DNA-Binding Studies of a Bifunctional Platinum Complex That Is a Luminescent Intercalator

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We have found that the mixed-ligand complex $Pt(trpy)OH^+$, where trpy denotes 2,2′:6′,2″-terpyridine, binds to DNA by competitive covalent and intercalative binding modes. Intercalation predominates in the kinetically controlled phase of the reaction, but after extended incubation the complex shifts essentially quantitatively to a covalently bound form. The intercalated form of the complex can exhibit dramatically enhanced photoluminescence except when the complex binds near a guanine residue which quenches the emission by means of an electron-transfer process. We have characterized the binding interactions by a variety of physical methods including absorbance, circular dichroic, and emission spectroscopies as well as viscometry. In addition, we have used DNA from salmon testis, *Micrococcus lysodeikticus*, and *Clostridium perfringens* as well as the synthetic forms poly(dA-dT)•poly(dA-dT), poly(dG-dC)•poly(dG-dC), and poly(dI-dC)•poly(dI-dC). For comparison, we have also carried out a number of parallel investigations with a well-characterized analogue, $Pt(trpy)HET^+$, where HET denotes 2-hydroxyethanethiol. The results are significant in several different respects. In the first place, this work establishes that platinum(II) terpyridines can be useful reporter probes because the photophysical properties vary dramatically with the local microenvironment. The $Pt(trpy)OH^+$ system is also interesting in that it is a good example of a bifunctional binding agent because the heteroaromatic ligand encourages intercalation while the hydroxide ligand acts as a good leaving group and ensures the possibility of covalent adduct formation. Finally, the results demonstrate how important the interplay between kinetics and thermodynamics can be during the evolution of the interaction of this type of reagent with DNA.

Introduction

Our group has been interested in the photochemistry and photophysics of four-coordinate metal complexes including copper(I) phenanthrolines,[1−3] copper(II) porphyrins,[4,5] and platinum(II) terpyridines.[6] Largely because of the availability of open coordination sites, the photophysics of these systems tend to be very sensitive to the local environment. For this reason, they are useful probes of host−guest phenomena, e.g., DNA-binding interactions.

Finding new ways to probe such systems is important because there are a variety of interactions possible between a small molecule and double helical DNA.[7−12] The simplest is capture by the ionic atmosphere of the macromolecule whereupon each system retains its normal solvation shell. More intimate binding occurs when the molecule approaches within van der Waals contact and docks in a groove of the DNA. Hydrophobic and/or hydrogen-bonding interactions are often important with this mode of binding as are steric and geometric factors. Thus, a crescent shape is a nice match for the contour of the DNA host. Covalent binding occurs when the drug bonds directly to one (or more) atoms, usually located on the surface of the DNA molecule. Perhaps the greatest degree of internalization occurs with intercalators. A classical intercalator contains a system of fused aromatic rings capable of entering the stack of base pairs at the core of ordinary B-form DNA.

We have used a number of physical methods including emission spectroscopy to study the binding of various copper(I) phenanthrolines and a copper(II) porphyrin with DNA.[4,13−17] Because of the importance of platinum(II) compounds as chemotherapeutic agents,[12,18,19] we have extended this work to a platinum(II) system $Pt(trpy)OH^+$, where trpy denotes 2,2′:6′,2″-terpyridine. Medicinally useful platinum-containing drugs such as cisplatin typically bind covalently to DNA.[20,21] However, due to the presence of the heteroaromatic ligand, platinum-

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(II) terpyridines are capable of intercalating into DNA.[22-24] Although Pt(trpy)OH^+ normally does not exhibit photoluminescence in aqueous media, the results can be quite different when DNA is present. In particular, the intercalated form of the complex is emissive when the nearby base pairs are adenines and thymines; guanine quenches the emission. However, even when we see emission, the intensity is strongly time dependent because the intercalated form of the complex slowly converts to a covalently bound form. The complex is bifunctional in the sense that the trpy ligand encourages the intercalative mode of binding whereas the hydroxide ligand is a good leaving group and ensures that covalent binding is also possible. The results are significant because they illustrate how sensitive the emission spectrum is to the mode of DNA binding and because they illustrate the profound influence reaction kinetics can have in shaping the course of DNA-binding.

