

***BtrI*, a novel restriction endonuclease, recognises the non-palindromic sequence 5'-CACGTC(-3/-3)-3'**

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ABSTRACT

The recognition sequence and cleavage positions of a new restriction endonuclease *BtrI* isolated from *Bacillus stearothermophilus* SE-U62 have been determined. *BtrI* belongs to a rare type IIQ of restriction endonucleases, which recognise non-palindromic nucleotide sequences and cleave DNA symmetrically within them.

Type II restriction endonucleases (ENases) include a group of 53 prototypes that recognise non-palindromic DNA sequences (ENases with recognition sequences that are interrupted by more than one base pair are not considered). Cleavage positions have been determined for 45 of these (1). Mainly, such ENases cleave DNA outside their recognition sites and are designated type IIS (2). There are only a few so-called type IIQ restriction enzyme prototypes that cut both DNA strands symmetrically within their non-palindromic recognition sequences (3).

Here we report a new member of this rare subgroup of type II ENases.

Bacillus stearothermophilus SE-U62 was grown at 55°C using Luria medium. *BtrI* has been isolated from disrupted cells using phosphocellulose, DEAE-cellulose, heparin-agarose and hydroxyapatite chromatographic steps. The final enzyme preparation was free of contaminating exo- and endonuclease activities. Optimal conditions for DNA cleavage with *BtrI* were 50 mM Tris-HCl (pH 7.6 at 25°C), 10 mM MgCl₂ and 100 mM NaCl at 60°C. The experimental patterns of cleavage of different DNAs by *BtrI* correspond to those predicted for a restriction enzyme recognising 5'-CACGTC-3' (Fig. 1).

The cutting positions of *BtrI* have been determined from analysis of cleavage products of ³²P-labelled oligodeoxyribonucleotides (Fig. 2). According to data presented in Figure 2 this enzyme splits DNA in the middle of the recognition sequence, forming blunt-ended DNA fragments. Interestingly, ligation of DNA fragments formed by *BtrI* leads not only to the formation of *BtrI* sites, but also to sites that can be recognised by *PmaCI* (CAC[^]GTG) and *AatII* (GACGT[^]C). A similar property has been described earlier for *BsrBI* and its isoschizomer, *AccBSI* (4).

Earlier, we proposed the type IIQ designation ('Q' means 'quasipalindromic') for this class of ENases (5). Type IIQ

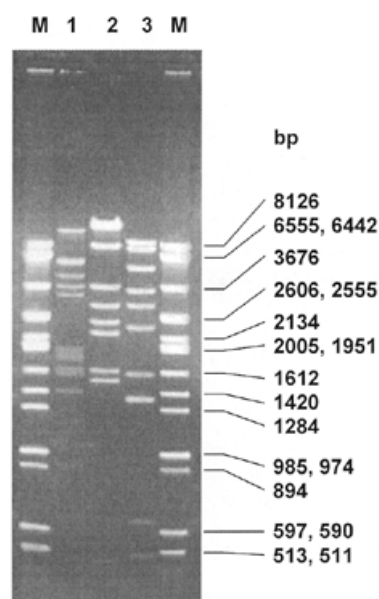


Figure 1. Viral DNA cleavage with *BtrI*. Lane 1, phage λ ; lane 2, phage T7; and lane 3, adenovirus type 2 DNAs. Size marker λ /*BmeI8I* are in lanes marked M. Electrophoresis was on a 1% agarose gel, run in Tris-borate buffer.

restriction enzymes cleave DNA symmetrically within their recognition sequence, which differs by one base pair from a palindrome. The origin of type IIQ enzymes may be the result of duplication and evolutionary changes in the genes of ancestor restriction enzymes recognising a palindromic DNA sequence. Recently this evolutionary scheme has been considered in detail for *Bpu10I*, one of the type IIQ enzymes discovered earlier (6).

We suggest that the reaction mechanism of DNA cleavage by restriction endonucleases may be considered to be quite conservative. Many restriction endonucleases can be placed in definite groups depending on their mode of DNA cleavage. We can see that there are common nucleotides in all enzymes of every group located in a definite position near the cleavage site. We observe this phenomenon for enzymes that cleave DNA producing 3' dinucleotide extensions, for example (7). These data are given in Table 1 including a new entry which has been discovered recently (1).

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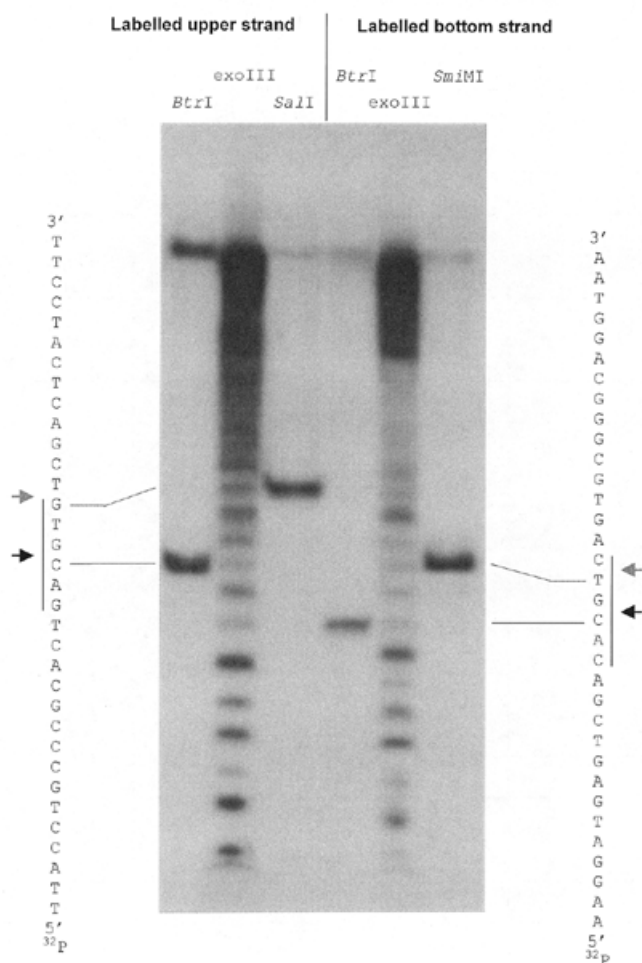


