Polyamine-induced Z-DNA conformation in plasmids containing $(dA\text{-}dC)_n \cdot (dG\text{-}dT)_n$ inserts and increased binding of lupus autoantibodies to the Z-DNA form of plasmids

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Blocks of potential Z-DNA-forming $(dA\text{-}dC)_n \cdot (dG\text{-}dT)_n$ sequences are ubiquitous in eukaryotic genomes. We examined whether naturally occurring polyamines, putrescine, spermidine and spermine, could provoke the Z-DNA conformation in plasmids pDHf2 and pDHf14 with 23 and 60 bp inserts respectively of $(dA\text{-}dC)_n \cdot (dG\text{-}dT)_n$ sequences using an e.l.i.s.a. Spermidine and spermine could provoke Z-DNA conformation in these plasmids, but putrescine was ineffective. For pDHf2 and pDHf14, the concentration of spermidine at the midpoint of B-DNA to Z-DNA transition was 25 μM, whereas that of spermine was 16 μM. Polyamine structural specificity was evident in the ability of spermidine homologues to induce Z-DNA. Inorganic cations, $Co(NH_3)_6^{3+}$ and $Ru(NH_3)_6^{3+}$, were ineffective. Our experiments also showed increased binding of anti-DNA autoantibodies from lupus patients as well as autoimmune MRL-*lpr/lpr* mice to pDHf2 and pDHf14 in the presence of polyamines. These data demonstrate that small blocks of $(dA\text{-}dC)_n \cdot (dG\text{-}dT)_n$ sequences could assume the Z-DNA conformation in the presence of natural polyamines. Increased concentrations of polyamines in the sera of lupus patients might facilitate immune complex-formation involving circulating DNA and anti-Z-DNA antibodies.

INTRODUCTION

Alternating purine/pyrimidine sequences undergo a conformational transition from their usual right-handed B-DNA form to a left-handed Z-DNA conformation under a variety of conditions (Pohl and Jovin, 1972; Wang et al., 1979; Nordheim and Rich, 1983; Rich et al., 1984; Wells, 1988). Among the nucleotides that are capable of forming Z-DNA, $(dA\text{-}dC)_n \cdot (dG\text{-}dT)_n$ sequences are particularly important because of their widespread distribution in mammalian genes (Hamada and Kakunaga, 1982; Hamada et al., 1982). Recent computer analysis of sixteen million nucleotide sequences of the genome of various organisms showed that the sequence motifs $(dA\text{-}dC)_n$ capable of adopting Z-DNA conformation and $(dA\text{-}dG)_n$ that can form triplex DNA are abundant in rodent and human genomes (Tripathi and Brahmachari, 1991). Another computer analysis of 300 well-characterized genes showed that Z-DNA-forming sequences are non-randomly distributed in the regulatory regions of human genes (Schroth et al., 1992). These data are consistent with a potential role for Z-DNA in the regulation of transcription and recombination. The use of Z-DNA-forming sequences as an indicator of negative supercoiling in transcribing genomic domains further supports the formation of Z-DNA during transcription (Rahmouni and Wells, 1992). Furthermore, Wittig et al. (1992) demonstrated the formation of Z-DNA in the promoter/enhancer regions of transcribing c-*myc* gene using an *in vitro* transcription system involving isolated nuclei in agarose microbeads. Conditions that favour Z-DNA formation in short blocks of $(dA\text{-}dC)_n \cdot (dG\text{-}dT)_n$ are important in understanding the role of Z-DNA in transcription.

Most of the studies on the induction of Z-DNA have been conducted on polynucleotides with alternating purine/pyrimidine sequences (Rich et al., 1984; Jovin et al., 1987). Among the polynucleotides, poly(dG-m^{5}dC)·poly(dG-m^{5}dC) has been the most versatile polymer, as it undergoes B to Z transition readily and the change can be documented using u.v. or c.d. spectroscopy (Behe and Felsenfeld, 1981; Thomas and Messner, 1988). On the other hand, poly(dA-dC)·poly(dT-dG) is quite resistant to the transition under most conditions used to provoke the B-DNA to Z-DNA transition in $(dG\text{-}dC)_n$ sequences (Woisard and Fazakerley, 1986). Our previous studies demonstrated a polyamine-induced Z-like conformation in poly(dA-dC)·poly(dG-dT) (Thomas and Messner, 1986). Haniford and Pulleyblank (1983) showed that plasmids with $(dA\text{-}dC)_n \cdot (dG\text{-}dT)_n$ sequences could undergo B-DNA to Z-DNA transition under the influence of negative supercoiling. The role of other exogenous and endogenous agents in inducing Z-DNA in these sequences has not been examined.

