Polyamine induced Z-conformation of native calf thymus DNA

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Received 14 April 1995

Abstract Conformational isomerization of native calf thymus DNA under the influence of spermine, spermidine and putrescine was monitored by UV absorption and immunospecific anti-Z-DNA antibodies. Immunological data indicated increased binding of anti-Z-DNA antibodies to polyamine-perturbed conformations of native DNA and double stranded poly(dG-dC). In the absence of polyamines, anti-Z-DNA antibodies did not bind to either polymers. Analysis of UV absorption studies indicates a left handed conformation of nDNA in the presence of polyamines. Moreover, we observed total aggregation of DNA in the presence of spermine on prolongued incubation. These perturbations in conformation were dependent on polyamine concentration. The results clearly suggest that certain regions of nDNA are sensitive to elevated levels of polyamines and are capable of undergoing B→Z transition.

Key words: Polyamine; Native DNA; Z-conformation; B→Z transition

1. Introduction

Polyamines like spermine, spermidine and putrescine are involved in regulating cell growth and differentiation [1,2]. Accumulating evidence indicates that these cations play an important role in the growth of hormone-responsive breast cancer [3] and their level is elevated in proliferating tissues [4]. Increased concentration of polyamines in the sera of active SLE patients is well documented [5]. Elevated polyamine levels have been observed in biological fluids of diseased states such as cancer, sickle cell anemia, cystic fibrosis and psoriasis [6]. One of the changes which has received the attention of researchers is the polyamine-induced left-handed Z-conformation of alternating purine-pyrimidine sequences in synthetic polymers or plasmids [6–8]. The complex formation between poly(dA-dC)·poly(dG-dT) and polyamines induces Z-conformation which significantly altered the immunogenicity of this polymer and produced anti-Z-DNA antibodies when challenged in experimental animals [9]. The B→Z transition has been studied in synthetic polymers like poly(dG-dC)·poly(dG-dC) and poly(dG-me^{5}dC)·poly(dG-me^{5}dC). A number of studies suggest that Z-DNA may exist in vivo [10–12]. We have shown earlier that B→Z transition in native calf thymus DNA depends on its microenvironment [13] and bromination in 4 M NaCl. It adopts a Z-/analogous conformation [14].

In earlier studies the effect of spermine and three pentamines, isolated from an extreme thermophile, on the stability, condensation and B→Z transition of calf thymus DNA was investigated. Aggregation of calf thymus DNA, monitored by an increase in absorbance at 320 nm, has been observed by these polycations [15].

In the present communication, the conformational isomerization of calf thymus DNA under the influence of varying concentrations of polyamines was monitored by UV absorption and immunospecific anti-Z-DNA antibody. We also observed aggregation of calf thymus DNA in the presence of spermine as a function of temperature and incubation time.

2. Materials and methods

2.1. Materials

Highly polymerized calf thymus DNA, anti-rabbit IgG alkaline phosphatase conjugate, sodium cacodylate, spermine, spermidine, and putrescine were obtained from Sigma, USA. Poly(dG-dC)·poly(dG-dC) was purchased from Pharmacia, Sweden and used without further purification. ELISA microtiter plates and modules were the product of NUNC (Denmark) and Dynatech (USA), respectively.

2.2. Antigen preparation and complex formation

Calf thymus DNA was purified free of proteins, RNA and single stranded regions [16]. Prototype Z-DNA was prepared from poly(dG-dC)·poly(dG-dC) brominated in sodium citrate buffer (20 mM sodium citrate, 1 mM EDTA, pH 7.0 containing 4 M NaCl) [17]. The sample was extensively dialysed against cacodylate buffer (1.0 mM sodium cacodylate, 50 mM NaCl, 0.15 mM EDTA, pH 7.4). Polyamines and nucleic acid polymers were dissolved in cacodylate buffer and dialysed extensively against the same buffer. Concentrated solutions of polyamines were prepared in buffer without EDTA. Calf thymus DNA and poly(dG-dC)·poly(dG-dC) were mixed with varying concentrations of different polyamines and incubated for 30 min at room temperature. Control samples contained solutions of polyamines without nucleic acids. For each point, triplicate sets of samples (control and experimental) were prepared and their absorbance was measured at 260 nm and 295 nm against cacodylate buffer. The absorbance was recorded on Shimadzu UV-240 spectrophotometer. Low level of variation was observed between triplicate samples. The arithmetic mean of control sample was subtracted from the arithmetic mean of experimental before the evaluation of data.

