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Dimethyl Sulfoxide Changes the Recognition Site Preference of Methyl-directed Site-specific DNA Endonuclease GlaI.

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ABSTRACT

Methyl-directed site-specific DNA endonuclease GlaI recognizes and cleaves methylated DNA sequence RCGY. GlaI activity in hydrolysis of recognition sequence containing two 5-methylcytosines is noticeably lower than in the case of the site with four or three 5-methylcytosines. We have shown that addition of DMSO to the reaction mixture significantly increases GlaI activity in hydrolysis of sites with two 5-methylcytosines but doesn't change a substrate specificity of the enzyme. A reaction buffer with DMSO may be used in epigenetic studies for GlaI digestion of eukaryotic genomes containing methylated CG pairs.
Key words: methyl-directed DNA endonuclease, site specificity, enzyme activity, dimethyl sulfoxide.

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INTRODUCTION

Methyl-directed site-specific DNA endonuclease (MD endonuclease) is a relatively new type of enzymes which hydrolyze only methylated DNA [1]. Currently there are 14 different prototypes of enzymes that cleave DNA sequences with 5-methylcytosines (5mC; http://mebase.sibenzyme.com). These enzymes cleave human and mammalian genomic DNAs [2-3] and may be used in epigenetics studies, in particular for determination of DNA methylation status of the genes regulation regions [4].

5mC-directed site-specific DNA endonuclease GlaI cuts DNA sequence 5'-R(5mC)↓GY-3'/3'-YG↑(5mC)R-5' as indicated by arrows producing blunt ends [5]. GlaI activity in hydrolysis of recognition sequence with different methylation patterns may significantly differ. A maximal activity of the enzyme is observed for a substrate 5'-G(5mC)G(5mC)-3'/3'-(5mC)G(5mC)G-5' and a minimal activity, only 5% from maximal one, is obtained in the cleavage of DNA sequence 5'-G(5mC)GC-3'/3'-CG(5mC)G-5' [6]. So, DNA sequence 5'-G(5mC)GC-3'/3'-CG(5mC)G-5', which is widely distributed in human and mammalian genomes, is a pure substrate for GlaI. It's a problem to achieve a complete digestion of genomic DNA with GlaI due to a weak enzyme's activity at these sites.

In this work we have studied influence of different buffers and DMSO on GlaI activity in DNA cleavage.

MATERIALS AND METHODS

MD endonuclease GlaI (8 U/µl), DNA methyltransferase SssI (2 U/µl), restriction endonuclease DriI (200 U/µl), plasmid pHspAI2 and phage λ DNAs were manufactured at SibEnzyme (Russia).

pHspAI2 plasmid (http://www.sibenzyme.com/soft-data/database) carries hspAIM gene encoding DNA methyltransferase HspAI which methylates a central cytosine in the sequence 5'-GCGC-3' on both DNA strands. Thus, plasmid pHspAI2 contains 20 sites 5'-G(5mC)GC-3'/3'-CG(5mC)G-5' and one sequence 5'-G(5mC)G(5mC)G(5mC)G(5mC)-3'/3'-(5mC)G(5mC)G(5mC)G(5mC)G-5' which presents three overlapping GlaI sites. The plasmid pHspAI2 was preliminary linearized with DriI and purified using standard phenol-chloroform procedure followed by ethanol precipitation. Phage λ DNA was preliminary methylated at CG sites with methyltransferase SssI in conditions recommended by manufacturer and purified using the same procedure.

A set of standard SibEnzyme restriction buffers was used with the following compositions:

Buffer B: 10 mM Tris-HCl (pH 7.6 at 25°C), 10 mM $MgCl_2$, 1 mM DTT;
Buffer G: 10 mM Tris-HCl (pH 7.6 at 25°C), 10 mM $MgCl_2$, 50 mM NaCl, 1 mM DTT;
Buffer O: 50 mM Tris-HCl (pH 7.6 at 25°C), 10 mM $MgCl_2$, 100 mM NaCl, 1 mM DTT;
Buffer W: 10 mM Tris-HCl (pH 8.5 at 25°C), 10 mM $MgCl_2$, 100 mM NaCl, 1 mM DTT;
Buffer Y: 33 mM Tris-acetate (pH 7.9 at 25°C), 10 mM magnesium acetate, 66 mM potassium acetate, 1 mM DTT.

