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Critical amount of oligovalent ion binding required for the B-Z transition of poly (dG-m^{5}dC)

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ABSTRACT

By working at very low Na^+ concentrations (1mM and less), the number of bound Mg(2+), cobalt hexamine(3+), and spermine(4+) necessary to induce the B-Z transition of poly (dG-m^{5}dC) has been directly measured. The results show that if as little as 1 cobalt hexamine(3+) or spermine(4+) is bound per 40-50 nucleotides the transition will occur. A greater fraction of bound Mg(2+) is required, 1 bound Mg(2+)/10 nucleotides. The dependence of the transition midpoint concentrations of oligovalent ions on Na^+ concentrations suggests that specific ion binding energies, not included in counterion condensation theory, are responsible for the transition.

INTRODUCTION

The left handed Z form of poly (dG-dC) and its methylated analogue poly (dG-m^{5}dC) is the stable structure in solution at any ionic strength if a sufficient concentration of di-, tri-, or tetra-valent ions is present (1). The critical concentration of +N ions is a strong function of ion type, charge, and monovalent salt concentration. In this paper, we take advantage of the fact that at very low ionic strengths and for low levels of oligovalent ion binding, virtually all added +N ions bind to DNA, if the polynucleotide concentration is much greater than the +N ion concentration. At low monovalent ion concentrations, therefore, one can directly measure the fraction of +N ions bound per polynucleotide phosphate, Θ_{+N}, at the midtransition point rather than rely on Manning theory or the Poisson-Boltzmann equation (see, for example, 2,3,4) to calculate a Θ_{+N}. We have applied this approach to determine Θ_{+N} for the B - Z transition of poly (dG-m^{5}dC) with Mg^{2+}, $Co(NH_3)_6^{3+}$, and spermine^{4+}. As an experimental check, observed Θ_{+N} values are shown to be independent of ionic strength over a range of low

monovalent ion concentrations and of polynucleotide concentration. Critical values of Θ_{+_N} vary from $1Mg^{2+}/10$ nucleotides to 1/(40-50) nucleotides for $Co(NH_3)_6^{3+}$ and spermine.

These low levels of binding and their ionic strength independence are inconsistent with a general electrostatic stabilization of the Z form relative to the B structure, as calculated, for example, by counterion condensation theory. The governing effect of oligovalent ions on the transition appears to be due to the surface chemistry of binding. Specific ion binding energy differences, for example, site binding or the interaction with polynucleotide hydration structure, would seem from the data to account for the stability of the Z form with added oligovalent ions. From this stand-point, +N ions shift the B - Z equilibrium in much the same way as has been proposed for various drugs (5,6), i.e., the binding free energy is greater for the Z form rather than the right handed B helix.

METHODS AND MATERIALS

The experiments were performed essentially as described previously (1). Synthetic poly(dG-m^5dC) was obtained from P-L Biochemical Co., and used without further purification. $Co(NH_3)_6Cl_3$ was a gift of Jon Widom and spermine.4HCl was purchased from Sigma.

Poly (dG-m^5dC) was exhaustively dialyzed against 0.5 mM Na cacodylate and 20 μM Na_2 EDTA in deionized distilled water, to ensure that any trace amounts of oligovalent counterions were removed. The Na^+ concentration was then varied by the addition of NaCl. To determine the room temperature transition midpoint concentration of oligovalent ions at a constant Na^+ concentration, poly (dG-m^5dC) was titrated in small increments of oligovalent ions, the temperature raised to 60° for 10 minutes to ensure conformational equilibrium, then the circular dichroism spectra were measured at room temperature. CD spectra of sample within the transition region showed no further change with time, over several days. Concentrations of poly(dG-m^5dC) were calculated using an extinction coefficient at 260 nm of 7.0×10^3 M^{-1} cm^{-1}.

A Jasco J-500A high sensitivity spectropolarimeter was used

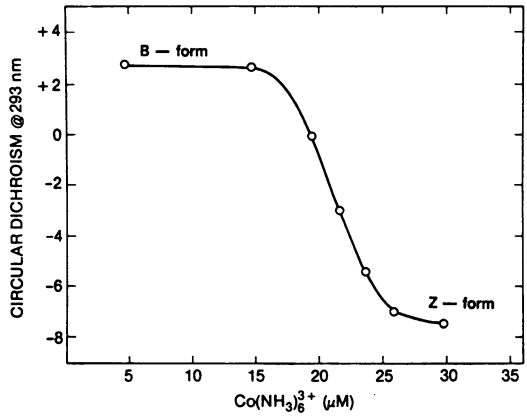

Figure 1: A typical CD titration curve of poly(dG-m^5dC) with $Co(NH_3)_6Cl_3$ at 0.1 M Na^+ is shown. The circular dichroism intensities at 293 nm are given in arbitrary units.

to measure CD spectra. The change in ellipticity at 293 nm was used to monitor the B-Z transition. Occasionally the transition was monitored by the change in absorbance at 290 nm, measured with a Cary 219 spectrophotometer. The two methods gave identical titration curves.

