Quantitative Evaluation of Electrostatic and Hydrogen-Bonding Contributions to Metal Cofactor Binding to Nucleic Acids

C. B. Black and J. A. Cowan*

Contribution from Evans Laboratory of Chemistry, The Ohio State University, 120 West 18th Avenue, Columbus, Ohio 43210

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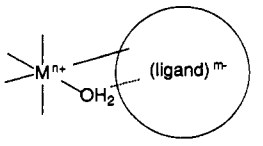
Abstract: The binding free energy (ΔG_b) of a hydrated alkali or alkaline earth ion to double-strand nucleic acids is dominated by electrostatic (ΔG_{es}) and hydrogen-bonding (ΔG_{hb}) contributions. We have estimated the relative magnitudes of these two terms by use of metal complexes of defined charge and hydrogen-bonding capability. A strategy is described where ΔG_b for $M^{n+}(aq)$ is compared with values obtained from substitutionally-inert cobaltic-ammine complexes of similar charge ($[Co(NH_3)_{6-z}X_z]^{n+}$, X = NH_3, NO_2^-). Values for the latter are dominated by the electrostatic term, and so ΔG_{hb} can be determined by direct comparison with the hydrated $Mg^{2+}(aq)$ or $Na^+(aq)$ ion of equivalent charge. Apparent binding affinities (K_a, M^{-1}) for a series of aquated metal ions ($Mg^{2+}(aq)$, $Na^+(aq)$) and cobalt coordination complexes ($Co(NH_3)_6^{3+}$, $[Co(NH_3)_5NO_2]^{2+}$, $[Co(NH_3)_4(NO_2)_2]^+$) to B- and A-configuration nucleic acids have been determined in 20 mM Tris (pH 7.0) by NMR line-shape analysis and the neighbor exclusion model of McGhee–von Hippel. B-conformer nucleic acids: $[Co(NH_3)_6]^{3+}$, 14 800 M^{-1}; $[Co(NH_3)_5NO_2]^{2+}$, 1500 M^{-1}; $[Co(NH_3)_4(NO_2)_2]^+$, 20 M^{-1}; $Mg^{2+}(aq)$, 12 800 M^{-1}; $Na^+(aq)$, 150 M^{-1}. A-conformer nucleic acids: $[Co(NH_3)_6]^{3+}$, 4200 M^{-1}; $[Co(NH_3)_5NO_2]^{2+}$, 250 M^{-1}; $[Co(NH_3)_4(NO_2)_2]^+$, undetermined; $Mg^{2+}(aq)$, 2500 M^{-1}; $Na^+(aq)$, 8 M^{-1}. Individual contributions from electrostatic attraction and hydrogen bonding have been evaluated and found to be additive for each specific configuration. The results are consistent with the expectations of polyelectrolyte theory. Stronger binding to B-conformers results from electrostatic terms, while the contribution from hydrogen bonding is apparently conformation independent. Variable temperature experiments demonstrate that the main factor dictating the exchange region for bound and free metal species derives from extensive hydrogen bonding to the polynucleotide.

Introduction

A quantitative measure of the magnitude and manner of metal binding to polynucleotides is relevant to understanding the molecular details of the catalytic and structural chemistry of metal ions in nucleic acid biochemistry. The importance of this topic is underscored by recent results in ribozyme and enzyme chemistry,[1-3] and longer standing issues concerning protein–DNA interactions and polyelectrolyte theory.[4,5] Previously, we have presented spectroscopic and thermodynamic arguments that support crystallographic evidence for outer-sphere binding of the fully hydrated alkali and alkaline earth metals ($Mg^{2+}(aq)$, $Na^+(aq)$, $K^+(aq)$) to oligonucleotides as the normal mode of interaction.[2a,3,6a] Electrostatic attractions (ΔG_{es}) and hydrogen bonding (ΔG_{hb}) constitute the two major thermodynamic contributors to the metal-binding energy (ΔG_b). The third minor component is direct coordination (ΔG_d).[10] In this paper we



$$\Delta G_b = \Delta G_{es} + \Delta G_{hb} + \Delta G_d$$

estimate the relative magnitudes of the two major terms (ΔG_{es} and ΔG_{hb}), which have not previously been determined, by use of metal complexes of defined charge and hydrogen-bonding capability. To this end we adopted a strategy where the binding free energy of $Mg^{2+}(aq)$ and $Na^+(aq)$ to oligonucleotides, which includes both electrostatic and hydrogen-bonding terms, was compared with binding energies for substitutionally-inert cobaltic-ammine complexes of similar charge ($[Co(NH_3)_{6-z}X_z]^{n+}$, X = NH_3, NO_2^-). When bound to inert Co(III) ions, the NH_3 ligands show little propensity for hydrogen-bonding interactions, and so the electrostatic term is the dominant contributor to the binding free energy. ΔG_{hb} can then be determined by direct comparison with the hydrated $Mg^{2+}(aq)$ or $Na^+(aq)$ ion of equivalent charge. We find that the electrostatic component shows a strong

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dependence on backbone conformation (A or B), while the contribution from hydrogen bonding is apparently conformation independent.

