# Determining Binding Constants of Metal Complexes to DNA by Quenching of the Emission of $Pt_2(pop)_4^{4-}$ (pop = $P_2O_5H_2^{2-}$)

**William A. Kalsbeck and H. Holden Thorp***

*Contribution from the Department of Chemistry, University of North Carolina, Chapel Hill, North Carolina 27599-3290*

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**Abstract:** The quenching of the emission of the excited state of $Pt_2(pop)_4^{4-}$ (pop = $P_2O_5H_2^{2-}$) by metal complexes that bind to DNA has been investigated. The quenching rate constant decreases by up to two orders of magnitude when excess DNA is added to the solution. This change in rate constant can be used to determine the binding constant of the quencher to DNA. This has permitted the determination of binding constants for metal complexes where the affinity is quite high ($\geq 10^5$ $M^{-1}$) or quite low ($\leq 1000$ $M^{-1}$). Uniquely, the precision of the determination is not dependent on the effect of DNA binding on the absorption spectrum of the metal complex, allowing for the determination of binding constants for metal complexes that exhibit small electronic changes upon binding. In addition, the method apparently does not depend on the loading level of metal complexes bound to DNA. This method has made possible the first study of the binding constant of a high-affinity ($K > 10^5$ $M^{-1}$), octahedral metallointercalator on buffer cation concentration. With use of polyelectrolyte theory, it can now be shown that there is a significant thermodynamic contribution to the binding affinity from forces other than electrostatics.

Determining the binding constants of metal complexes to DNA is of paramount importance in the development of cleavage agents for probing nucleic acid structure and other applications.[1-3] Difficulties in determining these binding constants arise because often the changes in the absorption spectrum of the metal complex upon binding are small compared to analogous changes in the spectra of organic molecules.[4,5] This occurs because only a portion of the metal complex may interact with the nucleic acid, and the effect of binding on the absorbance would thereby be smaller than with porphyrins or organic molecules where the entire chromophore interacts directly with the biomolecule.[6,7] In addition, the absolute magnitudes of extinction coefficients for metal-based chromophores are often much lower than those of organic species that bind to DNA.

Difficulties in measuring binding constants for metal complexes arise when binding constants are both quite high ($>10^5$ $M^{-1}$) and very low ($\leq 1000$ $M^{-1}$). In the case of high binding constants, equilibrium dialysis is difficult because detection of free metal complex is hampered by the relatively low extinction coefficients of metal complexes compared to organic species.[1,8,9] Equilibrium dialysis measurements are straightforward in the low binding constant region, but accumulation of enough reliable data points is quite time consuming. Absorbance titrations are difficult in general because of the relatively small changes in absorbance upon binding of metal complexes to DNA, especially in the visible region of the spectrum where there is no interfering DNA absorption.

There has been some discussion regarding the application of polyelectrolyte theory to the binding of cationic metal complexes to DNA.[3,5] In particular, the dependence of the binding constants of Λ- and Δ-$Ru(phen)_3^{2+}$ on solution ionic strength has been used to suggest that the binding of these complexes is primarily electrostatic (phen = 1,10-phenanthroline).[3] The $Ru(phen)_3^{2+}$ complex has a modest ($\sim 10^4$ $M^{-1}$) binding constant, which is straightforward to measure. To date, such a study has not been conducted on other intercalating complexes of ligands such as dipyridophenazine (dppz)[8] or phenanthrenequinone diimine (phi),[9] because complexes of these ligands have such strong affinities for DNA that binding constants are difficult to measure accurately with existing methodology. These complexes would be expected to exhibit a large contribution to the binding affinity from forces other than electrostatics

We have been investigating the photochemistry of $Pt_2(pop)_4^{4-}$ (pop = $P_2O_5H_2^{2-}$)[10] in aqueous solutions of DNA. We have reported previously that the excited state of $Pt_2(pop)_4^{4-}$ is capable of cleaving DNA by abstraction of hydrogen atoms from the sugar functionality of the nucleic acid.[11] The tetraanionic metal complex does not bind to DNA; however, the reaction does not involve a diffusable intermediate but direct attack of the excited state on the DNA sugar. Thus, the tetraanion must approach certain sites on the polyanion in order to abstract a hydrogen atom. It is therefore likely that the cleavage reaction will be dependent on novel DNA structures, and we hope to use this chemistry to develop new methodologies for understanding nucleic acid structure in solution. Because of the large electrostatic repulsion between the metal complex and the DNA polyanion, the rate of reaction is not fast enough to lead to detectable quenching of the intense $Pt_2(pop)_4^{4-*}$ emission.

We report here studies of the emission quenching of solution-bound $Pt_2(pop)_4^{4-*}$ by metal complexes that bind to DNA. These quenching processes occur by electron and energy transfer[10] and are therefore much faster than any quenching that may arise through hydrogen abstraction from DNA. The quenching rate constants vary by as much as two orders of magnitude on going from solutions containing no DNA to solutions where all of the quencher is bound to DNA. The dependence of the quenching

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rate constant on the DNA concentration can be used to determine binding constants for the quencher by using existing models to fit the data. Because of the large change in rate constant upon binding of the quencher to DNA, we are able to measure accurately binding constants that are both lower and significantly higher than those accessible by equilibrium dialysis and absorption titration. Using this method, we have been able to determine the dependence on ionic strength of the binding constant of Ru(tpy)(dppz)$OH_2^{2+}$ (tpy = 2,2′,2″-terpyridine),[12] which we have shown recently to bind to DNA by classical intercalation.[13] This study shows for the first time that polyelectrolyte theory is followed for high-affinity, octahedral metallointercalators and that there is a significant thermodynamic contribution to the binding affinity that does not arise from electrostatics.

