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BAL 31 nuclease as a probe in concentrated salt for the B-Z DNA junction

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ABSTRACT

The BAL 31 nuclease, an extracellular nuclease from *A. espejiana*, specifically recognizes and cleaves the salt induced conformational junction between B and Z-DNA. Short segments of (dC-dG) left-handed Z-helix, comprising ~1% of the total DNA, are specifically detected within two different recombinant plasmids. The BAL 31 enzyme is highly resistant to inactivation by the presence of high concentrations of a variety of electrolytes that stabilize left-handed helices, is active at physiological pH, and can be used to probe both linear and circular DNAs. Additionally, the nuclease cleaves left-handed $(dC\text{-}dG)_n$ only very poorly, if at all. Thus, the BAL 31 nuclease can be utilized as a probe for helical junctions and consequently for segments of left-handed DNA that might exist within predominantly right-handed naturally occurring genomes.

INTRODUCTION

The DNA polymer $(dC\text{-}dG)_n \cdot (dC\text{-}dG)_n$ exists in a left-handed conformation in high concentrations of salt (reviewed in 1, 2). Furthermore, segments of (dC-dG) in DNA restriction fragments and in recombinant plasmids adopt a left-handed conformation in high salt solution, while the neighboring regions of natural sequences remain in right-handed helices (3-10). Therefore, we wished to study the nature of the junction between left-handed Z-DNA and right-handed B-DNA that exists under conditions of high salt concentration.

Prior studies (6, 9, 10, 11) demonstrated that supercoiling causes the formation of left-handed structures in appropriate sequences under physiological conditions of salt and supertwist density. Furthermore, the single-strand specific S_1 nuclease specifically recognizes and cleaves the junction between right and left-handed regions (9, 10). However, this assay is inconvenient or impractical for some types of sequences that might adopt left-handed structures. Consequently, we have developed an assay that monitors the formation of junctions which are induced by salt. A variety of different salt conditions stabilize left-handed helices (7, 12).

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BAL 31 nuclease, an extracellular nuclease from Alteromonas espejiana, specifically recognizes non-duplex structures in nominally duplex DNAs (13-15) and readily hydrolyses single-stranded DNAs (14, 16). This nuclease has a remarkable resistance to inactivation in the presence of extremely high concentrations of electrolytes, retaining at least 40% of its maximal activity against single-stranded substrates in 4.5 M sodium chloride (14).

This paper shows that the BAL 31 nuclease recognizes and cleaves some features of the salt-induced junctions between B and Z-DNA. Thus, this assay can be utilized as a probe for junctions and consequently for segments of left-handed DNA that might exist within predominantly right-handed naturally occurring DNAs.

MATERIALS AND METHODS

DNA

Plasmid DNA was purified as described previously (16) from E. coli MO rec A^-. The plasmids used in this work, pRW751, pRW756 and pRW451, were characterized previously (4, 5). pRW751 is a pBR322 derivative which contains a 157 bp insert into the Bam HI site which is composed of the 95 bp E. coli lac operator DNA fragment flanked by alternating (dC-dG) stretches of 32 and 26 bp (Fig. 2). pRW756 is similar to pRW751 but contains only a single alternating (dC-dG) stretch of 32 bp (Fig. 3). pRW451 (5), the control plasmid, is a pBR322 derivative with a pBR322 fragment insert of 174 bp into the Bam HI site. ^{3}H-$(dG\text{-}dC)_n$ was prepared enzymatically using ^{3}H-dGTP and characterized as described (17). The specific activity was 12,500 cpm/μg. ^{3}H-Chick erythrocyte DNA was synthesized by nick translation with ^{3}H-dXTPs as precursors using established methods (18). The specific activity was 10,300 cpm/μg.

