A left-handed (Z) conformation of poly(dA-dC).poly(dG-dT) induced by polyamines

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ABSTRACT

Blocks of potential Z-DNA forming alternating purine-pyrimidine (APP) sequences are widely dispersed in native DNAs. We have studied the effects of naturally occurring polyamines on the conformation of a synthetic APP sequence, poly(dA-dC).poly(dG-dT) by circular dichroism spectroscopy. In the presence of micromolar concentrations of spermidine (125 μ M) and spermine (16 μ M), this polymer undergoes B to Z transition in low ionic strength (2 mM Na⁺) buffers. The concentration of polyamines required for B to Z transition increases with Na⁺ in the buffer and a straight line is obtained on plotting $\ln[\text{Na}^+]$ vs. $\ln[\text{spermidine}^{3+}]$. However, at concentrations of polyamines higher than those necessary to induce B to Z transition, Z-DNA converts to Ψ -DNA, an ordered, twisted, tight packing arrangement of the double helix. These results suggest a pathway for the transient formation of Z-DNA segments *in vivo* by interaction of the ubiquitous polyamines with naturally occurring blocks of APP sequences.

INTRODUCTION

The discovery of the left-handed Z-DNA by single crystal X-ray diffraction and spectroscopic techniques (1-4) has generated considerable interest in the structural polymorphism of DNA, and speculation on the role of altered DNA conformations in gene regulation (5-6). Earlier studies suggested that a strictly alternating block of guanine-cytosine (G-C) sequences is a prerequisite for the right-handed (B) to Z transition of DNA (7). Blocks of these sequences are only rarely found in native DNAs, and even in recombinant plasmids, they have a tendency to undergo deletion (8, 9). Similarly, the high ionic concentrations ($\sim 3\text{M}$ NaCl) required to induce the B to Z transition of poly(dG-dC).poly(dG-dC) make the *in vivo* existence of Z-DNA of questionable importance. However, later studies have shown that methylation of cytosine at the C-5 position, a phenomenon causally related to gene inactivation, dramatically reduces the salt concentration required to induce the B to Z transition of poly(dG-dC).poly(dG-dC) (10). In addition, the naturally occurring polyamines, putrescine ($\text{NH}_2(\text{CH}_2)_4\text{NH}_2$), spermidine ($\text{NH}_2(\text{CH}_2)_3\text{NH}(\text{CH}_2)_4\text{NH}_2$) and spermine ($\text{NH}_2(\text{CH}_2)_3\text{NH}(\text{CH}_2)_4\text{NH}(\text{CH}_2)_3\text{NH}_2$) were found

to induce the B to Z transition of poly(dG-m⁵dC).poly(dG-m⁵dC) at micromolar concentrations (10,11). This result is important since these polyamines are present in all living cells and their levels are elevated in rapidly proliferating cells. In mammalian cells, spermidine and spermine are present in millimolar concentrations (12, 13). Thus, the polyamine-induced Z-DNA formation provides a potential pathway for Z-DNA induction and stabilization under physiologically relevant conditions.

In contrast to the rare occurrence of blocks of (dG-dC)_n.(dG-dC)_n segments, blocks of another APP sequence, (dA-dC)_n.(dG-dT)_n, are widely dispersed in native DNAs (14, 15). These sequences undergo B to Z transition under negative supercoiling in physiological conditions (16). Covalent modification of poly(dA-dC).poly(dG-dT) by acetyl aminofluorene rendered it susceptible to salt-induced B to Z transition at 2M NaCl (17). Similar effects were observed on modification of the polymer by methylation, bromination and iodination (18). However, the unmodified polymer resisted B to Z transition under most conditions used to induce this transition in poly(dG-dC).poly(dG-dC) and its methylated derivative. In this paper, we report the effects of polyamines and Co(NH₃)₆³⁺ on the conformation of poly(dA-dC).poly(dG-dT). Our results show that poly(dA-dC).poly(dG-dT) undergoes B to Z transition at low ionic concentrations in the presence of spermidine and spermine. However, the Z-DNA thus formed is metastable and undergoes further structural reorganizations to give a circular dichroism (CD) spectrum similar to that found for Ψ-DNA, an ordered, twisted, packing arrangement of the double helix (20-24).

