Coordinatively Unsaturated Excited States and DNA-Binding Interactions

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ABSTRACT

We describe luminescence studies of a series of four-coordinate d^{10}, d^9 and d^8 systems. The phenanthroline complexes, $Cu(dmpp)_2^+$ and $Cu(bcp)_2^+$, emit in aqueous methanol solutions containing excess DNA or RNA because the interaction with the polynucleotides inhibits solvent-induced quenching. Hydrophobic forces strongly shape the binding which probably does not conform to a type of intercalation. In contrast, the copper porphyrin Cu(TMpyP4) intercalates into DNA that contains GC base pairs. It emits when intercalated but not when groove bound. Finally, we describe preliminary studies of emissive platinum(II) terpyridine complexes.

A. INTRODUCTION

We have been investigating the properties of the excited states of four-coordinate d^8, d^9, and d^{10} complexes of platinum(II), copper(II) and copper(I) centers, respectively. Changes in the coordination geometry and/or the coordination number in the excited state influence the excited state reactivity as well as the lifetime. As a result, studies of the photophysical properties provide information about the local environment. In the following we describe several studies involving these complexes, often in combination with double-stranded polynucleotides. Motivating factors are to understand DNA/drug interactions and to develop new reagents that specifically interact with DNA. Foregoing studies in this area include studies with metalloporphyrins [1,2] and phenanthroline complexes. In particular, Sigman and co-workers have carried out numerous studies with copper phenanthrolines [3,4], while Barton and co-workers have been heavily involved with studies of ruthenium(II) complexes of various phenanthroline ligands [5,6].

B. COPPER PHENANTHROLINES

Elegant work by Sigman and co-workers has shown that phenanthroline complexes of copper(I) act as chemical nucleases and that the mechanism involves a non-covalently bound form of the complex [3]. Sigman's cutting data suggest that the copper complex generally binds externally, on the surface of the DNA, within or about the minor groove. However, other work suggests that a more intimate mode of binding

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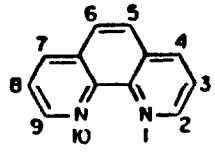
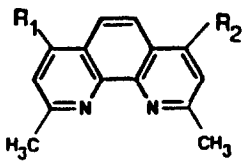
may obtain in which one of the phenanthroline ligands wedges between base pairs [7,8]. Partial intercalation is a term that describes this mode of binding. Our work, aimed at defining the nature of the non-covalently bound intermediates, involves a series of copper(I) complexes of phenanthroline ligands with methyl groups bound in the 2 and 9 positions (Figure 1). Although structurally analogous to complexes with nucleolytic activity, our complexes are much weaker reducing agents. As a result they do not readily activate molecular oxygen and, hence, do not exhibit significant nuclease activity. The methyl substituents also inhibit a flattening distortion that can quench the lifetime of the charge-transfer (CT) excited state [9]. Exciplex quenching is also possible and occurs when a donor solvent – or some other nucleophile – attacks what is formally a copper(II) center in the CT excited state [10-12]. A binding interaction with a macromolecule such as DNA may inhibit exciplex quenching either by sterically inhibiting the required reorganization of the coordination sphere or by reducing the local activity of the solvent.





Ligand	R_1	R_2
dmp	H	H
dmpp	Ph	H
bcp	Ph	Ph

Fig. 1. Phenanthroline ligands.

In the presence of DNA the CT absorption maximum of $Cu(dmp)_2^+$ undergoes a redshift of about 2 nm, and there is about a 10% decrease in the CT absorption intensity [12,13]. Although the complex binds to DNA, it remains solvent accessible because it exhibits virtually no detectable CT emission. These results are consistent with an external mode of binding, perhaps within the minor groove as proposed by Sigman for the analogous complex of 1,10-phenanthroline [3]. In accord with this model, the bound complex has no effect on the specific viscosity of salmon testis (ST) DNA [13].

