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Preferential site-dependent cleavage by restriction endonuclease PstI

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ABSTRACT

The four identical recognition sites for the restriction endonuclease PstI in purified plasmid pSM1 DNA I are cleaved at markedly different rates. The order and relative frequencies of cleavage at these four PstI sites have been determined from the order of appearance of partial cleavage products and from an analysis of production of specific unit length linear molecules. The same pattern of preferential cleavage is also found when linear, nicked circular, or relaxed closed circular forms of the same plasmid DNA are used as substrates for PstI. Inspection of the nucleotide sequences immediately adjoining each of the PstI sites suggests that the presence of adjacent runs of G-C base pairs confers significant resistance to cleavage.

INTRODUCTION

Type II restriction enzymes are endonucleases that recognize a defined DNA sequence, usually 4-7 bp in length, and effect a specific double strand break. It has been known for some time that at least some restriction enzymes cleave at certain of their identical recognition sites within a given DNA molecule more readily than at other sites.[1-5] We term this phenomenon preferential cleavage.

Davis and collaborators first observed that restriction endonuclease EcoRI preferentially cleaves the DNA's of bacteriophages lambda and P4.[1,2] Although the EcoRI sites in the middle of lambda linear DNA are cleaved much less frequently than the EcoRI sites near the ends, this bias does not hold for cleavage of P4 linear DNA by EcoRI.[2] Also, since the patterns of cleavage seen with lambda DNA's I and III were the same, these investigators concluded that the structural form of the DNA does not influence preferential cleavage. They therefore suggested that local nucleotide sequences influence preferential cleavage.

These results are in agreement with those of Rubin and Modrich[4], who digested small covalently closed supercoiled DNA's with EcoRI.

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These investigators showed that DNA I and DNA II of ColE1 are cleaved by EcoRI at the same rate. In addition, Rubin and Modrich[4] showed that double strand cleavage without dissociation of EcoRI from the substrate occurs to different extents with ColE1 DNA I and SV40 DNA I at 37°C. They concluded that sequences outside the EcoRI recognition site are responsible for these differences.

More recently, Nath and Azzolina[5] have confirmed that bacteriophage lambda DNA is preferentially cleaved by EcoRI as described by Thomas and Davis.[1] They further demonstrated that the BamHI and HindIII sites of lambda linear DNA show the same bias as the EcoRI sites: the sites near the ends are cleaved more frequently than those near the middle of the molecule. However, Nath and Azzolina[5] also reported that the linear form of plasmid pBR312 DNA is cleaved at least twice as rapidly as the circular form, suggesting that DNA structural type does indeed influence preferential cleavage.

In view of these somewhat contradictory results, we have reinvestigated the phenomenon of preferential double strand cleavage using the restriction endonuclease PstI with pSM1 plasmid DNA[6-8] as substrate. We find that PstI exhibits preferential cleavage with covalently closed circular supercoiled DNA I as substrate; that is, the rates of double strand scission at the four PstI sites of pSM1 DNA decrease in a specific order. The same basic pattern of preferential cleavage seen with pSM1 DNA I is also seen with covalently closed nonsupercoiled DNA I_0, with nicked circular DNA II, and with linear DNA III as substrates. The nucleotide sequence adjacent to the site at which cleavage is most highly inhibited is unique among the four PstI sites of pSM1 DNA in that runs of G-C base pairs are present at both the 5' and 3' sides of the site. We find no indication that proximity to either end of a linear DNA exerts a significant influence upon preferential cleavage.

MATERIALS AND METHODS

Bacterial strains and plasmids. Plasmid pSM1 in E. coli strain W677 has been described previously.[6-8] P. stuarti 164 was obtained from R. Roberts.

