. Under similar conditions the emission from the pyrr-T complex has a maximum at 560 nm and a lifetime of 440 ns. In aerated dichloromethane the excited-state lifetimes are longer, 2.6 and 1.6 μs for Pt(dma-T)CN$^+$ and Pt(pyrr-T)CN$^+$, respectively.

Influence of DNA on the Absorbance and Emission. When the dma-T complex interacts with double-stranded DNA in solution, two concentration-dependent phases of binding are evident in the absorbance data. Figure 5 contains

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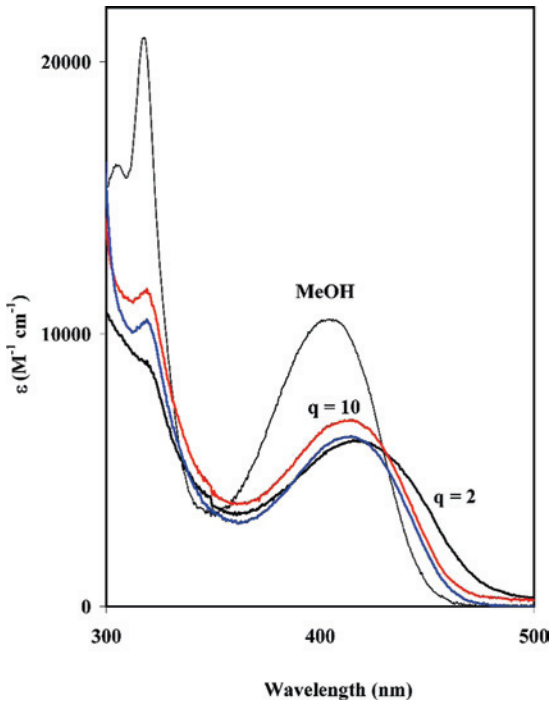

Figure 5. Absorbance spectra for a titration of Pt(dma-T)CN^+ with $[poly(dA\text{-}dT)]_2$. The DNA base-pair-to-platinum ratios are 30:1 (blue), 10:1 (red), 2:1 (thick black), and 0:1 (thin black). The solvent is MeOH for the 0:1 solution (no DNA present).

Table 2. Absorbance Data from Limiting Spectra ($q \geq 20$)

complex	host	$\Delta\lambda$, nm[a]	%H[b]
Pt(dma-T)CN^+	ST DNA	10	34
	$[poly(dG\text{-}dC)]_2$	8	33
	$[poly(dI\text{-}dC)]_2$	10	29
	$[poly(dA\text{-}dT)]_2$	11	41
Pt(pyrr-T)CN^+	ST DNA	11	33

[a] Bathochromic shift induced by interaction with DNA. Relative to MeOH control. [b] Hypochromism: the percent drop in absorbance induced at the band maximum.

illustrative results obtained with the $[poly(dA\text{-}dT)]_2$ host. At high loading, when the DNA base-pair-to-platinum ratio is low, for example, $q = 2$, the CT absorption band exhibits a strong hypochromic response and a strong bathochromic shift (Figure 5). Above 500 nm, a small wavelength-independent absorption also becomes apparent, presumably because of microparticulate formation. Consistent with that interpretation, the light-scattering effect disappears when excess DNA is present ($q \geq 30$), whereupon the CT absorption shifts to slightly shorter wavelengths as a limiting spectrum develops. Similar changes also occur in the region of the intraligand absorption. Analogous absorption changes occur in titrations involving other DNA hosts; see Table 2 for data obtained in the presence of excess DNA (at high q values). The results with ST DNA were so similar for the two complexes that the focus narrowed to the dma-T complex. All spectra obtained for the pyrr-T complex appear in the Supporting Information (Figures S1−S3).

Emission data reveal similar effects. Figure 6 shows results obtained in a titration of Pt(dma-T)CN^+ with $[poly(dI\text{-}dC)]_2$. In the absence of $[poly(dI\text{-}dC)]_2$ the emission signal is relatively weak. At $q = 2$ an intense, red-shifted emission grows in with a maximum of approximately 670 nm, but

