Selectivity of Polyamines in Triplex DNA Stabilization†

Thresia Thomas and T. J. Thomas*

Department of Environmental and Community Medicine and Program in Clinical Pharmacology, Clinical Research Center, University of Medicine and Dentistry of New Jersey–Robert Wood Johnson Medical School, New Brunswick, New Jersey 08903

Received August 6, 1993; Revised Manuscript Received October 11, 1993•

ABSTRACT: Triplex DNA has been recently studied as an anti-gene strategy to suppress the transcription of specific genes. A major challenge in this area is to stabilize triplex DNA at physiological conditions. We studied the effects of putrescine, spermidine, spermine, their synthetic homologs, and their acetyl derivatives on triple- and double-stranded structures formed from poly(dA) and poly(dT) by measuring their respective melting temperatures. In the presence of polyamines, the absorbance (A) versus temperature (T) profile showed two transitions: T_{m1}, corresponding to triplex → duplex + single-stranded DNA, and T_{m2}, corresponding to duplex melting. In the presence of 0.5 mM putrescine, T_{m1} and T_{m2} were 44.8 and 71 °C, respectively, in 10 mM sodium cacodylate buffer (pH 7.2). In contrast, triplex DNA was not detectable when the A versus T profile of the polynucleotides was monitored in the absence of putrescine. T_{m2} was also lower (55 °C) in the absence of putrescine. With 2.5 μM spermidine or 0.1 μM spermine, T_{m1} values were 42.8 and 54.4 °C and T_{m2} values were 65 and 82 °C, respectively. As the concentration of polyamine was increased, the difference between T_{m1} and T_{m2} decreased, and both melting transitions merged into one T_m, corresponding to the melting of triplex DNA to single strands. In a series of putrescine homologs, $H_2N(CH_2)_nNH_2$ where n = 2–6 (n = 4 for putrescine), $H_2N(CH_2)_3NH_2$ was the most effective diamine to stabilize the poly(dA)·2poly(dT) triplex. At 10 mM concentration, diaminopropane stabilized the triplex DNA such that the T_{m1} was 10 °C higher than that in the presence of an equimolar concentration of putrescine. Among a series of triamines $H_2N(CH_2)_3NH(CH_2)_nNH_2$ (where n = 2–8), spermidine (n = 4) was the most effective triplex-stabilizing agent. Similar structural specificity was found for N^1- and N^8-acetyl spermidines in stabilizing triplex DNA. In contrast, effects of these compounds on duplex DNA stabilization were relatively insensitive to changes in the length of the methylene bridging region. Our data demonstrate a differential structural effect of polyamines on stabilizing triplex versus duplex DNA and suggest novel strategies for developing triplex-based anti-gene therapeutic modalities.

Triple-helical DNA has been described in the literature for more than 3 decades, but the biological relevance of this DNA structure and its potential therapeutic implications have come to light only recently (Cooney *et al.*, 1988; Felsenfeld *et al.*, 1957; Giovannangél *et al.*, 1992; Glaser & Gabbay, 1968; Hélène & Tolulme, 1990; Le Doan *et al.*, 1987; Morgan & Wells, 1968; Moser & Dervan, 1987). Oligonucleotides with the potential to form triplex structure at the promoter region of growth regulatory genes, such as c-*myc*, interleukin-2 receptor, DNA polymerase α, and a progesterone-responsive gene, are being investigated to suppress the transcription of these genes and to develop new therapies for diseases including cancer, AIDS, and autoimmunity (Durland *et al.*, 1991; Giovannangeli *et al.*, 1992; Gee *et al.*, 1992; Gandhi *et al.*, 1993; Ing *et al.*, 1993). Recent research has shown that triplex-forming and antisense (for which the target is mRNA) oligonucleotides could be transported into cells and that they could exert their action in the cytoplasm and/or nuclei (Burch & Mahan, 1991; Stein *et al.*, 1993). However, several hurdles, including the low stability of triple-helical DNA under physiological conditions, remain to be solved before triplex-forming oligonucleotides (TFOs) can be utilized for therapeutic purposes (Wickstrom, 1992).

TFOs bind to DNA through the major groove in an antiparallel orientation forming strong T·A·T or G·G·C triplexes, with dissociation constants between 10^{-7} and 10^{-9} M (Rougée *et al.*, 1992). The presence of multivalent cations such as Mg^{2+} or spermine was essential for the stabilization of triplex DNA at physiological pH (Hampel *et al.*, 1991; Kohwi & Kohwi-Shigematsu, 1988; Mergny *et al.*, 1992; Moser & Dervan, 1987). Triplex DNA could form *in vivo* either by the binding of an oligonucleotide to a continuous stretch of purines or pyrimidines or as a part of an H-DNA (hinge DNA), which consists of folded regions of triple- and single-stranded DNA (Beltran *et al.*, 1993; Htun & Dahlberg, 1989; Kang *et al.*, 1992). Triplex-forming sequences consisting of purine·pyrimdine tracts are present in the regulatory regions of several genes and represent up to 1% of certain eukaryotic genomes (Hoffman-Lieberman *et al.*, 1986; Manor *et al.*, 1988). The stabilization of triplex DNA by polyamines is particularly important because of the ubiquitous presence of these molecules and the feasibility of modifying their structural features to provide efficient ligands to interact with DNA and stabilize unusual structures (Thomas *et al.*, 1985; Vertino *et al.*, 1987).