## Experimental Section

**Materials.** Calf thymus DNA was purchased from Sigma and used according to published procedures.[2] Phosphate buffer solutions were generated with use of $KH_2PO_4$ and $K_2HPO_4$ as described by Boyd.[14] Water was purified with a MilliQ purification system. The complexes $[Co(phen)_3](ClO_4)_3$,[15] $[Ru(phen)_3](ClO_4)_2$,[16] $[Ru(tpy)(bpy)OH_2](ClO_4)_2$,[17] $[Os(tpy)(bpy)OH_2](ClO_4)_2$,[17] $[Ru(tpy)(phen)OH_2](ClO_4)_2$,[18] $[Ru(tpy)(dppz)OH_2](ClO_4)_2$,[19] $[Ru(bpy)_2(dppz)](ClO_4)_2$,[8] $[Rh(phen)_3](ClO_4)_3$,[20] and $[Rh(phen)_2(phi)](ClO_4)_3$[21] were prepared as described (bpy = 2,2′-bipyridine).

**Measurements.** Emission spectra were measured in 1 $cm^2$ quartz cells using a Spex Industries FluoroMax spectrofluorometer. Samples for emission experiments were prepared by mixing stock solutions of $Pt_2(pop)_4^{4-}$, quencher, and calf thymus DNA ($\epsilon$(260 nm) = 6600 $M^{-1}$ $cm^{-1}$) and diluting the mixture with buffer to the appropriate concentrations. All DNA concentrations are given in terms of average values of nucleotide phosphates, as provided by the extinction coefficient. Each point in a binding constant titration was obtained on solutions that were freshly prepared from the stock solutions. The concentration of $Pt_2(pop)_4^{4-}$ was 8–10 $\mu$M. The excitation wavelength was 390 nm. Rate constants in the weak binding cases ($K$ < 10000 $M^{-1}$) were obtained by linear regression with eq 1. In the strong binding cases, Stern–Volmer behavior was not expected because of the dependence of the quenching rate constant on the total metal complex (quencher) concentration (eq 8). In these cases, an effective rate constant was calculated from a single quencher concentration. Complete binding constant determinations were repeated at several quencher concentrations, and no dependence of the binding constant on the quencher concentration was observed. In addition, the total dependence of $I^\circ/I$ on both the quencher and DNA concentrations could be accounted for by using eq 8. All fitting was performed with the Kaleidagraph software.

## Results and Discussion

**Quenching by $[Co(phen)_3]^{3+}$.** The binding and photochemistry of phenanthroline complexes of cobalt to DNA has been studied extensively.[2,22] Related complexes of rhodium have been shown to quench the $Pt_2(pop)_4^{4-*}$ emission efficiently.[23] Thus, we chose $Co(phen)_3^{3+}$ as the initial quencher to study.

Figure 1A shows the emission spectrum of $Pt_2(pop)_4^{4-}$ under various conditions in 50 mM phosphate buffer. The strong emission at 513 nm arises from the ($d\sigma^*$,$p\sigma$) excited state of the complex, for which detailed photophysical studies have been reported.[10,24] Upon the addition of 60 $\mu$M $[Co(phen)_3]^{3+}$ (Figure 1B), significant quenching of the $Pt_2(pop)_4^{4-*}$ emission is observed. This quenching is strongly attenuated by the addition of 5.0 mM DNA (Figure 1C). The dependences of the emission intensity ($I$) on the concentration of $Co(phen)_3^{3+}$ both with and without DNA are described by the Stern–Volmer equation (eq 1):

$$I^\circ/I = 1 + k_Q\tau[Q] \qquad (1)$$

where $I^\circ$ is the emission in the absence of $Co(phen)_3^{3+}$, $k_Q$ is the quenching rate constant, [Q] is the concentration of $Co(phen)_3^{3+}$, and $\tau$ is the emission lifetime, which has been reported previously to be 10 $\mu$s.[10,24] The quenching rate constants obtained from least-squares fitting are shown in Table I. At this ionic strength, addition of DNA reduces the rate of quenching by an order of magnitude. At these DNA and $Co(phen)_3^{3+}$ concentrations, all of the cobalt complex is bound to DNA. This result is consistent with a model where the cobalt complex is bound to DNA while the $Pt_2(pop)_4^{4-}$ complex is repelled from the DNA, because it is a hydrophilic tetraanion.

The rate constants for quenching of $Pt_2(pop)_4^{4-*}$ emission by $Co(phen)_3^{3+}$ with and without 5.0 mM DNA as a function of buffer ionic strength are shown in Table I. In the absence of DNA, the quenching rate constant increases with decreasing ionic strength. This is expected since the cobalt complex is a trication and the platinum complex is a tetraanion. In the presence of 5.0 mM calf thymus DNA, the opposite trend is observed: the rate constant decreases as the ionic strength decreases. These results are also consistent with binding of $Co(phen)_3^{3+}$ to DNA while $Pt_2(pop)_4^{4-}$ remains in the bulk solution. At lower ionic strength, the electrostatic repulsion between $Pt_2(pop)_4^{4-}$ and the DNA polyanion is maximized, and the largest attenuation of the quenching rate is observed. As the ionic strength is increased, the DNA–$Pt_2(pop)_4^{4-}$ repulsion decreases and the quenching rate constant increases.

