II-Q restriction endonucleases—new class of type II enzymes

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Received May 17, 1990; Revised and Accepted September 14, 1990

ABSTRACT

Unique restriction endonucleases Bpu10I and BsiI have been isolated from *Bacillus pumilas* and Bacillus sphaericus, respectively. The recognition sequences and cleavage points of these enzymes have been determinated as 5'-CC↑TNAGC-3' / 3'-GGANT↑CG-5' for Bpu 10I and 5'-C↑TCGTG-3' / 3'-GAGCA↑C-5' for BsiI.

Restriction endonucleases Bpu10I and BsiI represent a new class of enzymes which recognize non-palindromic nucleotide sequences and hydrolize DNA within the recognition sequence. Bpu10I and BsiI recognition sequences may be regarded as quasipalindromic and the enzymes may be designated as type II-Q restriction endonucleases.

INTRODUCTION

The majority of type II restriction endonucleases recognize and cleave the palindromic DNA sequences within the recognition site. Besides, a small group of enzymes is known to recognize asymmetrical five or six nucleotide sequences. However, in this case DNA is cleaved outside the recognition site [1]. Several enzymes of this group recognize six nucleotide sequences and hydrolyze DNA near the recognition site, e.g. BbvII ($GAAGACN_{2/6}$), Eco31I ($GGTCTCN_{1/4}$), Ksp6321 ($CTCTTCN_{1/4}$). The recognition sequences of these enzymes either do not contain the elements of palindromic symmetry or demonstrate them at positions 1 or 6.

The paper describes new restriction endonucleases (type II-Q endonucleases) which recognize asymmetrical sequences and hydrolize DNA within the recognition site.

Restriction endonucleases Bpu10I (CC↑TNAGC) and BsiI (C↑TCGTG) were isolated from *Bacillus pumilus* and Bacillus *sphaericus*, respectivly. The enzymes are named according to a traditional nomenclature [2].

MATERIALS AND METHODS

Restriction endonucleases Bpu10I and BsiI were isolated as described [3]. Substrate DNA was cleaved in 30 μl of the reaction mixture. The assay conditions for Bpu10I were: 20 mM Tris-HCl (pH 8.5), 10 mM $MgCl_2$, 100 mM NaCl, 37°C. The reaction mixture used for DNA cleavage by BsiI contained 20 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$ and 20 mM NaCl. Lambda CI857 DNA was cleaved by Bim19I, StyI, EcaI and VspI (the recognition sequences TTCGAA, CC(A/T)(A/T)GG, GGTNACC and ATTAAT, respectively) and the resulting hydrolyzates were used as markers of the fragment's length.

To determinate the recognition site of enzymes, FORTRAN program was run on IBM-370 computer.

The DNA cleavage sites for Bpu10I and BsiI were determinated by modified Maxam-Gilbert method [4].

RESULTS

Substrate specificity of Bpu10I and BsiI was deduced according to a well-known procedure. Figures 1 and 2 show the canonical cleavage of substrate DNA by Bpu10I and BsiI. First, the cleavage site of pBR322 DNA was determinated by mapping, enzymes BspI, MspI and Sau3AI were used. Bpu10I was shown to cleave pBR322 DNA at position 1580 ± 15, whereas BsiI was capable to cleave DNA at positions 2655 ± 15, 4030 ± 15, 4340 ± 15. Second, a computer search for recognition sequences localized only at intervals 1580 ± 30 for Bpu10I and 2655 ± 30, 4030 ± 30, 4340 ± 30 for BsiI was conducted among ABCDE, ABCDEF, $ABCN_{(1-6)}DEF$ sequences. The only sequence, CTCGTG, consistent with experimental data was detected for BsiI. As it was impossible to determine the Bpu10I recognition site in the initial phase of search, later we used experimental data for maximum lengths of the fragments from cleavage of lambda and T7 DNAs having longer sequences. As follows from Figure 1, the largest fragments contain 7500 ± 700 and 4900 ± 500 bp for lambda and T7 phages, respectively. Sequences were analysed if maximum lengths of the fragments were more (or equal) than experimental data. The results of the analysis performed with three substrates successively are represented in Table 1. Twenty six sequences which meet the requirements of search were mapped on lambda and T7 DNAs. The restriction maps were compared with those which were obtained by experiments for Bpu10I. In this way, the only recognition sequence, CCTNAGC, consistent with the experimental data was found (Table 2). The nucleotide sequence analysis of the Hind III-F-fragment of variolovaccine virus genome [5] showed that it had no more than one recognition site (CCTNAGC) located at the position where this DNA fragment was cleaved by Bpu10I.

