Conformational lability of poly(dG-m⁵dC):poly(dG-m⁵dC)

Fu-Ming Chen

Department of Chemistry, Tennessee State University, Nashville, TN 37203, USA

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ABSTRACT

The remarkable conformational lability of poly(dG-m⁵dC):poly(dG-m⁵dC) is demonstrated by the observation of an acid-mediated conformational hysteresis. An acid-mediated Z conformation that exists in solutions containing low sodium concentrations that would normally favor the B conformation is described in this report. This Z conformation is reached by an acid-base titration of a B-poly(dG-m⁵dC):poly(dG-m⁵dC) solution which is not far from the B-Z transition midpoint. The resulting Z conformation is thermally very stable, with direct melting into single strands at approximately 100 °C. In contrast, the B form DNA, initially in solutions of the same ionic strength but without exposure to acidic pH, exhibits a biphasic melting profile, with conversion into the Z form (with high cooperativity) prior to an eventual denaturation into single strands at around 100 °C. Cooling experiments reveal that such biphasic transitions are quite reversible. The transition midpoint for the thermally poised B to Z transformation depends strongly on the NaCl concentration and varies with sample batch. The acid-mediated Z form binds ethidium more weakly than its B counterpart, and the ethidium induced Z to B conversion occurs in a step-wise (non-allosteric) fashion without the requirement of a threshold concentration. The acid-mediated as well as the thermally poised Z conformations are reversed by the addition of EDTA, suggesting the involvement of trace amounts of multivalent metal ions.

INTRODUCTION

Since the discovery of Z form DNA by X-ray crystallography (1) and the confirmation by Raman spectroscopy (2) that the salt induced CD inversion of poly(dG-dC):poly(dG-dC) in solution observed by Pohl & Jovin (3) is the consequence of a B to Z transition, there has been increasing interest in the possible role of Z DNA in gene expression (4). The observation that methylation at the C-5 position greatly facilitates such B-Z interconversions (5) and the correlation of cytosine methylation with gene inactivation of eukaryotes (6) suggest that Z DNA may indeed play a role in the control of gene expression.

The ease in which poly(dG-m⁵dC):poly(dG-m⁵dC) can be converted to the Z form is apparent from the much lower NaCl requirement (0.7 M) (5) in

comparison with that of its unmethylated counterpart (2.35 M) for the conformational transformation (see the review in reference 4). Other more efficient agents have since been found which can effect such conversions for this methylated polymer at near physiological conditions (5). The conformational lability of poly(dG-m⁵dC):poly(dG-m⁵dC) is further illustrated by the recent observation that when NaCl is absent or its concentration is very low, this polymer exists in the Z form (7,8,9,10). This phenomenon appears to be unique to poly(dG-m⁵dC):poly(dG-m⁵dC), as experiments with the unmethylated polymer have failed to observe such a Z formation under similar low salt conditions. This gives further support to the possible connection of methylation, the B-Z transition, and gene regulation.

I report here another striking characteristic exhibited by poly(dG-m⁵dC):poly(dG-m⁵dC). This methylated polymer can exist in a Z form through acid-base titration under certain low salt conditions that normally favor the B conformation. Such an acid-mediated Z conformation was not observed for the corresponding unmethylated polymer under similar conditions. This fact further testifies to the unique conformational lability of this methylated polynucleotide.

MATERIALS AND METHODS

Poly(dG-m⁵dC):poly(dG-m⁵dC) and ethidium bromide were purchased from P-L Biochemicals and Sigma, respectively, and used without further purification. Stock solutions of poly(dG-m⁵dC):poly(dG-m⁵dC) were prepared either in 10 mM tris buffer of pH 8 or aqueous solution, containing 0.01 M or no NaCl (solution condition is to be specified for each experiment). An extinction coefficient of 7000 M⁻¹ cm⁻¹ at 255 nm was used for the concentration (per nucleotide) determination of this methylated polymer in the B conformation. Chelex 100 resin was purchased from Bio-Rad and the chelexed water was obtained either through a column or by adding the resin directly to water, shaking rigorously, waiting overnight, and then centrifuging. CD spectra were measured by a JASCO-J500A recording spectropolarimeter at various temperatures using water-jacketed cells. Absorbance measurements were made on a Cary 210 spectrophotometric system. Adjustment of pH was accomplished by introducing trace amounts of either 0.1 M NaOH or HCl directly into the spectro-cell, and pH value was measured by a micro pH electrode. The desired salt concentration was obtained by adding appropriate amounts of NaCl crystals or concentrated NaCl solution. Absorbance or CD intensity was monitored at

