Role of the Buffer Cation in Determining DNA Binding Constants for Metal Complexes

William A. Kalsbeck† and H. Holden Thorp*

Department of Chemistry, University of North Carolina, Chapel Hill, North Carolina 27599-3290

Received November 30, 1993

The DNA binding of small molecules, including metal ions, metal complexes, organic molecules, and peptides, is an area of great interest and activity.[1,2] Of course, understanding these binding interactions in detail requires a detailed knowledge of the polyion structure, solvation, and charge compensation. Polyelectrolyte theory describes the thermodynamic consequences of the binding of a cationic ligand to a polyanion.[3] The polyelectrolyte effect associated with cation binding reduces the exposure of the polyanion charge to the aqueous salt solution and is closely analogous to the hydrophobic effect observed for the binding of a nonpolar ligand to a nonpolar macromolecule.[4] One of the central concepts of polyelectrolyte theory is that of counterion condensation developed by Manning.[3,5,6] From a practical point of view, these principles of polyelectrolyte theory can be expressed as

$$\ln K_{obs} = \ln K^{\circ}_{t} + Z\xi^{-1}(\ln(\gamma\pm\delta)) + Z\psi \ln([M^+]) \quad (1)$$

where K_{obs} is the measured binding constant 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 $[M^+]$, ψ is a constant for a given DNA structure, and $\xi = 4.2$ and $\delta = 0.56$ for calf thymus DNA.[7] An experimental value for $Z\psi$ can be determined from the slope of a plot of $\ln K_{obs}$ vs $\ln[M^+]$, allowing for the calculation of K°_{t}. The magnitude of K°_{t} represents the contribution to binding from nonelectrostatic forces.[7,8]

We have recently reported a method for determining binding constants for metal complexes to DNA that involves quenching of the excited state of $Pt_2(pop)_4^{4-}$ (pop = $P_2O_5H_2^{2-}$).[9] The dinuclear platinum complex has a high-energy excited state that is readily quenched ($k_Q \sim 10^9$ M^{-1} s^{-1}) by many metal complexes. When DNA is added to the solution, the quenching rate constant is reduced by as much as 2 orders of magnitude if the quencher binds to DNA, because the $Pt_2(pop)_4^{4-}$ is electrostatically repelled from the polyanion. From the dependence of the quenching rate constant on the DNA concentration, the binding constant of the quencher can be determined. The sensitivity of this technique has allowed us to extend the range of binding affinities to values that are both significantly higher and lower than those conveniently measured using conventional methods.

Using the $Pt_2(pop)_4^{4-}$ method, we have previously determined the dependence on ionic strength of the binding constant of $[Ru(tpy)(dppz)OH_2]^{2+}$ (tpy = 2,2′,2″-terpyridine, dppz = dipyrido[3,2-*a*:2′,3′-*c*]phenazine),[9] which we have shown to bind to DNA by classical intercalation.[10,11] This study showed 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. The study was possible because quantitation of the high binding affinity of the metallointercalator was sufficiently convenient to permit repeated measurements as a function of cation concentration. We report here the ionic strength dependence of the binding constant of $[Ru(tpy)(bpy)OH_2]^{2+}$ (bpy = 2,2′-bipyridine), which exhibits a relatively small affinity for DNA.[12,13] It has been suggested that complexes of bpy bind to DNA only by electrostatic forces; however, this has not been shown using polyelectrolyte theory, because precise determination of the binding constant was complicated by the low affinity of bpy complexes. We report here that K°_{t} is indeed quite low for $[Ru(tpy)(bpy)OH_2]^{2+}$, but it is readily measurable using our technique. In addition, we have observed that the choice of buffer dramatically affects the measured thermodynamic binding energy, possibly denoting a difference in the nonelectrostatic binding affinities of the buffer cations.