Enzymes

The extracellular nuclease of Alteromonas espejiana (BAL 31 nuclease) (19) exists in two kinetically distinct forms which differ markedly in the rate of terminally directed hydrolysis of linear duplex DNA per unit of activity against single-stranded DNA (14). The form exhibiting the faster kinetics of exonucleolytic hydrolysis (F form) also has shown to be more effective in cleaving duplex DNA at sites of covalent alteration such as those introduced by reaction with carcinogenic or mutagenic agents (15). Recent studies (Wei, C.-F., Alianell, G.A., Bencen, G. and Gray, H.B., Jr., unpublished) have resulted in the separation and purification to homogeneity of both forms, using affinity chromatography on 5'-AMP covalently coupled to

agarose (14) followed by gel filtration chromatography on Sephadex G-100 Superfine. The F form nuclease used in these studies consists of a single polypeptide chain with a molecular weight of 108,000 as determined by electrophoresis in polyacrylamide gels containing sodium dodecyl sulfate in the presence of appropriate protein molecular weight standards. This preparation displayed only a single band upon electrophoresis in heavily loaded polyacrylamide gels containing SDS.

Alu I and Ava II were purchased from B.R.L. Eco RI was a gift of S.M. Stirdivant (this lab at the University of Wisconsin). Reaction conditions for these enzymes were as published (16).

Wheat germ topoisomerase was a generous gift of R. R. Burgess (University of Wisconsin). Populations of topoisomers of the plasmids were generated and characterized as described (20).

Nuclease Digestion.

Previous studies showed that BAL 31 nuclease will cleave supercoiled DNA but not closed circular duplex DNA which was relaxed (13-15, 19, 21). Thus, we studied relaxed plasmids. Populations of topoisomers of the plasmids with superhelical densities centered around zero under the conditions of treatment with BAL 31 nuclease were generated with wheat germ topoisomerase.

Plasmids (1-2 μg) were preincubated at 30°C for 1 hour in 45 μl of either high salt buffer (4.5 M NaCl, 12.5 mM $MgSO_4$, 12.5 mM $CaCl_2$, 20 mM Tris-HCl pH 8.1, 1 mM EDTA) or low salt buffer (0.6 M NaCl, 12.5 mM $MgSO_4$, 12.5 mM $CaCl_2$, 20 mM Tris-HCl pH 8.1, 1 mM EDTA). BAL 31 nuclease (87 units/ml) then was added and incubation was at 30°C for 30 minutes. The BAL 31 nuclease then was irreversibly inactivated by addition of one tenth of a volume of 0.5 M EDTA to chelate calcium ions (22), and the samples were dialysed against the appropriate restriction enzyme buffer. An aliquot of the sample was taken for analysis by agarose gel electrophoresis. The appropriate restriction enzyme (2 units) was added to the remainder of the sample which was incubated at 37°C for 6 hours. Analysis of the products of digestion was by electrophoresis on 4 or 5% polyacrylamide gels.

RESULTS

Effect of Salt on BAL 31 Nuclease

Prior studies (14) revealed that the BAL 31 nuclease is active in the presence of high concentrations of salt (7 M CsCl or 4.5 M NaCl). We wished to evaluate the effects of the salt concentrations used in these studies on the BAL 31 nuclease activity. In the low salt buffer (0.6M NaCl), the

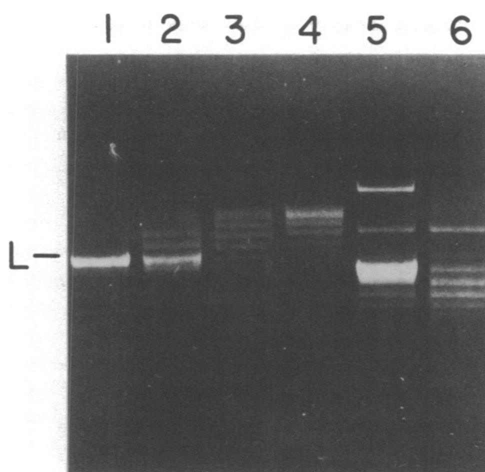

Fig. 1. Analysis by electrophoresis on a 1% agarose gel of the products of treatment of topoisomers of pRW751 (lanes 1 and 3), pRW451(lanes 2 and 4) and pRW756 (lanes 5 and 6) with BAL 31 nuclease under high salt (lanes 1,2, and 5) or low salt (lanes 3, 4, and 6) conditions. L: linearized plasmid. Salt conditions and other procedures were described in Methods.

nuclease preparation contained 870 units/ml of activity, assayed as described (14) using denatured calf thymus DNA as substrate. In the high salt buffer (4.5 M NaCl), the activity of the nuclease decreased to 70% of this value.