The dependence of the quenching rate constant on DNA concentration was also examined, and the results are shown in

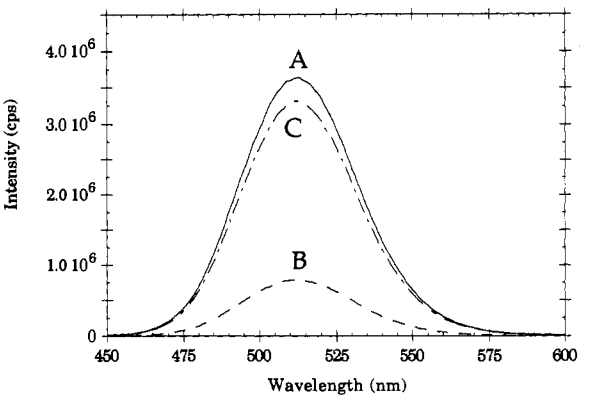


**Figure 1.** Emission spectra of (A) 10 $\mu$M $Pt_2(P_2O_5H_2)_4^{4-}$ in pH 7 buffer and with (B) 60 $\mu$M $Co(phen)_3^{3+}$ and (C) 5 mM calf thymus DNA and 60 $\mu$M $Co(phen)_3^{3+}$ added.

**Table I.** Ionic Strength Dependence of Quenching of $Pt_2(pop)_4^{4-*}$ by $Co(phen)_3^{3+}$

| buffer concn, mM | $k_Q$(buffer),[a] $M^{-1}$ $s^{-1}$ | $k_Q$(DNA), $M^{-1}$ $s^{-1}$ | $k_Q$(buffer)/$k_Q$(DNA) |
|---|---|---|---|
| 1 | $17 \times 10^9$ | $(5–9) \times 10^7$ | 200–300 |
| 5 | $11 \times 10^9$ | $7.5 \times 10^7$ | 147 |
| 25 | $5 \times 10^9$ | $9 \times 10^7$ | 55 |
| 50 | $2.7 \times 10^9$ | $2.6 \times 10^8$ | 10 |

[a] $k_Q$ values determined with eq 1.

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**Table II.** DNA Concentration Dependence of Quenching of $Pt_2(pop)_4^{4-*}$ by $Co(phen)_3^{3+}$

| [DNA],[a] mM | $k_Q$, $M^{-1}$ $s^{-1}$ | [DNA],[a] mM | $k_Q$, $M^{-1}$ $s^{-1}$ |
|---|---|---|---|
| 0 | $5.0 \times 10^9$ | 1.0 | $5.7 \times 10^8$ |
| 0.10 | $3.3 \times 10^9$ | 2.0 | $1.4 \times 10^8$ |
| 0.25 | $2.3 \times 10^9$ | 5.0 | $9.0 \times 10^7$ |
| 0.50 | $9.5 \times 10^8$ | | |

[a] Buffer concentration: 25 mM.

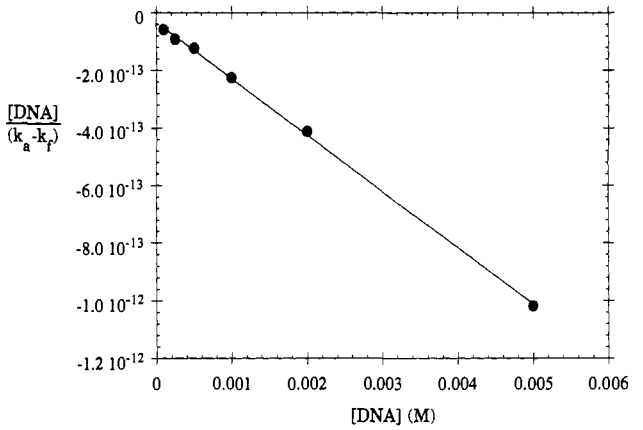


**Figure 2.** Plot of quenching data for $Co(phen)_3^{3+}$ and linear least-squares fit to eq 3, $K = 6.1 \times 10^3$ $M^{-1}$.

Table II. The rate constant decreases with increasing calf thymus DNA concentration. At a constant concentration of $Co(phen)_3^{3+}$, the amount of metal complex bound to DNA will increase as the DNA concentration increases.

The results in Table II suggested to us that the dependence of the quenching rate constant on DNA concentration could be used to determine the binding constant of $Co(phen)_3^{3+}$ to DNA. Since the change in quenching rate constant upon binding was at least an order of magnitude, it was clear that reliable measurements could be made over a wide range of DNA concentrations. Our model suggests that two different rate constants can be used to fit our data, one for quenching by $Co(phen)_3^{3+}$ free in solution ($k_f$) and a slower rate constant for quenching by $Co(phen)_3^{3+}$ bound to DNA ($k_b$), where $k_f$ and $k_b$ are given in Table I as $k_Q$(buffer) and $k_Q$(DNA), respectively. Fitting of absorption titrations has been performed with use of an identical model where two extinction coefficients, $\epsilon_b$ and $\epsilon_f$, are used for a chromophore bound to DNA and free in solution, respectively.[25] The ratio of the concentration of metal complex bound to DNA to metal complex free in solution is given by

$$\frac{(\epsilon_a - \epsilon_f)}{(\epsilon_b - \epsilon_f)} = \frac{C_b}{C_t} \qquad (2)$$

where $\epsilon_a$ is the extinction coefficient of the metal complex at a given DNA concentration, and $C_b$ and $C_t$ are the concentrations of the fully bound and free complex, respectively. Substitution of the appropriate quenching rate constants for the extinction coefficients and rearrangement results in eq 3, which serves as the starting point for binding constant calculations.