Figure 2. Determination of the cleavage positions of *BtrI*. An oligodeoxyribonucleotide duplex with a labelled upper or bottom chain was cleaved by *BtrI* and ENases with known cleavage positions *Sall* (G↓TCGAC) and *SmiMI* (CAYNN↓NNRTG). The same duplex cleaved with exonuclease III provided fragment length markers. Reaction products were separated by electrophoresis in a 20% polyacrylamide gel, 7 M urea, Tris–borate buffer. Recognition sequences of *BtrI* are shown by side lines. Cleavage positions are shown by grey (*Sall* and *SmiMI*) and black (*BtrI*) arrows.

A similar approach may be used for enzymes recognising interrupted palindromic DNA sequences (8). So, these data show that evolution of whole groups of ENase recognition sequences may be based on general cleavage mechanisms.

Based on this consideration we supposed (3) that type IIQ enzymes arose from some palindrome-recognising enzymes due to the maintenance of heterodimeric organisation and a slight change of target-recognising domain in one subunit in the course of evolution which, however, did not affect the cleavage mechanism. The formation of heterodimeric type IIQ enzyme from homodimeric type II enzyme may occur in three steps: (i) ancestor gene duplication; (ii) loss of ability to form homodimeric structure while keeping an ability of counteraction

between heterosubunits; and (iii) a slight change of the sequence recognised by one subunit.

Table 1. Comparison of recognition and cleavage sites of five enzymes from thermophilic bacilli

ENase	Recognition site	
	Upper chain	Bottom chain
<i>BstF5I</i>	5'– G G A T G N N [↓] –3'	5'– N [↑] C A T C C C –3'
<i>BsrDI</i>	5'– G C A A T G N N [↓] –3'	5'– N [↑] C A T T G C C –3'
<i>BtsI</i>	5'– G C A G T G N N [↓] –3'	5'– N [↑] C A C T G C C –3'
<i>BsmI</i>	5'– G A A T G C N [↓] –3'	5'– G [↑] C A T T C C –3'
<i>BsrI</i>	5'– A C T G G N [↓] –3'	5'– C [↑] C A G T –3'

Identical elements of recognition sites are in bold.

The list of known type IIQ restriction enzymes and their possible ancestor ENases are given in Table 2. We suggest that type IIQ enzymes have evolved from isoschizomers of corresponding well-known restriction enzymes by replacement of one base pair in their recognition sequence. Thus, in the last column we indicate known recognition sequences that may be transformed to the corresponding type IIQ enzymes by replacement of one base pair. However, *BsiI* and *BtrI* have a several possible ancestors but only one of them has a cleavage pattern similar to the cleavage pattern of the type IIQ enzyme.

So, we suggest *BsiI* has evolved from an *XhoI* isoschizomer rather than from a *PmaCI* isoschizomer, whereas *BtrI* has probably evolved from a *PmaCI*-like ENase.

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Table 2. Known IIQ type restriction enzyme prototypes

ENase	Recognition sequence	References ^a	Hypothetical recognition sequence of ancestor ENase (corresponding prototype) ^b
<i>AciI</i>	CCGC(-3/-1)	9	C [^] CGg (<i>HpaII</i>) g [^] CGC (<i>HinPI</i>)
<i>BbvCI</i>	CCTCAGC(-5/-2)	L.Ge, unpublished observations S.Krotee, M.Ganatra and R.Grandoni, unpublished observations	CCTnAGC (-5/-2) (<i>Bpu10I</i>)
<i>Bpu10I</i>	CCTNAGC(-5/-2)	10	CC [^] TNAGg (<i>SauI</i>) gC [^] TNAGC (<i>EspI</i>)
<i>BsiI</i>	CACGAG(-5/-1)	5	C [^] tCGAG (<i>XhoI</i>) CAC [^] GtG (<i>PmaCI</i>)
<i>GdiII</i>	CGGCCR(-5/-1)	11 M.Van Montagu, unpublished observations	C [^] GGCCg (<i>XmaIII</i>) y [^] GGCCR (<i>CfrI</i>)
<i>BtrI</i>	CACGT(-3/-3)	This article	CAC [^] GTg (<i>PmaCI</i>) gACGT [^] C (<i>AatII</i>)

^aREBASE data (1).^bDiffering nucleotides are in lower case.