2.3. Antibody preparation and purification

Anti-Z-DNA antibody was induced in rabbits by intramuscular injections of Z-DNA (50 μg/injection) complexed with methylated BSA and emulsified in Freunds' complete adjuvant. Subsequent injections were given in Freunds' incomplete adjuvant at weekly intervals for five weeks [18]. The induced antibodies were specific for Z-conformation [14]. Immunoglobulins were fractionated by 40% saturated ammonium sulphate precipitation of the antiserum. The crude immunoglobulins after extensive dialysis in PBS were subjected to DEAE Sephacel chromatography followed by affinity purification through a Sepharose 4B column linked with immunogen [18,19]. The homogeneity of the isolated anti-ZDNA IgG was confirmed by the observation of a single band in a polyacrylamide gel corresponding to a molecular mass of 150,000. The purified IgG was stored in small volumes at −20°C until use.

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Abbreviations: nDNA, native DNA; buffer, 0.1 mM cacodylate buffer, pH 7.4.

SSDI 0014-5793(95)00591-9

2.4. Enzyme-linked immunosorbent assay

Constant amounts of native DNA and poly(dG-dC)·poly(dG-dC) (50 μg/ml) were mixed with varying concentrations of polyamines (0–400 μg/ml) and incubated for 30 min at room temperature. All solutions were in cacodylate buffer without EDTA. After incubation, the nucleic acid/polyamine mixture was diluted to a nucleic acid concentration of 2.5 μg/ml in cacodylate buffer without EDTA. One hundred μl of the resulting complex were used to coat microtiter plates for 2 h at room temperature, or overnight at 4°C. ELISA was performed as reported earlier [20].

3. Results

Increased UV absorbance around 295 nm has been found to be related to structural perturbations in the classical B-DNA helix [21]. Lafer et al. [22] have earlier shown that double stranded poly(dG-dC) in 4 M NaCl attain the Z-conformation and an observed absorbance ratio (295/260) of 0.30. Later studies accepted the increased absorbance ratio as one of the criteria for Z-conformation [23]. Table 1 has been computed from UV spectroscopic behaviour of native calf thymus DNA complexed with 100 μg/ml each of spermine, spermidine, and putrescine. In the presence of polyamines the net absorbance of nDNA was increased and the data conclusively showed an increase in absorbance ratio in the presence of spermine, spermidine and putrescine. The percent relative increase was in the range of 19 to 27. Poly(dG-dC)·poly(dG-dC) in the presence of varying concentrations of spermine, under identical conditions, showed more than 60% increase in absorbance ratio when the polyamine concentration reached 40 μg/ml or higher (Table 2). In presence of spermine, aggregation of calf thymus DNA as a function of temperature and incubation time was evaluated. The absorbance ratio (260/320) of the mixture was computed to be 1.6. Almost complete aggregation was observed when the mixture was kept overnight at 4°C. After centrifugation at around 8,000 rpm the clear supernatant was completely devoid of absorbance at 260 nm indicating complete aggregation of DNA.

Fig. 1 shows the results of ELISA on B-DNA to Z-DNA transition of purified native calf thymus DNA in the presence of spermine, spermidine, and putrescine. The concentration of polyamines was varied while the antigen concentration was kept constant. The enzyme immunoassay was conducted as described by Thomas and Messner [6]. The experimentally induced antibodies against Z-DNA had almost no binding with native DNA when coated on microtiter plates in the absence of polyamines. Anti-Z-DNA antibodies in the presence of polyamines produced a characteristic color on ELISA. With increasing concentrations of polyamines, the antibody binding was found to increase. In this case both spermine and spermidine were almost equally effective in the conversion of B-DNA to the Z-conformation. Putrescine appears to be less effective.

For the purpose of comparison, double stranded poly(dG-dC) coated on microtiter plate was complexed with varying concentrations of polyamine. On the basis of anti-Z-DNA antibody data (Fig. 2) comparatively lesser amounts of polyamines were required to convert B-form of poly(dG-dC) to Z-conformation. Anti-Z-DNA antibodies showed no binding with double stranded poly(dG-dC) when coated on plates without polyamines. Various polyamines showed almost similar degree of effectiveness in B→Z transition. It has been shown earlier that sperminie is quite capable of inducing B→Z transition in double stranded poly(dG-dC) [24].

