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Diepoxybutane forms a monoadduct with B-form $(dG\text{-}dC)_n \cdot (dG\text{-}dC)_n$ and a crosslinked diadduct with the left-handed Z-form

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ABSTRACT

The characteristics of the reactions of DL-diepoxybutane (DEB) with $(dG\text{-}dC)_n \cdot (dG\text{-}dC)_n$ in the right-handed B-form or the left-handed Z-form were investigated. DEB does react with right-handed B-DNA since less salt is required to convert the modified B-form to Z-form than for the unmodified DNA. However, the product appears to be a monoadduct rather than the crosslinked diadduct formed with the Z-form. The modified B-form can be isolated, converted to a Z-form with 1mM $MnCl_2$, and then this activated complex further reacts intramolecularly to give the crosslinked Z-product. This modified Z-form cannot be reverted to the B-form unless the crosslink is cleaved with periodate. Only $MnCl_2$, and to a lesser extent $ZnCl_2$, was effective in facilitating the intramolecular conversion of the B-DNA monoadduct to the Z-DNA diadduct; 1mM $MgCl_2$ and 4M NaCl were ineffective suggesting that somewhat different types of modified left-handed conformations were generated by the different salts. DEB also cleaves DNA under our reaction conditions thus precluding studies with supercoiled recombinant plasmids harboring segments that adopt Z-structures.

INTRODUCTION

$(dG\text{-}dC)_n \cdot (dG\text{-}dC)_n$ (1) undergoes a cooperative structural transition from a right-handed B-form to a left-handed Z-form in the presence of a variety of salts and dehydrating agents as monitored by CD, Raman, phosphorus and proton NMR spectroscopy and other methods (reviewed in 2-13 and references cited therein). This sequence as well as other naturally occurring sequences adopts left-handed structures which are induced by physiological supercoil densities in recombinant plasmids (3, 4, 14-17).

Castleman et al. (18) recently reported that $(dG\text{-}dC)_n \cdot (dG\text{-}dC)_n$ can be stabilized in the Z conformation by a crosslinking reaction with DL-diepoxybutane (DEB) when the Z structure was induced by 1mM $MnCl_2$ and heat. When $(dG\text{-}dC)_n \cdot (dG\text{-}dC)_n$ in the B conformation was reacted with DEB, this modified polymer could adopt a B or Z conformation, depending on the salt concentration. On the other hand, when the DEB reaction was carried out with Z-form $(dG\text{-}dC)_n \cdot (dG\text{-}dC)_n$ [induced by 1mM $MnCl_2$ and heating to 60°C], the

modified polymer stayed in the Z conformation even after removal of $MnCl_2$. CD spectroscopy served to measure these changes with antibody binding as a confirmatory test. These reactions with $(dG\text{-}dC)_n \cdot (dG\text{-}dC)_n$ may be summarized as follows:

$$\text{a) B-DNA + DEB} \longrightarrow \text{B-DNA} \underset{\text{1mM phosphate}}{\overset{\text{4M NaCl}}{\rightleftharpoons}} \text{Z-DNA}$$

$$\text{b) Z-DNA + DEB} \longrightarrow \text{Z-DNA} \xrightarrow{\text{1mM phosphate}} \text{Z-DNA}$$

These observations raised the following questions. First, if the same type of DEB reaction, presumably crosslinking, took place in a) as in b), how could the modified B-DNA be converted to Z-DNA whereas the modified Z-DNA could not be converted back to B-DNA? Second, does DEB react with B-DNA at all (i.e., is DEB a Z-DNA specific agent). If so, what type of reaction occurs with B-DNA? Third, can DEB be employed as a specific probe for Z-DNA in plasmids (3, 4, 8, 14-17, 19) and chromosomes (20-22) as proposed (18).

Our studies indicate that B-DNA reacts with DEB to form a monoadduct which, after conversion of the DNA to a Z-form, further reacts to form a diadduct crosslink.

MATERIALS AND METHODS

Materials

All chemicals were reagent grade from Fisher (sodium cacodylate), Sigma (sodium periodate, DL-diepoxybutane), Baker (sodium chloride), and Mallinckrodt Chemical Works (manganese(II) chloride). $(dG\text{-}dC)_n \cdot (dG\text{-}dC)_n$ was prepared enzymatically and characterized as described (1) (generous gift from J.E. Larson, this laboratory).