Media and chemicals. Penassay broth (antibiotic medium No. 3) and Difco agar were obtained from Difco. Optical grade CsCl and Ethidium Bromide (EtdBr) were purchased from the Harshaw Chemical Co., and

Calbiochem, respectively. Agarose, Tris, yeast tRNA, EDTA, and bromophenol blue were purchased from Sigma Chemical Co. Acrylamide, N,N'-methylene-bisacrylamide, N,N,N'N'-tetramethyethylene diamine (TEMED), and ammonium persulfate were electrophoresis purity reagents obtained from Biorad Laboratories. 8-hydroxyquinoline was purchased from Aldrich.

Enzymes. T4 DNA ligase was obtained from New England Biolabs. Restriction endonculeases PstI, BglII, and HindIII were purchased from New England Biolabs or from Bethesda Research Laboratories. EcoRI was obtained from Miles Laboratories. All enzymes were used as recommended by the manufacturers. PstI was also purified from P. stuarti 164 by the method described by Smith et al.[9] Bacterial alkaline phosphatase (BAPF) was obtained from Worthington Biochemical Corporation.

Purification of Plasmid DNA. Cultures of W677/pSM1 were lysed by the cleared lysis method using Brij and deoxycholate[10] as described elsewhere.[11] Plasmid DNA was purified from two sequential EtdBr/CsCl density gradients, and EtdBr and CsCl were removed as described earlier.[11]

Preparation of different DNA structural forms. Linear pSM1 DNA III was obtained by digestion of purified DNA I to completion using EcoRI. The reaction was terminated by heating at 65°C for 10 min. Enzyme was removed by two extractions with distilled, neutralized phenol containing 0.1% 8-hydroxyquinoline equilibrated with 0.1M Tris, pH 8.0. The solution was then twice extracted with ether. Ether was removed using N_2 gas, and the DNA was precipitated with ethanol after adding tRNA (2 µg/ml total volume) as carrier.

pSM1 DNA I_0 was prepared using DNA topoisomerase I purified from HeLa cell nuclei as described previously,[12] followed by phenol and ether extraction.

pSM1 DNA II was obtained by rebanding purified DNA I in EtdBr/CsCl and removing the top (less dense) DNA band with a syringe needle. Removal of EtdBr by isopropanol extraction and CsCl by dialysis has been described earlier.[11]

Time course digestion of DNA with PstI. Approximately 3.0 µg pSM1 DNA in 40 µl reaction mixture [6 mM Tris-HCl (pH 7.4), 6 mM $MgCl_2$, 50 mM β-mercaptoethanol, 100 µg/ml bovine serum albumin] containing PstI at a ratio of 0.6 or 0.8 units/µg were incubated at 30°C. At 15 min intervals for a total of 120 min, 3 µl were removed from the tube and

added to 10 µl TE (0.01M Tris, pH 8.0, 0.001 M EDTA). These aliquots were then treated at 65°C for 10 min to terminate the reaction. All solutions, tubes, and plastic pipet tips used were sterilized to avoid contamination by nucleases. Gels were stained with 0.5 µg/ml EtdBr, illuminated with short wave ultraviolet light to permit visualization of DNA, and photographed using Kodak Royal Pan film. The negative was traced using a Joyce Loebl microdensitometer. The peaks from the tracing were cut out, weighed, and the mole fraction X_i of species i was calculated from the relationship $X_i = (w_i/M_i)/\Sigma(w_i/M_i)$, where w_i is the weight in mg of peak i from the densitometer tracing, M_i is the molecular weight in megadaltons of a particular DNA fragment i, and the sum is taken over all relevant DNA fragments.

The different monomers, dimers, and trimers produced by limited digestion by PstI were identified by two methods: by their estimated molecular weights determined from agarose gel electrophoresis with HindIII fragments of bacteriophage lambda DNA as known size markers and also by cleavage with restriction endonucleases unique for each PstI fragment. Cutting sites for EcoRI, SalI, BstEII and BglII lie in PstI fragments A, B, C, and D, respectively, of pSM1 DNA[13-15] (see Fig. 1).