Experimental Section

Materials. Aldrich Chemical Co. supplied 2,2′:6′,2″-terpyridine (trpy), $NaBF_4$, and [Pt(trpy)Cl]Cl·$2H_2O$. Sigma Chemical Co. supplied DNA from salmon testis, *Micrococcus lysodeikticus*, and *Clostridium perfringens*, abbreviated below as ST, ML, and CP DNA, respectively, as well as the synthetic forms poly(dA-dT)·poly(dA-dT), poly(dG-dC)·poly(dG-dC), and poly(dI-dC)·poly(dI-dC), abbreviated in the following as AT, GC, and IC DNA, respectively. The buffer material *N*-(2-hydroxyethyl)piperazine-*N*′-(3-propanesulfonic acid), or EPPS for short, and the ligand 2-hydroxyethanethiol, or HET, as well as the ribonuclease A and the 5′-IMP all came from Sigma, too. Pharmacia supplied the Sepharose CM CL-6B cation-exchange resin.

Methods. The procedure of Howe-Grant and Lippard[25] yielded [Pt(trpy)HET]BF_4, and the method of Aldridge et al.[6] gave the corresponding hydroxide complex [Pt(trpy)OH]BF_4. Treatment of the isolated solid with a minimum quantity of acetonitrile removed an absorbing impurity. We purified and sonicated the natural DNA (random-sequence DNA) as before,[4] but we used synthetic DNA as received. The absorbance in the UV in conjunction with published extinction coefficients[4] provided a value for the DNA-P, i.e., the DNA–phosphate, concentration. In the case of IC DNA the value of the absorptivity was $\epsilon_{251} = 6900\ M^{-1}\ cm^{-1}$.[26] We adopted eq 1 as a

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

measure of the effect the Pt(trpy)OH^+ complex had on the specific viscosity (η) of ST DNA in solution, where t was the flow time with the platinum complex and the DNA in solution, t' was the flow time of the control solution without the complex, and t_0 was the flow time of buffer alone. For these measurements, we used freshly mixed solutions with a DNA-P concentration of 500 μM and operated at 25 °C. A previously reported procedure for the analysis of luminescence decay curves yielded emission lifetimes.[6] In the ion-exchange chromatography studies, we loaded a DNA solution on a CM CL-6B column, preequilibrated with 0.05 M pH 9.0 EPPS, and eluted with the same buffer. For most of the spectroscopic studies the platinum concentration was around 15 μM. The reported luminescence spectra are difference spectra with a solution of DNA in buffer as the reference.

Instrumentation. The UV–visible data came from a Perkin-Elmer Lambda 4C spectrophotometer, while the CD spectra came from a JASCO J-600 spectropolarimeter. An SLM/Aminco SPF-500C spectrofluorometer yielded the luminescence data. The sonifier was a Vibracell VC50 from Sonics and Materials, and the viscometer was a Cannon-Manning Semi-Micro 25 E16 unit from Cannon Instruments Co. An earlier report described the apparatus used for measuring emission lifetimes.[6] The pH meter was a Radiometer Model PHM 64.

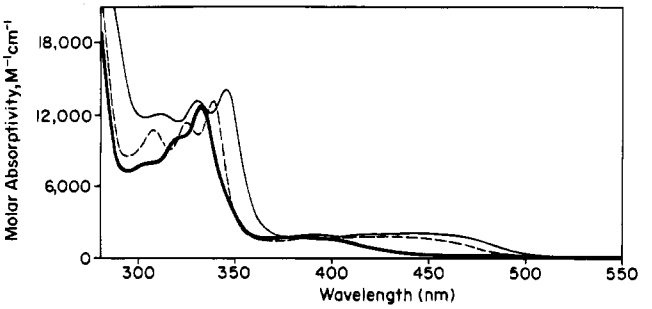

Figure 1. Absorption spectra of Pt(trpy)OH^+ at 25 °C in DMSO (—, thin line), in acetonitrile (- - -), and in 0.05 M pH 9 EPPS (—, thick line).