MATERIALS AND METHODS

Poly(dA-dC).poly(dG-dT) (Lot No. 517940, s_{20,w}=10.9) was purchased from Pharmacia, Inc. (Piscataway, N.J.). The polymer was dissolved in a buffer containing 0.15M NaCl, 1 mM Na cacodylate, 0.15 mM EDTA to form a stock solution of about 1 mg/mL. This solution was diluted to about 30 µg/mL in a buffer containing 1 mM NaCl, 1 mM Na cacodylate and 0.15 mM EDTA, pH 7.4 (1 mM NaCl buffer). The diluted solution was then dialyzed extensively from this buffer. For spectroscopic measurements at different ionic concentrations, small volumes of 4M NaCl solution was added to the dialyzed solution of poly(dA-dC).poly(dG-dT) in 1 mM NaCl buffer to adjust the concentration of NaCl in the buffer.

Putrescine, spermidine, spermine and N¹- and N⁸- acetyl spermidines were purchased from Sigma Chemical Co. (St. Louis, MO) and were used without further purification. Co(NH₃)₆Cl₃ was prepared by standard methods (25) and was

Figure 1. CD spectra of poly(dA-dC).poly(dG-dT) in 1 mM NaCl, 1 mM Na cacodylate, 0.15 mM EDTA, pH 7.4 + no additives (—); 4 mM putrescine (---); 125 μ M spermidine (— · —); and 15 μ M spermine (·····).

purified by recrystallization from ethanol three times. Concentrated solutions of the various polyamines and $\text{Co}(\text{NH}_3)_6^{3+}$ were prepared so that the volume of these solutions added to poly(dA-dC).poly(dG-dT) solution could be kept at a minimum (less than 10% of total volume). Before the spectroscopic measurements, poly(dA-dC).poly(dG-dT) and the various counterions were mixed and kept for at least 30 min. to attain equilibrium. UV spectra were recorded on a Beckman DU-8 spectrophotometer and molar concentrations were calculated using an extinction coefficient of 6800 at 258 nm. CD spectra were taken on a Jasco J41 spectropolarimeter with a microprocessor attachment.

RESULTS

Figure 1 shows the CD spectra of poly(dA-dC).poly(dG-dT) in the presence of putrescine, spermidine and spermine. In 1 mM NaCl buffer, there is only slight perturbations in the CD spectrum of poly(dA-dC).poly(dG-dT) up to a

Figure 2A. CD spectra of poly(dA-dC).poly(dG-dT) in 1 mM NaCl, 1 mM Na cacodylate, 0.15 mM EDTA, pH 7.4 + no additives (—); spermidine: 40 μM (---); 80 μM (---); 120 μM (---); 160 μM (.....).

Figure 2B. CD spectra of poly(dA-dC).poly(dG-dT) in 1 mM NaCl, 1 mM Na cacodylate, 0.15 mM EDTA, pH 7.4 + no additives (—); spermidine: 240 μM (----); 280 μM (---).

concentration of 4 mM putrescine. However, with the addition of about 125 μM of spermidine, the CD spectrum of poly(dA-dC).poly(dG-dT) undergoes considerable changes, with the appearance of a negative band at 294 nm and a shift in the positive band from 280 nm to 263 nm. These changes are characteristic of B to Z transition (7, 9-11). Similar changes are found with the addition of 15 μM spermine. The B to Z transition of poly(dA-dC).poly(dG-dT) is accompanied by an increase in the positive envelope of the CD spectrum. Figure 2 A and B shows the effects of increasing concentrations of spermidine on the CD spectrum of poly(dA-dC).poly(dG-dT). There are only minor changes in the CD spectrum of this polymer up to a concentration of about 100 μM spermidine. However, over a narrow concentration range of 125 to 150 μM spermidine, Z-DNA spectrum is formed, showing cooperativity of the B to Z transition of poly(dA-dC).poly(dG-dT). It may be mentioned here that the transition occurs at a lower