With regard to the role of Z-DNA in transcriptional regulation, polyamines, the cellular organic cations, are important because of their ubiquitous nature as well as their specificity and effectiveness in facilitating B-DNA to Z-DNA transition (Thomas et al., 1985; Basu and Marton, 1987; Thomas and Messner, 1988). Polyamine levels are exquisitely regulated by growth stimulatory and inhibitory agents (Tabor and Tabor, 1984; Russell, 1985; Pegg, 1986). Ornithine decarboxylase (ODC) is a key enzyme in the biosynthesis of polyamines. It is stimulated by hormones, growth factors and carcinogens, as well as tumour promoters (Russell, 1985; Sunkara et al., 1987). Although the induction of ODC and increase in polyamines are associated with cell proliferation, the cellular function of polyamines is not as yet fully defined. Previous studies (Porter and Bergeron, 1983; Thomas and Messner, 1988) suggested that polyamine-induced B to Z transition might be an important function of polyamines,

Abbreviations used: ODC, ornithine decarboxylase; AP2, *N*-(2-aminoethyl)-1,3-propanediamine; AP3, 3,3′-iminobispropylamine; AP4, *N*-(2-aminobutyl)-1,3-propanediamine (spermidine); AP5, *N*-(2-aminopentyl)-1,3-propanediamine; AP6, *N*-(2-aminohexyl)-1,3-propanediamine; AP8, *N*-(2-amino-octyl)-1,3-propanediamine; SLE, systemic lupus erythematosus.

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judging from the high degree of structural specificity of these compounds both in B to Z transition and in supporting cell growth.

As the biological function of Z-DNA may depend on the ability of endogenous agents to promote this conformation under physiologically compatible ionic conditions, we started to examine the efficacy of polyamines to provoke Z-DNA in recombinant plasmids with potential Z-DNA-forming sequences. In the first series of these experiments, we found that spermidine and spermine are capable of provoking the Z-DNA form of pDHg16, a plasmid containing a 23 bp insert of $(dG\text{-}dC)_n$ sequences (Thomas et al., 1991). In the present series of experiments, we further investigated the effect of these polyamines on the conformation of pDHf2 and pDHf14, plasmids containing a 23 and 60 bp insert respectively of $(dA\text{-}dC)_n \cdot (dG\text{-}dT)_n$ sequences. In contrast with the low occurrence of $(dG\text{-}dC)_n$ sequences in the mammalian genome, blocks of $(dA\text{-}dC)_n \cdot (dG\text{-}dT)_n$ sequences varying from 8 to 144 bases have been widely dispersed in the regulatory regions of mammalian genes (Tripathi and Brahmachari, 1991; Schroth et al., 1992).

An early indication of a physiological function of Z-DNA was the finding that antibodies binding to this form were present in the sera of patients with systemic lupus erythematosus (SLE or lupus), an autoimmune disease (Lafer et al., 1983; Sibley et al., 1984; Mackworth-Young and Schwartz, 1988; Thomas et al., 1988b). This finding was particularly important as attempts to produce anti-DNA antibodies with B-DNA were not successful (Tan, 1982; Stollar, 1986). Later it was found that Z-DNA is immunogenic in experimental animals (Lafer et al., 1981; Gunnia et al., 1991b). Furthermore, monoclonal anti-Z-DNA antibodies were produced from the spleen cells of unimmunized MRL-*lpr/lpr* mice that spontaneously developed a lupus-like syndrome at an early stage of their lifespan and died because of it (Bergen et al., 1987). Thus there are strong links between Z-DNA and lupus. In the present study, our major goal was to characterize the polyamine requirement for the induction and stabilization of Z-DNA in short blocks of $(dA\text{-}dC)_n$ sequences. In order to assess the pathogenic role of anti-Z-DNA antibodies in lupus, we characterized the conditions under which endogenous anti-Z-DNA antibodies recognized $(dA\text{-}dC)_n$ sequences. Our results showed a facile transition of these plasmids to the Z-DNA conformation in the presence of spermidine and spermine. Polyamine-induced Z-DNA conformation of pDHf2 and pDHf14 also showed increased binding toward autoantibodies present in the sera of SLE patients and that of autoimmune MRL-*lpr/lpr* mice.

EXPERIMENTAL

Recombinant plasmids

Plasmid DNAs, pDHf2 and pDHf14 with inserts of 23 and 60 bp respectively of $(dA\text{-}dC)_n \cdot (dG\text{-}dT)_n$ sequences on the parental plasmid pDPL6, were obtained from Dr. David Haniford of the University of Toronto. pDPL6 (2.2 kb) was constructed from pBR322 by removing the 14 bp Z-DNA-forming region (Haniford and Pulleyblank, 1983). All three plasmids were prepared by methods described by Pulleyblank et al. (1983) and characterized by agarose-gel electrophoresis. The superhelical densities of both pDHf2 and PDHf14 were −0.034, as determined by Keller's (1975) band-counting method. Marker populations of topoisomers with varying superhelical densities were generated by relaxing DNA with topoisomerase I (Bethesda Research Laboratories) in the presence of different concentrations of ethidium bromide.