Table 1
UV absorption characteristics of native calf thymus DNA complexed with polyamines

nDNA with:	Absorbance at		Absorbance ratio (295/260)	Percent relative increase in absorbance ratio
	260 nm	295 nm		
–	1.473	0.153	0.104	–
Spermine	1.484	0.196	0.132	26.92
Spermidine	1.455	0.185	0.127	22.12
Putrescine	1.425	0.176	0.124	19.23

The data represent the arithmetic mean of triplicate samples. The absorbance of polyamine (100 μg/ml) without nDNA was subtracted from the sample containing nDNA and polyamines.

Table 2
UV absorption characteristics of poly(dG-dC)·poly(dG-dC) complexed with varying concentration of spermine

Poly(dG-dC) with spermine (μg/ml)	Absorbance at		Absorbance ratio (295/260)	Percent relative increase in absorbance ratio
	260 nm	295 nm		
0	0.620	0.120	0.194	–
10	0.634	0.156	0.246	26.8
20	0.644	0.176	0.273	40.7
40	0.627	0.198	0.316	62.9
50	0.615	0.193	0.314	61.8
100	0.600	0.188	0.313	61.3

The data represent arithmetic mean of triplicate samples of control and experimental. The absorbance of spermine in each set was subtracted from the experimental value.

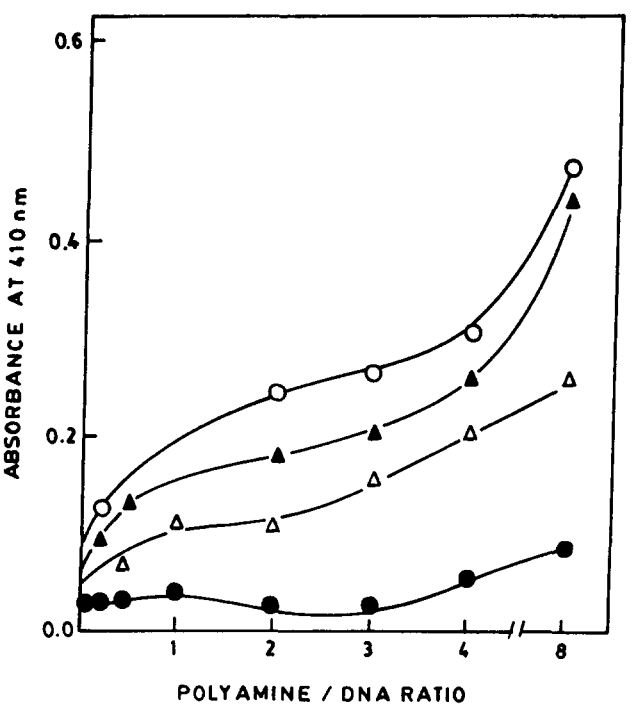

Fig. 1. Enzyme-immunoassay detection of anti-Z-DNA antibody binding to native DNA (●), and DNA complexed with varying amounts of spermine (○), spermidine (▲), and putrescine (△). The color was developed for 45 min and absorbance read at 410 nm.

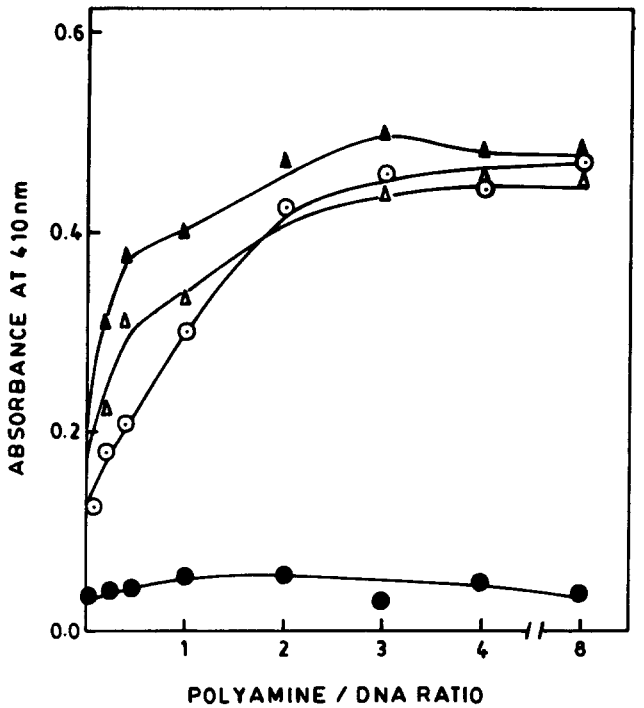

Fig. 2. Enzyme-immunoassay titration of anti-Z-DNA antibody binding to poly(dG-dC)·poly(dG-dC) (●), and the polymer mixed with varying amounts of spermine (○), spermidine (▲) and putrescine (△).