Finally, the sequences were tested for possible nucleotide variations. A canonic patterns of cleavage of lambda DNA by

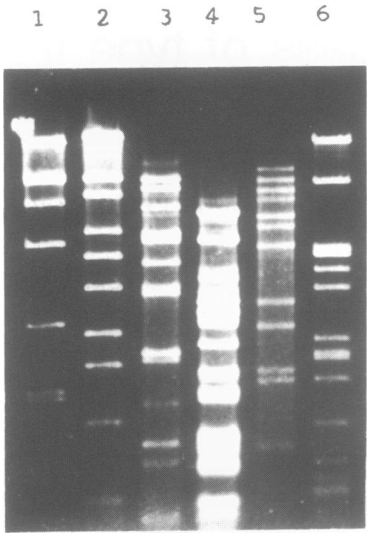

Figure 1. Electrophoresis of lambda and T7 DNA digests. Lane 1: BimI digest of lambda DNA; lane 2R: lambda DNA plus StyI; lane 3: lambda DNA plus Bpu10I; lane 4: T7 DNA plus Bpu10I; lane 5: lambda DNA plus EcaI; lane 6: lambda DNA plus VspI.

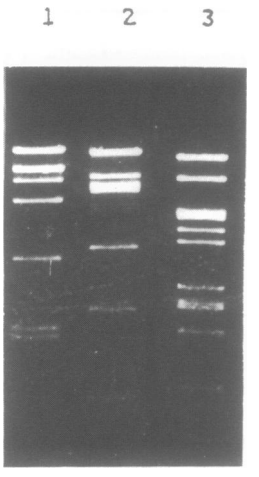

Figure 2. Electrophoresis of lambda DNA digests. Lane 1: BimI; lane 2: BsiI; lane 3: VspI.

Table 1. Determination of possible recognition sequences for Bpu10I.

Type of sites	The number of pBR322 DNA recognition sequences only at interval 1580 ± 30	The number of lambda CI857 DNA cleavage sites resulting in the fragment with sizes of more than 6800 bp for recognition sequences A	The number of T7 cleavage sites resulting in the fragment with sizes of more than 4400 bp for recognition sequences B
ABCDE	0	–	–
ABCDEF	7	4	3
$ABCN_1DEF$	7	4	3
$ABCN_2DEF$	7	5	3
$ABCN_3DEF$	9	7	7
$ABCN_4DEF$	8	6	6
$ABCN_5DEF$	7	4	3
$ABCN_6DEF$	5	2	1

Table 2. Theoretical and experimental lambda and T7 DNA fragments lengths for CCTNAGC and CCTNAG(C/G)

CCTNAGC	CI857 CCTNAG(G/C)	Bpu10I	CCTNAGC	T7 CCTNAG(G/C)	Bpu10I
7497	–	7500 700	4902	–	4900 500
6645	6645	6600 600	3805	–	4000 400
5293	5293	5700 600	2764	3247	3000 300
4095	4199	4400 450		3136	
	4095		2301	2614	2400 250
3977	3864	4100 400	2133	2095	2200 200
3864			2095		
3212	3298	3400 300	1998	1942	2000 200
	3212		1942		
2639	2639	2600 250	1669	1716	1700 170
2538	2538		1346	1352	1400 140
	2482				
1611	1611	1600 160			
1599	1599				
1508	1508	1500 150			
	1495				
1060	1060	1100 100			

BsiI is shown in Figure 2. The lengths of the fragments (20000 ± 2000, 7000 ± 700, 5800 ± 600, 5100 ± 500, 2500 ± 250, 1500 ± 150 and 800 ± 100 bp)generated by this enzyme were used as criteria for the analysis. The results of such an analysis are shown in Table 3 and confirm that the BsiI recognition sequence has no nucleotide variations. Parallel and simultaneous hydrolysis of lambda DNA by BsiI and XnoI (the recognition sequence CTCGAG) may serve as evidence in support of this idea. The comparison of the additional fragments of lambda DNA hydrolysis by BsiI and XnoI with those resulting from DNA cleavage by BsiI (Figure 3) shows that the CTCGAG sequence is not the recognition site for BsiI. Thus, BsiI recognizes CTCGTG (the complementary sequence CACGAG).