293 nm after each pH or salt adjustment and the spectrum measured after equilibrium was established. Melting experiments were carried out either by CD measurements with manual temperature adjustment or by monitoring absorbance and collecting data every 15 s with an Apple II microcomputer. For the absorbance measurements a heating rate of 0.5 °C per min was maintained by a Neslab RTE-8 refrigerated circulating bath and a EPT-4RC temperature programmer.

RESULTS

NaCl Titration

In agreement with observations made by others (8,9,10), our commercially obtained poly(dG-m⁵dC):poly(dG-m⁵dC) exists as a Z conformation (as indicated by CD) when dissolved in water or 10 mM tris HCl buffer (pH 8) in

Fig. 1. CD spectra at different temperatures for poly(dG-m⁵dC):poly(dG-m⁵dC) in 10 mM tris HCl/pH 8/10 mM NaCl solution.

Fig. 2. CD spectra at different temperatures for poly(dG-m⁵dC):poly(dG-m⁵dC) in 10 mM tris HCl/pH 8/5 mM NaCl solution. (A): in the absence of EDTA and (B): 1 mM EDTA added at 65 °C.

the absence of NaCl. Salt titrations with an 11 μ M poly(dG-m⁵dC):poly(dG-m⁵dC) solution in pH 8 buffer result in a very cooperative Z to B transition around 2.5 mM NaCl. The B conformation persists until around 0.7 M NaCl whereupon another cooperative transition results in the reappearance of the Z conformation. The low- and high-salt Z conformations exhibit nearly identical absorption and CD spectra.

Thermally Poised Conformational Transitions

No apparent conformational transitions, other than denaturation into single strands around 107 °C, have been observed when a low-salt Z DNA solution is heated. However, the B-form poly(dG-m⁵dC):poly(dG-m⁵dC), in solutions containing concentrations of NaCl not far from that of the low-salt B-Z transition midpoint, transforms to a Z conformation at higher temperatures prior to denaturation into single strands. The midpoint for such

Fig. 3. Thermal melting profiles of poly(dG-m⁵dC):poly(dG-m⁵dC) in pH 8 buffer containing 5 and 10 mM NaCl, respectively. (A): CD monitored at 293 nm and (B): CD monitored at 253 nm, solid diamond is that of solution with 1mM EDTA added.

thermally poised B to Z transitions is NaCl concentration dependent, the higher the NaCl concentration the higher the transition temperature. These features are illustrated by the temperature dependent CD spectra in Figures 1 and 2 for the 11 μ M poly(dG-m⁵dC):poly(dG-m⁵dC) solutions containing 10 and 5 mM NaCl, respectively.

Addition of 1 mM EDTA to the low-salt or thermally poised Z DNA solution results in a reversion to the B conformation. In the presence of EDTA the B form of poly(dG-m⁵dC):poly(dG-m⁵dC) melts around 100 °C in 5 mM NaCl solution, somewhat lower than the Z melting in the absence of EDTA (see Figs. 2B and 3B).

The low-salt B to Z transition as well as thermal denaturation characteristics are more easily seen by monitoring ellipticities at 293 or 253 nm as shown in Figure 3. It is interesting to note that although the low-salt

Fig. 4. (A): equilibrium CD spectra after jumping to various temperatures from a temperature slightly below 50 °C. (B): Arrhenius and van't Hoff plots for the thermally induced B to Z transitions.

