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# Interruptions of $(CG)_n$ sequences by GG, TG and CA need not prevent B to Z transition in solution

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## ABSTRACT

Oligonucleotides containing alternating purines-pyrimidines with AT base pairs have been shown to exist in the Z-form preferably in solid state. We report that oligodeoxyribonucleotides with GG, TG and CA interruptions in their alternating CG sequences can undergo B to Z transition in solution in the absence of any chemical modification or topological constraint. The sequences, d(CGCGCGGCGCGC) and d(CGTGCGCACG) have been synthesised and shown to adopt Z- conformation in presence of millimolar concentrations of $Ni^{2+}$ under low water activity conditions. Significance of GG, TG and CA interruptions in the B to Z transition is discussed.

## INTRODUCTION

Subsequent to the discovery of the Z-conformation of DNA in crystals of alternating CG sequences (1,2), several other oligonucleotides with alternating purine-pyrimidine sequences have been found to undergo B ---> Z transition under a variety of experimental conditions ( see reviews in ref. 3,4). Sequencing of several genomic DNAs has shown that long stretches of (CG) sequences are rather uncommon in genomic DNA other than those in the HTF island region (5). However, tandem repeats of TG sequences have been found to be abundant in mouse globin gene (6), yeast chromosomes (7), African green monkey genome (8), human genome (9) and higher plants (10). The TG repeats in intron of $\alpha$ - lactalbumin gene (11), 5' flanking sequences of somatostatin gene (12) and mouse metallothionein promoter I (13) have been attributed to be the possible site for the formation of Z- conformation. It has been shown that both $d(CG)_n$ and $d(TG)_n \cdot d(AC)_n$ sequences of medium length adopt Z-conformation when cloned in a negatively supercoiled plasmid (14,15). The

extent of supercoiling necessary for the stabilization of Z-DNA has been measured for different sequences of varying lengths (15-17). It has been shown from our laboratory that several sequences in the pBR322 Form V DNA, a highly negatively supercoiled molecule, adopt left handed helical conformation, presumably the Z-form (18,19), whereas such sequences remain in the B-form in Form I DNA. It may be emphasized that some of these sequences contain both AT and GC base pairs as well as purine-pyrimidine sequences which are not entirely alternating. Analysis of these Z-helicogenic sequences has enabled us to rank these sequences in their potential to adopt altered conformation when under the influence of supercoiling force (18). It has also been shown that a 14 base pair sequence with one GG interruption in pBR322 which adopts the Z-conformation under natural superhelical force (20), also adopts Z-conformation in pBR322 Form V DNA (18) where the molecule has zero linking number.

Sequences like d(CGCGTG) (21), d(CGCATGCG) (22,23) and several others (4) have been shown to adopt the Z-conformation in the solid state. However, unconstrained oligonucleotides where AT base pairs are also included as part of the alternating purine-pyrimidine sequence have so far not been reported to adopt Z-conformation in solution, unless some of the bases are chemically modified (24). Recently, sequences like d(TGCGCGCA) and d(CACGCGTG) have been reported to adopt partial Z conformation in solution using laser Raman spectroscopy (25). Concentration of the oligomers used in this study was several hundred fold higher than that required for $d(CG)_n$ ($n \geq 3$) sequences to adopt Z conformation in solution as judged by CD spectroscopy under similar ionic conditions (4). These studies apparently indicate that crystal lattice forces, strong intermolecular interaction, negative supercoiling or chemical modification is obligatory for B ---> Z transition in sequences having a short assorted array of alternating purine-pyrimidine bases.

In order to understand the role of specific DNA sequences in stabilizing the left-handed Z-structures, we have undertaken conformational studies of unmodified oligonucleotides having both CG and AT base pairs. Here, we report the synthesis and

structural studies of two oligonucleotides, d(CGCGCGGCGCGC) and d(CGTGCGCACG) with a view to understand the effect of GG, TG and CA interruptions in B ---> Z transition. We show that in solution unmodified alternating CG oligonucleotides with not only TG, CA and GG interruptions but also a G/G mismatch can undergo B ---> Z transition.