Gel electrophoresis. 1.0% agarose slab gels 15.5 cm X 14.0 cm X

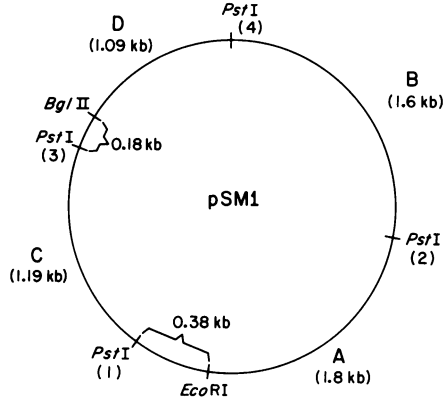


Figure 1.
Restriction map of pSM1 DNA showing the four PstI sites and the four fragments (A, B, C and D) that result from complete digestion with PstI. The number under each fragment letter is the size in kilobases of the fragment. The number in parentheses at each PstI site indicates the order in which the sites are cleaved by PstI (see text). The entire pSM1 DNA molecule is 5.67 kb in size.[14]

0.4 cm and 4.0% polyacrylamide slab gels (bis-acrylamide: acrylamide of 1:20) 15.5 cm X 17.0 cm X 0.2 cm were prepared with standard E buffer.[6] Polyacrylamide gels were allowed to polymerize at room temperature for approximately one hr before samples were loaded. Electrophoresis was carried out at 90-100 volts for 3-4 hr or until the tracking dye had migrated 75% of the length of the gel.

Analysis of PstI-cleaved unit length linear molecules. pSM1 DNA I was digested with limiting amounts of PstI and analyzed after electrophoresis in a 1.0% agarose gel stained with EtdBr. In those digests in which unit length linear molecules comprised a significant fraction (30%) of the digestion products, the linear molecules were excised from the gel and run into a 4.0% polyacrylamide gel. The unit length linear DNA was excised from the second gel after staining and was isolated by electrophoresis into dialysis tubing. The DNA solution in the tubing was 3 times ethanol precipitated, and the final precipitate was dried under vacuum, resuspended in 20 µl of reaction mixture for BglII and incubated at 37°C in the presence of excess BglII. The products of these BglII digests were visualized after electrophoresis at 4°C in a 0.75% agarose gel (28 cm X 14 cm X 0.15 cm) by EtdBr staining. The mole fractions of the 5.5, 4.3, and 3.2 kb fragments were determined from microdensitometer tracings using the relationship described above, where the sum was taken over only the larger of the two fragments produced from each linear DNA species by digestion with BglII.

Recombinant DNA techniques. Conditions for ligation using T4 ligase and transformation of $CaCl_2$-treated E. coli have been described elsewhere.[16] Plasmid DNA was purified from 30 ml cultures of transformants by the rapid purification technique described by Kahn et al.[17]

RESULTS

The plasmid pSM1 is a 5.67 kb[7] derivative of the 89.3 kb drug resistance plasmid R12.[6] Preferential cleavage of pSM1 DNA by PstI[9,18] was observed when the PstI to DNA ratio was lowered in an attempt to produce linear, unit length pSM1 DNA molecules instead of the four fragments (A, B, C and D) produced by limit digest[13] with this enzyme (Fig. 1). When aliquots of these non-limit digests were examined by agarose gel electrophoresis, the dimer composed of PstI fragments D and B (DB) was formed significantly more rapidly and persisted longer than

the other two visible dimers, AC and CD (Fig. 2, lanes i and j). Also, the monomer fragments A and C were initially present in excess compared to the B and D monomers (Fig. 2, lanes e and f). These observations indicated that the PstI site between fragments B and D was cleaved less rapidly than the other PstI sites. This preferential inhibition of cleavage at the D-B junction occurred with three preparations of PstI, both commercial and non-commercial, that were used in these experiments and does not seem to be a property of a particular preparation of PstI.