Polyamines—putrescine ($H_2N(CH_2)_4NH_2$), spermidine ($H_2N(CH_2)_4NH(CH_2)_3NH_2$), and spermine ($H_2N(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$)—are known to play important roles in cell proliferation and differentiation (Marton & Morris, 1987; Pegg, 1988; Tabor & Tabor, 1984). Cellular polyamine levels are exquisitely regulated by growth stimulatory and inhibitory agents. Ornithine decarboxylase is a key enzyme involved in the biosynthesis of polyamines (Heby & Persson, 1990). Increased accumulation of natural polyamines suppresses their own biosynthesis by feedback

† This work was supported, in part, by research Grant CA42439 from the National Institutes of Health and by the W. H. Conzen Chair of Clinical Pharmacology (Dr. James R. Seibold).

* Author to whom correspondence should be addressed.

• Abstract published in *Advance ACS Abstracts,* December 1, 1993.

0006-2960/93/0432-14068$04.00/0

control mechanisms, including the degradation of ODC as well as the stimulation of spermidine and spermine acetyl transferases. In eukaryotes, spermidine and spermine are present in millimolar concentrations and may be as high as 5 mM in the nucleus (Sarhan & Seiler, 1989). However, a major portion of intracellular polyamines exists bound to macromolecules, and the concentrations responsible for regulatory interactions might be in the micromolar range (Davis *et al.*, 1992). Exogenous polyamines and their analogs are readily transported into the cell (Feuerstein *et al.*, 1992).

Stabilization of double-stranded DNA by polyamines has been recognized for many years (Tabor, 1962; Thomas & Bloomfield, 1984). The effect of polyamines on the stability of the duplex DNA is governed by ionic charge, pH, the ionic concentration of the medium, and the acetylation status of the polyamine. Structural specificity effects were minimal in studies using synthetic homologs of the natural polyamines on duplex DNA stabilization. In contrast, structural specificity is an important feature in the induction and stabilization of left-handed Z-DNA by polyamines (Basu & Marton, 1987; Thomas *et al.*, 1985; Thomas & Messner, 1988; Vertino *et al.*, 1987). Hampel *et al.* (1991) showed that natural polyamines favor triplex DNA formation in neutral pH and suggested that polyamines could be important in stabilizing triplex DNA in chromosomal structures. Since the negative charge density of a triplex is higher than that of a duplex, polyamines should bind more tightly to the former and shift the equilibrium in favor of triplex formation. In the present study, we questioned whether the polyamines' structure plays a role in triplex DNA stabilization, in addition to their ability to interact with DNA by electrostatic forces. To address this question, we used a series of putrescine and spermidine homologs that differed in the number of methylenes in the bridging region separating the positively charged amino and imino groups, while keeping the same ionic charge (+2 for putrescine homologs and acetylspermidines and +3 for spermidine homologs). Our data show that chemical structural effects play an important role in stabilizing the poly(dA)·2poly(dT) triplex, whereas(dA)·poly(dT) duplex stabilization is largely governed by the number of positive charges on the polyamine.

MATERIALS AND METHODS

Polyamines. Putrescine·2HCl (1,4-diaminobutane, C4), spermidine·3HCl, and spermine·4HCl were purchased from Sigma Chemical Co. (St. Louis, MO). Putrescine homologs—1,2-diaminoethane (C2), 1,3-diaminopropane (C3), 1,5-diaminopentane (C5), and 1,6-diaminohexane (C6)—were also purchased in their hydrochloride forms from Sigma. Two lower homologs of spermidine, *N*-(2-aminoethyl)-1,3-propanediamine and 3,3′-iminobis(propylamine), were purchased as free amines from Aldrich Chemical Co. (Milwaukee, WI), converted to their hydrochlorides, and purified by recrystallization from ethanol (Thomas & Bloomfield, 1984). Higher homologs of spermidine, $H_2N(CH_2)_nNH(CH_2)_4NH_2$ (where n = 5–8), were obtained in their hydrochloride forms from Professor David Morris of the University of Washington (Seattle, WA), who has described the synthesis and characterization of these compounds (Jorstad *et al.*, 1980). For simplicity of nomenclature, these compounds are considered as derivatives of the aminopropyl group and are thus represented as AP2–AP8. N^1- and N^8-acetylspermidine·2HCl were purchased from Sigma. High-performance liquid chromatography (HPLC) analysis of these compounds showed no contaminating polyamines. Concentrated solutions of the polyamines were prepared in 10 mM sodium cacodylate buffer, the pH was adjusted to 7.2, and small volumes were added to polynucleotide solutions to make up to the necessary concentrations.

Polynucleotides. Poly(dA) and poly(dT) were purchased from Pharmacia, Inc. (Piscataway, NJ). The polynucleotides were dissolved in 10 mM sodium cacodylate buffer (pH 7.2) and 0.5 mM EDTA and dialyzed extensively from the same buffer. All experiments were conducted in this buffer. Concentrations of poly(dA) and poly(dT) were measured using molar nucleotide extinction coefficients of 8900 at 257 nm for poly(dA) and 9000 at 265 nm for poly(dT). To prepare triplex solution, poly(dA) and poly(dT) were mixed in a 1:2 molar ratio in 10 mM sodium cacodylate buffer, and the appropriate concentrations of polyamines, $MgCl_2$, and/or NaCl were added. The solutions were heated at 90 °C for 5 min, cooled to room temperature (22 °C), and allowed to equilibrate for 16 h at this temperature before use in melting temperature experiments.

Melting Temperature Measurement. Absorbance versus temperature heating curves were obtained using a Perkin-Elmer Lambda 2 spectrophotometer. The temperature of the five-cell holder was regulated by a thermoelectrically regulated programmer interfaced to an IBM PS2 computer. Melting profiles were obtained by increasing the temperature at a rate of 0.5 °C/min with the absorbance and temperature being recorded every 30 s. Melting temperatures (T_m) were taken as the temperatures corresponding to half-dissociation of the complexes, and the reproducibility was within ±0.5 °C. The first derivative, dA/dT (where A is absorbance and T is temperature), of the melting curve was computer generated and was also used for determining the melting temperatures of the triplex (T_{m1}) and duplex (T_{m2}) forms of the polynucleotides. Melting temperature values obtained from both methods did not differ by more than 0.5 °C.