Calf thymus DNA and polynucleotides

Calf thymus DNA was purchased from Worthington Biochemicals (Freehold, NJ, U.S.A.), dissolved in a buffer containing 150 mM NaCl, 1 mM sodium cacodylate and 0.15 mM EDTA, and dialysed from a buffer containing 50 mM NaCl, 1 mM sodium cacodylate and 0.15 mM EDTA (pH 7.2) (50 mM NaCl buffer) before use in e.l.i.s.a. experiments. Poly(dG-dC)·poly(dG-dC) was purchased from Pharmacia (Piscataway, NJ, U.S.A.) and was converted into its brominated derivative by methods described by Lafer et al. (1981). Poly(dA-dT)·poly(dA-dT) and poly(dA-dC)·poly(dG-dT) were also purchased from Pharmacia. All polynucleotides were used after dialysis from 50 mM NaCl buffer.

Chemicals and immunological reagents

Putrescine.2hydrochloride, spermidine.3hydrochloride and spermine.4hydrochloride were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.) and were used without further purification. H.p.l.c. analysis of these compounds showed no contaminating polyamines. Two lower homologues of spermidine, *N*-(2-aminoethyl)-1,3-propanediamine (AP2; please see Table 1 for chemical structure) and 3,3′-iminobispropylamine (AP3), were purchased as free amines (Aldrich Chemical Co., Milwaukee, WI, U.S.A.), converted into their hydrochlorides, and purified by recrystallization from ethanol (Thomas and Bloomfield, 1984). Higher homologues of spermidine (AP5 to AP8) were obtained in the hydrochloride form from Professor D. Morris of the University of Washington (Seattle, WA, U.S.A.). Concentrated solutions of the polyamines were prepared in double-distilled deionized water, and small volumes were added to DNA solutions to make up the necessary concentrations.

$Co(NH_3)_6^{3+}$ (99.999%) and $Ru(NH_3)_6^{3+}$ (99.99%) were obtained from Aldrich Chemical Co. and Aesar/Johnson Matthey (Ward Hill, MA, U.S.A.) respectively, and were used without further purification.

Protamine sulphate, alkaline phosphatase-conjugated affinity-purified polyvalent goat anti-mouse immunoglobulins and phosphatase substrate (*p*-nitrophenyl phosphate) were purchased from Sigma. Peroxidase-conjugated affinity-purified rabbit anti-human immunoglobulins and *o*-phenylenediamine were purchased from Cappel Biochemicals (Malvern, PA, U.S.A.) and Sigma respectively.

Monoclonal anti-Z-DNA antibody (Z22)

The monoclonal antibody Z22 (Nordheim et al., 1986) used in this study was a gift from Professor B. D. Stollar (Tufts University, Boston, MA, U.S.A.). A stock solution of Z22 was diluted in PBS containing 0.05% Tween 20 and 0.02% NaN_3 for the e.l.i.s.a.

Sera from patients with SLE

Sera were collected from 15 patients fulfilling the American College of Rheumatology criteria for SLE. We also collected sera from normal volunteers for control experiments.

Autoimmune mouse sera

We used sera from MRL-*lpr/lpr* mice at 20 weeks of age. These mice were originally obtained from The Jackson Laboratory (Bar Harbor, ME, U.S.A.) and were housed in our certified Vivarium as part of our on-going studies on the immunopathogenetic mechanisms of murine lupus (Gunnia et al., 1991a).

E.l.i.s.a.

The e.l.i.s.a. protocol used to detect Z-DNA has been published elsewhere (Thomas et al., 1988a; Thomas, 1991). In short, this method consisted of incubating the plasmids with the necessary concentrations of polyamines, coating this solution on a microtitre plate (Costar, Cambridge, MA, U.S.A.; catalogue no. 3590), and subsequently treating the plate with Z22, alkaline phosphatase-conjugated polyvalent immunoglobulins and the enzyme substrate. The microtitre plates were precoated with 0.0001% protamine sulphate to facilitate the binding of DNA. The enzyme–substrate reaction was stopped by the addition of 50 μl of 1 M NaOH/well, and the absorbance was read at 405 nm with a microplate reader (Molecular Devices, Menlo Park, CA, U.S.A.).

Binding of autoantibodies from SLE patients and MRL-*lpr*/*lpr* mice to plasmids in the presence and absence of spermidine and spermine was also determined by an e.l.i.s.a. protocol similar to that used for detecting Z-DNA (Thomas et al., 1991; Gunnia et al., 1991a). In these experiments, human or mouse sera were used instead of the monoclonal antibody Z22. Furthermore, the secondary antibody was raised against human and mouse immunoglobulins respectively for the assay of human and mouse sera.

