

Effects of natural and synthetic polyamines on the conformation of an oligodeoxyribonucleotide with the estrogen response element

Thresia Thomas^{1,4,*}, Gayathri D. Kulkarni², Michael A. Gallo⁴, Norma Greenfield³, Joan S. Lewis¹, Akira Shirahata⁵ and T. J. Thomas²

¹Department of Environmental and Community Medicine, ²Department of Medicine and ³Department of Neuroscience and Cell Biology, ⁴Environmental and Occupational Health Sciences Institute and The Cancer Institute of New Jersey, University of Medicine and Dentistry of New Jersey—Robert Wood Johnson Medical School, New Brunswick, NJ 08903, USA and ⁵Josai University, Sakado, Saitama 350-02, Japan

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ABSTRACT

We studied the effects of natural and synthetic polyamines on the conformation of an oligodeoxyribonucleotide (ODN1) harboring the estrogen response element (ERE) by circular dichroism (CD) spectroscopy and polyacrylamide gel electrophoresis. Putrescine and spermidine had no marked effect on the CD spectrum of ODN1. In contrast, spermine provoked and stabilized two characteristic changes in the CD spectrum. The first change was indicated by an increase in the intensity of the CD band at 280 nm at 0.5 mM spermine in Tris–HCl buffer containing 50 mM NaCl. This change appears to be related to changes in base tilt and conformational alterations similar to A-DNA. At 1–2 mM spermine, the CD spectrum was characterized by a loss of positive bands at 220 and 270 nm. This change might have contributions from polyamine-induced condensation/aggregation of DNA. Spectral measurements were also conducted in Tris–HCl buffer containing 150 mM NaCl to minimize contributions from condensation and aggregation of ODN1. Under these conditions, CD spectral changes were retained by (ODN1), although the magnitude of the change was diminished. In contrast, a control oligodeoxyribonucleotide (ODN2) having similar base composition did not show any significant change in the CD spectrum in the presence of 150 mM NaCl and 2 mM spermine. The changes in the CD spectrum of ODN1 were highly sensitive to polyamine structure, as evidenced by experiments using spermine analogs with altered number of $-\text{CH}_2-$ groups separating the amino and imino groups. Electrophoretic mobility shift analysis further showed ODN1 stabilization by spermine and its analogs. These data demonstrate the ability of an ODN containing ERE to undergo conformational transitions in the presence of polyamines and suggest

a possible mechanism for polyamine-mediated alterations in the interaction of estrogen receptor with ERE.

INTRODUCTION

The polyamines, putrescine [$\text{H}_2\text{N}(\text{CH}_2)_4\text{NH}_2$], spermidine [$\text{H}_2\text{N}(\text{CH}_2)_4\text{NH}(\text{CH}_2)_3\text{NH}_2$] and spermine [$\text{H}_2\text{N}(\text{CH}_2)_3\text{NH}(\text{CH}_2)_4\text{NH}(\text{CH}_2)_3\text{NH}_2$], are ubiquitous cellular cations that play important roles in cell proliferation and differentiation (1–3). Cellular polyamine levels are exquisitely regulated by growth stimulatory and inhibitory agents. In eukaryotes, spermidine and spermine are present in millimolar concentrations and may be as high as 5 mM in the nucleus (4). Although cellular functions of polyamines are not as yet precisely defined, polyamines are known to induce and stabilize unusual DNA conformations (5–8). A dramatic example of the ability of polyamines to alter DNA conformation is the conversion of alternating purine–pyrimidine (APP) sequences from their usual B-DNA form to Z-DNA, under physiologically compatible cationic concentrations (7–9). In addition to electrostatic interaction between cationic sites of polyamines and the negative DNA phosphate charges, several other modes of polyamine binding to DNA have been proposed (10–13). Thus polyamines may either bind to the major or minor groove of DNA, or form a zipper-like intra-strand hydrogen bonding network (10,13). These different interactions depend on the DNA sequence context and the presence of competing cations, leading to the stabilization of A-DNA, Z-DNA and triplex DNA (9–16). The ability of polyamines to modulate sequence-specific protein–DNA interactions was recently demonstrated for ER and a number of other gene-regulatory proteins (17–20).

We previously reported the involvement of polyamines in the mechanism of action of estrogens in breast cancer cells (21,22). Estrogenic action in these and other target cells is mediated by ER, a ligand activated transcription factor. Activation of gene expression occurs as estrogen receptor (ER) recognizes the *cis* acting GGTCANNNTGACC-sequence known as estrogen response element (ERE), a highly conserved palindromic sequence found in

*To whom correspondence should be addressed at: Clinical Research Center, Robert Wood Johnson Medical School, New Brunswick, NJ 08903, USA.
Tel: +1 908 418 8481; Fax: +1 908 418 8480

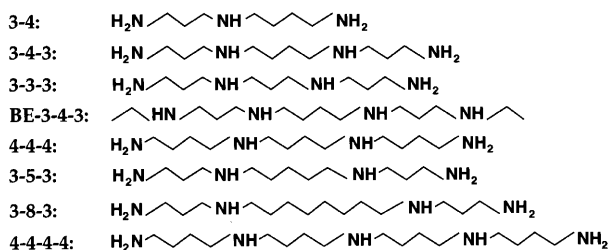


Figure 1. Chemical structure of polyamine analogs.

the regulatory regions of estrogen-responsive genes (23,24). The molecular mechanism of the ER-induced activation of gene expression is not as yet clear, but involvement of a non-B-DNA conformation has been suggested (25–27). We studied the effect of polyamines on ER binding to conformationally labile polynucleotides and found enhanced binding of ER to DNA at polyamine concentrations capable of provoking unusual conformations (19,28). With poly(dG-m⁵dC).poly(dG-m⁵dC) we found maximal ER binding to the polynucleotide at the early stages of the B-DNA to Z-DNA transition (20). Electrophoretic mobility shift assays confirmed enhanced binding of ER to ERE in the presence of polyamines (17,20).