RESULTS

Effect of Putrescine, Spermidine, and Spermine on the Melting Transitions of Poly(dA)·2Poly(dT). The formation of duplex and triplex DNAs from poly(dA)·2poly(dT) was examined by the method of continuous fractions (Job, 1928; Plum *et al.*, 1990; Thomas *et al.*, 1986). An inflection point at 0.5 mol fraction representing duplex DNA formation was observed both in the absence and presence of 0.5 mM putrescine (results not shown). But when triplex formation was examined by this method using molar fractions of the duplex DNA and a third strand of poly(dT), an inflection point representing triplex formation was obtained only in the presence of putrescine. We used melting temperature measurements to characterize the stability of duplex and triplex DNA in the presence and absence of various polyamines and inorganic cations.

Figure 1A shows the absorbance versus temperature profile and the first derivative of poly(dA) and poly(dT) mixed in a 1:2 ratio in 10 mM sodium cacodylate buffer. There was a sharp increase in absorbance centered at 55.5 °C showing the melting transition of the polynucleotide. In another set of experiments using poly(dA) and poly(dT) in a 1:1 ratio, the melting temperature was 55 °C, confirming that the melting temperature obtained in Figure 1A is that of duplex DNA.

Figure 1B shows the melting profile of poly(dA)·2poly(dT) in the presence of 10 mM sodium cacodylate buffer containing 0.5 mM putrescine. As the temperature increased, there was an increase in absorbance centered at 44.8 °C and then another transition centered at 71 °C. The first melting transition,

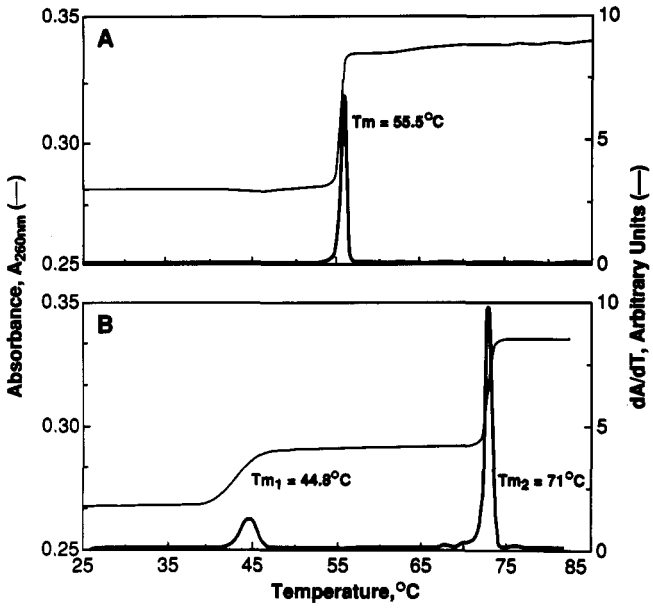

FIGURE 1: UV absorbance versus temperature profiles and the first derivative plots of poly(dA)·2poly(dT) in the absence (A) and presence (B) of 0.5 mM putrescine. Melting temperatures (T_m) corresponding to the melting of triplex (T_{m1}) and duplex (T_{m2}) forms of the polynucleotides are indicated in B. All melting temperature experiments were conducted in a buffer containing 10 mM sodium cacodylate (pH 7.2) and 0.5 mM EDTA.

designated as T_{m1}, corresponds to the melting of triplex DNA to duplex DNA and a single-stranded DNA: poly(dT)·poly(dA)·poly(dT) → poly(dA)·poly(dT) + poly(dT). The second melting transition of T_{m2} corresponds to the melting of poly(dA)·poly(dT). There was a 16 °C increase in T_{m2} compared to the melting of duplex DNA in Figure 1A. This increase is attributable to the stabilization of duplex DNA by putrescine, as reported previously (Tabor, 1962; Thomas & Bloomfield, 1984).

Figure 2A shows the effect of putrescine on T_{m1}, T_{m2}, and the difference between T_{m2} and T_{m1}. On increasing the putrescine concentration from 0.5 to 275 mM, T_{m1} and T_{m2} merged, with T_{m1} increasing by nearly 50 °C and T_{m2} by only 20 °C. The single transition above 275 mM corresponds to the triplex melting to single strands.

Figure 2B shows the influence of spermidine on T_{m1} and T_{m2}. The trends of T_{m1} and T_{m2} versus log[spermidine] curves are similar to that obtained in the case of putrescine. However, the concentration of spermidine needed to induce and stabilize triplex DNA was 200-fold lower than that of putrescine. At 2.5 μM spermidine, T_{m1} and T_{m2} were 42.5 and 65.4 °C, respectively. T_{m1} and T_{m2} merged into a single melting transition (80.5 °C) at 50 μM spermidine.

Similar studies were conducted with spermine concentrations ranging from 0.1 to 10 μM (results not shown). In the presence of 0.1 μM spermine, T_{m1} was 54 °C and T_{m2} was 82 °C. T_{m1} increased to 85 °C at 7.5 μM spermine and merged with T_{m2}. Spermine is thus 25-fold more efficient than spermidine in stabilizing triplex and duplex DNAs. This relative efficacy of polyamines, spermine > spermidine > putrescine, is a well-known feature of polyamine effects and reflects the combined influence of their ionic charge as well as chemical structure (Gosule & Schellman, 1976; Thomas & Bloomfield, 1984; Wilson & Bloomfield, 1979).

