AN ALTERNATIVE PROCEDURE FOR THE STRAND SEPARATION OF DNA FRAGMENTS

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1. Introduction

The separation of the complementary strands of DNA fragments is of great advantage in sequence analysis [1]. Strand separation can often be achieved by gel electrophoresis of the dissociated strands, possibly due to different conformations of the two strands acquired by intrastrand folding [2].

We have devised a different method for the separation of complementary DNA strands which takes advantage of the property of a number of restriction nucleases to produce fragments with protruding 5′ or 3′ ends [3]. Complementary strands of fragments obtained by digestion with 2 different restriction nucleases may thus differ in length. This difference can be as large as 9 nucleotides, e.g., in fragments cleaved by *Eco*RII and *Pst*I. An analysis of the resolving power of sequencing gels suggested that such a difference should be sufficient for the separation of DNA strands up to 300 nucleotides long. This observation is the basis for the preparative method of DNA strand separation described in this work.

2. Materials and methods

Bovine 1.711a satellite DNA (density in CsCl, 1.711 g/cm^3) was isolated from calf thymus DNA according to [4] using the modifications in [5]. Bovine 1.715 satellite DNA (satellite I; density in CsCl, 1.715 g/cm^3) was purified according to [6]. Restriction nucleases were purified as in [7]. DNA sequence analysis was done as in [1]. A fifth sequencing reaction specific for purines was used [8]. The preparative separation of complementary DNA strands was achieved in 0.5–0.7 mm thick 6% and 8% acrylamide gels containing 8.3 M urea. Other conditions of gel electrophoresis were as for sequencing gels [1].

3. Results and discussion

The DNA fragments used in this work were derived from bovine 1.711a and 1.715 satellite DNA [4]. The 1.711a satellite DNA has a repeat unit of 1413 basepairs of which there are ~35 000 copies in the bovine genome [9]. A 420 basepair fragment was isolated from a *Hin*fI digest of this satellite DNA. The fragment contains one *Sau*3A and two *Sst*I cleavage sites (fig.1) the location of which was confirmed by sequence analysis of the complete repeat unit [9]. By cleavage of this fragment with both *Sst*I and *Sau*3A four fragments are obtained all of which carry one protruding 5′- and one protruding 3′-end. According to the specificity of the restriction nucleases used, the lengths of the complementary strands of these fragments differ by 7 (A,D) and 8 (B,C) nucleotides. This should be sufficient to allow a strand separation according to chainlength in a denaturing polyacrylamide gel under conditions similar to those used for sequence analysis.

The 420 basepair *Hin*fI fragment was digested with both *Sau*3A and *Sst*I. The mixture of DNA fragments obtained was treated with alkaline phosphatase and terminally labelled with [^{32}P]phosphate [1]. The DNA sample was dissolved in 50 μl 66% formamide containing xylene cyanol and bromphenol blue as marker dyes, denatured for 2 min at 100°C, chilled in

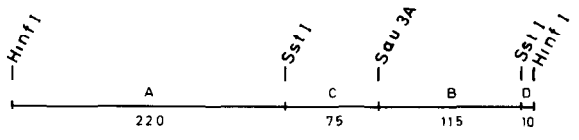

Fig.1. Location of the *Sst*I and *Sau*3A cleavage sites in the 420 basepair *Hin*fI fragment derived from bovine 1.711a satellite DNA. Distances between cleavage sites are given in basepairs.

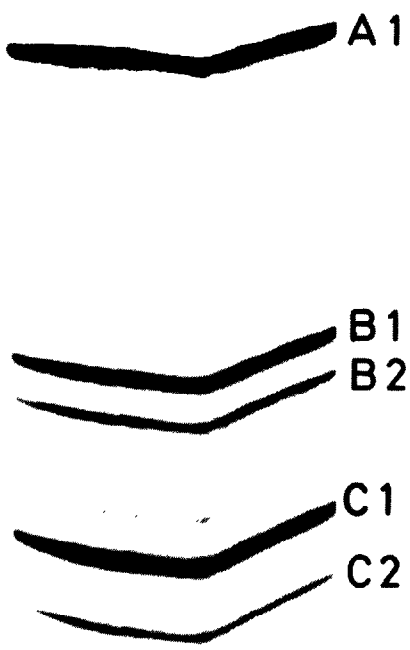

Fig.2. Autoradiogram of a strand separation gel. The 420 basepair *Hin*fI fragment (fig.1) was digested with *Sst*I and *Sau*3A, terminally labelled, denatured, and loaded on an 8% acrylamide gel (200 × 400 × 0.5 mm) containing 8.3 M urea as described in the text.