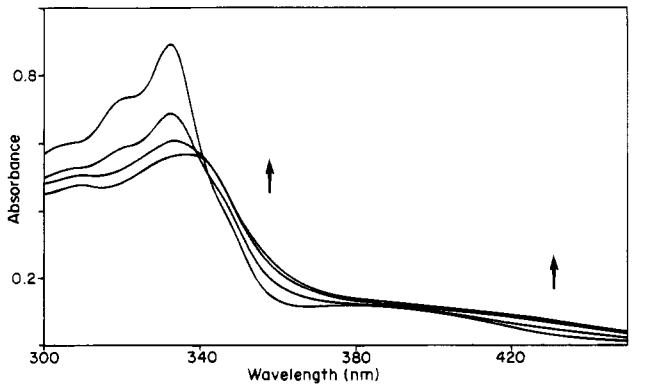

Figure 2. Absorption spectra of freshly prepared solutions of Pt(trpy)OH^+ and ST DNA in 0.05 M pH 9 EPPS at 25 °C. In order of decreasing absorbance at 330 nm, the DNA-P/Pt ratios were 0, 4, 10, and 20. The platinum concentration was 70 μM.

Results

Pt(trpy)OH^+ in Solution. The Pt(trpy)OH^+ ion shows limited stability in organic solvents. Thus, after the complex incubates in acetonitrile or DMSO for a period of hours, there is a diminution in the characteristic long-wavelength CT absorption band of the original complex that suggests loss of the hydroxide ligand. In contrast, in 0.05 M pH 9 EPPS buffer there is no significant change in absorbance for at least several days. According to the literature, Pt(trpy)X^+ systems, where X is a ligand anion such a chloride, tend to self-associate in aqueous solution so that the absorption spectrum is concentration dependent.[27] For the hydroxide complex, the association constant appears to be small because the absorbance data follow Beer's law up to a platinum concentration of at least 50 μM. Figure 1 presents absorbance data of freshly prepared solutions of Pt(trpy)OH^+ in three different solvents.

Interaction of Pt(trpy)OH^+ with Random Sequence DNA. Binding to DNA profoundly influences the absorption spectrum as well as other physical properties in aqueous solution. For example, Figure 2 shows a series of absorption spectra of Pt(trpy)OH^+ in the presence of varying amounts of ST DNA. At DNA-P/Pt ratios in the range 4–10, the interaction with DNA causes a decrease in the intraligand absorbance at 332 nm, an increase in absorbance in the region of 360 nm, and a bathochromic shift of the center of the charge-transfer absorption system from around 385 nm to around 410 nm. At higher DNA-P/Pt ratios the intraligand absorption bands also exhibit a bathochromic shift as well as broadening. With the addition

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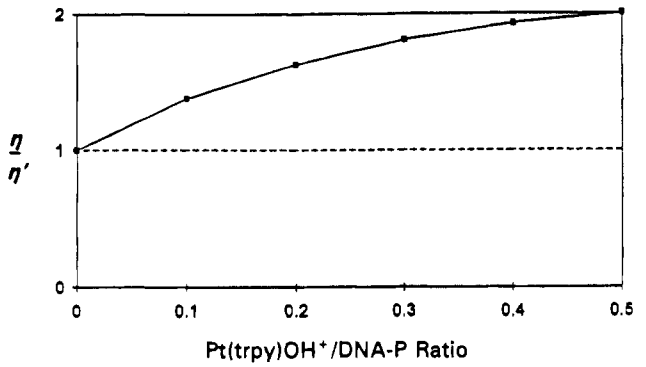


Figure 3. Relative viscosity ratio for ST DNA versus the Pt(trpy)-OH^+/DNA-P ratio immediately after the addition. The DNA-P concentration was 500 μM in 0.05 M pH 9 EPPS at 25 °C.

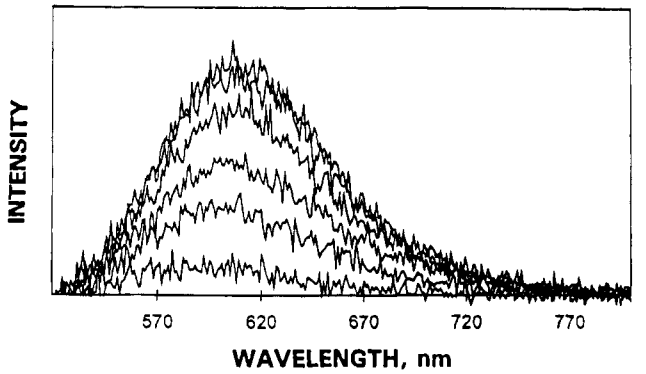


Figure 4. Uncorrected photoluminescence spectra of Pt(trpy)OH^+ in combination with ST DNA in 0.05 M pH 9 EPPS at 25 °C. In order of increasing emission intensity the DNA-P/Pt ratios were 0, 10, 25, 50, 75, and 100. The platinum concentration was 23 μM.

