The B Form to Z Form Transition of Poly(dG-m^{5}dC) Is Sensitive to Neutral Solutes through an Osmotic Stress†

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ABSTRACT: Several neutral solutes, ranging in size from methanol to a tetrasaccharide, stachyose, are shown to stabilize the left-handed Z form of the methylated polynucleotide poly(dG-m^{5}dC). The action of these solutes is consistent with an osmotic stress, that is, with their effect on water chemical potentials coupled to a difference in the number of associated water molecules between the B and Z conformations. The apparent difference in hydration between the two forms is, however, dependent on the particular solute used to probe the reaction. The effect of solutes is not consistent either with a direct binding of solute or with an indirect effect on electrostatics or ion binding through changes in the solution dielectric constant. The interplay of NaCl and neutral solute in modulating the B−Z transition suggests that salt also could be stabilizing the Z form through an osmotic stress.

Hydration is increasingly recognized as an important component of DNA structure, stability, and dynamics (Westhof, 1988; Berman, 1991, 1994). As a corollary to this, if there are differences in the numbers of water molecules interacting with different DNA structures, then conformational transitions will depend on the availability of water. The transition between the B and A forms of DNA in fibers, for example, is triggered by changes in relative humidity (Harmouchi *et al.*, 1990). It has been argued that the change in nucleic acid hydration linked to the change in water vapor pressure is a key energy difference between these two forms (Saenger *et al.*, 1986; Shakked *et al.*, 1989). The left-handed Z form of poly(dG-dC) and poly(dG-m^{5}dC) appears to be less hydrated than the right-handed B form [e.g., Jovin *et al.* (1987) and Westhof (1988)]. The B ↔ Z transition in solution is known to be sensitive to many experimental parameters, such as temperature, salt species and concentration, and alcohol concentration (Jovin *et al.*, 1987). No direct evidence has been presented, however, establishing a link between hydration and the B−Z equilibrium. We previously showed that both large "hydrophobic" cations and anions, such as tetraalkylammonium or tetraalkylcarboxylate, are especially effective at inducing the transition (McDonnell & Preisler, 1989). The strong effect of these ions suggested an indirect role acting through their effect on bulk water properties and differences in B−Z hydration energies.

Hydration changes between the B and Z forms in solution can be probed by measuring the dependence of the transition on water chemical potential. Solution water activity can be varied by addition of neutral solutes that are not expected to bind directly to the polynucleotides. If these solutes are excluded from the water surrounding polynucleotides, an osmotic stress is created that acts to favor conformations that exclude less solute. The magnitude of the effect will depend on both the water activity or, equivalently, solution osmotic pressure and the difference in the number of solute-excluding waters between the two conformations. The osmotic stress technique has explicitly been used to probe the effect of water activity on the conformational equilibria of several proteins (Kornblatt & Hui Bon Hoa, 1990; Colombo *et al.*, 1992; Rand *et al.*, 1993), the action of restriction nucleases on DNA (Robinson & Sligar, 1993), and the binding of drugs or proteins to DNA (Sidorova & Rau, 1995; Garner & Rau, 1995).

We find that a wide range of neutral solutes, in one series of polyols from methanol to sucrose and stachyose (a tetrasaccharide) and in a second series of the monohydroxyl alcohols methanol, ethanol, and 1-propanol, are effective in stabilizing the Z conformation. Using the virtually stoichiometric binding of cobalt hexammine to poly(dG-m^{5}dC) at low ionic strength and the subsequent linear dependence of the fraction of Z form on the amount of cobalt hexammine added (Chen *et al.*, 1984), the free energy difference between the B and Z forms is seen to scale linearly with solute osmolal concentration or, equivalently, water chemical potential. For each solute, no dependence of the transition midpoint osmotic stress on salt concentration is observed between about 1 and 100 mM NaCl. Differences in electrostatic double layer energies or ion binding caused by changes in dielectric constant due to the addition of solutes cannot be the basis for the transition. Rather, it seems that these solutes are probing differences in solute-excluding waters of hydration between the two conformations.

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Although the free energy difference between the B and Z forms varies linearly with the solute osmolal concentration for any particular neutral solute, the absolute effectiveness of each solute is quite dependent on both its size and chemical nature. Unlike the osmotic stress experiments on opening and closing of membrane channels (Zimmerberg & Parsegian, 1986; Vodyanoy *et al.*, 1993) or the oxygenation of hemoglobin (Colombo *et al.*, 1992), there are no well-defined water cavities in the B−Z transition that exclude solutes simply by all or none sterics. Monohydroxyl alcohols are more effective than polyols of similar size. Among the polyols, there is an approximate inverse molecular weight dependence of the transition midpoint osmolal concentration in 20 mM NaCl from glycerol to stachyose. The exclusion of neutral solutes from poly(dG-m^{5}dC) is consistent with either a "preferential hydration" (Timasheff, 1993) of the DNA, i.e., the exposed macromolecular surface prefers to interact with water rather than with solute, or a steric exclusion of solute from the DNA surface, i.e., a "crowding" effect (Zimmerman & Minton, 1993) simply based on the difference in water and solute size.