$$[DNA]/(k_a - k_f) = \frac{[DNA]}{(k_b - k_f)} + \frac{1}{K(k_b - k_f)} \qquad (3)$$

If eq 3 is operative, a plot of $[DNA]/(k_a - k_f)$ vs [DNA] should be linear and the slope divided by the $y$-intercept would give $K$, the binding constant. Figure 2 shows such a plot for quenching by $[Co(phen)_3]^{3+}$. The binding constant obtained from a linear least-squares fit, $6.1 \times 10^3$ $M^{-1}$, is in good agreement with values

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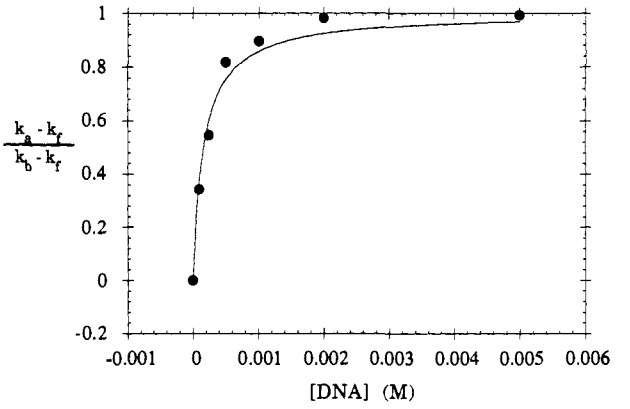


**Figure 3.** Plot of quenching data for $Co(phen)_3^{3+}$ and nonlinear least-squares fit to eq 4, $K = 6.2 \times 10^3$ $M^{-1}$.

previously reported for other tris(phenathroline) complexes of trivalent metal ions.[2,26]

Although the linear representation of the binding data shown in Figure 2 provides the appropriate binding constant, it is not necessarily the best format for evaluating the quality of the data. For this reason, eq 3 was solved for $C_b/C_t$ to give eq 4.

$$C_b/C_t = \frac{(k_a - k_f)}{(k_b - k_f)} = \frac{K[DNA]}{K[DNA] + 1} \qquad (4)$$

A plot of $(k_a - k_f)/(k_b - k_f)$ vs [DNA] and a non-linear least-squares fit are shown in Figure 3. The binding constant obtained from this fit is $6.2 \times 10^3$ $M^{-1}$, which is identical with that obtained from the linear fit to eq 3. Equation 4 will hereafter be used for determining binding constants, because the form of Figure 3 offers a better opportunity to evaluate the quality of the data and because we will introduce an alternative to eq 4 below that is in the same format.

**Quenching by Other Metal Complexes.** Complexes of numerous metal ions have now been studied for their DNA binding and cleavage properties.[1,2,5,22,26] We have synthesized a number of aquaruthenium(II) complexes that bind to DNA and cleave DNA upon oxidation to oxoruthenium(IV) or hydroxoruthenium(III).[12,13,18,19,27] Related ruthenium(II) complexes are known to quench the emission of $Pt_2(pop)_4^{4-*}$ efficiently,[10] and the binding properties of these complexes have been extensively investigated.[1,3-5,28] Many known ruthenium(II) complexes have been identified as having binding constants that are difficult to determine because they are quite large or very small.[1,8]

Binding data obtained from the quenching of $Pt_2(pop)_4^{4-*}$ by $[Ru(bpy)_3]^{2+}$ are shown in Figure 4. The binding constant determined with eq 4 is $6.8 \times 10^2$ $M^{-1}$, identical with the value reported previously, which was determined by equilibrium dialysis.[1] Binding constants for related bipyridine complexes determined by the same method are given in Table III. The ability to determine binding constants for such weak binding complexes is an advantage of this method. Previous attempts to determine the binding constant for $[Ru(bpy)_3]^{2+}$ by absorption titration were unsuccessful.[1]

Binding constants at the other extreme, those which are very large, are also very difficult to determine accurately.[8,9] This is because the complex binds to a large extent, even at very low DNA concentrations. Relatively small changes in the absorption spectrum of the metal complex are difficult to follow under these conditions. Emission spectroscopy is often more sensitive to

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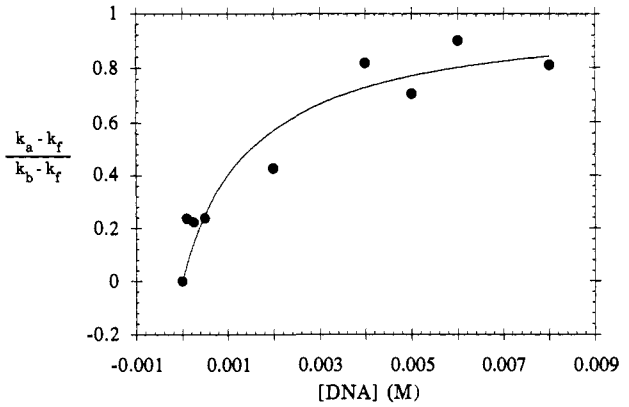


**Figure 4.** Plot of quenching data for $Ru(bpy)_3^{2+}$ and nonlinear least-squares fit to eq 4, $K = 6.8 \times 10^2\ M^{-1}$.