In order to further characterize the structural and conformational variables contributing to ER–ERE binding, we have analyzed the effects of natural and synthetic polyamines on the conformation of ODN1 consisting of the ERE and its flanking sequences. Our results indicate that spermine and certain synthetic analogs provoke and stabilize conformational alterations in ODN1.

MATERIALS AND METHODS

Chemicals and oligonucleotides

Putrescine–2HCl, spermidine–3HCl and spermine–4HCl were obtained from Sigma Chemical Co. (St Louis, MO). All spermine analogs, 1,11-diamino-4,8-diazaundecane (3-3-3), 1,14-diamino-5,10-diazatetradecane (4-4-4), *N,N'*-bis(3-aminopropyl)-1,5-diaminopentane (3-5-3), *N,N'*-bis[3-aminopropyl]-1,8-diaminooctane (3-8-3), as well as 1,19-diamino-5,10,15-triazanonadecane (4-4-4-4) were synthesized as described earlier (29–31). The structures of all spermidine analogs were confirmed by elemental analysis as well as NMR, HPLC and Mass spectrometry. Figure 1 shows the chemical structures of the compounds.

ODNs were purchased from Oligos, Etc., Inc. (Wilsonville, OR). The ERE consensus sequence from the vitellogenin gene and its flanking sequences, consisting of 41 bases was used for this study (32). A 38 base complementary sequence was chosen to allow for efficient end-labeling (33,34). The base sequence of ODN1 is given below:

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5' GAT CCAGGT CAGAGT GAC CTG AGC TAA AAT AAC ACA TTC AG 3'
3' GGT CCA GTC TCA CTG GAC TCG ATT TTA TTG TGT AAG TC 5'
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An ODN having similar distribution of purines and pyrimidines and GC content, arranged in a scrambled sequence was used as a control (ODN2):

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5' AAA GCT CGC TTC CTG AAG ACG TTC TCG AAG AGA AAT CTC TT 3'
3' CGA GCG AAG GAC TTC TGC AAG AGC TTC TCT TTA GAG AA 5'
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The ODNs were dissolved in a buffer containing 10 mM Tris–HCl (pH 7.5) and 50 mM NaCl and dialyzed three times against the same buffer before use.

Measurement of CD spectra

The CD spectra of the ODN solutions were recorded with an AVIV model 62D spectropolarimeter fitted with a 5-cell, thermally regulated cell holder and cells with 1 cm path-length. An equimolar mixture of the two strands was used at 66 μmol of nucleotide residue/l in 10 mM Tris–HCl (pH 7.5) buffer containing 50 mM NaCl. Molar extinction coefficient (ϵ) for each ODN at 260 nm was determined as described by standard methods (35,36). The single stranded oligomer extinction coefficients, per mole of nucleotide residue, were 10 243 and 9210 M⁻¹ cm⁻¹, for upper and lower strands of ODN1, respectively. The extinction coefficients, for upper and lower strands of the ODN2 were 9512 and 9736 M⁻¹ cm⁻¹, respectively. After the addition of small volumes (5–10 μl) of polyamine stock solutions in the same buffer, the ODN solution was boiled for 10 min, cooled and allowed to equilibrate at 22°C for 2 h and at 4°C for 1 h. Spectra were recorded at 4°C unless otherwise indicated. The molar ellipticity was calculated from the equation, $[\theta] = 100 \times \theta/cl$, where θ is the relative intensity, c the molar concentration of nucleotide residue and l the path-length of the cell in centimeters (8). CD measurements were made at a series of concentrations of the natural and synthetic polyamines. Repetitive measurements were made at concentrations that marked distinct changes in the spectrum. Representative results from two to three measurements are presented.

Polyacrylamide gel electrophoresis

ODN1 was labeled with ³²P by end-labeling with polynucleotide kinase (34). The labeled ODN1 was purified by gel electrophoresis and electro-elution. ³²P-labeled ODN1 (20 nmol nucleotide/l) was used to examine the effect of polyamines on ODN1. Samples were prepared in 10 mM Tris–HCl (pH 7.5) containing 50 mM NaCl and appropriate concentrations of polyamines were added. Samples were then boiled and allowed to cool as in the CD studies. Samples were analyzed by 12% polyacrylamide gel electrophoresis. Gels were run at 4°C in Tris-borate buffer (89 mM Tris base, 89 mM boric acid, 2 mM EDTA, pH 8.3) for 3 h at 230 V. The gels were dried and exposed to X-ray film for 24–48 h at –70°C.