## MATERIALS AND METHODS

Deoxynucleosides, tetrazole, dimethoxytrityl chloride, trimethyl silyl chloride and diisopropyl ethyl amine were purchased from Sigma. Other reagents and solvents used in the synthesis were of analytical grade. Solvents were purified before use. NaCl and $NiCl_2$ used in CD studies were from Merck (India) and cobalt hexamine chloride (HCC) was from Fluka.

### Preparation of oligonucleotides

5',N- protected deoxynucleosides were prepared according to Jones' procedure (26). Respective phosphoramidites were prepared using standard procedure (27) and checked by $^{31}P$ NMR for purity. Oligonucleotides were synthesised manually in an open column (28) using published procedure (27) with minor modifications. The decanucleotide was purified on reversed phase HPLC and the dodecanucleotide was purified on a preparative gel of 20% polyacrylamide containing 8 M urea and tris borate EDTA buffer, pH 8.3 (29).

### CD and UV measurements :

CD spectra were recorded using Jasco J-500A automatic recording spectropolarimeter. UV measurements were carried out with Beckman DU 8B spectrophotometer. Mean residual ellipticity was calculated as reported earlier (30). In CD melting experiments, % helix was calculated taking the maximum negative rotation (at 293 nm for Z- form and 248 nm for B- form ) at $10^{\circ}$ C as the representative of 100 % helix. The cooperative transition shown in figure 6 was assumed to reflect helix to coil equilibrium. This two state model was used to calculate the thermodynamic parameters, viz., enthalpy, entropy and free energy according to published procedure (31).

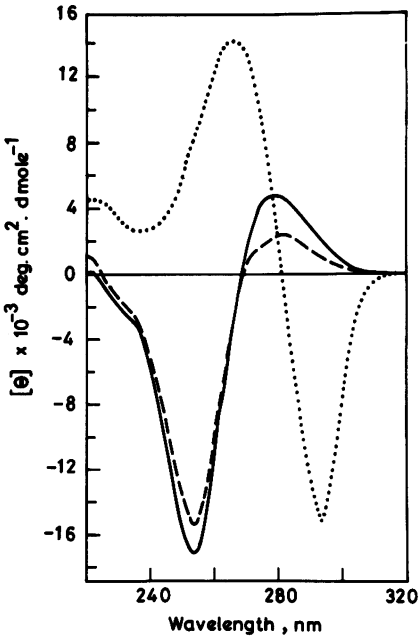



Figure 1. CD spectra of d(CGTGCGCACG) in 2 mM sodium cacodylate, 0.1 mM EDTA, pH 7.4 : 100 mM NaCl (———) ; 5 M NaCl (----) ; 5 M NaCl, 10 mM $NiCl_2$ (.....).

## RESULTS

Figure 1 shows the CD spectra of d(CGTGCGCACG). In low salt concentration (0.1 M NaCl) the oligomer gives a positive band at 280 nm and a negative band at 256 nm which are characteristic of B- DNA (32).In presence of 5 M NaCl a decrease in the positive band is observed. On addition of 10 mM $NiCl_2$ to this 5 M NaCl solution, the CD spectrum gets inverted, showing a negative band at 293 nm and a positive band at 265 nm, characteristic of the Z- conformation (4,32,33).

Figure 2 is the CD spectra of d(CGCGCGGCGCGC) which shows that the dodecamer exists in B-conformation in low and high salt solutions, like the decanucleotide. In presence of 11 mM $NiCl_2$, the dodecanucleotide also adopts Z-conformation as evident from the negative CD band at 294 nm and a positive band at 265 nm.