There are several indications that even NaCl might be acting osmotically to induce the B−Z transition, not electrostatically as is commonly assumed (Pack *et al.*, 1986; Fenley *et al.*, 1990; Klement *et al.*, 1990). The neutral solute-induced transition is insensitive to salt concentration below about 0.1 M NaCl, i.e., at concentrations small enough that the contribution of salt to the solution osmotic pressure is small compared with that of solute. Moreover, at the transition midpoint, the required osmolal concentration of NaCl (without added neutral solute) is comparable to that for a neutral solute such as stachyose. Finally, comparable increases in sucrose or salt concentrations are necessary for the transition of poly(dG-dC) relative to poly(dG-m^{5}dC).

MATERIALS AND METHODS

Chemicals. Ultrapure sucrose and glycerol were purchased from BRL/Gibco. Sorbitol, arabitol, and stachyose were obtained from Sigma Chem. Co.; glucose, ethylene glycol, methanol, and ethanol were obtained from J. T. Baker Chemical Co.; 1-propanol was obtained from Aldrich; and cobaltic hexammine chloride was obtained from Kodak. All were analytical grade and used without further purification. Solute solution osmotic pressures (osmolal concentrations) were obtained from standard chemical tables or measured using a Wescor model 5100C vapor pressure osmometer [see Parsegian *et al.* (1995)]. Double-distilled water was used for all solutions.

Polynucleotides. Poly(dG-m^{5}dC)·poly(dG-m^{5}dC) and poly(dG-dC)·poly(dG-dC) were purchased from Pharmacia Biotechnology. The polynucleotides were further treated by standard phenol/chloroform extraction, followed by ethanol precipitation. Samples were dissolved in 10 mM Tris-HCl (pH 7.5) and 1 mM ethylenediaminetetraacetic acid (EDTA) (TE buffer) and exhaustively dialyzed against TE buffer to ensure removal of contaminating higher valence cations. Both polynucleotides were very polydisperse, with an average molecular mass of about 10^6 Da for poly(dG-m^{5}dC) and about 5×10^5 Da for poly(dG-dC), as estimated from agarose gel electrophoresis. The conversion between absorbance at 260 nm and concentration was taken as 17.1 $(mg/ml)^{-1}$ for poly(dG-m^{5}dC) and 16.5 $(mg/ml)^{-1}$ for poly(dG-dC).

Circular Dichroism. Circular dichroism (CD) spectra of the polynucleotides were obtained as described previously (Chen *et al.*, 1984) using either a Jasco J-500A or J-600 high-sensitivity spectropolarimeter, equipped with a water-jacketed sample holder, maintained at 20 °C. Polynucleotide solutions, with $A_{260} \sim 0.25$, and at particular osmolyte and salt concentrations, were first heated to 50 °C for 15 min and then held in the sample holder for an additional 15 min. The B−Z transition was monitored by the change in signal intensity at 290 nm using the salt and solute solution without polynucleotide as the reference. In some experiments, signals were monitored for about 15 min to ensure that equilibrium had been reached. In general, equilibration was quite rapid. Occasionally, however, samples had to be heated to 50 °C a second time to reach a stable signal, especially samples in the midtransition range of salt and solute concentrations. Full circular dichroism spectra (220−320 nm) and UV spectra were always taken at the start and end of the titration experiment, and in some titrations, CD spectra were taken at each intermediate salt or solute concentration.

The B−Z titrations with cobaltic hexammine shown in figures 2 and 3 give a transition midpoint in the absence of solute of about 1 $Co(NH_3)_6^{3+}$/8 base pairs (bp). This is a factor of 2−3 times larger than reported by us previously (Chen *et al.*, 1984) and is due to a much larger residual 10 μM EDTA concentration from the dilution of the poly(dG-dm^{5}C) stock solution. The cobalt hexammine concentration necessary for the transition, however, still scales linearly with the polynucleotide concentration, and the ratio $[Co(NH_3)_6^{3+}]$/[poly(dG-dm^{5}C)] at the transition midpoint is constant at this low salt concentration (data not shown).

RESULTS

Sucrose Can Induce the Z Form at Low Salt Concentrations. Figure 1 shows the dependence of the circular dichroism signal at 290 nm on sucrose concentration for poly(dG-m^{5}dC) at 20 °C, in 20 mM NaCl, 10 mM Tris (pH 7.5), and 1 mM EDTA. This change in signal is characteristic of the B → Z transition for poly(dG-m^{5}dC) (Behe & Felsenfeld, 1981). The midpoint sucrose concentration (3.9 osm) at this salt concentration is independent of EDTA concentration between 0.1 and 2 mM, indicating the transition is not due to contaminating di- or trivalent cations. The inset of Figure 1 shows the limiting circular dichroism spectra at 3.0 and 5.05 osm (45 and 55% w/w, respectively) sucrose. The CD spectrum at 3.0 osm sucrose is unchanged from the B form spectrum in the absence of sucrose. The spectrum at 5.05 osm sucrose is virtually identical to the Z form spectrum in 1.0 M NaCl (data not shown).