RESULTS

Effects of polyamines on the conformation of pDHf2 and pDHf14

Figure 1 shows the results of our e.l.i.s.a. experiments with pDHf2 and pDHf14 complexed with spermidine. The plasmids were incubated with different concentrations of spermidine for 2 h before addition to the microtitre plates. At low concentrations (< 10 μM) of spermidine, there was no binding of Z22 toward the plasmids and the absorbance value at the end of the e.l.i.s.a. experiment was essentially zero. This experiment also showed that low concentrations of protamine sulphate used to facilitate the binding of plasmid DNA to the microtitre plate had no effect on the conformation of pDHf2 and pDHf14 as determined by e.l.i.s.a. The superhelical density of the plasmids was -0.03, a value lower than that necessary to provoke the Z-DNA conformation (Hamada et al., 1982). At concentrations of 15–50 μM spermidine, however, there was a positive colour reaction due to the binding of Z22 to the plasmid coated on the microtitre plate. The optimum value of the absorbance was 0.3 for pDHf14 and 0.45 for pDHf2 and occurred at a spermidine concentration of 50 μM. From the absorbance versus polyamine concentration plots, we determined the concentration of spermidine at the midpoint of the B-DNA to Z-DNA transition of the plasmids and the results are presented in Table 1. Previous experiments showed that the midpoint concentrations thus determined from e.l.i.s.a. experiments were comparable with those determined by u.v. and c.d. spectroscopic experiments (Thomas et al., 1988a). Our data showed no significant difference in the amount of spermidine required to convert the 23 or 60 bp plasmid to the Z-DNA form. In both cases, there was reduction in absorbance values when the spermidine concentration reached 75 μM.

We conducted several control experiments to ascertain that the binding of Z22 to spermidine-treated pDHf2 and pDHf14 was directed to the Z-DNA conformation of the plasmid. Experiments with pDPL6 (Figure 1) showed no colour reaction up to 100 μM spermidine. Previous experiments also failed to detect any reactivity of Z22 with pDPL6 in the presence of spermidine up to 1 mM concentration (Thomas et al., 1991). These data demonstrate that the insert $(dA\text{-}dC)_n \cdot (dG\text{-}dT)_n$ sequence is an essential requirement for pDHf plasmids to assume the Z-DNA conformation. In other control experiments, we coated microtitre plates with spermidine and found no reactivity of this polyamine toward Z22, thereby confirming that the binding of the antibody was not directed toward spermidine itself. Furthermore, there was no reactivity of Z22 to spermidine-treated calf thymus DNA or poly(dA-dT)·poly(dA-dT), DNA polymers known to be resistant to Z-DNA formation in the presence of polyamines. In addition, Z22 could not react with poly(dA-dC)·poly(dG-dT) in the presence of spermidine, although we previously reported a Z-DNA-like conformation in this polynucleotide using c.d. spectroscopy (Thomas and Messner, 1986). In a positive control experiment with Br-poly(dG-dC)·poly(dG-dC), there was a positive colour reaction even in the absence of spermidine and the absorbance was 1.5. It is known that Br-poly(dG-dC)·poly(dG-dC) is capable of existing in the Z-DNA form at low NaCl concentrations (Lafer et al., 1983; Thomas et al., 1991).

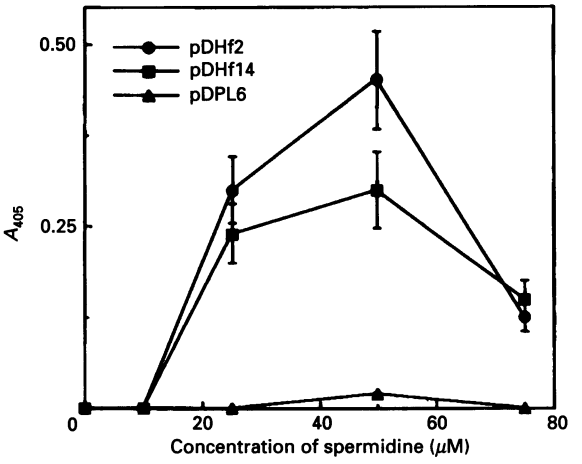

Figure 1 E.l.i.s.a. of pDHf2 (●), pDHf14 (■) and pDPL6 (▲) treated with different concentrations of spermidine

Plasmid DNAs (22 μM) were incubated for 2 h with the indicated concentrations of spermidine in a buffer containing 1 mM sodium cacodylate, 50 mM NaCl and 0.15 mM EDTA (pH 7.4). The plasmid/spermidine solutions were subsequently loaded on microtitre plates and treated with monoclonal anti-Z-DNA antibody Z22, alkaline phosphatase-conjugated immunoglobulins and the enzyme substrate. Error bars indicate $\pm$S.D. ($n = 3$–4).

Table 1 Midpoint concentrations of polyamines in B-DNA to Z-DNA transition of plasmids pDHf2 and pDHf14

Midpoint concentrations were determined using the buffer containing 1 mM sodium cacodylate, 50 mM NaCl and 0.15 mM EDTA (pH 7.4). Means $\pm$ S.D. from three to five experiments are given. N.B. AP4 is spermidine. ND, not determined.