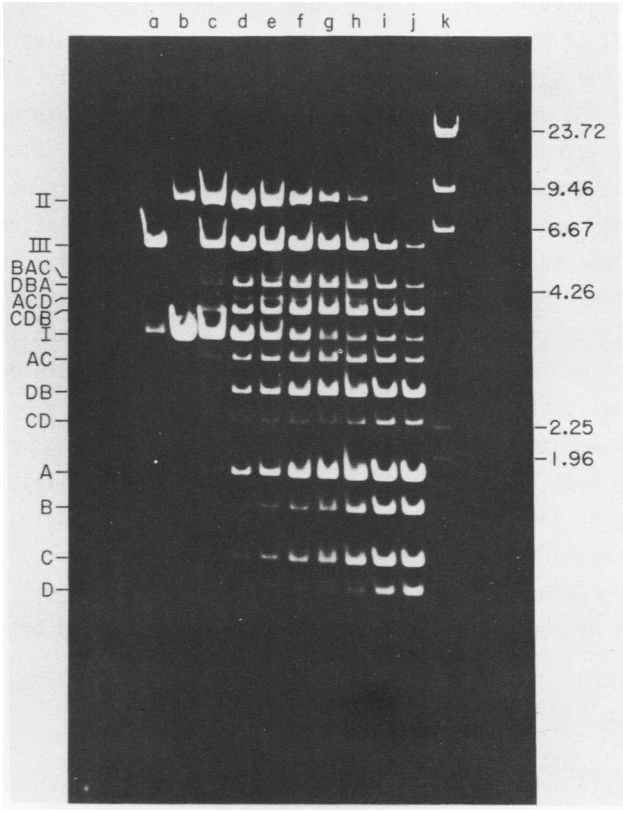


Figure 2.
1.0% agarose gel showing time course of cleavage of pSM1 DNA I by PstI. Purified pSM1 DNA I was incubated at 30°C with PstI (N.E. Biolabs). Aliquots were withdrawn at 15 min intervals, heat inactivated, and analyzed by agarose gel electrophoresis. The numbers on the right represent the sizes in kilobases of λ DNA HindIII fragments (lane k). Purified pSM1 DNA's I, II, and III are shown in lane a. Aliquots withdrawn at 0, 15, 30, 45, 60, 75, 90, 105 and 120 min are in lanes b, c, d, e, f, g, h, i and j, respectively.

Determination of the order of cleavage of the PstI sites of DNA I. The relative rates of cleavage at the remaining three PstI sites of pSM1 DNA I were next examined. We first determined which of the four PstI sites on pSM1 DNA I was the first to be cleaved by PstI using the procedure described below. In addition to four PstI sites, pSM1 DNA contains one cleavage site for BglII.[14] Therefore, after pSM1 DNA is cut by PstI once to produce a specific unit length linear molecule, subsequent cleavage with BglII[19] will produce two fragments whose sizes depend upon which PstI site had been cleaved originally. If the first PstI site cleaved is located at the B-A junction, digestion with BglII will result in the production of two fragments of sizes 2.5 and 3.2 kb. Alternatively, if the first PstI site cleaved is located at the A-C, C-D, or D-B junctions, digestion with BglII will produce fragments of 1.4 and 4.3 kb, 0.2 and 5.5 kb, or 0.9 and 4.8 kb, respectively (Fig. 3).[13,14]

After limited digestion with PstI, linear unit length pSM1 DNA molecules were isolated and treated with excess BglII. The products of this digestion were examined after electrophoresis in an agarose gel and quantitated as described in Materials and Methods. Out of eight possible fragments, five fragments of sizes 5.5, 4.3, 3.2, 2.5, and 1.4 kb were detected on the gel (Fig. 4, lane a). With the exception of the smallest expected fragment (0.2 kb), which presumably migrated off the gel, the fragments observed would have been produced by BglII cleavage