Table 3. Possible substitutions of bases in recognition site CTCGTG

Position	Possible nucleotides		Identical bases
	pBR322	lambda CI857	
1	C	C	C
2	T	T	T
3	CT	AC	C
4	G	G	G
5	T	T	T
6	G	G	G

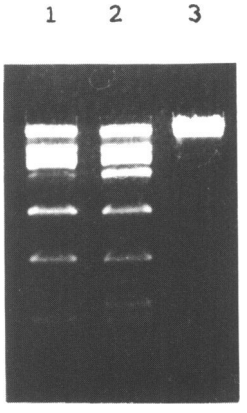

Figure 3. Comparison of BsiI and XnoI cleavage patterns. Lane 1: BsiI; lane 2: BsiI plus XnoI; lane 3: XnoI.

Possible substitutions of base-pair in every position of the Bpu10I recognRition site are shown in Table 4 for four substrates DNAs. As follows from Table 4, the CCTNAG(C/G) sequence might be regarded as the recognition site. However, by comparing the length of the fragments of DNA cleavage at this site with experimental data (Table 2) we may see that CCTNAGC is the Bpu10I recognition sequence.

To determine the DNA cleavage site for Bpu10I and BsiI, the DNA sequence was subjected to direct analysis at the recognition site. The corresponding regions of pBR322 DNA containing the recognition sites were examined. In case of BsiI pBR322 DNA (7 μg) was successively cleaved by MspI, RsaI and Sau3AI. The reaction products were labelled with the large fragment of *E.coli* DNA-polymerase I (the Klenow fragment) and α-^{32}P dCTP. The mixture was fractionated by 6% polyacrylamide gel electrophoresis. The 400 bp DNA fragment was extracted from the gel and sequenced (Figure 4a).

By analyzing the second strand of another site the same amount of pBR322 DNA was cleaved by EcoRI and TaqI. The fragments were labeled with α-^{32}-P dCTP and subjected to electrophoresis. After having been extracted from 5% gels the 340 bp fragment was analyzed (Figure 4b).

Since BsiI recognizes the non-palindromic site, we performed an additional analysis of DNA cleavage site in one of strands. For this purpose pBR322 DNA was cleaved by EcoRI and PstI. The resulting DNA fragments were labeled with α-^{32}P dATP.