Buffer «B100» (10 mM Tris-HCl pH 7.6 at 25°C, 50 mM KCl, 0.1 mM EDTA, 200 µg/ml BSA and 1 mM dithiothreitol) was used in order to obtain serial dilutions of GlaI. 1 µl of original or diluted enzyme preparation was added to 1 µg of DNA in 20 µl of restriction buffer containing or not containing dimethyl sulfoxide (DMSO, «Sigma», USA). Incubation was performed at 30°C for 30 min (pHspAI2/DriI) or 1 hour (methylated λ DNA). Products of GlaI hydrolysis were analyzed by electrophoresis in TAE buffer in 1% or 2% agarose gel.

RESULTS AND DISCUSSION

Earlier we studied cleavage of oligonucleotide duplexes with GlaI and obtained a table of the enzyme activity in cleavage of various substrates [6]. A maximal GlaI activity was observed in hydrolysis of DNA sequence with four 5-methylcytosines 5'-G(5mC)G(5mC)-3'/3'-(5mC)G(5mC)G-5'. GlaI cleaves sequences 5'-R(5mC)G(5mC)-3'/3'-YG(5mC)G-5' and 5'-A(5mC)GT-3'/3'-TG(5mC)A-5' about 4 times slower. Other variants of the recognition sites including 5'-G(5mC)GT-3'/3'-CG(5mC)A-5' and 5'-G(5mC)GC-3'/3'-CG(5mC)G-5' are hydrolyzed 10-20 times slower than a site with four 5mC. In this work we have studied GlaI activity in hydrolysis of methylated plasmid and phage DNAs and DMSO influence on these substrates cleavage.

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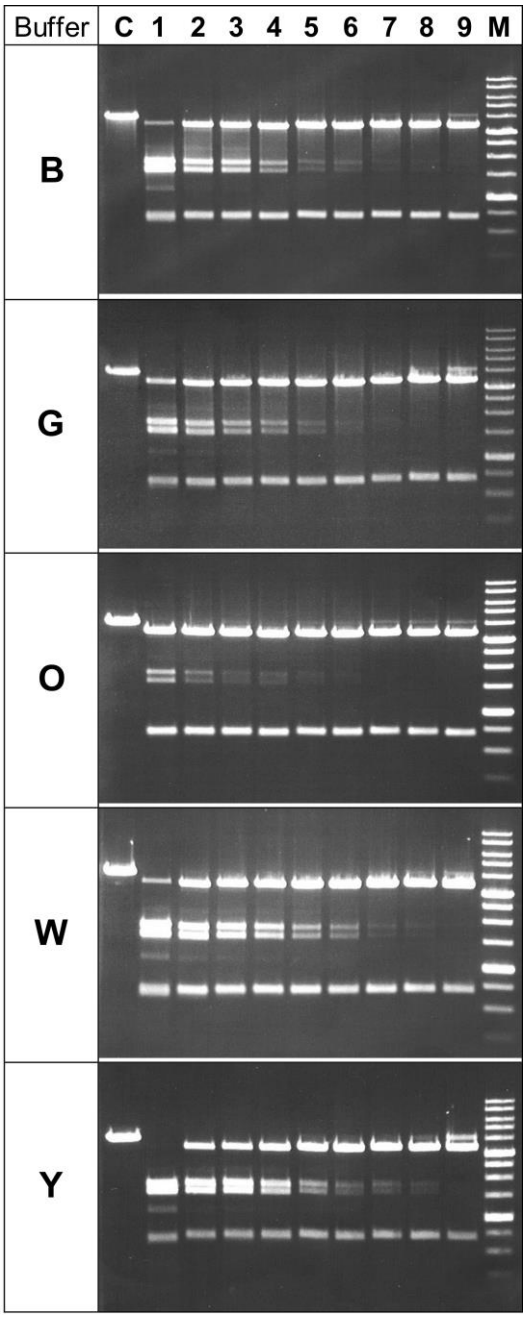
A sequence with three overlapping sites, containing four 5mC in its central part, is the best substrate and GlaI activity in this site cleavage is maximal. GlaI activity in hydrolysis of all other sites is much lower. Figure 1 shows results of pHspAI2/DriI (plasmid linearized with restriction endonuclease DriI) hydrolysis with GlaI in five SE buffers. In all buffers we observe a very high activity in hydrolysis of DNA sequence with overlapping GlaI sites. For all buffers we see an original linearized form of plasmid only in the last lanes (lane 7-9) where the most diluted enzyme was added. At the same time we don't see a complete hydrolysis of pHspAI2/DriI in the used buffers even in a first lane. Based on the data of Figure 1 we have selected buffer Y as an optimal buffer for the forthcoming experiments.