CD and UV Spectroscopy

CD spectra were recorded at room temperature with a Jasco J500A spectropolarimeter as described previously (7); UV spectra were obtained with a Varian 2290 spectrophotometer. Since the extinction coefficient of $(dG\text{-}dC)_n \cdot (dG\text{-}dC)_n$ after DEB reaction was unknown, the exact concentrations of these polymers were uncertain. To calculate the molar CD spectra, it was assumed that the concentrations of the DNA samples were not changed by the experimental procedures used. This assumption is reasonable since the concentration of $(dG\text{-}dC)_n \cdot (dG\text{-}dC)_n$ was not changed by dialysis using the same buffer system (data not shown). The reproducibility of the data was approximately ± 10%.

Reaction of $(dG-dC)_n \cdot (dG-dC)_n$ with DEB

$(dG-dC)_n \cdot (dG-dC)_n$ was reacted with DEB using a slight modification of the procedure of Castleman et al. (18). A 0.87 OD/ml solution of $(dG-dC)_n \cdot (dG-dC)_n$ was incubated at 37°C with 6.7% (v/v) DEB in 3mM sodium cacodylate, 0.1mM EDTA, pH 7.0 buffer ('buffer A'). After various time intervals, the reaction mixtures were dialyzed overnight against 1500 volumes of 'buffer A'. These DNA samples were used for sodium chloride titration studies as well as for the following reactions. The DEB modified polymer solution was made 1mM in $MnCl_2$ and was then incubated at 37°C. Aliquots were withdrawn at various time intervals and dialyzed overnight at 4°C against 1500 volumes of 10mM sodium cacodylate, 2mM EDTA, pH 7.0 ('buffer B') to remove $MnCl_2$, followed by dialysis against 'buffer A' for 6 hours.

Sodium Periodate Reaction with DEB Treated $(dG-dC)_n \cdot (dG-dC)_n$

The DEB reacted polymers prepared as above were reacted with $NaIO_4$ (4 mg/ml) for 16 h in the dark and then dialyzed against 'buffer A' to remove unreacted sodium periodate (18).

NaCl Titration of DEB Modified $(dG-dC)_n \cdot (dG-dC)_n$

For the titration of the DEB modified polymer with NaCl, a 5.0 M NaCl solution in 3mM cacodylate, pH 7.0 buffer was used for the additions. After each addition of NaCl, the solution was allowed to equilibrate for 10 minutes or until no further measurable CD changes occurred.

RESULTS

NaCl Titration of DEB Modified $(dG-dC)_n \cdot (dG-dC)_n$

Castleman et al. (18) demonstrated that DEB reaction with B-form $(dG-dC)_n \cdot (dG-dC)_n$ caused little change in its CD spectrum and that the reacted DNA adopted a typical Z-form spectrum when the NaCl concentration was raised to 4M. These authors were unable to conclude if DEB, in fact, reacted with right-handed B-form $(dG-dC)_n \cdot (dG-dC)_n$. Thus, we performed NaCl titration studies monitored by CD with $(dG-dC)_n \cdot (dG-dC)_n$ reacted with DEB at different time intervals to attempt to resolve this uncertainty.

B-form $(dG-dC)_n \cdot (dG-dC)_n$ in 'buffer A' was reacted with DEB at 37°C and unreacted DEB was removed by dialysis against the same buffer. Fig. 1A shows a titration curve where the DEB reaction time was 1.5 h. 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 changes. To find the B to Z transition midpoint, ΔE at 292 and at 250 nm was plotted against the NaCl concentration (Fig. 1B).