When we introduce a phenyl substituent into the 4 position of the dmp ligand, the results are more complicated, in part because the solubility decreases [14]. Here we use a mixed solvent that includes methanol (MeOH). In 20% MeOH the complex dissolves and binds to DNA or RNA [14]. Changes occur in the CT absorption maximum as above, and we observe CT emission from the bound complex (Figure 2). Spectral results and viscometry data show that an aggregation phenomenon involving the copper complex and the polynucleotide occurs at low nucleotide-to-copper (DNA-P/Cu) ratios. However, increasing the DNA-P/Cu ratio disperses the aggregates.

There is no change in the specific viscosity due to the DNA at high DNA-P/Cu ratios. Thus, the dmpp complex is not a classical intercalator.

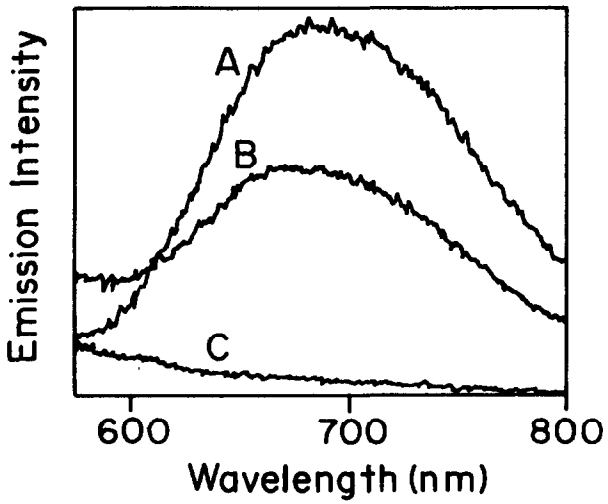


Fig. 2. Uncorrected emission spectra measured in 20% MeOH at room temperature. The samples contained ST DNA with DNA-P/Cu = 50. The solutions contain $Cu(bcp)_2^+$ (A), $Cu(dmpp)_2^+$ (B) or $Cu(dmp)_2^+$ (C).

Binding to DNA induces still stronger emission when the phenanthroline contains phenyl substituents in both the 4 and the 7 positions (Figure 2). In most of our studies of the bcp complex we have used a solvent containing 33% MeOH. Aggregation occurs at low DNA-P/Cu ratios, but excess DNA promotes dispersion of the chromophore [12,13,15]. We also observe a viscosity enhancement in the presence of excess ST DNA; however, the enhancement is probably too great to be ascribed to intercalative binding [13]. To explain these results, we have proposed that $Cu(bcp)_2^+$ is capable of extending the effective length of the polymer molecules in solution by bridging between two or more DNA molecules. This type of adduct can be viewed as a natural successor to the aggregates that occur at low DNA-P/Cu ratios.

In summation, the dmpp and bcp complexes form emissive adducts with B form DNA or A form RNA, and the emission yields are comparable. Hydrophobic interactions are obviously central to the binding phenomena, at least those that support CT emission in solution. The effect can probably be traced to the release of solvent molecules from the surface of the phenanthroline ligand and the DNA surface as adduct formation occurs. Local melting of the nucleotide structure apparently allows dmpp and bcp complexes to internalize into the macromolecule enough that solvent-induced quenching of the CT emission becomes inefficient. Bridged adducts involving

more than one duplex may also be important. Viscometry results are inconsistent with classical intercalation.

C. COPPER(II) PORPHYRINS

The cationic porphyrin Cu(TMpyP4) has a structure more suited to intercalation (Figure 3). In order to interpret the DNA-binding results, we first discuss how the luminescence of a copper(II) porphyrin depends upon the solution environment.