The exonuclease activity of the enzyme, as judged by its ability to remove nucleotides from the termini of linear duplex phage PM2 DNA, was also determined under the two salt concentrations. In the high salt buffer, the observed exonucleolytic hydrolysis rate was 26% of the value in low salt buffer. If these rates are normalized by the specific activity against single-stranded DNA under the two sets of conditions, the effect of the higher salt concentration was to decrease the exonucleolytic hydrolysis rate to 39% of the value at the lower salt concentration.

To the best of our knowledge, the BAL 31 nuclease is the only single strand specific endodeoxyribonuclease that retains activity under such high salt conditions.

BAL 31 Nuclease Treatment of Plasmids

Figure 1 shows the effect of treatment of pRW751 and pRW756, which contain (dC-dG) tracts that adopt left-handed structures in high salt (3-5) as well as the control plasmid pRW451 with BAL 31 nuclease under either high salt or low salt conditions. Treatment of pRW751 with BAL 31 nuclease under high salt conditions (lane 1) resulted in extensive linearization of the plasmid.
However, the same plasmid was totally resistant to BAL 31 nuclease treatment under low salt conditions (lane 3), no linear molecule being produced. A similar situation was demonstrated for pRW756; treatment of this plasmid with

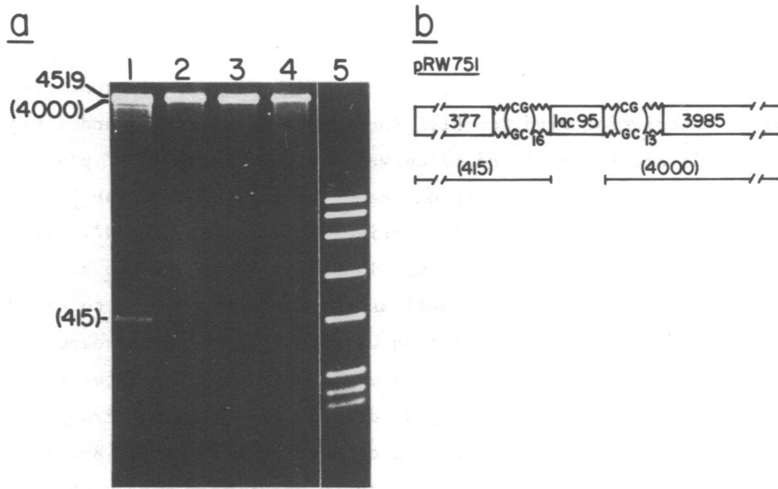

Fig. 2. Panel a. Analysis by electrophoresis on a 5% polyacrylamide gel of the products of treatment of pRW451 (lanes 3 and 4) and pRW751 (lanes 1 and 2) with BAL 31 nuclease followed by restriction with Eco RI. Initial BAL 31 treatment was under either high salt (lanes 1 and 3) or low salt (lanes 2 and 4) conditions. Sizes of BAL 31 specific bands are denoted in brackets. Lane 5 is a restriction digest of pBR322 used as size markers. Panel b. Mapping of BAL 31 specific bands produced by treatment of pRW751 with BAL 31 nuclease under high salt conditions followed by restriction with Eco RI. Sizes based on the sequence of pRW751 are shown within the drawing, and the sizes of the BAL 31 specific bands determined from Fig. 2a are shown underneath (in brackets).

BAL 31 nuclease under high salt conditions again led to extensive linearization (lane 5), whereas the plasmid was totally resistant to BAL 31 nuclease under low salt conditions (lane 6). With the control plasmid pRW451, treatment with BAL 31 nuclease under low salt conditions had no effect (lane 4) and treatment with the nuclease under high salt conditions (lane 2) led to the production of only a small amount (<10%) of linear plasmid.