Interdependence of Cobalt Hexammine Binding at Low Salt and Solute Concentrations Necessary for the B−Z Transition. Figure 2 shows a $Co(NH_3)_6^{3+}$ titration of the B−Z transition of poly(dG-m^{5}dC) with no added sucrose and with 1.8 osm (~34% w/w) sucrose, at 20 °C, both in 10 mM TrisCl (pH 7.5). We have previously shown (Chen *et al.*, 1984) that virtually every added $Co(NH_3)_6^{3+}$ binds to poly(dG-dm^{5}C) at low salt concentrations and induces a fixed fraction of Z form. Less cobaltic hexammine is needed to induce the Z form at 1.8 osm sucrose than with no sucrose. Without added $Co(NH_3)_6^{3+}$, the CD spectra show only B form DNA for both 0 and 1.8 osm sucrose. The fraction of B form decreases linearly with total $Co(NH_3)_6^{3+}$ concentration for both sucrose concentrations.

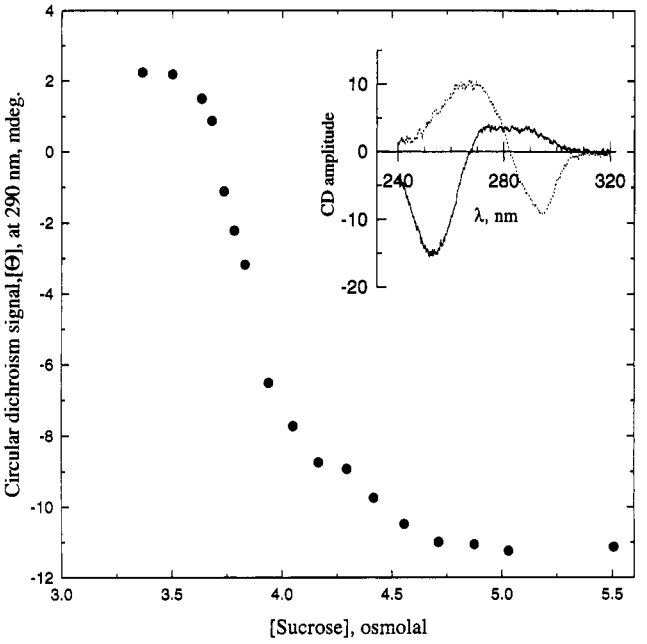

FIGURE 1: Circular dichroism signal at 290 nm of poly(dG-m^{5}dC), in 20 mM NaCl, 10 mM Tris-HCl (pH 7.5), and 1 mM EDTA and at 20 °C, is shown as a function of sucrose osmolal concentration. Each point represents a single sample made from concentrated poly(dG-m^{5}dC) stock in 10 mM Tris-HCl, 1 mM EDTA (TE buffer), concentrated NaCl solution, and 65% (w/w) sucrose in TE buffer. Clear plateaus are seen below about 3.5 osm sucrose (~46% w/w) and above about 5.0 osm (~55%). The midpoint transition concentration is about 3.9 osm sucrose. The figure inset shows the limiting CD spectra at 3.35 and 5.05 osm sucrose.

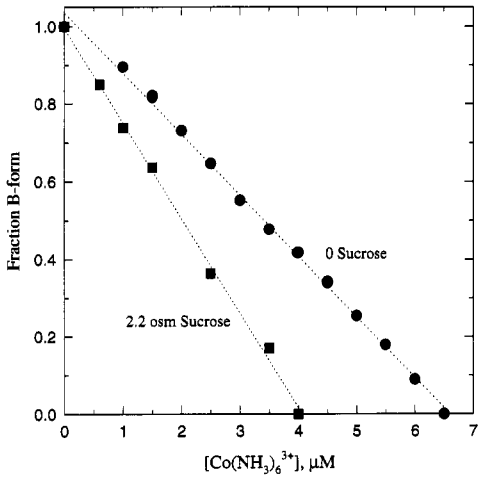

FIGURE 2: Fraction B form is shown as a function of cobaltic hexammine added for two sucrose concentrations, 0 (●) and 2.2 osm (39% w/w) (■). Poly(dG-m^{5}dC), dialyzed against 10 mM Tris-HCl (pH 7.5) and 0.1 mM EDTA, was diluted into 10 mM Tris and the appropriate sucrose concentration to a final concentration of about 45 μM bp. The residual EDTA concentration was about 10 μM. The sample was titrated with a concentrated solution of $Co(NH_3)_6Cl_3$ (20 mM) and the CD signal at 290 nm measured. The normalized fraction B form was determined from the starting (no cobaltic hexammine) and end point CD signals. The change in sucrose concentration due to added $Co(NH_3)_6Cl_3$ is negligible. The approximate linear dependence of fraction B form on $Co(NH_3)_6^{3+}$ concentration is a result of essentially stoichiometric binding of trivalent ion.

Figure 3 shows the interdependence between cobalt hexammine concentration added per base pair and the osmolal solute concentration, for methanol, ethanol, glycerol,