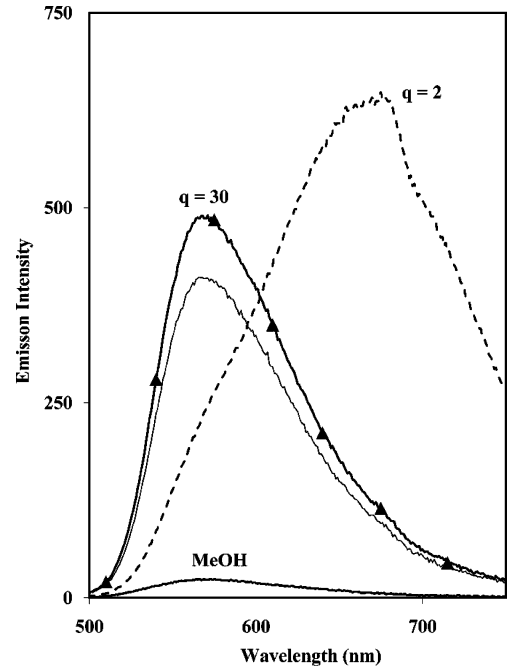

Figure 6. Room-temperature emission spectra from a titration of Pt(dma-T)CN^+ with $[poly(dI\text{-}dC)]_2$ at $q = 2$ (dashed line), 10 (thin line), and 30 (▲). The solvent is MeOH for the $q = 0$ spectrum (thick line).

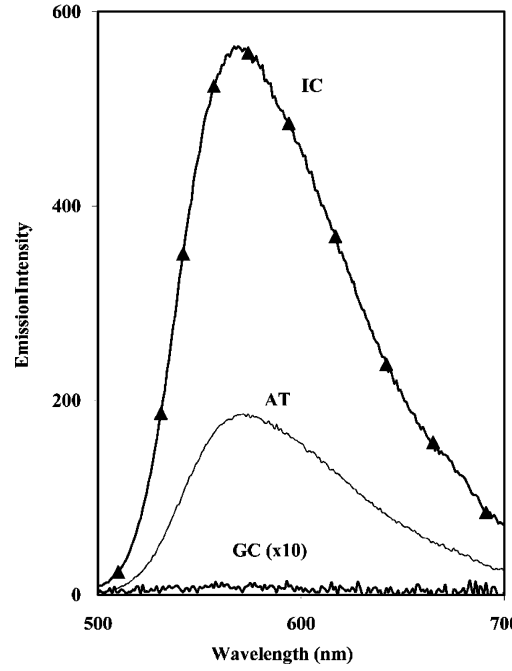

Figure 7. Emission spectra of Pt(dma-T)CN^+ bound to excess $[poly(dI\text{-}dC)]_2$ (▲), $[poly(dA\text{-}dT)]_2$ (thin line), and $[poly(dG\text{-}dC)]_2$ (thick line, ×10).

when excess DNA is in solution, the emission weakens and the maximum shifts back to 570 nm.

The limiting emission intensity (obtained at high q values) varies considerably with the nature of the DNA host. When excess ST DNA is present, for example, the emission from the bound form of Pt(dma-T)CN^+ is only about half as intense as the control signal obtained in MeOH. See Figure 7 for a comparison of limiting emission spectra. In aerated buffer solution the emission from the dma-T complex exhibits a lifetime of 11 ± 0.5 μs, when excess [poly(dI-

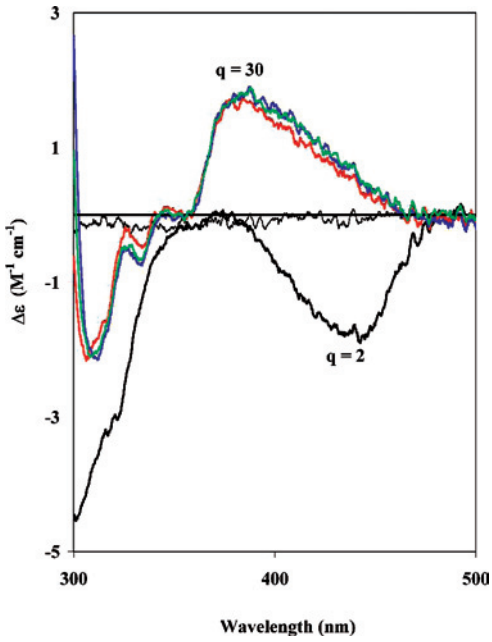

Figure 8. Induced CD spectra of Pt(dma-T)CN$^+$ obtained in a titration with ST DNA at $q = 30$ (blue), 20 (green), 10 (red), and 2 (thick black). The $q = 0$ spectrum (thin black) is for buffer alone.

dC)]$_2$ is present. When [poly(dA-dT)]$_2$ is the host, the lifetime is 9 ± 1 μs, although the residuals suggest that the decay may not be strictly single exponential.