Thus, treatment of $(dC\text{-}dG)_n$ containing plasmids with BAL 31 nuclease under conditions where these blocks will adopt the Z-conformation resulted in extensive linearization of the plasmids, whereas only a small amount of linearization occurred with the plasmid that does not contain any $(dC\text{-}dG)_n$. All three plasmids were totally resistant to BAL 31 nuclease treatment under conditions where the $(dC\text{-}dG)_n$ blocks remained in the B-conformation.

Mapping of Cleavage Sites

Determination of the specificity of cleavage of the plasmids by the BAL

31 nuclease was important for establishing this approach as an assay for salt-induced junctions. Figure 2a shows the effect of treatment of pRW751 and pRW451 with BAL 31 nuclease under either high or low salt conditions followed by restriction with Eco RI. For treatment of pRW451 under high or low salt conditions (lanes 3 and 4) as well as for treatment of pRW751 under low salt conditions (lane 2), only one band corresponding to the Eco RI linearized plasmid is evident. Alternatively, treatment of pRW751 with BAL 31 nuclease under high salt conditions (lane 1) followed by Eco RI produces a BAL 31-specific band of 415 bp as well as a band corresponding to the linearized plasmid. Close examination of the 4.5kbp band corresponding to the linearized plasmid reveals it to be a doublet. Mapping shows that these BAL 31-specific bands (Fig. 2b) are $(dC\text{-}dG)_n$ block terminated fragments produced by cleavage by the nuclease at or near the "internal" B-Z junctions. (The term "internal" junction will be used to describe a B-Z junction near the lac region that, after BAL 31 nuclease and restriction enzyme cleavage, gives a restriction fragment which terminates in a (dC-dG) tract.) If the BAL 31 nuclease recognized and cleaved all B-Z junctions, we should expect to also find the fragments (510 and 4095 bp in length) which do not have (dC-dG) termini. These fragments were not observed under any digestion conditions (Fig. 2 and results not shown). It is apparent that these fragments which do not have (dC-dG) termini are degraded by the BAL 31 exonuclease activity. The BAL 31 exonuclease activity degrades GC-rich regions of DNA more slowly than regions of lower GC content (14, 22) and it degrades $(dG\text{-}dC)_n$ sequences in the left-handed structure even more slowly (Fig. 4, see below).

Figure 3a shows further mapping of the cleavage of pRW751 and pRW756 by BAL 31 nuclease under high salt conditions. The three plasmids pRW751, pRW756, and pRW451 were treated with BAL 31 nuclease under high salt conditions followed by restriction with Ava II. For pRW451, only the 1743, 1585, and 345 bp Ava II restriction fragments are evident. However, with pRW751, the 1585 bp Ava II band, which contains the $(dC\text{-}dG)_n$ blocks, is considerably less intense and a number of BAL 31 specific bands can be seen. The major BAL 31 bands are 1048 and 440 bp, and there are several minor bands of between 1180 and 910 bp. Similarly for pRW756, there are two major BAL 31 specific bands of 768 and 443 bp as well as the Ava II bands of 1743, 1183 and 345 bp. The mapping of these BAL 31 specific bands is presented in Figure 3b. For pRW751, the two major BAL 31 specific bands (1048 and 440 bp) map to $(dC\text{-}dG)_n$ terminated fragments resulting from cleavage at or near the "internal" B-Z junctions. The minor BAL 31-specific bands represent