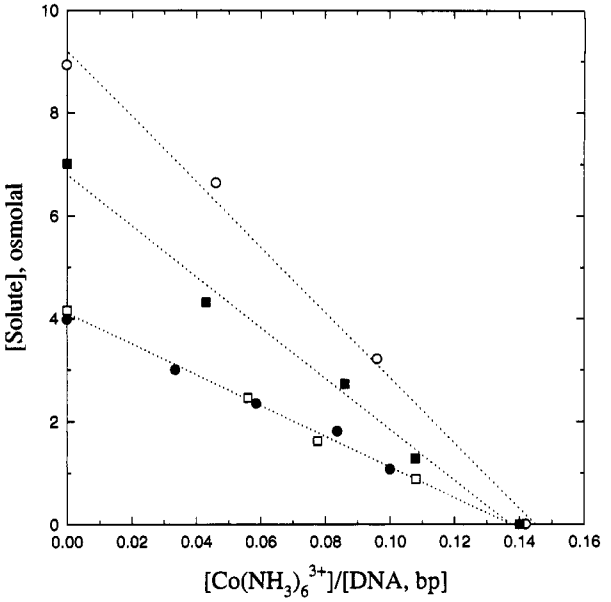

FIGURE 3: Dependence of solute osmolal concentration on cobaltic hexammine bound per bp at the B−Z transition midpoint of poly(dG-m^{5}C) is shown for ethanol (□), methanol (■), glycerol (○), and sucrose (●). Each point represents the result of a $Co(NH_3)_6Cl_3$ titration at a fixed solute concentration as described in Figure 2. The points for 0 Co^{3+}/bp were determined by titration with solute in 10 mM Tris-HCl (pH 7.5) and 1 mM EDTA at 20 °C as described in Figure 1.

and sucrose, at the B−Z transition midpoint in 10 mM TrisCl, at 20 °C. For each solute, there is an apparent linear dependence of osmolal concentration (equivalently, of the water chemical potential) on the number of $Co(NH_3)_6^{3+}$ ions added per base pair (essentially, on the number of $Co(NH_3)_6^{3+}$ ions bound per base pair). The magnitude of the sensitivity to water activity, however, is solute specific. At the same cobaltic hexammine concentration, much less sucrose or ethanol is needed than methanol or glycerol.

This linear dependence of solute concentration on $Co(NH_3)_6^{3+}$ binding is consistent with an osmotic stress acting on the B−Z transition, i.e., that there is a difference in the number of solute-excluding water molecules associated with the two conformations. Free energy differences are then dependent on this change in water and the bulk water chemical potential. The solute specificity within the osmotic stress explanation simply represents a dependence of the number of solute-excluding water molecules on the solute size and nature.

The Osmotic Effectiveness is Strongly Dependent on Solute Nature and Size. Figure 4 shows the dependence of the solute osmolal concentration at the B−Z transition midpoint of poly(dG-m^{5}dC) at 20 °C, in 20 mM NaCl, 10 mM Tris (pH 7.5), and 1 mM EDTA, on solute molecular weight. For the homologous set of polyol solutes, glycerol, arabitol, glucose, sorbitol, sucrose, and stachyose (a tetrasaccharide), there is a marked decrease in the solute concentration necessary for the B−Z transition with increasing molecular weight (MW). The inset of Figure 4 shows that the B−Z midpoint solute osmolal concentration varies approximately linearly with 1/MW for these solutes. The monohydroxyl alcohols, ethanol and 1-propanol, are clearly different. Both are much more effective than similarly sized ethylene glycol and glycerol. The chemical nature of the solute is also important in effecting the B−Z transition.

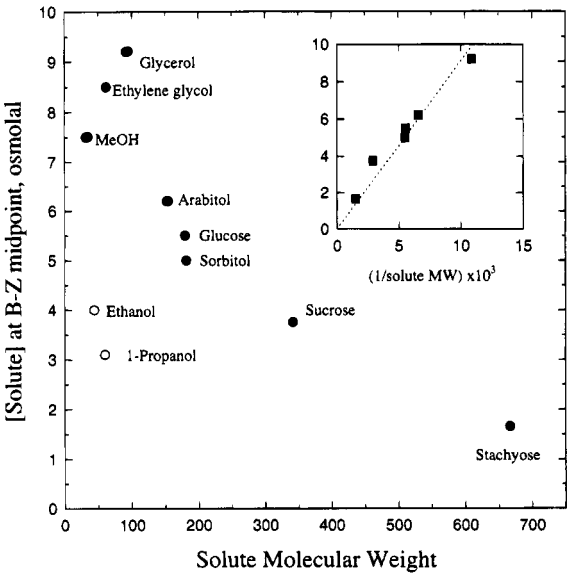

FIGURE 4: Dependence of osmolal concentration at the B−Z transition midpoint of poly(dG-m^{5}C) on solute molecular weight is shown for several monohydroxy alcohols and polyol solutes. Each point was obtained from CD data in 10 mM Tris-HCl (pH 7.5) and 1 mM EDTA at 20 °C, as described in Figure 1. The inset shows the dependence of midpoint osmolality on 1/MW for the polyols from glycerol to stachyose. The inverse molecular weight dependence suggests that the difference in the number of larger solute-excluding water molecules between the B and Z forms scales linearly with the size of the solute for polyols.