Experimental Methods

Synthesis of (A·dT)$_{20}$ Hybrid Substrate and DNA Oligonucleotides. Substrate RNA·DNA hybrid was synthesized from dT_{20} (4 mg) and poly (rA) (4 mg) (Pharmacia). The components were dissolved in buffer (50 mM Tris, 100 mM KCl, pH 7.5) and annealed (42–44 °C) for 15 min. After precipitation the resulting pellet was taken up in nuclease buffer and incubated with 500 U mung bean nuclease (30 min, 37 °C), and the hybrid was isolated following normal protocols.[7b] The purity of the 20-mer hybrid was verified by comparison with appropriate molecular weight markers on 20% homogenous PAGE. Low molecular weight DNA oligonucleotides were prepared by sonication of poly-DNA (Boehringer Mannheim) and size selected by agarose gel electrophoresis prior to excising a band of the required size range (100 bp ≤ 200 bp).[11,12] The DNA was isolated and dissolved in 20 mM Tris (pH 7.0).[7b] Oligonucleotide samples were equilibrated in this buffer mixture by at least three cycles of ethanol precipitation. Both hybrid and DNA were stored at −20 °C in 20 mM Tris buffer. Concentrations are defined by phosphate equivalents (determined from a weighed mass of sample).

^{25}Mg NMR Experiments. ^{25}MgO (98 atom % ^{25}Mg) was obtained from Oak Ridge National Laboratory. A stock solution of $^{25}Mg^{2+}$ (~0.25 M) was prepared from the oxide following titration with 1.0 M $HClO_4$ until the solution stabilized at pH 7.0, and the concentration was quantitated by atomic absorption (Perkin-Elmer).[10] Experimental procedures were similar to those described previously for studies of tRNA and 5S rRNA.[6,10] ^{25}Mg NMR spectra were recorded at 18.374 MHz on a Bruker MSL 300 spectrometer. Spectra were obtained without sample spinning using the RIDE pulse sequence to reduce the effects of acoustic ringing.[13] Normally, two left shifts were applied to the data prior to Fourier transformation. A standard 0.3 M $MgCl_2$ solution gave a signal with a line width $\Delta\nu_{1/2}(^{25}Mg^{2+})_{free}$ = 2.5 Hz, in close agreement with literature precedent.[14] No broad resonances were detected by use of larger sweep widths and short preacquisition delays (50 μs). Integrations against an AMP(Mg) standard of similar line width demonstrated that all of the signal intensity could be accounted for. Typical spectral parameters were as follows: spectral digitization, SW = 2000 Hz, SI = 1 K, TD = 1 K; pulse width = 90° (30 μs); acquisition time = 256 ms; preacquisition delay = 200 μs. A line broadening of 5 Hz was used. Titration experiments were carried out at 298 K, with successive additions from the stock solution of $^{25}Mg^{2+}$. In the later portion of the titration, when a good signal/noise was attainable within a reasonable time period, additions of Mg^{2+} were made from a 1 M $MgCl_2$ solution containing natural abundance $^{25}Mg^{2+}$. In all cases the concentrations of stock solutions were accurately determined by atomic absorption. Variable temperature experiments (from 5 to 55 °C) were performed with a fixed $[Mg^{2+}]$/[ligand] ratio, and values for the line width $\Delta\nu_{1/2}(^{25}Mg^{2+})$ obtained before and after heating were similar. The sample was observed to precipitate from solution at temperatures above 55 °C.