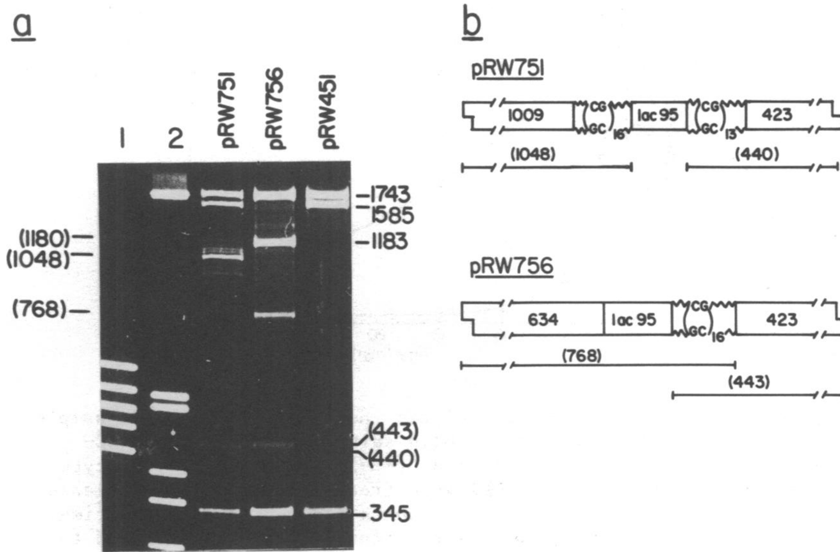

Fig. 3. Panel a. Analysis by electrophoresis on a 4% polyacrylamide gel of the products of treatment of pRW751, pRW756, and pRW451 with BAL 31 nuclease under high salt conditions followed by restriction with Ava II. Lanes 1 and 2 are restriction digests of pBR322 used as size markers. Sizes of BAL 31 specific bands are indicated in brackets.
Panel b. Mapping of BAL 31 specific bands produced by treatment of pRW751 and pRW756 with BAL 31 nuclease under high salt conditions followed by restriction with Ava II. Sizes based on the sequence of the plasmid are shown within the drawing in each case, with the sizes of the BAL 31 specific bands, as determined from Fig. 3a, shown underneath (in brackets).

fragments that do not terminate in $(dC\text{-}dG)_n$ as well as $(dC\text{-}dG)_n$ terminated fragments produced by cleavage at the junction furthest from each AvaII site. The former fragments are present in only small amounts due to their susceptibility to BAL 31 exonucleolytic cleavage. The latter fragments appear as only minor bands due to their susceptibility, under the high salt conditions, to further cleavage at each of their three B-Z junctions. For pRW756, the two major BAL 31-specific bands (768 and 443 bp) map to $(dC\text{-}dG)_n$ terminated fragments resulting from BAL 31 nuclease cleavage at or near the B-Z junction distal to an Ava II site. The corresponding fragments resulting from cleavage by the BAL 31 nuclease proximal to an Ava II site that might also be expected are not clearly evident due to BAL 31 exonuclease activity on the fragments which do not contain (dC-dG) termini (discussed above).

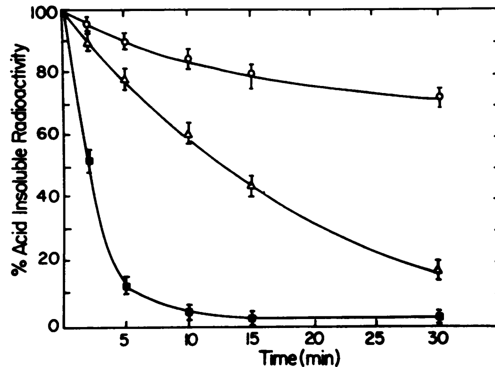

Fig. 4. Analysis of BAL 31 exonucleolytic activity. Tritiated samples (1-5 μg) of $(dC-dG)_n \cdot (dC-dG)_n$ under high salt condition (-O-), $(dC-dG)_n \cdot (dC-dG)_n$ under low salt condition (-Δ-), and chick erythrocyte DNA under low salt conditions (-■-) were treated with BAL 31 nuclease (1-10 units) and the loss of acid insoluble counts was monitored with time. Correction was made for the known lower exonucleolytic activity of the nuclease under the higher salt conditions. Furthermore, studies were performed at the appropriate higher enzyme levels. CD studies on the $(dC-dG)_n$ samples used in these determinations revealed the presence of a Z-conformation in high salt and a B-structure in low salt. Other details are in Materials and Methods.

