Modification methylase M.*Sau*3239I from *Streptomyces aureofaciens* 3239

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By chromatography on phosphocellulose and Heparin-Sepharose the modification methylase M.*Sau*3239I was detected and partly purified from cells of *Streptomyces aureofaciens* 3239. Methylation by this enzyme protects DNA from cleavage by the restriction endonuclease R.*Sau*3239I. The enzyme catalyzes methylation of adenine to *N*-6-methyladenine in the 5′-CTCG^{m}AG-3′ recognition sequence.

Streptomyces aureofaciens; DNA methylation; Modification methylase

1. INTRODUCTION

While more than 1100 site-specific endonucleases of type II, and of them 146 with original cleavage specificity, are known [1], only 117 corresponding modification methylases, and of them only one, M.*Sal*I produced by a Streptomycete, was described [2]. This can be considered to be a result of difficulties in the isolation of modification enzymes which are very unstable. Up to now we have isolated in our Institute three site-specific endonucleases from Streptomycetes: *Sau*I [3], *Sau*3239I [4,5], an isoschizomer of *Xho*I, and *Sau*BMKI [6], an isoschizomer of *Nae*I. In this paper the characterization of the corresponding modification methylase M.*Sau*3239I is reported.

To the present time the corresponding modification methylase to restriction endonuclease R.*Xho*I was not isolated and characterized but the corresponding modification methylases to its two isoschizomers M.*Bsu*MI [7] and M.*Pae*R7I [8] were isolated. The first enzyme methylates the internal cytosine and the second one methylates adenine in the recognition sequence. Because of this difference in specificity it seemed to be of interest to identify the site of nucleotide modification of the recognition sequence by the corresponding modification methylase of a further isoschizomer.

2. MATERIALS AND METHODS

2.1. *Microorganisms and cultivation*

Streptomyces aureofaciens, strain 3239 which was used as a source of the modification methylase M.*Sau*3239I was grown at 28°C on a rotary shaker on a medium containing in 1000 ml sucrose (3.0 g), dextrine (15.0 g), urea (0.1 g), NaCl (0.5 g), K_2HPO_4 (0.5 g), $MgSO_4$ (0.5 g), peptone (5.0 g), $FeSO_4$ (0.01 g) and beef extract (1.0 g), pH 7.2. The cells of *S. aureofaciens* were harvested after 10 h of cultivation, 3 times washed with PEM buffer, pH 7.4 (10 mmol/l Na-phosphate, 10 mmol/l EDTA, 10 mmol/l 2-mercaptoethanol) and kept frozen at −17°C.

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2.2. *Chemicals*

The restriction endonuclease *Sau*3239I and λDNA were prepared by the Department of Experimental Production (Institute of Molecular Biology, Slovak Academy of Sciences). *S*-adenosyl-L-methionine (SAM) and P11 phosphocellulose were from Serva, ^{3}H-labeled SAM was a product of Amersham, heparin-Sepharose was a product of Pharmacia and high-molecular DNA from bovine spleen was from the Department of Biochemistry (Faculty of Science, Comenius University, Bratislava).

2.3. *Modification methylase assay*

The usual specific 'protection' assay was used for the detection of methylase activity. The reaction mixture (20 μl) contained 20 mmol/l Tris-HCl, pH 7.5, 50 mmol/l NaCl, 1 mmol/l EDTA, 78 μmol/l SAM and 0.7 μg λDNA. After incubation at 37°C for 3 h $MgCl_2$ was added to 10 mmol/l final concentration and the mixture incubated with 5 U of restriction endonuclease *Sau*3239I for another hour. 10 μl of a mixture of 50% glycerol, 0.05% Bromphenol blue and 0.1 mol/l EDTA were added to stop the reaction. Electrophoresis of DNA was performed on 0.8% agarose slabs (0.3 × 12.5 × 6 cm) containing 0.5 μg/ml ethidium bromide [9].

2.4. *Determination of methylated base*

λDNA (40 μg) or DNA from bovine spleen (60 μg) were methylated by the isolated enzyme preparation after chromatography on a Heparin-Sepharose column in a reaction mixture (200 μl) containing 10 mmol/l Tris-HCl (pH 7.5), 50 mmol/l NaCl, 1 mmol/l EDTA and 5.5 nmol [^{3}H]SAM (spec. act. 1.45×10^{5} Bq/nmol). After 3 h of incubation at 37°C the reaction was stopped with 200 μl 10% TCA. Precipitated DNA was washed 5 times with 0.5 ml 5% TCA and 3 times with cold 95% ethanol. After drying DNA was hydrolyzed with 0.4 ml concentrated HCOOH in sealed tubes at 175°C for 30 min. Hydrolysates dried over NaOH pellets were dissolved in 30 μl deionized water, mixed with 5-methylcytosine, *N*-6-methyladenine and other DNA bases and analyzed by paper chromatography on Whatman No.1 paper in the solvent system butanol/water/aqueous ammonia (86:13:1).