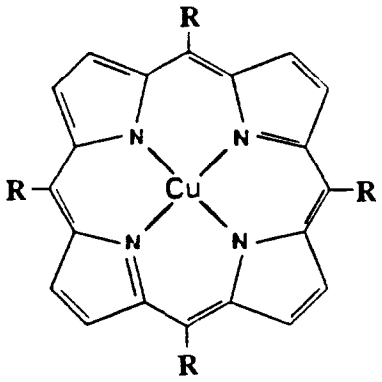
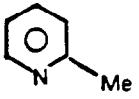
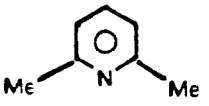


Fig. 3. Copper(II) porphyrin with aryl substituents in the 5, 10, 15, 20 ring positions. For TPP the substituent is a phenyl group, for TMpyP4 a N-methyl pyridinium-4-yl group.

The emission that has been observed from copper(II) porphyrins has been associated with the lowest energy $^3\pi\pi^*$ state of the porphyrin ligand [16,17]. Due to the unpaired spin in the d shell of the copper center, there are actually two closely spaced emitting multiplet levels, the tripdoublet and tripquartet states that are in thermal equilibrium with each other. The wavelength of the emission maximum is typically not very solvent sensitive, but in the case of copper(II) porphyrins the emission intensity and the lifetime are quite solvent dependent. Thus, the excited state lifetime of Cu(TPP) is 29 ns in toluene but <40 ps in pyridine [18]. Kim et al. proposed that the quenching mechanism in pyridine involves the formation of a five-coordinate adduct with a relatively low lying CT excited state [18]. We think that deactivation occurs via a d-d state of the five-coordinate form [15,19]. The addition of an axial ligand raises the energy of the d_z^2 orbital and lowers the energy of the $d_z^2 \rightarrow d_{x^2-y^2}$ excited state that probably mediates decay back to the ground state. To characterize the quenching mechanism further, we have begun quenching studies of Cu(TPP) in toluene. The data presented in Table 1 demonstrate that bulkier Lewis bases are less effective quenchers consistent with a mechanism involving coordination at an axial position.

TABLE 1
Quenching constants with Cu(TPP)

Donor	k_q, $M^{-1}s^{-1}$
	2.3×10^9
	1.7×10^9
	2.3×10^6

The water soluble Cu(TMpyP4) system is four coordinate in aqueous solution and exhibits negligible emission intensity, presumably due to like quenching by the solvent. Previous studies have established that TMpyP4 complexes can bind to DNA either externally via one of the grooves or by intercalation [1,2]. We reasoned that if the copper porphyrin were to intercalate into DNA, the axial positions would be blocked and quenching impeded.

In fact, Cu(TMpyP4) exhibits a broad emission with a maximum at ca. 775 nm when any of several different types of DNA are present in solution. In the presence of excess DNA Figure 4 shows the emission intensity varies with the content of GC base pairs. This is consistent with intercalative binding since TMpyP4 complexes without axial ligands tend to intercalate in GC-rich regions of DNA [2]. Luminescence studies have also provided new information about the way in which Cu(TMpyP4) partitions between binding sites [19].

D. PLATINUM(II) TERPYRIDINES

For our studies of d^8 systems we have focused on platinum(II) terpyridines. The 2,2':6',2" terpyridine ligand (trpy) is attractive because it shows a strong preference for a planar geometry and can therefore be expected to discourage tetrahedral distortions likely to promote non-radiative decay. In addition, the extended pi system of the trpy ligand should provide for low-lying CT states with useful photochemical and photophysical properties.