Data Analysis. NMR line-shape analysis programs employed in this study were obtained from Dr. T. Drakenberg (Lund, Sweden) and have been described in greater detail elsewhere.[6] The program was modified by using an equation $((r/M_f) = K_a(1 - nr)[(1 - nr)/(1 - (n - 1)r)]^{(n-1)})$ that accounts for binding of multiple ions according to the neighbor exclusion model of McGhee–von Hippel.[24] This relates the apparent binding constant (K_a), free metal ion concentration (M_f), fraction of the binding sites occupied (r), and the site size (n, number of binding sites formally blocked by a metal ion M). Association constants (K_a) and activation energies (ΔG^*) were determined following procedures described previously.[6b,15] Briefly, several parameters are required to fully define the line shape of a resonance. These include the chemical shifts of free and bound ions (normally $\delta(^{25}Mg^{2+})_{free} \sim \delta(^{25}Mg^{2+})_{bound}$). The relative populations of $(Mg^{2+})_{free}$ and $(Mg^{2+})_{bound}$ are dependent on $[Mg^{2+}]$, [ligand], the site size (n), and the binding constant (K_a), and so these parameters are introduced into the analysis routine. The temperature dependence of k_{off} is assumed to follow standard transition-state theory ($k_{off} = (kT/h)e^{-\Delta G^*/RT}$]. This manifests itself through the dependence of $\Delta\nu_{1/2}(^{25}Mg^{2+})$ on the relative rate of exchange between free and bound $^{25}Mg^{2+}$. Several problems arising in ^{25}Mg NMR studies on larger biological macromolecules have been outlined in detail elsewhere.[6,10] Under conditions where $\tau_c\omega_o \leq 1.5$, such as found for most small biological macromolecules and all smaller substrate ligands, the ^{25}Mg NMR line shape has Lorentzian form (the relaxation properties of all possible transitions are effectively equivalent).[16] The analysis program employed does not, however, assume a Lorentzian line form. For large molecules that bind Mg^{2+} strongly ($K_a > 10^4$ M^{-1}), the resonance from the Mg^{2+} complex may be too broad to detect. This is not the case in this study. Integration of resonances demonstrated that all of the signal was observable for all metal/ligand ratios; even when less than 1 equiv of $^{25}Mg^{2+}$ was added. All resonances were readily fit assuming Lorentzian behavior (Figure 1), and data was analyzed by an iterative least-squares fit of digitized NMR spectra as described previously.[6,10] A correlation time (τ_c) of 10 ns was assumed for bound Mg^{2+}(aq) a value consistent with previous measurements on oligonucleotides from our laboratory. This lies at the upper limit for Lorentzian behavior.[15] While it is possible that the bound metal ion possesses a small degree of internal rotational freedom (*i.e.* $\tau_c(Mg^{2+})_{bound} < \tau_c(DNA)$), this is unlikely to be significant, otherwise Lorentzian signals would be commonly observed in NMR studies of magnesium binding to larger polynucleotides. Furthermore, the association constant (K_a) and off-rate (k_{ex}) would not be affected by internal motion, although the quadrupole coupling constant (χ_B) might be

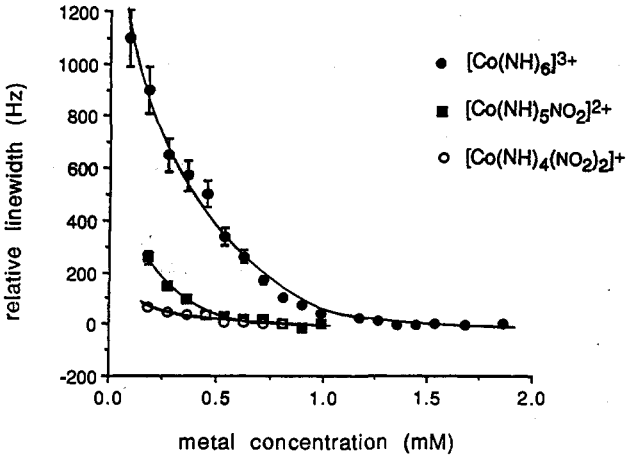

Figure 1. Example of cobalt titration curves obtained from binding studies with $(dA)_n$·$d(T)_n$ at 298 K (pH 7). Binding was evaluated by monitoring line broadening of the ^{59}Co NMR resonance in solutions of varying metal: DNA concentration ratios. Relative line widths were obtained by subtracting the natural line width obtained from the complex in free aqueous solution from the value obtained in the presence of nucleotide. General solution conditions in 20 mM Tris (pH 7.0): [nucleotide] varied from 2.5 to 2.1 mM and $[Co^{n+}]$ varied from 0.09 to 1.8 mM. Spectra were obtained in H_2O solutions to avoid interference from line broadening resulting from partial deuteration of NH_3 ligands.[21a] Experimental points are shown relative to a theoretical curve obtained by joining calculated points from the fitting analysis (see Experimental Methods and refs 6 and 10 for further details). The analysis program accounts for binding of multiple ions by the neighbor exclusion model of McGhee and von Hippel.

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underestimated. We assume one metal-binding site per phosphate for the purpose of data fitting, which is similar to values that we have experimentally determined for low molecular weight tRNA and rRNA molecules.[6,10] This value refers to the total number of available binding sites and does not imply that all are necessarily populated at any given concentration of Mg^{2+}. Data was analyzed by an iterative least-squares fit of digitized NMR spectra. Zero filling was employed where necessary to better define the peak. Typically, each spectrum was defined by 30 points, and the best combination of ΔG^*, χ_B, and K_a was found by an iterative procedure. Consideration of the variation of $\Delta\nu_{1/2}(^{25}Mg^{2+})$ with $[Mg^{2+}]/[\text{ligand}]$ and temperature gives values for the association constant (K_a), quadrupole coupling constant (χ_B), activation free energy (ΔG^*), and the off-rate (k_{off}).[15] Titration and variable temperature (VT) data are analyzed cyclically to give the best fit because of the interdependence of equations containing K_a, χ_B, and ΔG^* as variables.[6,14,15] The analysis routine, considering all data sets, gives rise to unique values for each of the aforementioned parameters; however, the individual enthalpic and entropic components of the activation energies for the monophosphate complexes are most likely unreliable since ΔH^* is defined by the slope and inflection in the slow–intermediate exchange region of the VT plot. For this reason the relative values of ΔH^* and ΔS^* are not considered. Most ions will lie in similar structural environments, and so we would not expect a wide distribution of exchange rates that might introduce error in the determination of K_a and ΔG^*. The very small number of unusual binding sites that may exist do not significantly influence the overall results derived from the analysis. As a result of the low sensitivity of the ^{25}Mg nucleus for NMR studies, we estimate errors in the range ±10–20%.