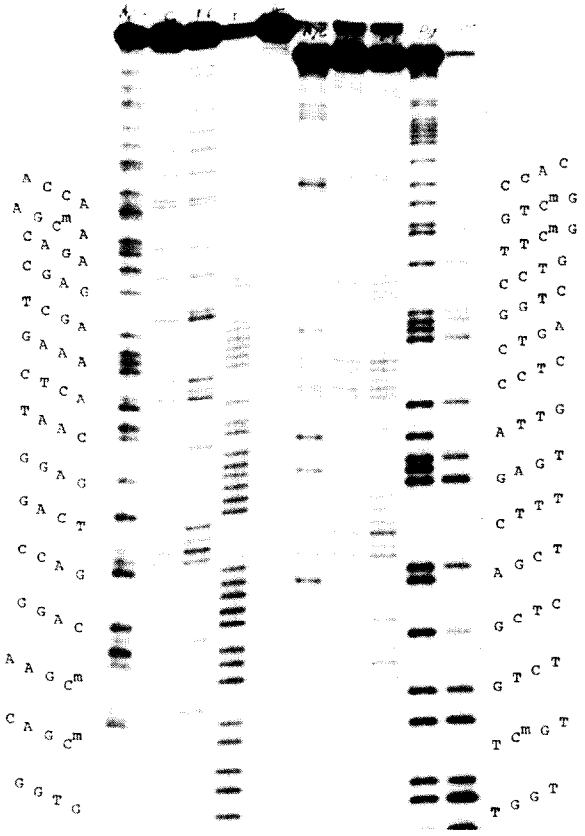

Fig.3. Autoradiogram of an 8% sequencing gel of fragments B1 (left of the gel) and B2 (right of the gel). C^m designates 5-MeCyt.

ice and applied to an 8% polyacrylamide gel containing 8.3 M urea. The electrophoresis was done at 1200 V until xylene cyanol passed 27 cm.

The autoradiogram of the gel is shown in fig.2. There are 3 groups of bands corresponding to fragments A–C. The smallest fragment D (fig.1) had run off the gel. In the pattern observed minor bands were present in addition to those two expected for each fragment. This may be due to a sequence heterogeneity in the satellite DNA or more likely to the nicking activity of *Sau*3A cleaving at several sequences similar to the canonical recognition site [7,10]. However, this does not interfere with the demonstration of the strand separation method.

Individual bands were cut out from the gel, the fragments were eluted and submitted to sequence analysis. Sequencing gels of B1 and B2 are shown in fig.3. The gels could be read without ambiguity, and it is clear from the sequences determined that B1 and B2 correspond to the complementary strands of fragment B. It was similarly shown by sequence analysis that C1 and C2 correspond to complementary strands.

The different labelling of the complementary strands (fig.2) is most likely due to the fact that the longer strands carry the protruding 5′-end of the original fragment, the shorter ones the recessed 5′-end which is less accessible to polynucleotide kinase [1]. A1 was shown by sequence analysis to correspond to the longer strand of fragment A. The strand complementary to A1 has not been found, possibly because of the nicking activity of *Sau*3A. Some of the minor fragments seen in fig.2 may correspond to cleavage products of this strand. Alternatively, the recessed 5′-end of fragment A may not have been labelled.

The method can also be used for the preparation of unlabelled complementary strands of DNA fragments obtained with appropriate pairs of restriction nucleases. This is shown for a 260 basepair *Eco*RI–*Sst*I fragment isolated from bovine 1.715 satellite DNA (fig.4).

Previous methods for the separation of comple-

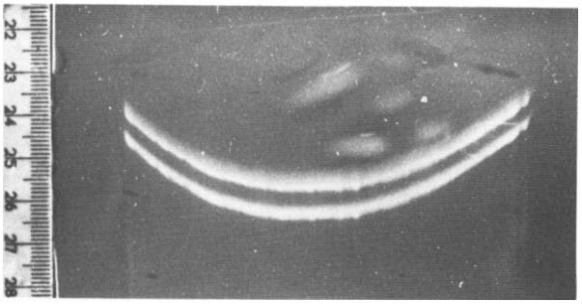

Fig.4. Preparative separation of complementary strands of a 260 basepair *Eco*RI–*Sst*I fragment obtained from bovine 1.715 satellite DNA. Dimensions of the gel 120 × 400 × 0.7 mm; xylene cyanol migrated ~70 cm. The photograph was obtained after staining with ethidium bromide under UV illumination.

mentary DNA strands by gel electrophoresis [1,2] have quite often been unsuccessful (see, e.g., [11]). Since sequencing DNA according to Maxam and Gilbert [1] is greatly facilitated by strand separation of the DNA fragments, alternative methods of strand separation are highly valuable. The usefulness of the alternative procedure described here has been demonstrated by sequencing fragments of a bovine satellite DNA. This procedure requires DNA fragments to be generated by restriction nucleases producing protruding 5′- and 3′-ends. It does not depend, however, on the base composition or the secondary structure of the individual strands and therefore should be widely applicable.

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