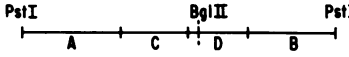
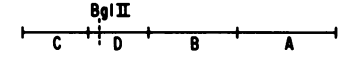
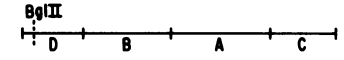
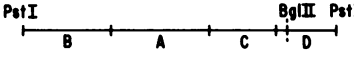
Location of PstI Site Cleaved	PstI-Generated Linear Molecules	Fragment Sizes Subsequent to BglII Cleavage	Mole Fraction of Larger Fragment
B-A Junction		3.2 + 2.5	0.25
A-C Junction		4.3 + 1.4	0.57
C-D Junction		5.5 + 0.2	0.18
D-B Junction		4.8 + 0.9	nd

Figure 3.
Schematic representation of the four linear unit length pSM1 DNA's each derived from a single PstI cleavage event. The four PstI fragments of pSM1 are lettered A-D.[14] Linear pSM1 DNA molecules each resulting from one cleavage event by PstI at the B-A, A-C, C-D, or D-B junctions are shown. The location of the single cleavage site for BglII is indicated for each linear species.

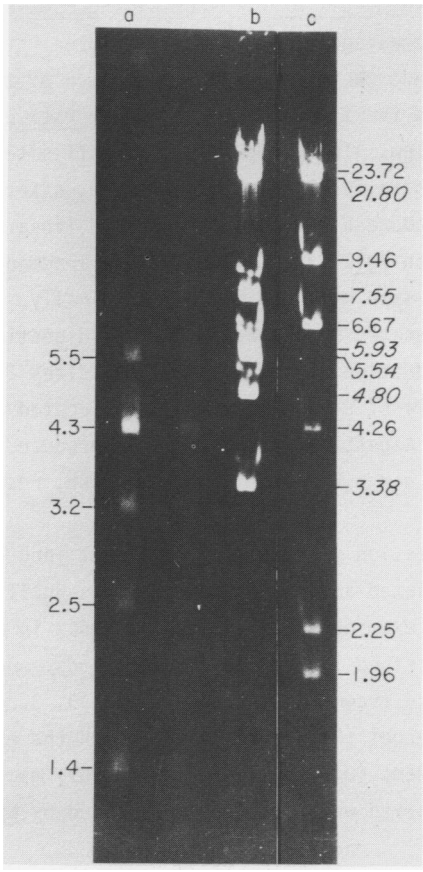


Figure 4.
A 0.75% agarose gel of cleavage products resulting from successive cleavage by PstI and BglII. PstI-derived unit length pSM1 DNA was prepared as described in Materials and Methods. The numbers to the left of the Figure are the sizes in kilobases of pSM1 DNA fragments generated after BglII cleavage of linear molecules. The numbers to the right of the Figure are the sizes in kilobases of DNA fragments produced by cutting λ DNA with EcoRI (lane b, italicized) or HindIII (lane c). The fragments (4.8 and 0.9 kb) expected from BglII cleavage preceded by PstI cleavage at the D-B junction were not present in detectable quantities.

preceded by one PstI cut at the B-A, A-C, or C-D junctions (Fig. 3).

The 4.8 and 0.9 kb fragments expected after PstI cleavage at the D-B junction followed by BglII cleavage were not present in measurable amounts. This finding supports our initial observation, based on the persistence of the DB dimer, that the PstI site at the D-B junction is

cleaved least frequently (Fig. 2). It was also apparent that the most abundant fragment produced by BglII cleavage of unit length pSM1 linear molecules was 4.3 kb in size (Fig. 4, lane a). This is one of the two fragments expected if the first PstI cut is at the A-C junction. Therefore, the PstI site cleaved most frequently was at the A-C junction.

