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Restriction endonuclease *Bso*FI is sensitive to the 5′-methylation of deoxycytidines in its recognition sequence

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ABSTRACT

The isoschizomeric restriction endonucleases *Fnu*4HI and *Bso*FI cleave DNA at 5′-GC↓NGC-3′ sequences. *Fnu*4HI has been shown to be inhibited by 5′-CG-3′ methylation in the sequences 5′-G^{m}CGGC-3′ or 5′-GCGG^{m}CG-3′. We have now investigated the methylation sensitivity of *Bso*FI by testing its activity on plasmid DNA 5′-CG-3′ methylated with the M.SssI DNA methyltransferase or on synthetic $(CGG)_n$ repetitive oligodeoxyribonucleotides which have been partly or completely C methylated. The data demonstrate that *Bso*FI cannot cleave at its recognition sequence when it is completely 5′-CG-3′ methylated. These enzymes have proven to be useful in analyses of the methylation status in $(CGG)_n$ repeats of the human genome.

Methylation patterns in mammalian DNA at 5′-CG-3′ dinucleotides have been studied extensively using restriction endonucleases that are not able to cleave DNA in the presence of 5′-methylated deoxycytidines (5-^{m}C) in their recognition sequences. The isoschizomers *Fnu*4HI and *Bso*FI cut the DNA sequences 5′-GC↓NGC-3′ and are therefore useful to analyze 5′-CG-3′ methylation of important repetitive sequences, like 5′-d$(CGG)_n$-3′ repeats. Such repeats have been found to be unstable in that they can extensively expand in distinct regions of the human genome (1–3). High levels of methylation in the expanded repeats have been shown by genomic sequencing and by using the restriction endonuclease *Fnu*4HI (3,4) which has been reported to be unable to cut the sequences 5′-G^{m}CGGC-3′ and 5′-GCGG^{m}CG-3′ (5). However, the methylation sensitivity of the isoschizomer *Bso*FI has not yet been studied.

Here we report investigations on the influence of 5′-CG-3′ methylation on the activity of *Bso*FI using either unmethylated or methylated synthetic, repetitive oligodeoxyribonucleotides or *in vitro* methylated pBluescript SK(+) DNA as substrate. This plasmid DNA contains 23 *Bso*FI recognition sites: Ten of these sites can be methylated by DNA methyltransferase M.SssI from *Spiroplasma species* which methylates specifically deoxycytidine residues in all 5′-CG-3′ dinucleotides (6). Methylated or unmethylated plasmid DNA was incubated with *Bso*FI, *Hpa*II, *Hha*I or *Msp*I (Fig. 1). Restriction endonucleases *Hpa*II or *Hha*I were used as controls, because they were unable to cut DNA in the presence of CG-specific DNA methylation, whereas restriction endonuclease *Msp*I was not inhibited (5). Complete cleavage was observed with *Msp*I only (Fig. 1, lane 7), whereas *Hpa*II and *Hha*I cut the DNA poorly, because the DNA had been methylated by M.SssI (Fig. 1, lanes 1 and 5). Comparisons of the complex *Bso*FI cleavage patterns between unmethylated and methylated plasmid DNA revealed that only those 5′-G^{m}CGG^{m}CG-3′ sequences, which had been completely methylated by M.SssI, were not cut by *Bso*FI (Fig. 1, lanes 3 and 4).