RESULTS

Figure 1 shows a typical CD titration curve for poly (dG-m^5dC) with $Co(NH_3)_6^{3+}$ at .1 M Na^+. Since the curve is stable with time (see above), it is assumed to represent the equilibrium titration curve. We choose for the critical oligovalent ion concentration the interpolated concentration that gives an ellipticity at 293 nm halfway between the B and Z baselines.

Qualitatively, the three ions we study here, Mg^{2+}, $Co(NH_3)_6^{3+}$, and $spermine^{4+}$ all give the same features for the dependence of the critical Θ_{+N} on [Na^+]. We will only, therefore, show graphs for $Co(NH_3)_6^{3+}$. Figure 2a shows the midpoint transition concentrations of $Co(NH_3)_6^{3+}$ as a function of [Na^+] for 3 concentrations of poly (dG-m^5dC) (35, 70, and 140 μM in nucleotide phosphates). Three regions of behavior are apparent. At very low ionic strengths, the

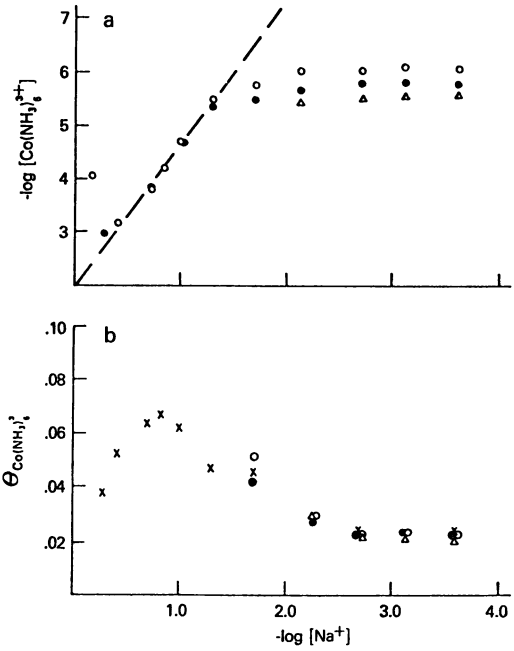

Figure 2a: A log-log plot of the dependence of the transition midpoint concentration of cobalt hexamine(3+) on Na^+ concentration is shown. The different symbols correspond to different poly(dG-m^5dC) concentrations: (o) 35μM, (●) 70μM, and (▲)140μM in nucleotide phosphate. The dashed line shows the linear region of the transition.

Figure 2b: The critical number of cobalt hexamine(3+) bound per nucleotide is determined in two ways. The transition concentrations at low ionic strength are directly converted to θ by dividing by the nucleotide concentration. The different concentrations of poly(dG-m^5dC) are represented by the same symbols as in fig. 2a. Results of a Manning counterion condensation calculation are shown by (x).

concentration of $Co(NH_3)_6^{3+}$ necessary for the transition is independent of $[Na^+]$, but dependent on polynucleotide phosphate concentration. Within a range of intermediate salt concentrations (between about .01 and .6 M Na^+), log $[Co(NH_3)_6^{3+}]_{crit}$ is about linearly proportional to log $[Na^+]$ and independent of polynucleotide concentration. Above about .6M, Na^+ by itself is about sufficient to induce the Z form and an apparent synergism between $Co(NH_3)_6^{3+}$ and Na^+ is observed.

Figure 2b shows the low ionic strength data of 2a normalized by the polynucleotide concentration. It is apparent that this ratio is independent of DNA-phosphate concentration and ionic strength. At these low monovalent ion concentrations, if the polynucleotide concentration is doubled, then the amount of $Co(NH_3)_6^{3+}$ necessary to induce the transition also must be doubled.