BAL 31 Exonuclease Poorly Cleaves $(dC-dG)_n$

Figure 4 shows the digestion of right-handed $(dC-dG)_n$, left-handed $(dC-dG)_n$ and chick erythrocyte DNA by BAL 31 exonuclease. Tritiated samples of each of the DNAs were treated with the nuclease under the appropriate conditions and the loss of acid-insoluble material was monitored. We showed above that the BAL 31 nuclease possesses less activity under the higher salt concentration than it possesses under the lower salt concentration, and a suitable correction was made. It can be seen that the BAL 31 exonuclease will only slowly digest $(dC-dG)_n$ polymer compared with random sequence DNA. This is in good agreement with previous observations that the BAL 31 exonuclease degrades GC-rich regions of DNA more slowly than regions of lower GC content (14, 22) and supports well our reasoning for the detection of mainly $(dC-dG)_n$ terminated fragments following BAL 31 treatment and restriction analysis (Figs. 2 and 3). Additionally, it is apparent that exonucleolytic degradation of $(dC-dG)_n$ under conditions where it adopts a left-handed helix is much slower than under conditions where the polymer is right-handed, even after correction for the lower activity of the enzyme under the higher salt conditions.

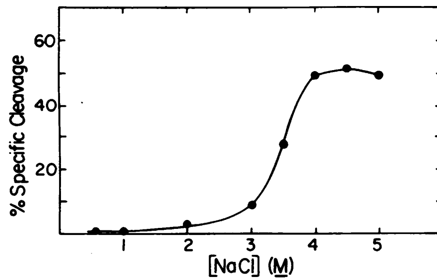

Fig. 5. The effect of NaCl on the amount of specific cleavage of pRW751 by BAL 31 nuclease. pRW751 (1-2 μg) in 12.5 mM $MgSO_4$, 12.5 mM $CaCl_2$, 20 mM Tris-HCl pH 8.1, 1 mM EDTA, and 0.6-5.0 M NaCl was treated with BAL 31 nuclease for 30 min. at 30°C. The BAL 31 was then inactivated with EDTA, the DNA restricted with Ava II and the resulting fragments analyzed on a 2% agarose gel. The gel was stained with ethidium bromide, and the DNA was visualised under ultra violet light. A photographic negative of the gel was traced with a Joyce-Loebl microdensitometer and the area under each peak was determined under conditions of known linear response. Other details are as described in Results.

Effect of NaCl on Specific Cleavage

Figure 5 presents the data derived from treatment of pRW751 with BAL 31 nuclease under different NaCl conditions (0.6-5.0 M). Following treatment with BAL 31 nuclease, the DNA then was digested with Ava II to monitor specific cleavage. The products of this treatment were analyzed on a 2% agarose gel. The amount of specific cleavage by the BAL 31 nuclease in the region of the $(dC\text{-}dG)_n$ blocks of pRW751 was determined from the ratio of the 1585 bp Ava II fragment (containing the $(dC\text{-}dG)_n$ blocks) to the 1743 bp fragment (see Fig. 3) relative to that for pRW451 subjected to identical treatment. The amount of specific cleavage is plotted against the sodium chloride concentration at which the BAL 31 treatment was performed. It is apparent that the appearance of cleavable junctions in the plasmid is a cooperative phenomenon with a midpoint, under the conditions of BAL 31 treatment, of 3.5 M NaCl. This result is in good agreement with the induction of left-handed helices in the $(dC\text{-}dG)_n$ blocks of pRW751 as a function of salt concentration as monitored by the observed relaxation of the plasmid (5). Direct comparison of these results, however, is difficult due to the presence of different salts in the two types of experiments.