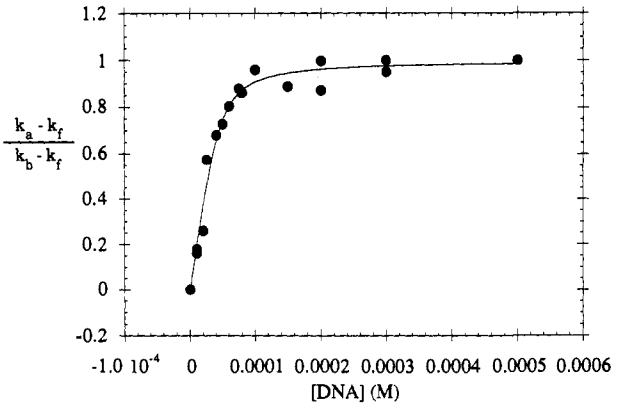


**Figure 5.** Plot of quenching data for $Ru(tpy)(dppz)OH_2^{2+}$ and nonlinear least-squares fit to eq 6, $K = 7.3 \times 10^5\ M^{-1}$.

**Table III.** Binding Constants[a] for Low-Affinity Metal Complexes

| complex | $K$,[b] $M^{-1}$ | $K$,[c] $M^{-1}$ |
|---|---|---|
| $Co(phen)_3^{3+}$ | $6.2 \times 10^3$ [d] | |
| $Rh(phen)_3^{3+}$ | $2.9 \times 10^3$ | $5.0 \times 10^3$ [e] |
| $Ru(bpy)_3^{2+}$ | $6.8 \times 10^2$ | $7.0 \times 10^2$ [f] |
| $Ru(tpy)(bpy)(OH_2)^{2+}$ | $6.6 \times 10^2$ | |
| $Os(tpy)(bpy)(OH_2)^{2+}$ | $5.5 \times 10^2$ | |
| $Ru(phen)_3^{2+}$ | $4.8 \times 10^3$ | $6.2 \times 10^3$ [g] |
| $Ru(tpy)(phen)(OH_2)^{2+}$ | $3.9 \times 10^3$ | |

[a] All binding constants refer to 50 mM ionic strength unless otherwise noted. [b] Determined using $Pt_2(pop)_4^{4-*}$ quenching and fitting to eq 3, this work. [c] Determined using equilibrium dialysis. [d] 25 mM buffer concentration. [e] Reference 26. [f] Reference 1. [g] Reference 28.

**Table IV.** Binding Constants for High-Affinity Metal Complexes

| complex | $K$,[a] $M^{-1}$ | $K$, $M^{-1}$ |
|---|---|---|
| $Ru(tpy)(dppz)(OH_2)^{2+}$ | $7.3 \times 10^5$ | $6.6 \times 10^5$ [b,c] |
| $Ru(bpy)_2(dppz)^{2+}$ | $4.0 \times 10^6$ | $>10^6$ [d,e] |
| $Rh(phen)_2(phi)^{3+}$ | $3.3 \times 10^6$ | $\sim 10^7$ [b,g] |

[a] Binding constants determined by $Pt_2(pop)_4^{4-*}$ quenching using fitting by eq 6 in 50 mM, pH 7 buffer. [b] Determined by absorption titration fit to eq 6. [c] Reference 29. [d] Determined using equilibrium dialysis. [e] Reference 8. [g] Reference 9.

binding,[3,29] but many complexes emit only weakly or not at all. The high intensity and long lifetime of the $Pt_2(pop)_4^{4-*}$ emission make quenching measurements possible even with micromolar concentrations of quencher. Thus, our method potentially allows for accurate measurements of $C_b/C_f$, even at high ratios of metal complex to DNA.

In order to determine high binding constants, a more sophisticated mathematical model than eq 4 is required. Equation 4 makes the assumption that the DNA concentration is much greater than the metal complex concentration,[25] which is not the case for tight binding species. For the metallointercalating species discussed here, appreciable binding occurs at [DNA]/metal complex ratios as low as 2:1.[8,9,12,13] An expression for $C_b$ based on neighbor-exclusion binding has been used by Bard to determine binding constants using electrochemistry.[2] This expression is conveniently written as:

$$C_b = \frac{b - \left(b^2 - \frac{2K^2C_t[\mathrm{DNA}]}{s}\right)^{1/2}}{2K} \qquad (5a)$$

$$b = 1 + KC_t + \frac{K[\mathrm{DNA}]}{2s} \qquad (5b)$$

where $s$ is the size of the binding site in base pairs and $K$ is the binding constant of the species of interest. An expression for $C_b/C_t$ can be obtained by substituting eq 5 into eq 2 to give:

$$\frac{(k_a - k_f)}{(k_b - k_f)} = \frac{b - \left(b^2 - \frac{2K^2C_t[\mathrm{DNA}]}{s}\right)^{1/2}}{2KC_t} \qquad (6)$$

A plot of $(k_a - k_f)/(k_b - k_f)$ vs DNA concentration can then be subjected to a two-parameter fit by using eq 6.

Complexes of the dppz ligand have been reported to bind to DNA by classical intercalation.[8,12,13,30] Because of the avid affinity of these complexes for DNA, attempts at precisely quantitating the binding constant have been unsuccessful.[8,12,30] Two dppz complexes have been studied recently, $Ru(tpy)(dppz)OH_2^{2+}$ and $Ru(bpy)_2(dppz)^{2+}$, and the best estimate for the binding affinity of these complexes is $\sim 10^6\ M^{-1}$ from electrochemical, equilibrium dialysis, and absorption titration experiments.[8,12] Figure 5 shows a titration plot for $[Ru(tpy)(dppz)(OH_2)]^{2+}$ obtained by quenching of $Pt_2(pop)_4^{4-*}$. The binding constant obtained, 730 000 $M^{-1}$, is in good agreement with previous estimates for dppz complexes. The value for $s$ of 1.9 is in good agreement with that of 2 determined by viscometric titration.[13] In addition, we have recently reported measurements of the binding constant and site size for $Ru(tpy)(dppz)OH_2^{2+}$ by emission and absorption titrations which, though less precise, are in good agreement.[29] The binding constants for $Ru(tpy)(dppz)OH_2^{2+}$ and other high-affinity metallointercalators are shown in Table IV along with estimates from other methods.