RESULTS

Conformational changes of ODN1 in the presence of polyamines

Figure 2 shows the CD spectra of ODN1 in 10 mM Tris–HCl (pH 7.5) and 50 mM NaCl in the presence of different concentrations of putrescine, spermidine or spermine. In the absence of polyamines, the CD spectrum had typical features of B-DNA, with positive bands at 220 and 270 nm and a negative band at 245 nm. These CD bands result from nondegenerate interactions of the transition dipole of the bases to the electronic transitions of the asymmetric sugar. Generally, CD bands are affected by the interaction of the planar bases to the furanose ring as well as by the stacking interactions between the bases. The CD bands at 220 and 245 nm have been attributed to $\pi \rightarrow \pi^*$ transitions whereas those at wavelengths higher than 265 nm might have contributions from $n \rightarrow \pi^*$ transitions (37,38). As shown in Figure 2A and B, putrescine (0.5–50 mM) and spermidine (0.5–10 mM) exerted relatively minor effects on the CD spectrum of ODN1. In contrast, there was a remarkable change in the CD spectrum of ODN1 in

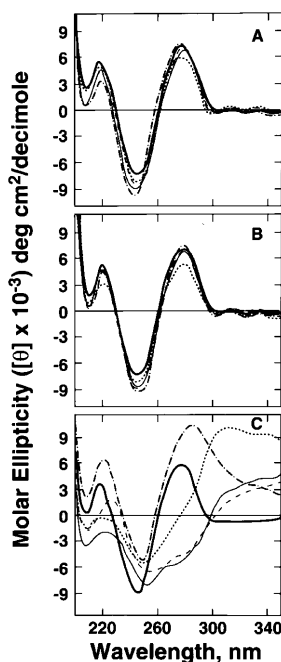


Figure 2. Effect of putrescine, spermidine and spermine on the CD spectrum of ODN1. ODN1 solutions were prepared in 10 mM Tris-HCl (pH 7.5) and 50 mM NaCl in the presence or absence of different concentrations of polyamines. Line representations are as follows: (A) 0 (—), 0.5 (---), 1 (-·-·-), 10 (—) and 50 (·····) mM putrescine. (B) 0 (—), 0.5 (---), 1 (-·-·-), 5 (—) and 10 (·····) mM spermidine. (C) 0 (—), 0.5 (-·-·-), 1 (·····), 1.5 (---) and 2 (—) mM spermine.

the presence of 0.5 mM spermine (1:7.5 ratio of DNA phosphate to spermine). The band intensity at 220 and 270 nm increased and the positive wavelength maximum shifted from 270 to 280 nm region. At 1 mM spermine, the positive band at 220 nm was reduced in intensity and the 280 nm band shifted to 300 nm. At 1.5–2 mM concentrations, the positive band at 300 nm was also reduced in intensity and a negative band centered at 255 nm became prominent. The CD spectra of ODN2 was also recorded in Tris buffer containing 50 mM NaCl and increasing concentrations of spermine. The major difference in the spectrum of ODN2 compared with ODN1 was the absence of an increase in ellipticity at 260–300 nm in the presence of 0.5 mM spermine. However, there was a gradual decrease in positive ellipticity at 260 nm between 0.5 and 2 mM spermine (results not shown). In other control experiments, the CD spectrum of ODN1 was unchanged in the presence of 0.5–2 mM $MgCl_2$.

Spermine-induced changes in the CD spectrum of ODN1 and ODN2 at low ionic strength (50 mM NaCl) might include contributions from condensation and aggregation of ODNs. We therefore examined the absorbance of ODNs at 350 nm to detect the extent of Raleigh scattering. Absorbance values of 0.1–0.2 optical units were found with ODN solutions in 10 mM Tris-HCl buffer containing 50 mM NaCl and 0.5–2 mM spermine. To circumvent the complexities caused by aggregation of ODN, we further studied the properties of ODN1 and ODN2 in 10 mM Tris-HCl buffer containing 150 mM NaCl. Under these conditions the absorbance of both ODN solutions at 350 nm was zero in the presence of 0.5–2 mM spermine.

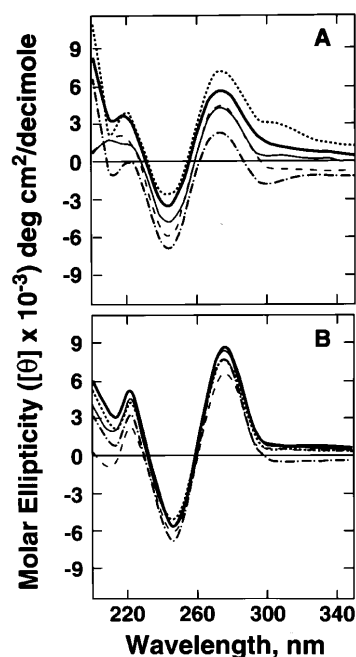


Figure 3. Effect of spermine on the CD spectra of ODN1 (A) and ODN2 (B) in 10 mM Tris-HCl (pH 7.5) and 150 mM NaCl. ODN solutions were prepared in the presence of different concentrations of spermine. Line representations are as follows: 0 (—), 0.5 (·····), 1 (—), 1.5 (---) and 2 (-·-·-) mM spermine.

Figure 3A and B shows the effects of spermine on the CD spectra of ODN1 and ODN2, respectively. An increase in the positive band intensity at 280 nm was observed in the CD spectrum of ODN1 at 0.5 mM spermine. At higher concentrations of spermine there was a decrease in the positive band, concomitant with an increase in the negative ellipticity. Another feature of this spectrum is the presence of a new negative band at 295 nm. In contrast, there was no significant change in the CD spectrum of ODN2. This result would suggest that the conformational changes provoked by spermine in ODN1 are dependent on the base sequence distribution.