Experimental Section

Materials. Calf thymus DNA was purchased from Sigma and used according to published procedures.[14] Phosphate buffer solutions were generated using KH_2PO_4 and K_2HPO_4 as described by Boyd.[15] Tris-HCl buffer solutions were generated by combining the appropriate amounts of HCl and Trizma base (Sigma). Water was purified with a MilliQ purification system. The complexes $K_4[Pt_2(P_2O_5H_2)_4]$ and $[Ru(tpy)(bpy)OH_2](ClO_4)_2$ were prepared by literature methods.[16,17]

Measurements. Emission spectra were measured in 1-cm square quartz cells using a Spex Industries FluoroMax spectrofluorometer. The emission spectra were recorded at 1 nm intervals from 450–600 nm using: an excitation wavelength of 390 nm, an integration time of 0.05–0.075 s, and excitation and emission slit widths of 0.5 and 1.0 mm, respectively. The emission spectra were corrected for photomultiplier tube response using correction factors provided by the instrument manufacturer. Samples for emission experiments were prepared by mixing stock solutions of $[Pt_2(pop)_4]^{4-}$, quencher, and calf thymus DNA and diluting 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 (ϵ(260 nm) = 6600 M^{-1} cm^{-1}). 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 μM, and that of $[Ru(tpy)(bpy)OH_2]^{2+}$ was 20 μM. Effective rate constants were calculated from peak emission intensities obtained at a single quencher concentration by application of the Stern–Volmer equation. Binding constants for $[Ru(tpy)(bpy)OH_2]^{2+}$ were calculated by the previously described method.[9]

Results and Discussion

The DNA binding constants for many bpy complexes have been determined.[9,13,14,16] The measured binding constants, typically less than 1000 M^{-1}, are 3 to 4 orders of magnitude smaller than those of metallointercalators, such as phi and dppz complexes, and about 10 times lower than phen complexes.[9,13] This low affinity for DNA has been attributed to a binding interaction that is almost entirely due to electrostatic forces.[13] If this is the case, K°_{t} would be expected to be nearly zero.[17]

To investigate this hypothesis, the dependence of the DNA binding of $[Ru(tpy)(bpy)OH_2]^{2+}$ on the salt concentration was

† Present address: Department of Chemistry, University of California, Riverside, California 92521-0403.

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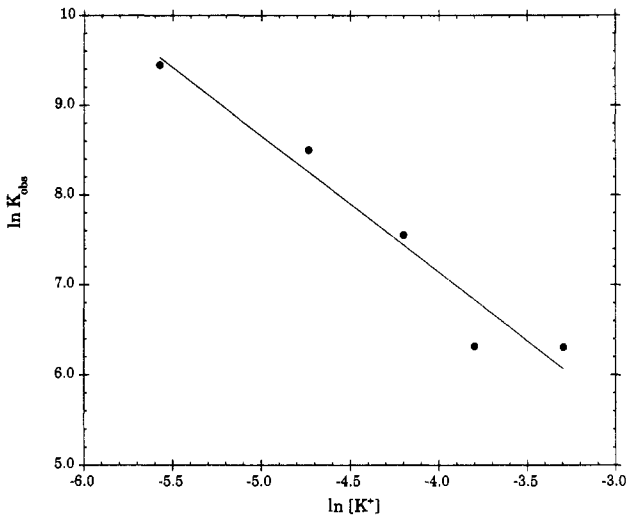

Figure 1. Dependence of the observed binding constant for $[Ru(tpy)(bpy)OH_2]^{2+}$ on the concentration of potassium ions in phosphate buffer. The x axis reflects the actual concentration of potassium ions and not the ionic strength.

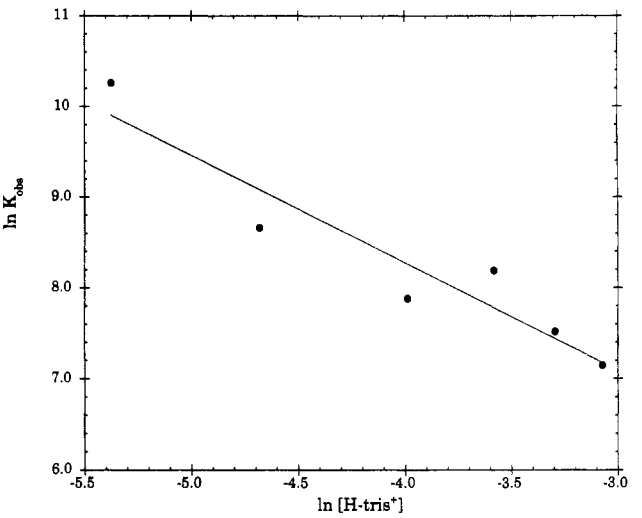

Figure 2. Dependence of the observed binding constant for $[Ru(tpy)(bpy)OH_2]^{2+}$ on the concentration of H–Tris$^+$ ions in tris buffer.