It is clear from eq 6 that the amount of quenching will depend on the metal complex concentration. Thus, for determining binding constants for high-affinity molecules with eq 6, actual Stern–Volmer rate constants are not used. Instead, an effective rate is calculated by using a single quencher concentration and eq 1. We have observed that the binding constants obtained are not a function of the absolute concentration of the metal complex. However, the metal complex concentration must be low enough that a significant number of data points can be obtained where all of the metal complex is not bound to DNA. We have found with these high-affinity ($K \sim 10^6\ M^{-1}$) complexes that 5–20 $\mu$M concentrations give acceptable numbers of data points for confident fitting.

From eqs 1 and 6, it should be possible to derive an equation for fitting the dependence of the quenching on the concentration of the metal complex at constant DNA concentration. Again, we use a model of two rate constants for quenching of $Pt_2(pop)_4^{4-*}$, one for metal complex free in solution ($k_f$) and one for

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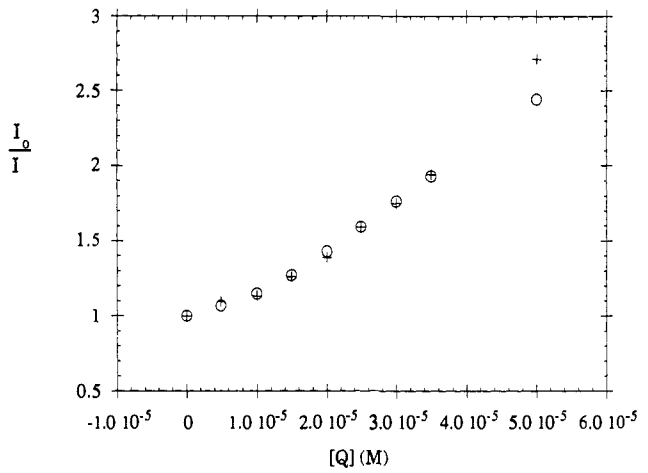


**Figure 6.** Measured (+) and calculated (O) values of $I^0/I$ vs $[Rh(phen)_2(phi)]^{3+}$ in the presence of 0.1 mM DNA. Calculated values were obtained with eq 8, with $K = 3.8 \times 10^6$ M$^{-1}$ and $s = 4.7$.

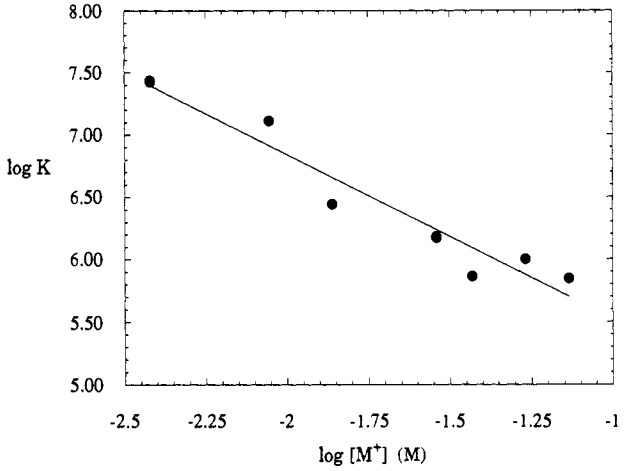


**Figure 7.** Plot of log $K$ vs log $[M^+]$ and linear least-squares fit, slope = −1.31.

metal complex bound to DNA ($k_b$). Thus, the modified quenching equation can be written as:

$$I^\circ/I = 1 + \tau(k_f C_f + k_b C_b) \quad (7)$$

where $C_f$ is the concentration of metal complex free in solution. We would then expect a plot of $I^\circ/I$ vs metal complex concentration to give a two-phase dependence where at low concentrations of metal complex most of the metal complex is bound to DNA and the limiting rate constant will be $k_b$. As the metal complex concentration is increased, increasing amounts of metal complex will remain free in solution until a second phase is reached where $k_f$ is the limiting rate constant.

Such a plot is shown in Figure 6, which gives $I^\circ/I$ vs quencher concentration for $[Rh(phen)_2(phi)]^{3+}$ at a constant DNA concentration of 0.1 mM. By substituting eq 5 into eq 7, $I^\circ/I$ can be described in terms of the binding parameters.

$$\frac{I^\circ}{I} = \left[\left(\left(\left(\frac{b - \left(b^2 - \frac{2K^2C_t[DNA]}{s}\right)^{1/2}}{2KC_t}\right)(k_b - k_f)\right) + k_f C_t\right)\tau\right] + 1 \quad (8)$$

Also shown in Figure 6 are calculated $I^\circ/I$ values determined from eq 8 and the quencher concentrations, binding constant, and site size for $Rh(phen)_2(phi)^{3+}$ given in Table IV. These simulations were performed for several different DNA concentrations, and good agreement between observed and calculated $I^\circ/I$ values was obtained in all cases. At high concentrations of $[Rh(phen)_2(phi)]^{3+}$, eq 8 underestimates the amount of quenching somewhat. At these concentrations, the metal complex is in excess compared to the number of binding sites, so a large amount of rhodium is not bound to DNA. Here, there is probably increased quenching because of ion pairing between the tricationic rhodium complex and $Pt_2(pop)_4^{4-}$. For determining binding constants, the important concentration regime is at low metal complex concentrations, where outstanding agreement between measured and calculated quenching ratios is observed. The binding constant and site size could also be separately determined by fitting plots such as that shown in Figure 5 to eq 8. The binding constants and site sizes obtained using eq 8 agree very well with those obtained from fitting the DNA concentration dependence.