We next examined the effects of a series of spermine analogs on the conformation of ODN1. These studies were conducted in 10 mM Tris-HCl buffer containing 50 mM NaCl to detect the full range of interactions as compared with that of spermine (Fig. 2). Our results on the effects of spermine analogs, 3-3-3, 3-5-3 and 3-8-3 on the CD spectrum of the oligomer are presented in Figure 4. The analog 3-3-3, with one $-CH_2-$ group less than spermine, induced conformational changes very similar to those provoked by spermine (Fig. 4A). With 3-3-3, the conformational variant with high positive band (280 nm) was induced at 0.5 mM concentration, whereas the variant with negative band centered around 255 nm was induced at 2 mM. The effect of analog 3-5-3, which has one $-CH_2-$ group more than that of spermine was remarkably different from that of spermine in inducing conformational changes in ODN1 (Fig. 4B). With this analog, there was no change in the CD spectrum at 0.5 mM concentration. The positive band at 270 nm shifted to 300 nm and its intensity increased with the analog concentration. This shift was associated with an increase in the intensity of the negative band at 245 nm. This spermine analog did not induce the second spectral change with a broad negative CD band centered at 255 nm at the highest

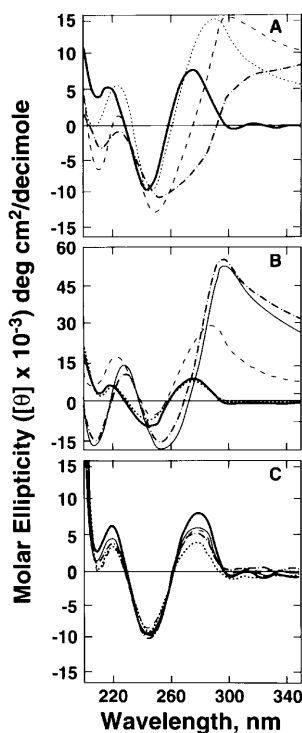


Figure 4. Effects of analogs 3-3-3, 3-5-3 and 3-8-3 on the CD spectrum of ODN1. ODN1 solutions were prepared in 10 mM Tris-HCl (pH 7.5) and 50 mM NaCl. Line representations are as follows: (A) 0 (—), 0.5 (.....), 1 (---) and 2 (- - -) mM 3-3-3. (B) 0 (—), 0.5 (.....), 1 (---), 1.5 (- - -) and 2 (—) mM 3-5-3. (C) 0 (—), 0.5 (—), 1 (---), 1.5 (- - -) and 2 (.....) mM 3-8-3.

concentrations tested. As shown in Figure 4C, we found that the analog 3-8-3, with an 8 carbon chain instead of the 4 carbon chain of spermine. In contrast, there was a decrease in the intensity of the positive CD band in the presence of this analog, similar to the changes observed in calf thymus DNA in the presence of putrescine (39). Thus the distance between the imino groups in spermine analogs is critical to the ability of polyamines to facilitate conformational changes in ODN1.

We also examined the effects of analog 4-4-4 and a comparable pentamine (4-4-4-4) on the conformation of ODN1. Figure 5A and B shows the effects of 4-4-4 and 4-4-4-4 on the CD spectrum of the oligomer. Analog 4-4-4 was effective in inducing the positive band at 280–300 nm, but it did not provoke the transition leading to the broad negative band even at 2 mM concentration. In contrast, 4-4-4-4 was highly effective in inducing the negative CD band, requiring a 10-fold lower concentration than spermine. However, 4-4-4-4 did not stabilize the conformer with high positive band in the 280–300 nm region. This result underscores the importance of positive charges in facilitating conformational transitions in ERE.

We next examined the effect of bis(ethyl)spermine on the conformation of ODN1. Bis(ethyl)spermine and related compounds have been studied as anticancer agents in several laboratories (40). Figure 6 shows the effect of bis(ethyl)spermine on the CD spectrum of ODN1. This compound had no significant effect on the CD spectrum up to 1 mM concentration. At 1.5 and 2 mM concentrations, there was an increase in the intensity of the band at 270 nm, but there was no shift of the peak to longer

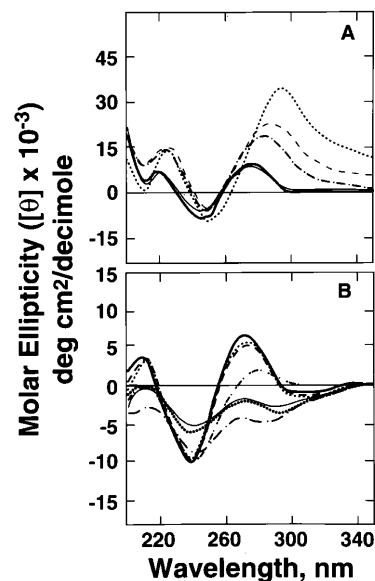


Figure 5. Effect of 4-4-4 and 4-4-4-4 on the CD spectrum of ODN1. ODN1 solutions were prepared in the presence of 10 mM Tris-HCl (pH 7.5) and 50 mM NaCl. Line representations are as follows: (A) 0 (—), 0.5 (—), 1 (- - -), 1.5 (- - -) and 2 (.....) mM 4-4-4. (B) 0 (—), 0.01 (.....), 0.025 (- - -), 0.05 (.....), 0.1 (- - -), 0.25 (●●●) and 0.5 (—) mM 4-4-4-4.

