Left Handed Z-DNA Helices and B-Z Junctions

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INTRODUCTION

DNA is a flexible molecule that adopts a variety of conformations. Left-handed helices have been demonstrated in synthetic DNA polymers (reviewed in Ref. 1-2) and in segments of DNA restriction fragments (3) and recombinant plasmids (4-8). Other DNA conformations such as cruci forms and bent structures have also been demonstrated. Thus DNA micro heterogeneity has been demonstrated in a variety of systems (9-11). The role of the static and dynamic structures and properties of DNA in gene expression has been reviewed (1,12).

Left-handed DNA in Polymers and Restriction Fragments

In 1972 Pohl and Jovin indicated that under high salt conditions, $(dC\text{-}dG)_n\cdot(dC\text{-}dG)_n$ underwent a marked structural transition as revealed by circular dichroism (CD) spectroscopy (13). Subsequently, X-ray crysta llographic studies of oligomers of $(dC\text{-}dG)_n\cdot(dC\text{-}dG)_n$ revealed that the high salt form of $(dC\text{-}dG)_n\cdot(dC\text{-}dG)_n$ was a left handed helix (14). Phosphorus NMR (15), proton NMR (16), CD (17), and Raman spectroscopic (18) studies indicated that this left handed Z-type DNA conformation can also exist in solution. A family of left handed conformations exists, since in a wide variety of conditions the CD spectra are substantially different quantitatively but similar in shape (19). Methylation at C5 position of cytosines facilitated the B to Z transition of $(dC\text{-}dG)_n\cdot(dC\text{-}dG)_n$ polymer or this sequences in restriction fragments (20).

Reaction of Diepoxybutane with B form or Z form $(dC\text{-}dG)_n\cdot(dC\text{-}dG)_n$

Diepoxybutane (DEB) reacts with DNA to form covalent adducts at N7 position of guanines (21, 22). We studied the reaction of DEB with B form or Z form $(dC\text{-}dG)_n\cdot(dC\text{-}dG)_n$. Fig. 1 A shows

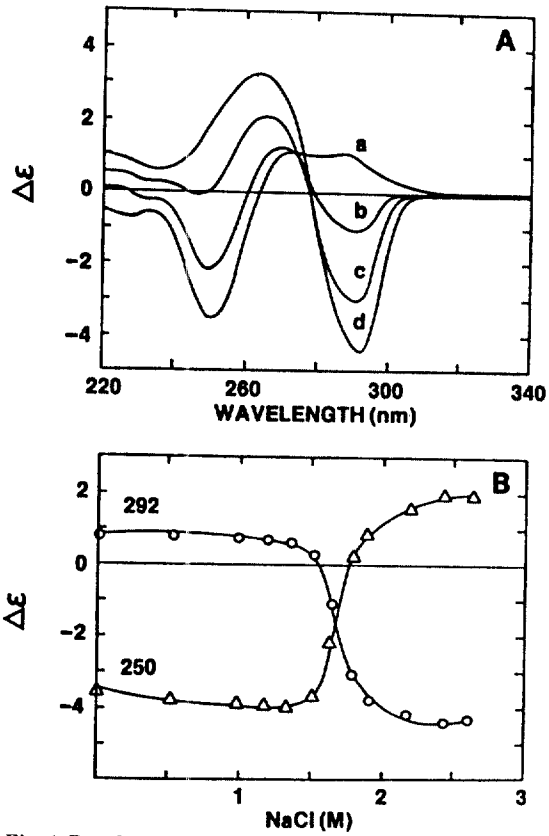

Fig. 1. Panel A. CD spectra of DEB reacted $(dC\text{-}dG)_n\cdot(dC\text{-}dG)_n$ at different NaCl concentrations. B form $(dC\text{-}dG)_n\cdot(dC\text{-}dG)_n$ was reacted with DEB for 1.5 h and unreacted DEB was removed by dialysis as described (24). The treated DNA was then titrated with NaCl.concentrations (M) were: a, 0; b, 1.64; c, 1.73; d, 2.42.

Panel B. Effect of NaCl on the molar ellipticity of DEB reacted B form $(dC\text{-}dG)_n\cdot(dC\text{-}dG)_n$. Circles, $\triangle E_{292}$, triangles, $\triangle E_{250}$. From ref. 24.

