Proc. Int. Symp. Biomol. Struct. Interactions, Suppl. J. Biosci.,
Vol. 8, Nos 3 & 4, August 1985, pp. 563-578. © Printed in India.

Left handed DNA in synthetic and topologically constrained form V DNA and its implications in protein recognition

Y. S. SHOUCHE, P. K. LATHA, N. RAMESH, K. MAJUMDER, V. MANDYAN and SAMIR K. BRAHMACHARI*
Molecular Biophysics Unit, Indian Institute of Science, Bangalore 560012, India

Abstract. We have investigated structural transitions in Poly(dG-dC) and Poly(dG-Me^{5}dC) in order to understand the exact role of cations in stabilizing left-handed helical structures in specific sequences andthe biological role, if any, of these structures. From a novel temperature dependent $Z \rightleftharpoons B$ transition it has been shown that a minor fluctuation in Na^+ concentration at ambient temperature can bring about B to Z transition. Forthe first time, wehave observed a novel $Z \rightleftharpoons B \rightleftharpoons Z$uble transition in poly(dG-Me^{5}dC) as the Na^+ concentration is gradually increased. This suggests that a minor fluctuation in Na^+ concentration in conjunction with methylation may transform small stretches of CG sequences from one conformational state to another. These stretches could probably serve as sites for regulation. Supercoiled formV DNA reconstituted from pBR322 and pβG plasmids have been studied as model systems, in order to understand the nature and role of left-handed helical conformation in natural sequences. A large portion of DNA in form V, obtained by reannealing the two complementary singlestranded circles is forced to adopt left-handed double helical structure due to topological constraints (L_k = 0). Binding studies with Z-DNA specific antibody and spectroscopic studies confirm the presence of left-handed Z-structure in the pβG and pβR322 form V DNA. Cobalt hexamine chloride, which induces Z-form in Poly(dG-dC) stabilizes the Z-conformation in form V DNA even in the non-alternating purine-pyrimidine sequences. A reverse effect is observed with ethidium bromide. Interestingly, both topoisomerase I and II (from wheat germ) act effectively on form V DNA to give rise to a species having an electrophoretic mobility on agarose gel similar to that of open circular (form II) DNA. Whether this molecule is formed as a result of the left-handed helical segments of form V DNA undergoing a transition to the right-handed B-form during the topoisomerase action remains to be solved.

Keywords. Poly(dG-Me^{5}C); $Z \rightleftharpoons B \rightleftharpoons Z$; form V; topoisomerase.

Introduction

The role of secondary structures of DNA, in maintaining the fidelity and versatality of functions of DNA *in vivo* is becoming increasingly important. The initial theoretical work of Sasisekharan and co-workers (Sasisekharan *et al.*, 1978; Sasisekharan and Pattabiraman, 1978) and subsequent discovery of left-handed Z-DNA in $d(CG)_3$ and $d(CG)_2$ crystals (Wang *et al.*, 1979; Wing *et al.*, 1980) has initiated a series of studies on the factors responsible for structural transitions in DNA. *In vivo* DNA exhibits different biological functions for which the presence of various cations in millimolar quantities is obligatory. The B-Z transition in several of the alternating purine-

* To whom correspondence is to be addressed.
Abbreviations used: Z_t, Z- Conformationwhen Na^+ concentration is less than 15mM; Z_k , Z-conformation when Na^+ concentration is more than 600 mM; CD, circular dichroism; VUV, vacuum ultraviolet; Ab, antibody; L_k, linking number; W_r, Writhing number; T_r, twist right handed; T_l, twist left handed; T_t, transition mid-point °C.

pyrimidine sequences have been shown to be promoted by cations, ligands, solvent changes, topological stress and chemical modification of the polymer (Pohl and Jovin, 1972; Ramesh and Brahmachari, 1983; Klysik *et al.*, 1981, Sasisekharan and Brahmachari, 1981, Behe and Felsenfeld, 1981). One of the most common modifitions believed to be associated with gene inactivation is the methylation at the C5 position of the cytosine residue in d(CG) sequences. Such modification of cytosine is widely present in eukaryotic genome and also in prokaryotic viral DNA. Methylation of cytosine in Poly(dG-dC) has been shown to decrease the requirement of cation concentration necessary to bring about B → Z transition (Behe and Felsenfeld, 1981). Hence Poly(dG-dC) and Poly(dG-Me^5dC) make excellent model systems for studies on the functional role of B⇌Z transitions. We have shown from a temperature dependent Z⇌B transition that a minor fluctuation in Na^+ concentration at ambient temperature can bring about a conformational change from B to Z form. We have also observed a novel temperature dependent **Z⇌B⇌Z** double transition in Poly(dG-Me^5dC) as the Na^+ion concentration is gradually increased.

The demonstration of left handed segment in specific sequences under various experimental conditions has made it imperative to search for the existence of such structures in the natural sequences. Studies on form V DNA (Stettier *et al.*, 1979) have proved beyond doubt that under topological constrain even non-alternating purine-yrimidine residues in natural DNA sequences could form stable left handed helical structures and could coexist with the right handed segments. Here we present our studies on p*β*G and pBR322 form V DNA and its interaction with proteins.

Materials and methods

Polynucleotide sample preparation

Sodium salt of Poly(dG-dC) and Poly(dG-Me^5dC) were obtained from Pharmacia-PL-Biochemicals. Sodium content of the Poly(dG-dC) was estimated (flame photometrically) to be 3 mM in 1 mg/ml aqueous stock solution. The solution of Poly(dG-dC) $A_{258\ nm} \simeq 7{\cdot}0$ was diluted with absolute alcohol to get the desired alcohol concentration. For salt titration, Poly(dG-Me^5dC) was extensively dialysed against 0·5 mM sodium cacodylate buffer (pH 7·0) before use. A polynucleotide solution of $A_{255\ nm} = 0{\cdot}65 - 0{\cdot}75$ was used for all the experiments. The required salt concentration was obtained by the addition of either solid or solution of NaCl (Analytical grade). Appropriate volume correction was made.