Figure 1: GlaI activity in five standard SibEnzyme buffers. C – control DNA of pHspAI2 plasmid linearized with DriI. 1 – the same substrate treated with 8 U of GlaI. 2-9 – serial 2-fold dilutions of GlaI. M - DNA fragments length marker "SE 1 kB".



It is well-known that substrate specificity of some DNA endonucleases depends on composition of reaction mixture. In particular, DMSO is known for a long time as a substance capable to change a substrate specificity of DNA endonucleases stimulating the enzyme's star activity. Addition of DMSO to the reaction mixture at concentration 5-30% results in DNA hydrolysis at new sites for a number of enzymes [7].

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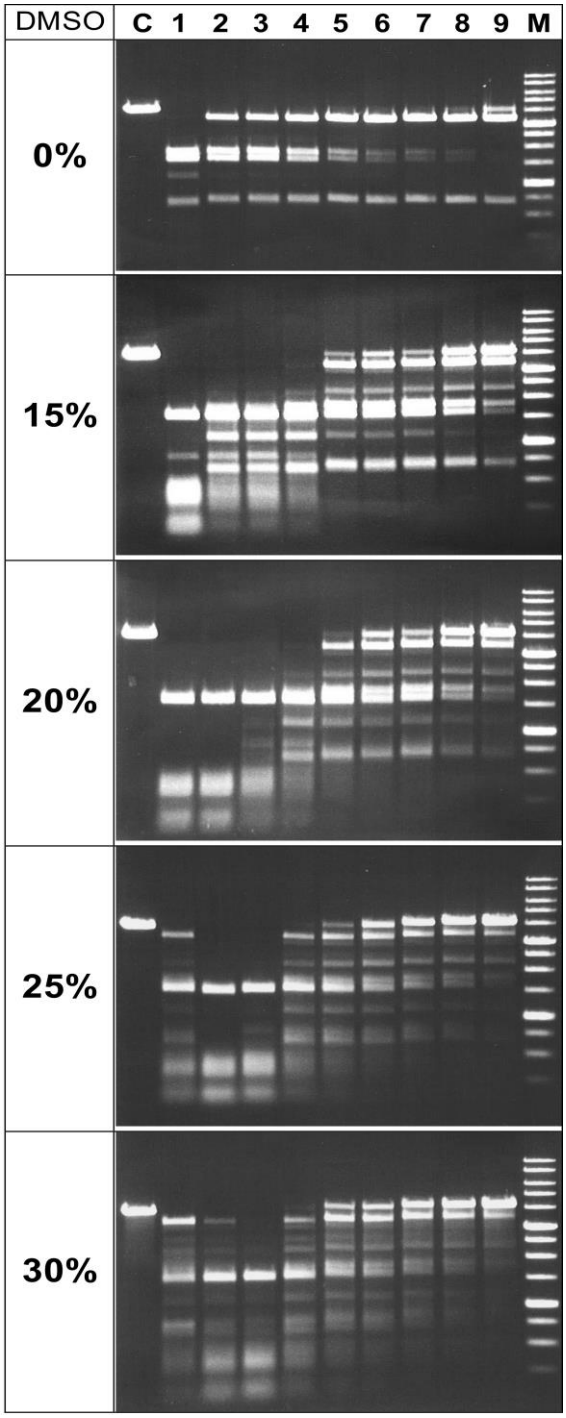
Modulation of site-specificity with DMSO is interesting for DNA cleavage with MD endonucleases which cleave methylated sites with a different activity depending on the methylation pattern. We have studied influence of DMSO on GlaI activity in hydrolysis of pHspAI2/DriI. Figure 2 demonstrates the results of the plasmid cleavage with Gla in buffer Y in the presence of different concentration of DMSO. As it can be seen from the electrophoregrams, the addition of DMSO significantly changes patterns of the plasmid DNA cleavage with GlaI. We see much higher activity of GlaI in all enzyme's dilutions at 15-20% of DMSO which results in formation of a big number of smaller DNA fragments. A picture of GlaI hydrolysis in the absence of DMSO (lanes 1 and 2) roughly corresponds to a pattern of DNA cleavage with GlaI in the presence of 15-20% DMSO (lanes 4 and 5, respectively).

