Relaxation of *Pvu*II recognition sequence

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The substrate specificity of *Pvu*II endonuclease is relaxed in the presence of dimethyl sulfoxide. The new recognition sequences cleaved in pBR322 DNA have been found to be CCGCTG, CATCTG, CAGATG, CAGGTG and CAGCGG.

Restriction endonuclease *Recognition sequence* *Specificity relaxation*

1. INTRODUCTION

The specificity of several restriction endonucleases is relaxed by modifying the reaction conditions [1-3]. In most cases the nucleotide sequences of new sites correspond to shortened sequences derived from the standard site [1,4,5].

In the presence of DMSO, the specificity of *Pvu*II endonuclease is relaxed and the enzyme cuts pBR322 DNA at several sites (>15). This secondary enzyme activity is referred to as *Pvu*II*, as designated for *Eco*RI* [1]. We have determined the new recognition sequences of *Pvu*II* and find that it cleaves degenerate sequences derived from the normal sequence (CAG↓CTG) : CCG↓CTG, CAT↓CTG, CAG↓ATG, CAG↓GTG and CAG↓CGG at the positions indicated by the arrows, producing fragments with flush ends.

2. MATERIALS AND METHODS

*Pvu*II restriction endonuclease was purchased from New England Biolabs Inc. pBR322 DNA was purchased from Boehringer Mannheim. Standard conditions of reaction for *Pvu*II were: 6 mM Tris-HCl (pH 7.5), 6 mM $MgCl_2$, 50 mM NaCl and 6 mM 2-mercaptoethanol. Modified conditions of reaction were: standard conditions + 15% (v/v) DMSO.

The sequences present at the 5′-termini of the *Pvu*II* fragments were analysed as follows: pBR322 DNA (20 μg) was digested with *Pvu*II endonuclease, in the presence of 15% DMSO, and the multiple fragments obtained were dephosphorylated at their 5′-ends by treatment with alkaline phosphatase and then labelled with ^{32}P using polynucleotide kinase and [γ-^{32}P]ATP [6]. Excess ATP was eliminated by filtration through a Sephadex G-50 column. To obtain DNA molecules labelled at only one of the 5′-ends, the labelled DNA fragments were cleaved with *Hin*fI endonuclease and separated by 8% polyacrylamide gel electrophoresis. The desired fragments were eluted from the gel and sequenced by the method of Maxam and Gilbert [7].

Abbreviations: bp, base pairs; DMSO, dimethyl sulfoxide

3. RESULTS AND DISCUSSION

Under the standard reaction conditions, *Pvu*II endonuclease converts circular pBR322 DNA to the linear form (fig.1, track 2). In the presence of DMSO, *Pvu*II hydrolyzes pBR 322 DNA at multiple sites. Fig.1 illustrates these results: the maximum effect is obtained with 15% DMSO (fig.1, track 5).

By terminal labelling with polynucleotide kinase and [γ-^{32}P]ATP of *Pvu*II* fragments, DNA

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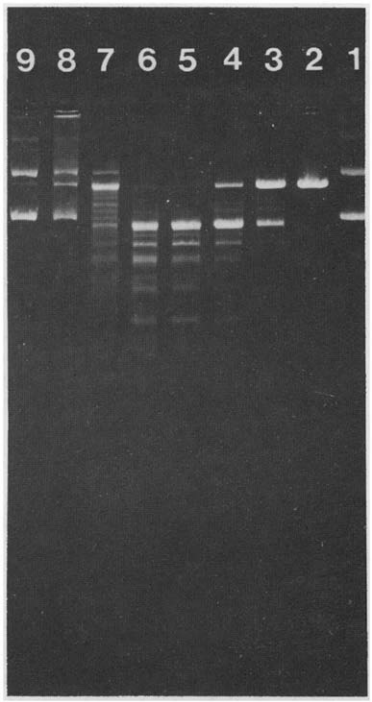