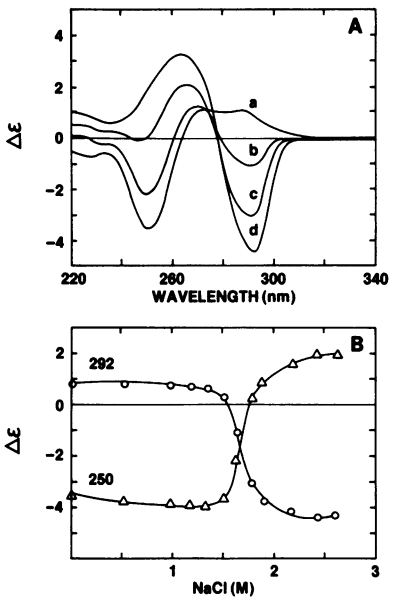

Fig. 1. Panel A. CD spectra of DEB reacted $(dG\text{-}dC)_n \cdot (dG\text{-}dC)_n$ at different NaCl concentrations. B form $(dG\text{-}dC)_n \cdot (dG\text{-}dC)_n$ was reacted with DEB for 1.5 h in 'buffer A' and unreacted DEB was removed by dialysis as described in Methods. The treated DNA was then titrated with NaCl as described in Methods. The NaCl concentrations (M) were: a, 0; b, 1.64; c, 1.78; d, 2.42. Panel B. Effect of NaCl on the molar ellipticity of DEB reacted B form $(dG\text{-}dC)_n \cdot (dG\text{-}dC)_n$. Circles, ΔE_{292}, triangles, ΔE_{250}.

The transition midpoint was 1.68 M NaCl in this case. This transition midpoint was considerably lower than for the unmodified polymer (2.6 M NaCl) (6) and hence serves as direct evidence for the DEB modification of the B-form polymer.

Fig. 2 shows the results from similar studies conducted at six different DEB reaction times (0.5 to 8 h). Panel B reveals that the sodium chloride concentration at the transition midpoint decreased as the reaction time with DEB increased. Thus, we conclude that DEB does react with right-handed B-DNA in addition to, as reported previously (18), left-handed $(dG\text{-}dC)_n \cdot (dG\text{-}dC)_n$. Furthermore, these results are in agreement with the methylation studies of Moeller *et al*. (23) which revealed that alkylation of the N7 position of guanines in $(dG\text{-}dC)_n \cdot (dG\text{-}dC)_n$ caused a reduction in the salt concentration required to cause the B to Z transition.

Attempts to chemically determine the extent of reaction of DEB with the DNA were thwarted by the unavailability of isotopically labeled DEB and by our lack of understanding of which DEB isomer (D or L or meso) is most reactive with the different DNA conformations. Attempts to chromatographically identify G mono- and diadducts were unsuccessful using the general methods described (24) as well as several other acid degradation procedures and paper chromatography systems. However, reaction with less than 10% of Gs would

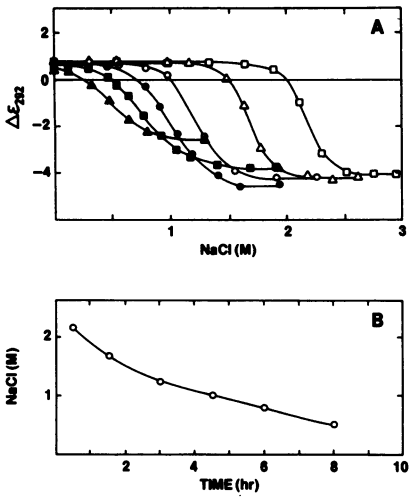

Fig. 2. Panel A. Effect of reaction time of $(dG\text{-}dC)_n \cdot (dG\text{-}dC)_n$ with DEB on B to Z transition. The NaCl titration curves were constructed under the conditions shown in Fig. 1 using the DEB reacted $(dG\text{-}dC)_n \cdot (dG\text{-}dC)_n$ except the reaction time with DEB was varied. (□), 0.5h; (Δ), 1.5h; (o), 3h; (●), 4.5h; (■), 6h; (▲), 8h. Panel B. Effect of DEB reaction time with $(dG\text{-}dC)_n \cdot (dG\text{-}dC)_n$ on the NaCl concentration required for the midpoint in the B to Z transition. The midpoint of the transition for unreacted $(dG\text{-}dC)_n \cdot (dG\text{-}dC)_n$ is 2.6 M (6).

have been difficult to detect. Thus, we conclude that a small extent of reaction of DEB with each DNA molecule accounts for these profound effects.