Preparation of form V DNA

Plasmids p*β*G and pBR322 were purified by the procedure of Manniatis (Manniatis *et al.*, 1982). *E. coli* strain containing pBG plasmid was a gift from ch. Weissman. Form V DNA was prepared following the procedure of Weissman and co-workers (Stettler *et al.*,1979). Purified supercoiled DNA was digested in the presence of 0·3 mg/ml ethidium bromide with 0·2 *μg* of DNAase I per 500 *μ*g of DNA for 20min to achieve 80% conversion of form I DNA to form II (nicked open circular) DNA. In the case of pBR322 DNA we have also used *EcoRI* in the presence of 100 *μ*g/ml ethidium bromide

for conversion of form I to form II. This procedure avoids multiple cuts on a single plasmid molecule. Single stranded circular DNA and single stranded linear DNA were separated by alkaline sucrose density gradient (5–20% for pβG and 10–30% for pBR322) at 35,000 rpm in SW41 rotor for 13 h. at 15°C. Fractions containing single stranded circles were pooled, neutralized and annealed at 60°C for 20 min. This annealed form V DNA was further purified on 5–20 % neutral sucrose density gradient in SW65 rotor at 60,000 rpm for 135 min at 15°C. Fractions containing form V as judged by electrophoresis were pooled and precipitated. Form V samples stored in Tris-DTA buffer pH 8·0 at 4°C were dispersed by incubating for 10 min at 50°C before using for any experiment. Electrophoresis were carried out in 1 % agarose in TAE buffer (pH 7·8) with 4V/cm.

Z-DNA specific antibody

Z-DNA antibody used was purified IgG raised in goat and was a generous gift from Dr. B. D. Stollar. In each antibody binding assay 0·3 μg of DNA was incubated with (1–12 μg) of antibody in 20 μl of buffer containing 0·2 M NaCl, 60 mM sodium phosphate, 30 mM EDTA pH 8·0 for 1 h at room temperature.

Topoisomerase I and II

Topoisomerases were isolated from wheat germ (manuscript in preparation) and reactions were performed following standard procedure (Dynan *et al.*, 1981).

CD measurements

CD measurements were carried out using a JASCO J-20 automatic recording spectropolarimeter with MLS(GDR) constant temperature circulating bath. Temperature of the cell holder was monitored using a bent thermometer. Constant temperature of the sample was obtained byprolonged equilibration, usually for 30 min. The mean residue ellipticity [θ] was calculated as described elsewhere (Latha *et al.*, 1983) by assuming ε_{258} = 7100 M^{-1} cm^{-1} for Poly(dG-dC), ε_{255} = 7000 M^{-1} cm^{-1} for Poly(dG-Me^5dC) and ε_{260} = 6600 M^{-1} cm^{-1} for form V DNA.

UV measurements

All UVmeasurementswere carried out ona Beckman DU8B spectrophotometer with a built-in temperature controller.

Results and discussion

Effect of cation on temperature dependent Z ⇌ B transitions in Poly(dG-dC)

It was believed until recently that B→ Z transition in Poly(dG-dC) induced by Na^+ ion is isoenthalpic (ΔH = 0). We have reported for the first time the existence of a temperature dependent reversible Z→ B transition of Poly(dG-dC)in a medium of low dielectric constant (aqueous alcohol) (Latha *et al.*, 1983).

CD spectrum of B-form of Poly(dG-dC) in water at 27°C is shown in figure 1. In the

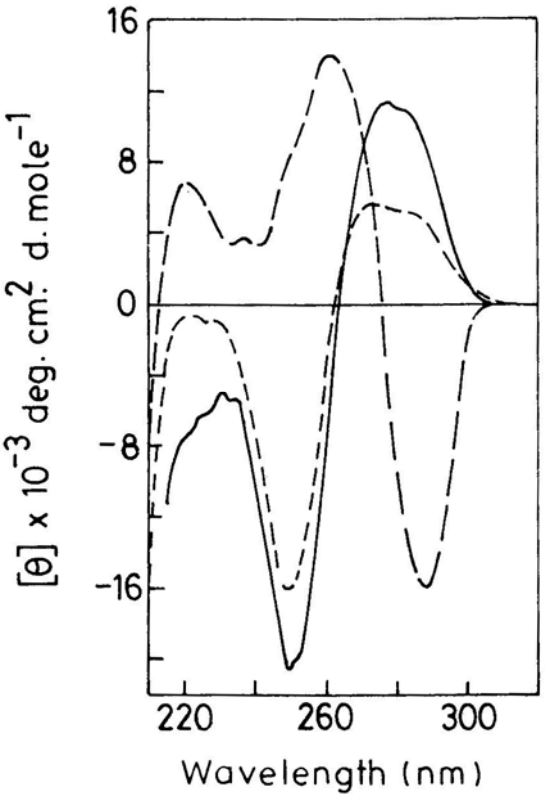

Figure 1. CD spectra of Poly(dG-dC): (– – –) in water at 27°C; (— —) in 60 % alcohol at 27°C and (——) at 71°C.

presence of 60 % (v/v) alcohol at 27°C, Poly(dG-dC) exhibits a Z-DNA like spectrum with a strong negative band at ~ 290 nm (figure 1). On raising the temperature to 71°C a characteristic B-DNA like CD spectrum was obtained. Figure 2 shows the variation of $[\theta]_{290\ nm}$ with temperature for Poly(dG-dC) in 60% (v/v) alcohol. The effect of temperature on the Z-DNA obtained by 60 % (v/v) alcohol in the presence of an additional 1·5 mM NaCl is also shown in figure 2. It can be seen that the transition to B-form was complete by 47°C. The temperature induced Z→ B transition both in the absence and in the presence of NaCl is highly co-operative and the temperature range over which the transition takes place is narrow (of the order of 10°C). The temperatures at the mid point of Z→ B transition (T_t) are 64·5±0·5°C and 39·5±0·5°C respectively (figure 2). Thus we have shown that millimolar quantities of NaCl present in the medium can bring down the transition temperature significantly. Recently, it has been suggested by Larsen and Weintraub that alteration in secondary structure of DNA caused by an embryonic Na^+ gradient may be responsible for the differential S1 nuclease sensitivity observed for the same DNA sequences (Larsen and Weintraub, 1982). Therefore, the shift in transition temperature with increasing salt concentration