Fig.1. Effect of DMSO on *Pvu*II* activity under standard conditions. 0.5 μg pBR322 DNA was digested with 30 units of *Pvu*II endonuclease in the absence or presence of DMSO for 6 h, 30 min at 37°C. (2) 0%; (3) 5%; (4) 10%; (5) 15%; (6) 20% (7) 30%; (8) 50%; (9) 60% (v/v). (1) undigested pBR322 DNA.

molecules are obtained with two labelled 5′-ends, whereas the Maxam and Gilbert technique [7] requires DNA fragments with only one labelled terminus. To obtain a number of DNA fragments labelled at only one of the 5′-ends, the *Pvu*II* fragments were cleaved with a second restriction enzyme. We used *Hin*fI restriction endonuclease, which cleaves several *Pvu*II* fragments. The fragments which were not cleaved with *Hin*fI were not examined.

On the basis of the 5′-terminal nucleotide sequence of the multiple *Pvu*II* fragments determined by the method of Maxam and Gilbert (shown in fig.2 for 3 fragments), we found that *Pvu*II* endonuclease activity cleaves the following recognition sequences:

CAG↓CTG, standard site.
CCG↓CTG, substitution of A by C at the second position of the site (fig.2A).
CAT↓CTG, substitution of G by T at the third position of the site.
CAG↓ATG, substitution of C by A at the fourth position of the site.
CAG↓GTG, substitution of C by G at the fourth position of the site (fig.2B).
CAG↓CGG, substitution of T by G at the fifth position of the site (fig.2C).

The nucleotide sequences of the regions of pBR322 DNA containing cleavage sites for endonuclease *Pvu*II* are presented in table 1.

From the above examples of recognition sequences, we conclude that under modified reaction conditions, *Pvu*II* recognizes degenerate sequences which derive from the normal sequence. In contrast to results obtained previously [1,4,5], the specificity relaxation of *Pvu*II does not involve the loss of selectivity to both terminal nucleotides of the hexanucleotide site. Our results indicate the involvement of loss of specificity at positions internal to the hexanucleotide sequence CAGCTG. In each recognition sequence, only one nucleotide of the hexanucleotide site is substituted. The process appears to be similar to that described for *Bam*HI [8]; relaxation occurs at the internal hexanucleotide sequence.

From the present data, it seems likely that the other *Pvu*II* sites, which were not examined, correspond to degenerate sites derived from the normal sequence.

Finally, since the cleavage specificity of *Pvu*II* is altered even under DMSO concentrations lower than 5% (v/v), in concentrations which are not sufficient to cause the modification of the enzyme conformation, the relaxation could be due to the removal of water molecules in the vicinity of the DNA, lowering the specificity of the restrictase as

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Fig.2. 5′-terminal sequences of *Pvu*II* fragments. (A) 300 bp long fragment located between positions 3062 and 3362, labelled at position 3062. (B) 85 bp long fragment located between positions 766 and 851, labelled at position 766. (C) 188 bp long fragment located between positions 2186 and 2374, labelled at position 2186.