^{59}Co NMR Experiments and Data Analysis. ^{59}Co NMR spectra were recorded at 71.212 MHz on a Bruker MSL 300 spectrometer. Spectra were obtained using the WALTZ pulse sequence in H_2O solutions to avoid interference from isotopic shifts and with no sample spinning. Typical spectral parameters were as follows: spectral digitization, sweep width = 16 130 Hz, frequency domain (SI) = 1 K, time domain (TD) = 1 K; pulse width = 90° (15 μs); acquisition time = 32 ms; delay time between spectra = 400 ms; preacquisition delay = 31 μs. A line broadening of 10 Hz was used in data processing. Titration experiments were carried out at 295 K in 20 mM Tris (pH 7.0), with successive additions from 0.1 M stock solutions of cobalt complexes (Figure 1). Variable temperature experiments were carried out over a temperature range from 8 to 45 °C with a fixed [complex]/[nucleotide] ratio.

An analysis program similar to that employed for $^{25}Mg^{2+}$ was used after appropriate adjustments to account for the distinct nuclear spin of ^{59}Co ($I = 7/2$). Integration of resonances demonstrated that all of the signal was observable for all metal/ligand ratios. Variable temperature experiments demonstrated essentially no variation of $\Delta\nu_{1/2}(^{59}Co)$ with temperature, and so for the purposes of data fitting we assumed a modest activation free energy ΔG^* of 3 kcal mol^{-1}. Since ΔG^* for Mg^{2+}(aq), which lies in the slow exchange region, is ~15 kcal mol^{-1}, this is an appropriate assumption. Parameters obtained from best fits to the data show little variation with ΔG^* in the range 1–5 kcal mol^{-1}.

Quadrupole coupling constants (χ_B) for the bound and free forms of the complex were found to be similar, as expected from previous work on outer-sphere ion pairs.[17] Significant rotational freedom of the bound complex ($\tau_c \approx 1$ ns)[18] was also assumed inasmuch as the cobalt-bound amines are relatively poor hydrogen bond donors. This assumption does not influence the magnitude of the binding constant K_a, but may affect χ_B. However, the value of χ_B determined with this assumption is consistent with literature values for these complexes obtained in solution,[17] and so the assumption appears to be valid. Longitudinal relaxation times (T_1) were found to be relatively insensitive to binding, as expected from model studies.[17,19] The T_1 of 180 ms determined in aqueous solution varied by only a small amount (165–124 ms) over the range of Co/nucleotide concentrations investigated.

^{23}Na NMR Experiments and Data Analysis. ^{23}Na NMR spectra were recorded at 79.39 MHz on a Bruker MSL 300 instrument in a fashion similar to that described above after appropriate adjustments to account for the distinct nuclear spin of ^{23}Na ($I = 3/2$). Nucleotide samples were dissolved in 20 mM Tris (pH 7.0), and typical spectral parameters were as follows: spectral digitization, sweep width = 3185 Hz, frequency domain (SI) = 8 K, time domain (TD) = 8 K; pulse width = 90° (23 μs); acquisition time = 1.29 s; delay time between spectra = 0.41 s; preacquisition delay = 157 μs. A line broadening of 2 Hz was used in data processing. Earlier approaches reported in the literature describe the use of relaxation data (T_1 values) as the experimentally determined parameter. This requires the assumption that $[Na^+] \gg$ [nucleotide].[20] This was not valid in our study, and so this approach was not used. As for the case of Mg^{2+}(aq) described earlier, the rotational correlation times used in data analysis were asssumed to be close to the Lorentzian limit.[6,10] That is, we assume that the motion of the bound aquated metal ion is both restricted and correlated with the motion of the nucleotide. This is justified by comparison with observations made from previous studies on Na^+ binding to polynucleotides, where deviations from Lorentzian behavior were observed. This can only arise if the motion of the metal ion and nucleotide are strongly correlated.[4a] Variable temperature experiments were carried out over a temperature range from 8 to 45 °C with a fixed $[Na^+]$/[nucleotide] ratio.