Induced CD Spectra. The two phases of binding are also evident in the induced CD spectral data; see Figure 8 for a titration of Pt(dma-T)CN$^+$ with ST DNA. The complex itself is intrinsically achiral and does not exhibit a CD signal in the absence of host; however, at high loading of Pt(dma-T)CN$^+$ on ST DNA, a negative induced CD signal occurs in the CT region of the spectrum with the extremum at about 440 nm. In contrast, when excess ST DNA is present, the induced CD signal is positive in the same wavelength domain and the maximum shifts to about 385 nm. Figure 9 contains an overlay of induced CD spectra obtained from Pt(dma-T)CN$^+$ in the presence of different DNA hosts under conditions of high and low loading.

Aging Effects. One aging study simply involved monitoring a sample containing ST DNA and Pt(dma-T)CN$^+$ at a DNA-base-pair-to platinum ratio of 14:1 over a four-day period. There was no significant change in the absorbance, emission, or induced CD spectrum. These results contrast sharply with those reported earlier for Pt(trpy)OH$^+$, where major spectral changes were apparent after overnight incubation because of a shift from intercalative to covalent binding.[6] A second experiment involved incubating different platinum complexes with 25 equiv of 5′-AMP in methanol. In the mass spectrum with the Pt(dma-T)Cl$^+$ complex, the signal for the complex with 5′-AMP as the coligand was three times as intense as that of the chloride precursor after 24 h. In contrast, experiments with Pt(dma-T)CN$^+$ revealed no sign of the displacement of cyanide over the same time interval. The mass spectrum did show loss of the parent ion, perhaps because of precipitate formation, but there was no sign of the formation of a complex with 5′-AMP as the coligand.

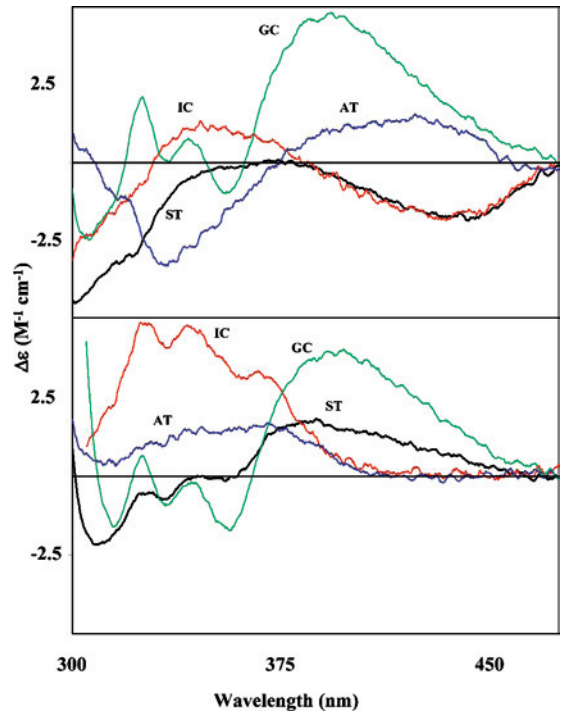

Figure 9. Induced CD spectra of Pt(dma-T)CN$^+$. Top: At high loading ($q = 2$) with poly(dG-dC)]$_2$ (green); [poly(dI-dC)]$_2$ (red); [poly(dA-dT)]$_2$ (blue); and ST DNA (black). Bottom: At low loading ($q = 20$) with poly(dG-dC)]$_2$ (green); [poly(dI-dC)]$_2$ (red); [poly(dA-dT)]$_2$ (blue); and ST DNA (black).

Viscometry. The two phases of binding of Pt(dma-T)CN$^+$ to ST DNA were equally apparent from viscometry measurements. During this series of experiments, the concentration of DNA remained essentially fixed, and the concentration of platinum increased. Figure 10 reveals that the specific viscosity increased, up until a platinum-to-base-pair ratio of R ≈ 0.25, consistent with intercalation of the ligand into the DNA macromolecule.[25,26] At higher loading of Pt(dma-T)CN$^+$, another type of binding took over, and the specific viscosity actually began to decrease.