B to Z transition temperature is 20 °C higher in the 10 mM NaCl solution than in the 5 mM NaCl solution (57 vs. 77 °C), the temperatures for the melting of Z conformations into single strands are almost identical (~ 107 °C). The Z conformation appears to melt directly into single strands rather than transforming into other duplex conformations prior to melting, as suggested by the appearance of an isoelliptic point (see Fig. 1B).

The thermally poised B-Z interconversions are quite reversible, enabling the measurement of thermally induced B to Z transition kinetics at various temperatures by using the same sample. This was accomplished by lowering the temperature to slightly below 50 °C (B-form) in a 5 mM NaCl/pH 8 solution and then jumping to the desired temperatures to measure the kinetics (single exponential decay) as well as the equilibrium CD spectra. Such measurements enabled the construction of Arrhenius and van't Hoff plots for obtaining the

Fig. 5. (A) Representative CD spectra during acid-base titrations for an 11 μ M poly(dG-m⁵dC) : poly(dG-m⁵dC) / 0.01 M NaCl solution without buffer. A 5-cm water-jacketed cylindrical cell was used. (B) Plot of 293 nm molar ellipticity vs. pH during a typical acid-base titration. The numbers represent the order of titration.

activation energy (170 kJ/mole) and enthalpy change (450 kJ/mole), respectively, for such B-Z transitions (Fig. 4).

Hysteresis in Acid-Base Titration.

In one of our aqueous solution experiments without buffer, a rather curious phenomenon was uncovered. An 11 μ M poly(dG-m⁵dC) : poly(dG-m⁵dC) solution (pH 7.2) of 0.01 M NaCl initially exhibited a characteristic B form CD spectrum. Upon lowering the pH to around 4.5, however, a slow change in CD spectrum was observed which reached equilibrium after several hours. The resulting CD spectrum was characteristic of a Z form except for the somewhat reduced intensities. If the pH of the solution was then increased stepwise, in increments of 0.5 pH units or less, to the original value, the intensity of the 293 nm negative CD (characteristic of the

Z conformation) steadily increased and reached its maximum around neutral pH instead of going back to the original B conformation. The Z conformation remained evident after several days.

The hysteresis in the pH titration is graphically illustrated in Figure 5A. An initial measurement of pH indicates that the solution is roughly neutral and the CD spectrum exhibits typical B characteristics (broad positive 280 and strong negative 253 nm bands). Changing the solution to a slightly basic condition by adding trace amounts of 0.1 M NaOH does not alter the B form CD spectrum. Upon lowering the pH to around 5.3, however, a CD spectrum characteristic of Z DNA appears (negative 293 nm band) with a first-order half-life of around 1/2 hour. Further lowering of pH to 3.7 results in a substantial intensity decrease as a consequence of partial denaturation, although the negative impression at 293 nm is still evident. Raising the pH to around 6 results in an enhancement of this band. Further increase to pH 9 not only does not result in reversion to the original B form but, instead, completes conversion to the Z form, as judged by the amplitude of the negative 293 nm band. The two-state nature of such interconversion is suggested by the existence of an isoelectric point at 274 nm, if the partially acid-denatured pH 3.7 spectrum is excluded.

The striking feature of this hysteretic pH titration is made more apparent by plotting the molar ellipticity at 293 nm vs pH as shown in Fig. 5B with all 11 titrated points included.

In addition to the characteristic CD inversion, a B to Z conformational transition is also known to be accompanied by a reduced absorbance at 260 nm and a concomitant intensity enhancement at the 290 nm wing (3). Some representative absorption spectra for 18 μ M poly(dG-m⁵dC):poly(dG-m⁵dC) in 0.01 M NaCl solution without buffer during an acid-base-salt titration are shown in Figure 6A. The prominent appearance of the 290 nm wing after the acid-base titration (curve 2) is apparent, and its near identical spectral features with those of the high salt Z conformation (curve 4) are consistent with the formation of a Z DNA as suggested by the CD evidence presented above. The reversion of this acid-mediated Z conformation to the B form (characterized by the absorbance increase at 260 nm and a concomitant decrease of the 290 nm wing) can be achieved by increasing the NaCl concentration, as shown by the 0.05 M NaCl spectrum (curve 3). It is also interesting to note that the absorption spectra after the acid-base titration (curves 2,3,4) exhibit measurable absorbance above 310 nm, possibly the consequence of light scattering due to