DEB Reacted B-form $(dG\text{-}dC)_n \cdot (dG\text{-}dC)_n$ Undergoes an Intramolecular Reaction

Z-form $(dG\text{-}dC)_n \cdot (dG\text{-}dC)_n$ can be stabilized by a crosslinking reaction with DEB (18). We demonstrated (Figs. 1 and 2) that DEB does react also with B-form $(dG\text{-}dC)_n \cdot (dG\text{-}dC)_n$. However, this B-form product can undergo the salt induced B to Z transition (18) whereas the Z-form product cannot undergo the Z to B transition. Thus, we hypothesized that DEB crosslinks Z-form $(dG\text{-}dC)_n \cdot (dG\text{-}dC)_n$, however, it cannot crosslink B-form $(dG\text{-}dC)_n \cdot (dG\text{-}dC)_n$ but only forms a monoalkylation product. The experimental scheme employed to test this notion is shown in Fig. 3.

B-form $(dG\text{-}dC)_n \cdot (dG\text{-}dC)_n$ in 'buffer A' was incubated at 37°C with 6.7% DEB for 6 h and then dialyzed against 'buffer A' to remove unreacted DEB. This DEB reacted polymer undergoes the B to Z transition at lower NaCl concentrations than unreacted $(dG\text{-}dC)_n \cdot (dG\text{-}dC)_n$ as shown in Fig. 2. For the experiment outlined in Fig. 3, the Z conformation was generated by $MnCl_2$. The treated polymer underwent a B to Z transition instantaneously (within seconds) upon addition of $MnCl_2$ to 1mM as monitored by CD (data not shown). Heating to 60°C for 15 min was not required to induce the B to Z transition as was the case for the unmodified $(dG\text{-}dC)_n \cdot (dG\text{-}dC)_n$. The modified DNA in 1mM $MnCl_2$ was then incubated at 37°C. If the incubation time was zero, (i.e. $MnCl_2$ was removed by dialysis without further incubation), there was no detectable change of the CD spectra compared to the DEB reacted B form (dG-

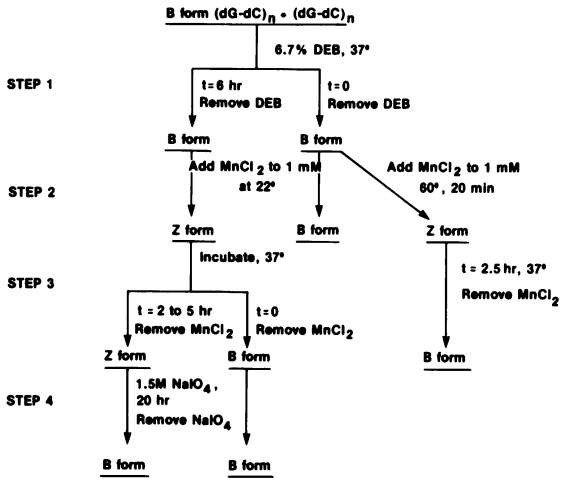

Fig 3. Experimental scheme. Left side (experimental). Step 1; the B form $(dG\text{-}dC)_n \cdot (dG\text{-}dC)_n$ in 'buffer A' was incubated at 37°C with 6.7% DEB for 6 h and unreacted DEB was removed by dialysis against 'buffer A'. Step 2; $MnCl_2$ was added to 1 mM. Step 3; this solution was incubated at 37°C. After 0, 2, 3, 5 hours of incubation, aliquots were withdrawn and $MnCl_2$ was removed by dialysis against 'buffer B' and subsequently 'buffer A'. The CD spectra of these samples revealed Z form DNA except for the one with no incubation (shown in Fig. 4). Step 4; each DNA sample was then reacted with sodium periodate (4 mg/ml) for 16 h in the dark at room temperature and dialyzed against 'buffer A' 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.

