

Dependence of site-specific endonuclease Glal activity on quantity and location of methylcytosines in the recognition sequence 5'-GCGC-3'

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[Tomilova J.E.](#), [Chernukhin V.A.](#), [Degtyarev S.Kh](#)

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The activity dependence of site-specific endonuclease Glal that recognizes and hydrolyzes only methylated DNA sequence 5'-GCGC-3' on the quantity and location of 5-methylcytosines in enzyme's recognition sequence has been studied. A significant DNA cleavage has been observed for oligonucleotides duplexes containing four and three 5-methylcytosines or two internal modified bases. The cleavage efficiency is maximal for DNA duplex with four 5-methylcytosine and decreases when a number of methylated bases are lower. Glal hydrolyzes recognition sequences with 5-methylcytosines but not with N4-methylcytosines.

The most of bacterial site-specific DNA endonucleases are restriction endonucleases. Restriction endonucleases (restriction enzymes) and DNA-methyltransferases (methylases) of the same specificity form the so called restriction-modification (R-M) systems. It is supposed that restriction endonucleases (restriction enzymes) protect the cell against the penetration of foreign DNA (for example, phage DNA), whereas methylases protect the host DNA against cleavage with own restriction enzyme by modifying the recognition sequence. Even the name of enzyme "restriction endonuclease" originated from the experimentally observed phenomenon of the restriction of bacterial phage propagation in microorganisms [1]. Recently we discovered site-specific endonucleases which hydrolyze only cytosine-methylated DNA and exist in bacterial cells without corresponding methylases [2], [3]. Based on the current classification [4] these enzymes may be placed into IIM group of site-specific endonucleases, i.e. endonucleases recognizing only methylated DNA. Only three specificities of endonucleases have been described to present day in IIM group. Enzyme *DpnI* and its isoshizomers recognize the DNA sequence 5'-G(6mA)[^]TC-3' in which adenine is methylated [5]. Two other endonucleases do not have isoshizomers and hydrolyze DNA methylated at the cytosine base in position C5: *BisI* recognizes and hydrolyzes sequence 5'-G(5mC)[^]NGC-3' [2], *Glal* - sequence 5'- G(5mC)[^]GC-3' [3]. Because of several cytosines presence in the recognition sequences of two latter IIM endonucleases, the enzymes activity of *BisI* and *Glal* might depend on the number and location of modified bases in the site. The goal of the

present work was to study the efficiency of DNA cleavage with endonuclease *GlaI* depending on cytosines methylation in recognition site 5'-GCGC-3'.

MATERIALS AND METHODS

Restriction endonucleases, T4 polynucleotide kinase and buffer solutions manufactured by SibEnzyme (Russia) were used in the experiments. Oligodeoxyribonucleotides of the following composition, which served as a substrate for endonuclease *GlaI*, were synthesized at SibEnzyme Ltd. (Russia):

G1: 5'-CTATGAACGTTTTTCGCGCTGACGGACCGTATC-3'
G2: 5'-GATACGGTCCGTCAGCCGAAAACGTTTCATAG-3'
G3: 5'-CTATGAACGTTTTTCG(5mC)GCTGACGGACCGTATC-3'
G4: 5'-GATACGGTCCGTCAG(5mC)GC GAAAACGTTTCATAG-3'
G5: 5'-CTATGAACGTTTTTCGCG(5mC)_TGACGGACCGTATC-3'
G6: 5'-GATACGGTCCGTCAGC(5mC)_GAAAACGTTTCATAG-3'
G7: 5'-CTATGAACGTTTTTCG(5mC)G(5mC)_TGACGGACCGTATC-3'
G8: 5'-GATACGGTCCGTCAG(5mC)G(5mC)_GAAAACGTTTCATAG-3'
G9: 5'-CTATGAACGTTTTTCG(4mC)G(4mC)_TGACGGACCGTATC-3'
G10: 5'-GATACGGTCCGTCAG(4mC)G(4mC)_GAAAACGTTTCATAG-3'

Oligonucleotides under an even number are complementary to oligonucleotides under an odd number. All oligonucleotide duplexes have the same primary structure and differ from each other in the presence or the absence of methylcytosine in sequence GCGC (site GCGC, recognized by *GlaI*, underlined).

Isolation of plasmid DNA pHspAI was performed using the QIAGEN GmbH kit (Germany) according to the manufacturer's protocol.

Preparation of a substrate for endonuclease *GlaI*

One of the chains of oligonucleotide duplex was labeled at 5'-end using T4-polynucleotide kinase and γ [³²P]ATP. After oligonucleotide purification, complementary unlabeled oligonucleotide was added, the tube was heated at 95°C for 5 minutes followed by cooling to room temperature on the table.

Hydrolysis of oligonucleotide duplexes with endonuclease *GlaI*

Hydrolysis reaction was conducted in 10 μ l of the reaction mixture containing SE-buffer "Y" (33 mM Tris acetate pH 7.9 (at 25°C), 10 mM Mg(CH₃COO)₂, 66mM KCH₃COO, 1mM DTT) and oligonucleotide duplex at the concentration of 62.5 nM at the temperature of 37°C for 1 hour. Experiments shown in Fig. 6 were performed in *GlaI* buffer (Tris-HCl pH 8.5 (at 25°C), 5 mM MgCl₂, 10 mM NaCl, 1.5 mM β -mercaptoethanol) at the temperature of 30°C).

The minimal quantity of the enzyme, which is enough for complete hydrolysis of the oligonucleotide duplex G7*/G8 in *GlaI* buffer at the temperature of 30°C was taken as a unit of enzyme activity.

The determination of hydrolysis degree for oligonucleotide duplexes

Electrophoresis of the hydrolysis products was carried out in denaturing 20% PAAG with 7 M urea in tris-borate buffer. Gel autoradiography was performed using the Cyclone Storage System (Packard Instrument Co., USA). For each labeled product, DLU (Digital Light Units) was determined, which is proportional to the intensity of [³²P] isotope radiation minus the background. Hydrolysis percentage was determined as a proportion of DLU for the obtained reaction product divided the sum DLU falling on the rest of initial DNA and the obtained DNA fragment. Data treatment was performed using the OptiQuant program V. 0.3.00 (Packard Instrument Co., USA).