$NiCl_2$ titration profiles of the two oligomers in presence of 5 M NaCl are given in figure 3. The mid point of B---> Z transi-

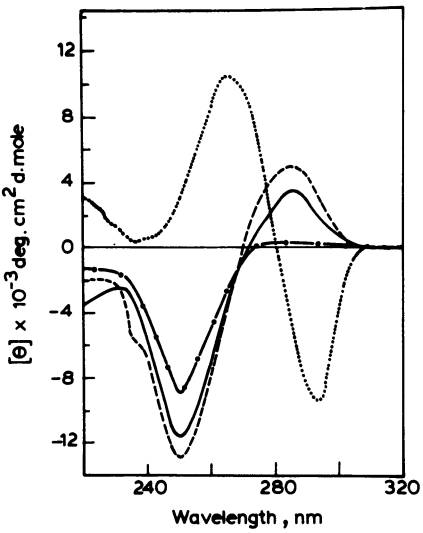



Figure 2. CD spectra of d(CGCGCGGCGCGC) in 2 mM sodium cacodylate, 0.1 mM EDTA, pH 7.4 : without NaCl (---- ); 5 M NaCl (——— ); 5 M NaCl, 5 mM $NiCl_2$ (—•—•—); 5 M NaCl, 11 mM $NiCl_2$ (.....).

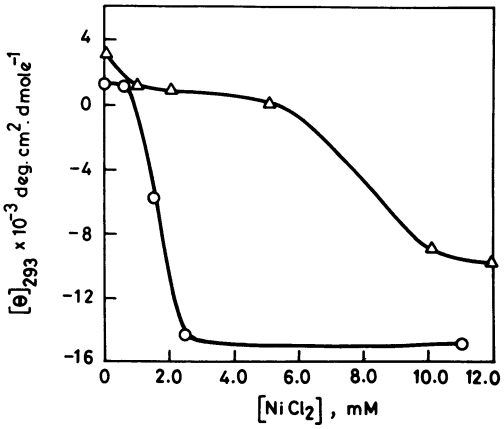



Figure 3. $NiCl_2$ titration of d(CGCGCGGCGCGC) (—△— )& d(CGTGCGCACG) (—O— ) in 2 mM sodium cacodylate 0.1 mM EDTA, pH 7.4 , 5 M NaCl.

tion for d(CGTGCGCACG) is 1.5 mM $NiCl_2$ and the transition is complete at 3 mM $NiCl_2$. The dodecanucleotide has the mid point of transition at 7.5 mM $NiCl_2$ and the transition is complete by 11 mM $NiCl_2$. Figure 4 is the reversed titration profile of

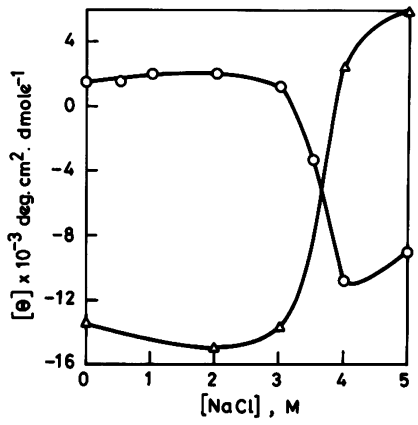



Figure 4. NaCl titration of d(CGTGCGCACG) in 2 mM sodium cacodylate, 0.1 mM EDTA, pH 7.4 and 10 mM $NiCl_2$. Molar ellipticity at 293 nm (—○—) ; molar ellipticity at 253 nm (—△—).

d(CGTGCGCACG), with NaCl in presence of 10 mM $NiCl_2$. It shows that $Ni^{2+}$ can induce B to Z transition only in presence of high NaCl concentration. Here the mid point of transition is at 3.5 M NaCl and at 4 M NaCl the transition is complete. A similar trend is seen in the case of d(CGCGCGGCGCGC).