Discussion

Pt(dma-T)CN$^+$ and Pt(pyrr-T)CN$^+$. The Pt(pyrr-T)CN$^+$ complex is similar to Pt(dma-T)CN$^+$ which has been studied previously in organic media.[15] The first crystal structures of either ion appear herein and are worth noting because of the paucity of structures available for platinum(II) terpyridine complexes with a cyanide coligand.[36] The platinum complexes segregate into dimers in both cases, but the Pt(dma-T)CN$^+$ dimer is unusual because the Pt−C≡N axes are practically orthogonal to each other. Head-to-tail packing is the norm in the solid state.[34−36] Absorbance data indicate that the complexes also tend to dimerize or form higher aggregates in aqueous solution; however, the complexes conform to Beer's law in methanol where they apparently exist as monomers. The dma-T and pyrr-T complexes are attractive because of the intense CT absorption. Critical to the absorption is the excellent conjugation between the electron-donating substituent and the terpyridine ligand; indeed, the C−N−C plane of the substituent twists only a few degrees away from the best plane through the C and N

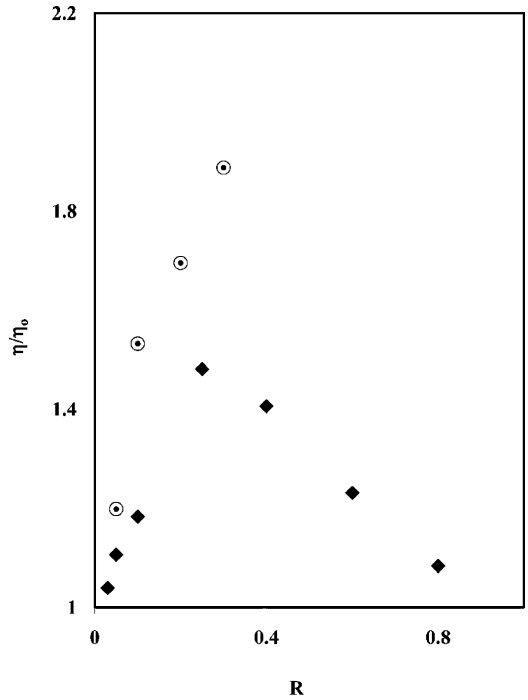

Figure 10. Standard reduced viscosity ratios as a function of the platinum-to-DNA base pair ratio R for Pt(dma-T)CN^+ interacting with ST DNA in 0.05 M pH 7.8 tris buffer (◆, this work). Other data from the literature pertain to 5,15-di(*N*-methylpyridinium-4-yl)porphyrin (⊙, ref 41).

atoms of the terpyridine core. Viewed as an oscillator, each complex has a relatively long dipole length because of the coupling between intraligand charge-transfer (ILCT) and metal-to-ligand charge-transfer (MLCT) absorptions.[15,42] The complexes also exhibit intense, long-lived emission in solution, though the emission is solvent dependent. Thus, the emitting state of Pt(dma-T)CN^+ has a shorter lifetime in methanol (0.45 μs) than in dichloromethane (2.6 μs), where both measurements pertain to aerated solutions. The lifetime is shorter in methanol because of solvent-induced exciplex quenching induced by the donor solvent.[12,43] The more ^{3}ILCT character in the excited state, the less effective is the exciplex quenching by Lewis bases.[43] The photophysical properties of the pyrr-T and dma-T complexes proved to be remarkably similar. The expectation was that the reactive excited-state of Pt(pyrr-T)CN^+ might exhibit enhanced ILCT character because the pyrrolyl group is supposed to be a better π donor.[44]

Incorporating cyanide as the coligand also markedly affects the reactivity. Ordinarily, substitution is kinetically facile in platinum terpyridine complexes,[45,46] as witnessed by the fact that 5′-AMP readily displaces the chloride ligand of Pt(dma-T)Cl^+. There is no evidence the corresponding replacement occurs with Pt(dma-T)CN^+, presumably because cyanide is the thermodynamically preferred ligand. Accordingly, covalent binding is not likely to be a factor in DNA-binding studies.

Low Loading on DNA. By analogy with Pt(trpy)HET^{+}[47] and Pt(trpy)OH^+,[6] Pt(dma-T)CN^+ and Pt(pyrr-T)CN^+ should be capable of intercalating into B-form DNA. In line with this expectation, introducing an excess of DNA induces bathochromic shifts in the absorption maxima as well as hypochromic responses in the CT and IL absorption intensities of both complexes. Another indication of intercalative binding[3,6,48] is the increase in the specific viscosity of sonicated ST DNA that attends the uptake of the dma-T complex (Figure 10). An increase in viscosity is diagnostic of intercalative binding when DNA undergoes rod-like diffusion because incorporaton of the ligand enhances the length and rigidity of the host.[49] Often, the specific viscosity increases by as much as 100%, as shown for 5,15-di-(*N*-methylpyridinium-4-yl)porphyrin in Figure 10.[41] Interestingly, the specific viscosity increase is only about 50% with the binding of Pt(dma-T)CN^+ at $R \approx 0.25$, or a ratio of four base pairs per platinum (Figure 10). The fall off in the higher loading regime occurs because of a shift in binding motif, vide infra.