The relative mole fraction of each of the four possible PstI-derived linear species can be estimated by determining the relative amount of either one of the two fragments that result from BglII cleavage, since the mole fractions of these two fragments are equal. Accordingly, the mole fraction of the 4.3 kb fragment is 0.57 and the mole fractions of the 3.2 and 5.5 kb fragments are 0.25 and 0.18, respectively. Therefore, on the average, the PstI site cleaved second most frequently is at the B-A junction, and the site cleaved third most frequently is at the C-D junction. The PstI site at the D-B junction is cleaved very rarely in comparison to the other sites. These results are summarized in Figure 3.

In this analysis we have assumed that the mole fractions of each of the four PstI-derived unit length linear species provide a direct measure of the relative rate of scission at the single PstI site which is cleaved during formation of each unique linear molecule. This assumption is valid even though the relative net amounts of each of the four linear species present at any time are determined by the rates both of formation (as described) and of degradation (by cleavage at the three remaining PstI sites internal to each linear molecule) because PstI scissions subsequent to the initial PstI cleavage event would degrade the three remaining unit length linear molecules uniformly. For example, excluding the unit length linear molecule formed by PstI cleavage at the A-C junction, there are three unit length linear molecules which contain this junction at an internal position. The rates at which these three unit length linear molecules are lost due to PstI cleavage at the internal A-C junction are expected to be the same because the location of a specific site within a DNA molecule appears not to influence the susceptibility to cleavage (unpublished results). Because the same reasoning applies to the other three PstI sites of pSM1 DNA, the relative proportions of the four unit length linear species at early times are expected to indicate the relative order of the frequencies of initial PstI cleavage at the four PstI sites. This

conclusion holds in spite of the fact that subsequent internal cleavage by PstI might also have occurred.

Time course of PstI cleavage of DNA. To confirm the order of cleavage deduced from the analysis of linear molecules, the course of digestion was followed over time using purified pSM1 DNA I and limiting PstI. Aliquots of the digests were examined on an agarose gel (Fig. 2), and a photographic negative of this gel was analyzed as described in Materials and Methods.

Figure 5 shows the relative amounts of trimer, dimer, and monomer fragments which appeared during the course of the reaction. The rates of formation of the four possible trimer fragments were in the order CDB>DBA>ACD>BAC (Fig. 5a). The trimer fragments that contain both the B and the D fragments (CDB and DBA) were formed much more readily than the trimers that did not contain both B and D (ACD and BAC). This again supports the observation made previously that the PstI site at the D-B junction is cleaved relatively infrequently.

Also, of the two trimers that contain both B and D, at any given time the amount of the trimer that lacks the A fragment (CDB) was greater than that which lacks the C fragment (DBA). The amount of CDB present would be expected to be greater than the amount of DBA if the PstI sites flanking the A fragment (at the A-C and B-A junctions) were more readily cleaved than are the sites flanking the C fragment (at the A-C and C-D junctions). Since the A-C junction flanks both the A and C fragments, it would seem that cleavages at the B-A and C-D junctions are rate limiting in the production of CDB and DBA, respectively. Furthermore, the amount of CDB would be greater than that of DBA if the site at the B-A junction was cleaved more frequently than the site at the C-D junction. This deduced order is in agreement with the order of cleavage determined from analysis of linear molecules, as described in the previous section.

The dimer fragments present during the course of the reaction in order of decreasing abundance were DB>>AC>CD (Fig. 5b). The DB dimer was present in great excess, and this again shows that the PstI site at the D-B junction is cleaved least frequently of the four PstI sites of pSM1 DNA. The AC and CD dimers were next most abundant. The AC dimer was present in amounts greater than the CD dimer at early times in the reaction because the production of the CD dimer requires a PstI cut at the D-B junction, and this cut is only rarely made.