Hydrolysis of plasmid DNA with endonuclease *GlaI*

The reaction was conducted in 80 µl of the reaction mixture containing SE-buffer "Y" (33 mM Tris acetate pH 7.9 (at 25°C), 10 mM Mg(CH₃COO)₂, 66mM KCH₃COO, 1mM DTT) and 0.8 µg of plasmid pHspAI DNA [3] at the temperature of 37°C for 1 hour. Hydrolysis products were separated in 1.2% agarose gel in tris-borate buffer.

Statistical treatment of results

Treatment was performed using the program Origin 7.0. Microsoft Office Excel 2003 was used for graphical representation of results.

RESULTS AND DISCUSSION

Recently we discovered a new site-specific endonuclease *GlaI*, which does not cleave widely used DNA substrates (lambda and T7 DNAs, adenoviral DNA, plasmids pUC19, pBR322) and hydrolyzes only the plasmid DNA containing a gene of DNA-methyltransferase HspAI [3]. Enzyme M.HspAI methylates internal cytosine at position 5 in the sequence 5'-GCGC-3' [3]. This fact allowed us to suggest and then to prove that site-specific endonuclease *GlaI* cleaves sequence 5'-G(5mC)GC-3'/3'-CG(5mC)G-5'. However, the question about the influence of methylation of the other cytosines in the recognition sequence on enzyme *GlaI* activity was not clear. In this work we have studied *GlaI* activity dependence on the quantity and location of methylated cytosines in enzyme's recognition sequence 5'-G(5mC)GC-3'/3'-CG(5mC)G-5'. Unlike all known restriction endonucleases, it does not seem feasible to produce DNA substrates for *GlaI* in the form of plasmid, phage or other native DNA having recognition sites methylated in a different ways. In this connection, we used chemically synthesized oligonucleotide duplexes containing methylcytosine in different positions of the recognized sequence 5'-GCGC-3'/3'-CGCG-5' to study the properties of endonuclease *GlaI*.

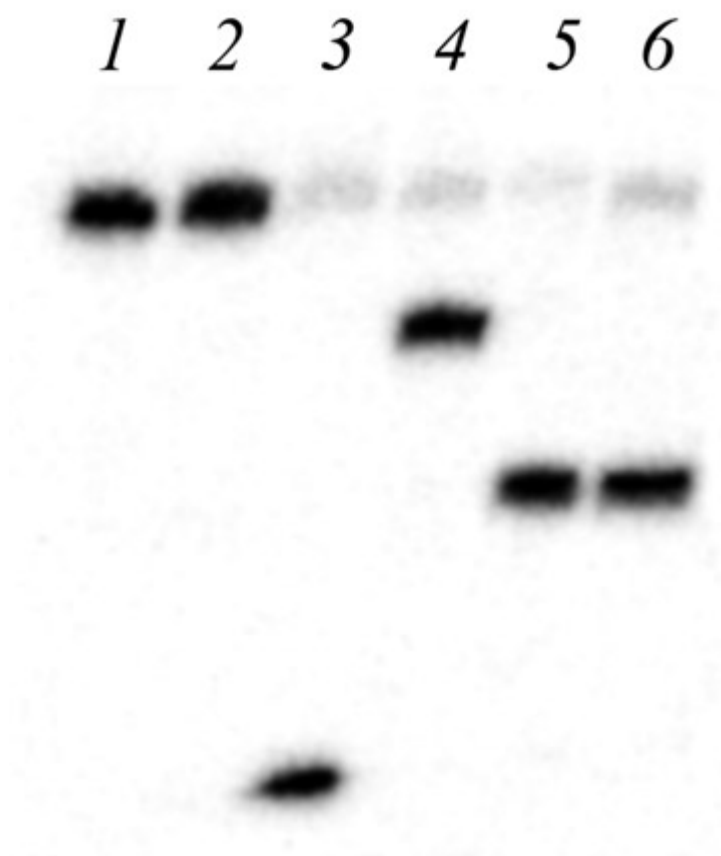


Fig. 1 Cleavage of the oligonucleotide duplex G7*/G8 with different endonucleases. Runs: 1 - DNA duplex without the enzyme, 2 - hydrolysis with restriction endonuclease *HspAI*; 3 - hydrolysis with restriction endonuclease *AcII*; 4 - hydrolysis with restriction endonuclease *RsrII*, 5, 6 - hydrolysis with endonuclease *GlaI* (10 units and 2 units, respectively).

To study the dependence of enzyme *GlaI* activity on the quantity and location of modified cytosines in the recognition sequence, we performed the comparative study of hydrolysis efficiency of different methylated oligonucleotides. Both chains of the recognition site of endonuclease *GlaI* contain a total of four cytosines; each of them can be methylated. Two of them are internal and form a central dinucleotide CG, and two are external ones. It is known that recognition sequence 5'-GCGC-3'/3'-CGCG-5' possesses palindromic symmetry i.e. repeats itself at turning 180 degrees round the central axis. The location of methylated cytosines in the recognition site can also form a palindrome and be symmetrical. Table 1 presents the structure of the recognition site of *GlaI* in oligonucleotide duplexes used in this work and their shorthand notation.

Oligonucleotide duplex	Position of C5-methylcytosines in the <i>GlaI</i> recognition sequence.	Duplex's definition
G1*/G2, G2*/G1	5' - GCGC - 3' 3' - CGCG - 5'	—
G1*/G4, G2*/G3	5' - GC G C - 3' 3' - CG (m5C) G - 5'	—T
G3*/G2, G4*/G1	5' - G (m5C) GC - 3' 3' - C G CG - 5'	—T
G1*/G6, G2*/G5	5' - G CGC - 3' 3' - (m5C) GCG - 5'	T—
G5*/G2, G6*/G1	5' - GCG (m5C) - 3' 3' - CGC G - 5'	—T
G1*/G8, G2*/G7	5' - G C G C - 3' 3' - (m5C) G (m5C) G - 5'	T—T
G7*/G2, G8*/G1	5' - G (m5C) G (m5C) - 3' 3' - C G C G - 5'	—T—T
G3*/G4, G4*/G3	5' - G (m5C) G C - 3' 3' - C G (m5C) G - 5'	—T—
G5*/G6, G6*/G5	5' - G CG (m5C) - 3' 3' - (m5C) GC G - 5'	T—T
G3*/G6, G4*/G5	5' - G (m5C) GC - 3' 3' - (m5C) G CG - 5'	T—T
G5*/G4, G6*/G3	5' - GCG (m5C) - 3' 3' - CG (m5C) G - 5'	—T—T
G3*/G8, G4*/G7	5' - G (m5C) G C - 3' 3' - (m5C) G (m5C) G - 5'	T—T—T
G7*/G4, G8*/G3	5' - G (m5C) G (m5C) - 3' 3' - C G (m5C) G - 5'	—T—T—T
G5*/G8, G6*/G7	5' - G C G (m5C) - 3' 3' - (m5C) G (m5C) G - 5'	T—T—T
G7*/G6, G8*/G5	5' - G (m5C) G (m5C) - 3' 3' - (m5C) G C G - 5'	T—T—T
G7*/G8, G8*/G7	5' - G (m5C) G (m5C) - 3' 3' - (m5C) G (m5C) G - 5'	T—T—T—T