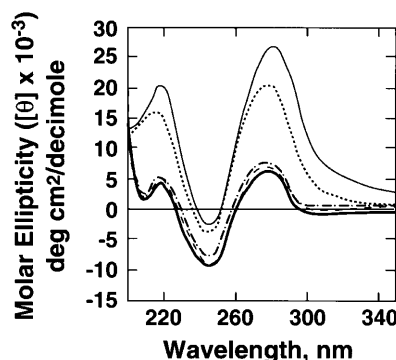


Figure 6. Effect of bis(ethyl)spermine on the CD spectrum of ODN1. ODN1 solutions were prepared in the presence of 0 (—), 0.5 (- - -), 1 (- - -), 1.5 (.....) and 2 (—) mM bis(ethyl)spermine.

wavelengths, as observed for spermine and its unsubstituted analogs. Thus steric hindrance of the alkyl group on the pendant amino group could alter the interaction of the spermine analog with the ERE.

In order to understand the contributions of single-stranded loops to the conformational characteristics observed, we further examined the CD spectrum of the two single-stranded ODNs comprising ODN1. At 0.5–2 mM concentrations, spermine suppressed the positive (270 nm) and negative (250 nm) bands of the upper strand of ODN1 (Fig. 7A). There was also a decrease in the CD band at 270 nm in the case of the lower strand of ODN1 (Fig. 7B). However, spermine binding to single-stranded ODNs did not induce spectral features similar to those found in ODN1 in the presence of spermine. Thus, CD spectral features of duplex

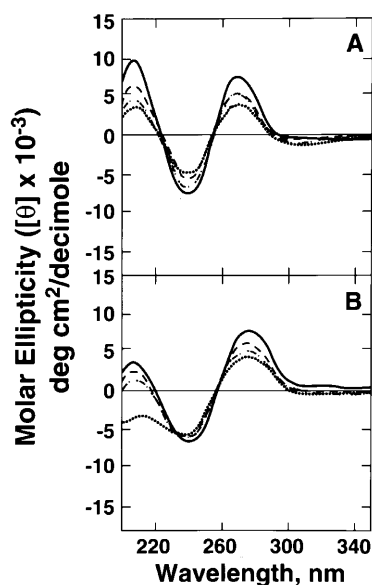


Figure 7. Effect of spermine on single-stranded ODNs. The upper (A) and lower (B) strand of the ODN1 were prepared in 10 mM Tris-HCl (pH 7.5) and 50 mM NaCl in the presence of different concentrations of spermine. The concentrations of spermine were 0 (—), 0.5 (---), 1 (-·-) and 2 mM (·····).

ODN1 in the presence of spermine do not originate from single-stranded loops.

Analysis of polyamine interactions with ERE by gel electrophoresis

We also used polyacrylamide gel electrophoresis to examine the structural features of ODN1. Since duplex formation from single strands is an oligomer concentration-dependent process, we conducted the annealing reaction at different concentrations of unlabeled ODN1. The same amount (20 000 c.p.m., 20 nmol of nucleotide/l) of radioactive ODN1 was mixed with different concentrations of cold oligonucleotide before boiling and re-annealing. Our results (Fig. 8) indicate that ODN1 existed in the duplex form in control samples without any polyamines at 10–30 $\mu\text{g/ml}$ concentration, the range needed for CD studies. At lower oligomer concentrations, single-stranded and duplex forms were found in the absence of polyamines. In contrast, in the presence of 0.5 mM spermine, all of the ODN1 could be detected in the duplex form even at the lowest concentration. Thus spermine stabilized duplex ODN1 at nM oligomer concentrations, conditions that precluded duplex formation in Tris buffer containing 50 mM NaCl.

Figure 9 shows the electrophoretic mobility shift analysis of ODN1 treated with various concentrations of spermine and bis(ethyl)spermine. ^{32}P -labeled ODN1 was used at 20 000 c.p.m. without added unlabeled ODN1. Aggregated ODN1 species was detected at the origin of the gel in the presence of high concentrations of spermine, but not with bis(ethyl)spermine. In the presence of spermine, a trailing band was also observed just above the duplex oligomer band. Similar studies using samples at 30 $\mu\text{g/ml}$ unlabeled ODN1 did not show aggregated form at the origin or any slow moving species of the oligomer in the presence of spermine (results not shown). Thus aggregates stable to the

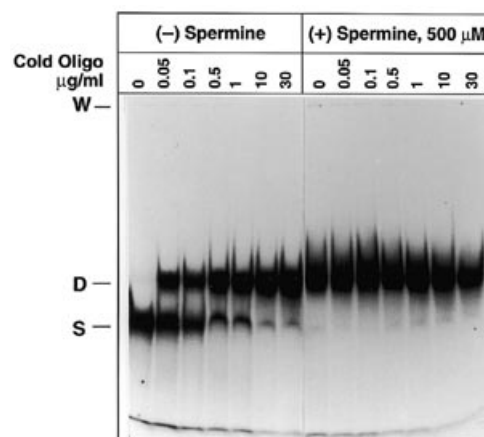


Figure 8. Polyacrylamide gel electrophoresis to determine the duplex stabilization effects of spermine. The ^{32}P -labeled ODN1 (20 nmol of nucleotide residue/l) was used with indicated concentrations of nonradioactive ODN1. Mobilities of duplex (D) and single-stranded (S) species were identified by labeling the single-stranded ODNs individually and analyzing their mobility in comparison to that of the labeled duplex. The origin of the gel is marked by 'W'.