Polyamines	Chemical structure	Midpoint concentration (μM) pDHf2	pDHf14
AP2	$H_2N[CH_2]_2NH[CH_2]_3NH_2$	ND	85 ± 8
AP3	$H_2N[CH_2]_3NH[CH_2]_3NH_2$	ND	60 ± 7
AP4	$H_2N[CH_2]_4NH[CH_2]_3NH_2$	25 ± 5	25 ± 3
AP5	$H_2N[CH_2]_5NH[CH_2]_3NH_2$	ND	45 ± 5
AP6	$H_2N[CH_2]_6NH[CH_2]_3NH_2$	ND	90 ± 10
AP8	$H_2N[CH_2]_8NH[CH_2]_3NH_2$	ND	150 ± 4
Spermine	$H_2N[CH_2]_3NH[CH_2]_4NH[CH_2]_3NH_2$	15 ± 5	18 ± 4

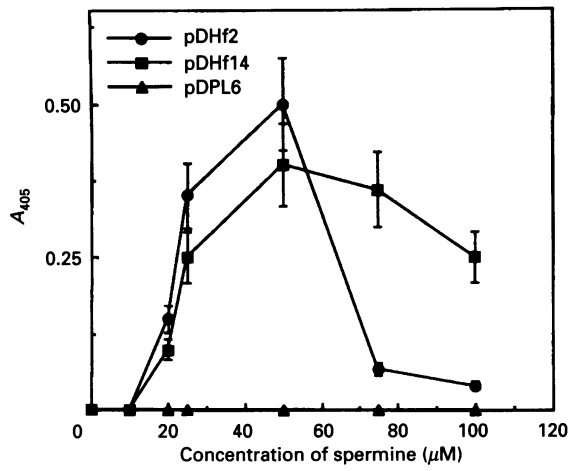

Figure 2 E.l.i.s.a. of pDHf2 (●), pDHf14 (■) and pDPL6 (▲) treated with different concentrations of spermine

Plasmid DNAs (22 μM) were incubated for 2 h with the indicated concentrations of spermine in a buffer containing 1 mM sodium cacodylate, 50 mM NaCl and 0.15 mM EDTA (pH 7.4). The plasmid/spermine solutions were subsequently loaded on microtitre plates and treated with monoclonal anti-Z-DNA antibody Z22, alkaline phosphatase-conjugated immunoglobulins and the enzyme substrate. Error bars indicate ±S.D. ($n = 3$–4).

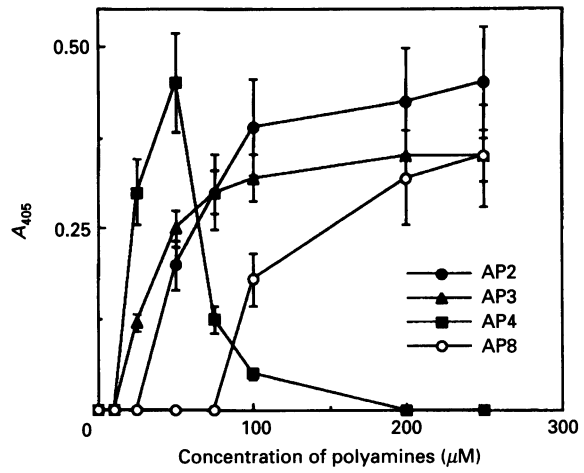

Figure 3 E.l.i.s.a. of pDHf14 in the presence of spermidine or its homologues

●, AP2; ▲, AP3; ■, AP4 (spermidine); ○, AP8. Experimental conditions were as described for Figure 1. Error bars indicate ±S.D. ($n = 3$–4).

Although we conducted similar experiments with pDHf2 and pDHf14 complexed with putrescine up to concentration of 5 mM, there was no reactivity of Z22 to the plasmids, thereby showing that this bivalent amine is not capable of provoking the Z-DNA conformation of these plasmids.

In the next set of experiments, we examined the effect of spermine on the conformation of both pDHf2 and pDHf14 using the e.l.i.s.a. technique. Figure 2 shows that at concentrations of 15–50 μM spermine, both plasmids recognized Z22 and a positive colour reaction was obtained. The optimum absorbance in this case was 0.5. The midpoint concentrations of spermine for pDHf2 and pDHf14 were not significantly different (Table 1). There was no reactivity of the control plasmid, pDPL6, complexed with spermine, thus demonstrating that the interaction of $(dA\text{-}dC)_n \cdot (dT\text{-}dG)_n$ inserts with spermine was the cause of the Z-DNA form.

Effect of spermidine homologues and inorganic trivalent cations

Under physiological pH and ionic concentrations, spermidine is a tripositive cation and hence its effect on the conformation of pDHf2 and pDHf14 might be governed by the ionic nature of these cations. In several studies involving polyamines with DNA, including condensation (Gosule and Schellman, 1978; Thomas and Bloomfield, 1983), stabilization of duplex structure (Thomas and Bloomfield, 1984), as well as aggregation (Srivenugopal et al., 1987), there was no dominant effect of polyamine structure. Interestingly, however, in cases involving the induction and stabilization of unusual DNA structures, such as Z-DNA and triplex DNA, a remarkable structural specificity was observed (Thomas and Messner, 1988; Thomas and Thomas, 1993). Therefore, in order to differentiate the role of ionic and structural effects on the ability of polyamines to provoke the B-DNA to Z-DNA conformation transition of pDHf2 and pDHf14, we investigated the effects of spermidine homologues on the conformational transition of pDHf2 and pDHf14. Figure 3 shows the results of our e.l.i.s.a. experiment with three of these homologues and spermidine. The midpoint concentrations determined for all homologues are presented in Table 1. Compared with spermidine (AP4), AP2, AP3, AP5, AP6 and AP8 were 3-, 2-, 2-, 4- and 6-fold less efficient at provoking the Z-DNA conformation of pDHf14, as measured by their midpoint concentrations of B-DNA to Z-DNA transition. Another interesting observation from this study is that a decrease in anti-Z-DNA antibody binding occurred with spermidine at high concentrations, but not with any of the homologues. These results clearly show structural specificity in the interaction of tervalent polyamines with $(dA\text{-}dC)_n \cdot (dG\text{-}dT)_n$ inserts in Z-DNA formation.