DISCUSSION

The analysis of DNA-cation binding has been greatly facilitated by the development of Manning's counterion condensation theory (for review, see 2). In particular, the binding competition between monovalent and di- or tri-valent ions has been quite successfully treated within Manning's framework. In general, Mg^{2+}, for example, binds to DNA more strongly than Na^+ primarily because for every one Mg^{2+} that binds, two Na^+ ions are released. This leads to a favorable entropy for Mg^{2+} binding that will be dependent on Na^+ concentration. The lower the bulk Na^+ concentration, the more favorable will be the entropy gained in releasing bound Na^+ ions. In 1mM Na^+ and low levels of Mg^{2+} binding, Krakauer (7) reported an equilibrium constant (defined by Manning (2) as the ratio of Mg^{2+} bound/DNA-phosphate, Θ, to the free Mg^{2+} concentration) of about 10^6. For small ratios and very low Na^+ concentrations, this means that virtually all added Mg^{2+} will be present as DNA bound counterion. For example, with $[Mg^{2+}]_{total} = 5 \times 10^{-4}$ M, $[Na^+] = 0.5$ mM, and [DNA-phosphate] = 50×10^{-4} M, using eqns 6 and 7 of Wilson and Bloomfield (8), modifying C_2, (free Mg^{2+} concentration) as $C_{2,total}$ - [DNA-P], we calculate $\Theta = .0995$ and $C_{2,bound} \; ([DNA\text{-}P]) / C_{2,free} \sim 140$, or 99.5% of the total Mg^{2+} is bound.

Titration of poly(dG-m^5dC) with di-, tri-, or tetra-valent counterions at low Na^+ concentrations will provide a direct measure of the number of oligovalent ions that must be bound in order to induce the B-Z transition, provided two experimental criteria are met. First, for a given DNA concentration, a plateau value for the amount of oligovalent counterion added is reached, i.e., for sufficiently low monovalent ion

TABLE 1
Oligovalent Ion Binding Parameters for the
B-Z Transition of Poly (dG-m^{5}dC)

Ion	θ_{crit}[a], exp	θ_{crit}[b], Manning	d log $C_{+N,crit}$/d log Na^+ [c]
Mg^{2+}	.10 ± .01	.24	1.95
$Co(NH_3)_6^{3+}$	.023 ± .002	.065	2.85
Spermine^{+4}	.024 ± .003	.13	3.60

a Directly measured at 1mM Na^+

b Calculated from eqs 6 and 7 of Wilson and Bloomfield (8) at .1 M Na^+

c Measured in the linear region of the titration curve

concentrations, the amount of added di-, tri-, or tetra-valent ion to induce the B-Z transition must be independent of the +1 ion concentration. Secondly, the plateau value must be independent of the DNA-phosphate concentration. The data in figure 2b show that, for the B-Z transition, both these requirements are fulfilled.

Table 1 contains a summary of the critical Θ values necessary to induce the Z form at room temperature for the three oligovalent ions we have studied. All correspond to low levels of binding, ranging from 1/40-50 DNA-P for $Co(NH_3)_6^{3+}$ and spermine^{4+} to 1/10 DNA-P for Mg^{2+}.

The region of the curve in fig 2a showing an approximately linear dependence of log $C_{Co}3+$ on log $C_{Na}+$ is an example of a more classical DNA- counterion binding curve, in which most of the added oligovalent ion is free (unbound). Within Manning's framework, for small Θ ratios, the slope of this line should be the charge Z of the oligovalent ion. For the $Co(NH_3)_6^{3+}$ data of fig 2a, the slope is 2.8. The slopes for other oligovalent ions are also given in table 1. All are consistent with small ratios.

Within the Manning formalism, one can calculate Θ values in this intermediate salt by, for example, eqns 6 and 7 of reference 8. These calculated Θ_{+N} values are shown in figure 2b for $Co(NH_3)_6^{3+}$ and also listed in table 1 for all three ions at .1M Na^+ This figure is typical of the data for spermine and Mg^{2+}. Manning theory would suggest that significantly higher ratios are necessary to induce the Z form at intermediate salt

concentrations than at very low ionic strengths. While there is substantial evidence showing that counterion condensation theory correctly predicts slopes of lines for ion binding experiments and maximal Θ ratios for mono- and di-valent ions it is less certain that the theory can be used to calculate reliable absolute binding ratios in competition experiments. Granot and Kearns (4), for example, find that the Manning theory underestimates the number of bound Mn^{2+} ions in this intermediate salt. The binding competition of spermidine with either Na^{+} or Mg^{2+} (9) is, on the other hand, significantly overestimated by the counterion condensation formalism (see, for example, 3). Whether Θ_{crit} values are in fact higher in the intermediate range or the calculated Θ overestimates the actual binding is not known. The low ionic strength plateau, however, provides a direct measure of Θ_{crit}, if an actual plateau is reached and Θ is independent of DNA concentration.