Fig. 6. (A) Representative absorption spectra during an acid-base-salt titration for an 18 μ M aqueous poly(dG-m dC):poly(dG-m dC)/0.01 M NaCl solution without buffer. Spectra were measured with a Cary 210 spectrophotometric system at 25 $^{\circ}$ C using a cuvette of 1-cm pathlength. NaCl concentration was changed by adding appropriate amounts of solid NaCl with manual shaking. (B) Plot of A_{295}/A_{260} vs. pH during an acid-base titration.

some aggregate formation. Krueger & Prairie (8) have also observed slight aggregation in their aqueous Z DNA solution without NaCl and noticed that upon centrifuging at 12000 g for 6 min., the light scattering can be eliminated but with a concomitant 10% reduction in both UV and CD intensities. Although the connection between the low-salt Z form and the Z^* DNA is not clear, it is interesting to note that one of the characteristics of Z^* DNA is the sedimentability through this type of centrifugation (11).

The hysteresis resulted from acid-base titration can more clearly be seen by plotting A_{295}/A_{260} vs. pH as illustrated in Figure 6B. The original solution at pH 7 has an absorbance ratio of 0.23, somewhat higher than the characteristic 0.13 of the B conformation (3). Upon acidifying the

Fig. 7. Ethidium bromide titrations of $11 \mu\text{M}$ poly(dG-m⁵dC):poly(dG-m⁵dC) solutions without buffer: B form with 10 mM NaCl at 25 °C (solid square), acid mediated Z form with 10 mM NaCl at 25 °C (open square), thermally induced Z form with 5 mM NaCl at 35 °C (open cross). Water-jacketed cylindrical cell of 5-cm pathlength was used.

solution to pH 3.7, rapid increase in the absorbance ratio is seen. Gradual alkaline back titration is accompanied by an initial reduction and the eventual leveling of the absorbance ratio from 0.52 to around 0.42 (characteristic of the Z form) for pHs above 5. Absorption ratios higher than 0.42 at pHs below 4.5 are understandable in terms of combined contributions from Z formation, base-protonation of the duplex, and acid denaturation, as all these processes cause enhancement of this ratio.

Although this Z conformation can also be formed in 20 mM NaCl, it was not observed in solutions containing 30 mM or more NaCl. This suggests that the acid mediated Z conformation can only exist in a very narrow NaCl concentration range not far from the low-salt Z to B transition midpoint. The ability to form the acid-mediated Z conformation, however, is sample and batch dependent. For example, in one sample batch only a partial Z conformation can be obtained after returning to neutral pH even though Z conformation is evident in the acidic condition. The Z conformational reduction, however, is a very slow process and takes hours (or overnight) to accomplish.

Transition to the B conformation from this acid-mediated Z form through ethidium bromide titration suggests a step-wise (non-allosteric) conversion. Such a transformation is complete when the [DNA base pair]/[ethidium] ratio reaches around 7 in a 10 mM NaCl solution at 25 °C (Fig. 7), in agreement with the more quantitative results of Walker et al. (12) on the ethidium titration of Z-poly(dG-m⁵dC):poly(dG-m⁵dC) in 2 mM MgCl₂ solution.

In one sample containing 5 mM NaCl, poly(dG-m⁵dC):poly(dG-m⁵dC) exists as a B conformation at temperatures below 25 °C but can be thermally induced into Z-form around 30 °C with a high degree of cooperativity. Ethidium titration of this thermally induced Z DNA at 35 °C indicates that binding of one drug molecule can convert approximately 18 base pairs from Z to the B form (see also Fig. 7).