Table 1 Structure of oligonucleotide duplexes

Fig. 2 presents gel autoradiography after electrophoretic separation of hydrolysis products of oligonucleotide duplexes G7*/G8, G7*/G4, G7*/G6, G3*/G8, G5*/G8, G3*/G4 with different qualities of endonuclease *GlaI*.

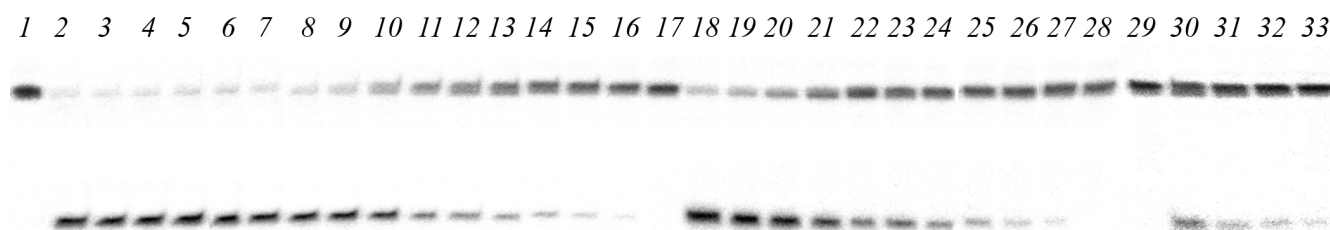


Fig. 2 Cleavage of oligonucleotide duplexes with endonuclease *G/ai*.

Runs: 1-6 - duplex G7*/G8, 7-11 - duplex G7*/G4, 12-16 - duplex G7*/G6, 17 - 22 - duplex G3*/G8, 23-28 - duplex G5*/G8, 29-33 - duplex G3*/G4. *G/ai*, 8 units - 2, 7, 12, 18, 23, 30. *G/ai*, 4 units - 3, 8, 13, 19, 24, 31. *G/ai*, 2 units - 4, 9, 14, 20, 25, 32. *G/ai*, 1 unit - 5, 10, 15, 21, 26, 33. *G/ai*, 0.5 unit. - 6, 11, 16, 22, 27.

Experimental data were processed as indicated in "Materials and Methods". The obtained numeric values for duplexes G7*/G8, G3*/G8, G7*/G4, G8*/G7, G8*/G3 and G4*/G7 are presented in Fig.3, those for duplexes G7*/G6, G6*/G7, G8*/G5 and G5*/G8 - in Fig.4 in the form of dependence of the portion of hydrolyzed oligonucleotide duplexes (per cents) on the quantity of enzyme *G/ai* added. Similar results for duplexes G3*/G4, G4*/G3 and G7*/G2 are presented in Figure 5. Fig.4 also presents data on the cleavage of oligonucleotides G3*/G8 and G4*/G7 for convenience of comparative analysis.