Figure 3. CD spectra of poly(dA-dC).poly(dG-dT) in 1 mM NaCl, 1 mM Na cacodylate, 0.15 mM EDTA, pH 7.4 + 160 μ M spermidine as a function of time.

concentration of spermidine if EDTA is avoided in the buffer. Similar EDTA effects were observed in the low salt B to Z transition of poly(dG-m⁵dC).poly(dG-m⁵dC) (26). When the concentration of spermidine is increased above that required for B to Z transition, a 20-25 fold increase in the intensity of the positive band of the CD spectrum is observed. This CD spectral change is characteristic of the formation of Ψ -DNA (20-24). The B $\rightarrow$ Z $\rightarrow$ Ψ transitions are reversible, and the B-DNA CD spectrum can be restored on making the solution 0.1M with NaCl.

Figure 3 shows the time dependence of the CD spectral transitions of poly(dA-dC).poly(dG-dT) in the presence of 160 μ M of spermidine. The transition to Z form is almost instantaneous under these ionic conditions. On incubation at 22°C, the intensity of the negative band ($\Delta\epsilon_{294}$) increases and then levels off after about an hour, showing the completion of B to Z transition (Figure 4). However, if $\Delta\epsilon_{262}$ is plotted against time, such a leveling off is not observed.

Figure 4. Effect of reaction time on $\Delta\epsilon_{294}$ (Δ) and $\Delta\epsilon_{262}$ ($\bullet$). Conditions of spectral recordings are given in figure 3.

On the other hand, there is a continuous increase in $\Delta\epsilon_{262}$, showing the conversion of Z-DNA to Ψ -DNA.

The concentration of spermidine required to induce B to Z transition of poly(dA-dC).poly(dG-dT) is dependent on the NaCl concentration. Figure 5 shows

Figure 5. Effect of NaCl concentration on spermidine concentration required to induce B to Z transition of poly(dA-dC).poly(dG-dT) in 1 mM NaCl, 1 mM Na cacodylate, 0.15 mM EDTA, pH 7.4.

Figure 6. CD spectra of poly(dA-dC).poly(dG-dT) in 1 mM NaCl, 1mM Na cacodylate, 0.15 mM EDTA, pH 7.4 + no additives (—); spermine: 4 μM (---); 8 μM (-.-.-); 12 μM (....); 16 μM (— — —); 20 μM (— · — · —); and 24 μM (——).

this dependence, plotted as $\ln [\text{Na}^+]$ vs. $\ln [\text{spermidine}^{3+}]$. A straight line is obtained, demonstrating a pattern predicted by the counterion condensation theory developed by Manning (27) and modified by Wilson and Bloomfield (28). Similar ionic dependence has been reported in the condensations of B-DNA with spermidine (28) as well as in the B to Z transition of poly(dG-m⁵dC).poly(dG-m⁵dC) with $\text{Co}(\text{NH}_3)_6^{3+}$ (10).

Figure 6 shows the effect of spermine on the conformation of poly(dA-dC).poly(dG-dT) in 1 mM NaCl buffer. The changes in the CD spectra are similar to that in the presence of spermidine up to a concentration of 16 μM spermine, when the Z(Ψ)-DNA CD spectrum is observed. However, on increasing the concentration of spermine above this level, the positive band of the spectrum undergoes reduction, with slight shift of the peak maximum to higher wavelength, 263 to 270 nm. The negative band at 294 nm also disappears. These changes can be interpreted as a Ψ to A transition (29). It may be mentioned here that the Z, Ψ and A DNA structures formed in the presence of polyamines show

Figure 7. CD spectra of poly(dA-dC).poly(dG-dT) in 1 mM NaCl, 1mM Na cacodylate, 0.15 mM EDTA, pH 7.4 + no additives (—); $\text{Co}(\text{NH}_3)_6^{3+}$: 100 μM (.....); and 200 μM (---).

considerable tailing of the CD spectrum up to 350 nm. This indicates that poly(dA-dC).poly(dG-dT) undergoes intermolecular collapse and aggregation during these transitions (30).