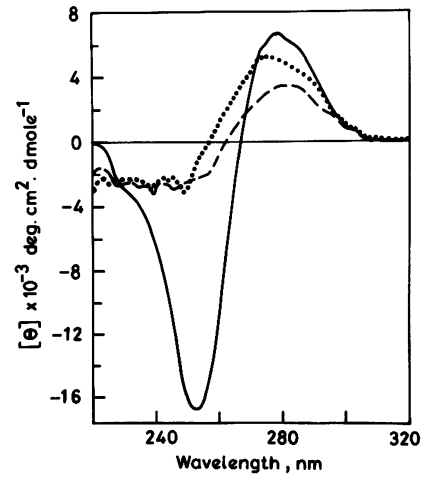



Figure 5. CD spectra of d(CGTGCGCACG) in 10 mM NaCl, 0.1 mM EDTA, pH 7.4 : 60% ethanol (———); 60% ethanol, 2.5 mM $NiCl_2$(– – – –); 5 M NaCl, 2.5 mM $NiCl_2$ at 60° C (.....).

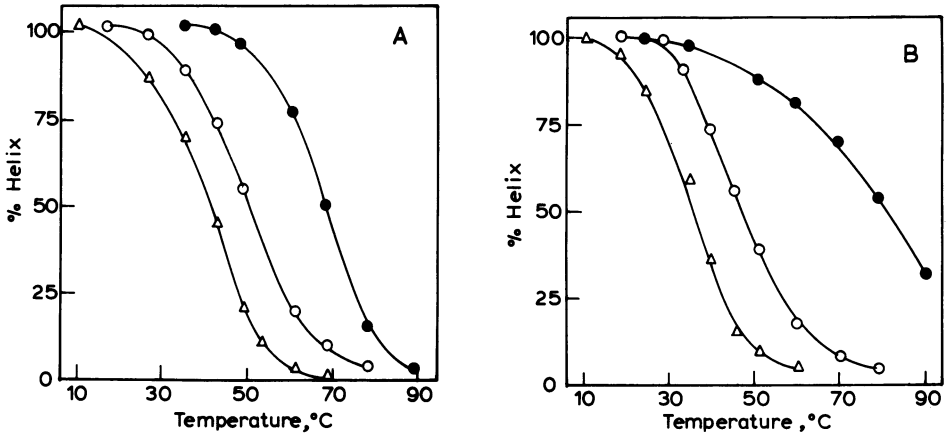



Figure 6. a) CD melting profile of d(CGTGCGCACG) in 2 mM sodium cacodylate, 0.1 mM EDTA, pH 7.4 with 100 mM NaCl (—●—), 5 M NaCl (—○—), 5 M NaCl and 3.0 mM $NiCl_2$ (—△—).
b) CD melting profile of d(CGCGCGGCGCGC) in 2 mM sodium cacodylate, 0.1 mM EDTA, pH 7.4 with 100 mM NaCl (—●—), 5 M NaCl (—○—), 5 M NaCl and 11 mM $NiCl_2$ (—△—).

In figure 5, CD spectrum of d(CGTGCGCACG) is shown in presence of ethanol, a known Z-inducing agent. The decanucleotide does not adopt Z-conformation in presence of 60 % ethanol and gets denatured when $NiCl_2$ is added to it.

CD melting profiles of the deca and dodecamer in presence of different salt concentrations are shown in figure 6. The decamer has Tm 68°C and 50°C in presence of 100 mM and 5 M NaCl respectively and its Z- form, in presence of 5 M NaCl and 3 mM $NiCl_2$, melts at 45°C. The dodecamer does not melt completely in presence of 100 mM NaCl and shows a decrease in Tm (46°C) in 5 M NaCl. Like the decamer this molecule also shows a further decrease in Tm (36°C) when in the Z-form. Using Figure 6, equilibrium constants at different temperatures were calculated. The plot, ln k vs. 1/T ( not shown ) was used to calculate $\Delta H$, $\Delta S$ and $\Delta G$ values for the helix - coil transition of these oligomers. These thermodynamic parameters are given in table 1.