Structural Specificity Effects of Polyamines on Triplex and Duplex DNA Stabilization. We next examined the relative importance of polyamine structure and ionic state in stabilizing triplex and duplex forms of poly(dA)·2poly(dT)

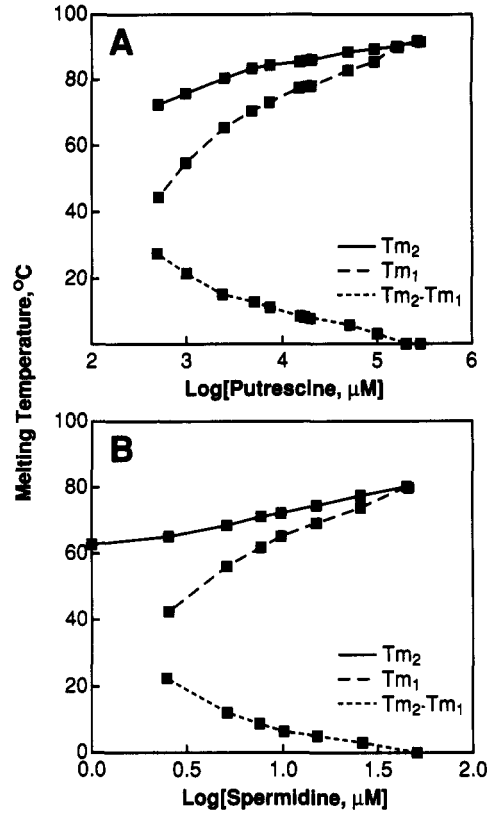

FIGURE 2: Effect of putrescine (A) and spermidine (B) concentrations on the melting temperatures of triplex (T_{m1}) and duplex (T_{m2}) forms of DNA. The differences between T_{m2} and T_{m1} at each polyamine concentration are also plotted.

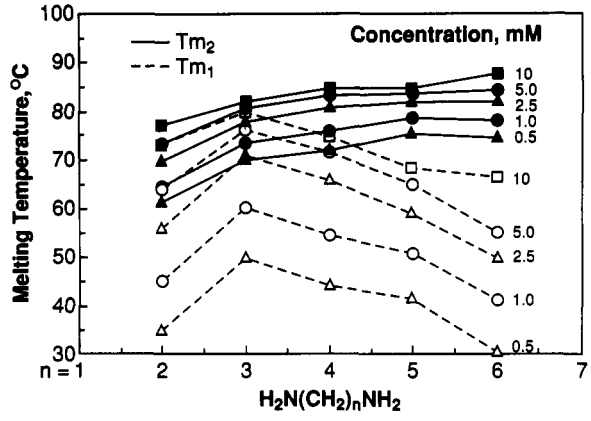

FIGURE 3: Structural specificity effects of putrescine homologs on the melting temperatures of triplex (T_{m1}) and duplex (T_{m2}) forms of DNA. The number of methylene groups (n) in putrescine homologs is plotted against the melting temperature at different diamine concentrations.

using four homologs of putrescine, $H_2N(CH_2)_nNH_2$, where n = 2–6 (n = 4 for putrescine). Figure 3 shows the values of T_{m1} and T_{m2} plotted against the methylene chain length, n. At the indicated concentrations of the diamines, T_{m1} and T_{m2} were derived from the same absorbance versus temperature profile. At the five concentrations (0.5, 1, 2.5, 5, and 10 mM) that we studied, there was a remarkable structural specificity effect of diamines on T_{m1}, showing an initial increase from diaminoethane to diaminopropane and a subsequent decrease for diaminobutane (putrescine) and higher homologs. Diaminopropane exerted the highest stabilizing effect on triplex DNA at all concentrations studied. In contrast, a marked increase in T_{m2} occurred from diaminoethane to diaminopropane only.

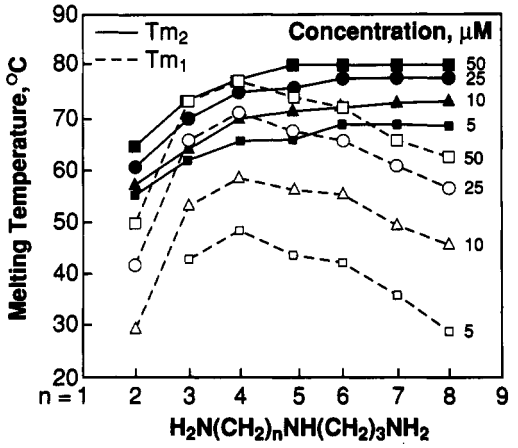

FIGURE 4: Structural specificity effects of spermidine homologs on the melting temperatures of triplex (T_{m1}) and duplex (T_{m2}) forms of DNA. The number of methylene groups (n) on the variable arm of spermidine homologs is plotted against the corresponding melting temperatures at different triamine concentrations.

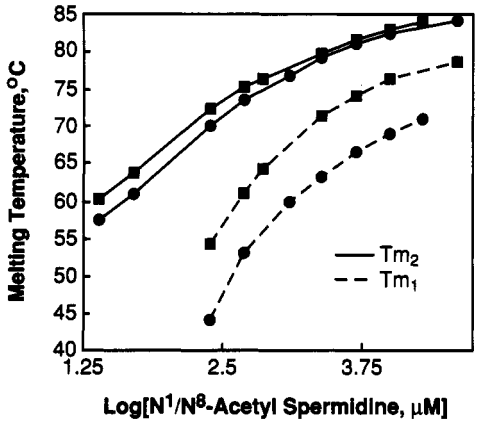

FIGURE 5: Effect of N^1- (●) and N^8-acetylspermidines (■) on the melting temperatures of triplex (T_{m1}) and duplex (T_{m2}) forms of DNA.