Since N^1 - and N^8 - acetyl spermidines were shown to induce B to Z transition of poly(dG-m⁵dC).poly(dG-m⁵dC) (11), we have studied the effects of these counterions on the B to Z transition of poly(dA-dC).poly(dG-dT). Both these compounds were not effective in inducing any conformational transitions in the polymer other than slight perturbations in the CD spectrum. In contrast, $\text{Co}(\text{NH}_3)_6^{3+}$, an efficient promoter of B to Z transition of poly(dG-m⁵dC).poly(dG-m⁵dC) could induce only a B to A transition with poly(dA-dC).poly(dG-dT) (Figure 7).

DISCUSSION

The results presented in this paper show that the natural polyamines, spermidine and spermine, induce a conformational transition of poly(dA-dC).poly(dG-dT), characterized by a blue shift of the positive CD band from 280

to 263 nm, with the concomitant formation of a negative band at 294 nm. With the addition of polyamines, the trough at 240 nm of B form poly(dA-dC).poly(dG-dT) also reaches a minimum. This transition occurs over a narrow concentration range of polyamines. The transition is reversible and the B-DNA CD spectrum can be recovered with the addition of NaCl. These changes are consistent with that observed in CD spectra on B to Z transition of poly(dG-dC).poly(dG-dC) and its derivatives (7-11) as well as that of poly(dA-dC).poly(dG-dT), modified with acetyl aminoflourene, in the presence of high concentrations of NaCl (17). Similar spectral changes were also found with poly(dG-m⁵dC).poly(dG-m⁵dC) in the presence of spermidine, spermine, Co(NH₃)₆³⁺ (10) and N¹- and N⁸- acetyl spermidines (11). From a comparison of these spectral transitions, we conclude that poly(dA-dC).poly(dG-dT) undergoes B to Z transition in the presence of spermidine and spermine. This conclusion derives further support from the recent CD and ³¹P NMR studies of Woisard and Fazakerley (31). These investigators have shown that the CD spectrum of poly(dA-dC).poly(dG-dT) undergoes inversion at low ionic concentrations (2 mM Na⁺) in the presence of [tris(2-aminoethyl)amine] zinc(II) chloride (ZnN4) and ZnN4 + spermine, similar to that reported here. Under these conditions, the ³¹P NMR peak of B-DNA at -4.2 ppm splits into two peaks, at -1.7 and -4.1 ppm, another characteristic feature of Z-DNA formation (32). Vorlickova et al. (33) have also described a Z-like conformation of poly(dA-dC).poly(dG-dT) in the presence of high concentrations of CsF.

The Z form of poly(dA-dC).poly(dG-dT) formed in the presence of polyamines is labile and undergoes inter and intramolecular collapse and aggregation leading to Ψ-DNA. The Ψ-DNA is considered to be an ordered, twisted, self-assembly of the polymer to a supramolecular form. Previous studies have shown that Ψ-DNA could be formed from B-DNA under a variety of conditions, including the addition of organic polymers, ethanol and salt, certain histones and polyamines (20-24). The formation of Ψ-DNA from Z-DNA was also demonstrated by Zacharias et al. (34) as an intermediate in B to Z transition of poly(dG-dC).poly(dG-dC) in the presence of sodium acetate in aqueous solution.

The formation of a Z-like form of poly(dA-dC).poly(dG-dT) in aqueous solutions in the presence of polyamines has several important implications. Firstly, blocks of (dA-dC)_n.(dG-dT)_n sequences are widely dispersed in native DNAs (5, 14-15, 35-37). The functional significance of the presence of these sequences is unknown, but their existence in the transcriptional enhancer elements of SV 40 (5), human immunoglobulin gene (35), rat somatostatin gene (36), etc. suggests that these potential Z-DNA forming sequences and the