Of course, in order to develop a useful method for determining binding constants, the uncertainty in the determined binding constants must be assessed. Determining errors statistically from titration plots underestimates the amount of uncertainty and is strongly dependent on the method of fitting. For example, using linear fits to determine binding constants for weak binding complexes (Figure 2) always gives high (>0.99) correlation coefficients. When the data are replotted and fit to eq 4, however, a more realistic picture of the error is obtained (Figure 3).

The primary errors in determining the binding constant are not apparent upon inspection of the titration curves. Complete determinations made on different sets of solutions more accurately reflect the error inherent in the measurement. On the basis of numerous trials for several of the metal complexes discussed here, we have determined the error in the measurement of binding constants to be ±30%. The data in Figure 7 discussed below were all from completely different determinations of binding constants, and the degree of scatter accurately reflects the error in the measurement. Estimates of binding constants for high-affinity metal complexes have previously been within at best an order of magnitude;[8,9] thus, an error of ±30% represents a signficant advance in terms of precision.

**Salt Dependence of Ru(tpy)(dppz)$OH_2^{2+}$ Binding.** The role of buffer cation concentration in determining the binding constant of cations to DNA can be quantitated by using polyelectrolyte theory.[31] This theory has been shown to describe the binding of $Ru(phen)_3^{2+}$ to DNA, but this complex is not a high-affinity metallointercalator, such as a dppz or phi complex.[3] To date, a similar study has not been conducted for such a high-affinity metal complex because of the difficulty in accurately determining large ($\geq 10^6$ M$^{-1}$) binding constants for metal complexes. Using our method of $Pt_2(pop)_4^{4-*}$ quenching, we can determine these large binding constants with sufficient precision to permit such a study.

The binding constant for Ru(tpy)(dppz)$OH_2^{2+}$ was determined by using our method as a function of buffer salt concentration. As the salt concentration is decreased, the binding constant for the complex increases dramatically, up to $27 \times 10^6$ M$^{-1}$ at 5 mM ionic strength from $0.73 \times 10^6$ M$^{-1}$ at 50 mM ionic strength. As we have shown in Table I, the difference between quenching rate constants with and without DNA increases as the ionic strength decreases. An increased binding constant as a result of lower ionic strength would ordinarily make the measurement more difficult; however, this is compensated in our method by the increased resolution provided by a larger change in quenching rate constant upon binding.

The dependence of the binding constant on the concentration of the monovalent buffer cation is shown in Figure 7. The plot shown gives log $K$ versus the logarithm of the monovalent ($Na^+$ or $K^+$) cation concentration. The linear dependence observed is

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**Table V.** Comparative Energetics of Binding to DNA

| compd | $K_{obs}$/ $10^4$,[a] $M^{-1}$ | $Z\Psi$[b] | $K°_t$/ $10^4$, $M^{-1}$ | $\Delta G°_t$,[c] kcal/mol |
|---|---|---|---|---|
| $Ru(tpy)(dppz)OH_2^{2+}$ | 73 | 1.32 | $2.7 \pm 1.1$[b] | $-5.9 \pm 0.3$ |
| ethidium[d] | 49.4 | 0.75 | 6.1 | −6.5 |
| $\Delta$-$Ru(phen)_3^{2+}$ [d] | 0.97 | 1.38 | 0.02 | −3.1 |
| $\Lambda$-$Ru(phen)_3^{2+}$ [d] | 1.07 | 1.24 | 0.03 | −3.4 |

[a] Measured in $\mu$ = 50 mM phosphate buffer. [b] Determined from the least-squares fit of eq 9 to the data in Figure 6. $Z\Psi$ is the absolute value of the slope in Figure 7. [c] Determined from $\Delta G°_t = -RT \ln K°_t$. [d] Reference 3.

predicted from polyelectrolyte theory, which states that the logarithm of $K$ should depend on buffer cation concentration as:[31]

$$\ln K_{obs} = \ln K°_t + Z\xi^{-1}(\ln(\gamma_{\pm}\delta)) + Z\Psi(\ln([M^+])) \quad (9)$$

where $K_{obs}$ is the measured binding concentration at a monovalent cation concentration of $[M^+]$, $Z$ is the charge on the metal complex, $K°_t$ is the "thermodynamic" binding constant, $\gamma_{\pm}$ is the mean activity coefficient at cation concentration $[M^+]$, and $\xi$ = 4.2 and $\delta$ = 0.56 for calf thymus DNA.[31] The magnitude of $K°_t$ represents the contribution to binding from non-electrostatic forces.