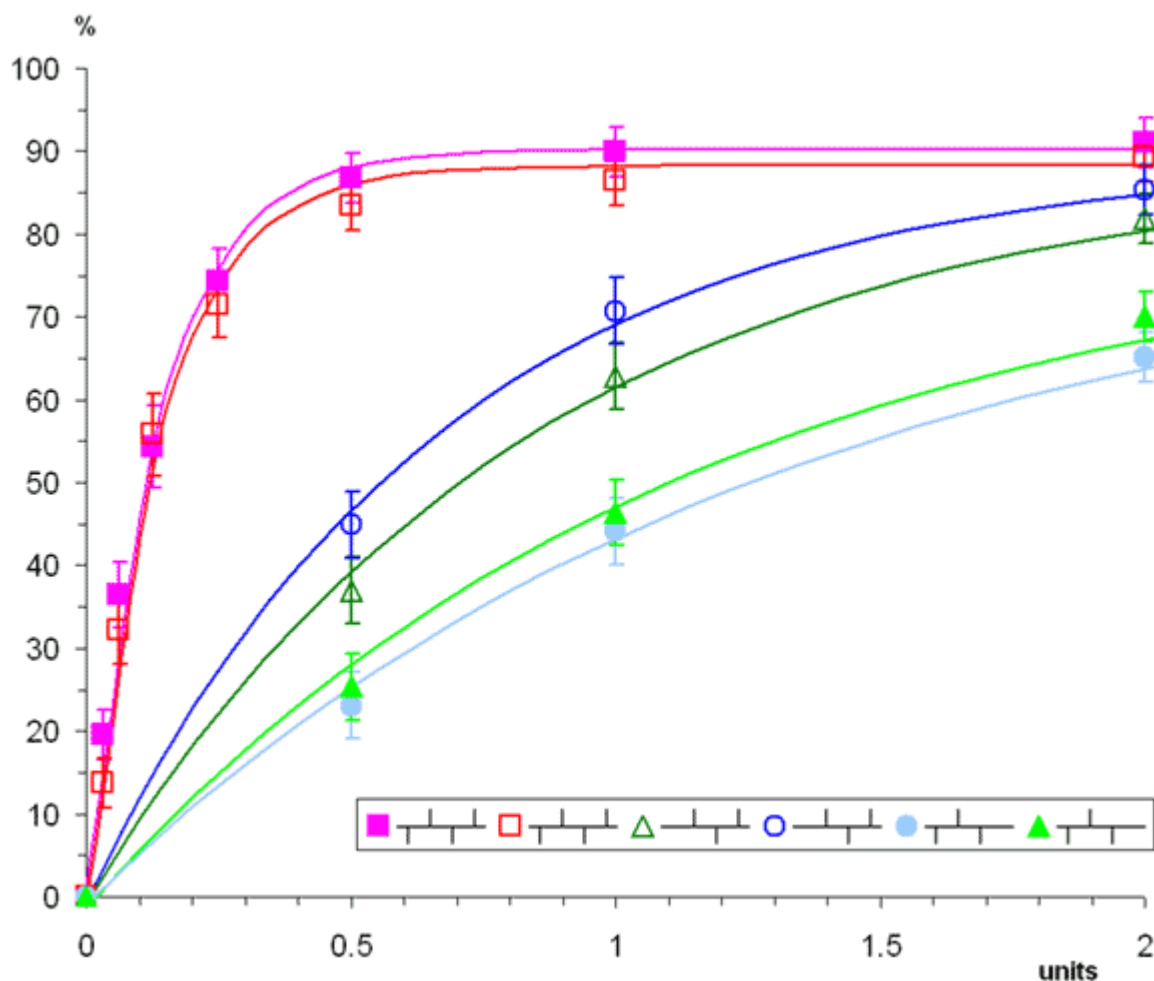


Fig. 3. Dependence of the portion of hydrolyzed duplexes in % (axis Y) on the quantity of endonuclease *G/ai* units added to the reaction mixture (axis X). ϕ - duplex G7*/G8, £ - duplex G8*/G7, ~ - duplex G3*/G8, TM - duplex G8*/G3, p - duplex G4*/G7, r - duplex G7*/G4.

As Figure 3 shows, the highest enzyme activity is observed in the presence of four 5-methylcytosines (fully symmetric duplex G7*/G8) in the recognition site. Asymmetrical sites with three methylated cytosines two of which are internal are hydrolyzed less effectively. The cleavage rates of DNA chains with two methyl groups differ insignificantly in these duplexes (G3*/G8 and G4*/G7). For both oligonucleotides, hydrolysis of the complementary DNA chain carrying one methyl group with enzyme *G/ai* occurs with somewhat lower and also practically equal activity.

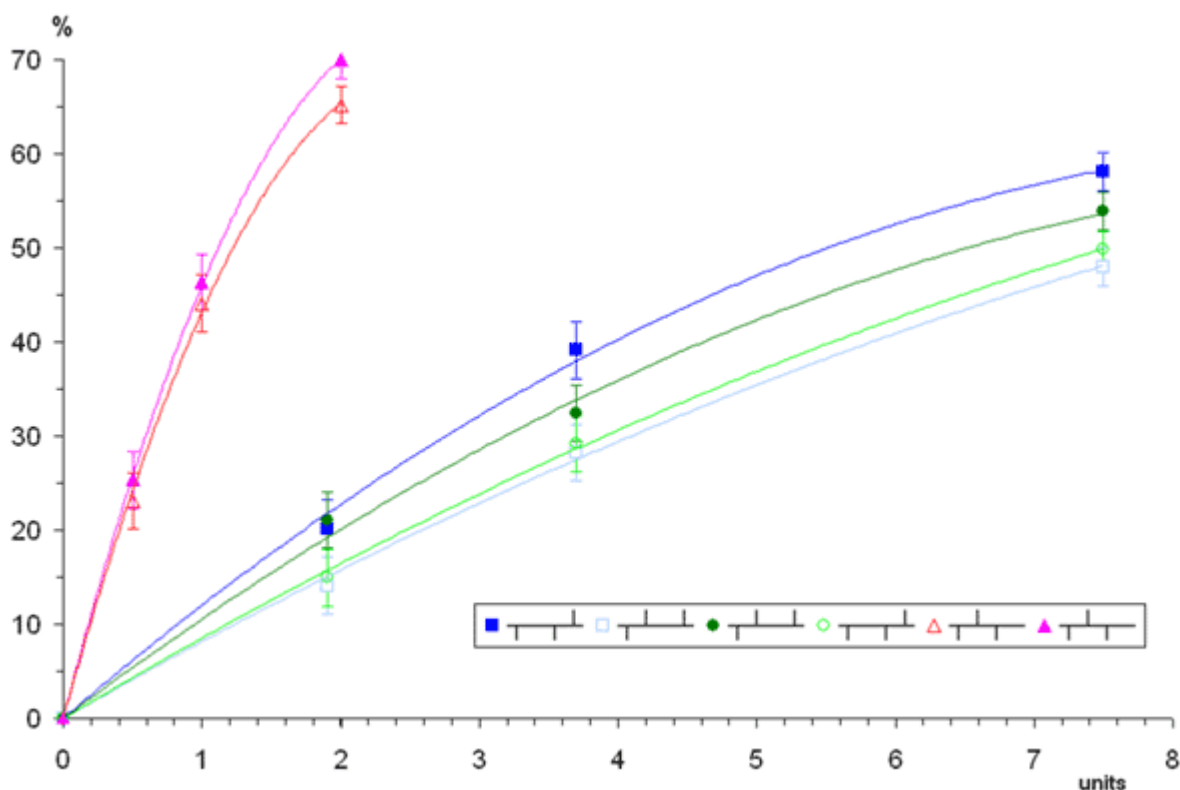


Fig.4. Dependence of the portion of hydrolyzed duplexes in % (axis Y) on the quantity of endonuclease *G/ai* units added to the reaction mixture (axis X). $\blacktriangle$ - duplex G4*/G7, $\blacktriangledown$ - duplex G3*/G8, $\blacksquare$ - duplex G5*/G8, $\square$ - duplex G8*/G5, $\bullet$ - duplex G7*/G6, $\circ$ - duplex G6*/G7.

From the data presented in Fig.4, it is seen that a significantly lower efficiency of hydrolysis is observed for asymmetrical oligonucleotide duplexes with three methylcytosines, two of which are external (G7*/G6, G6*/G7, G8*/G5 and G5*/G8). However, in this case the cleavage rates of all DNA chains in these duplexes are close to each other. And finally, a still lower enzyme activity is observed for a cleavage of the symmetrical duplex containing two internal methylated cytosines - duplex G3*/G4 (Fig 5). Oligonucleotide duplex carrying two methylcytosines in one chain is a bad substrate and is cleaved only with a very high *G/ai* concentrations (G7*/G2 in Fig.5 and Fig.8). We did not observe any visible hydrolysis of duplexes containing two external 5-methylcytosines, one external and one internal 5-methylcytosines in different chains and duplexes with one 5-methylcytosine (data are not shown).