Figure 2: GlaI activity in buffer Y containing different DMSO concentrations. Lane description as on Figure 1.



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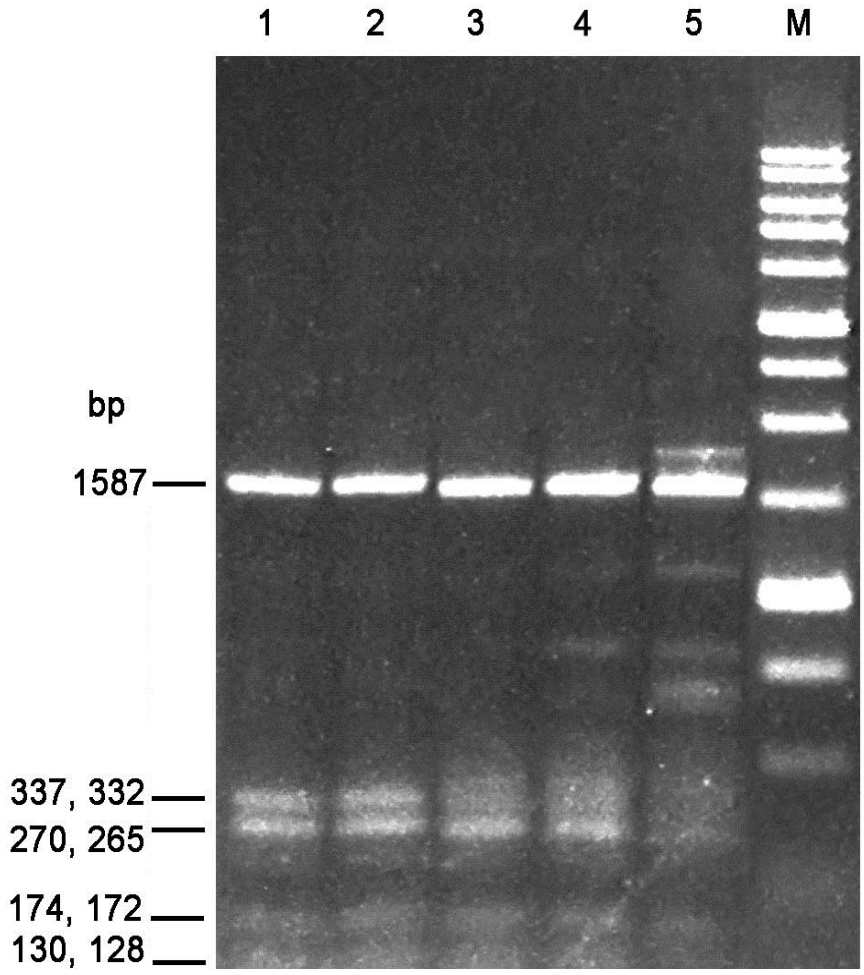
A pattern of DNA cleavage with GlaI in the presence of 20% DMSO (lane 1 and 2) may be considered as a picture of complete digestion of substrate pHspAI2/DriI. We have calculated a theoretical picture of pHspAI2/DriI cleavage at all sites GCGC. The calculated lengths of the corresponding DNA fragments are indicated at left on Figure 3. Figure demonstrates results of pHspAI2/DriI hydrolysis with GlaI in a presence of 20% DMSO. Electrophoresis was performed in 2% agarose to achieve a better resolution of DNA cleavage pattern. Data of Figure 3 shows that a final picture of plasmid DNA hydrolysis with GlaI in a presence of 20% DMSO exactly corresponds to a theoretical pattern of the linearized plasmid DNA cleavage at sites GCGC. This pattern is the same at three dilutions of GlaI and this results show that an excess of GlaI in a presence of DMSO doesn't produce GlaI star activity. Thus, a presence of DMSO significantly increase GlaI activity in cleavage of 5'-G(5mC)GC-3'/3'-CG(5mC)G-5' sites but doesn't change a substrate specificity of GlaI and we don't observe any star activity in DNA hydrolysis with the enzyme.