Photoluminescence results also provide convincing evidence for intercalation of the dma-T complex. Intercalation into DNA affects the emission by blocking access to the platinum center and preventing solvent-induced exciplex quenching,[6,12] analogous to results obtained with copper-containing cationic porphyrins.[26,50] By virtue of almost completely enshrouding the platinum complex, intercalative binding can also inhibit quenching by molecular oxygen in solution. Not withstanding which type of quencher inhibition is more important, there is a dramatic increase in luminescence intensity from Pt(dma-T)CN^+ when it binds to $[poly(dI\text{-}dC)]_2$, and the lifetime of the emission extends to 11 μs. In order of increasing quantum yield, Figure 7 reveals the trend is $[poly(dI\text{-}dC)]_2 > [poly(dA\text{-}dT)]_2 \gg [poly(dG\text{-}dC)]_2$. As in the case of the previously studied system Pt(trpy)OH^+,[6] the results correlate with the reduction potential of the purine base. The potentials (in V vs SCE) are 0.85−0.9,[51,52] 1.05,[51] and 1.16[53] for guanine, adenine, and inosine, respectively. The values are approximate because they relate to the isolated bases; nevertheless, they clearly suggest that electron-transfer quenching by guanine should be most efficient. The important parameter is the reduction potential of the excited state. On the assumption that the zero-zero transition occurs on the high energy edge of the emission spectrum where the intensity is 10% of the maximum,[54] the photoexcited state of the bound form of Pt(dma-T)CN^+ stores about 2.38 V of energy. The excited-state reduction potential

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is therefore around 1.08 V versus SCE because the dma-T complex has a ground-state reduction potential of −1.30 V versus SCE.[15] Accordingly, electron-transfer quenching should be approximately thermoneutral for adenine and uphill for inosine. The results with the ST DNA host are intriguing because the emission intensity is about the same as with [poly(dG-dC)]$_2$, despite the fact that ST DNA only contains 41% G≡C base pairs.[55] Long range electron-transfer quenching by guanine could account for the weakness of the signal. Alternatively, the complex may preferentially intercalate next to G≡C base pairs, as reported for Pt(trpy)HET$^+$.[47]

Induced CD results are consistent with the latter interpretation. A key observation is that the sign of the induced CD signal varies with the host in the region of the intraligand absorptions (300−350 nm). More specifically, the net signal is positive when Pt(dma-T)CN$^+$ binds to [poly(dA-dT)]$_2$ or [poly(dI-dC)]$_2$, but negative when [poly(dG-dC)]$_2$ acts as the host (Figure 9). The response is even more specific in the CT absorption region, where interaction with excess [poly(dA-dT)]$_2$ or [poly(dI-dC)]$_2$ induces little or no CD signal but interaction with [poly(dG-dC)]$_2$ induces a relatively strong positive band that maximizes at 395 nm. Coupling between appropriate transition dipole moments is normally responsible for an induced CD signal;[56,57] hence, there must be unusually strong coupling among one or more excited states of guanine and the CT state of the complex. The results obtained with ST DNA are interesting because they may reveal whether the binding of Pt(dma-T)CN$^+$ is base dependent. In fact, the induced CD signal is distinctly positive at about 400 nm when Pt(dma-T)CN$^+$ interacts with ST DNA; however, the apparent $\Delta\varepsilon$ value is only about half as large as that observed with [poly(dG-dC)]$_2$. The intensity is difficult to interpret because there is no easy way of predicting how the number of nearest neighbor guanines affects intensity. Because ST DNA contains 41% G≡C base pairs, there will be a G≡C base pair on one or the other side of the chromophore about 65% of the time, even if the dma-T complex intercalates randomly among base pairs. Sandwiching between two G≡C base pairs is less probable, with statistical odds of about 6:1 against. The evidence for preferential binding next to G≡C base pairs is actually clearer in the region of the intraligand transitions. Indeed, at the shorter wavelengths the induced CD signal nearly tracks that induced by binding to [poly(dG-dC)]$_2$, down to exhibiting the same type of well resolved vibronic structure. In view of that agreement there can be little doubt but that Pt(dma-T)CN$^+$ preferentially intercalates in sites that contains at least one, if not two G≡C base pairs.