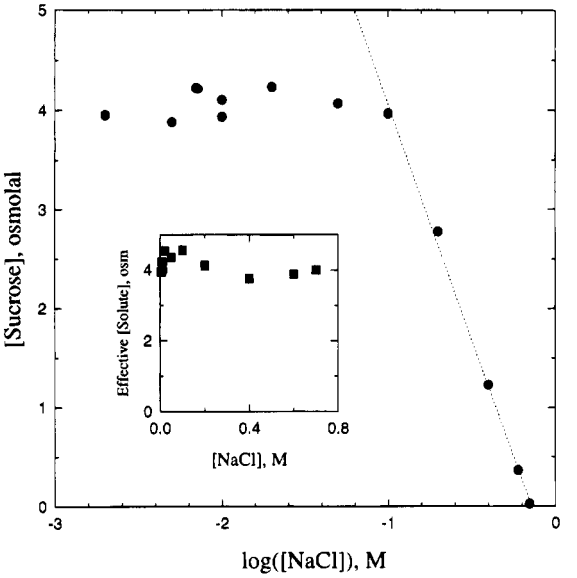

FIGURE 5: Osmolal concentration of sucrose at the B−Z transition midpoint of poly(dG-m^{5}C) at 20 °C, with 10 mM Tris-HCl (pH 7.5) and 1 mM EDTA, is shown as a function of the NaCl concentration. Each point is the result of CD measurements of a titration of DNA in a fixed concentration of NaCl with sucrose, as in Figure 1. The data show a salt-independent region between about 1 and 100 mM Na^+. The salt-dependent region above 0.1 M Na^+ can be analyzed in two ways. The slope of the dotted line shown in the figure gives the ratio of the change in Na^+ binding to the change in water binding accompanying the transition. Alternatively, the figure inset shows the effective total solute osmolal concentration, summing the contribution from salt and sucrose. This assumes that salt acts osmotically rather than electrostatically and that salt is about 3 times as effective as sucrose, i.e., that the B−Z transition occurs at 1.3 osm NaCl (0.7 M) compared with 4 osm sucrose.

The B−Z Transition with Sucrose Is Independent of Salt below 0.1 M. Figure 5 shows the dependence of sucrose osmolal concentration on salt concentration at the B−Z transition midpoint of poly(dG-m^{5}dC), at 20 °C and with 10 mM Tris and 1 mM EDTA. A 7 mM Na^+ sample was made entirely from Na_3EDTA; the lack of significant deviation for this data point again indicates that the sucrose was essentially free of contamination with di- or trivalent cations. Below about 100 mM NaCl, no effect of salt on the transition sucrose concentration is observed, suggesting that no extra Na^+ binding accompanies the B−Z transition at these salt concentrations. A similar insensitivity to salt concentration is also seen for glycerol, methanol, and ethanol between 10 and 100 mM NaCl (data not shown).

Above 0.1 M NaCl, however, the midpoint osmolal concentration of sucrose decreases as salt concentration is increased. No added sucrose is, of course, necessary at about 0.7 M NaCl, the transition concentration for salt. As will be discussed in more detail later, this interplay of salt and sucrose concentrations can be interpreted in two ways. If a difference in the direct binding of Na^+ to the B and Z forms is assumed, then the slope of the line shown in Figure 5 can give the change in ion binding accompanying the transition. Alternatively, if salt is considered to act on the transition through water activity analogous to the neutral solutes, then an effective total solute osmolal concentration can be calculated and is shown in the inset of Figure 5. This effective osmotic stress is nearly constant over the entire range of salt concentrations examined.

More Sucrose Is Required for the Transition of Poly(dG-dC) Than for Poly(dG-m^{5}dC). In general, poly(dG-dC) requires significantly higher concentrations of NaCl or divalent ions to induce the Z form than does poly(dG-m^5-dC). For example, about 2.3 M NaCl is needed at 20 °C for the transition of poly(dG-dC) compared with only about 0.7 M for poly(dG-m^{5}dC) (Behe & Felsenfeld, 1981). Figure 6 shows comparative sucrose vs cobalt hexammine B−Z transition plots for poly(dG-m^{5}dC) and poly(dG-dC) at 10 mM Tris (pH 7.5) and 20 °C, as described in Figure 3. The sucrose concentration necessary for the B−Z transition of poly(dG-dC) in the absence of cobalt hexammine can only be estimated by extrapolation as about 7.5 osm, some 2-fold higher than for poly(dG-m^{5}dC).

DISCUSSION

The B ↔ Z transition of poly(dG-dm^{5}dC) is sensitive to the presence of all the neutral monohydroxyl alcohols, polyols, and sugars we have examined here. The efficacy of each solute is dependent on both solute size (larger solutes are more effective) and chemical nature (monohydroxyl alcohols are more effective than polyols of similar size). The approximate linear dependence of the solute osmolal concentration on the number of cobalt hexammine ions bound at low ionic strengths (Figures 3 and 6) restricts the possible interpretations of the mechanism of solute action. If the difference in binding free energies of a $Co(NH_3)_6^{3+}$ ion between the B and Z forms, $\Delta G_{B\text{-}Z,Co}$, is independent of the number of ions already bound, then this figure indicates a linear compensation at the B−Z transition midpoint between differential ion binding energies, $n_{Co}\Delta G_{B\text{-}Z,Co}$, and water chemical potentials. This suggests that these solutes are acting osmotically, modulating the B−Z transition indirectly through changes in water activity and differences in water binding between the B and Z forms. Before discussing the