Effect of Other Conditions on Specific Cleavage

The sensitivity of pRW751 to specific endonucleolytic cleavage by BAL 31 nuclease was tested under other conditions that have been shown (7) to induce

the formation of left-handed DNA in the $(dC\text{-}dG)_n$ segments of this plasmid. It was found (data not shown) that pRW751 is specifically cleaved by BAL 31 nuclease at the junction regions (similar to Figs. 2 and 3), if the nuclease treatment is carried out in the presence of 5 mM $MnCl_2$ and 25% ethylene glycol. Previous work (7) showed that these conditions induce the $(dC\text{-}dG)_n$ segments of pRW751 to adopt a left-handed conformation. Thus, we conclude that the specific cleavage by BAL 31 nuclease is due to the formation of left-handed helices within right-handed DNA.

Lack of Cleavage of Transition Intermediate

A possible alternate explanation is that the BAL 31 nuclease cleaves a transient intermediate structure associated with B-Z transition, rather than some stable feature of the B-Z junction. In order to investigate this possibility, we carried out the B-Z transition in the presence of the BAL 31 nuclease. BAL 31 nuclease was added to $^3H\text{-}(dG\text{-}dC)_n$ in low salt buffer and the loss of acid insoluble material with time was monitored. (Reaction conditions were similar to those used in Fig. 4.) An appropriate amount of high salt buffer then was added to induce the B-Z transition and the acid insoluble material was further determined. In parallel, the CD spectra of an identical sample were monitored to confirm that the $(dG\text{-}dC)_n$ was undergoing the expected B-Z transition. We found no increase in loss of acid insoluble material that would be expected if a transient intermediate was cleaved by the nuclease. Additionally, cleavage of $^3H\text{-}(dG\text{-}dC)_n$ by BAL 31 nuclease was studied over the salt concentration range (1.5 to 3.0 M) that causes the B-Z transition. Again, no cleavage of an intermediate was observed. Thus, any transiently occuring intermediate associated with the B-Z transition is not cleaved by the BAL 31 nuclease.

DISCUSSION

The BAL 31 nuclease specifically recognizes and cleaves some features of the junctions between left-handed and right-handed DNA. This behavior does not occur when these $(dC\text{-}dG)_n$ block containing plasmids are treated with BAL 31 nuclease under conditions where the $(dC\text{-}dG)_n$ blocks remain in a B-conformation nor does it occur when a control plasmid, which is lacking the $(dC\text{-}dG)_n$ blocks, is treated with BAL 31 nuclease under either condition. The major BAL 31-specific fragments produced correspond to segments terminating in the $(dC\text{-}dG)_n$ blocks.

Since the BAL 31 nuclease is the second single-strand specific nuclease (9) shown to specifically cleave B-Z junctions, it is tempting to hypothesize

that the the junctions (10) contain non-paired nucleotides. Model building studies suggest that steric constraints require at least one base-pair "slip out" within a B-Z DNA junction (23). However, the substrate requirements for both the S_1 and BAL 31 nucleases are uncertain at this level of sophistication. A detailed understanding of the properties of B-Z conformational junctions must await further studies.

The BAL 31 nuclease is an important probe for B-Z junctions since it can be used on non-supercoiled viral and cellular DNAs. At the present time, S_1 nuclease as a B-Z junction probe is restricted to supercoiled DNAs due to its salt inactivation. Furthermore, the parallel use of both the S_1 and BAL 31 nuclease assays provides assurance of the presence of a left-handed structure since different stabilizing agents, supercoiling and salt, respectively, are employed. This minimizes the possibility that other types of structural aberrations (1, 24, 25) are being recognized. The S_1 nuclease is a more precise probe since it does not contain the substantial exonucleolytic activity found in the BAL 31 nuclease. However, the BAL 31 nuclease is optimally active at physiological pH (14), is tolerant of a wide variety of reaction conditions (14), and can be utilized to probe both linear and circular DNAs for high salt induced conformational changes.

These studies confirm the previous observations (14,22) that the BAL 31 nuclease degrades GC-rich segments more slowly than regions of lower GC content. Furthermore, the (dC-dG) tracts are degraded even more slowly when in a left-handed structure (Fig. 4); however, detectable degradation does occur. We feel that it is highly unlikely that the nuclease is cleaving a _bona fide_ left-handed duplex structure but instead is degrading frayed ends or (dC-dG) regions which are transiently in a right-handed conformation.