## DISCUSSION

The oligonucleotide d(CGCGCG) is known to undergo B ---> Z transition in solution, however, d(GCGCGC) remains in the B-

TABLE-1. Enthalpy, entropy and free energy for helix to coil transition

| Oligomer | Salt conc. | Confor-mation | ΔH | ΔS | ΔG at 25°C |
|---|---|---|---|---|---|
| | 5 M NaCl | B | -128.9 | -343.3 | -26.6 |
| d(CGCGCG-GCGCGC) | 5 M NaCl+ 11 mM $NiCl_2$ | Z | -108.2 | -293.6 | -20.7 |
| | 5 M NaCl | B | -107.7 | -280.2 | -24.3 |
| d(CGTGC-GCACG) | 5 M NaCl+ 3 mM $NiCl_2$ | Z | -120.8 | -330.9 | -22.2 |

( Δ H and ΔG are in Kcal/mol and ΔS is in cal/mol.deg )

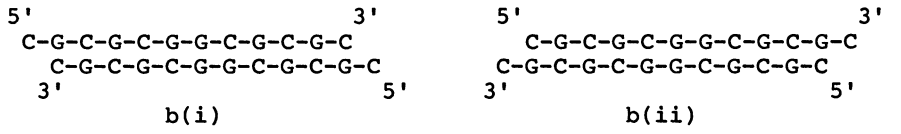
form even in 5 M NaCl (32). NaCl induced B ---> Z transition in both d(CGCGCGCGCGCG) and d(GCGCGCGCGCGC) are reported (34). The decanucleotide d(CGTGCGCACG) is perfectly complementary and alternating in purine - pyrimidine. CD studies show that this decamer does not undergo B ---> Z transition at high concentration of NaCl , HCC or ethanol. Interestingly, B to Z transition is observed in presence of 5 M NaCl and 3 mM $NiCl_2$. This indicates that $Ni^{2+}$ has a strong influence on the conformation of this molecule in presence of high NaCl concentration since $Ni^{2+}$ itself could not bring about the B to Z transition. Inhibition of B to Z transition in the absence of $Ni^{2+}$ may be attributed to the additional hydration at A/T base pairs. $Ni^{2+}$ is known to coordinate with the N - 7 of purines (35,36) and by doing so it may disrupt the water structure and stabilise the syn conformation of the purines. These events could result in the B to Z transition in the molecule.

Unlike several other oligonucleotides studied so far, d(CGCGCGGCGCGC) is non selfcomplementary but has the potential to form concatamers in low salt solutions as shown below.

```
 5'                      3' 5'                      3'
..C-G-C-G-C-G-G-C-G-C-G-C C-G-C-G-C-G-G-C-G-C-G-C..
            ..C-G-C-G-C-G-G-C-G-C-G-C C-G-C-G-C-G-G-C-G-C-G-C..
            3'                        5' 3'                    5'
```

(a)

It is observed that d(CGCGCGGCGCGC) has a mobility in nondenaturing 20% polyacrylamide gel, equivalent to that of a molecule with 12 b.p. Also, attempts to ligate this molecule with T4 DNA ligase met with very little success. The inefficiency of ligation, in conjunction with the above observation led us to rule out the existence of the molecule in the concatamer form (a). Considering the base complementarity, the following will be the most favourable forms for this molecule, since other duplex forms would result into large number of base pair mismatches.