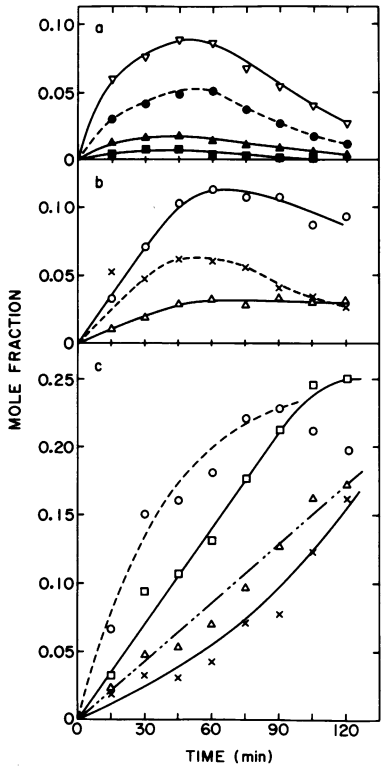


Figure 5.

Production of trimer, dimer, and monomer fragments from pSM1 DNA I cut by PstI. The gel in Fig. 2 was photographed and the negative was traced and analyzed as described in Materials and Methods. For determination of the mole fractions of partial digestion products as described in Materials and Methods, the sum is taken over all bands in each lane of the gel. In the 1.0% agarose gel shown in Fig. 2, the dimer BA migrates at the same position as DNA I, so the amount of BA could not be determined in these reactions.

A, trimer fragments: ▽-▽, CDB; ●---●, DBA; ▲-▲, ACD; and ■-■, BAC.
B, dimer fragments: O-O, DB; X---X,AC; and Δ-Δ, CD
C, monomer fragments: O---O, A; Δ-Δ, B; □-□, C; and X--X, D.

The monomer fragments generally appear, in decreasing abundance, in the order A>C>B>D (Fig. 5c). This order is consistent with the ordering of the cleaved sites obtained from analysis of the kinetics of appearance of trimer, dimer, and unit length linear DNA molecules; that is, if the order of cleavage of the different PstI sites is the A-C junction >> the B-A junction > the C-D junction >>> the D-B junction,

the monomers should appear in the order A>C>B>D.

Construction of recombinant plasmids using non-limit digests of pSM1 DNA. The order of cleavage of pSM1 DNA by PstI determined by gel analysis as described above is supported by experiments in which non-limit PstI digests of pSM1 DNA were ligated to DNA of the plasmid vector, pKA01.[16,20] The pKA01 DNA, which contains a single PstI site,[20] had been completely cleaved with PstI and treated with bacterial alkaline phosphatase prior to ligation.[16] Following transformation with the ligated DNA, plasmid DNA from many individual transformants was isolated by the rapid plasmid purification procedure[17] and analyzed by agarose gel electrophoresis following limit digestion with PstI. Not all possible combinations of PstI fragments of pSM1 were found in these recombinant plasmids. In particular, not a single recombinant plasmid was recovered which contained any multimeric combination of PstI fragments requiring cleavage at the D-B junction. All other possible combinations of fragments (those not requiring cleavage at the D-B junction) were recovered, however.

Influence of DNA structural form on preferential cleavage. pSM1 DNA's I_0 (relaxed closed circular), II (nicked circular), and III (linear) were prepared and purified as described in Materials and Methods. These DNA's were cleaved with limiting PstI, and the products were analyzed by the same procedure described above for DNA I. DNA I_0 was cleaved to determine if the presence of superhelical turns affects preferential cleavage. DNA III was used because it has been reported that proximity to termini enhances cleavage in linear DNA.[5] Cleavage of these three structural forms of the DNA by PstI revealed, however, no differences between linear and circular DNA's (data not shown). The rates of production and also the relative abundances of different trimer, dimer, and monomer fragments were the same as for pSM1 DNA I.

DISCUSSION

We have demonstrated that purified pSM1 DNA is preferentially cleaved by PstI and that the basic pattern and rate of cleavage appear not to be influenced by the superhelicity or linearity of the substrate. These results are in agreement with those reported by Thomas and Davis[1] and Rubin and Modrich[4] for cleavage of lambda, P4, and ColE1 DNA's with EcoRI. However, because a different enzyme was used in these previous studies, we cannot be certain that the mode of action of the two

restriction enzymes is the same. Our results do not allow us to determine whether the preferential effects of PstI cleavage occur at the level of site recognition (and binding) or of DNA scission .