Figure 3: GlaI cleavage of linearized pHspAI2 plasmid DNA at 20% DMSO concentration at better resolution. Lanes 1 – 16 U of GlaI. Lanes 2-5 – serial 2-fold dilutions of GlaI. M – DNA fragments length marker "SE 1 kB". Theoretically calculated lengths of the GlaI fragments are shown at left.



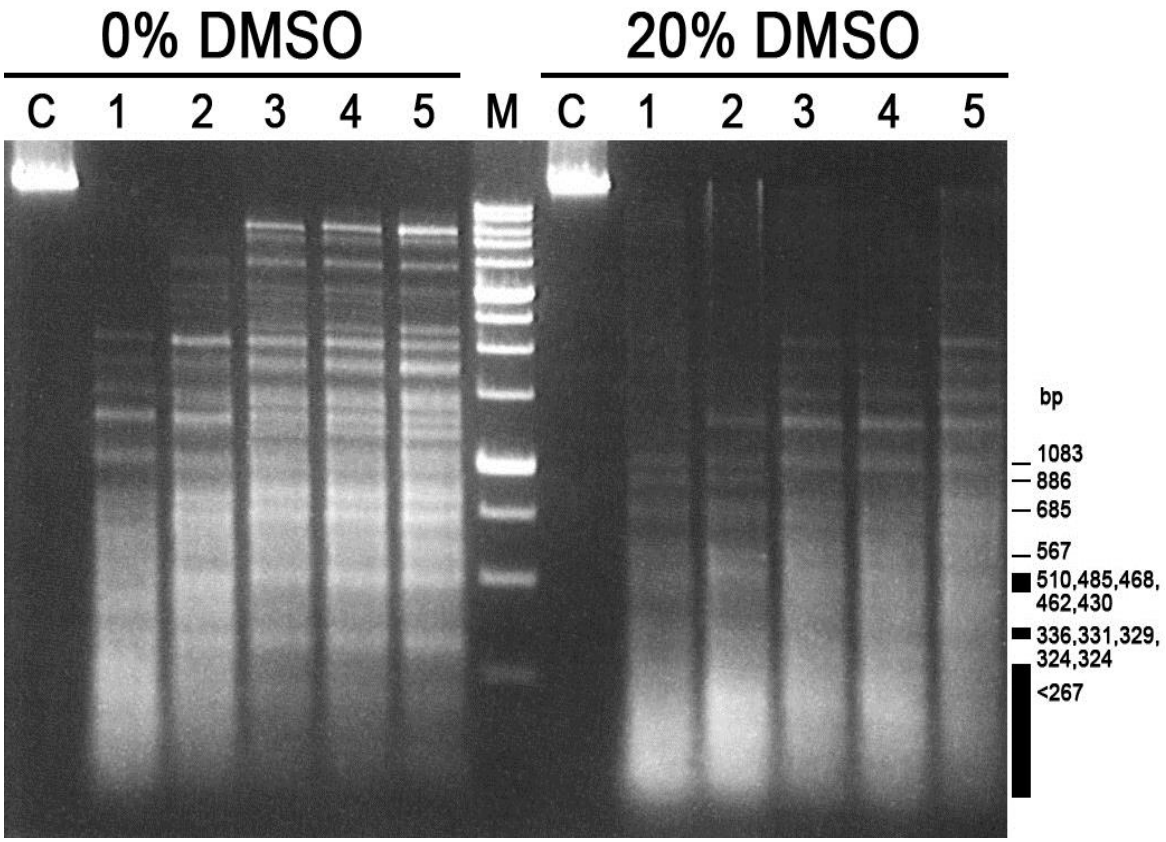
Surprisingly, but on the other side Figure 2 shows that addition of DMSO significantly decrease GlaI activity in the cleavage of the sequence with three overlapping sites. We see the linearized form of plasmid DNA in lanes 5-9 in the presence of DMSO and only in lane 9 in the control (no DMSO). So, GlaI cleaves a region with three overlapping sites 16 times slower in the presence of DMSO.