The acid mediated Z form of poly(dG-m⁵dC):poly(dG-m⁵dC) is thermally quite stable and melts directly into single strands at around 107 °C without prior transformation into a B or other duplex form in a 10 mM NaCl solution of 11 μM DNA concentration. The original B conformation under the same NaCl concentration, however, exhibits a biphasic melting profile as detailed in previous sections. The high temperature Z form can be reverted back to the B form by lowering the temperature. The preference of Z conformation at higher temperatures and the reversibility of such thermally poised B-Z interconversions are in agreement with some recent observations made on this polymer under different solution conditions (13,14,15). As expected, binding of ethidium to B-poly(dG-m⁵dC):poly(dG-m⁵dC) inhibits the thermally induced B to Z transition. For example, the presence of 0.5 μM ethidium in an 11 μM poly(dG-m⁵dC):poly(dG-m⁵dC)/5 mM NaCl solution results in a 30 °C increase in the B to Z transition midpoint and a concomitant reduction in transition cooperativity, as evidenced by a 3-fold reduction in the estimated van't Hoff enthalpy change (not shown).

Addition of 1 mM EDTA (disodium salt) to the acid-mediated Z solution results in an instantaneous conversion to the B form, and in the presence of 1 mM EDTA the original B solution fails to form the Z conformation through acid-base titration. The polymer in the resulting 5 mM NaCl solution (aqueous) containing EDTA melts around 95 °C without first converting to the Z conformation.

A curious conformational dependence on DNA concentration was also noticed during our experiments. In one sample batch, the B conformation appears to be preferred for DNA concentrations below 30 μM while the Z-form is favored at higher concentrations. For example, poly(dG-m⁵dC):poly(dG-m⁵dC) possesses a characteristic Z form CD in 0.33 mM/0.01 M NaCl solution while dilutions from this concentration to 11 and 23 μM at 25 °C result in conversion to the B form in 0.01 M NaCl solutions with half times of roughly 1 and 3 min., respectively. The possibility that this apparent concentration dependence arises from the fact that the free ion concentrations in the stock solution are significantly different from those of diluting buffer, as a

consequence of DNA binding, was tested by dialyzing a stock poly(dG-m⁵dC):poly(dG-m⁵dC) solution (pH 7.2) containing 0.01 M NaCl against a 0.01 M NaCl solution and the dialysate then used to prepare dilute DNA solutions. Indeed, the resulting CD spectra do not appear to exhibit any apparent concentration dependence, all showing Z-form characteristics with a slight B conformational presence as indicated by the reduced positive intensities around 250 nm.

To further investigate the possible role of polyvalent cations in the low-salt Z formation and the acid-mediated hysteresis, comparative studies were also carried out by adding the stock solution (0.4 mM aqueous poly(dG-m⁵dC):poly(G-m⁵dC) solution): (1) directly into regular double distilled water, (2) into an overnight-chelexed water, and (3) into water and the DNA solution then chelexed overnight. A new sample batch was used in these experiments and Chelex-100 resin was employed in chelex treatments.

At 25 °C and moderately basic pH (~10), a 10 µM aqueous solution (no chelex treatment) of poly(dG-m⁵dC):poly(dG-m⁵dC) exhibits a Z conformation, as judged from the CD spectrum. Lowering the temperature to 0 °C, however, results in ~80% B formation. The thermally poised B to Z transition midpoint is around 10 °C and thermal denaturation occurs around 97 °C. The denaturation and B-Z transitions are quite reversible. Salt induced Z to B transition midpoint is roughly 2 mM in NaCl at 25 °C. Acid-base titration of this aqueous B-poly(dG-m⁵dC):poly(dG-m⁵dC) solution containing 2.5 mM NaCl results in Z conformation through the afore-mentioned hysteresis. Results using chelexed water for solution preparation do not appear to be very different from the ones just described, suggesting that the multivalent cation contamination in water may not be significant.

The chelex-treated 10 µM aqueous poly(dG-m⁵dC):poly(dG-m⁵dC) solution (pH ~9.7 upon removal of the resin), on the other hand, exhibits predominant B characteristics at 25 °C with some Z contribution. This solution exists as nearly complete B conformation at 20 °C, can thermally be converted to the Z conformation with a transition mid-point of about 33 °C, and melts into single strands at around 94 °C. Acidifying the solution to pH 6.9 results in a near complete Z formation at 25 °C. Upon returning to the original basic pH, however, the solution converts into a near 100% B form (with no discernable Z contribution) after an overnight wait. This acid-base treated solution increases the thermally poised B to Z transition temperature by about 5 degrees but does not affect the melting

temperature of Z to single-strand transformation.

