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A Comparative Study for Supercoiled Pm2 DNA in Which Action Performed By Mung Bean Nuclease

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Abstract – Single strand specific mung bean nuclease was used to probe for regions of altered secondary structure in supercoiled PM2 DNA. Supercoiled DNA is cleaved >10,000 times faster than the relaxed topoisomer. Catalytic quantities of enzyme convert supercoiled DNA to both nicked-circular and unit length linear forms at pH 5 but to predominantly the nicked-circular form near neutral pH. At the elevated enzyme concentrations required to cleave relaxed DNA, unit length linear DNA and smaller fragments are produced from pH 5 to 7.

One nick per supercoiled DNA molecule is introduced at pH 6.6. The nicks are repairable by DNA ligase and are not strand-specific. Snake venom phosphodiesterase selectively cleaves the strand opposite the nicks, permitting restriction endonuclease mapping. The nicks occur at three specific sites. Sites at 0.75 and 0.76 map units are cleaved with equal frequency, while a site at 0.82 is cleaved less frequently. The former sites map near one of the eight known early denaturation regions of PM2 DNA, while the latter does not. Since most early denaturation sites are not cleaved, sites other than these dA + dT-rich regions may be the preferred locations of strand unwinding and separation in supercoiled PM2 DNA.

It is shown that local denaturation can be a natural consequence of supercoiling, even in environments where base pairing of linear DNA is energetically favored. Any change in the molecular total twist from its unstressed value is partitioned between local denaturation and smooth twisting in both the native and coil regions so as to minimize the total conformational free energy involved. Threshold degrees of torsional deformation are found for the existence of stable, locally melted conformations. As these thresholds are surpassed, the number of denatured bases increase smoothly from zero.

Existing experimental evidence regarding denaturation in supercoiled DNA is in good agreement with the predictions of this theory. In addition, from existing data one can estimate the partitioning of superhelicity between twisting and writhing. Possible consequences of stress-induced strand separation on the accessibility of the DNA to enzyme attack are discussed. Control of local melting by DNA topoisomerases and DNA gyrases could regulate diverse events involved in transcription, replication, recombination, and repair.

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INTRODUCTION

An endonuclease isolated from mung bean sprouts preferentially cleaves denatured as opposed to native DNA and thus has been called “single strand specific.” Mung bean nuclease has been purified to homogeneity and the activity on various regions of DNA characterized. Known singlestranded regions of DNA such as single-stranded tails and internal single-stranded gaps are preferentially cleaved, while nicks are relatively resistant. Transient, localized unwinding of duplex DNA structure may result in susceptible sites both within and at the ends of a linear molecule. Hydrolysis at these transiently single-stranded regions is slow relative to that of single-stranded DNA and is highly dependent on reaction conditions which affect the secondary structure of DNA . Model DNA hetero duplexes whose mismatched bases may be accommodated in a stacked helical structure are cleaved at extremely low efficiency when only one base is mismatched and at greater efficiency as the number of adjacent mismatched bases is increased.

We wanted to assess the feasibility of using mung bean nuclease to probe the structureo f negatively supercoiled DNA near neutral pH, away from the acidic pH optimum of the enzyme. Neutral pH is advantageous since it more closely approximates the pH that both DNA and proteins which interact with DNA experience *in uiuo.* In addition, spontaneous nicking of DNA near neutral pH is negligible compared to that at acidic pH. At acidic pH, mung bean nuclease introduces a limited number of nonrandom cleavages in both supercoiled and linear

forms of PM2 DNA. The locations of these sites were not determined.

In this paper, we demonstrate that mung bean nuclease is catalytically active on supercoiled PM2 DNA near neutral pH. We quantify the preference for the supercoiled over the relaxed topoisomer as a function of pH and characterize the products formed. We map the nicks introduced into supercoiled PM2 DNA near neutral pH utilizing venom phosphodiesterase as are agent to selectively cleaveth e strando pposite the nicks. Finally, we compare the locations of single strand character detected by mung bean nuclease to the locations of the known early melting sites in PM2 DNA.