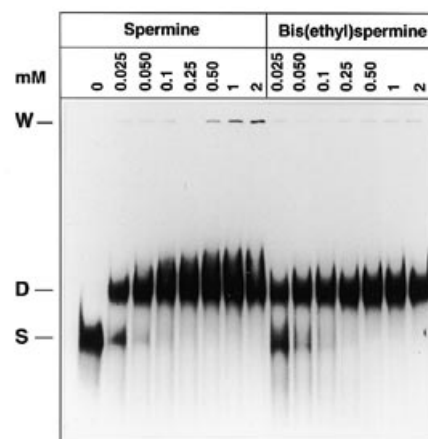


Figure 9. Polyacrylamide gel electrophoresis to determine the duplex stabilization and aggregation effects of polyamines on ODN1. ^{32}P -labeled ODN1 was used in the presence of indicated concentrations of spermine or (bisethyl)spermine. Mobilities of duplex (D) and single-stranded (S) species were identified by labeling the single-stranded ODNs individually and analyzing their mobilities relative to that of the duplex. The origin of the gel is marked by 'W'.

electrophoresis conditions occur beyond the ratio of DNA phosphate to spermine (1:7.5–1:30) utilized in the CD studies.

DISCUSSION

Results presented in this report demonstrate that a natural tetramine, spermine, is capable of provoking unique conformational changes in an ODN harboring the ERE. In the presence of 0.5 mM spermine, the positive band at 270 nm undergoes a red shift and an increase in intensity. Spectral changes qualitatively similar to this conformational variant are also induced by several spermine analogs, 3-3-3, 3-5-4, 4-4-4 and bis(ethyl)spermine. Among the analogs tested, bis(ethyl)spermine was unique in inducing a large

increase in intensity of the CD band in the absence of a concomitant red shift. At 2 mM spermine, a major CD spectral feature was the presence of a negative band centered around 255 nm. This spectral change was also induced by 3-3-3 and the pentamine 4-4-4-4. The results on the efficacy of 3-3-3 on ODN1 are consistent with previous reports on the optimum interactions of aminopropyl groups in stabilizing Z-DNA, triplex DNA and A-DNA (15,39,41). The high efficacy of the pentamine is indicative of the importance of the extra 4-carbon chain and an additional positive charge in provoking conformational alterations in the ERE. Studies in the presence of 150 mM NaCl indicate that ODN1 is very sensitive to polyamine-induced CD spectral changes whereas ODN2 had no marked conformational alterations in the presence of spermine. Our results suggest that spermine and higher polyamine analogs are capable of provoking changes in the base orientation and backbone phosphate configuration of ODN1.

The intensity of the CD band at 275 nm reflects the helix winding angle, such that the higher the intensity, the smaller the winding angle (42). Thus the initial interaction of ERE oligonucleotide with spermine and several analogs appears to produce underwinding in comparison to the B-DNA form of the duplex. Bases may also be tilted as in the A-DNA conformation (10,43). Although the formation of A-DNA in the presence of spermine was initially suggested on the basis of electrostatic interactions with DNA phosphate (10,39), X-ray crystallographic studies indicate binding of spermine to the bases in the deep groove of the A-DNA octamer, GTGTACAC (44). Studies using CD and NMR spectroscopy also showed an A-DNA conformation in an oligonucleotide (ACCGCGGT) in the presence of spermine (43). Since the distance between phosphate groups in the same chain of A-DNA is shorter than that of B-DNA, the preferential interaction of spermine to A-DNA involves the aminopropyl group of spermine (39). Thus major groove binding, as well as intra-chain binding to phosphate groups, might be involved in the interaction of spermine with ODN1.

At 1–2 mM spermine, the 275 nm band attributed to the A-DNA conformation is reduced in intensity. This change might be partly due to polyamine induced condensation of DNA as low ionic strength buffers promote condensation and aggregation of DNA (45–47). It has also been demonstrated that these polyamines facilitate the formation of liquid crystalline DNA in which the arrangement of DNA is not random (48,49). Short duplexes in the condensates/aggregates might have stacked structures which are aligned side by side, probably with a chiral twist. More detailed investigations are needed to dissect the molecular organization of these structures. However, complications due to polyamine-induced supramolecular organization of ODN1 in low ionic strength buffer could be circumvented by using 150 mM NaCl in the buffer. The observation of CD bands at 245, 275 and 295 nm in the ERE oligonucleotide in the presence of 2 mM spermine is unusual and may be comparable to that of topologically constrained plasmid DNA (38). With ODN2, polyamine-induced effects are completely abolished by the presence of 150 mM NaCl. Thus differential modes of polyamine action appear to be manifested in the two ODNs.