Results and Discussion

In this paper we report studies directed toward evaluating the chemistry and bonding interactions that underlie metal binding to nucleic acids. These studies are directly relevant to an understanding of the catalytic and structural chemistry of metal ions in nucleic acid biochemistry. By use of metal complexes of defined charge and hydrogen-bonding capability, we have developed a strategy that allows a detailed and quantitative evaluation of the relative magnitudes of the electrostatic and hydrogen-bonding contributions (ΔG_{es} and ΔG_{hb}, respectively) to the binding free energy of M^{n+} to double-strand nucleic acids. This is achieved by direct comparison of the binding free energy of Mg^{2+}(aq) and Na^+(aq) to oligonucleotides, which includes both electrostatic and hydrogen-bonding terms, with the binding energies for substitutionally-inert cobaltic-ammine complexes of similar charge ($[Co(NH_3)_{6-z}X_z]^{n+}$, X = NH_3, NO_2^-). The rationale, results, and implications for these studies are detailed in the discussion that follows.

Evaluation of Kinetic and Thermodynamic Parameters. Previous studies of the interaction of Mg^{2+}(aq) with $d(G\text{-}C)_n$, $d(A\text{-}T)_n$, $d(G)_n{\cdot}d(C)_n$, and $d(A)_n{\cdot}d(T)_n$ by use of ^{25}Mg NMR spectroscopy have demonstrated distinct binding behavior for G/C *vs* A/T DNA.[11] For this reason our attention is restricted for the most part to dA·dT oligonucleotides and A·dT hybrids. In contrast to prior studies,[4,25-26] we routinely use systematic NMR line-shape analysis to determine binding parameters.[6] After accounting for variations in solution ionic strength, our results do, however, show relatively good agreement with data obtained from other laboratories, for example, results for $Co(NH_3)_6^{3+}$ binding to DNA,[21] Mg^{2+}(aq) interactions with RNA,[22] and other Na^+(aq) and Mg^{2+}(aq) binding data for DNA and RNA.[20,23] This provides confidence in the line-shape analysis approach. Inasmuch as the electrostatic attraction of the polynucleotide backbone for positively charged metal ions or complexes will be slightly different at the beginning relative to the later stages of the titration, we view binding in terms of the McGhee–von Hippel neighbor exclusion model, assuming a set of identical but mutually dependent binding sites.[12,24] An analysis under these terms provides a good fit to the data (Figures 1 and 2), which is not improved by assuming more than one binding constant. We consider the binding affinities in Table 1 to be *effective binding constants* (K_{eff}).

Evaluation of Electrostatic and Hydrogen-Bonding Contributions to Metal Cofactor Binding. The binding parameters (K_a, M^{-1}) reported for $Co(NH_3)_6^{3+}$ in Table 1 show good correlation with values for Mg^{2+}(aq) binding to double-strand DNA (B-conformation) and double-strand RNA (A-conformation).[6,10,21-23] The relative trend of K_a(B-conformer) > K_a(A-conformer) is also retained. The apparent larger effective binding constant for a B- relative to an A-conformation reflects the distinctive size and structure of the major and minor grooves and possibly also distinct local charge densities for these two conformers. The distinct binding affinities are not due to the smaller size of the

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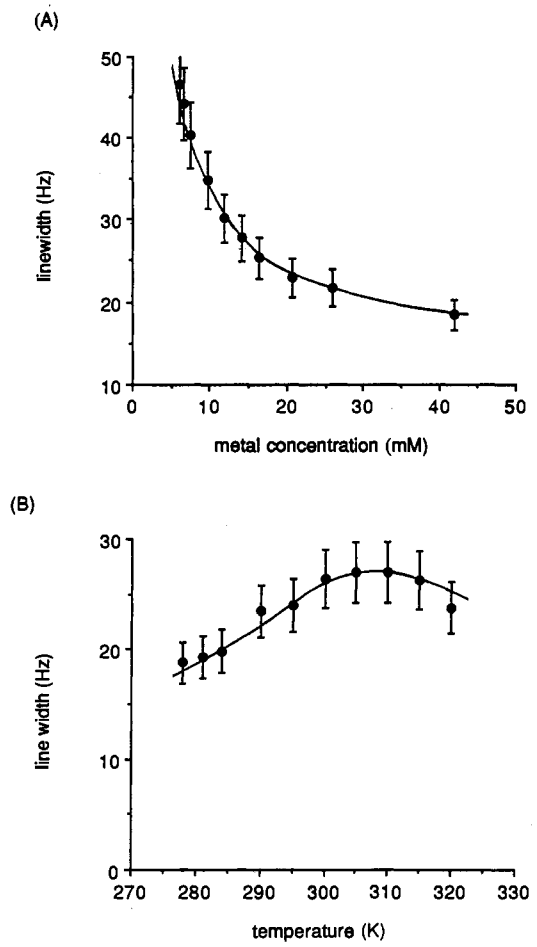