of CP DNA, qualitatively similar changes occur in the absorption spectrum, but there is less broadening of the intraligand bands at DNA-P/Pt = 20. A Scatchard plot of the data involving ST DNA yields a rough estimate of the association constant, $K \approx 7 \times 10^4\ M^{-1}$. Furthermore, the analysis of the intercept suggests that the bound form of the hydroxide complex occupies about five bases, or two and a half base pairs. This accords with the results of Lippard and co-workers reported for the corresponding HET complex.[23] We also combined Pt(trpy)HET^+ with ST DNA and obtained similar spectral shifts. In particular, the center of the CT absoprtion shifted from ca. 475 nm to ca. 540 nm with the addition of the DNA. In the presence of ST DNA the Pt(trpy)OH^+ complex also gives a CD spectrum. The spectrum shows three peaks in the near-UV region: positive maxima at 424 nm and 348 nm and a negative extremum at 308 nm. Finally, Figure 3 shows that the specific viscosity of ST DNA effectively doubles upon loading with the hydroxide complex.

The initial binding interaction with DNA also leads to the appearance of an emission signal from Pt(trpy)OH^+, but the emission intensity varies strongly with the composition of the DNA. With ST DNA the emission intensity increases up to DNA-P/Pt ≈ 75 (Figure 4). By comparison, the addition of CP DNA gives larger emission enhancements; however, the emission increase plateaus at DNA-P/Pt ≈ 25. In contrast, with ML DNA there is no emission enhancement at all. Thus, for excitation at 342 nm, where the absorbance remains relatively constant after the addition of DNA, the emission intensity I varies as follows across this series:

$$0 \approx I(\mathrm{ML}) < I(\mathrm{ST}) < I(\mathrm{CP})$$

We have monitored the luminescence decay in the presence of

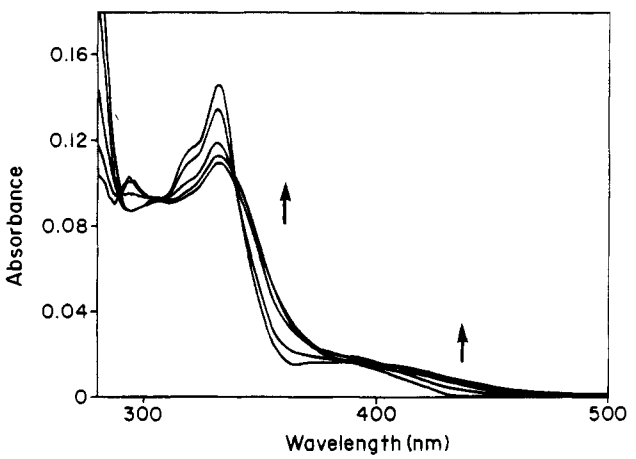


Figure 5. Absorption spectra of freshly mixed solutions containing 10 μM Pt(trpy)OH^+ and IC DNA in 0.05 M pH 9 EPPS at 25 °C. In order of decreasing absorbance at 330 nm the DNA-P/Pt ratios were 0, 4, 10, 20, 40, and 50.

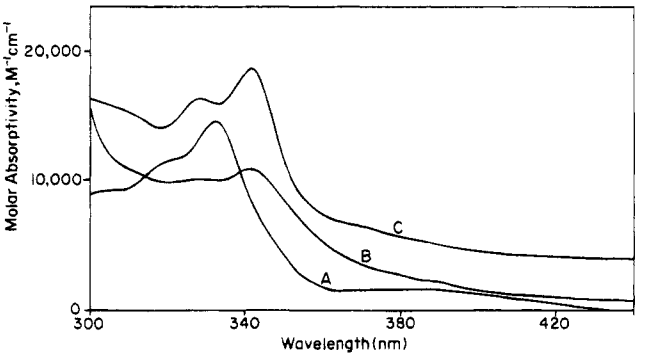


Figure 6. Absorption spectra of Pt(trpy)OH^+ in 0.05 M pH 9 EPPS at 25 °C: (A) without DNA; (B) with IC DNA at DNA-P/Pt = 40 after overnight incubation; (C) after 1 h in the presence of 25 equiv of 5′-IMP. The platinum concentration was 10 μM, and the offset in (C) was 2600 $M^{-1}\ cm^{-1}$.

excess ST DNA, but the curve is not a single exponential.