Bacteriophage PM2 was first isolated from the marine pseudomonad, Pseudomonas BAL-31 (Espejo and Canelo, 1968). The DNA from this lipid-enveloped phage is a covalently close circular (ccc) double-stranded DNA (Camerini-Otero and Franklin, 1975). In vivo, the phage DNA is closely associated with ~ 300 molecules of a 6500 dalton polypeptide (protein IV) (Schafer and Franklin, 1975).

The remainder of the phage particle is composed of three other proteins (I, II and III) and an asymmetric phospholipid bilayer. The DNA isolated from PM2 has a high super helical density of σ= -0.12. This value was determined using ethidium bromide fluorescence (Lee and Morgan, 1978) and is the highest reported value for a natural ccc DNA molecule.

PM2 DNA was also reported to undergo an undefined cooperative transition with increasing salt, as monitored by binding to nitrocellulose (Kuhnlein et al., 1980). This finding, as well as the high salt medium requirement for host growth, made PM2 DNA an attractive candidate for containing Z-DNA regions at the naturally occurring superhelical density.

This report shows that the isolated superhelical form of PM2 DNA contains naturally occurring regions of Z-DNA. Antibodies specific for Z-DNA are shown to bind to PM2 form I, but not form II or form III, as demonstrated by competitive inhibition in a radioimmunoassay (RIA) and gel electrophoresis of the antibody:DNA complex. Direct visualization of the complexes by electron microscopy demonstrated the specificity and distribution of antibody binding along the PM2 DNA molecule in reference to the HpaII restriction site. These results are confirmed by topological analysis of the phage DNA.

METHODOLOGY

Mung Bean nuclease was isolated and purified as described by Kowalski et al. . Restriction endonucleases were purchased from New England BioLabs. Bacterial alkaline phosphatase was obtained from Worthington Biochemicals. Polynucleotide kinase was obtained from P-L Biochemicals. DNA : Bacteriophage PM2 DNA was purified as previously described. The DNA was >90% supercoiled as determined by electrophoresis on 1% agarose gels. The superhelical density of the DNA was -0.084.

Cleavage of Supercoiled PM2 DNA with Mung Bean Nuclease : Supercoiled PM2 DNA was cleaved in the absence and presence of NaCl. In the absence of NaCl the reaction mixture contained 10 mM Tris-HCl (pH 7.0), 32 }ig of PM2 DNA in a volume of 288 \il. In the presence of NaCl the reaction mixture contained the preceding components plus 0.1 M NaCl in the same volume.

After preincubation for 15 min at 37 C, mung bean nuclease (32 jil, diluted in 10 mM Tris-HCl, pH 7.0, 0.005% Triton X-100) was added and the mixture incubated at 37 C. The mung bean nuclease concentration in the reaction mixture was 0.1 units per ml in the absence of NaCl and 2 units per ml in the presence of NaCl. Reaction at 2 units per ml in the absence of NaCl gave the same site specificity as 0.1 units per ml. After 30 min incubation, which was sufficient to cleave all the supercoiled DNA, 0.1 M Na2EDTA (35 jil) was added and the mixture was cooled to 0 C. The enzyme was removed by extraction with phenol (saturated with 10 mM Tris-HCl (pH 7.4) 1 mM Na2EDTA).

DNA Sequencing : DNA fragments containing a single 5' end label were generated by cleavage of end-labeled DNA with restriction enzymes, and were isolated and purified according to the procedures described above. DNA was sequenced using the Maxam and Gilbert procedure. Products of the sequencing reactions were denatured by heating at 90 C for 2 min in a solution containing 1 mg per ml xylene cyanole, 1 mg per ml bromophenol blue, 98% formamide and 1 mM Na2EDTA and electrophoresed in 10% or 20% polyacrylamide-8 M urea slab gels (0.4 mm thick, 18 cm wide and 40 cm long) at 1400 volts for 6 h (10% gel) or 24 h (20% gel).

Each set of samples was loaded at two different times on each gel (10%:0 h and 3 h or 20%:0 h and 16 h) in order to examine different regions of the same DNA sequence. Mung bean nuclease cleavage products were denatured and electrophoresed in the same way. The gels were wrapped in Saran wrap and exposed to X-ray film both with and without intensifying screen (Cronex, Dupont) at -70 C for appropriate time periods.