a titration curve of $(dC\text{-}dG)_n \cdot (dC\text{-}dG)_n$ after DEB reaction. As the NaCl concentration was increased, the Z characteristics of the CD spectra increased, and finally reached the point where increasing NaCl concentration did not cause any further change. The transition midpoint was 1.68M NaCl which was considerably lower than for the unmodified polymer (2.6M NaCl) (13), and hense serves as direct evidence for the DEB modification of the B form polymer. These results are in good agreement with the methylation studies of Moeller *et al.* (23) which revealed that alkylation of the N7 position of guanines in $(dC\text{-}dG)_n \cdot (dC\text{-}dG)_n$ caused a reduction in the salt concentration required to induce the B to Z transition (24). Castleman *et al.* (22) reported that $(dC\text{-}dG)_n \cdot (dC\text{-}dG)_n$ can be stabilized in the Z conformation by a crosslinking reaction with DEB. Our result showed that DEB does react also with B form $(dC\text{-}dG)_n \cdot (dC\text{-}dG)_n$. However, this B form product can undergo the salt induced B to Z transition where as the Z form product cannot undergo the Z to B transition (22,24). Thus we hypothesized that DEB crosslinks Z from $(dC\text{-}dG)_n \cdot (dC\text{-}dG)_n$, however, it cannot crosslink B form $(dC\text{-}dG)_n \cdot (dC\text{-}dG)_n$ but only form a mono adduct. Fig. 2 shows the experimental scheme to test this notion. B form $(dC\text{-}dG)_n \cdot (dC\text{-}dG)_n$ in 3 mM Na/cacodylate, 0.1 mM EDTA, pH 7 buffer was incubated with 6.7% DEB for 6h then unreacted DEB was removed by dialysis against the same buffer. The treated polymer under went a B to Z transition instantaneously upon addition of $MnCl_2$ to 1 mM as monitored by CD. The modified DNA in 1 mM $MnCl_2$ was then incubated at 37°C. If the incubation time was zero, there was no detectable change of the CD spectra compared to the DEB reacted B form $(dC\text{-}dG)_n \cdot (dC\text{-}dG)_n$. After more than 2h of incubation, stable Z form CD spectra were obtained even if $MnCl_2$ was removed by extensive dialysis. Thus, the DNA was locked in a left handed Z structure by an apparent intramolecular cross-linking reaction (data not shown).

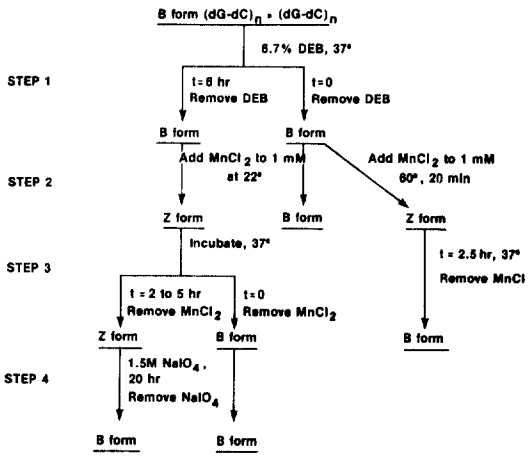

Fig. 2. Experimental scheme. *Left side* (experimental). Step 1: the B form $(dG\text{-}dC)_n \cdot (dG\text{-}dC)_n$ was incubated at 37°C with 6.7% DEB for 6h and unreacted DEB was removed by dialysis. Step 2; $MnCl_2$ was added to 1 mM. Step 3; this solution was incubated at 37°C. After 0, 2, 3, 5 hours of incubation, aliquots were withdrawn and $MnCl_2$ was removed by dialysis. The CD spectra of these samples revealed Z form DNA except for the one with no incubation. Step 4; each DNA sample was then reacted with sodium periodate (4mg/ml) for 16 h in the dark at room temperature and dialyzed to remove sodium periodate. The CD spectrum of each DNA sample at this point was that of B form DNA. *Right side* (control). A parallel control experiment was performed. The same procedure was used except the incubation time with DEB in Step 1 was zero. From ref. 24.

Left handed DNA in plasmids and the B-Z junctions

Alternating (pu-py) sequences adopt left handed conformation in recombinant plasmids. Supercoiling is a sensitive indicator of the B to Z transition, even in a small portion of the plasmids. When one turn of right handed primary helix is converted to one turn of left handed helix in a covalently closed plasmid, two supercoil turns will be lost. Thus, this is an extremely sensitive assay for conformational transitions within plasmids (25.26). Two-dimensional gel electrophoresis has been proved powerful for determining the supercoil relaxation due to conformational transitions (7,8,24). Fig. 3 shows two demensional gel electrophoretic analyses on a family of topoisomers of pRW756 containing $(dC\text{-}dG)_{16}$ sequence (panel a) and pBR322 control (panel b). A discontinuity in the gel mobility found for pRW756 at 15 negative

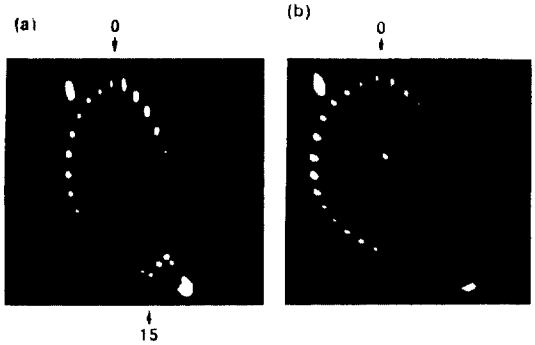

Fig. 3. Two-dimensional gel electrophoretic analyses of topoisomers of pRW756 (panel a) and pBR322 (panel b). The analyses were performed as described (39). 0, relaxed topoisomer. Topoisomers to the right of the relaxed topoisomer contain positive supercoils and those to the left contain negative supercoils in the first dimension. 15, topoisomer with 15 negative supercoil turns. From ref. 39.

supercoil turns represents B to Z transition of $(dC\text{-}dG)_{16}$ sequence. This kind of discontinuity in the gel mobility was not observed in panel b with pBR322 (24).