Polyacrylamide gel electrophoresis studies were conducted to examine whether the oligomer remained in the duplex form at the spermine concentrations that induced conformational transitions. These studies indicated that aggregates stable to electrophoresis conditions were sparse in the ODN1 and it required a higher ratio

of polyamine to DNA phosphate than that used in the CD studies. On the other hand, CD spectral bands extended to the long wavelength region (300–350 nm) in many cases. Liquid crystalline organization and bundling of the molecules may contribute to the observed CD changes in the long wavelength region (50,51). This type of organization occurs in polynucleotides or in supercoiled plasmids in the presence of 10 mM MgCl₂ and 20–30% ethanol (51). The CD spectra of these structures are characterized by high molar ellipticities (in the range of 50–100). In our studies only certain synthetic analogs of spermine [4-4-4, 3-5-3 and bis(ethyl) spermine] yield molar ellipticities >10. The higher order structures may represent participation of polyamines in the organization of DNA, relevant to the packaging and function of DNA in the cell. A correlation between polyamine-induced DNA condensation and increase in transcription was reported using an *Escherichia coli* -pBR322 system (52). The differential effects of spermine analogs on the structure and conformation of specific DNA sequences might be important in developing of polyamine analogs for breast cancer therapy.

X-ray crystallographic studies on the interaction of spermine to an ODN containing GTG sequence indicate that spermine stabilizes base pair opening in this region (53). ODN1 has a GTG sequence in the palindromic region. It is thus possible that the effects of spermine on ERE conformation has contributions from this type of local distortions. Furthermore, tracks of adenine containing four or more nucleotides are prone to sequence-directed bending (54). Polyamine binding and charge neutralization are likely to induce bending of ODN1. Further studies are needed to assign specific CD spectral changes to localized alterations in the conformation of ERE.

Sequence-specific DNA binding proteins add to the complexity of conformational variants possible *in vivo*. Recent studies have revealed the involvement of multiple ER-associated proteins in the gene regulatory function of ER (54,55). It is likely that polyamines act in concert with ER-associated accessory proteins to stabilize specific DNA conformations. In this regard, putrescine and spermidine may also play important roles because ER–DNA binding is facilitated by these polyamines as well, even though spermine had maximal effect (17,19,20). Biologically, the consequences of these and other functions of polyamines are manifested in the reversible inhibition of cell growth in the presence of inhibitors of polyamine biosynthesis and certain polyamine analogs (1,2,40).

In summary, our studies demonstrate the ability of spermine to provoke conformational changes in an ODN commonly used for studies of ER–ERE interactions. The conformational variant, occurring at 0.5 mM spermine, has the features of A-DNA. At higher concentrations of spermine, a chirally packed condensed form of ERE is produced. The implications of these changes on ER–ERE interactions need to be investigated further. The ODN containing ERE and spermine present a dynamic system for studies on gene regulation. Modulation of DNA structure and conformation appears to be fundamental to gene regulation by polyamines.