RESULTS

Enzymatic Cleavage Rate *of* Supercoiled and Relaxed *DNA as* a Function *of* pH-A fluorometric assay for covalently closed circular DNA was used to measure the rate of the first endonucleolytic cleavage (single or double strand break). Although the activity is optimal at acidic pH , cleavage of supercoiled DNA is readily measurable at neutral and alkaline pH values at elevated enzyme concentrations. Initial rate measurements with supercoiled DNA at pH 5 and pH 8. The enzyme concentration at pH 8.0 (filled circles) is 75,000 times

that at pH 5.0 (open circles). Providing less than 50% of the substrate was cleaved and chemical compounds which stabilize the activity at acidic pH values were present (see under "Materials and Methods"), the cleavage of closed circular DNA substrate was linear with time and enzyme concentration at all pH values tested.

PM2 DNA was examined for its ability to compete with the binding of a polyclonal IgG preparation of anti-Z-DNA IgG to left-handed ^{32}P-labelled poly[d(G-m^{5}C)J. Two different assay conditions were tested. The 'high salt' condition was designed to maximize the extent of the Z-conformation in PM2 DNA by using high ionic strength and elevated temperature.

The 'low salt' assay was used to detect the presence of 'physiological' Z-DNA at physiological temperature and ionic conditions. The former assay was carried out in 4.0 M NaCl, 40 mM Tris-HCl (pH 7.2), 4.0 mM EDTA at 54°C and the latter assay was performed in 0.2 M NaCl, 40 mM Tris-HCl (pH 7.2), 4 mM MgCl2 at 37°C.

Although a ting bean nuclease cleavage sites have been mapped on the PM2 genome, these sites have not been located in restriction fragments of suitable size for nucleotide sequence analysis. Given an error of ±0.01 map units, it was predicted that the mung bean nuclease site at 0.75 map units would be located in Hind III fragment 4 (0.72-0.76 map units) and the site at 0.70 would be located in Hind III fragment 3 (0.63-0.72 map units). Confirmation of these predictions was necessary before attempting nucleotide sequence analysis. Supercoiled PM2 DNA was cleaved once per molecule by mung bean nuclease in the absence and presence of NaCl (see Materials and Methods). The products, mainly nicked circular and some linear DNA, were digested by restriction endonuclease Hind III and the resultant fragments ^{32}P-labeled at their 5' ends. The end-labeled DNA fragments were then denatured and electrophoresed in a 10% polyacrylamide-8 M urea gel.

DISCUSSION

Though the optimal pH is acidic, catalytic quantities of mung bean nuclease efficiently cleave supercoiled PM2 DNA under neutral pH conditions. The reaction is highly preferential for supercoiled DNA, since 28,000 times more enzyme is required to cleave the relaxed toposiomer at the same rate.

Supercoiled DNA is converted to singly nicked circular DNA. The nicked-circular DNA is resistant to linearization in the presence of at least 40 times the amount of enzyme required for complete conversion. The efficiency and limited nature of this reaction makes mung bean nuclease a useful alternative to pancreatic DNase I for the preparation of singly nicked PM2 DNA molecules. Since the nicks possess 3'-OH and 5"P termini, these molecules serve as substrates for DNA ligase , exonuclease 111, and DNA polymerase.

It is interesting that the single strand specific endonuclease activity of venom phosphodiesterase shows a similar preference (10,000-fold) fosr upercoiled over relaxedP M2 DNA. In contrast to mung bean nuclease hydrolysis of supercoiled DNA, however, the nicked-circular DNA produced does not accumulate but is rapidly linearized, presumably facilitated by gap formation by the associated 3' "+ 5' exonuclease activity. Since efficient linearization occurs whether the initial nick is generated by the enzyme itself, by DNase I, or by mung bean nuclease, venom phosphodiesterase appears to be an excellent reagent for specific cleavagofe the strando pposite nicks containing 3'-OH and 5'-P termini in duplex DNA.