Regardless of the type of DNA involved, marked changes in the physical properties of the Pt(trpy)OH^+ adduct occur over a period of hours. The changes in the absorption spectrum include a bathochromic shift in the intraligand absorption region and a hypsochromic shift in the charge-transfer absorption bands; vide infra. For samples that luminesce, there is a concomitant loss of emission intensity. In addition, the chromatographic properties of the DNA adduct change dramatically. Thus, shortly after the exposure of the complex to DNA, a simple elution through a cation-exchange column effectively strips the platinum complex from the biopolymer. However, the same column fails to separate the platinum terpyridine chromophore from the DNA-containing fractions if the sample has had a chance to equilibrate overnight.

Interaction with Artificial DNA. Combination with AT, GC, or IC DNA induces similar changes in the absorption spectrum of Pt(trpy)OH^+ as with ST DNA. Figure 5 shows spectra obtained right after the addition of IC DNA. In addition, the absorption spectrum evolves in the same way with time. Figure 6 presents data obtained with IC DNA after incubation and, for the sake of comparison, a spectrum of the adduct formed with 5′-IMP.

The luminescence results with Pt(trpy)OH^+ give additional insight into the ways in which the composition of the DNA influences the photophysics and the binding. Thus, very substantial emission enhancements occur with the addition of AT or IC DNA, but there is absolutely no enhancement with

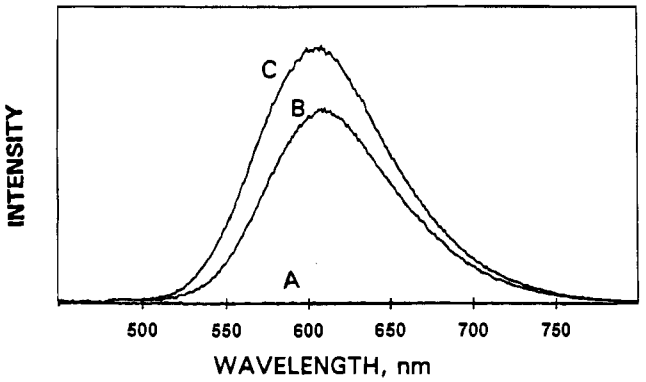
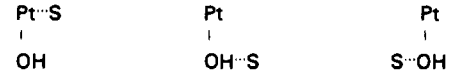


Figure 7. Uncorrected emission spectra of $Pt(trpy)OH^+$ in 0.05 M pH 9 EPPS at 25 °C at DNA-P/Pt = 20: (A) with GC DNA; (B) with AT DNA; (C) with IC DNA. In each case the absorbance at the excitation wavelength of 342 nm was 0.09.

GC DNA. With IC DNA the emission intensity plateaus by DNA-P/Pt = 50, but the signal continues to increase slightly at this ratio with the addition of AT DNA. Figure 7 shows how the emission intensity varies for three different types of DNA at DNA-P/Pt = 20. In the presence of excess AT DNA, the emission decays with a lifetime of ca. 95 ns under nitrogen or in air-saturated solution. The lifetime is somewhat longer (135 ns) with IC DNA as the host. Emission data also reveal a strong preference for intercalation next to GC base pairs. Thus, the emission intensity from a solution containing the hydroxide complex and a 50:50 mixture of GC and AT DNA is only about 12% of that found with pure AT DNA at the same DNA-P/Pt ratio. And at a 2:1 AT-to-GC ratio the emission intensity is only 25% as great as it is with the AT DNA control. As with ST DNA, the emission intensity decreases over time as the platinum complex shifts to an alternate mode of binding, and the emission decrease is more rapid with IC DNA than it is with AT DNA. We also investigated $Pt(trpy)HET^+$ in combination with IC DNA, but the complex gave no detectable emission at DNA-P/Pt = 20.