structural changes associated with them in the presence of polyamines might have a role in gene expression. In this context, it is important that DNA isolated from DNA/anti-DNA immune complexes of systemic lupus erythematosus (SLE), a prototype autoimmune disease in man, showed an enrichment of APP sequences (37), thereby implying that the formation of Z-DNA, which is more immunogenic than B-DNA, might be involved in the production of anti-DNA autoantibodies which occur in SLE patient sera (38, 39). Secondly, polyamines are found in all living cells (12, 13) and are thought to play an important, but not yet known, role in gene regulation (12). Spermidine and spermine are present in millimolar quantities in eukaryotic cells. Higher levels of polyamines are often found in rapidly growing cells, including tumor cells (13), as well as in SLE patients (40). Thus the ubiquitous presence of the potentially Z-DNA forming (dA-dC)_n-(dG-dT)_n sequences and the polyamines provides a rationale for the immunologically detected segments of Z-DNA in native genomes. The formation of Ψ-like form from Z-DNA in the presence of polyamines is also significant since supramolecular organization of DNA is an essential step in the assembly of DNA in virus capsids and chromosomes. The observation of Ψ-DNA formation from poly(dA-dC).poly(dG-dT) is compatible with the tendency of Z-DNA formed from poly(dG-dC).poly(dG-dC), and poly(dG-m⁵dC).poly(dG-m⁵dC) to undergo compaction into toroidal or spheroidal structures in the presence of multivalent ions (26, 41).

Even though APP sequences are known to undergo conformational transitions, accompanied by condensation or aggregation, specific ion effects play an important role in these transitions. Poly(dG-m⁵dC).poly(dG-m⁵dC) and poly(dA-dT).poly(dA-dT) undergo B to Z and B to X transitions, respectively, in the presence of spermidine and Co(NH₃)₆³⁺ (10, 42). In these two cases, Co(NH₃)₆³⁺ is about an order of magnitude more effective than spermidine. Similar ion specific effects were observed in the collapse of native B-DNA with these counterions (43). In contrast, the present study shows that Co(NH₃)₆³⁺ is not an effective cation to provoke B to Z or B to Ψ transitions of poly(dA-dC).poly(dG-dT), whereas spermidine and spermine are effective promoters of these transitions. The N¹- and N⁸- acetyl spermidines, which were effective with poly(dG-m⁵dC).poly(dG-m⁵dC) (11), are also ineffective in the case of poly(dA-dC).poly(dG-dT). The reason for these variations might lie in the different modes of binding of counterions with polynucleotides, dictated by the charge density and flexibility of the counterions as well as the negative charge spacing on the polynucleotides (44).

A mechanism for the ability of polyamines to induce conformational

transitions in APP sequences has not been established despite extensive research on the interaction of polyamines with polynucleotides in recent years (45, 46). Since the polyamines are positively charged under physiological pH, it is reasonable to assume that DNA phosphate charge neutralization is the first step in the interaction of polyamines with DNA. Earlier studies on the condensation of B-DNA with polyamines showed that neutralization of DNA phosphate charge to the extent of about 89%, as calculated from Manning's polyelectrolyte theory (27), was a prerequisite to induce the collapse of DNA into compact structures (28). However, B to Z transition of poly(dG-m⁵dC).poly(dG-m⁵dC) was achieved at much lower phosphate charge neutralization by Co(NH₃)₆³⁺ and spermidine (10). In both cases, an interionic dependence predicted by the polyelectrolyte theory was observed (10, 28), giving a straight line on plotting ln [Na⁺] vs. ln [spermidine³⁺]. We find a similar dependence of ln [Na⁺] vs. ln [spermidine³⁺], in B to Z transition of poly(dA-dC).poly(dG-dT). The concentrations of polyamines required to induce B to Z transition in poly(dA-dC).poly(dG-dT) are much higher than those necessary for poly(dG-m⁵dC).poly(dG-m⁵dC). This is not surprising since drastic conditions, such as the presence of about 19% ethanol (6) or the presence of NiCl₂ at very high polymer concentration (47) were required to induce B to Z transition of poly(dA-dC).poly(dG-dT) in the presence of NaCl.

In conclusion, these results identify the conditions under which poly(dA-dC).poly(dG-dT) can undergo B to Z and Z to Ψ transitions in aqueous solution in the presence of polyamines. Since (dA-dC)_n.(dG-dT)_n sequences and polyamines are widely dispersed in the cell, these results provide a potential pathway by which genomic DNA may assume the Z-form under favorable conditions. Whether such a mechanism is operative or realized in vivo and its effects on gene regulation are yet to be understood.

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