Figure 2. (A) Example of sodium titration curves obtained from binding studies with ds RNA at 295 K (pH 7). Binding was evaluated by monitoring line broadening of the ^{23}Na NMR resonance in solutions of varying metal: DNA concentration ratios. General solution conditions in 20 mM Tris (pH 7.0): [nucleotide] varied from 6.2 to 5.9 mM and [Na^+] varied from 6.1 to 42 mM. (B) Variation of line width $\Delta\nu_{1/2}(^{23}Na^+)$ with temperature. General solution conditions in 20 mM Tris (pH 7.0): [nucleotide] = 6.1 mM, [Na^+] = 15 mM (pH 7). Variable temperature experiments were conducted from 8 to 45 °C, and values for $\Delta\nu_{1/2}(^{23}Na^+)$ obtained before and after heating were similar. An activation energy $\Delta G^* \sim 13.0(\pm 0.5)$ kcal mole^{-1} was determined. In both A and B the experimental points are shown relative to a theoretical curve obtained by joining calculated points from the fitting analysis (see Experimental Methods and refs 6 and 10 for further details). The analysis program accounts for binding of multiple ions by the neighbor exclusion model of McGhee and von Hippel.

hybrid employed relative to ds DNA. Results with longer hybrids lead to similar conclusions. When bound to inert Co(III) ions the NH_3 ligands show little propensity for hydrogen-bonding interactions, and so the decrease in K_a observed for $Co(NH_3)_6^{3+}$ > $[Co(NH_3)_5NO_2]^{2+}$ > $[Co(NH_3)_4(NO_2)_2]^+$ can be attributed almost entirely to the change in the charge of these complexes. A substantially lower hydrogen-bonding capability for bound NH_3 over H_2O would be expected from several lines of evidence. (1) Klemperer and co-workers have demonstrated NH_3 to be a hydrogen bond acceptor rather than donor.[8] It is evident, however, that the donor capability is greater in those cases where the nitrogen center bears a substantial positive charge, such as the ammonium ion or protonated amines, or the amide nitrogen in peptides bonds (due to extensive conjugation with the carbonyl) that facilitates hydrogen bonding in proteins. In those instances where significant positive charge is not located directly on the nitrogen, such as amines that are complexed to a metal ion, the hydrogen-bonding capability is less evident. Preferential interactions between chiral anions and specific $Co(en)_3^{3+}$ enantiomers have been identified in both solution and the solid state.[9] These

Table 1. Summary of Metal-Binding Constants for Nucleic Acids in B- and A-Configurations

metal complex[c]	$K_a(M^{-1})$[a,b] B-conformer[d]	(n)[e]	$K_a(M^{-1})$[a,b] A-conformer[f]	(n)[g]
$[Co(NH_3)_6]^{3+}$	14 800	2.9	4200	3.2
$[Co(NH_3)_5NO_2]^{2+}$	1500	1.9	250	2.2
$[Co(NH_3)_4(NO_2)_2]^+$	20	1.2	*h*	
Mg^{2+}(aq)	12 800	2.2	2500	2.3
Na^+(aq)	150	1.1	8	1.2

[a] Estimated errors lie in the range ±10–20%. Nucleotide concentrations are determined in terms of phosphate equivalents. Samples were prepared in 20 mM Tris (pH 7.0). [b] Binding affinities were estimated from NMR line-shape analyses as described in the text.[7a] [c] The cobalt complexes, $Mg(H_2O)_6^{2+}$, and $Na(H_2O)_6^+$ are similar in size. [d] Results obtained with $(dA)_n{\cdot}(dT)_n$, $n \leq 200$. [e] Site size estimated for B-DNA. [f] Results obtained with ds RNA and $(A)_x{\cdot}(dT)_x$ hybrids (x = 20–100). Within experimental error there is no significant difference. [g] Site size estimated for A-hybrid. [h] Binding was too weak to measure.

observations have been rationalized in terms of hydrogen bond formation between the amine N–H's and oxygen acceptors on the complex anion, although the strength of these H-bond interactions and the contribution of favorable steric factors and/or crystal-packing forces are not yet well-defined. (2) Cleland and co-workers have noted that the geometric isomers of $Cr(NH_3)_{4-x}(H_2O)_xATP$ derivatives show distinct binding affinities for an enzyme pocket. This observation cannot be readily explained by electrostatic attractions, since these complexes differ only in the cis/trans arrangements of NH_3 and H_2O ligands.[27] Rather, these results most likely reflect the variation of hydrogen-bonding capability according to the relative disposition of NH_3 and H_2O in the coordination sphere of the chromium ion and the available hydrogen bond acceptors in the enzyme pocket. (3) The absence of significant hydrogen bonding by $Co(NH_3)_6^{3+}$ is fully consistent with the absence of a nonelectrostatic contribution to the binding energy (see the analysis in terms of polyelectrolyte theory presented below). A substantive hydrogen-bonding component also explains the comparable binding affinity of the divalent Mg^{2+}(aq) ion for B-DNA, relative to the trivalent $Co(NH_3)_6^{3+}$ complex. Assuming a conservative value for a typical H-bond energy of ~1–3 kcal mol^{-1}, it is clear that formation of H-bond networks can result in a substantial contribution to binding energies.