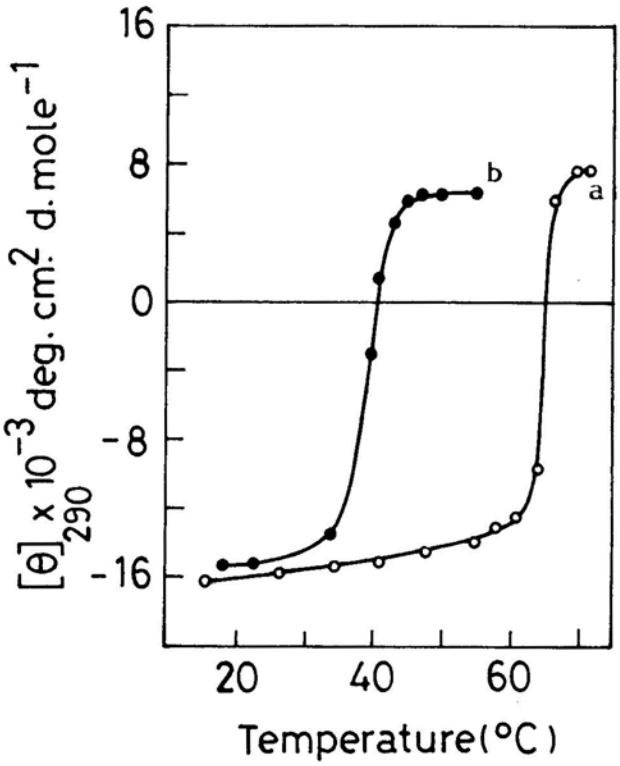

Figure 2. Temperature dependence of Z-form of Poly(dG-dC) in 60 % alcohol:
Curve (a): Without NaCl;
Curve (b): With an additional 1·5 mM NaCl.

at the millimolar level could open up the possibility of a Z-B equilibrium that is controlled by a salt gradient at ambient temperature.

Z⇌B⇌Z transition in Poly(dG-Me^{5}dC)

The effects of a variety of cations on Poly(dG-Me^{5}dC) have been examined (Behe and Felsenfeld, 1981). All the studies were carried out in the presence of 50 mM NaCl. We have probed into the conformational states of Poly(dG-Me^{5}dC) in the presence of very low salt concentrations *i.e.* below 50 mM, the results of which are presented in figures 3 to 5.

Effect of NaCl

Figure 3 shows the CD spectra of Poly(dG-Me^{5}dC) dialysed against 0·5 mM sodium cacodylate (pH 7·0) in the absence of NaCl, with 30 mM NaCl and 1·4 M NaCl. The respective UV spectra are given in figure 4. Judging from the ellipticity at 290 nm and 255 nm, Poly(dG-Me^{5}dC) exists in the Z-form both in the presence of 1·4 M NaCl and in its absence, whereas at an intermediate salt concentration (30 mM) it has the B-

conformation (figure 3). This observation is further supported by the presence of the characteristic shoulder in the UV spectra for the above Z-structures (Pohl and Jovin 1972) and its absence for the B-form (figure 4).

The UV difference spectrum between the B- and Z-forms of Poly(dG-Me^{5}dC) (220-320 nm region) in 30 mM NaCl and in the absence of NaCl respectively is shown in figure 4. This difference spectrum is exactly similar to that obtained with the B- and Z-forms of the polynucleotide at 30 mM and 1·4 M NaCl. The difference spectra exhibit a maximum around 295 nm as expected for a characteristic difference spectrum between B- and Z-forms. The ratio of A_{260} to A_{295} for the low salt and high salt Z-forms are identical (~ 2·5) whereas for the B-form it is higher (~ 4·5).

Figure 5 gives the variation of molar ellipticity at 290 nm with the salt concentration for Poly(dG-Me^{5}dC). For the sake of comparison, a similar plot of the degree of transition *versus* NaCl concentration for Poly(dG-dC) is given in the same figure. It can be seen from figure 5 that Poly(dG-dC) exists in the B-form upto 2 M NaCl. On the other hand, for NaCl concentrations up to 15 mM, Poly(dG-Me^{5}dC) exists completely in the Z-form and then undergoes a co-operative transition to the B-form with a midpoint of transition around 20 mM Further it continues to remain in the B-form for

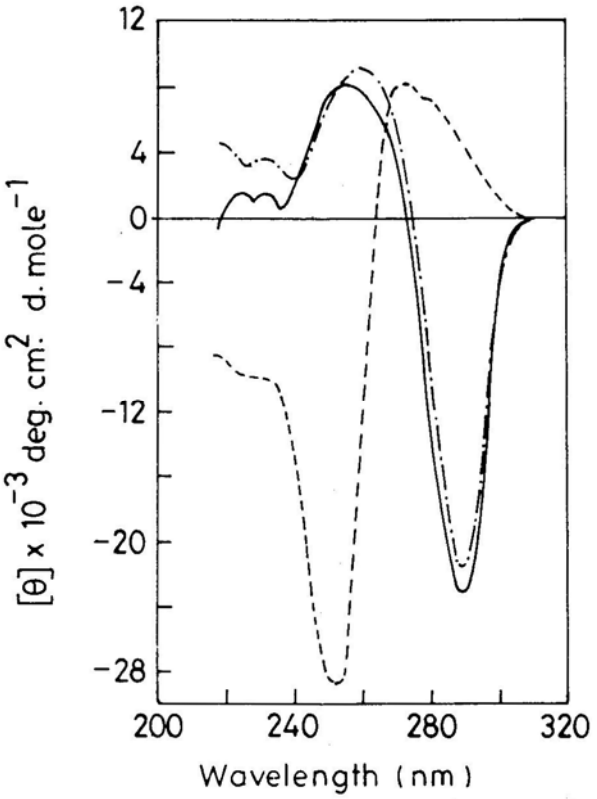

Figure 3. CD of Poly(dG-Me^{5}dC): (——) no NaCl; (– – – –) 30 mM NaCl; (— · — ·) 1·4 M NaCl.