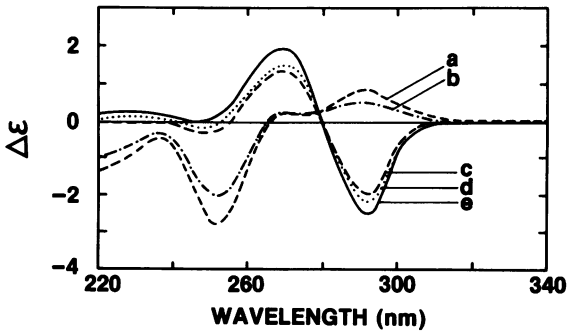

Fig. 4. CD spectra of DEB reacted $(dG\text{-}dC)_n \cdot (dG\text{-}dC)_n$ prepared in Fig. 3 (left side, experimental). The DNA sample for each spectrum was: a, after Step 1 and an identical spectrum was obtained after Step 3 when t=0; c, after Step 3 when t=2h; d, after Step 3 when t=3h; e; after Step 3 when t=5h; b, spectrum after Step 4 (identical spectra were observed for all periodate treated DNAs).

$dC)_n \cdot (dG\text{-}dC)_n$ (Fig. 4). After more than 2 hours of incubation, stable Z-form CD spectra were obtained even if $MnCl_2$ was removed by extensive dialysis against 'buffer B' followed by 'buffer A'. Thus, the DNA was locked in a left-handed Z structure by an apparent crosslink reaction. With longer incubation times, the Z characteristics of the CD spectra became more obvious (Fig. 4). It is probable that the reaction reached an end point after 5 h incubation since changes in ΔE_{292} were smaller after 2 h compared to the 0 to 2 h period and the ellipicity reached a plateau after 5 h.

Thus, the DEB reaction with B-form $(dG\text{-}dC)_n \cdot (dG\text{-}dC)_n$ generated some type of activated complex which further reacted to crosslink the DNA only after the Z-form was induced by $MnCl_2$.

To further understand the properties of the crosslinked Z-structures, ethidium bromide (EB) binding determinations were conducted on all DNAs after Step 3 (Fig. 3). Fluorescence enhancement studies revealed that the modified B-form did bind EB whereas the modified Z-form DNAs did not bind. Thus, the presence of EB was not sufficient to cause a Z to B transition for the crosslinked DNAs. Pohl et al. (25) showed that the salt-induced cooperative B to Z transition for $(dG\text{-}dC)_n \cdot (dG\text{-}dC)_n$ was reversed by addition of EB. Thus, this demonstrates that the crosslink is an effective stabilizer of the Z-structure.

A control experiment was carried out as shown in the right portion of Fig. 3. This control verified that there was, in fact, no free DEB in the reaction at Step 3 (Fig. 3) which would give a crosslinking reaction. Identical conditions were used for the control study except DEB was removed by dialysis against 'buffer A' without incubating the reaction mixture at 37°C. If this dialysis step did not completely remove DEB, a crosslinking reaction should have been observed after addition of $MnCl_2$ and incubation at 37°C for 2.5 h as shown previously (18). No crosslinking occurred since the CD spectra for B-form DNA was observed after removal of $MnCl_2$ by the procedures shown in Fig. 3 (data not shown). Therefore, this shows that unreacted DEB was removed effectively by the dialysis in Step 1 (Fig. 3).

Periodate Cleavage of Crosslinks (Step 4)

Each of the crosslinked Z-form DNA samples, which were prepared by the methods shown in Fig. 3, was reverted from Z to B by reaction with sodium periodate, consistent with the previous investigation (18). The CD spectra of the products of the sodium periodate reactions are shown in Fig. 4.

Interestingly, it is possible to recycle the periodate generated B-form (after Step 4, Fig. 3 left side) back through steps 2 and 3 to again form