Figure 4 shows our results on the structural specificity of spermidine homologs. Among a series of seven triamines, spermidine was the most effective compound in stabilizing the triplex form of poly(dA)·2poly(dT). The efficacy of spermidine (n = 4 on the variable methylene bridging region) in stabilizing triplex DNA was clear from T_{m1} values that peaked at this chain length at all concentrations of the triamines studied. The nearest homologs of spermidine, AP3 and AP5, had stabilizing effects comparable to that of spermidine. The lower (AP2) and higher (AP6, AP7, and AP8) homologs were much less effective than spermidine in their ability to stabilize triplex DNA. In contrast, T_{m2} was less sensitive to changes in the length of the methylene bridging region, particularly for the higher homologs of spermidine. AP2 was the least effective triamine in stabilizing duplex DNA, and this could be attributed to incomplete protonation of this molecule (Thomas & Bloomfield, 1984).

Effect of Spermidine Acetylation on Triplex DNA Stabilization. Figure 5 shows the influence of the acetylation of spermidine on the ability of this polyamine to stabilize triplex DNA. Acetylation of the primary amino group of spermidine produces two monoacetyl derivatives, N^1- and N^8-acetylspermidine, that differ in positive charge separation by one methylene group. In Figure 5, effects of these two compounds on T_{m1} and T_{m2} are plotted against their concentrations. At identical concentrations of N^1- and N^8-acetylspermidines, the duplex melting temperatures (T_{m2}) of poly(dA)·poly(dT) were

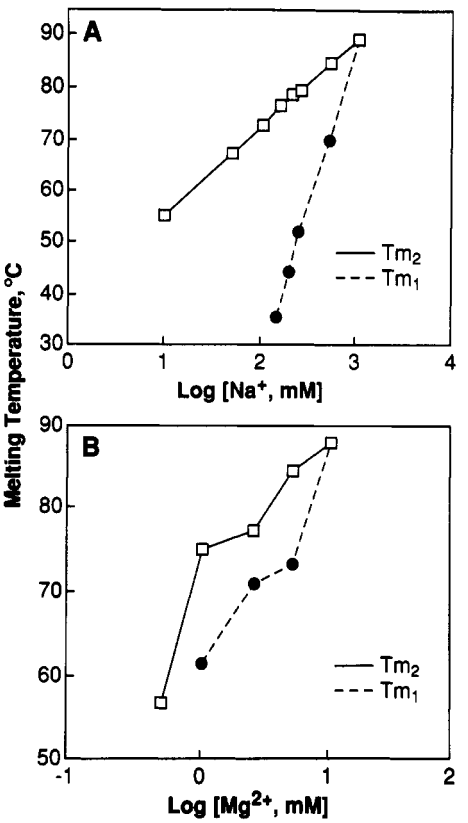

FIGURE 6: Effect of NaCl (A) and $MgCl_2$ (B) concentrations on the melting temperatures of triplex (T_{m1}) and duplex (T_{m2}) forms of DNA.

similar. In contrast, N^8-acetylspermidine had a greater stabilizing effect on triplex DNA than that exerted by N^1-acetylspermidine. At identical concentrations, T_{m1} of the triplex in the presence of N^8-acetylspermidine was 7.5–10.2 °C higher than that in the presence of N^1-acetylspermidine. This result confirms our observation with putrescine homologs that the diaminopropane moiety has the optimal stabilizing effect on triplex DNA. N^8-Acetylspermidine has a functional diaminopropane structure, while N^1-acetylspermidine has a diaminobutane structure.

Effect of NaCl and $MgCl_2$ on T_{m1} and T_{m2}. Figure 6A,B shows the effects of NaCl and $MgCl_2$, respectively, on T_{m1} and T_{m2} of poly(dA)·2poly(dT). Increasing concentrations of both of these inorganic cations stabilized triplex and duplex forms of the polynucleotide. T_{m1} and T_{m2} merged into one T_m in the presence of 1000 mM NaCl or 10 mM $MgCl_2$. These data also show that diamines are more efficient than $MgCl_2$ in stabilizing triplex DNA. For example, triplex DNA was not detectable at 0.5 mM and lower concentrations of Mg^{2+}, whereas triplex stabilization occurred at 0.5 mM putrescine with a T_{m1} of 44.8 °C (Figure 1B). With 0.5 mM diaminopropane, T_{m1} was 50.2 °C. Thus, polyamines are more efficacious than inorganic cations in stabilizing triplex DNA.

Effect of NaCl on the Polyamine Effect. Figure 7 shows the effect of different concentrations of NaCl on the T_{m1} and T_{m2} of poly(dA)·2poly(dT) in the presence of 5 mM putrescine. As the concentration of NaCl increased, there was a decrease in T_{m1} up to 100 mM NaCl, indicating the competitive displacement of putrescine by NaCl and the resulting destabilization of the triplex DNA. At 150 mM NaCl and 5 mM putrescine, T_{m1} was 58 °C, whereas in the presence of 150 mM NaCl alone, T_{m1} was 36 °C (Figure 6A). Thus at high concentrations of NaCl, triplex stabilization occurred

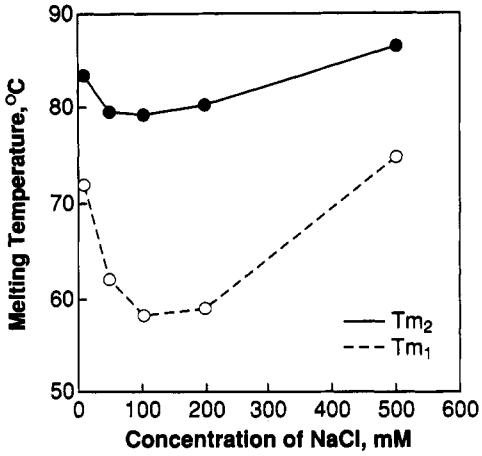

FIGURE 7: Effect of NaCl on the stabilization of triplex (– –) and duplex (—) forms of poly(dA)·2poly(dT) in the presence of 5 mM putrescine.

through the combined effects of both putrescine and NaCl. This result also demonstrates that putrescine could stabilize the triplex form of poly(dA)·2poly(dT) at physiologically compatible ionic concentrations.