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REFERENCES

- 1 Tabor, C.W. and Tabor, H. (1984) *Annu. Rev. Biochem.* **53**, 749–790.
- 2 Pegg, A.E. (1988) *Cancer Res.* **48**, 759–774.
- 3 Marton, L.J. and Morris, D.R. (1987) In McCann, P.P., Pegg, A.E. and Sjoerdsma, A. (eds), *Inhibition of Polyamine Metabolism. Biological Significance and Basis for New Therapies*. Academic Press, New York, pp. 79–105.
- 4 Sarhan, S. and Seiler, N. (1989) *Biol. Chem. Hoppe-Seyler* **370**, 1279–1284.
- 5 Gosule, L.C. and Schellman, J.A. (1976) *Nature* **259**, 333–3335.
- 6 Wilson, R.W. and Bloomfield, V.A. (1979) *Biochemistry* **18**, 2192–2196.
- 7 Behe, M. and Felsenfeld, G. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 1619–1623.
- 8 Thomas, T.J. and Bloomfield, V.A. (1985) *Biochemistry* **24**, 713–719.
- 9 Thomas, T.J. and Messner, R.P. (1988) *J. Mol. Biol.* **201**, 463–467.
- 10 Zhurkin, V.B., Lysov, Y.P. and Ivanov, V.I. (1980) *Biopolymers* **19**, 1415–1428.
- 11 Egli, M., Williams, L.D., Gao, Q. and Rich, A. (1991) *Biochemistry* **30**, 11388–11402.
- 12 Jain, S., Zon, G. and Sundaralingam, M. (1989) *Biochemistry* **28**, 2360–2366.
- 13 Schmid, N. and Behr, J.P. (1991) *Biochemistry* **30**, 4357–4361.
- 14 Feuerstein, B.G., Williams, L.D., Basu, H.K. and Marton, L.J. (1991) *J. Cell. Biochem.* **46**, 37–47.
- 15 Thomas, T. and Thomas, T.J. (1993) *Biochemistry* **32**, 14068–14074.
- 16 Musso, M., Thomas, T., Shirahata, A., Sigal, L.H., Van Dyke, M.W. and Thomas, T.J. (1997) *Biochemistry* **36**, 1441–1449.
- 17 Sabbah, M., Ricousse, S.L., Redeuilh, G. and Baulieu, E.E. (1992) *Biochem. Biophys. Res. Commun.* **185**, 944–952.
- 18 Pangiotides, C.A., Artandi, S., Calame, K. and Silverstein, S.J. (1995) *Nucleic Acids Res.*, **23**, 1800–1809.
- 19 Thomas, T. and Kiang, D.T. (1988) *Nucleic Acids Res.* **16**, 4705–4720.
- 20 Thomas, T., Gallo, M.A., Klinge, C.M. and Thomas, T.J. (1995) *J. Steroid Biochem. Mol. Biol.* **54**, 89–99.
- 21 Thomas, T., Trend, B., Butterfield, J.B., Jänne, O.A. and Kiang, D.T. (1989) *Cancer Res.* **49**, 5852–5857.
- 22 Thomas, T. and Kiang, D.T. (1987) *Biochem. Biophys. Res. Commun.* **148**, 1338–1345.
- 23 Katzenellenbogen, J.A., O'Malley, B.W. and Katzenellenbogen, B.S. (1996) *Mol. Endocrinol.* **10**, 119–131.
- 24 Beato, M., Herrlich, P. and Schütz, G. (1995) *Cell* **83**, 851–857.
- 25 Lannigan, D.A., Koszewski, N.J. and Notides, A.C. (1993) *Mol. Cell. Endocrinol.* **94**, 47–54.
- 26 Lannigan, D.A., Tomashek, J.J., Obourn, J.D. and Notides, A.C. (1993) *Biochem. Pharmacol.* **45**, 1921–1928.
- 27 Lannigan, D.A. and Notides, A.C. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 863–867.
- 28 Thomas, T. and Thomas, T.J. (1993) *J. Receptor Res.* **13**, 1115–1133.
- 29 He, Y., Suzuki, T., Kashiwagi, K., Kusama-Eguchi, K., Shirahata, A. and Igarashi, K. (1994) *Eur. J. Biochem.* **221**, 391–398.
- 30 Shirahata, A., Hosoda, H., Takahashi, N., Beppu, T., Niitsu, M. and Samejima, K. (1995) *Biol. Pharm. Bull. (Tokyo)* **18**, 355–359.
- 31 Samejima, K., Takeda, Y., Kawase, M., Okada, M. and Kyougoku, Y. (1984) *Chem. Pharm. Bull. (Tokyo)* **32**, 3428–3435.
- 32 Burch, J.B.E. (1984) *Nucleic Acids Res.* **12**, 1117–1135.
- 33 Murdoch, F.E., Grunwald, K.A.A. and Gorski, J. (1991) *Biochemistry* **30**, 10838–10844.
- 34 Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.D., Smith, J.A., Seidman, J.G. and Struhl, K. (1987) *Current Protocols in Molecular Biology*. Wiley Interscience, pp. 3.1.1–3.3.15.
- 35 Fasman, G.D. (ed.) *Handbook of Biochemistry and Molecular Biology* (1975) 3rd edn, *Nucleic Acids*, Vol. 1, CRC Press, Cleveland, OH, pp. 589.
- 36 Gray, D.M., Hung, S.-H. and Johnson, K.H. (1995) *Methods Enzymol.* **246**, 19–35.
- 37 Goto, S. (1984) *Biopolymers* **23**, 2211–2222.
- 38 Johnson, W.C. Jr. (1996) In Fasman, G.D. (ed.), *Circular Dichroism and the Conformational Analysis of Biomolecules*. Plenum Press, New York, pp. 433–468.
- 39 Minyat, E.E., Ivanov, V.I., Kritzyn, A.M., Minchenkova, L.E. and Schyolkina, A.K. (1978) *J. Mol. Biol.* **128**, 397–409.
- 40 Marton, L.J. and Pegg, A.E. (1995) *Annu. Rev. Pharmacol. Toxicol.* **35**, 55–91.
- 41 Thomas, T.J., Canellakis, Z.N. and Bloomfield, V.A. (1985) *Biopolymers* **24**, 725–729.
- 42 Lyubchenko, Y.L., Shlyakhtenko, L.S., Appella, E. and Harrington, R.E. (1993) *Biochemistry* **32**, 4121–4127.
- 43 Robinson, H. and Wang, A.H.-J. (1996) *Nucleic Acids Res.* **24**, 676–682.
- 44 Jain, S., Zon, G. and Sundaralingam, M. (1989) *Biochemistry* **28**, 2360–2364.
- 45 Thomas, T.J. and Bloomfield, V.A. (1983) *Biopolymers* **22**, 1097–1106.
- 46 Wilson, R.W. and Bloomfield, V.A. (1979) *Biochemistry* **18**, 2192–2196.
- 47 Subirana, J. and Vives, J.L. (1981) *Biopolymers* **20**, 2281–2283.
- 48 Rau, D.C. and Parsegian, V.A. (1992) *Biophys. J.* **61**, 246–259.
- 49 Pelta, J., Durand, D., Doucet, J. and Livolant, F. (1996) *Biophys. J.* **71**, 48–63.
- 50 Reich, Z., Ghirlando, R. and Minsky, A. (1991) *Biochemistry* **30**, 7828–7836.
- 51 Reich, Z., Levin-Zaidman, S., Gutman, S.B., Arad, T. and Minsky, A. (1994) *Biochemistry* **33**, 14177–14184.
- 52 Baeza, I., Gariglio, P., Rangel, L.M., Chavez, P., Cervantes, L., Arguello, C., Wong, C. and Montanez, C. (1987) *Biochemistry* **26**, 6387–6392.
- 53 Tari, L.W. and Secco, A.S. (1995) *Nucleic Acids Res.* **23**, 2065–2073.
- 54 Hagerman, P.J. (1990) *Annu. Rev. Biochem.* **59**, 755–781.
- 55 Halachmi, S., Marden, E., Martin, G., MacKay, H., Abbondanza, C. and Brown, M. (1994) *Science* **264**, 1455–1458.
- 56 Zeiner, M. and Gehring, U. (1995) *Proc. Natl. Acad. Sci. USA* **92**, 11465–11469.