With poly(dG-m^{5}dC)·poly(dG-m^{5}dC), tervalent inorganic cations $Co(NH_3)_6^{3+}$ and $Ru(NH_3)_6^{3+}$ were excellent promoters of the Z-DNA conformation (Thomas and Thomas, 1990). In contrast, these metal complexes had no effect on the conformation of poly(dA-dC)·poly(dG-dT) (Thomas and Messner, 1986). We therefore conducted e.l.i.s.a. experiments with pDHf2 and pDHf14 complexed with $Co(NH_3)_6^{3+}$ and $Ru(NH_3)_6^{3+}$ to examine their effect on the conformation of these plasmids. There was no colour reaction in these cases up to 250 μM, thereby showing that the polyamine structure, rather than the trivalent ionic state, was important in the induction of the Z-DNA conformation of these plasmids.

Binding of sera from SLE patients of pDHf2 and pDHf14 in the presence of spermidine

In the next set of experiments we examined whether spermidine-induced Z-DNA conformation of plasmids have increased affinity toward natural anti-DNA antibodies present in the sera of SLE patients and that of autoimmune mice. In these experiments, we treated plasmids with different concentrations of spermidine and then coated them on microtitre plates. The plates were then treated with sera from SLE patients or MRL-*lpr*/*lpr* mice. The plates were further coated with the appropriate enzyme-conjugated antibody and then treated with the enzyme substrate. Figure 4 shows representative results of the binding of sera from

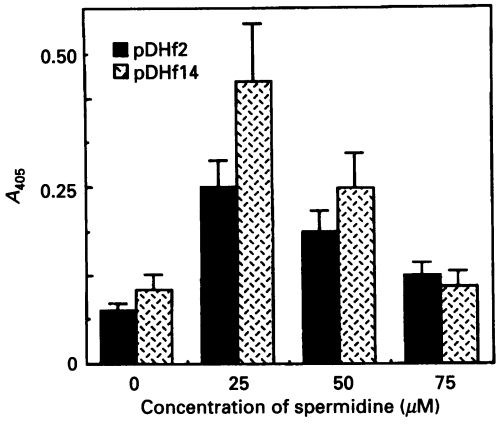

Figure 4 Effect of spermidine on the binding of sera from SLE patients to pDHf2 and pDHf14

Plasmid DNAs were incubated for 2 h with the indicated concentrations of spermidine in a buffer containing 1 mM sodium cacodylate, 50 mM NaCl and 0.15 mM EDTA (pH 7.4). The plasmid/spermidine solutions were coated on microtitre plates and subsequently treated with SLE-patient sera (1:400 in PBS containing 0.05% Tween 20) followed by alkaline phosphatase-conjugated rabbit anti-human immunoglobulins and the enzyme substrate. Similar results were obtained for sera from ten SLE patients.

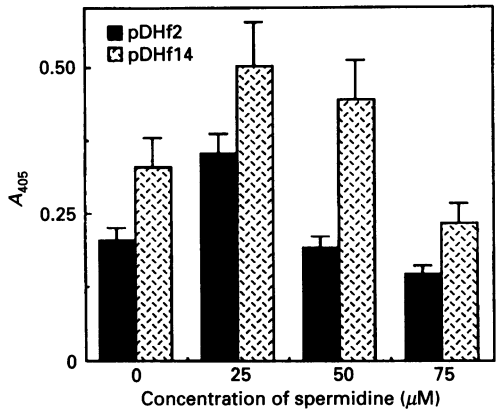

Figure 5 Effect of spermidine on the binding of MRL-*lpr/lpr* mouse sera to pDHf2 and pDHf14

Plasmid DNAs were incubated for 2 h with the indicated concentrations of spermidine in a buffer containing 1 mM sodium cacodylate, 50 mM NaCl and 0.15 mM EDTA (pH 7.4). The plasmid/spermidine solutions were coated on microtitre plates and subsequently treated with pooled mouse sera (1:200 in PBS containing 0.05% Tween 20) followed by alkaline phosphatase-conjugated mouse immunoglobulins and the enzyme substrate. Error bars indicate ±S.D. from triplicate experiments.

SLE patients to pDHf2 and pDHf14 plasmids in the presence and absence of spermidine. Autoantibodies present in SLE-patient sera bound to plasmids even in the absence of spermidine, indicating the presence of anti-DNA antibodies in this autoimmune disease. More strikingly, however, there was an increase in the binding of these autoantibodies to plasmids with spermidine concentrations up to 25 μM and then a decrease. We found the same trend in the binding of monoclonal anti-Z-DNA antibody with pDHf2 and pDHf14 in the presence of spermidine. A low level of binding was also found with the control plasmid, pDPL6. However, there was no significant increase in binding of sera from SLE patients to pDPL6 in the presence of spermidine compared with that in its absence (results not shown). Control experiments with normal human sera showed no binding to the plasmid in the presence or absence of the polyamine. Thus the increased binding appears to be a consequence of the conversion of the plasmid into the Z-DNA form.