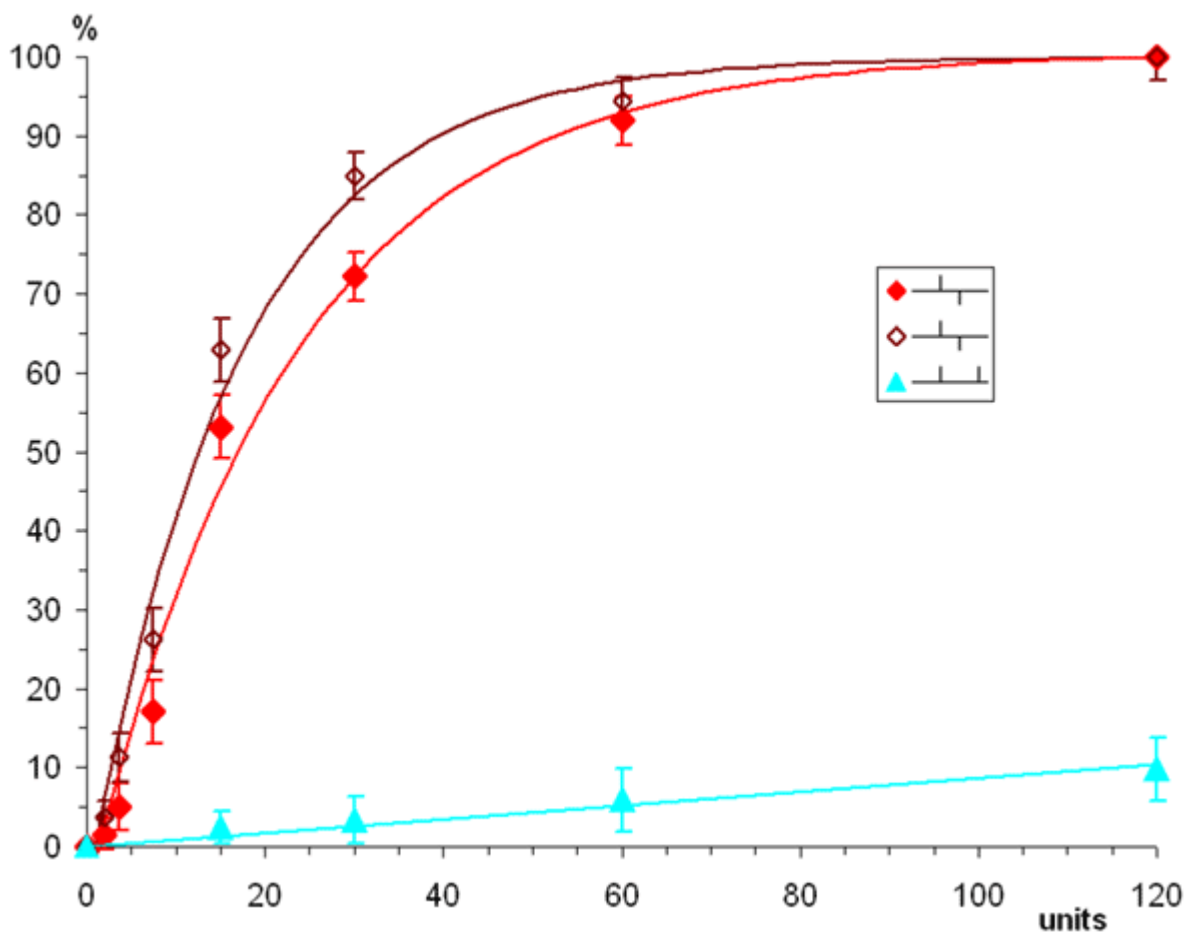


Fig. 5. Dependence of the portion of hydrolyzed duplexes in % (axis Y) on the quantity of endonuclease *G/ai* units added to the reaction mixture (axis X). ♦ - duplex G*3/G4, ◊ - duplex G4*/G3, ▲ - duplex G7*/G2.

Fig. 6A presents the data on the dependence of hydrolysis of duplexes G7*/G8 and G4*/G7 on time in buffer "Y" (SibEnzyme) and *G/ai* buffer at 30°C. As the Figure shows, the enzyme activities on these substrates and in these buffers do not differ. Figure 6B presents the data on the enzyme activity in *G/ai* buffer on different substrates. As the Figures 6A and 6B show, the enzyme activity in hydrolysis of the duplex containing four methylcytosines in the recognition site (duplex G7*/G8) is 2 - 3 times higher than the enzyme activity at cleaving duplexes with one external non-methylated cytosine. (duplex G4*/G7). The cleavage of duplex G7*/G6, in which two external and one internal cytosine are methylated, occurs at 6-7 times lower activity than the activity on substrate G7*/G4. Data presented in Fig. 5 (as compared with Fig. 4) and 6B show that the enzyme activity on duplex G3*/G4 is approximately 2 times lower than the activity on substrate G7*/G6. The enzyme activity on duplex G7*/G2 is observed only at very high

concentrations of the enzyme (Fig.5 and Fig.6B) and makes up less than 0.1% of the enzyme activity on duplex G7*/G8.