The mung bean nuclease nicks in supercoiled PM2 DNA occur at three specific sites at a frequency at one nick per DNA molecule with no apparent strand specificity. Since several previous studies with supercoiled DNA showed a correlation between sites reactive to single strand specific agents and sites of early denaturation, it was interesting to compare the locations of the mung bean nuclease cleavages to the locations of the early denaturation sites determined by Brack et al.

Purified IgGs which specifically bind to Z-DNA have been used as probes to detect the presence of the Z-conformation in polytene chromosomes (Nordheim et al., 1981; Arndt-Jovin et al., 1983). In addition, studies involving cloned purine-pyrimidine inserts utilized these antibodies to demonstrate the right-to-left helical transition under physiological density (Nordheim et al., 1982c). Recently, a group of anti-ZDNA antibodies with varying specificities for the helix determinants have been characterized (Zarling et al., 1983). One of these polyclonal antibodies, T-4, a sequence-independent anti-Z-DNA IgG, was used to probe for the presence of left-2126

handed regions in PM2 DNA under high salt/high temperature or in physiological conditions. Both the results obtained by competitive RIA and the agarose gel electrophoresis demonstrate antibody binding to PM2 DNA under both ionic conditions. In both assays the IgGs do not bind to linear or relaxed PM2 DNA; thus, the binding is absolutely dependent on the superhelical state of the DNA. A similar superhelical dependency was observed for the Z-state in plasmids containing cloned (d(C-G)n or d(A-C)n d(G-T)n inserts (Peck et al., 1982; Nordheim and Rich, 1983; Haniford and Pulleyblank, 1983).

This paper has shown that the location of mung bean nuclease
cleavage sites in supercoiled PM2 DNA can change

with ionic environment and temperature. This report shows that the nature of the sequences cleaved by mung bean nuclease in supercoiled PM2 DNA change with ionic environment. In the absence of NaCl, a long dA+dT-rich sequence is cleaved. Previous studies mapped a major cleavage site at 0.75 map units or at 0.75 and 0.76 map units depending upon the resolution of the agarose gel.

The fine mapping results presented here show that there are actually multiple cleavages spanning 135 bp from 0.7451-0.7591 map units. The dA+dT-content of this region is enriched (74%) compared to that of the PM2 genome (57%).

REFERENCES

- Sung, S., and Laskowski, M., Sr. (1962) J. Biol. Chem. 237, 506-511 Johnson. P. H.. and Laskowski, M., Sr. (1970) J. Biol. Chem. 245,891-898
- Kowalski, D., Kroeker, W. D., and Laskowski, M., Sr. (1976) Biochemistry 15, 4457-4463
- Ghangas, G. S., and Wu, H. (1975) J. Biol Chem. 250, 4601-4606
- Kroeker, W. D., and Kowalski, D. (1978) Biochemistry 17, 3236-3243
- Chan, H. W., Dodgson, J. B., and Wells, R. D. (1977) Biochemistry 16, 2356-2366
- Kroeker, W. O., Kowalski, D., and Laskowski, M.. Sr. (1976) Biochemistry 15, 4463-4467
- Dodgson, J. B., and Wells, R. D. (1977) Biochemistry 16, 2374- 2379
- Nordheim,A. and Rich,A. (1983) Proc. NatI. Acad. Sci. USA, 80, 1821-1825.
- Griffith,J.P. and Christiansen,G. (1978) Annu. Rev. Biophvs. Bioeng., 7, 19-35.
- Beard, P., Morrow, J.F. and Berg, P. (1973) J. Virol. 12, 1303-1313.
- Dasgupta, S., Allison, D.P., Snyder, C.E. and Mitra, S. (1977) J. Biol. Chem. 252, 5916-5923.
- Germond, J.E., Vogt, V.N. and Hirt, B. (1974) Eur. J. Biochem. 43, 591-600.
- Funnell, B.E. and Inman, R.B. (1979) J. Mol. Biol. 131, 331-340.
- Tong, B.Y. and Battersby, S.I. (1979) Nucleic Acids Res. 6, 1073-1079.
- von Hippel, P.H. and Felsenfeld, G. (1964) Biochemistry 3, 27-39.
- Wingert, L. and von Hippel, P.H. (1968) Biochem. Biophys. Acta. 157, 114-126.