4. Discussion

The barrier of interconversion of double stranded DNA between left handed and right handed helical forms is dependent upon the base sequence, the presence of covalently modified nucleotides, and various environmental factors including the concentrations of metal ions and polyamines [25]. Protonated amines are among the most effective cations that induce the left handed Z-form in certain polynucleotides [26,27] and cause condensation of DNA [28]. The importance of spermidine and spermine lies in their requirement for normal cell growth and differentiation. Their interaction with nucleic acids may be responsible in part for the biological function of polyamines [1,2].

In the present communication the specific binding of anti-Z-DNA antibody to polyamine–DNA complex is quasi-evidence for the conformational isomerization of native B-epitopes into the Z-conformation in the presence of increasing concentrations of spermine, spermidine and putrescine. Poly(dG-dC)·poly(dG-dC) taken as a reference polymer showed similar epitope polymerization. Anti-Z-DNA antibody showed no binding with poly(dG-dC)·poly(dG-dC) and nDNA when the polynucleotides were coated on the microtiter plate in the absence of polyamines. Monoclonal anti-Z-DNA antibodies and polyclonal antibodies that are highly specific for the Z-DNA conformation have been used to detect the presence of left handed segments in natural DNAs [29–33]. And solid-phase enzyme immunoassay is a very sensitive technique to study the B-DNA to Z-DNA conformational transition of polynucleotides [34].

In UV spectroscopy, the native B-DNA to Z-DNA transition was monitored by recording the absorbance ratio (295/260) in the presence of polyamines. Taking into consideration the ratio observed in case of poly(dG-dC)·poly(dG-dC) (average 0.315) and the concentration of spermine required to achieve this conversion (40 μg/ml), native calf thymus DNA showed around 15% isomerization into Z-conformation (average absorbance ratio 0.125 at 100 μg/ml of spermine). Earlier studies from this laboratory have shown a similar potentiality of calf thymus DNA to attain Z-conformation in a changed microenvironment [13].

We observed the aggregation of nDNA in the presence of spermine on prolongued incubation. Almost complete aggregation was observed when the mixture was kept overnight at 4°C. On centrifugation at around 8,000 rpm the clear supernatant was completely devoid of absorbance at 260 nm confirming complete DNA aggregation. In the case of calf thymus DNA, earlier studies observed only aggregation but not transition to the Z-conformation [15]. Behe and Felsenfeld [26] reported that poly(dG-dC)·poly(dG-dC) undergoes aggregation at higher concentrations of polyamines. Pentamines isolated from the thermophile, *Thermus thermophilus*, caused aggregation of DNA at a much lower concentration than that of spermine [15]. Spermine is able to induce many different conformational changes in DNA [35–38]. The natural polyamines are capable of altering the immunogenicity of polynucleotides by mechanisms involving the stabilization of the Z-DNA conformation [9]. This study points out the transition of native DNA to the Z-conformation in the presence of polyamines, and on prolongued incubation aggregation has been observed. Of special significance is the induction of the Z-conformation in the presence of spermine. Although the specific biological role of spermine is not fully clarified, its importance in the regulation of cell growth and differentiation is well documented [1,2]. A higher affinity of spermine for poly(dG-dC)·poly(dG-dC) compared to poly(dA-dT)·poly(dA-dT) has been observed [39]. Specific structural changes in alternating A-T sequences has been observed in the presence of spermine and its analogues [40]. It has also been observed that a tract of pur-pyr six base pairs long is sufficient to assess a stereospecific binding to one spermine molecule [41].

The ability of spermine to induce a B→Z transition in native calf thymus DNA might be important in chromatin condensation. Moreover, tracts of alternating A-T sequences in the genome of many species, often close to or at eukaryotic promoters [42] indicate that specific structural changes in these sequences may have a role in the regulation of transcription and spermine may take an important part in such regulation.

Acknowledgements: This work was supported in part by a research grant (BT/TF/9/36/90) to R.A. from the Department of Biotechnology, Government of India.

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