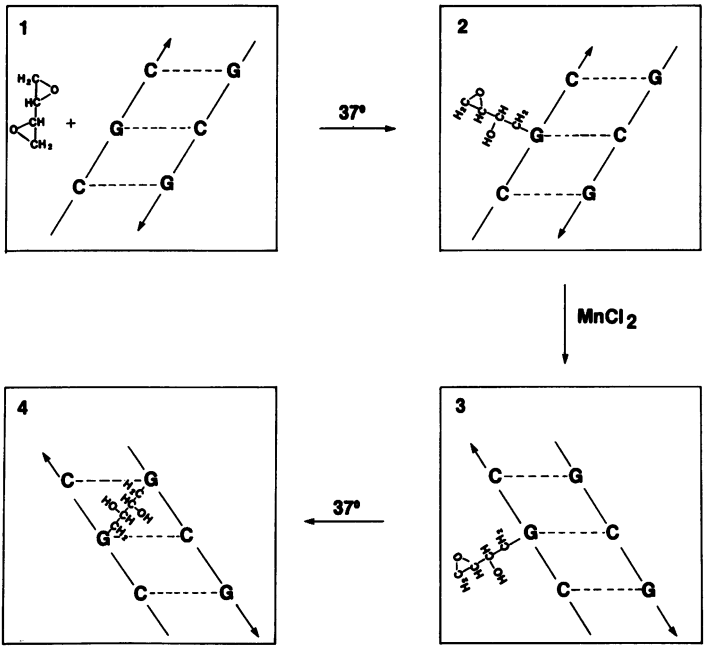

Fig. 5. Proposed mechanism. Panel 1; Right-handed B form $(dG-dC)_n \cdot (dG-dC)_n$ reacts with DEB at 37°C to form a monoalkylation product at guanine (Panel 2). Panel 3: B form monoalkylated product undergoes a B to Z transition in 1 mM $MnCl_2$ at room temperature. Panel 4; The second alkylation step is now possible and the reaction proceeds at 37°C. A bidentated product is formed to give a stable crosslinked Z form DNA which cannot undergo a Z to B transition. If the DNA is in a Z form initially (panel 3), it readily forms the crosslinked product (18). Panels 1 and 2 represent DNA in a right-handed structure whereas panels 3 and 4 represent left-handed structures.

crosslinked Z-form. If the B-form DNA, after the periodate reaction, is made 1mM in $MnCl_2$ and incubated at 37°C for 3 h, the recycled DNA is locked into the Z-form again. Thus, apparently only a portion of the monoadduct further reacted to give the diadduct in the first cycle. Also, these results infer, as suggested above, that only a small number of diadducts may be required to stabilize the left-handed Z-structures.

Structural Interpretation

In summary, the results described herein and previous studies (18) demonstrated that DEB crosslinks left-handed Z-DNA, stabilizing the Z-form even when the ionic conditions required to generate the Z structure are removed. Alternatively, DEB does react with B-form $(dG-dC)_n \cdot (dG-dC)_n$ to give a somewhat altered B structure which readily undergoes the salt-induced

B to Z transition. This modified DNA (activated complex), in the absence of DEB in the reaction, undergoes a time dependent further reaction to give crosslinked Z-DNA which is otherwise identical to Z-DNA crosslinked with DEB (18).

We propose (Fig. 5) the following explanation of the results. When B-form $(dG\text{-}dC)_n \cdot (dG\text{-}dC)_n$ was reacted with DEB, the product was mostly monoalkylation with little or no crosslinking (panel 2). Alkylation of a neighboring guanine by the other end of the DEB molecule does not occur. After the Z form was induced by 1mM $MnCl_2$ (panel 3), a second alkylation step took place to form the crosslinked product (panel 4). A structural difference between the right-handed B and left-handed Z-DNAs must be responsible for this behavior (Discussion).

DISCUSSION

DEB reacts with right-handed B-DNA to form a monoadduct whereas it reacts with left-handed Z-DNA to form a diadduct (Fig. 5). The monoadduct "activated B-DNA complex" reacts intramolecularly after the salt induced conversion to a modified Z-structure to give the crosslinked Z-product. DEB reacts principally at the N7 position of guanines (24, 26) and the atomic coordinates of B and Z-DNA structures are known (3, 13, 27). Fig. 6 shows the geometries of two bp in the B and Z conformations for an oligomer where crosslinking probably occurs. It might be expected that the experimental results could be explained by examining the differences in these bp geometries but this is not the case. The distances between the pairs of guanosine N7 atoms are quite similar (as shown in Table I of ref. 18), and, as seen in Fig. 6, there are no substantial differences in the orientations of the bonds that would connect DEB to the guanine rings in the two conformations. Thus, we are unable to rationalize the experimental results based on an examination of the structural differences between the two conformations.