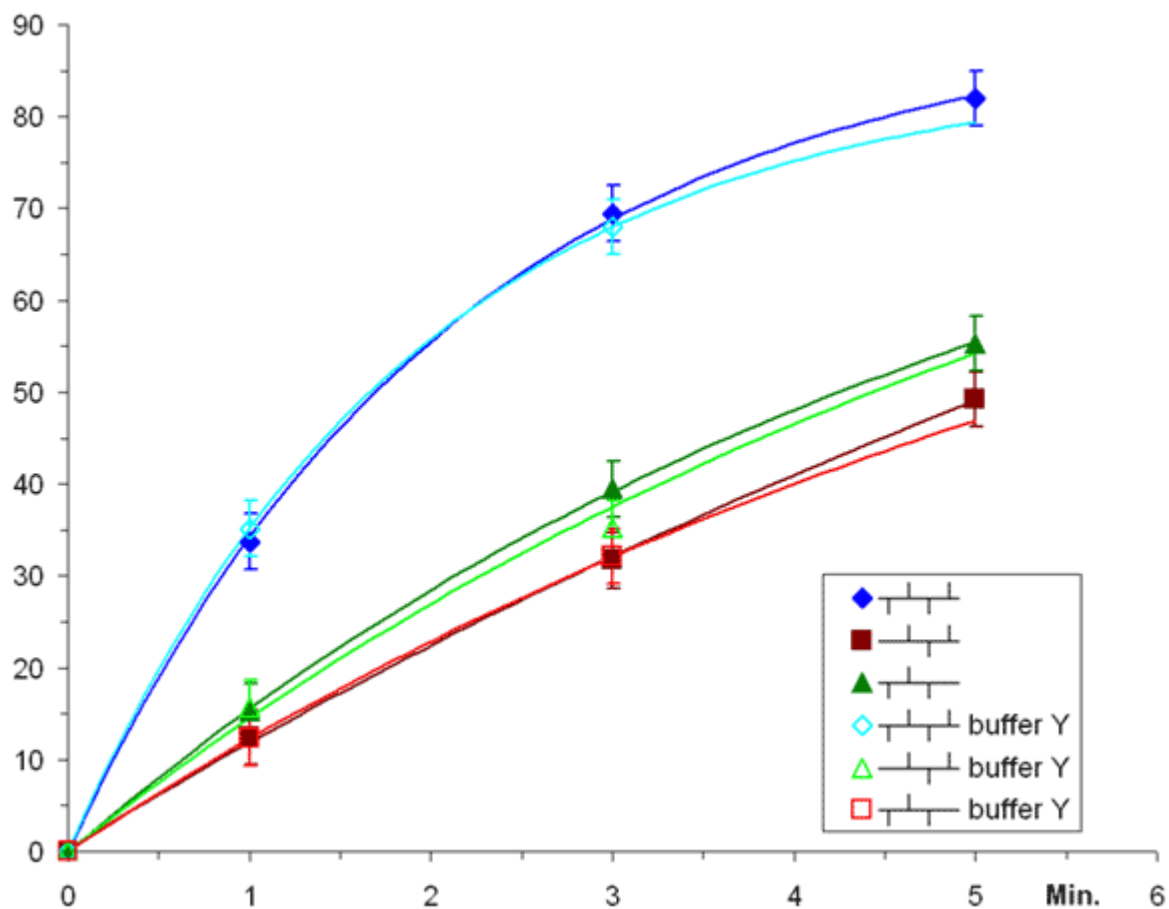


Fig.6A. Time-dependence of the portion of hydrolyzed oligonucleotide duplexes with different methylation degrees of the recognition site. Time-dependence of the portion of hydrolyzed duplexes G7*/G8 (blue diamonds and cyan squares), G7*/G4 (green triangles and red squares) and G4*/G7 (green triangles and red squares) in % (axis Y) on time in buffer "Y" (SibEnzyme) and *GlaI* buffer at 30°C.

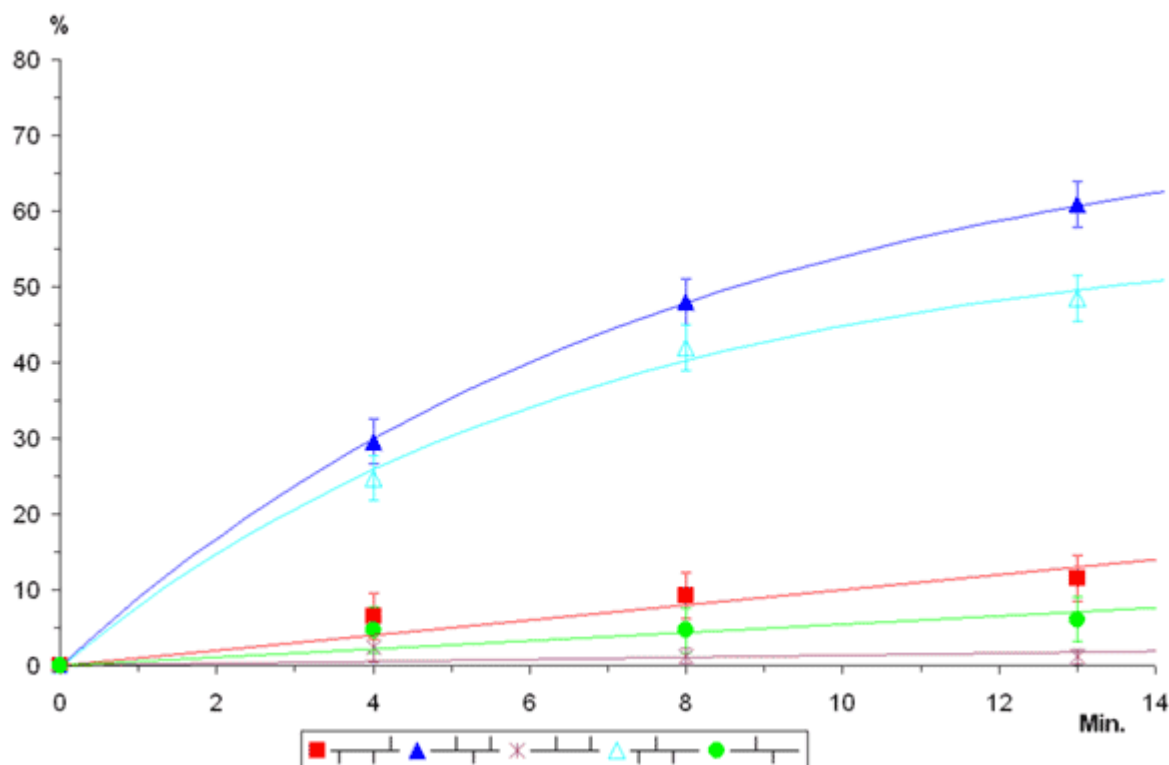


Fig.6B. Time-dependence of the portion of hydrolyzed oligonucleotide duplexes with different methylation degrees of the recognition site. Time-dependence in % of the portion of hydrolyzed duplexes G7*/G6 - ϕ , G7*/G4 - ϕ , G7*/G2 - $\hat{U}$, G4*/G7 - ϕ and G4*/G3 - $\sim$ in *GlaI* buffer at 30°C

Table 2 summarizes the obtained data on the enzyme activity on different methylated substrates. The Table shows that *GlaI* effectively cleaves DNA sequence 5'-GCGC-3'/3'-CGCG-5' containing 4 or 3 modified bases when both internal cytosines are methylated. Sites with two external and one internal cytosine as well as with two internal modified cytosines are cleaved much worse. Oligonucleotide with two modified bases on one chain is cleaved insignificantly and only at larger concentrations of the enzyme.