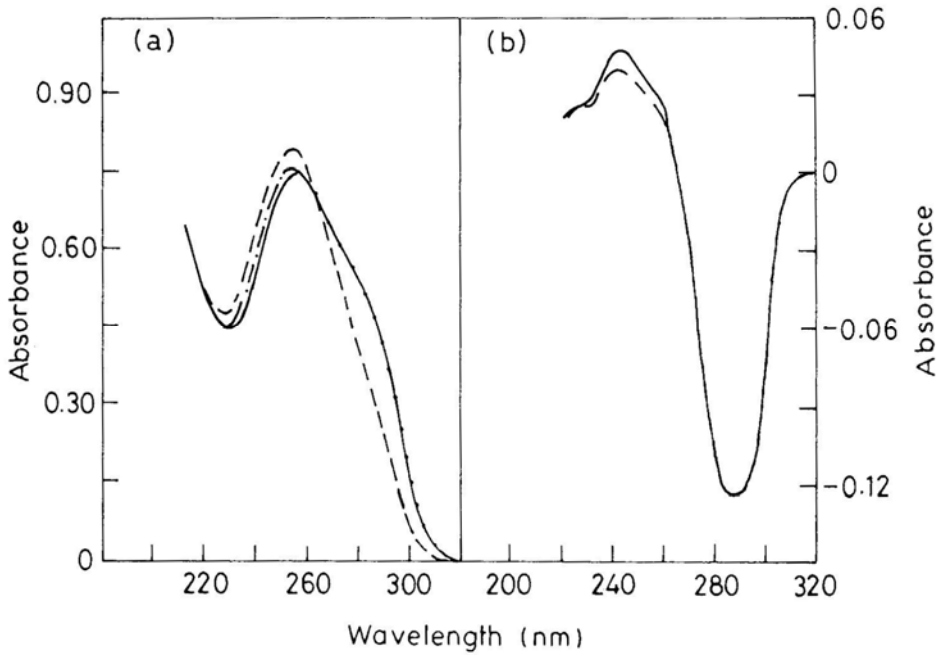

Figure 4. (a): UV spectra of Poly(dG-Me^{5}dC): (——) no NaCl; (– – – –) 30 mM NaCl; (—· —· —) 1·4 M NaCl.
(b): difference UV spectra between B- and Z-forms of Poly(dG-Me^{5}dC).
(——) 30 mM NaCl; no NaCl.
(– – –) 30 mM NaCl; 1·4 M NaCl.

over a large range of NaCl concentrations. The second transition, to the Z-form, has a midpoint around 780 mM. A plot of $[\theta]_{255\ nm}$ against NaCl concentration also gives similar results (data not shown).

Methylation of cytosine residues at carbon-5 stabilizes the Z-conformation (Behe and Felsenfeld, 1981) and a transition to the Z-form relieves the torsional strain in negatively supercoiled DNA under physiological conditions (Klysik *et al.*, 1983). Contrary to the earlier observations that the Z-form in Poly(dG-Me^{5}dC) exists only in NaCl concentrations as high as 1 M, we have observed that even very low NaCl concentrations can stabilize the Z-conformation. It is generally believed that the B-form of DNA is stablized by the ordered water structure around the DNA molecule as evidenced from the crystallographic studies on d(CGCGAATTCGCG) (Drew and Dickerson, 1981). These explanations hold good for the high salt transition observed earlier. However, it is difficult to explain the stabilization of the Z-form at such low NaCl concentration as observed by us, in terms of the decreased water activity. We suggest that it may be the specific ion binding that may be responsible for the stabilization of the low salt Z-form. Interestingly, we have observed that Cs^+ is more effective in bringing about the $Z_I \rightarrow$B transition than Na^+. Mg^{2+} is not found to have any efect in the low salt Z-DNA and the low salt Li-DNA exists only in the B-form (manuscript in preparation). This is in complete agreement with our earlier observation

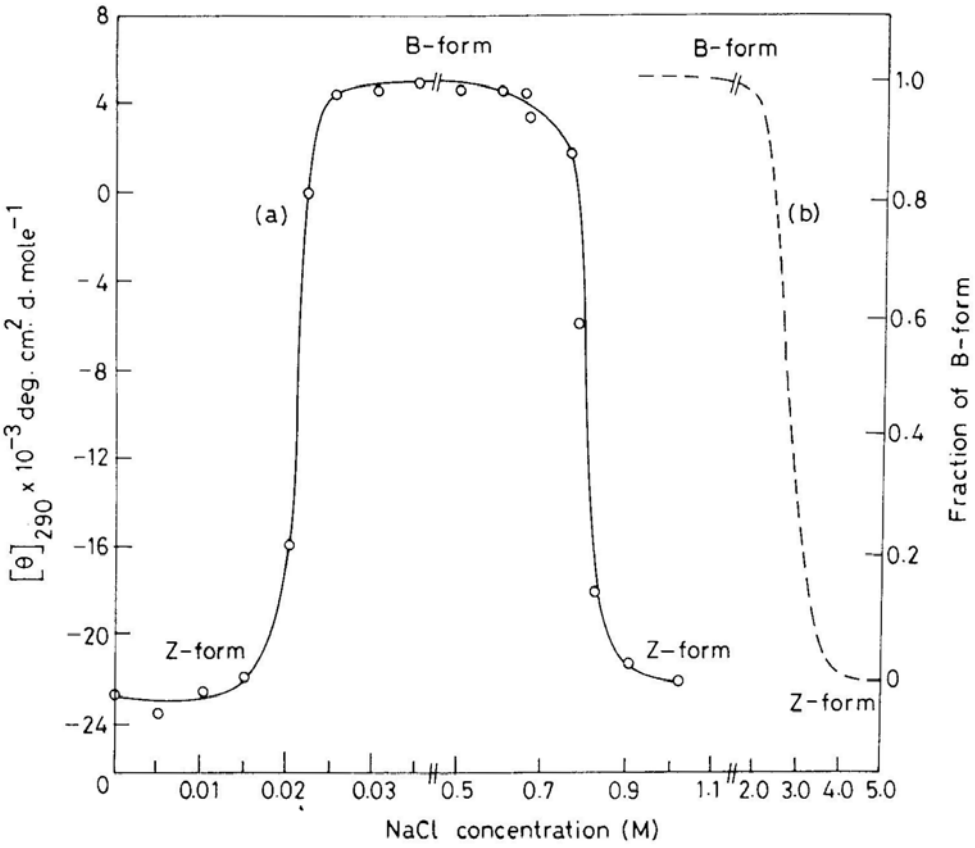

Figure 5. Curve (a): Variation of $[\theta]_{290\ nm}$ of Poly(dG-Me^{5}dC) with NaCl concentration. Curve (b): B→ Z transition in Poly(dG-dC) as a function of NaCl concentration.

that Li^+ locks the structure of Poly(dG-dC) in the B-form. Addition of alcohol does not bring about B → Z transition (Ramesh and Brahmachari, 1983).