DISCUSSION

Data presented in this paper demonstrate that natural polyamines and their synthetic homologs are excellent promoters of the triplex form of poly(dA)·2poly(dT) at physiological pH. In the presence of 150 mM NaCl, 5 mM putrescine increased the T_{m1} of poly(dA)·2poly(dT) by 22 °C compared to that in its absence. Our results also show a remarkable structural specificity of divalent and trivalent polyamines in stabilizing triplex DNA. Among a series of diamines and triamines, diaminopropane and spermidine were the most efficient compounds in the induction and stabilization of triplex DNA. N^8-Acetylspermidine is more effective than N^1-acetylspermidine in stabilizing triplex DNA. A common structural determinant of these compounds is the trimethylene bridging region separating two positive charges. These findings are important in understanding the ligand structural requirements for stabilizing triplex DNA and may contribute to the development of novel therapeutics based on TFO-mediated suppression of transcription.

Our results on the effect of polyamines on the stability of duplex DNA are consistent with previous studies by us and others (Tabor, 1962; Thomas & Bloomfield, 1984). The ionic state of the polyamines is important in determining their effectiveness in increasing the melting temperature of the duplex DNA, but no significant influence of polyamine structure was observed with a series of putrescine or spermidine homologs. For example, Tabor (1962) reported that a series of diamines from diaminoethane (C2) to diaminooctane (C8) exerted comparable effects on the T_m (ranging from 81.9 to 84.2 °C in the presence of 1 mM concentrations of diamines) of calf thymus DNA. There was no significant structural specificity effect for AP4–AP8 on the melting of calf thymus DNA at different NaCl concentrations (Thomas & Bloomfield, 1984). It was, however, found that AP2 and AP3 were less effective than spermidine in stabilizing duplex DNA (Thomas & Bloomfield, 1984). These differences were attributed to partial protonation of these compounds and/or their inability to make effective contacts with the phosphate groups of DNA (Suwalski *et al.*, 1969; Tabor, 1962; Thomas & Bloomfield, 1984). Our current data on the effect of these compounds on poly(dA)·poly(dT) duplex melting are similar. In contrast, we report a remarkable structural specificity of these compounds in triplex DNA stabilization. Furthermore, divalent polyamines are more efficient stabilizers of triplex DNA than an inorganic cation, Mg^{2+}.

The structural specificity of spermidine homologs on triplex DNA reported here is comparable to that observed in the case of Z-DNA induction and stabilization by polyamine homologs and derivatives (Basu & Marton, 1987; Thomas *et al.*, 1985; Vertino *et al.*, 1987). Thomas and Messner (1988) found a 40-fold difference between the concentrations of spermidine and AP8 at the midpoint of the B-DNA to Z-DNA transition of poly(dG-m^{5}dC)·poly(dG-m^{5}dC). With respect to the two acetyl derivatives, N^8-acetylspermidine with a charge separation of three methylene groups was 2-fold more efficient than N^1-acetylspermidine in Z-DNA formation (Thomas *et al.*, 1985). In our experiments with triplex DNA, we found the same order of efficacy in elevating T_{m1} as was observed for provoking Z-DNA. These structural effects may reflect relative affinities of various polyamines for DNA duplex and triplex forms. Padmanabhan *et al.* (1991) showed that the relative affinities of 1,2-diaminopropane and putrescine for duplex DNA were higher than those of 1,5-diaminopentane and 1,6-diaminohexane by using ^{23}Na relaxation experiments.

The effect of the ionic charge of polyamines on their ability to interact with DNA and to stabilize unusual structures such as triplex DNA cannot be discounted. The efficacy of these compounds to increase triplex melting temperature increases with their ionic charge: spermine > spermidine > putrescine. Acetylation further reduces their efficacy to raise T_{m1}. There is a minimum in the plot of T_{m1} versus NaCl at constant putrescine concentration (Figure 7). As suggested by Manning (1978) and Record *et al.* (1978) for counterion effects of duplex DNA stabilization, the triple helix might be stabilized relative to duplex DNA and single-stranded coils by counterion condensation because the negative charge density on triplex DNA is higher than that on the latter two forms. As $[Na^+]$ increases, the multivalent cations are displaced by Na^+ due to ion competition for the available negative charges on DNA. At the same time, there is a weaker destabilization by the diffuse Debye–Hückel ion atmosphere composed predominantly of univalent ions. The balance of these complex interactions results in an initial decrease in T_m with $[Na^+]$, reaching a minimum followed by a rise as the univalent cations come to dominate the charge cloud around the DNA phosphate charges (Thomas & Bloomfield, 1984; Wilson & Bloomfield, 1979).