Thus, the presence of DMSO increases GlaI activity in the cleavage of sites 5'-G(5mC)GC-3'/3'-CG(5mC)G-5' and decreases GlaI activity in hydrolysis of a highly methylated region of overlapping sites. As a result DNA cleavage with GlaI in the presence of 15-20% DMSO allows to achieve a complete digestion of substrate DNA with differently methylated sites.

It's interesting that according to Figure 2 we see a higher GlaI activity of diluted enzyme (lanes 2 and 3) than non-diluted one in the presence of 25% and 30% DMSO (lane 1). We can speculate that high DMSO concentrations stimulate formation of inactive complexes of the enzyme if GlaI concentration is high enough.

Plasmid DNA is a relatively small substrate and we have tested a methylated lambda DNA to make sure that GlaI doesn't display any star activity in hydrolysis in presence of DMSO of much longer DNA with modified not only GCGC sites but methylated CG dinucleotides. The substrate was prepared by treating the phage λ DNA with the DNA methyltransferase SssI modifying the cytosine in dinucleotide 5'-CG-3'/3'-GC-5' in both strand. All GlaI recognition sites 5'-R(5mC)GY-3'/3'-YG(5mC)R-3' are presented in this substrate. Figure 4 demonstrates the results of the methylated lambda DNA hydrolysis with GlaI in an absence and presence of 20% DMSO. As it can be seen from Figure 4 the presence of 20% DMSO significantly increases GlaI activity in hydrolysis of CG-methylated λ phage DNA. Pattern of DNA hydrolysis on lane 4 in the presence of DMSO roughly corresponds to a picture of DNA without DMSO (lane 1 at 0% DMSO). Thus, based on the results of plasmid and phage DNA hydrolysis (Figures 2 and 4) we can conclude that in a presence of 20% DMSO GlaI activity in cleavage of sites 5'-R(5mC)GY-3'/3'-YG(5mC)R-5' is increased 8 times.

Figure 4: Cleavage of methylated λ phage DNA with GlaI in the absence and presence of DMSO. C – control untreated DNA. Other designations as on Figure 3. Theoretically calculated lengths of the GlaI fragments are shown at right.



Thus, our results show that an addition of 20% DMSO to the reaction mixture increases GlaI activity in a cleavage of sites 5'-R(5mC)GY-3'/3'-YG (5mC) R-5' and allows to achieve a complete hydrolysis of methylated long DNA substrates with GlaI.

CONCLUSIONS

An addition of 10-25% DMSO to the reaction mixture increases GlaI activity in a cleavage of sites 5'-R(5mC)GY-3'/3'-YG(5mC)R-5' about 8 times. A presence of DMSO doesn't change a substrate specificity of GlaI and doesn't produce any star activity in DNA hydrolysis with the enzyme. The described method of increasing the efficacy of DNA digestion with GlaI allows to achieve a complete hydrolysis of long methylated DNA substrates.

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