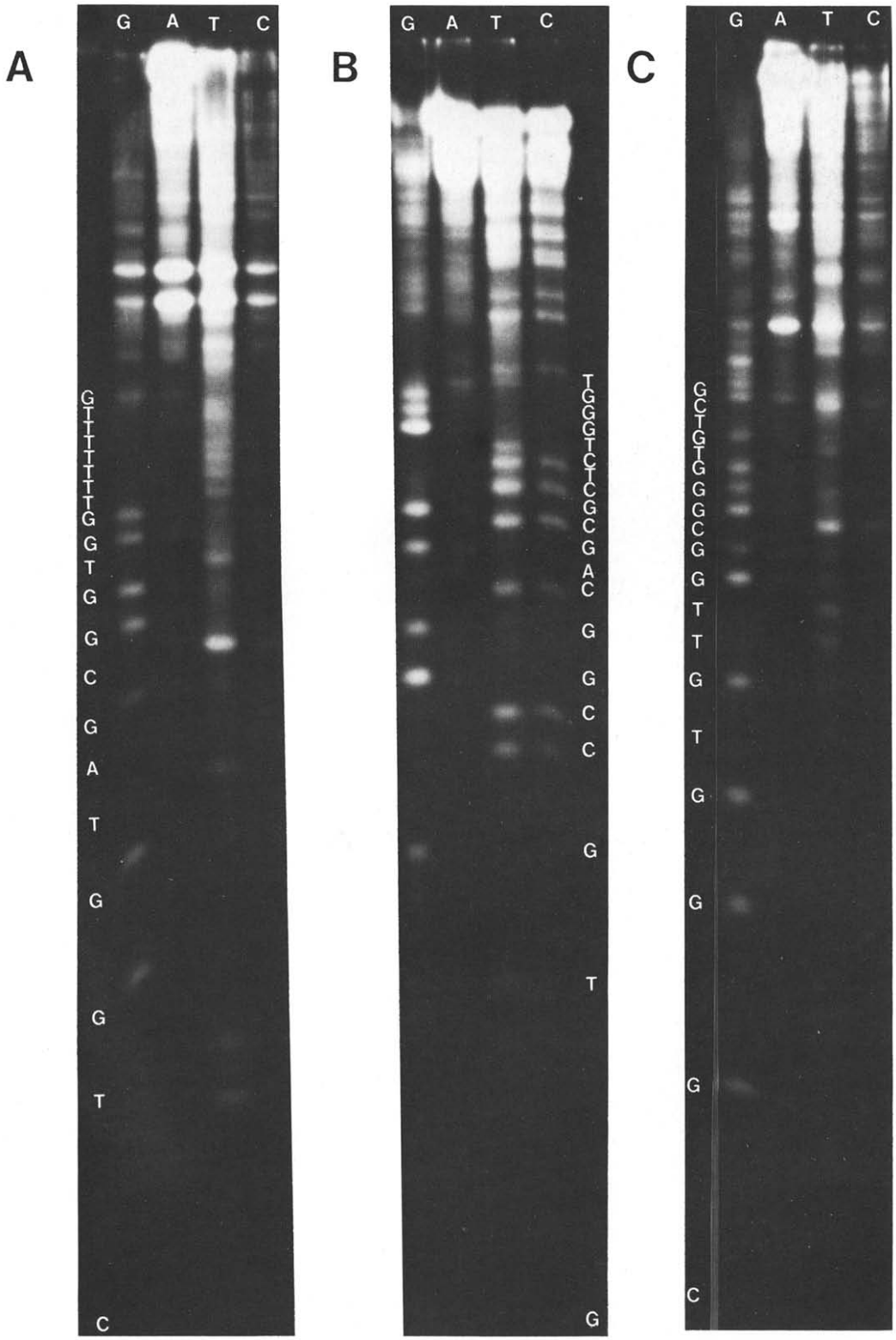
A
G A T C
GTTTTTTTTGGTGGCGATTGGGGTC
B
G A T C
TGGGTCTCGCGACGGCCCGGT
C
G A T C
GCTGTGGGCGGTTGTGGGGC

Table 1

Nucleotide sequences in pBR322 DNA containing a cleavage site for *Pvu*II*

422
GCGCCACAG↓GTGCGGTTG
765
GTAGGACAG↓GTGCCGGCA
1140
ACTGGACCG↓CTGATCGTC
1713
CACCTACAT↓CTGTATTAA
2025
GGCAGACAT↓CTGTGAATC
2067
TTACCGCAG↓CTGCCTCGC
2186
GCGCGTCAG↓CGGGTGTTG
2321
ACCGCACAG↓ATGCGTAAG
2816
GCCCGACCG↓CTGCGCCTT
3061
AAACCACCG↓CTGGTAGCG
3406
GCTTACCAT↓CTGGCCCCA

was suggested by Malyguine et al. [2]. However, our results do not completely rule out the possibility that the relaxation of the specificity of *Pvu*II endonuclease could be due to a conformational change of the *Pvu*II protein [1].

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