Our findings on the structural specificity of polyamines in triplex DNA stabilization raise new questions on the mechanism of polyamine–DNA interaction. Electrostatic interactions between the positively charged amino or imino groups of polyamines on DNA phosphate groups are insensitive to the variations in the methylene groups. Molecular modeling and molecular mechanical calculations on polyamine–DNA interactions have suggested a binding mode involving the docking of polyamines in either the major or minor groove of DNA (Feuerstein *et al.*, 1986; Suwalski *et al.*, 1969). Experimental evidence for either mode of interactions is scant. Single-crystal X-ray crystallography (Egli *et al.*, 1991) of oligonucleotide–spermine complexes showed a complex mode of interactions involving the binding of the amino groups of spermine in the major groove of DNA. At least three spermine molecules were bound to a single hexanucleotide, with each hexanucleotide, in turn, making contact with three spermine molecules. Using photoaffinity cleavage with polyaminobenzenediazonium salts, Schmid and Behr (1991) showed

strong minor grove and weak A,T preferences of the polyamines. They further suggested fast crawling of the polyamine within the minor groove due to individual NH_2^+ jumping between multiple equidistant and isoenergetic bidentate hydrogen-bonding sites. In triplex DNA, the major groove is occupied by the third strand and, hence, docking of polyamines in the major groove might be blocked, rendering the minor groove the preferred site of interaction.

Triplex stabilization has important applications in the mapping of large genomes (Strobel & Dervan, 1990). Triplex formation could be used to limit the specificity of restriction enzymes to recognition sequences, thereby permitting the orchestrated cleavage of large genomic DNA and enabling isolation and mapping of selected regions of chromosomal DNA (Strobel *et al.*, 1991). Lee *et al.* (1987) have shown that monoclonal antibodies to triplex DNA recognize the presence of this structure in chromosomal loop regions. Triplex DNA might be important in transcriptional regulation *in vivo*, possibly through RNA triplex formation and interference with the binding of transcription factors at the promoter regions of certain genes (Celano *et al.*, 1992; Roberts & Crothers, 1992). Recent studies show that the hereditary persistence of fetal hemoglobin is associated with point mutations that disrupt intramolecular DNA triplex formation (Ulrich *et al.*, 1992).

Our findings also raise the possibility that diaminopropane or its derivatives might be useful in selectively stabilizing triplex DNA *in vivo*. Cooney *et al.* (1988) showed that treatment of cells with an oligonucleotide with the potential to form triplex structure at the promoter region of the c-*myc* protooncogene inhibited the expression of this gene without affecting other genes. "Footprinting analysis" showed the presence of triple-helical DNA structure at the promoter sites of the c-*myc* gene. In addition to polyamines, benzo[*a*]-pyridoindole, ethidium bromide, acridine, and psoralen also stabilize triplex DNA (Giovannangéli *et al.*, 1992; Grigoriev *et al.*, 1993; Scaria & Shafer, 1991; Sun *et al.*, 1989) and are being studied for site-specific binding to gene promoters.

In summary, the data presented here provide a quantitative measure of the effects of salt, ionic charge, and polyamine structure, as well as the acetylation status of polyamines, on the stability of triplex DNA as measured by thermal helix–coil transition temperatures. These data might be useful in defining the experimental conditions necessary to stabilize triplex DNA and to realize the potential usefulness of triplex-based therapy as an anti-gene strategy. Our laboratory is currently undertaking studies to use this technology to inhibit steroid hormone-mediated proliferation of breast cancer cells.

ACKNOWLEDGMENT

We thank Mr. Yong-Ping Yan for conducting the melting temperature measurements. We are also grateful to Professor David Morris for the higher homologs of spermidine.

REFERENCES

Basu, H. S., & Marton, L. J. (1987) *Biochem. J. 244*, 243–246.

Beltran, R., Martinez-Balbas, A., Bernues, J., Bowater, R., & Azorin, F. (1993) *J. Mol. Biol. 230*, 966–978.

Burch, R., & Mahan, L. (1991) *J. Clin. Invest. 88*, 1190–1196.

Celano, P., Berchtod, C. M., Kizer, D. L., Weeraratna, A., Nelkin, B. D., Baylin, S. B., & Casero, R. A., Jr. (1992) *J. Biol. Chem. 21*, 15092–15096.

Cooney, M., Czernuszewicz, G., Postel, E. H., Flint, S. J., & Hogan, M. E. (1988) *Science 241*, 8227–8231.

Davis, R. W., Morris, D. R., & Coffino, P. (1992) *Microbiol. Rev. 56*, 280–290.

Durland, R. H., Kessler, D. J., Gunnell, S., Duvic, M., Pettitt, B. M., & Hogan, M. E. (1991) *Biochemistry 30*, 9246–9255.

Egli, M., Williams, L. D., Gao, Q., & Rich, A. (1991) *Biochemistry 30*, 11388–11402.

Felsenfeld, G., Davies, D. R., & Rich, A. (1957) *J. Am. Chem. Soc. 79*, 223–224.

Feuerstein, B. G., Pattabiraman, N., & Marton, L. J. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 5948–5952.

Feuerstein, B. G., Szollsi, J., Basu, H. S., & Marton, L. J. (1992) *Cancer Res. 52*, 6782–6789.

Gandhi, V., Chapman, A., Huang, P., & Plukett, W. (1993) *Proc. Am. Assoc. Cancer Res. 34*, 349.

Gee, J. E., Blume, S., Snyder, R. C., Ray, R., & Miller, D. M. (1992) *J. Biol. Chem. 267*, 11163–11167.

Giovannangéli, C., Rougée, M., Garestier, T., Thuong, N. T., & Hélène, C. (1992) *Proc. Natl. Acad. Sci. U.S.A. 89*, 8631–8635.

Glaser, R., & Gabbay, E. J. (1968) *Biopolymers 6*, 243–254.

Gosule, L. C., & Schellman, J. A. (1976) *Nature 259*, 333–3335.

Grigoriev, M., Praseuth, D., Guieysse, L., Robin, P., Thuong, N. T., Helene, C., & Harel-Bellan, A. (1993) *Proc. Natl. Acad. Sci. U.S.A. 90*, 3501–3505.