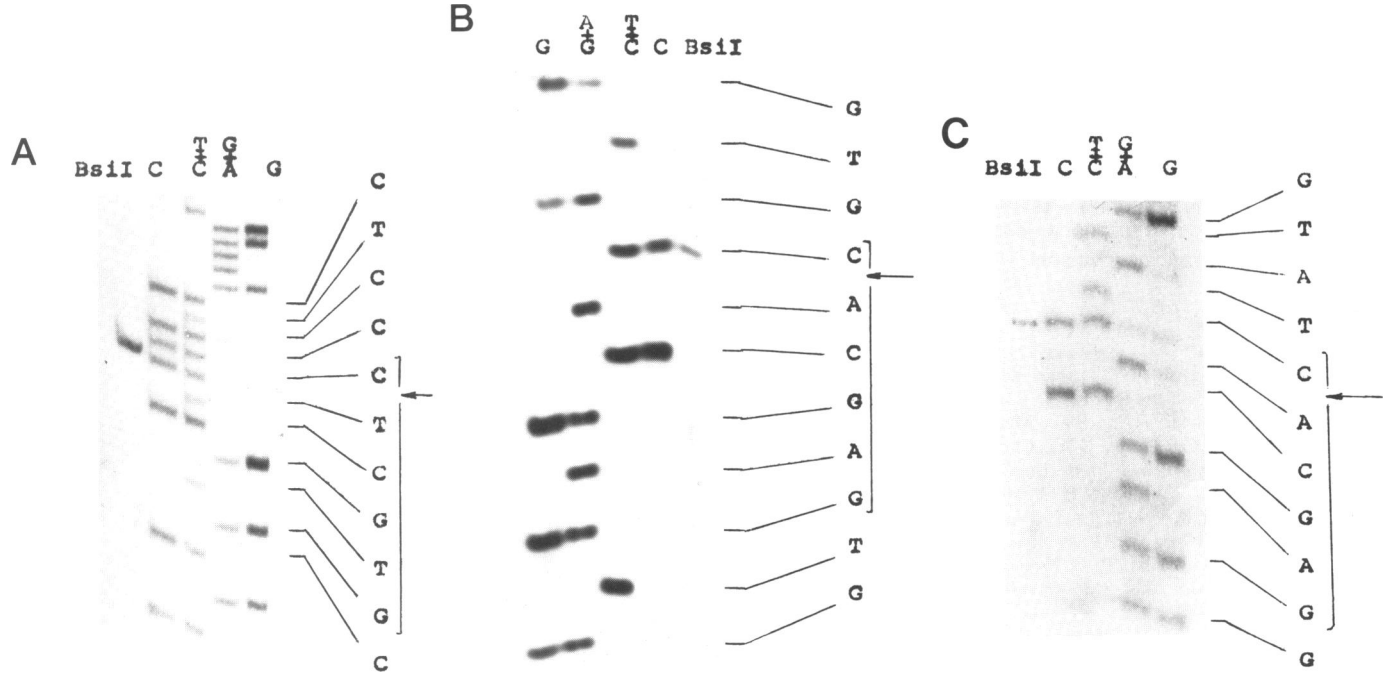

Figure 4. Sequencing of α^{32}-P-labeled pBR322 DNA fragments. Determination of the BsiI cleavage site. A) Sequencing of the MspI–RsaI fragment. B) Sequencing of the EcoRI–TaqI fragment. C) Sequencing of the EcoRI–PstI fragment.

Table 4. Possible substitutions of bases in the recognition site CCTNAGC

Position	Possible nucleotides				Identical bases
	pBR322	lambda CI857	T7	HindIII-F-fragment	
1	GCA	N	TGC	CG	CG
2	GC	TGC	N	G	G
3	CA	N	N	A	A
4	N	N	N	N	N
5	TC	TCA	TGC	T	T
6	C	C	TCA	TC	C
7	GC	N	GC	GA	C

N—random nucleotide.

Then the 750 bp DNA fragment was sequenced (Figure 4c).

It follows from the data in Figure 4 that BsiI recognizes and cleaves the DNA sequence $^{5'\text{-CITCGTG-3'}}_{3'\text{-GAGCAIC-5'}}$, as indicated by arrows.

It has been shown analogously that Bpu10I hydrolyzes the sequence $^{5'\text{-CCITNAGC-3'}}_{3'\text{-GGANTICG-5'}}$, as indicated by arrows [6].

DISCUSSION

Thus, restriction endonucleases Bpu10I and BsiI are unique enzymes. They have not isoschisomers and represent a new class of enzymes which recoRgnize non-palindromic nucleotide sequences and hydrolyze DNA within the recognition sequence. The investigations of the properties of this enzymes shows that they do not need cofactors, except for Mg^{2+} ions, and are not stimulated by ATP and S-adenosylmethonine. Therefore, BsiI and Bpu10I refer to type II restriction endonucleases, but recognize the unusual sites which are similar to the palindromic sequences CTCGAG (XhoI and its isoschizomers) and CCTNAGG (SauI and its isoschizomers), respectively. BsiI and Bpu10I recognition sequences differ from the corresponding palindromes in no more than one base which results in asymmetry in one nucleotide pair. The recognition sequences have the identical G+C content in every position and the restriction endonucleases hydrolyze DNA within these sequences in a similar fashion. Bpu10I and BsiI recognition sites may be regarded as quasi-palindromic and the endonucleases may be designated as type II-Q restriction enzymes.

It is suggested that BsiI and Bpu10I isolated from bacterial strains were derived from the enzymes with the recognition sites CTCGAG and CCTNAGG, respectively. Their origin was the result of the evolutionary changes in genomes of the corresponding microorganisms.

REFERENCES

1. Roberts J.R. (1988). Nucl. Acids Res. 16, Suppl. r271–r313.
2. Smith H.O., Nathans D. (1973). J.Mol.Biol. 81, 419–423.
3. Pirrotta V., Bickle T.A. (1980). Methods Enzymol. 65, 88–95
4. Prikhod'ko G.G., Petrov N.A., Chizikov V.E. Degtyarev S.K. (1988). Biotekhnologiya 4, 618–620. (Russ.)
5. Micryukov N.N., Chizikov V.E., Prikhod'ko G.G., Urmanov I.K., Serpinsky O.I., Blinov V.M., Nikulin A.E., Vasilenko S.K. (1988). Biotekhnologiya 4, 442–449. (Russ.)
6. Degtyarev S.K., Zhilkin P.A., Prikhod'ko G.G., Repin V.E., Rechkunova N.I. (1989). Mol. Biol. (Moskow) 23, 1051–1056. (Russ.)