Discussion

Solvent Effects on the CT State. As solvent, water has a dramatic effect on the CT absorption spectrum of $Pt(trpy)OH^+$. Aldridge et al. reported that the low-energy metal-to-ligand CT absorption bands of $Pt(trpy)OH^+$ occurred at similar energies in acetonitrile and methylene chloride.[6] However, Figure 1 shows that the CT absorption occurs at significantly higher energies in water. The reduction in dipole moment that occurs upon formation of the CT excited state is certainly one factor determining the hypsochromic shift in the CT absorption.[6,28] However, specific interactions with the solvent may also be important. The following abbreviated structures depict the most likely possibilities:



where the solid line designates the platinum–hydroxide bond and the dotted line indicates the interaction with solvent. One obvious possibility is that water can act as electron donor to platinum or to the proton on the hydroxide ligand. However, these interactions should occur in acetonitrile and DMSO, too. The unique property of water is its hydrogen-bonding ability as indicated by the relatively high acceptor number it has in the Gutmann scale.[29] There are two obvious centers for hydrogen bond formation with the platinum complex. One is the platinum itself by virtue of the electron density in the dz^2 orbital. Structure reports have confirmed the existence of this type of adduct with platinum(II) in the solid state.[30] More conventionally, there is a possible donor–acceptor interaction with the hydroxide oxygen. This interaction could be significant because, as Caulton has recently stressed, coordinated ligands like hydroxide, that have multiple lone pairs, retain significant donor ability when the central metal has filled $d\pi$ orbitals.[31] In keeping with this explanation, there are reports that water has a similar effect on CT transitions of ruthenium(II) polypyridines when a bridging ligand such as cyanide is also present in the coordination sphere.[32,33] The shift to higher energy occurs because formation of the hydrogen bond is favorable in the ground state but unfavorable in the CT excited state due the decrease in the electron density at the metal center.

The relatively high energy of the CT state may also explain why the complex shows little or no emission in water. Frequently, the CT excited states of polypyridine complexes deactivate via metal-centered (d–d) excited states,[6,34,35] and these states become more accessible at ordinary thermal energies when the CT states shift to higher energy. In the case of platinum(II), formation of the d–d state involves the population of the metal–ligand σ antibonding orbital, conventionally the $x^2 - y^2$ orbital. Consequently, the lengths of the metal–ligand bonds increase in the excited state. If depopulation of the dz^2 orbital occurs as well, even greater structural reorganization may occur due to the addition of ligands along the z axis.[36] Such significant structural changes generally entail a crossing or avoided crossing with the ground-state potential energy surface, and this greatly facilitates radiationless decay.[37] Therefore, in a solvent like water where the d–d states are thermally accessible, relaxation to the ground state tends to be a very efficient process.

The fact that the wavelength maxima of the intraligand absorption bands are solvent dependent is noteworthy. Normally, $\pi-\pi^*$ transitions are not particularly solvent sensitive because there is rarely much change in the dipole moment. In the case of $Pt(trpy)OH^+$, however, these states evidently couple strongly with the CT excitations because both types of transitions shift in parallel.

Initial Intercalation. The parallels among the physical data strongly suggest that, in the initial stage of reaction with DNA, $Pt(trpy)OH^+$ intercalates like $Pt(trpy)HET^+$. Thus, as we have reported above for $Pt(trpy)OH^+$, Jennette et al. showed that combining the HET complex with calf thymus DNA induced hypochromism in the UV absorbance of the trpy ligand and a redshift of the CT maximum from ca. 480 nm to ca. 530 nm.[22] Also in accord with our results, the interaction with DNA

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induced maxima at 352, 420, and 530 nm in the CD spectrum of the HET complex as well as an increase of ca. 80% in the specific viscosity of DNA at maximal loading.[22] Subsequent studies yielded estimated binding constants for the HET complex of the order of 10^4-10^5 M^{-1}, depending on the buffer, the ionic strength, and the composition of base pairs.[23] Consistent with the nearest-neighbor exclusion principle for intercalative binding, the analysis showed that the complex had a "footprint" of a little larger than 2 base pairs because saturation occurred when the complex occupied about 44% of the slots between base pairs.[23] Fiber diffraction data subsequently confirmed that the minimum spacing between bound platinum atoms was 10.2 Å,[38,39] and Wang et al. established that the HET complex is an intercalator when they reported the crystal structure of the Pt(trpy)HET^+ adduct with the $[dCpG]_2^{2-}$ miniduplex.[24]