The basic question to be answered is: how is Θ_{crit} related to the energetics of the B-Z transition? We can suggest two somewhat different appoaches to this problem. One is that the Z form is just electrostatically less favorable than the B structure. The added oligovalent counterions then, through general ion condensation, lower the effective charge density sufficiently to reduce the unfavorable electrostatic energy difference below the presumed free energy contributions from other sources favoring the Z form. Within the condensed counterion framework, the general electrostatic free energy depends both on what is on the surface, through the effective charge density and entropic losses due to ion condensation, and on the charge shielding by the diffuse ion atmosphere, calculated from the bulk ionic strength. Alternatively, the effect of +N ions on the B-Z transition can be viewed solely as a DNA surface phenomenon. A critical number of oligovalent ions must be bound on the surface to induce the transition. The most straight forward method to formalize this approach is to assume that the equilibrium constant for binding a +N ion to Z DNA is greater than to the B form. This binding energy difference would arise from specific interactions between the +N ion and the DNA surface, as, for example, discrete site binding (as phosphate

bridging) or a differential stabilization of DNA surface hydration. Rose et al. (10) have found, for example, from NMR studies that specific site binding of Mg^{2+} to DNA is more important than for Na^+, dominating the $^{25}Mg^{2+}$ NMR spectrum.

Within the Manning framework, the two alternatives can be midpoint, i.e., at Θcrit. If additional NaCl is added, a general electrostatics calculation would predict that the equilibrium would shift towards the Z form. Although some +N ions will be competed off the surface by the additional Na^+ and the effective charge density will increase slightly, the increase in ion atmosphere charge shielding through an increased ionic strength will more than compensate energetically. Any time ions, regardless of charge, are added, electrostatic energies will decrease within the counterion condensation model. If, however, a critical number of +N ions must be surface bound to induce the transition to Z DNA, then the addition of +1 ions will drive the transition back to the B form. Experimentally, if more Na^+ is added to the midpoint salt conditions for the $Co(NH_3)_6^{3+}$ induced transition, with $[Na^+]$ <0.6M, for example, the equilibrium is shifted to the B form. It would appear then that differences in specific binding energies for oligovalent counterions between B and Z forms, not included in general counterion condensation, are responsible for the stabilization of the Z form. That specific surface chemistry is important in governing the B-Z transition is readily apparent from the behavior of alkaline earth metals with poly (dG-dC) (1), where the critical concentrations of divalent ions at room temperature vary from 0.7 M for Mg^{2+} to 0.04 M for Ba^{2+}.

In the limit of an infinitely long polymer (to eliminate length dependent cooperativity effects), the low ionic strength results can be simplistically treated within a site binding model. If ΔG_{int} is the intrinsic free energy difference per base pair between B and Z forms at low monovalent ion concentrations, ΔG_{N+} is the free energy difference per base pair for binding a +N ion, and $2\Theta_{+N}$ is the directly measured, critical number of +N ion bound per base pair at the room temperature transition midpoint, then $\Delta G_{int} + (2\Theta_{+N})\Delta G_{+N} = 0$. In comparing two oligovalent ions (+N and +M), the ratio of

the counterion binding free energy differences, is given by the inverse ratio of the critical values, $\Delta G_{+N}/\Delta G_{+M} = \Theta_{+M}/\Theta_{+N}$. From the data in Table 1, $Co(NH_3)_6^{3+}$ and spermine^{4+} are bound to the Z form relative to the B structure with about four times more energy than Mg^{2+}.

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REFERENCES

1. Behe, M. and Felsenfeld, G. (1981) Proc. Natl. Acad. Sci. USA 78, 1619-1623.
2. Manning, G. S. (1978) Quart. Rev. Biophys. 11, 179-246.
3. Wilson, R. W., Rau, D. C. and Bloomfield, V. A. (1980) Biophys. J. 30, 317-326.
4. Granot, J. and Kearns, D. R. (1982) Biopolymers 21, 219-232.
5. Merado, C. M. and Tomasz, M. (1977) Biochemistry 16, 2040-2046.
6. Santella, R. M., Grunberger, D., Weinstein, I. B. and Rich, A. (1981) Proc. Natl. Acad. Sci. USA. 78, 1451-1455.
7. Krakauer, N. (1971) Biopolymers 10, 2459-2490.
8. Wilson, R. W. and Bloomfield, V. A. (1979) Biochemistry 18, 2192-2196.
9. Rubin, R. L. (1977) J. Bacteriol. 129, 916-925.
10. Rose, D. M., Bleam, M. L. and Record, Jr., M. T. (1980) Proc. Natl. Acad. Sci. USA. 77, 6289-6292.