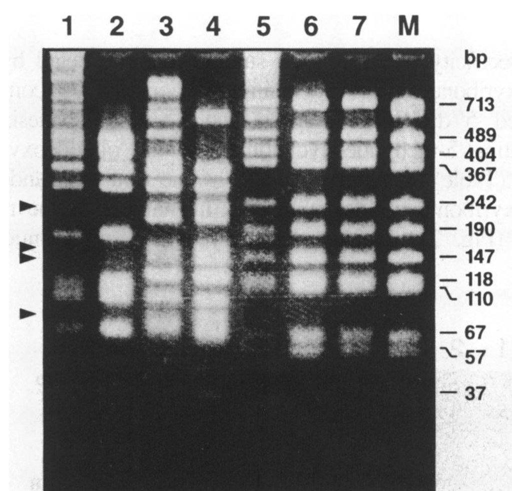

Figure 1. Methylated or unmethylated pBluescript SK(+) DNA was cut with different methylation-sensitive restriction endonucleases. In detail, 10 μg DNA was incubated overnight with 4 U of M.SssI (New England Biolabs) and 3.2 mM *S*-adenosyl-L-methionine at 37°C. After stopping the reaction (20 min at 65°C), 2 μg M.SssI-treated DNA was incubated either with 10 U of *Bso*FI at 55°C (New England Biolabs) or with 20 U of *Msp*I, *Hpa*II or *Hha*I (all Boehringer Mannheim) according to the manufacturers' protocols. Reaction products were analyzed by electrophoresis on a 3.5% NuSieve agarose gel. Lanes 1, 3, 5 and 7 contained methylated plasmid DNA cut with *Hha*I, *Bso*FI, *Hpa*II or *Msp*I, respectively. Lanes 2, 4 and 6 contained products obtained with unmethylated DNA using *Hha*I, *Bso*FI and *Hpa*II, respectively. The arrows indicate those cleavage products which decrease in intensity upon digestion with *Bso*FI due to 5′-CG-3′ methylation (lanes 3 and 4). Lane M contained unmethylated DNA cut with *Msp*I which was also used as a size marker.

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Table 1. Sequences of the oligodeoxyribonucleotides used in this study

Oligodeoxy-ribonucleotide	Nucleotide sequence	Cleavage by *Bso*FI	Cleavage products, lengths in base pairs
$(CGG)_{17}$ds	5′-$(CGG\ CGG)_8$ CGG-3′	completely cleavable	3–5
	3′-$(GCC\ GCC)_8$ GCC-5′		
$(MGG)_{17}$ds	5′-$(^{m}CGG\ ^{m}CGG)_8\ ^{m}CGG$-3′	not cleavable	–
	3′-$(G^{m}CC\ G^{m}CC)_8\ G^{m}CC$-5′		
8MCGGds	5′-$(CGG\ C^{m}GG)_8$ CGG-3′	completely cleavable	5–7
	3′-$(GCC\ G^{m}CC)_8$ GCC-5′		
4MCGGds	5′-$(CGG)_6\ (^{m}CGG)_4\ (CGG)_7$-3′	partly cleavable	3–15
	3′-$(GCC)_6\ (G^{m}CC)_4\ (GCC)_7$-5′		

^{m}C = 5-methyldeoxycytidine

The specificity of *Bso*FI was studied in more detail by using oligodeoxyribonucleotides containing 17 partly or completely methylated 5′-d(CGG)-3′ repeats which were synthesized by incorporating 5-methyldeoxycytidine into the oligodeoxyribonucleotides (Table 1). After cleavage of the double-stranded (ds) oligodeoxyribonucleotides with *Bso*FI, analyses of the reaction products (Fig. 2) revealed that only oligodeoxyribonucleotide $(MGG)_{17}$ds (see Table 1), which contained the sequence 5′-$G^{m}CGG^{m}CGG$-3′, escaped cleavage (Fig. 2, lane 3). However, the unmethylated oligodeoxyribonucleotide $(CGG)_{17}$ds (Fig. 2, lane 2) or partly methylated oligodeoxyribonucleotides containing the sequence 5′-$G^{m}CGGCGG$-3′ (lane 6 and 8) as well as hemimethylated oligodeoxyribonucleotides (data not shown) were cut completely to very small fragments (Table 1).

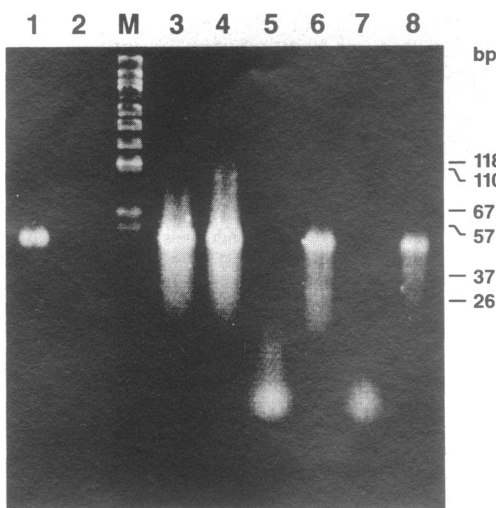

Figure 2. Unmethylated or synthetically methylated repetitive oligodeoxyribonucleotides (2 µg) were cleaved with 5 U of *Bso*FI for 1 h at 55°C. Reaction products were analyzed on 5% NuSieve agarose gels. Lanes 2, 3, 5 and 7 contained the double-stranded oligodeoxyribonucleotides $(CGG)_{17}$ds, $(MGG)_{17}$ds, 8MCGGds, and 4MCGGds (Table 1), respectively, treated with *Bso*FI, whereas lanes 1, 4, 6 and 8 showed the uncut control oligodeoxyribonucleotides in the same order. Lane M contained pBluescript SK(+) DNA cut with *Msp*I as size marker.

We conclude that cleavage of DNA by *Bso*FI is inhibited by 5′-CG-3′ methylation of its recognition sequence. However, *Bso*FI differs from its isoschizomer *Fnu*4HI in that inhibition requires complete C-specific methylation of its recognition sequence.

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