When the 5' phosphate labelled compound is subjected to S1 nuclease digestion, most of the labelled oligomer disappears, indicating the presence of protruding ends as in b(i). Gap filling with DNA polymerase ( Klenow fragment) using $\alpha^{32}P$ labelled dCTP and dGTP separately shows that incorporation of labelled cytosine is only 4 % to that of guanosine. This indicates that a major population of the molecule must be existing in the b(i) form. These studies do not provide direct evidence for the presence of b(ii), nevertheless, its existence cannot be ruled out. Therefore it appears to be quite likely that the molecule will exist in b(i) and b(ii) forms, both having protruding cytosines at the ends and a G/G mismatch in the middle, as predominant species. This was further confirmed by digestion with restriction enzymes , Hha I and Fnu D II. Despite the presence of more than one recognition site for Hha I (GCGC) and Fnu D II (CGCG), neither the molecule itself nor its gap filled form is recognised and cleaved by these enzymes. Cleavage site at the ends of the molecule may not be recognised due to the lack of proper flanking sequences and sites in the middle are not recognised because of the presence of G/G mismatch. If the arrangement of duplex had been a concatamer without any mismatch , like (a), both the enzymes would have acted on this molecule.

The dodecanucleotide d(CGCGCGGCGCGC) did not adopt Z- conformation under the influence of Z- inducing agents, e.g., NaCl, HCC and ethanol, as in the case of d(CGTGCGCACG). Surprisingly, this molecule also undergoes B ---> Z transition in presence of $Ni^{2+}$ ion and 5 M NaCl like the decanucleotide, shown above. The presence of GG interruption and G/G mismatch in this molecule necessitates a higher concentration of $Ni^{2+}$, 4 times than that for the decamer, for inducing B to Z transition. This interruption might provide an unusual Z-Z junction in the molecule. Molecular model for Z-Z junction has been worked out and shown to be energetically reasonable with good geometry and base stacking (37). The GG interruption in this molecule breaks the continuity in the purine - pyrimidine alteration and divides it into two domains, CGCGCG and GCGCGC, first being a Z- helicogenic and second a B- helicogenic part. In another molecule with two helical domains, d(TATATACGCGCG), only the Z- helicogenic segment has been shown to undergo B ---> Z transition in high concentrations of NaCl (38). In this case, however, the continuity in the alternation of purines and pyrimidines is not lost unlike in d(CGCGCGGCGCGC). Our present study shows that even the Z- helicogenic stretch of this dodecamer does not adopt Z- conformation in the presence of 5 M NaCl or 2 mM HCC. This can be accounted for by the G/G mismatch and 5'-protruding cytosine in the Z-helicogenic domain which does not allow the B ---> Z transition.

Synergistic effect of ethanol and $MgCl_2$ in bringing about the B ---> Z transition in poly(dG-dC) is well documented (39).A combination of $Mg^{2+}$ and ethanol was unable to induce B ---> Z transition in d(CGCGCGGCGCGC) and d(CGTGCGCACG). Attempts to induce Z- conformation under the combined influence of NaCl and HCC or that of ethanol and $NiCl_2$ did not succeed. This indicates that the mode of interaction of $Ni^{2+}$ with the DNA is different from that of HCC. Also, it shows that ethanol is not a substitute for NaCl in decreasing the water activity around the DNA molecule required for $Ni^{2+}$ induced B ---> Z transition. Sharp negative band at 293 nm, comparable to half band width of poly(dG.dC), in both the oligomers is an indication of a highly ordered structure of these molecules.

Titration profile of 5 M NaCl solution of decamer and dodecamer with $NiCl_2$ (Fig 3) shows that transition in the case of the dodecamer is much less cooperative. CD spectra of these sequences show the absence of a well defined isosbestic point in the transition. The absence of isosbestic point during B ---> Z transition has been reported for NaCl induced transition in poly(dG-dC) also (40).This suggests that transition from B-form to Z-form induced by $NiCl_2$, is not a simple one step process. It may involve one or more intermediates.