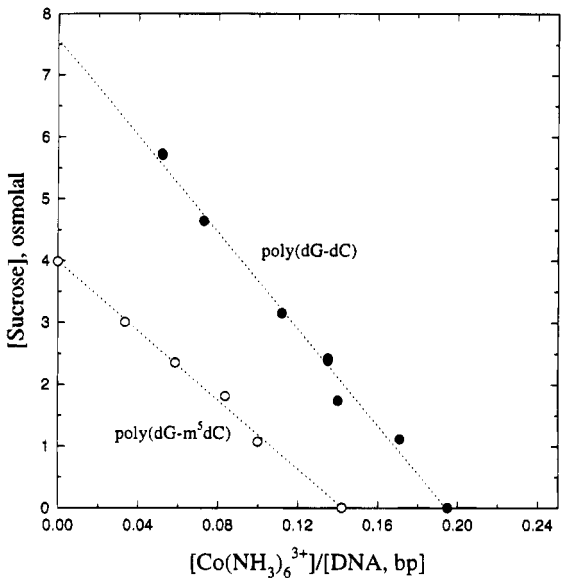

FIGURE 6: Ratio of cobaltic hexammine to DNA concentrations at the B−Z transition midpoint of poly(dG-m^{5}C) and poly(dG-C) is shown for a range of sucrose osmolal concentrations. The experimental conditions are the same as in Figure 2: 10 mM Tris-HCl (pH 7.5), 10 μM EDTA (from polynucleotide dilution), and 20 °C. The poly(dG-m^{5}C) data are identical to the data with sucrose in Figure 3. Without added $Co(NH_3)_6^{3+}$, it was not possible to observe a transition of poly(dG-dC) at the highest sucrose concentration tested (5.5 osm). The data can be extrapolated, however, to an estimated midpoint of 7.5 osm sucrose.

energetics of an osmotic action, however, we will first discuss two other possible modes of action, a difference in the direct binding of solutes to the B and Z conformations and an indirect effect of solutes changing salt−DNA interactions.

The free energy change due to a difference in the direct binding of solutes to DNA sites of the B and Z forms would typically be expected to vary linearly with the solute chemical potential or, to first order, with log[solute]. At the large solute concentrations used here (compared to DNA concentrations), the concentration dependence would appear linear only in the limit of very weak binding in which the fraction of occupied sites is small. This would require, however, that the difference in binding, for example, of sucrose and ethanol or of stachyose and 1-propanol to the B and Z forms of DNA be remarkably and improbably similar, given the differences in structure and chemical properties and that a weak binding ($<kT$/bp) is sufficient to induce the Z form. Although a difference in weak, direct binding of solutes to the B and Z forms can not be rigorously disproved, it seems very unlikely.

Alternatively, solutes may affect the B−Z transition indirectly through salt−DNA interactions. The change in dielectric constant due to added neutral solutes is the most straightforward way to link solutes with changes in electrostatic energies. The solute specificities shown in Figure 4 are, in fact, qualitatively consistent with changes in dielectric constant. The bulk solution dielectric constant varies approximately linearly with the volume fraction of solute, so that an approximately constant solute weight concentration at the B−Z transition midpoint for homologous polyols and sugars implies an approximately constant dielectric constant, to first order. The change in solution dielectric constant also depends on the chemical nature of the solute, so that, at a fixed weight concentration, monohydroxyl alcohols are more effective in lowering the dielectric constant than polyols or sugars. An indirect action of solutes through salt−DNA interactions, however, also requires that the B−Z transition be dependent on salt activity. The absence of a salt concentration dependence below about 0.1 M NaCl (figure 5) means that solutes are not affecting the transition through the change in dielectric constant coupled to classical double layer or ion binding electrostatics.

The final possibility is that these solutes can affect the B−Z transition indirectly through water−DNA interactions coupled to their effect on water activities or osmotic pressures. Several different formalisms have been developed to describe this linkage (Parsegian *et al.*, 1995). If the solutes are too large to enter water-filled spaces associated with the macromolecule and if there is a difference in the number of water molecules that sterically exclude solute between two conformations of the macromolecule, then increasing solute concentration will favor the conformation that sequesters fewer water molecules from solute. For this limiting case of steric exclusion, incremental changes in the free energy difference, dG_{osm}, between the two conformations due to the difference in the number of solute-excluding water molecules, Δn_w, are related to incremental changes in water chemical potential, $d\mu_w$, or, equivalently, to changes in solute osmolal concentration, $d[solute]_{osm}$, by

$$dG_{osm} = -kT\Delta n_w d\mu_w = kT\Delta n_w \frac{d[solute]_{osm}}{55.6} \quad (1)$$

There is no dependence of Δn_w on solute size (beyond some minimum) or chemical nature for an ideal steric exclusion. An osmotic effect due to strictly steric exclusion of solutes has been seen in the opened−closed equilibrium of membrane channels (Vodyanoy *et al.*, 1993).

The DNA surfaces of the B and Z forms, however, are well exposed to the bulk solution. There are no defined, water-filled cavities that clearly exclude solutes. Nevertheless, solutes can still act osmotically; i.e., the energy perturbation due to solute varies linearly with water chemical potential, if there is a difference between water and solutes in their interactions with the DNA surface. Both Timasheff and co-workers (Timasheff, 1993) and Eisenberg (Eisenberg, 1994) have made direct measurements of the interaction of solutes with protein and nucleic acid surfaces. Many solutes, such as sucrose, are excluded from these surfaces, and the observed exclusion varies linearly with solute concentration. The magnitude of the exclusion often depends on both the size and chemical nature of the solute.