The stabilization of B conformation upon more elaborate chelex treatment, as evidenced by the increase in the thermally poised B to Z transition mid-point, the reduction in the Z contribution at room temperature, and the higher [NaCl] requirement for the Z to B transition, is consistent with the interpretation that the multivalent cations are responsible for the low-salt Z formation. The presence of minor Z contribution at room temperature even after exhaustive chelex treatment also suggests that the trace ion chelating ability of Chelex-100 is not as powerful as that of EDTA. The formation of a near complete B instead of returning to the original B conformation with minor Z contribution after acid-base titration of the chelexed solution suggests that the sodium ions introduced in the back titration are more important in favoring the B formation than the possible trace metal ion contamination which should favor the Z formation. This interpretation is also supported by the negligible effect on the Z to single-strand melting temperature, while a 5 °C increase is observed in the thermally induced B to Z transition midpoint for the chelexed and acid-base titrated solution. The lower Z to single-strand melting temperature for the chelexed solution also suggests that this melting depends on the amount of multivalent cations present in the solution.

Attempts were also made to carry out acid-base titrations in the presence of Chelex in order to chelate out the possible multivalent cation contamination during such titration. The presence of Chelex does not greatly interfere with the spectral measurements, as Chelex settles rapidly to the bottom after shaking. When the solution is changed to moderately acidic conditions, the tendency for the Z formation is initially apparent (as indicated by the negative going 293-nm CD) but is eventually overshadowed by a slower B formation. The initial tendency to form Z conformation is consistent with the fact that base protonation facilitates Z formation, and the gradual return to the B conformation is the consequence of stronger affinity of Chelex towards H^+ than towards the sodium ion which results in the release of Na^+ into the solution (relative binding affinity and slower second order kinetics are suggested by the supplier). Although the absence of an acid-mediated hysteretic effect is also consistent with the chelation of possible contaminating multivalent cations during the titration, such an interpretation is complicated by the sodium ions release from the resin.

The role of acidity and multivalent cation contamination on the observed hysteresis was also investigated by alternately adding trace amounts of acid

and base (total amounts roughly similar to the ones which caused the observed hysteresis) to an aqueous (non-chelexed) poly(dG-m⁵dC):poly(dG-m⁵dC) solution containing 2.5 mM NaCl (B-form) at pH 10. Such additions do not cause the pH to deviate far from the original value. The resulting solution was let stand for overnight but no Z formation was observed, suggesting that base protonation may be essential for the observed hysteresis and the multivalent cation contamination may be insignificant.

DISCUSSION

The basis for the existence of the low-salt Z conformation most likely is due to the presence of trace amounts of multivalent cations in the solution which bind strongly to the Z form DNA, and have not yet been displaced by the low concentration of sodium ion. This hypothesis appears to be reasonable in view of the extreme effectiveness of the di- and tri-valent cations (5), especially their amine complexes (14), in causing B to Z transitions. Indeed, Woisard et al. (14) have found that the presence of nM quantities of zinc-amine complex can induce B to Z transition in low NaCl solutions, and the van't Hoff enthalpy change for the thermally poised B to Z transition is nearly identical to ours. Chen et al. (21) have also observed that as little as one hexaamminecobalt or spermine bound per 40-50 nucleotides can cause the B to Z transition of poly(dG-m⁵dC):poly(dG-m⁵dC) at low ionic strength. The fact that the addition of EDTA to the low-salt Z (see also reference 7) or to the acid-mediated Z DNA solution results in an instantaneous transformation to the B form, as well as the fact that the presence of EDTA in the solution inhibits the formation of an acid-mediated Z conformation, give further credence to such an hypothesis. The additional facts that dialysis with the buffer solution slightly decreases the Z contribution in the stock solution, that a 15-30 fold dilution with the undialyzed buffer results in B conformation in one sample batch, and that a chelexed aqueous DNA solution at 25 °C exhibits predominant B conformation in contrast to the Z preference in the unchelexed solution are all consistent with the notion that the multivalent cations are originally present in the lyophilized polymer of Z conformation. It should be noted, however, that Fruerstein et al. (10) have observed the formation of Z-form poly(dG-m⁵dC):poly(dG-m⁵dC) in the presence of EDTA under low NaCl concentrations.