Under the combined influence of 60 % ethanol and 3 mM $NiCl_2$ these oligomers undergo denaturation. CD melting profiles of these oligomers clearly indicate that the B- conformation is destabilised in high NaCl concentration due to disruption of the spine of water along the minor groove (41) resulting in the lowering of Tm of both the molecules. The Z- form in both the molecules is less stable than B- form as evident from the further downward shift in Tm in presence of $NiCl_2$ along with 5 M NaCl. It is interesting to note that Z to coil transition by heating is not through B- conformation unlike poly(dG-dC), and poly (dG-d $^5$mC) (4,19) and the process is reversible. As evident from the thermodynamic data presented in table 1, both the oligomers are more stable in B-form when compared with the Z-form, assuming that coil state of the respective oligomers in different ionic conditions is energetically similar. The perfect alternating purine pyrimidine sequence of the decamer makes its Z-conformation more stable than that of the dodecanucleotide which has a GG break in CG alternation and also a G/G mismatch.

Here we show that unconstrained short oligonucleotides having an assorted array of alternating purine-pyrimidine sequence without any base modification or strong intermolecular interactions, can undergo B ---> Z transition in solution. However, it may be noted that the transition here is not as facile as in the case of the corresponding $d(CG)_n$ (n = 5) sequence (34). It requires extremely low water activity (5 M NaCl) as well as the presence of a transition metal ion which binds to the bases ($Ni^{+2}$ ). For its efficient binding to the N - 7 of purines and subsequent stabilization of the syn conforamtion (35,36), $Ni^{2+}$ may be requiring the bases that are

relatively free of $H_2O$ molecules. Such a situation may be attained under the dehydrating conditions prevalent in high NaCl concentration. This may be the reason why $Ni^{2+}$ alone did not induce B -->Z transition as shown by the reversed titration (fig 4) and transition is brought about only with the prior presence of sufficiently low water activity condition.

It is not unlikely that the B<--->Z equilibrium, for the two oligomers studied here, exists even in 5 M NaCl and the GG interruption in d(CGCGCGGCGCGC) and TG and CA interruptions in d(CGTGCGCACG) might be preventing the propagation. Hence, the population of Z-conformation under the influence of NaCl and HCC might be too small to be detected spectroscopically. The absence of a B ---> Z transition in pd(CGCGCG) has been reported earlier from our laboratory (42). It has been proposed that the extra phosphate present at the 5' position may be inhibitory for the nucleation step of the B ---> Z transition. In the case of the dodecamer b(i) form, the 5' phosphate at the first CG base pair could be one of the factors preventing the B ---> Z transition.

Critical cation balance in controlling DNA structural transition and its role in protein-DNA interaction has been investigated by several workers (43,44,45). Also there are many classes of proteins that interact with DNA and have sequence specificity and structure sensitivity (18,46,47). In vivo existence of Z- DNA and its inhibitory role in Eco R I methylase has been convincingly demonstrated recently (48). Therefore, the possibility of a combined influence of proteins and ions on DNA structure and function cannot be eliminated. Proteins, like RNA polymerase with transition metal ions might induce structural transitions by providing metal ions to coordinate with the specific sites of bases which are known to induce conformational changes in DNA. This could be possible if the protein factor / membrane factor render a low water activity condition to a specific stretch of DNA. Low energy structural transition in a DNA molecule can be brought about by negative supercoilng or by lowering of water activity, an event which could occur in an interaction with a DNA binding protein (49). Such interactions in the regions discriminated by proteins could cause local destabilisation, favouring the structural transition promoted by

the protein involved. Such a metal ion - protein induced mechanism for structural transition could be envisaged even at the physiological ionic concentrations. Structural and functional differences have been observed between the two intrinsic zinc ion concentrations of E.coli RNA polymerase and the transition metal ion has been shown to play a role in the catalytic mechanism of the enzyme (50,51). These studies indicate that the Z-potential of a particular region of DNA is a sequence dependent property. A particular sequence may undergo structural transition under a particular condition of low water activity and transition metal ion influence. This dual control on structural transition of a DNA stretch may be of fundamental importance to DNA - structure dependent biological functions.

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