The results of fitting eq 9 to the data in Figure 7 are given in Table V. The slope of the plot provides an experimental measure of $Z\Psi$. The parameter $\Psi$ is the number of counterions associated with each DNA phosphate, and it has been shown that $\Psi$ = 0.88 for calf thymus DNA.[31] The theoretical value for $Z\Psi$ for a 2+ ion would therefore be 1.76. Our value for $Ru(tpy)(dppz)OH_2^{2+}$ is somewhat lower than the theoretical value; however, Chaires et al. have determined nearly identical values for $\Delta$- and $\Lambda$-$Ru(phen)_3^{2+}$.[3] Slightly lower values of $Z\Psi$ can be attributed to changes in DNA hydration or coupled anion release from the metal complex upon binding. Results from the analyses of $Ru(phen)_3^{2+}$ and ethidium binding by Chaires et al. are given for comparison in Table V.

Also given in Table V are values for $K°_t$ and $\Delta G°_t$ for binding of $Ru(tpy)(dppz)OH_2^{2+}$ to DNA. These quantities represent the binding energetics in the absence of electrostatic forces. The values for $Ru(tpy)(dppz)OH_2^{2+}$ are clearly much larger than those for $Ru(phen)_3^{2+}$ and approach quite closely the values for ethidium. Strikingly, $\Delta G°_t$ is only 0.6 kcal/mol greater for ethidium than for $Ru(tpy)(dppz)OH_2^{2+}$. We have shown previously that $Ru(tpy)(dppz)OH_2^{2+}$ gives results in viscometry, helical unwinding, and oxidation kinetics experiments that are similar to those for ethidium and demonstrate binding by classical intercalation.[13] These results, combined with those of Barton et al.[8,30] and the buffer cation concentration dependence described here, build a very strong case for intercalation by dppz complexes. Recently, David and Barton have demonstrated specific intercalation of $Rh(phen)_2(phi)^{3+}$ using NMR.[32]

The confirmation that the binding of these metal complexes to DNA is governed by eq 9 provides insight into the interplay of electrostatic and other forces in controlling the binding affinities. In general, the $Z\xi^{-1}(\ln(\gamma_{\pm}\delta))$ term is small compared to the other two, so the binding affinity is basically a sum of the electrostatic $Z\Psi$ term and the $K°_t$ term. For complexes that bind solely by electrostatics, $\ln K_{obs}$ should be essentially the same as the $Z\Psi$ term. If $Z\Psi \sim -1.3$, as it is in $Ru(phen)_3^{2+}$ and $Ru(tpy)(dppz)OH_2^{2+}$, it is straightforward to calculate that this is indeed the case for $Ru(bpy)_3^{2+}$ and $Ru(tpy)(bpy)OH_2^{2+}$. This indicates that at 50 mM salt concentration, electrostatic forces account for $\sim 10^2$ $M^{-1}$ in binding affinity (for dications), and the remainder of the binding affinity arises from non-electrostatic forces. On going from bpy complexes to dppz complexes, $\Delta G°_t$ increases from essentially zero to about 6 kcal/mol. This 6 kcal/mol is responsible for the difference in binding affinity between $10^2$ $M^{-1}$ for bpy complexes and $10^6$ $M^{-1}$ for dppz complexes. In the case of dppz complexes that are known to be metallointercalators, it is clear that intercalation is responsible for the increased binding affinity. For phen complexes, which have been shown to exhibit $\Delta G°_t$ values of about 3 kcal/mol,[3] the contribution to the binding affinity arising from non-electrostatic forces is not as high as that for the dppz complexes, but it is readily measurable, and the binding affinity is certainly greater than the $10^2$ $M^{-1}$ expected for a complex that binds solely by electrostatics.

(32) David, S. S.; Barton, J. K. *J. Am. Chem. Soc.* **1993**, *115*, 2984.

## Conclusions

The $Pt_2(pop)_4^{4-}$ excited state has a significantly high energy (56.7 kcal/mol) excited state for a metal complex.[10] Thus, quenching by electron or energy transfer is likely to occur with most inorganic complexes and many organic molecules that bind to DNA. One of the advantages of the method is that the difference in quenching does not depend strongly on the type of interaction of the metal complex with the DNA; the difference in $k_b$ and $k_f$ is similar for weak binders and intercalating complexes. In absorption titrations, the extent of the interaction of the metal complex with the DNA determines the change in the absorption spectrum brought about by binding. This ultimately will govern the precision of the binding constant determined. Thus, quenching of $Pt_2(pop)_4^{4-}$ can be used to determine binding constants of molecules or complexes with absorption spectra that are not greatly altered by DNA binding. This has made it possible to determine binding constants for very weak ($K < 1000$ $M^{-1}$) binding complexes such as $Ru(bpy)_3^{2+}$ and $Ru(tpy)(bpy)OH_2^{2+}$.

Another advantage of the method comes from the ionic strength dependence. As shown in Table I, the difference between $k_b$ and $k_f$ increases as the ionic strength is decreased. Thus, even though binding constants for cations are higher at low ionic strength, it is still straightforward to make the measurement by $Pt_2(pop)_4^{4-*}$ quenching. This has made possible the first study of the binding constant of a high-affinity metal complex as a function of buffer concentration (Figure 6).

Absorption spectra of many small molecules that bind to DNA are affected by intermolecule interactions when there are a large number of small molecules bound to DNA. These interactions can cause a nonlinear dependence of the absorption spectrum on the concentration of bound molecules at high loading levels. If the $Pt_2(pop)_4^{4-}$ is solution bound, then the only factor controlling the quenching rate constant is the *number* of metal complexes bound to DNA. The average distance between the metal complexes should not influence the degree of quenching. This is apparent in Figure 5, where eq 8 accounts for the dependence of the quenching on the metal complex concentration, including at high loading levels.

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