There are several different molecular interpretations for this exclusion of solutes from exposed macromolecular surfaces. At one extreme, the difference in the size between a solute and a water molecule can result in an effective volume exclusion of solute from the macromolecule due to a distance of closest approach and a consequent crowding effect (Zimmerman & Minton, 1993; Zimmerman, 1993; Garner & Burg, 1994). Crowding will affect reactions that change the shape or the exposed surface area of macromolecules, favoring those conformations that lower solute-excluded volume. Water only enters the formulation since the change in excluded volume is necessarily a water-filled volume. Only the size of the solute is important in defining the magnitude of the effect. Any difference in macromolecular solvation between water and solute is assumed to be

small compared with volume exclusion energies. At the other extreme, Timasheff (1993) has discussed solute exclusion in terms of a preferential hydration due to an increased protein "surface tension" in the presence of solutes; i.e., interactions of macromolecular surfaces with water are much lower in energy than interactions with solute. Solute exclusion would be expected to depend on both solute size and chemical nature, as well as on the nature of the macromolecular surface.

Regardless of the mechanism of solute exclusion from exposed macromolecular surfaces, the effect can be formally analyzed in the same way as ideal steric exclusion of solutes from macromolecular cavities or channels using eq 1. The parameter Δn_w, however, is now operationally defined as the change in the effective number of solute-excluding water molecules between two macromolecular conformations. For transitions involving changes in solute-exposed surface area, the apparent water release will depend not only on the differences in hydration between conformations but also on the solute size and chemical nature; Δn_w will not have a simple interpretation in terms of the hydration differences between the B and Z forms alone. The slopes in Figures 3 and 6 represent the trade-off at the transition midpoint, $f_B = 0.5$, between $Co(NH_3)_6^{3+}$ binding energies and the osmotic work

$$\frac{d[\text{solute}]_{osm}}{dn_{Co}}\Big|_{f_{B=0.5}} = -\frac{55.6\Delta G_{B-Z,Co}}{\Delta n_w} \quad (2)$$

The observation that the Z form is favored at higher osmolal solute concentrations, or at lower water activities, would then indicate that this conformation has fewer associated water molecules that exclude solutes than the B form. This result is qualitatively consistent with previous observations that the Z form, like the A form, is favored over the B form under conditions of low relative humidity in fibers and crystals (Arnott *et al.*, 1980). Observations like this have led to speculation that changes in hydration are energetically significant in determining DNA conformation (Saenger *et al.*, 1986; Shakked *et al.*, 1989). The entropy of the B−Z transition in solution has also been interpreted as possibly showing a release of bound water. Higher temperatures favor the Z form (Roy & Miles, 1983; Behe *et al.*, 1985; Chaires & Sturtevant, 1986) even though several experimental measurements of configurational freedom, X-ray crystal structure isotropic, thermal B factors (Drew & Dickerson, 1981; Holbrook *et al.*, 1986), hydrogen exchange rates (Ramstein & Leng, 1980; Pilet & Leng, 1982), and global flexibilities estimated by light scattering (Thomas & Bloomfield, 1983), as well as computer simulations of configurational fluctuation (Irikura *et al.*, 1985), indicate that the Z form is the conformationally more restricted structure. Since higher salt concentrations favor the Z form, the implied extra ion binding is also inconsistent with an increased entropy of the Z form. The only solution component left to account for the increased entropy is the water. An entropy favoring the Z form due to changes in DNA hydration is consistent with the apparent release of solute-excluding water in the B−Z transition inferred here.

The difference in exclusion of solutes between the B and Z forms shows both a size dependence and a dependence on the chemical nature of the solute. The approximate 1/MW dependence for polyol transition pressures (Figure 4) means that the transition occurs at an approximately constant polyol weight concentration of about 45%. A constant weight fraction, insensitive to molecular weight, for the transition is consistent with the "available volume theory" for the exclusion of rodlike solutes (Zimmerman & Minton, 1993), commonly used for calculating "crowding" effects, and seen experimentally for protein solubilities in dextran solutions (Laurent, 1963). The large dependence of exclusion on the solute chemical nature (polyols vs monohydroxy alcohols), however, means that the effect is more than just hard sphere exclusion. Even within one chemical family such as the polyols, it is not possible to distinguish a molecular weight dependent solute exclusion due to simple hard sphere sterics from exclusion due to a simple additivity of repulsive interactions between individual solute groups and the macromolecular surface. From this standpoint, a correlation between solute nature and dielectric constant comparing monohydroxyl alcohols and polyols would not be surprising. Repulsive interactions with the highly polar DNA surface should depend on the solute polarity.

We can estimate the number of solute-excluding water molecules (Δn_w) probed by sucrose from the width of the B−Z transition shown in Figure 1. If the plot is transformed to fraction B form, f_B, vs osmolal concentration of sucrose, then, at the transition midpoint, standard helix−coil theory for infinite chains (Engel, 1983) can be used to estimate

$$\frac{df_B}{d[\text{sucrose}]_{osm}}\Big|_{f_{B=0.5}} \approx \frac{\Delta n_w}{4(55.6\sigma^{1/2})} \quad (3)$$

where σ is the cooperativity parameter of the transition, estimated as about 10^{-4} (Szu & Charney, 1985; Chaires & Sturtevant, 1986). We can then estimate from Figure 1 that Δn_w is about −2.5 water molecules/bp for the B−Z transition of poly(dG-m^{5}dC) as probed by sucrose.