On the other hand, it is also clear that Pt(trpy)OH^+ forms another type of adduct, particularly at high DNA-P/Pt ratios. For example, the UV data in Figure 2 show that the system never really achieves a limiting spectrum and that the spectrum continues to evolve with the addition of excess DNA. Because of the chromatographic results and because of the nature of the spectral changes, we ascribe this to competitive formation of a covalently bound adduct. Thus, the most significant absorbance changes occur in the region from about 340 to 360 nm, where an absorbance increase occurs when a neutral ligand like a purine base replaces the coordinated hydroxide; vide infra. The fact that the covalently bound adduct is more apparent in solutions with a high DNA-P/Pt ratio suggests that the two modes of binding do not occur readily in the same region of DNA. This is reasonable since some melting of the duplex structure is probably necessary for effective covalent adduct formation, and segments of DNA that contain intercalators tend to be more rigid, higher melting domains. Thus, at high DNA-P/Pt ratios when there are long stretches of free duplex available, covalent binding becomes more feasible.

Excited-State Dynamics. For some molecules intercalation into DNA results in a dramatic enhancement in the photoluminescence yield,[40-42] and this is certainly the case with Pt(trpy)OH^+ as well. Here the bathochromic shift that occurs in the CT band system upon intercalation may be the key to the phenomenon because the short-lived d−d states become less accessible. In part, the energy shift is probably due to electronic coupling between the chromophore and the π system of DNA. However, displacing the complex from the protic solvent environment probably also has an important effect. Yet another contributing factor may be the inhibition of quenching reactions involving axial coordination sites as is the case with intercalated Cu(TMPyP4).[4] The latter effect may be less important with Pt(trpy)OH^+ because it has an excited-state lifetime of 170 ns in the coordinating solvent acetonitrile.

A very different type of quenching occurs when GC or ML DNA is the host, and the difference in the emission behavior observed with AT, GC, and IC DNA clearly implicates guanine as the quencher. This suggests the possibility of electron-transfer quenching because guanine is a relatively good reducing agent (Table 1) and because there are several reported examples of electron-transfer quenching by guanine.[43-47] Electron transfer is possible because Pt(trpy)OH^+ is a much better oxidizing agent in the excited state than it is in the ground state on account of the d-orbital vacancy created by the CT excitation. The sum of (1) the energy difference (in V) between the excited state and the ground state and (2) the ground-state potential provides an estimate of the reduction potential attributable to the excited state. However, under the conditions we investigated, the ground-state process was irreversible. Therefore, as an approximation we adopt the first reduction potential of Ru(trpy)$_2^{2+}$ (−1.2 V vs SCE[48]), since this is an example of the reduction of a trpy ligand coordinated to a divalent metal center. The energy of the emission maximum in the corrected emission spectrum, i.e. 2.0 V, serves as a crude estimate of the energy available. Together, these values suggest that the excited-state reduction potential is around 0.8 V and that electron-transfer from guanine is a feasible quenching mechanism. We originally included IC DNA in the study in order to test this hypothesis because, in the absense of the electron-releasing amino substituent, the purine ring system is a weaker reducing agent (Table 1). In fact, with IC DNA the intercalated complex emits nicely with a longer excited-state lifetime than it has with AT DNA. Although the decrease in lifetime is small in going to AT DNA, this may indicate that adenine is a weak quencher as well. Lecomte et al. have shown that adenine mononucleotide (AMP) quenches the excited state of Ru(tap)$_3^{2+}$, although much less efficiently than GMP.[49]

Table 1. Reduction Potentials, V vs SCE

base	$E_{1/2}$	base	$E_{1/2}$
$G^{+/o}$	0.85[a]	$A^{+/o}$	1.05[a,c]
	0.9 ∓ 0.05[b]	$I^{+/o}$	1.16[d]

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Electron-transfer quenching by guanine has a profound influence on the photophysical properties of the intercalated complex. Like the analogous complex Pt(trpy)HET^+ [23] and the porphyrin system Cu(TMPyP4),[4,50,51] Pt(trpy)OH^+ preferentially intercalates next to guanine−cytosine base pairs, and this exacerbates the effect. Since there is no emission from Pt(trpy)OH^+ bound to GC or ML DNA, the quenching is evidently complete when guanine is present in the binding site. With ST DNA guanine is not always in the binding site because almost 60% of the base pairs are adenine−thymine base pairs.[52] Even so, neighboring guanine residues inevitably influence the emission lifetime because long-distance electron-transfer quenching is possible. Since the rate of electron transfer falls off roughly exponentially with distance, the lifetime should vary according to the local sequence, and electron-transfer quenching is clearly one explanation for the observed multiphasic emission decay.