Apart from the effects of tertiary structure, at least five features of duplex structure could produce differential cleavage rates at various restriction sites. These include: 1) proximity to the ends in a linear DNA molecule[5]; 2) variation in DNA sequence within the recognition sites for enzymes such as HinfI and BstEII; 3) nearby regions of unusual DNA secondary structure such as Z DNA[21,22]; 4) variations in the DNA sequences adjacent to particular recognition sites; and 5) the presence of nearby special duplex structures such as loops, hairpins, or cruciforms. These factors could affect both the accessibility of a recognition site to the restriction enzyme and the stability of that site with regard to base-base interactions.

The first three of these factors are unimportant in the present case because 1) proximity to duplex termini did not influence preferential cleavage (pSM1 DNA's I and III have the same pattern and rate of preferential cleavage); 2) all PstI recognition sites have identical sequences; and 3) no runs of alternating G-C base pairs long enough to have unusual duplex structure, such as Z DNA,[21,22] are adjacent to any PstI site in pSM1 DNA (Table 1).

Factors four and five could, however, contribute to the

Table 1 Nucleotide Sequences Adjacent to PstI Sites of pSM1 DNA

Junction	Nucleotide Sequence
D-B	TGATGCGCTCCTGCAGGGGCTGTGT ACTACGCGAGGACGTCCCCGACACA
B-A	TAATCAATATCTGCAGTTTATGCTGG ATTAGTTATAGACGTCAAATACGACC
A-C	AACATGGCAACTGCAGTTCACTTACA TTGTACCGTTGACGTCAAGTGAATGT
C-D	CAGAAAACTGCTGCAGATGACCGGAG GTCTTTTGACGACGTCTACTGGCCTC

Nucleotide sequences of pSM1 DNA are described elsewhere.[14,23] The PstI recognition sequence is underlined, and the sites of scission are indicated by arrows.

preferential cleavage reported here. As described above, the site in pSM1 DNA least susceptible to PstI cleavage lies at the D-B junction. This site is located only 16 bp away from a possible stem-loop structure containing a loop of 2 bases and a stem 14 bp in length.[14] The three remaining PstI sites are located at least 150 bp away from possible regions of unusual secondary structure.[14,23] Any actual influence of such putative stem-loop structures upon preferential cleavage is, however, rendered unlikely by the identical cleavage rates for supercoiled and nonsupercoiled DNA's. It has been reported[24] that supercoiling is required for cruciform formation.

The PstI site at the D-B junction is also unique among the four PstI sites in that the immediately adjacent nucleotide sequences are unusually rich in G-C pairs (Table 1). G-C pairs are thermodynamically more stable than A-T pairs. Also, although only deoxynucleotide hexamers and octamers of sequence (CpGpCpGpCpGp) are known to form left-handed Z DNA structures,[21,22] we cannot rule out the possibility that a tetramer of this sequence could also form an atypical helical structure. A tetramer of alternating G-C base pairs is situated close to the D-B junction. It is, therefore, possible that the high G-C content of the nucleotide sequences adjoining the D-B junction contributes to the marked resistance to PstI cleavage exhibited by this site either by increasing the thermodynamic stability or by distorting the normal duplex structure at this site.

The thermodynamic data presently available[25,26] do not, however, permit reliable stability calculations for DNA in solution. Further experiments are in progress employing other restriction enzymes in order to determine the contributions of special structures and of relative thermodynamic stability to preferential cleavage. However, on the basis of our results as well as those of other investigators, preferential cleavage is likely to occur with many or all restriction endonucleases. This phenomenon could greatly limit the ability to purify or to clone certain multimeric restriction fragments.

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