However, our studies revealed that the intramolecular crosslinking of DEB modified $(dG\text{-}dC)_n \cdot (dG\text{-}dC)_n$ depended on the salt conditions used to stabilize the Z conformation. If 1mM $MgCl_2$ or 4M NaCl was used in place of $MnCl_2$ (Step 2 in Fig. 3) to stabilize the left-handed conformation (which was formed as determined by CD), only a B-form spectrum was found after removal of the salt, thus indicating that crosslinking did not occur. We interpret these results to indicate that somewhat different types of left-handed structures were generated by different salt conditions, in agreement with

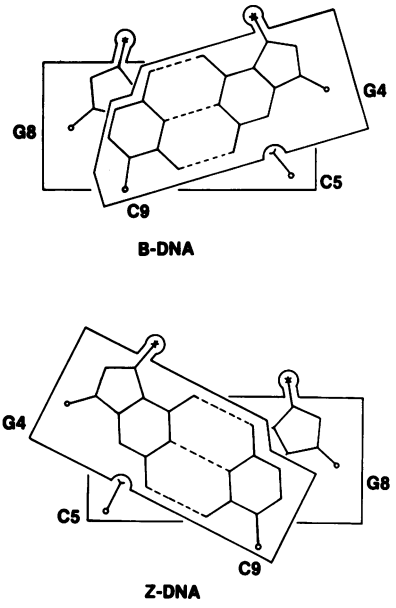

Fig. 6 Basepair configurations in B-DNA (top) and Z-DNA (bottom), showing the locations of the C1 and C4 atoms of the potential DEB crosslinks (asterisks). Note that the B to Z transition requires not only a change in the handedness of the helix, but also that all basepairs be flipped over (13, 30). The bp designations refer to geometry in the following oligomer.

$C^1—G^2—C^3—G^4—C^5—G^6$

$G^{12}-C^{11}-G^{10}-C^9—G^8—C^7$

previous solution studies (7). If 1mM $ZnCl_2$ was used, the crosslinking reaction took place but much slower than with $MnCl_2$ (data not shown). Subtle differences between the left-handed structures stabilized by the different salt conditions probably account for this behavior. Little information is available from crystallography studies about the different Z-form structures stabilized by different salts, thus precluding a detailed structural interpretation. However, we cannot exclude the unlikely possibility that the salt effect is on the DEB reaction per se rather than on the structure of the left-handed DNAs. Future studies with DEB analogs as molecular rulers may provide valuable insights into these uncertainties.

To evaluate if DEB was a useful general probe for different types of Z-DNA structures, such as $(dT\text{-}dG)_n \cdot (dC\text{-}dA)_n$ which exists on the 3' side of the mouse kappa immunoglobulin gene (15, 17) or the IVS2 sequence from human fetal globin genes (16), we attempted to crosslink pRW756 (28, 29). However, under all reaction conditions tested, the supercoiled DNA was readily nicked by DEB (~80% relaxation in 1 h)(data not shown). This behavior was expected since DEB reacts at the N7 position of G, thereby labilizing the glycosidic bond to give a depurinated site which is susceptible to chain scission. Thus, it is unlikely that DEB can be generally employed as a small molecule probe for left-handedness in supercoiled plasmids or chromosomal DNAs.