The results presented here seems to suggest that methylation in conjunction with fluctuations in Na^+ concentration in millimolar level may bring about a reversible $\mathbf{B \rightleftharpoons Z}$ transition in small stretches of alternating CG sequences in natural DNA. These stretches could probably then serve as sites for regulation.

Effect of temperature

The temperature dependent $\mathbf{Z \rightleftharpoons B}$ transitions in Poly(dG-dC) has prompted us to look into the effect of temperature on the B→Z transitions in Poly(dG-Me^{5}dC) also.

We have looked into the temperature dependent B→Z transitions in the regions around the two midpoints of transitions *i.e.* $Z_l \rightarrow$ B and $Z_h \rightarrow$ B. Figure 6 shows the plots of percentage Z-DNA as a function of temperature when the NaCl concentrations in the medium are 20 mM, 30 mM, 625 mM and 700 mM respectively. It is evident from figure 6 that higher temperatures always stabilize the Z-form in Poly(dG-Me^{5}dC). Transition temperature (T_t) increases while going from 20 mM-30 mM whereas it

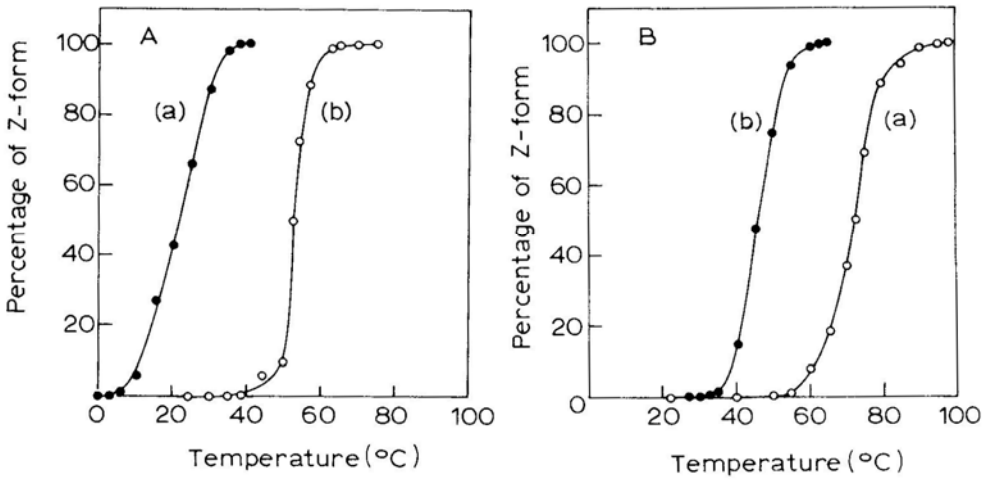

Figure 6. Temperature dependent B→ Z transitions in Poly(dG-Me^{5}dC) in the presence of various NaCl concentrations.
(A): 20 mM (a); 30 mM (b).
(B): 625 mM (a); 700 mM (b).

decreases on increasing the salt concentration from 625 mM to 700 mM. A phase diagram was obtained when the midpoints are plotted against NaCl concentrations (figure 7). We could not observe any thermally driven transition in the temperature range that is feasible for aqueous solutions when the salt concentrations were between 50 mM and 600 mM or below 10 mM or above 800 mM.

It is interesting to note that higher temperatures favour the Z-form in Poly(dG-Me^{5}dC) at all the salt concentrations studied here, whereas in the case of Poly(dG-dC), B-form is favoured. The crystal structures of $d(CG)_3$ and $d(Me^5CG)_3$ are nearly identical except for the small distortions in the torsion angles in the latter due to the presence of the methyl groups on the cytosine residues (Fujii *et al.*, 1982). It is fascinating to note how the presence of a methyl group at the 5th position of cytosine residue makes the Z-conformation in alternating CG sequences entropicallyfavourable at higher temperature.

Left handed DNA in natural sequences

Supercoiled form V DNA reconstituted from pBR322 and pβG plasmids have been used as model systems in order to understand the nature and role of left handed helical conformation in natural sequences. A large portion of DNA in form V, obtained by reannealing the two complementary single stranded circles is forced to adopt left handed double helical structures due to topological constraints (L_k = 0). In this molecule every right handed helical turn is either compensated by a left helical turn or left handed supercoil. Following the procedure of Weissman and co-workers (Stettler *et al.*, 1979) we have prepared form V DNA from pBR322 and *p*βG plasmids.