Attempts to observe the acid-mediated hysteresis in the presence of known amounts of Mg⁺⁺ or Co³⁺, however, were not successful. It is likely

that other more effective transition metal ions are responsible for the observed phenomenon. Although a very delicate balance between the multi- and mono-valent cation concentrations in the solution is essential for the observation of an acid-mediated hysteresis, the extraordinary sensitivity of poly(dG-m⁵dC):poly(dG-m⁵dC) conformation in regard to temperature should not be overlooked in such an experiment. Our experience suggests that in order to observe such an hysteresis, [Na⁺] and temperature should be very close to the transition midpoints.

The observation of a pH dependent Z conformation is of interest, in light of previous observations (16,17) that base protonation facilitates B-Z interconversions for poly(dG-dC):poly(dG-dC). The existence of an acidic pathway dependent Z form poly(dG-m⁵dC):poly(dG-m⁵dC) suggests that base protonation not only facilitates B-Z interconversions but also has a more profound effect on this polymer than on its unmethylated counterpart. Although the basis for the observed hysteresis in the acid-base titration is not understood, it most likely is the consequence of strong binding of protonated Z DNA to some multivalent cations already in the solution which somehow resist being dislodged by the Na⁺ even after returning to neutral pH.

Since poly(dG-m⁵dC):poly(dG-m⁵dC) is extremely sensitive to the cation concentration under these low salt conditions, one may argue that the observation of an acid-mediated hysteresis is possibly the consequence of the added Na⁺ during back titration with NaOH. The Z formation, however, is contrary to the observation that under low salt conditions the increased sodium ion concentration stabilizes the B rather than the Z conformation (8,9,10). Although the possibility of polyvalent cation contamination during the acid-base titration cannot be ruled out, our experimentation with the Chelex treatment seems to indicate that if such contamination exists, its Z conformation stabilizing effect is more than compensated for by the B conformation stabilization effect of the Na⁺ introduced during the NaOH addition.

On the practical side, the ability to form a Z conformation through acid-base titrations suggests that the ligand binding studies with these two forms can be carried out under conditions of nearly identical ionic strength, thus, eliminating any possible effects due to high salt on the Z DNA binding. For example, ethidium titrations with the B- and the acid-mediated Z-poly(dG-m⁵dC):poly(dG-m⁵dC) indicate much weaker binding ability toward the Z form than to the B form, and the drug induced Z to B transition

is step-wise, suggesting that the requirement of a threshold drug concentration and the greater transitional cooperativity observed at high salt (22) may be the consequence of high sodium ion concentration in the solution.

Although extensive acid-base titrations have previously been carried out, the existence of metastable conformations and striking hystereses have only been observed in polynucleotide solutions containing purine and pyrimidine in separate strands [poly(U):poly(A):poly(U) (18); poly(dG):poly(dC) (19); poly(dA-dG):poly(dC-dT) (20)]. These observed hystereses have been attributed to multiplex formation through G:G and C:C⁺ base pairings. It is not clear, however, that similar mechanisms are operative in the herein observed acid-mediated Z conformation of poly(dG-m⁵dC):poly(dG-m⁵dC), since no Z formation of homopolymers have thus far been uncovered.

The remarkable conformational lability of poly(dG-m⁵dC):poly(dG-m⁵dC) as revealed by its extreme sensitivity toward the presence of trace metal ions, salt concentration, temperature, ligand binding, as well as sample history makes it highly unlikely that nature does not utilize such versatility in some way. The fact that methylation at the 5 position of cytosine has been implicated in the gene inactivation of eucaryotes makes it almost certain that conformational transition plays a significant role in regulatory processes, although the actual mechanism remains unclear.

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