HhaI methylase has been used to detect d(GCGC) sequence in the left handed Z conformation, since this enzyme methylates all the enzyme sites in the plasmid but the region of left handed conformation (27). Anti-Z antibodies were also used to define the regions with Z-DNA (5,28).

Several DNA sequences including (dC-dG), $(dT\text{-}dG)_n \cdot (dC\text{-}dA)_n$ from mouse kappa immunoglobin genes (7), and mixtures of these sequences in intervening sequences in human fetal globin genes (8) adopt left-handed Z structures in recombinant plasmids at supercoil densities which exist in vivo. Since the majority of sequences in these plasmids is right-handed, B-Z junctions must exist. Little is known about the structure of B-Z junctions. Raman spectroscopy (3), ^{31}P NMR (29), and circular dichroism (19,29) studies on restriction fragments which contained B-Z junctions revealed no unusual features that could be attributed to the conformational interfaces. The only direct probes reported for the B-Z junctions are S_1 (7,8,30,31) and Bal 31 (32) nucleases which recognize and cleave at B-Z junctions. However, what type of structural abnormalities were recognized by these enzymes is still uncertain (33,34).

Bromoacetaldehyde as a probe for structural abnormalities

Bromoacetaldehyde (BAA) has been used widely as a probe for nonpaired adenines and cytosines in DNAs and RNAs (35-39). BAA forms covalent adducts at 1, and N_6 of adenine or 3, and N_4 of cytosine which are involved in hydrogen bonding of base pairs, therefore once the adducts are formed, base pairing at these positions is prohibited. The location of these "wedged open" regions may be mapped, by linearization of the DNA with restriction enzymes followed by S_1 nuclease cleavage (37,39). Fig. 4 shows the effect of supercoiling on the BAA reactions at the cruciforms and at the B-Z junctions of pRW756 containing $(dC\text{-}dG)_{16}$ sequence. The superhelical density at half maximum cleavage of the cruciforms was approximately -0.067 in good agreement with other studies (9,31) on the relationship between the superhelical density and cruciform formation. However, BAA reaction at the B-Z junctions in this plasmid was negligible when $\sigma < 0.09$. Studies with S_1 nuclease (30) and two dimensional gel analyses (39) revealed that $(dC\text{-}dG)_{16}$ sequence in pRW756 adopts a Z conformation when $\sigma > 0.04$. Therefore it is demon-

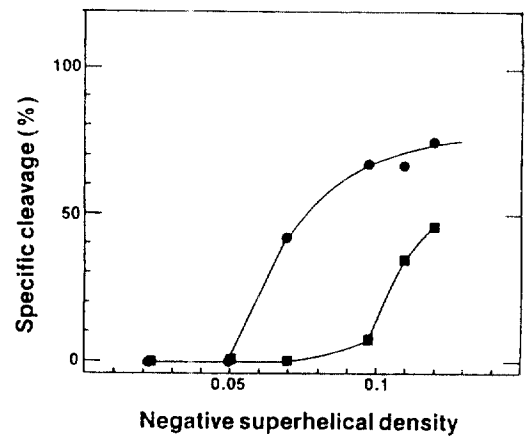

Fig. 4. Specific cleavage of pRW756 due to bromoacetaldehyde reaction as a function of mean negative superhelical density. A photographic negative was traced using a Joyce-Loebl microdensitometer. The loss of intensity (in per cent) of the specific fragments served to quantitate the BAA reaction at the pBR322 cruciforms and at the two B-Z junctions (squares). From ref. 39.

strated that B-Z junctions are present in pRW756 at $\sigma > 0.04$ but are not detected by BAA reaction at physiological superhelical densities (0.05-0.07).

Bromoacetaldehyde reaction with other plasmids

To evaluate the potential for BAA to react with other structural features, we performed similar studies on pCol-Md, pGF3, pß1BR16 and pHT△5′-186. pCO1-Md and pHT 5′-186 contain S_1 nuclease sensitive direct repeats which have been postulated to form slipped structures (40,41); since these slipped structures contain non-paired bases, they might be expected to be BAA reactive. The two other plasmids (pGF3 and pß1BR16) contain S_1 nuclease sensitive dG·dC tracts (42,43). In all four cases, no BAA sensitivity was observed in the insert although the vector cruciforms were reactive, thus serving as positive control.

Possible biological role of left handed DNA

It has been implicated that Z-DNA may play a role in the transcriptional enhancer of virus SV40 (44). Methylation of cytosine C5 in (dC-dG) sequences in vivo which is associated with gene inactivation in eukaryotes may be related to the Z-DNA since Z conformation is favored for the methylated $(dC\text{-}dG)_n$ sequence (20). Other biological properties that may be related to left handed DNA or B to Z transition include the following; Protein recognition or binding site; Effect on the supercoiling thus affect gene expression; Effect on chromosome structure; Termination of DNA replication and/or transcription; DNA recombination intermediate.

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