Several polyelectrolyte theories have been used to calculate the salt dependence of the free energy difference between the B and Z forms (Pack *et al.*, 1986; Fenley *et al.*, 1990; Klement *et al.*, 1990). None predicts the insensitivity to salt concentrations between 1 and 100 mM NaCl seen in Figure 5. Electrostatics makes an apparently negligible contribution to the free energy difference at these ionic strengths.

At salt concentrations above 0.1 M, the interplay of salt and sucrose concentrations can be interpreted in two ways. If the salt sensitivity is assumed due to changes in ion binding, then the linkage of salt and water activities at the B−Z transition midpoint can be expressed as

$$\frac{d[\text{sucrose}]_{osm}}{d\log[\text{NaCl}]}\Big|_{f_{B=0.5}} \approx \frac{55.6\Delta n_{Na^+}}{2.303\Delta n_w} \quad (4)$$

where we have approximated salt activity by its molar concentration, [NaCl]. The change in number of bound ions is Δn_{Na^+}. The slope observed in Figure 5 could then be interpreted as indicating that one extra Na^+ ion binds to the Z form for every 30 sucrose-excluding water molecules released ($\Delta n_w/\Delta n_{Na^+} \approx 30$).

An alternative interpretation of these results, given the insensitivity of the transition to salt at concentrations below 0.1 M, is that salt is not acting electrostatically, but rather osmotically, much as the neutral solutes. Quite apart from its requirement for charge neutralization, if NaCl as a species is excluded from the hydrating water of DNA, then salt will

act osmotically. Indeed, at high salt concentrations, Reisler *et al.* (1977) report an exclusion of salt from DNA that is independent of salt concentration, in contrast to electrostatic predictions. If NaCl acts osmotically, in the same way as the neutral solutes examined here, then the sucrose osmolal concentration dependence on salt concentration would reflect the extra contribution to the total effective osmotic pressure from NaCl. The 0.7 M salt concentration (1.4 M ions) necessary to induce the Z form of poly(dG-m^{5}dC) in the absence of sucrose (Behe & Felsenfeld, 1981) corresponds to about a 1.3 osm concentration. If the action of NaCl in the B−Z transition was purely osmotic, then NaCl would be about as effective as stachyose (Figure 4). The inset of Figure 5 shows the effective sucrose osmotic pressure, assuming that NaCl contributes osmotically and that its equivalent sucrose concentration is about 6[NaCl]; i.e., 0.7 M salt is osmotically equivalent to 4 osm sucrose.

Although this view of the effect of NaCl is unconventional, the similar increases in solute and salt concentrations necessary for the B−Z transition of poly(dG-dC) compared with that of poly(dG-m^{5}dC) are consistent with this interpretation. We estimate from extrapolation of the data in Figure 6 that about a 2-fold greater sucrose osmolal concentration (from 3.9 to 7.5 osm) is necessary for the transition midpoint of poly(dG-dC) compared with that of poly(dG-m^{5}dC). In comparison, using the salt and ethanol midpoint concentrations reported by Behe and Felsenfeld (1981), the B−Z transition of poly(dG-dC) compared with that of poly(dG-m^{5}dC) requires about a 5-fold greater ethanol osmolal concentration (from 4.5 to 21.9 osm) or about a 4-fold greater NaCl concentration (from 1.3 to 5.4 osm salt). These results are suggestive of an osmotic action for salts in the B−Z transition, as the concentrations and changes in concentrations of NaCl needed for the transition are not that different from ethanol or sucrose. The data are not sufficiently precise, however, to distinguish unambiguously between electrostatic and osmotic interpretations.

CONCLUSIONS

There is increasing interest in the role of hydration in determining the structure and dynamics of DNA, both experimentally and computationally (Berman, 1994). Changes in hydration associated with different DNA conformations are difficult to measure in dilute solution using techniques that probe bound water directly. Typically, these waters are not bound tightly enough to be greatly different from the bulk water they directly contact. The osmotic stress technique simply relies on the thermodynamic consequences of a change in hydration linked with a change in water chemical potential to modulate the energy difference between alternate conformations. The osmotic stress results with membrane channels (Vodyanoy *et al.*, 1993), with hemoglobin oxygenation (Colombo *et al.*, 1992), or with the specific−nonspecific *Eco*RI−DNA binding competition (Sidorova *et al.*, 1995) showed negligible dependence on solute size (after some minimum) and chemical nature. Concluding that these solutes affected the reactions through water activity and differences in hydration is straightforward (though not unambiguously proven). The results on the B−Z transition, however, show a blurring of this interpretation when these waters are not clearly sequestered sterically from the probing solutes. Even though each solute affects the B−Z reaction in a manner consistent with an osmotic stress, there is marked sensitivity to the solute identity. The definition of hydration depends on the particular solute probing the reaction. This coupling between solute-excluding water molecules and solute size and chemical identity seems unavoidable for macromolecular surfaces well-exposed to bulk solution. Although we cannot give a number for the change in hydration accompanying the B−Z transition independent of the solute, the results presented here demonstrate the potential applicability of osmotic stress to other DNA conformational transitions and provide a base line for interpreting future results. An experimental estimate of the contribution of hydration, so often neglected, to macromolecular structure, stability, and recognition will rely on correlating changes in hydration with changes in free energy.

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