High Loading Regime. The story is completely different in the high loading ($q \approx 2$) regime where every host, but [poly(dG-dC)]$_2$, supports a completely different type of binding. As already noted, a shift in binding motif is clearly evident from the drop in standard reduced viscosity that occurs when the platinum concentration is as high as one for every four base pairs of ST DNA in solution. The emission results extend to other hosts and are even more telling. Figure 6 reveals how the emission intensifies and shifts to longer wavelength with [poly(dI-dC)]$_2$ at a base-pair-to-platinum ratio of $q = 2$. Two lines of evidence suggest that aggregation of the complex explains both effects. One is that analogous long-wavelength emission routinely shows up in spectra of frozen solutions containing platinum(II) terpyridines, except at very low platinum concentrations. A large body of evidence indicates that stacking effects and/or platinum−platinum interactions are responsible for the red-shifted emission.[12,34,40] In addition, DNA has already been shown to be an effective template for the aggregation of monopositive[8,58] and dipositive[59,60] platinum(II) polypyridine complexes in aqueous solution. Charge considerations are important, but on balance, they are favorable for the aggregation of the dma-T complex. Of course, Coulombic repulsions work to disperse the complexes in solution because of the cationic nature of the platinum complex itself. The countervailing factor is that the electrostatic field of the DNA macromolecule naturally attracts cations and promotes aggregation of the platinum complexes. In fact, the unit charge of Pt(dma-T)CN$^+$ is probably ideal for aggregation because work with porphyrins reveals that the lower the charge, the more likely it is for a hydrophobic cation to aggregate on the surface of DNA.[61]

The induced CD spectra are comparatively rich at a $q = 2$ ratio and bring more insight to bear on the binding motifs. Figure 9 shows that induced CD spectra obtained at $q = 2$ are quite distinct from those obtained at $q = 20$ with every host except [poly(dG-dC)]$_2$. For any other host at a DNA-base-pair-to-platinum ratio of $q = 2$, the induced CD signal crosses the baseline a little below 390 nm, right about where a CD maximum appears when Pt(dma-T)CN$^+$ binds to [poly(dC-dG)]$_2$. The appearance of a crossover point in the CD spectrum is a classical indication of aggregation because closely spaced, chirally related chromophores typically undergo exciton coupling and exhibit a relatively intense, bisignate signal where the isolated system would have a simple extremum.[26,62] The distinctive property of the [poly(dG-dC)]$_2$ host is its relatively high melting temperature. It therefore seems plausible to infer that aggregated forms are more likely to deposit on relatively plastic hosts.

Conclusions

In summary, results from a number of physical methods provide detailed insight into the DNA-binding interactions of Pt(dma-T)CN$^+$ as a function of the base-pair-to-platinum ratio. Luminescence and CD measurements, in particular, supply some of the most useful information. Marked varia-

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tions in the charge-transfer region of the induced CD spectrum occur when there is a change in the binding motif. So long as DNA is in excess, the dma-T complex binds strictly as an intercalator, regardless of the base content. However, the complex preferentially intercalates next to G≡C base pairs. The intercalated form of the photoexcited complex is quite resistant to quenching by solvent and/or oxygen, and the emission lifetime extends to 11 μs when [poly(dI-dC)]$_2$ is the host. But the host itself is a potent quenching agent if G≡C base pairs are present because of the reducing strength of guanine residues. With flexible hosts like [poly(dA-dT)]$_2$, a second mode of binding comes into play at high drug loading, for example, at a DNA base-pair-to-platinum ratio of 2:1. Under such conditions, the complex accumulates on the surface of the DNA host and apparently stacks upon itself, much like it does in the crystal lattice. Covalent bonding is normally an important binding motif for platinum(II) terpyridines interacting with DNA,[3,6] but there is no sign of this effect with Pt(dma-T)CN$^+$. The platinum−carbon bond is probably too strong for cyanide to act as a good leaving group.

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Supporting Information Available: Complete X-ray crystallographic files in CIF format for [Pt(dma-T)CN]OTf and Pt(pyrr-T)CN]OTf. Absorbance, emission, and induced CD spectra of the Pt(pyrr-T)CN$^+$ complex in titrations with ST DNA. This material is available free of charge via the Internet at http://pubs.acs.org.

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