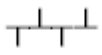
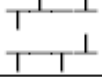
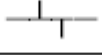
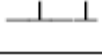
Oligonucleotide duplex	Duplex's definition	Enzyme's activity
G7*/G8 G8*/G7		100%
G3*/G8 G8*/G3		35-45%
G4*/G7 G7*/G4		35-45%
G5*/G8 G8*/G5		5-6%
G7*/G6 G6*/G7		5-6%
G4*/G3 G3*/G4		2-2.5%
G7*/G2 G2*/G7		<0,1%

Table 2 Glal activity on methylated oligonucleotide duplexes

As DNA methyltransferase capable of methylating internal cytosines in position C5 in sequence GCGC was found in bacterial strains, the comparison of hydrolysis efficiency of symmetrically methylated oligonucleotide duplexes G3*/G4 and G7*/G8 is of special interest. Table 2 shows that the enzyme activity in hydrolysis of the duplex containing all the four methylated cytosines (G7*/G8) is approximately 45 times more than that for the duplex containing only two internal methylated cytosines (G3*/G4).

As it is seen from the results of the different substrates cleavage in Fig.3-5, the site environment practically does not influence the cleavage efficiency of oligonucleotide duplexes. In fact, though in G3 and other odd numbers of oligonucleotides the pyrimidine cluster precedes the recognition sequence, while in G4 and other even numbers of oligonucleotides there is group of purines before the recognized sequence, differences in the enzyme activity at cleaving even and odd chains in equally methylated oligonucleotide duplexes are not revealed.

As it follows from the obtained results, the cleavage efficiency of oligonucleotide duplexes with enzyme *Glal* depends on the following points:

1. The number of methyl groups in the recognition sequence. The greater the number of methyl groups in the recognition site, the higher the hydrolysis rate is. The above data demonstrate that only sites containing from two to four methylated cytosines are hydrolyzed. In the first case, a significant level of hydrolysis is observed only if internal cytosines are methylated. The maximal hydrolysis rate is

observed when four 5-methylcytosines are present in the recognition site.

2. Methylation of internal cytosines in the site is more important for hydrolysis efficiency than that of external ones. This statement is substantiated by the fact that, at the same quantity of methyl groups, a site in which two internal and one external cytosine are modified is hydrolyzed considerably better than a site in which two external and one internal cytosine are methylated. Besides, hydrolysis is absent if two external cytosines in the site are methylated, whereas hydrolysis occurs if two internal bases are methylated.

Previously we investigated enzyme *GlaI*-cleavage of plasmid pHspAI containing the gene of DNA methylase HspAI recognizing and modifying sequence 5'-GCGC-3'/3'-CGCG-5' with the formation of two internal methylated cytosines [3]. It is interesting to compare the activity of enzyme *GlaI* at cleaving plasmid DNA and oligonucleotide substrates. Fig. 7 shows the dependence of cleavage of plasmid pHspAI with different quantities of the enzyme. The comparison of the obtained experimental data with the calculated hydrolysis pattern [3] shows that practically complete hydrolysis of 0.29 picomoles of plasmid DNA (4.72 picomoles of recognition sites) is observed at adding 120 units of enzyme to the reaction mixture.

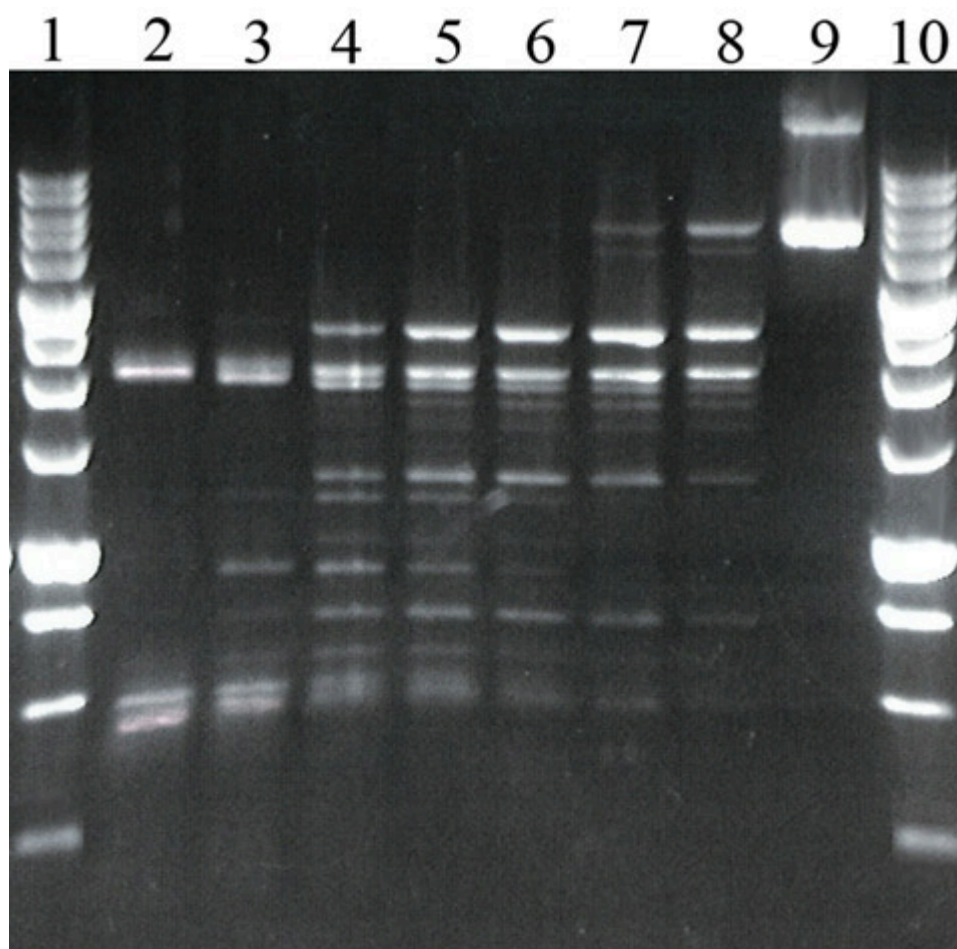


Fig. 7 Hydrolysis of plasmid pHspAI with different quantities of *GlaI*. The concentration of the sites 5'-G(5mC)GC-3'/3'-CG(5mC)G-5' - 59 nm.Runs: 1, 10 - marker 1 kb, 2-8 - plasmid pHspAI treated with *GlaI* (2 - 120 units, 3 - 60 units, 4 - 30 units, 5 - 15 units, 6 - 7.5 units, 7 - 3.75 units, 8 - 1.9 units), 9 - initial plasmid pHspAI.