We obtained similar results with sera from MRL-*lpr/lpr* mice (Figure 5). In this case also, there was an increase in the binding of the sera to the plasmid with increasing concentrations of spermidine up to 25 μM, and then a decrease. The final absorbance value was comparable with that in the absence of polyamines. Furthermore, we found no binding of the sera of normal Balb/c mice to pDHf2 and pDHf14 with or without spermidine.

DISCUSSION

Our data demonstrate that natural polyamines, spermidine and spermine, are capable of provoking a Z-DNA conformation in short segments of $(dA\text{-}dC)_n \cdot (dT\text{-}dG)_n$ present in plasmids. Supercoiled plasmids are convenient model systems to study conformational transitions of naturally occurring $(dA\text{-}dC)_n$ sequences in chromosomal domains where transcription is believed to occur. The effect of polyamines on B-DNA to Z-DNA transition of plasmids is structure-specific, but has little effect on insert sequence length within the 23 to 60 bp range. With $(dA\text{-}dC)_n \cdot (dG\text{-}dT)_n$-inserted plasmids, the midpoint concentrations of spermidine and spermine are 10-fold lower than that of a plasmid with a $(dG\text{-}dC)_n$ insert (Thomas et al., 1991). Interestingly, Z22 could not recognize polyamine-treated poly(dA-dC)·poly(dG-dT). In our e.l.i.s.a. experiments, the maximum response of pDHf2 was higher than that of pDHf14, as measured by the absorbance values at optimum polyamine concentrations. A possible explanation is that microstructural alterations with lower affinity for Z22 might occur in long stretches of (dA-dC)·(dG-dT) sequences. As $(dA\text{-}dC)_n \cdot (dG\text{-}dT)_n$ sequences are widely dispersed in the mammalian genome (Tripathi and Brahmachari, 1991; Schroth et al., 1992), our result suggests a common utilization of Z-DNA conformation in genomic functions such as transcription and recombination. Our studies further demonstrate that polyamines enhance the binding of anti-DNA antibodies to plasmids containing $(dA\text{-}dC)_n$ sequences, suggesting that polyamines could facilitate the interaction of DNA with anti-DNA antibodies and thus contribute to immune complex-formation *in vivo*.

Previous studies (Hamada and Kakunaga, 1982; Hamada et al., 1982) using Southern-blot analysis revealed the presence of 50000 blocks of $(dA\text{-}dC)_n$ sequences in human haploid genome. These results are complemented by computer search analysis (Tripathi and Brahmachari, 1991; Schroth et al., 1992), demonstrating that these sequences are non-randomly distributed in the regulatory regions of mammalian genes. Furthermore, Rahmouni and Wells (1992) provided evidence for the formation of Z-DNA during transcription as a mechanism to reduce the free energy of supercoiling. Wittig et al. (1992) have shown the presence of Z-DNA in transcribing the c-*myc* gene, the expression of which is highly regulated by polyamines (Celano et al., 1989). As polyamine biosynthesis is intimately related to cell proliferation (Tabor and Tabor, 1984; Russell, 1985; Pegg, 1986; Sunkara et al., 1987), it is possible that polyamine-induced Z-DNA formation might play a pivotal role in controlling transcription. The formation of Z-DNA is very sensitive to spermidine and spermine concentrations in that a slight increase from the optimum concentration converts DNA into conformational states that are not recognized by anti-Z-DNA antibodies. This would suggest that alterations in the conformation of the plasmid to a non-Z-DNA form might occur at elevated levels of

spermidine and spermine. Thus polyamines may exert a regulatory role in cell growth by inducing unusual conformational transitions that are important in transcription.

The influence of Z-DNA-forming sequences on transcription has been examined by other experimental models as well. Hamada et al. (1984) constructed fusion genes containing $(dA\text{-}dC)_n$ sequences with simian virus 40 promoter and chloramphenicol acetyltransferase gene. The results of this study showed that $(dA\text{-}dC)_n$ sequences enhanced gene expression by 2- to 10-fold depending on the length of the insert sequence and location of the insert. In contrast, Naylor and Clark (1990) reported down-regulation of the chloramphenicol acetyltransferase gene using $(dA\text{-}dC)_n$ inserts in a pituitary cell line. Delic et al. (1991) also found that $(dA\text{-}dC)_{40}$ sequences exerted inhibitory effects on transcription. Studies on *Drosophila hydei* indicated Z-DNA formation related to developmental time points, between two transcription units, one of which was down-regulated (Jimenez-Ruiz et al., 1991). Thomas and Kiang (1988) found that the binding of gene-regulatory proteins such as oestrogen receptor to DNA was enhanced by the presence of polyamines. Thus it seems that $(dA\text{-}dC)_n$ sequences with the potential to form Z-DNA have both stimulatory and inhibitory influences on transcription. This effect may depend on the presence of cell-type-specific transcription factors as well as the level of intracellular polyamines. Polyamine concentrations in the cell are reported to be in the range 1 to 5 mM, but recent studies indicate that most of the intracellular polyamines remain bound to macromolecules or sequestered (Watanabe et al., 1990; Davis et al., 1992). The concentration of polyamines necessary to achieve regulatory function is believed to be in the low micromolar range.