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REFERENCES

1. Wells, R.D., Larson, J.E., Grant, R.C., Shortle, B.E., and Cantor, C.R. (1970) J. Mol. Biol. 54, 465-497.
2. Wells, R.D., Brennan, R., Chapman, K.A., Goodman, T.C., Hart, P.A., Hillen, W., Kellogg, D.R., Kilpatrick, M.W., Klein, R.D., Klysik, J., Lambert, P.F., Larson, J.E., Miglietta, J.J., Neuendorf, S.K., O'Connor, T.R., Singleton, C.K., Stirdivant, S.M., Veneziale, C.M., Wartell, R.M., and Zacharias, W. (1982) Cold Spring Harbor Symposia 47, 77-84.
3. Rich, A. (1982) Cold Spring Harbor Symposia 47, 1-12.
4. Wang, J.C., Peck, L.J., and Becherer, K. (1982) Cold Spring Harbor Symposia 47, 85-92.
5. Wells, R.D., Goodman, T.C., Hillen, W., Horn, G.T., Klein, R.D., Larson, J.E., Muller, U.R., Neuendorf, S.K., Panayotatos, N., and Stirdivant, S.M. (1980) Prog. Nucleic Acid. Res. Mol. Biol. 24, 167-267.
6. Pohl, F., and Jovin, T.M. (1972) J. Mol. Biol. 67, 375-396.
7. Zacharias, W., Larson, J.E., Klysik, J., Stirdivant, S.M., and Wells, R.D. (1982) J. Biol. Chem. 257, 2775-2782.
8. Klysik, J., Stirdivant, S.M., Larson, J.E., Hart, P.A., and Wells, R.D. (1981) Nature 290, 672-677.
9. Thamann, T.V., Lord, R.C., Wang, A.H.J., and Rich, A. (1981) Nucleic Acids Res. 9, 5443-5457.
10. Wartell, R.M., Klysik, J., Hillen, W., and Wells, R.D. (1982) Proc. Natl. Acad. Sci. USA 79, 2549-2553.
11. Patel, D.J., Canuel, L.L., and Pohl, F.M. (1979) Proc. Natl. Acad. Sci. USA. 76, 2508-2511.
12. Mitra, C.K., Sarma, M.H., and Sarma, R.H. (1981) Biochemistry 20, 2036-2041.
13. Crawford, J.L., Kolpak, F.J., Wang, A.H.-J., Quigley, G.J., van Boom, J.H., van der Marel, R., and Rich, A. (1980) Proc. Natl. Acad. Sci. USA 77, 4016-4020.
14. Singleton, C.K., Klysik, J., Stirdivant, S.M., and Wells, R.D., (1982) Nature 299, 312-316.
15. Singleton, C.K., Kilpatrick, M.W., and Wells, R.D. (1984) J. Biol. Chem. 259, 1963-1967.
16. Kilpatrick, M.W., Klysik, J., Singleton, C.K., Zarling, D.A. Jovin, T.M., Hanau, L.H., Erlanger, B.F., and Wells, R.D. (1984) J. Biol. Chem. 259, 1268-1274.
17. Haniford, D.B., and Pulleyblank, D.E. (1983) Nature 302, 632-634.
18. Castleman, H., Hanau, L.H., and Erlanger, B.F. (1983) Nucleic Acids Res. 11, 8421-8425.
19. Stirdivant, S.M., Klysik, J., and Wells, R.D., (1982) J. Biol. Chem. 257, 10159-10165.
20. Nordheim, A., Pardue, M.L., Lafer, E.M., Moeller, A., Stollar, B.D., and Rich, A. (1981) Nature 294, 417-422.
21. Jovin, T.M., van de Sande, J.H., Zarling, D.A., Arndt-Jovin, D.J., Eckstein, F., Fuldner, H.H., Greider, C., Grieder, I., Hamori, E., Kalisch, B., McIntosh, L.P., and Robert-Nicoude, M. (1982) Cold Spring Harbor Symposia 47, 143-154.
22. Hill, R.J., and Stollar, B.D. (1983) Nature 305, 338-340.

23. Moeller, A., Nordheim, A., Nichols, S.R., and Rich, A. (1981) Proc. Natl. Acad. Sci. USA 78, 4777-4781.
24. Lawley, P.D., and Brookes, P. (1967) J. Mol. Biol. 25, 143-160.
25. Pohl, F.M., Jovin, T.M., Baehr, W., and Holbrook, J.J. (1972) Proc. Natl. Acad. Sci. USA 69, 3805-3809.
26. Otvos, L., and Elekes, J. (1975) Tetrahedron Lett. 29, 2481-2484.
27. Dickerson, R.E., Drew, H.R., Connor, B.N., Kopka, M.L., and Pjura, P.E. (1983) Cold Spring Harbor Symposia 47, 13-24.
28. Klysik, J., Stirdivant, S.M., and Wells, R.D. (1982) J. Biol. Chem. 257, 10152-10158.
29. Kang, D.S., and Wells, R.D. (1985) J. Biol. Chem. 260, 7782-7790.
30. Harvey, S.C. (1983) Nucleic Acids Res. 11, 4867-4878.