As it is seen from the data presented in Fig.5, for the case of oligonucleotide duplex G3*/G4, at the concentration of the sites 5'-G(5mC)GC-3'/3'-CG(5mC)G-5' of 62.5 nM, a significant hydrolysis of 0.62 picomoles of the sites is reached at adding 120 and more units of enzyme activity. From the comparison of these results it follows that under conditions of our experiments, the efficiency of the recognition sequence 5'-G(5mC)GC-3'/3'-CG(5mC)G-5' hydrolysis with *GlaI* in oligonucleotide duplex is approximately 8 times lower than in plasmid DNA.

Previously it was shown that the modification of cytosines in bacterial cells occurs with the formation of either 5-methylcytosine or N4-methylcytosine [6,7]. We checked the possibility of hydrolysis of DNA, in which sequence 5'-GCGC-3' is methylated in position NC4. For this purpose, we used duplex G9*/G10, which differs from duplex G7*/G8 only in that in the site 5'-GCGC-3' all the four cytosines are methylated in position NC4, but not C5.

As Figure 8 shows, *GlaI* does not hydrolyze duplex G9*/G10, containing the site 5'-G(m4C)G(m4C)-3'/3'-(m4C)G(m4C)G-5'. The presence of cytosines methylated in position NC4, probably, does not influence the efficiency of DNA cleavage. The hydrolysis rate of duplex G7*/G10 in which two cytosines in one DNA chain are methylated in position C5, and two cytosines in the other chain - in position NC4, does not differ from the cleavage rate for duplex G7*/G2, in which only two 5-methylcytosines are present in one of the chains.

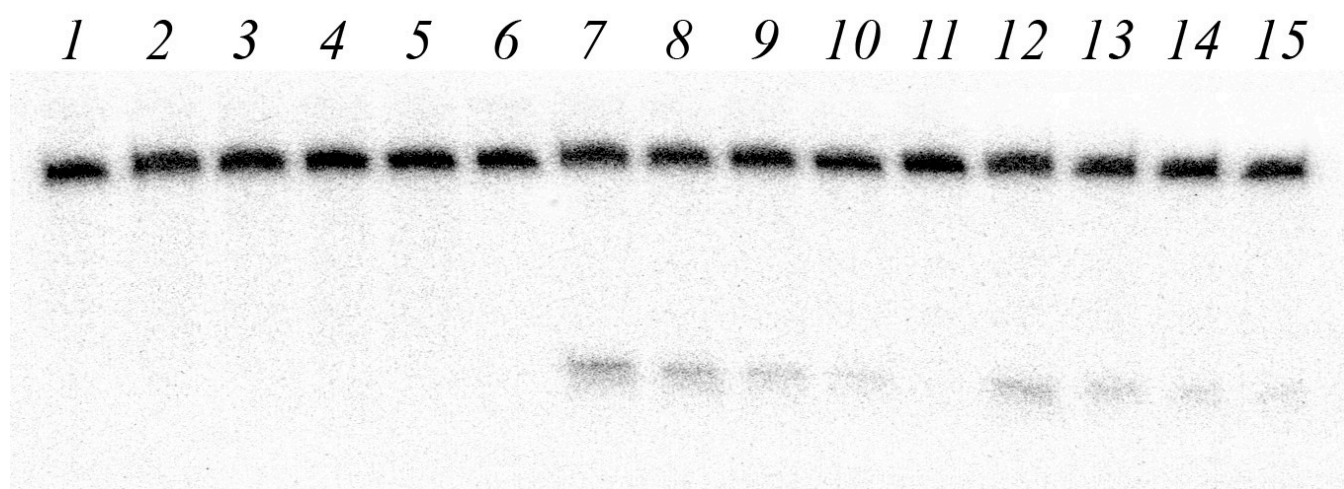


Fig. 8 The influence of NC4-modification of the site 5'-GCGC-3' on the ability of *GlaI* to hydrolyze DNA. Runs: 1 - duplex G9*/G10; 2-5 - duplex G9*/G10, treated with *GlaI* (120, 60, 30, 15 units respectively); 6 - duplex G7*/G10; 7-10 - duplex G7*/G10, treated with *GlaI* (120, 60, 30, 15 units respectively); 11 - duplex G7*/G2; 12-15 - duplex G7*/G2, treated with *GlaI* (120, 60, 30, 15 units respectively).

The question of the role of IIM endonucleases in the functioning of a bacterial cell still remains disputable. One of possible functions of enzymes of this type is the protection against the invasion of foreign methylated phage DNA [2], [3]. And while for enzymes *DpnI* and *BisI* such role is quite possible, in the case of *GlaI* the function of protection against such DNA seems unlikely. Currently known bacterial and phage methylases, which modify sequence 5'-GCGC-3', methylate only internal cytosine in the recognition site [8]. As a result of this modification, the site 5'-G(5mC)GC-3'/3'-GCG(5mC)G-5' is produced for which the hydrolysis rate with enzyme *GlaI* is significantly lower than that for the site with four methyl groups. That's why the following two questions remain unanswered:

1. Does high efficiency of hydrolysis of the sites GCGC containing three or four methyl groups with site specific endonuclease *GlaI* play any biological role?
2. Is modification of the site GCGC at which three or four cytosines are methylated in the recognition site possible in a bacterial cell?

It should be noted that methylases modifying cytosine in the site 5'-CG-3' have been found in many eukaryotic organisms. Based on the current Human Epigenome Project the study of CG methylation in chromosomal DNA is in progress now in many scientific institutions [9]. At present, the main method of the methylation status determination is the method of methyl-specific PCR [10]. As following from the data given above *GlaI* may find an application in the new methods of the methylation status determination either in the small pieces of eukaryotic DNA or of the whole genome.

There are two basic properties characterizing restriction endonucleases: the recognition sequence and positions of DNA cleavage. As it is shown above, in the case of type IIM site-specific endonucleases the quantity and the distribution of 5-methylcytosines in the recognition site also play a significant role in determination of the enzyme specificity and activity. Thus, when describing the main properties of type IIM site-specific endonucleases it's necessary to indicate three parameters characterizing these enzymes: the recognition sequence, the DNA cleavage positions and the location of 5-methylcytosines in the recognition sequence.

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ACCEPTED ABBREVIATIONS

* - ³²P -labeled oligonucleotide