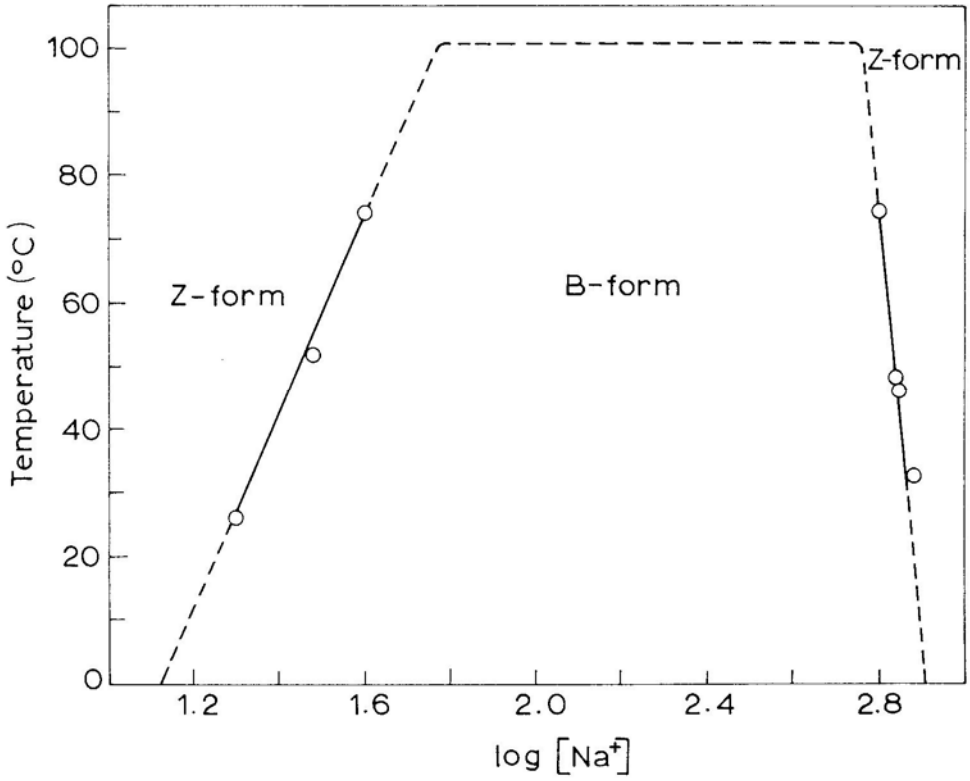

Figure 7. Dependence of T_t on NaCl concentration.

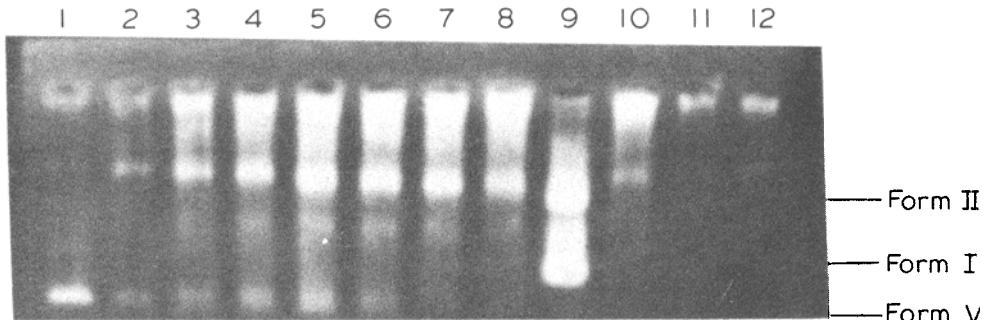

Figure 8. Fractions from alkaline sucrose density gradient.
Track 9 Control pBR322 DNA.

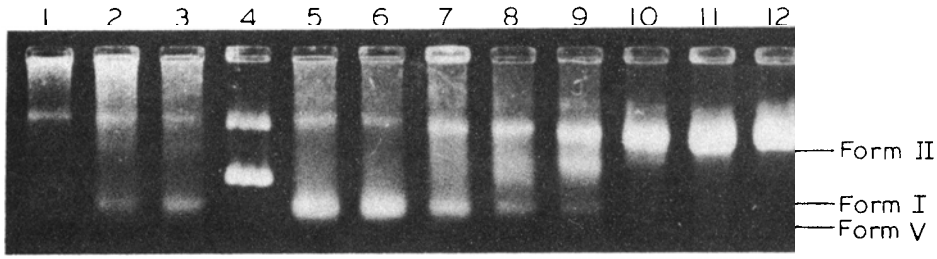

Figure 9. Fractions from neutral sucrose density gradient.
Track 4 Control pBR322 DNA.

Characterization of form V DNA

The purified form V DNA was identified by agarose gel electrophoresis. Figure 8 shows the gel pattern for pBR322 form V DNA obtained from 10–30 % alkaline sucrose density gradient. Form V DNA band moves faster than control, form I DNA. The neutral sucrose density gradient profile for pBR322 form V DNA is shown in figure 9. Similar profiles were obtained with pβG (data not shown).

Superhelical nature of the form V DNA was confirmed by 2D gel electrophoresis (figure 10) following the method used for identification of supercoiled form of pBR322 DNA (Hintermann *et al.*, 1981).

Both the form V samples exhibited a broad UV-melting and characteristic CD spectra with a negative band around 290 nm. Detailed CD and UV-melting studies will be reported elsewhere.

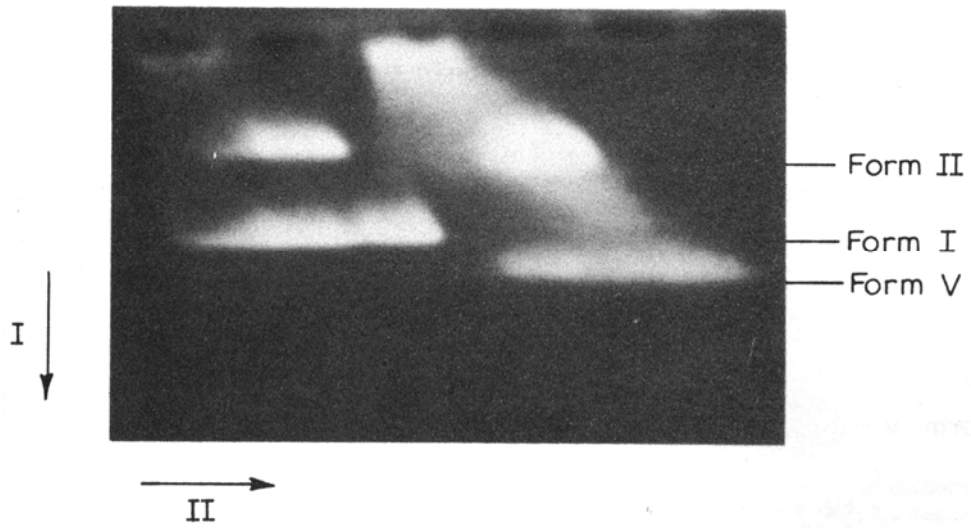

Figure 10. 2D electrophoresis of pBR322 form V DNA.