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REFERENCES

1. 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.
2. Zimmerman, S.B. (1982) Annual Rev. Biochem. 51, 395-427.
3. Klysik, J., Stirdivant, S.M., Larson, J.E., Hart, P.A., and Wells, R.D. (1981) Nature 290, 672-677

4. Klysik, J., Stirdivant, S.M. and Wells, R.D. (1982) J. Biol. Chem. 257, 10152-10158.
5. Stirdivant, S.M., Klysik, J. and Wells, R.D. (1982) J. Biol. Chem. 257, 10159-10165.
6. Wells, R.D., Miglietta, J.J., Klysik, J., Larson, J.E., Stirdivant, S.M., and Zacharias, W. (1982) J. Biol. Chem. 257, 10166-10171.
7. Zacharias, W., Larson, J.E., Klysik, J., Stirdivant, S.M. and Wells, R.D. (1982) J. Biol. Chem. 257, 2775-2782.
8. Wartell, R.M., Klysik, J., Hillen, W. and Wells, R.D. (1982) Proc. Natl. Acad. Sci. USA 79, 2549-2553.
9. Singleton, C.K., Klysik, J., Stirdivant, S.M., and Wells, R.D. (1982) Nature 299, 312-316.
10. Singleton, C.K., Klysik, J., and Wells, R.D. (1983) Proc. Natl. Acad. Sci., in the press.
11. Peck, L.J., Nordheim, A., Rich, A. and Wang, J.C. (1982) Proc. Natl. Acad. Sci. 79, 4560-4564.
12. van de Sande, H., Zarling, D., and Jovin, T. (1982) The EMBO Journal 1, 115-120.
13. Legerski, R.J., Gray, H.B. Jr., and Robberson, D.L. (1977) J. Biol. Chem. 252, 8740-8746.
14. Gray, H.B. Jr., Winston, T.P., Hodnett, J.L., Legerski, R.J., Nees, D.W., Wei, C. -F. and Robberson, D.L. (1981) in Gene Analysis and Amplification. Vol. 2. Structural Analysis of Nucleic Acids, Chirikjian, J.G. and Papas, T.S., eds., pp 169-203, Elsevier North-Holland, Amsterdam.
15. Wei, C. -F. Legerski, R.J., Robberson, D.L., Alianell, G.A. and Gray, H.B., Jr. (1982) in DNA Repair: A Laboratory Manual of Research Procedures. Vol. 2, Friedberg, E.C. and Hanawalt, P.C., eds., Marcel Dekker, Inc., New York (in press).
16. Hardies, S.C., Patient, R.K., Klein, R.D., Ho, F., Reznikoff, W.S. and Wells, R.D. (1979) J. Biol. Chem. 254, 5527-5534
17. Wells, R.D., Larson, J.E., Grant, R.C., Shortle, B.E. and Cantor, C.R. (1970) J. Mol. Biol. 54, 465-497.
18. Rigby, P.W.J., Dieckmann, M., Rhodes, C. and Berg, P. (1977) J. Mol. Biol. 113, 237-251.
19. Gray, H.B., Jr., Ostrander, D.A., Hodnett, J.L., Legerski, R.J. and Robberson, D.L. (1975) Nucleic Acids Res. 2, 1459-1492.
20. Singleton, C.K., and Wells, R.D. (1982) Anal. Biochem. 122, 253-257.
21. Lau, P.P. and Gray, H.B. Jr. (1979) Nucleic Acids Res. 6, 331-357.
22. Legerski, R.J., Hodnett, J.L. and Gray, H.B. (1978) Nucleic Acids Res. 5, 1445-1463.
23. Drew, H.R. and Dickerson, R.E. (1981) J. Mol. Biol. 152, 723-736.
24. Singleton, C.K. and Wells, R.D. (1982) J. Biol. Chem. 257, 6292-6295.
25. Larsen, A., and Weintraub, H. (1982) Cell 29, 609-622.