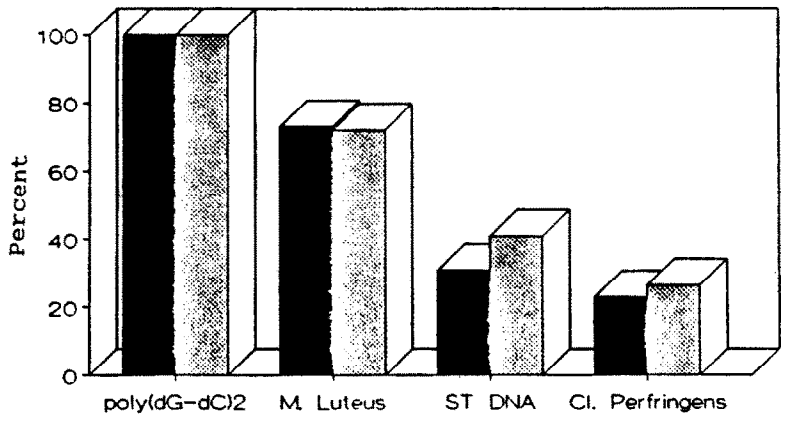


Fig. 4. Emission intensity from Cu(TMpyP4) with different types of DNA at room temperature and DNA-P/Cu = 100. The shaded bar denotes the emission intensity relative to that observed with poly(dG-dC)·poly(dG-dC). The unshaded bar registers the percentage of GC base pairs.

The commercially available chloride salt of $[Pt(trpy)Cl]^+$ dissolves as a monomer in acetonitrile and gives a series of moderately intense absorption bands, assignable as CT transitions, below the $\pi\pi^*$ transitions of the trpy ligand. Table 2 lists the CT maxima. As far as we can tell, the chloride complex does not emit in acetonitrile solution at room temperature. We detect a weak emission in methanol; however, the excitation spectrum does not match the absorption spectrum and the decomposition product turns out to be the methoxide complex. Table 2 contains the absorption maxima of this complex as well as those of the thiocyanate analogue. The methoxide complex and the thiocyanate complex also exhibit emission in acetonitrile at room temperature. (We added 10% MeOH for the methoxide studies to suppress hydrolysis by adventitious water.) The lifetimes and the emission maxima are also in Table 2. Figure 5 presents the corrected emission spectrum and the excitation spectrum of the methoxide complex.

Although these studies are preliminary, they establish that platinum terpyridines can be emissive. Exciplex quenching is evidently not very facile because the complexes emit in a donor solvent like acetonitrile. The photophysical properties and the CT spectrum of the trpy complex are quite sensitive to the nature of the ligand supplying the fourth donor atom. Studies designed to get at these issues are underway.

TABLE 2
Spectral data in acetonitrile

Complex	λ_{max}^{abs} (ε, M^{-1} cm^{-1})				λ_{max}^{em}	τ
$[Pt(trpy)Cl]^+$	347 (8200)	377 (2200)	390 (1960)		—	—
$[Pt(trpy)NCS]^+$	373 (3500)	387 (3800)	400 (4000)		588	3.5ns
$[Pt(trpy)OMe]^+$	380 (1420)	420 (1020)	~450	~470sh	654	180ns

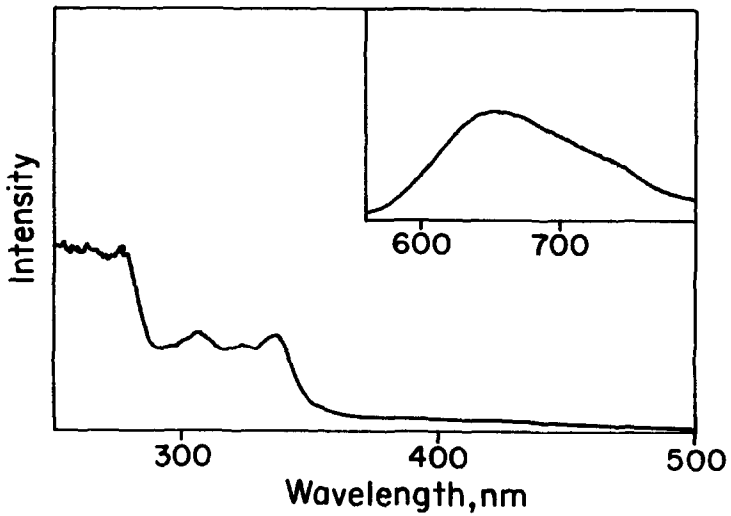


Fig. 5. Excitation spectrum of $[Pt(trpy)OMe]^+$ in methanol at room temperature. Insert: corrected emission spectrum.

E. ACKNOWLEDGEMENT

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