examined. The ionic strength of the phosphate buffer was varied from 0.005 M to 0.05 M, and the resulting plot of ln K_{obs} vs. ln[K^+] is shown in Figure 1. Note that the plot in Figure 1 is in terms of the concentration of potassium ions, not the ionic strength of the buffer. The low binding affinity of this complex requires much higher concentrations of DNA for the binding constant determination, thereby increasing the difficulty of the experiment and the degree of scatter in the data relative to analogous plots we have published for high-affinity complexes.[9] Nevertheless, the slope obtained (–1.5) is similar to those for $[Ru(tpy)(dppz)OH_2]^{2+}$ and $[Ru(phen)_3]^{2+}$. From the data in Figure 1, $K\,^\circ_t$ can be calculated to be 4.2 ± 1.2 M^{-1}, which is significantly smaller than that of $[Ru(phen)_3]^{2+}$ and $[Ru(tpy)(dppz)OH_2]^{2+}$[8,9] and only a factor of 2 higher than that for which we could no longer make a meaningful measurement. This result strongly supports the idea that complexes of bpy, such as $[Ru(tpy)(bpy)OH_2]^{2+}$, bind to DNA almost solely by means of electrostatic interactions. Similar conclusions have been drawn from similar experiments on the binding of $Co(NH_3)_6^{3+}$.[17]

During these experiments, we observed that in a Tris–HCl buffer system (Tris = tris(hydroxymethyl)aminomethane), the DNA binding constant of $[Ru(tpy)(bpy)OH_2]^{2+}$ was somewhat higher than in potassium phosphate buffer of the same cation concentration. Shown in Figure 2 is the plot of ln K_{obs} vs ln-[H–Tris$^+$] obtained for $[Ru(tpy)(bpy)OH_2]^{2+}$ in Tris–HCl buffer. The DNA binding constants are indeed consistently larger in tris than in phosphate buffer. The slope of –1.2 is also similar to those observed for nearly all of the divalent ruthenium complexes studied so far. In fact, the slopes observed for all the divalent ruthenium complexes studied thus far fall in the range of 1.35 ± 0.15, which indicates a very similar contribution to the binding affinity from electrostatics for all the complexes. Equation 1 can be used to calculate a $K\,^\circ_t$ from Figure 2 of 53 ± 17 M^{-1}, which is considerably larger than the value of 4.2 M^{-1} calculated for potassium phosphate solution.

The thermodynamic binding parameters for $[Ru(tpy)(bpy)OH_2]^{2+}$ in tris and phosphate buffers are given in Table 1. The parameter $\Delta G\,^\circ_t$ represents the free energy of binding in the absence of electrostatic effects. This quantity also shows a dramatic dependence on the composition of the buffer system, with average values of –0.8 kcal/mol in potassium phosphate and –2.3 kcal/mol in Tris–HCl. Chaires et al. have shown that the DNA binding of $[Ru(phen)_3]^{2+}$ is entropically driven by release of counterions from the DNA.[18] In the two buffer systems used here, these counterions are K^+ and H–Tris$^+$. These two cations are quite distinct: the alkali metal cation will be hydrated by at least a primary sphere of six water molecules, while H–Tris$^+$ is not likely to be as strongly solvated.

The difference in DNA binding exhibited by $[Ru(tpy)(bpy)OH_2]^{2+}$ in the different buffers probably arises from a different ability of the cations to compete with the metal complex for binding, which would require that K^+ exhibit a greater affinity for DNA than H–Tris$^+$. Along these lines, Anderson and Record have found that the affinity of Na^+ for DNA is several times greater than that of tetraalkylammonium ions.[19] One explanation for this could lie in the degree of hydration of the two cations, since the K^+-bound water molecules can form several hydrogen bonds with the polyphosphate backbone of DNA.[20] These hydrogen bonds must be broken for the counter ions to be released. Thus, the potassium ions will bind more strongly to DNA than the H–Tris$^+$ ions, thereby making displacement of the cation by the metal complex more difficult. The 1.5 ± 0.4 kcal/mol difference measured for the thermodynamic free energy of binding of $[Ru(tpy)(bpy)OH_2]^{2+}$ to DNA therefore gives a measure of the thermodynamic binding affinity for the hydrated potassium ion relative to H–Tris$^+$. This value is in good agreement with a recent estimate of the thermodynamic affinity for Na^+ and Mg^{2+} (1.3 ± 0.2 kcal/mol) made by Black and Cowan by NMR methods.[21] Interestingly, $\Delta G\,^\circ_t$ is apparently the same for Na^+, K^+, and Mg^{2+}.