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In contrast, DNA appears to have little effect on the photophysics of Pt(trpy)HET$^+$, as we find no detectable emission from the complex dissolved in water or in buffer containing IC DNA. This may be the consequence of an intrinsically short-lived CT excited state because we have found that the photophysical properties of Pt(trpy)X$^+$ systems vary enormously with the identity of ligand X.[6] In the case of the HET complex, the nature of the lowest energy CT state may differ to the extent that the excitation originates from a sulfur-centered molecular orbital due to the ease of oxidation of the thiolate moiety.[53] However, Eisenberg and co-workers have shown that mixed-ligand−platinum(II) complexes involving dithiolate ligands and diimine ligands frequently exhibit photoluminescence.[54,55] We obviously have a good deal more to learn about the factors that control the lifetimes of platinum(II) complexes in solution.

Shift to Covalent Binding. Despite the fact that Pt(trpy)-OH$^+$ has a high affinity for intercalation into DNA, the absorbance and emission results show that over a period of hours the complex switches to a more favorable mode of binding. The relative affinity is evident from the spontaneity of the conversion and the fact that the final adduct survives elution through a cation-exchange column. As Jennette et al. proposed for the analogous Pt(trpy)Cl$^+$ complex,[27] we ascribe this to covalent bond formation with DNA. The most telling observation is that the absorption spectrum of the final adduct is virtually identical to that of the adduct with the mononucleotide IMP. In each case the shift of the CT absorption to higher energy is consistent with the inductive effect expected for the replacement of an anionic ligand with a neutral donor. The energy of the CT state and its proximity to d−d states may explain why there is no evidence of CT emission from the covalently bound adduct of DNA. The site of attachment to DNA remains to be established, but in view of the literature on covalent adducts of platinum amines,[12,20,21] the most likely site is the N7 nitrogen of a purine residue. The fact that covalent adduct formation is more rapid with IC DNA than with AT DNA seems consistent with this hypothesis because pK_a data for the corresponding mononucleotides show that the N7 nitrogen of inosine is a stronger base.[56]

Conclusions

We have mapped the complex pathway by which a platinum(II) complex that contains both a heteroaromatic ligand and a good leaving group reacts with DNA. The unusual photophysical properties of the complex have provided useful insights because they are exquisitely sensitive to the microenvironment. Thus, Pt(trpy)OH$^+$ shows no significant emission in water due to solvent effects that elevate the energy of the luminescent charge-transfer state and facilitate deactivation via metal-centered excited states. Replacement of the hydroxide ligand with a neutral ligand has a similar effect. However, Pt(trpy)-OH$^+$ rapidly intercalates into DNA, and this shifts the CT excited state to lower energy. As a consequence, the intercalated form is capable of exhibiting photoluminescence except that electron-transfer quenching by guanine can severely limit the process. On a longer time scale, Pt(trpy)OH$^+$ loses the hydroxide ligand and forms a covalent bond with DNA much like therapeutically useful platinum complexes, such as cisplatin. In essence, the presence of the trpy ligand encourages intercalation of Pt(trpy)OH$^+$ while the lability of the hydroxide ligand ensures a means of covalent bond formation. Recently, Thorp and co-workers have found that mixed-ligand complexes of ruthenium(II) exhibit the same two modes of binding to DNA.[57] One of the most interesting aspects of our study is that it illustrates how complex the course of the interaction with DNA can be when the reactant is polyfunctional. Even a simple intercalator generally binds via a series of steps.[10] These include coulombic capture by the DNA, diffusion along a groove, and then insertion between base pairs. This chain of events repeats as the reagent shuffles among intercalation sites and achieves a lower free energy.[58] With Pt(trpy)OH$^+$ the preferred sites contain at least one guanine−cytosine base pair. Ultimately, however, the terpyridine complex forms an even more stable adduct when it sheds the hydroxide ligand and forms a covalent bond with the DNA molecule.

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