Our experiments provide an estimate of the electrostatic contributions to binding energy. Comparison of binding affinities (K_a) for Mg^{2+}(aq) or Na^+(aq) with data for inert cobalt complexes of similar charge ($[Co(NH_3)_5NO_2]^{2+}$ and $[Co(NH_3)_4(NO_2)_2]^+$, respectively) affords an opportunity to quantitatively evaluate the influence of hydrogen bonding from waters of solvation. By use of the thermodynamic relationship $\Delta G_b = -RT \ln K_a$, electrostatic contributions were estimated from K_a for the inert complex with equivalent charge (n+) and subtracted from ΔG values for the aquated species M^{n+}(aq) to determine the binding contribution from hydrogen bonding. This data is summarized in Table 2. The approach is straightforward, and the results are entirely self-consistent. Comparison of binding data for B- and A-conformers (Table 2) suggests that the major difference in binding energies derives not from hydrogen bonding but rather from the electrostatic term. The structural variations of these two configurations most likely result in distinct negative charge densities (q^-) and intersite distances (r^{+-}) in the equation defining the electrostatic interaction energy, E = (constant)$(q^+q^-)/r^{+-}$.

Analysis in Terms of Polyelectrolyte Theory. In support of the analysis presented above, we compare our conclusions against the results obtained from polyelectrolyte theory. Binding constants can be analyzed in terms of polyelectrolyte theory according to eq 1,[25] where Z is the charge on the metal species, ψ is the

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Table 2. Summary of Electrostatic and Hydrogen Bond Contributions to Metal Complex Binding Energy

	B-conformer $-\Delta G_b$ (kcal mol^{-1})	A-conformer $-\Delta G_b$ (kcal mol^{-1})
M^{3+} (electrostatic)[a]	5.7(±0.4)	4.9(±0.4)
M^{2+} (electrostatic)[a]	4.3(±0.3)	3.3(±0.3)
M^{+}(electrostatic)[a]	1.8(±0.2)	
M^{n+}(aq) (hydrogen bonding)[b]	1.3(±0.2)[c]	1.2(±0.2)[c]

[a] Estimated from the binding affinities (K_a) for the cobalt complexes in Table 1. The temperature was taken as 298 K. [b] An average value from the energy difference summarized as $\Delta G_b(M^{n+},aq) - \Delta G_b(Co^{n+})$. [c] These values are in good agreement with predictions from polyelectrolyte theory (see text). For magnesium data eq 1 can be used to estimate a value of ~1.0 kcal mol^{-1} for a B-conformer and ~0.7 kcal mol^{-1} for an A-conformer.

$$\ln K_{eff} = \ln K_t^\circ + Z\xi^{-1}\{\ln(\gamma_\pm\delta)\} - Z\psi\{\ln [M^+]\} \quad (1)$$

fraction of ions associated with each phosphate, K_{eff} is the effective binding constant for a monovalent cation concentration $[M^+]$, K_t° is the "thermodynamic" binding constant that reflects nonelectrostatic interactions, $\gamma_\pm$ is the mean activity coefficient at a cation concentration $[M^+]$, and terms ξ and δ are constants (for B-DNA, $\xi = 4.2$, $\delta = 0.56$). Parameter K_t° reflects the contribution of nonelectrostatic interactions to the binding of metals or ligands to a polyanion. In our case we ascribe K_t° to hydrogen bonding, an estimate of which can be made using eq 1) and data in Table 1. For example, for divalent cations binding to B-DNA it is known that $Z\psi \sim 1.76$. Rearranging eq 1 in the form $\ln K_t^\circ = \ln K_{eff} - Z\xi^{-1}\{\ln(\gamma_\pm\delta)\} + Z\psi\{\ln [M^+]\}$, a value for $\Delta G_t^\circ = -RT \ln K_t^\circ = 1.0$ kcal mol^{-1} can be calculated, which is close to the value estimated (~1.1–1.3(±0.2) kcal mol^{-1}] by comparison of binding constants from aquated ions (Mg^{2+}(aq) and Na^+(aq)) and cobalt–ammine derivatives bearing the same charge. In the same way, published data from Record and co-workers for $Co(NH_3)_6^{3+}$ binding to calf-thymus DNA show a small intercept for a plot of ln K_{eff} versus ln $[Na^+]$, indicating that the association of $Co(NH_3)_6^{3+}$ is primarily electrostatic in nature (the nonelectrostatic term is small; $\ln K_t^\circ + Z\xi^{-1}\{\ln(\gamma_\pm\delta)\} \sim 0.1$), and that the hydrogen-bonding contribution is negligible.[21] In contrast, equivalent experiments for Mg^{2+}(aq) binding to polynucleotides demonstrate a larger nonelectrostatic component. For example, the intercept from a plot of ln K_{eff} versus ln $[Na^+]$ for poly(A)·poly(U) is nonzero (that is, $\ln K_t^\circ + Z\xi^{-1}\{\ln(\gamma_\pm\delta)\} \sim 1.3$).[26] The constants ξ, ψ, and δ are similar for B-DNA and A-RNA, and so we may readily calculate K_t° and the contribution to the binding energy. This gives an energy of ~0.9 kcal mol^{-1}, which compares reasonably well with the value of 1.2 kcal mol^{-1} noted in Table 2.