Effect of cobalt hexamine on antibody binding

Presence of Z-DNA conformation in pBR*ß*G 2·17 form V DNA has been confirmed by Z-DNA specific antibody binding studies (Pohl *et al.*, 1982). We have observed that Z-DNA specific antibody binds to form V DNA from pBR322 and *pß*G. Figure 11 shows the decrease in electrophoretic mobility for form V DNA on binding to the antibody. A ratio of 0·3 μg DNA: 10 μg A b reduced the electrophoretic mobility of form V to that of the supercoiled DNA. No change in the mobility was observed for the supercoiled DNA control even after incubation with the highest concentration of antibody used.

It is known that polyvalent cations like $Co(NH_3)_6^{3+}$ stabilizes left handed Z-conformation in various synthetic polynucleotides. In order to see whether this cation can stabilize Z-DNA in non-alternating purine-pyrimidine sequences in natural DNA, we have studied the binding of Z-DNA antibody to form V in the presence of cobalt hexamine. An increase in antibody binding to form V was observed when incubated in the presence of 0·02 mM $Co(NH_3)_6Cl_3$ (figure 12). The mobility of form V DNA becomes equal to that of the supercoiled DNA at an antibody concentration of 6 μg as against the 10 μg in the absence of cobalt hexamine ion for identical concentration of DNA.

It is quite evident from the analysis of sequence of pBR322 that there is less than 2% alternating CG containing sequences whereas it has been estimated from VUV CD studies that atleast 40 % of the sequences are in the left handed conformation in pBR322 form V DNA (Brahms *et al.*, 1982). Earlier we have shown that the amine function ofcobalt hexamine can stabilize Z-DNA structure through hydrogen bonding (Ramesh and Brahmachari, 1983). This hydrogen bond interaction may be through the pendant oxygens of the two polynucleotide chains or through N7, O6 of G and phosphate of the next residue in the 5′ direction. Interestingly in the latter case, the orientation of N7 and O6 of G and phosphate of the next residue in the 5′-direction is such that it can favourably interact with the 3 amine functions of the cobalt complex, in two perpendicular planes. Thus the *syn*-conformation of G in the Z-helix facilitates the binding and thereby stabilizes it at a very low concentration of hexamine-cobalt

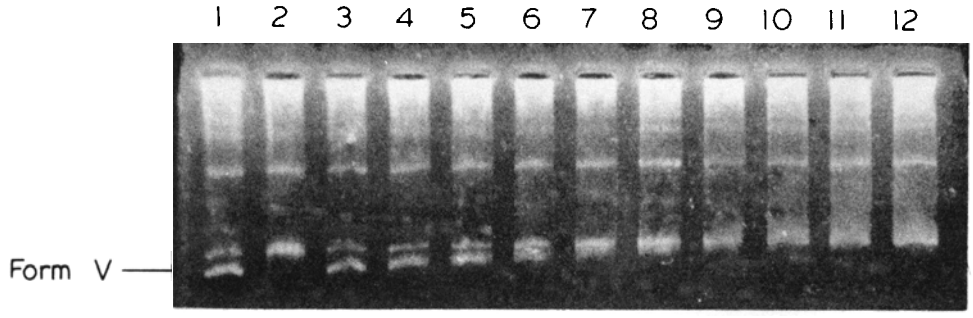

Figure 11. Z-DNA antibody binding to pBR322 form V DNA. Track 1 form V DNA, Track 2 pBR322 DNA, Tracks 3-12, 0·3 μg of form V DNA with 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 μg of Z-DNA antibody.

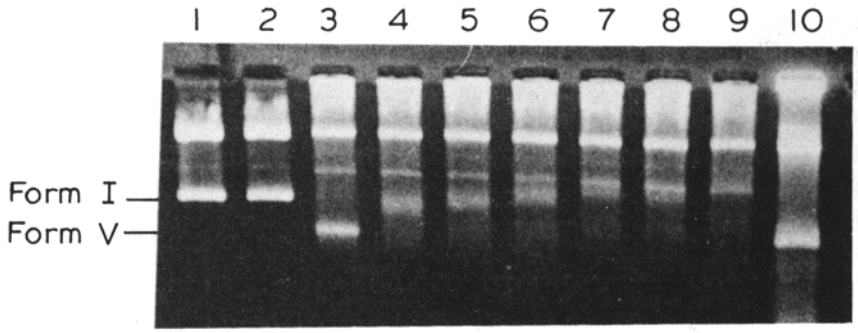

Figure 12. Z-DNA antibody binding to pBR322 form V DNA in presence of 0·02 mM of cobalt hexamine. Track 1 pBR322 DNA, Track 2, 0·3 μg of pBR322 DNA with 10 μg of Z-DNA antibody, Tracks 3-9, 0·3 μg of form V DNA with 0, 2, 4, 6, 8, 10, 12 μg of Z-DNA antibody, Track 10, 0·3 μg of form V DNA with 12 μg of Z-DNA antibody and treated with 1 % SDS before loading on the gel. The reaction mixture in all the above cases, the electrophoresis buffer, gel contain 0·02 mM cobalt hexamine.

complex. Higher binding of Z-DNA antibody to form V in the presence of 0·02 mM cobalt hexamine argues in favour of cobalt hexamine being able to stabilize lefthanded Z-orm even in non-alternating purine-pyrimidine sequences.