Conclusions

Two important conclusions can be drawn from the data in Table 1. The first is apparent from inspection of the parameters for the three ruthenium complexes in alkali cation buffers. Since $Z\psi$ has been shown to be similar for all three complexes, the contribution of electrostatic forces to the binding of all three is the same. The bpy complex, with a $\Delta G\,^\circ_t$ of –0.8 kcal/mol, is quite close to binding solely by electrostatic forces. Recent studies by Chaires et al. have used polyelectrolyte theory to demonstrate that binding of phen complexes is primarily electrostatic.[8] However, comparison of the $\Delta G\,^\circ_t$ values reveals that the value for the phen complex is actually halfway between those for bpy and dppz. Thus, while electrostatic forces may contribute a large

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Table 1. Thermodynamic Parameters for Binding of Metal Complexes to DNA

complex	buffer cation	K (M^{-1})[a]	$K°_t$ (M^{-1})	$\Delta G°_t$ (kcal/mol)
$[Ru(tpy)(bpy)OH_2]^{2+}$	K^+	660	4.2 ● 1.2	−0.8 ± 0.2
$[Ru(tpy)(bpy)OH_2]^{2+}$	H–Tris$^+$	1300	53 ± 17	−2.3 ± 0.2
Λ-$[Ru(phen)_3]^{2+}$ [b]	Na^+	11000	300	−3.4
$[Ru(tpy)(dppz)OH_2]^{2+}$ [c]	K^+/Na^+ [d]	700000	27000	−5.9 ± 0.3

[a] Ionic strength = 50 mM; thermodynamic parameters were determined in terms of actual concentrations of K^+ and H–Tris$^+$ cations. [b] Reference 8. [c] Reference 9. [d] Identical results obtained with either Na^+ or K^+ buffer cations.

portion of the binding affinity of phen complexes, the contribution due to nonelectrostatic forces is fully half that of dppz complexes, which have been shown by numerous techniques, including viscometry and helical unwinding,[11,22] to be true metallointercalators. Conversely, the $\Delta G°_t$ for the phen complex is clearly much larger than that for the bpy complex, which does indeed bind almost solely because of electrostatics. This particular experiment does not reveal the nature of the forces that contribute to $\Delta G°_t$ for phen complexes, but our results certainly support some sort of partial intercalation binding mode, as proposed elsewhere.[8,23,24]

The second important conclusion that can be drawn from Table 1 is evident upon comparing the binding affinities for $[Ru(tpy)(bpy)OH_2]^{2+}$ in the two buffer systems. Clearly, the nature of the buffer cation can dramatically affect the measured binding affinities for cationic complexes. In addition, our study implies that the binding affinity for K^+ is 1.5 kcal/mol larger than that of H–Tris$^+$, which probably arises from hydrogen bonding of the waters of hydration to DNA phosphates. Careful comparison of the electrostatic and nonelectrostatic contributions to the binding affinities of hydrated cations may be particularly important in studies of reactions of nucleic acids where hydrated cations play a critical role, as in ribozyme chemistry.[25]

The ability to make the detailed comparisons of binding thermodynamics discussed here is dependent on obtaining high-precision information on binding affinities. Affinities for both low- and high-affinity functionalities, such as bpy and dppz, are very difficult to quantitate using conventional methods, especially for metal complexes.[9,10,13,22] The data in Table 1 indicate that our method of $Pt_2(pop)_4^{4-}$ quenching provides a resolution of about ±0.2 kcal/mol in ΔG terms, which is more than sufficient to permit the detailed comparisons made here. Future studies will involve quantitation of affinities for other physiologically relevant moieties, such as hydrated metal ions.

Acknowledgment. H.H.T. thanks the National Science Foundation for a Presidential Young Investigator Award and the David and Lucile Packard Foundation for a Fellowship in Science and Engineering.

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