Hampel, K. J., Crosson, P., & Lee, J. S. (1991) *Biochemistry 30*, 4455–4459.

Heby, O., & Persson, L. (1990) *Trends Biochem. Sci. 15*, 153–158.

Helénè, C., & Toulme, J. J. (1990) *Biochim. Biophys. Acta 1049*, 99–125.

Hoffman-Lieberman, B., Lieberman, D., Troutt, A., Kedes, L. H., & Cohen, S. N. (1986) *Mol. Cell. Biol. 8*, 4174–4184.

Htun, H., & Dahlberg, J. E. (1989) *Science 243*, 1571–1576.

Ing, N. H., Beekman, J. M., Kessler, D. J., Murphy, M., Jayaraman, K., Zendegui, J. G., Hogan, M. E., O'Malley, B. W., & Tsai, M.-J. (1993) *Nucleic Acids Res. 21*, 2789–2796.

Job, P. (1928) *Ann. Chim. (Paris) 9*, 113–134.

Jorstad, C. M., Harada, J. J., & Morris, D. R. (1980) *J. Bacteriol. 141*, 456–463.

Kang, S., Wohlrab, F., & Wells, R. D. (1992) *J. Biol. Chem. 27*, 19435–19442.

Kohwi, Y., & Kohwi-Shigematsu, T. (1988) *Proc. Natl. Acad. Sci. U.S.A. 85*, 3781–3785.

Le Doan, T., Perrouault, L., Chassignol, M., Thuong, N. T., & Helene, C. (1987) *Nucleic Acids Res. 15*, 8643–8659.

Lee, J. S., Burkholder, G. D., Latimer, L., Haug, B. L., & Braun, R. P. (1987) *Nucleic Acids Res. 15*, 1047–1060.

Manning, G. S. (1978) *Q. Rev. Biophys. 11*, 179–246.

Manor, H., Rao, B. S., & Mortune, R. G. (1988) *J. Mol. Evol. 27*, 96–101.

Marton, L. J., & Morris, D. R. (1987) in *Inhibition of Polyamine Metabolsim. Biological Significance and Basis for New Therapies* (McCann, P. P., Pegg, A. E., & Sjoerdsma, A.) pp 79–105, Academic Press, New York.

Mergny, J. L., Duval-Valentin, G., Nguyen, C. H., Perrouault, L., Faucon, B., Rougée, M., Montenay-Garesteier, T. M., Bisagani, E., & Helene, C. (1992) *Science 256*, 1681–1684.

Morgan, A. R., & Wells, R. D. (1968) *J. Mol. Biol. 37*, 63–80.

Moser, H. E., & Dervan, P. B. (1987) *Science 238*, 645–650.

Padmanabhan, S., Brushaber, V. M., Anderson, C. F., & Record, M. T. (1991) *Biochemistry 30*, 7550–7559.

Pegg, A. E. (1988) *Cancer Res. 48*, 759–774.

Roberts, R. W., & Crothers, D. M. (1992) *Science 258*, 1463–1465.

Record, M. T., Jr., Anderson, C. F., & Lohman, T. M. (1978) *Q. Rev. Biophys. 11*, 103–178.

Rougée, M., Faucon, J. L., Barcelo, F., Giovannangéli, C., Garestier, T., & Hélène, C. (1992) *Biochemistry 31*, 9269–9278.

Sarhan, S., & Seiler, N. (1989) *Biol. Chem. Hoppe-Seyler 370*, 1279–1284.
Scaria, P. V., & Shafer, R. H. (1991) *J. Biol. Chem. 266*, 5417–5423.
Schmid, N., & Behr, J.-P. (1991) *Biochemistry 30*, 4357–4361.
Stein, C. A., Tonkinson, J. L., Zhang, L. M., Yakubov, L., Gervasoni, J., Taub, R., & Rotenberg, S. A. (1993) *Biochemistry 32*, 4855–4861.
Strobel, S. A., & Dervan, P. B. (1990) *Science 249*, 73–75.
Strobel, S. A., Lynn, A., Doucette-Stamm, Riba, L., Housman, D. E., & Dervan, P. B. (1991) *Science 254*, 1639–1642.
Sun, J.-S., Francois, J.-C., Montenary-Garrestier, T., Saison-Behmoara, T., Roig, V., Thuong, N. T., & Hélène, C. (1989) *Proc. Natl. Acad. Sci. U.S.A. 86*, 9198–9202.
Suwalski, M., Traub, W., Shmueli, U., & Subirana, J. A. (1969) *J. Mol. Biol. 42*, 363–373.
Tabor, H. (1962) *Biochemistry 1*, 496–501.
Tabor, C. W., & Tabor, H. (1984) *Annu. Rev. Biochem. 53*, 749–790.
Thomas, T. J., & Bloomfield, V. A. (1984) *Biopolymers 23*, 1295–1306.
Thomas, T. J., & Messner, R. P. (1988) *J. Mol. Biol. 201*, 463–467.
Thomas, T. J., Canellakis, Z. N., & Bloomfield, V. A. (1985) *Biopolymers 24*, 725–729.
Thomas, T., Nandi, U. S., & Podder, S. K. (1986) *Indian J. Biochem. Biophys. 23*, 192–196.
Ulrich, M., Gray, W. J., & Ley, T. M. (1992) *J. Biol. Chem. 267*, 18649–18658.
Vertino, P. M., Bergeron, R. J., Cavanaugh, P. F., Jr., & Porter, C. W. (1987) *Biopolymers 26*, 691–703.
Wickstrom, E. (1992) *Trends Biotechnol. 10*, 281–287.
Wilson, R. W., & Bloomfield, V. A. (1979) *Biochemistry 18*, 2192–2196.