Effect of ethidium bromide on antibody binding to form V DNA

Ethidium bromide is known to unwind the right handed duplex and thus cause decrease in supercoiling of form I DNA (Bauer, 1978). It has been reported earlier that up to 1 mg/ml concentration ofethidium bromide, the electrophoretic mobility of pβG form V DNA remains unchanged (Stettier *et al.*, 1979). We have carried out Z-DNA antibody binding experiments in the presence of increasing concentration of ethidium bromide. It has been observed that with increase in ethidium bromide concentration there is decrease in antibody binding to form V at a fixed ratio of Ab to DNA (figure 13). This

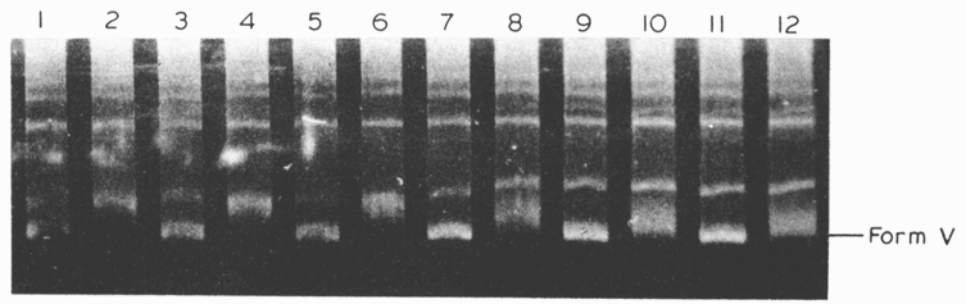

Figure 13. Z-DNA antibody binding to pBR322 form V DNA in presence of ethidium bromide. Tracks 1, 3, 5, 7, 9,11 —0·3 μg of form V DNA with 0·0, 0·1, 0·2, 0·6,1·2,2·0 μg of ethidium bromide. Tracks 2,4,6,8,10,12-0·3 μg of form V DNA, 10 μg of Z-DNA antibody with 0·0, 0·1, 0·2, 0·6, 1·2, 2·0 μg of ethidium bromide.

clearly indicates that in the presence of higher concentration of ethidium bromide there is a decrease in the extent of Z-conformation in form V. But this is not associated with increase in supercoiling. If ethidium bromide was binding to a left handed Z-segment and converting it to right handed B-form like in Poly(dG-dC), then there should have been an increase in the negative supercoiling of the form V molecule. Only way this can be explained is that the ethidium bromide is binding preferentially to right handed segment and unwinding the duplex thereby reducing the topological strain which in turn facilitates conversion of left handed Z-segment to right handed form. This is reflected in the decreased antibody binding to form V in the presence of ethidium bromide.

Action of topoisomerases on form V DNA

Eukaryotic topoisomerases I and II are known to relax supercoiled DNA in the absence and presence of ATP. In order to test the possibility as to whether the enzymes can recognize reconstituted superhelical DNA with large segments of left handed helical structure, we have studied the action of these enzymes on form V DNA. Interestingly, both topoisomerases I and II (from wheat germ) act effectively on form V DNA. Figure 14 shows that after the action of topoisomerase I, the form V molecules gets completely relaxed and exhibits mobility on agarose gel identical to that of the form II obtained by relaxing the superhelical pBR322 (DNA). Form V DNA which to start with had $L_k = 0$ and $-W_r = (T_R - T_L)$ was found to get linked ($L_k > 0$) on treatment with topoisomrase I. This has been confirmed by alkaline gel electrophoresis. Topoisomerase II was found to have similar effect on both pBR322 and pβG form V DNA. To explain the

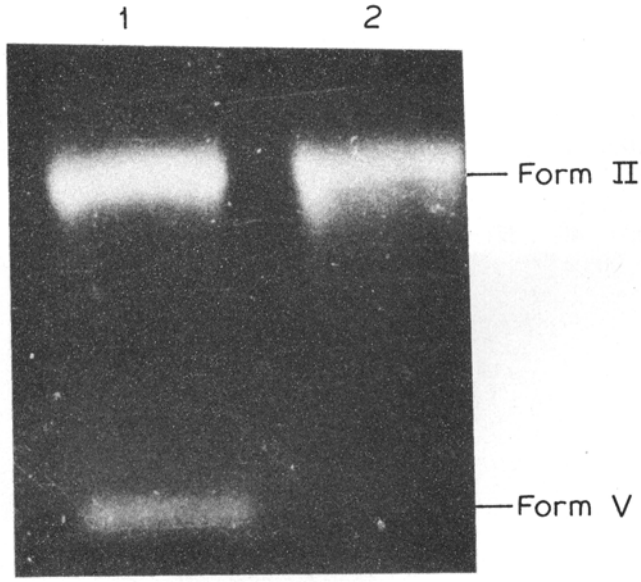

Figure 14. Action of topoisomerase I on pBR322 form V DNA. Track 1 Control pBR322 form V DNA, Track 2 pBR322 form V DNA treated with topoisomerase I.

identical electrophoretic mobility of relaxed form V DNA with that of form II DNA two possibilities are seen. In one all the left handed helical segments undergo transition to right handed form during the action of topoisomerase and thereby generating molecule identical to that ofcovalently closed form II DNA. Complete conversion with change in handedness is rather unlikely. The other alternative being topoisomerase releases the topological strain in form V by nicking and closing without complete change in secondary structure (*i.e.* $W_r = 0$ and $L_k = T_R - T_L$). In that case, in spite of having different L_k but identical topology both the relaxed forms show similar electrophoretic mobility. It will be interesting to determine the conformation of topoisomerase acted form V DNA. Work in this direction is in progress.

Conclusion

The presence of potential Z-DNA forming sequences in human genome (Kilpatrick *et al.*, 1984), viruses (Nordheim and Rich, 1983), transcriptionally active but replicatively inactive region of the Stylonychia mytilus Macronucleus (Lipps *et al.*, 1983), the finding ofZ-DNA binding protein in *Drosophila* (Nordheim *et al.,* 1982), and the detection of left handed Z-DNA segment in supercoiled plasmid under physiological conditions (Nordheim *et al.*, 1982) have opened several possibilities for structural transitions in DNA to have a functional role *in vivo.* The concept of monotonous double helical structure is inadequate to explain the complex nature of regulatory controls that are involved through specific nucleic acid-protein interaction. Variation in sequence specific double helical structure is likely to become a rule rather than exception and left handed DNA like Z-DNA may be merely the beginning.

Acknowledgements

The authors wish to thank Prof. V. Sasisekharan for stimulating discussions and Ms. Sunitha for technical assistance. Financial assistance from Department of Science and Technology, and Indian National Science Academy, New Delhi are acknowledged.

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