The structural specificity of polyamines was not recognized in early studies when polyamine–DNA interaction was thought to be independent of the DNA sequence and polyamine structure (Shapiro et al., 1969). More recent studies, however, point out that the binding constants of polyamines with G-C and A-T sequences are different (Igarashi et al., 1982). Using N^1- and N^8-acetylspermidines, Thomas et al. (1985) found that a difference in the charge separation by one methylene group had a major effect on the ability of these compounds to induce the Z-DNA conformation. The effects of backbone structure and pendant groups were also studied by Vertino et al. (1987) as well as Basu et al. (1990). In these cases, the ability of polyamines to interact with DNA roughly correlated with their ability to induce conformational alterations and/or aggregation of DNA. Taken together, these data implicate that site-specific binding of polyamines with DNA is important in the induction and stabilization of unusual DNA conformations, and DNA appears to be a prime target for the growth-regulatory effects of polyamines.

Electrostatic interactions between the cationic sites of polyamines and the negative charges on DNA phosphates were initially thought to be the driving force in polyamine–DNA interactions (Manning, 1978). However, recent reports indicate a different picture. Liquori et al. (1967) made molecular models that suggested a minor groove binding of polyamines with DNA. Based on molecular mechanical minimization calculations, Feuerstein et al. (1986) suggested a model that could fit spermine into the major groove of DNA. Single-crystal X-ray analysis of spermine–oligonucleotide complexes by Gessner et al. (1989) and Egli et al. (1991) have shown a more complex picture that involves amino groups of spermine spanning the opening of the major groove of DNA. At least three spermine molecules are bound to a single oligonucleotide, each oligonucleotide molecule in turn making contact with three spermine molecules. These studies were conducted with (dG-dC) oligomers; however, Jain et al. (1989) made a single-crystal analysis of spermine complexed with A-type $(GTGTACAC)_2$ and found that the spermine molecule was bound at the floor of the deep groove by interacting with bases and assumed an 'S' shape. Thus, many different modes of binding have been proposed for polyamine–DNA interaction. It should be noted here that the DNA structures are not all the same in the above examples. Induction and stabilization of unusual DNA conformations is a major consequence of polyamine binding with purine/pyrimidine as well as polypurine/polypyrimidine sequences. Our present study demonstrates that even small stretches of $(dA\text{-}dC)_n \cdot (dG\text{-}dT)_n$ sequence can be provoked to undergo the B-DNA to Z-DNA conformational transition under conditions of natural superhelical density of the plasmid.

The immunological abnormalities of SLE appear to have links to increased polyamine levels as well as Z-DNA (Lafer et al., 1983; Thomas et al., 1988b; Gunnia et al., 1991a). Van Helden (1985) found that circulating DNA from SLE patients have a high prevalence of alternating purine/pyrimidine sequences with the potential to form Z-DNA. Puri et al. (1978) found that SLE patients have increased polyamine levels in their serum compared with normal controls. Thus there is a possibility that polyamine-induced Z-DNA is one of the antigenic molecules that activate the production of antinuclear antibodies. Indeed, we found that immunization of experimental animals with poly(dA-dC)·poly(dG-dT) complexed with polyamines could produce anti-DNA antibodies including one reacting with Br-poly(dG-dC) in the Z-DNA form (Gunnia et al., 1991b). The finding that anti-DNA antibodies in lupus serum recognize short blocks of $(dA\text{-}dC)_n$ sequences in the presence of polyamines indicates that such blocks could act as an autoantigen under appropriate conditions. In addition, the continued presence of DNA antigens in the serum of SLE patients could facilitate immune complex-formation in the presence of polyamines which in turn could produce systemic pathogenic effects. For example, kidney damage in patients and animals with lupus is believed to be due to the deposition of immune complexes by the infiltrating antibodies (Stollar, 1986; Tan, 1982).

In summary, we report that a facile transition of short segments of $(dA\text{-}dC)_n$ sequences to the Z-DNA form occurs in the presence of polyamines. In view of the ubiquitous occurrence of blocks of these sequences and the recent demonstration of Z-DNA involvement in transcriptional regulation, our data suggest that polyamines act in concert with transcription factors in facilitating DNA conformational changes and initiation of transcription. The specificity of polyamine-induced Z-DNA conformation, in terms of DNA sequence, polyamine concentration and polyamine structure suggests that this interaction is an integral part of the cellular function of polyamines, responsible for their effects on cell growth and differentiation. Polyamine-induced Z-DNA formation appears to have pathogenic effects especially if it occurs in the serum circulating DNA.

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