From the aforementioned analysis, we can understand the difference in nonelectrostatic interactions for the binding of Mg^{2+}(aq) and $Co(NH_3)_6^{3+}$ with double-strand nucleotides in terms of outer-sphere coordination where hydrogen bonding from metal-bound solvent waters can contribute to the binding energy. Previously, we have demonstrated that Mg^{2+}(aq) will typically coordinate to double-strand nucleotides as an outer-sphere complex, retaining its inner solvation shell.[6,10] Binding of fully hydrated Mg^{2+}(aq) has been predicted by the counterion condensation model of Manning.[4d] Outer-sphere binding has also been experimentally demonstrated in crystallographic studies by Rich and co-workers.[28] Other specific examples are described in detail in ref 2a. We have further detailed the thermodynamic rationale for outer-sphere coordination in a recent publication.[11]

In summary, our results are fully consistent with previous studies of metal ion binding to polynucleotides obtained by a variety of physicochemical methods and theoretical models. Furthermore, the results are consistent with expectations from polyelectrolyte theory. Our approach does, however, provide an experimental method of probing the molecular details of the chemistry underlying these binding interactions.

Variable Temperature Behavior. Previously, we have tried to address the interesting question of what factors determine the profile of a variable temperature plot for metal ion exchange; that is, what factors govern the transition temperature where a metal complex will move from slow, through intermediate, to fast exchange? Such plots reflect the switch from a chemical-exchange-dominated line width (slow), to a relaxation-dominated line width (fast). The observed trends do not depend on whether an inner- or outer-sphere coordination mode is adopted, since proteins generally bind Mg^{2+} by direct inner-sphere contacts from the protein residues to the metal ion but still lie in the slow exchange region at ambient temperature.[2a,6,14] Furthermore, the exchange region cannot be correlated with K_a since both ADP^{3-} and ATP^{4-} bind Mg^{2+} with $K_a > 10^3$ M^{-1}, in contrast to the weaker complex with RNA, but lie in the fast exchange region. A possible explanation is that the exchange region for metal binding is determined by the total number of binding contacts to the aquated Mg^{2+} center, either inner or outer sphere.[10] Both RNA and protein complexes typically form ≥4 binding contacts with $Mg(H_2O)_n^{2+}$,[28,29] which is not the case for low molecular weight phosphate ligands.

These inert cobalt complexes allow further evaluation of the factors regulating metal exchange rates with the nucleic acid. Previously, we reported exchange in the slow exchange region for Mg^{2+}(aq) interacting with oligonucleotides at ambient temperature ($k_{ex} \sim 2 \times 10^3$ s^{-1}).[6,10] This contrasted with exchange in the fast region observed for Mg^{2+}(aq) binding to individual nucleotide mono-, di-, and triphosphates.[10] We rationalized this observation in terms of a hydrogen bond network formed between waters of solvation on Mg^{2+} and base heteroatoms or oxygens on the ribose–phosphate backbone.[10] This network provides a sufficiently large activation barrier to inhibit facile release of M^{n+}(aq). The cobalt complexes employed in this study show essentially no variation of ^{59}Co NMR line width with temperature (even for trivalent $Co(NH_3)_6^{3+}$). These complexes fall in the fast exchange region, which we attribute to the inability of the coordinating NH_3 ligands to engage in substantial hydrogen bond interactions. That is, the activation barrier for chemical exchange is low, and so rapid exchange is both expected and observed.

Concluding Remarks. The specificity of polynucleotide interactions with proteins, antibiotics, and other biological ligands in part reflects the relative affinities of metal cofactors and binding ligands.[10,25] Studies directed toward evaluating the molecular details of metal binding to polynucleotides are relevant to understanding the catalytic and structural chemistry of metal ions in nucleic acid biochemistry. In this paper we have demonstrated the rational use of chemical probe ions to explore the chemistry underlying these binding interactions.

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