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3. RESULTS AND DISCUSSION

3.1. *Purification of modification methylase M.*Sau*3239I*

5 g of frozen *S. aureofaciens* cells suspended in 10 ml of 10 mmol/l PEM, pH 7.4, buffer were disrupted by an MSE sonicator and centrifuged at 1.3×10^6 m·s^{-2} for 90 min. Prior to phosphocellulose chromatography NaCl was added to the supernatant to 0.1 mol/l final concentration. This supernatant was applied on a P11 cellulose column (2.5 × 14 cm) equilibrated with 10 mmol/l PEM buffer. Elution was carried out by a 500 ml linear gradient (1–2 mol/l) of NaCl in the same buffer. *Sau*3239I methylase activity was eluted in a broad peak between 1.6–1.8 mol/l NaCl as determined by conductivity. The pooled active phosphocellulose fractions were dialysed against 10 mmol/l PEM buffer, applied slowly to a Heparin-Sepharose column (1 × 9 cm) and eluted with a 200 ml linear NaCl gradient (0–2 mol/l) in the same buffer; methylase activity appeared after 1.5 mol/l NaCl.

After chromatography on Heparin-Sepharose the enzyme preparation was very unstable. Even after concentration and in the presence of bovine serum albumin or 50% glycerol, dithiothreitol or dithioerythritol, activity of the preparation, kept at −17°C, was retained only for 4 days whether NaCl was or was not removed. In contrast to other methylases [10–13] methylase M.*Sau*3239I is eluted from phosphocellulose at high ionic strength. Activity determination by protection assay is, under these conditions, made possible by the fact that the activity of restriction endonuclease *Sau*3239I is not affected even by high concentrations of NaCl [14].

3.2. *Assessment of methylated base*

For the assessment of the methylated DNA base the purified enzyme preparation after Heparin-Sepharose chromatography and ^{3}H-labeled SAM of high specific activity were used. After hydrolysis of methylated DNA and the analysis of bases by paper chromatography high radioactivity was found only in the spot of *N*-6-methyladenine (Table I). As methylase M.*Sau*3239I protects DNA from cleavage by restriction endonuclease R.*Sau*3239I it can be presumed that methylation takes place in the 5′-CTCG^{m}AG-3′ sequence in a similar way as was described for M.*Pae*R7I [8].

Up to now isoschizomers of R.*Xho*I with corresponding methylases modifying adenine in CTCG^{m}AG ([8] and this paper) and internal cytosine in CT^{m}CGAG [7] in the specific recognition sequence are known. This fact as well as findings that the methylation by heterologic methylases of the leftmost cytosine in ^{m}CTCGAG [15], the internal one in CT^{m}CGAG [16] and of adenine in CTCG^{m}AG [17] in the recognition site prevents DNA cleavage by the restriction endonuclease R.*Xho*I, make this group of enzymes an interesting object of study in comparative enzymology.

Table I

Tritium incorporated into bases of M.*Sau*3239I methylated DNA (the numbers are averages of two measurings)

Base	cpm	
	λDNA	Beef spleen DNA
Guanine	65	48
Cytosine	63	60
5-Methylcytosine	65	53
Adenine	65	55
Thymine	70	68
N-6-Methyladenine	1524	1456
Average of background	72	61

REFERENCES

[1] Roberts, R.J. (1989) Nucleic Acids Res., Sequences Suppl. 17, r347–r387.
[2] Nelson, M. and McClelland, M. (1989) Nucleic Acids Res., Sequences Suppl. 17, r389–r415.
[3] Timko, J., Horwitz, A.H., Zelinka, J. and Wilcox, G. (1981) J. Bacteriol. 145, 873–877.
[4] Gašperík, J., Godány, A., Hostinová, E. and Zelinka, J. (1983) Biológia (Bratislava) 38, 315–319.
[5] Šimbochová, G., Timko, J., Zelinková, E. and Zelinka, J. (1986) Biológia (Bratislava) 41, 357–365.
[6] Timko, J., Turňa, J. and Zelinka, J. (1988) in: Metabolism and Enzymology of Nucleic Acids (Zelinka, J. and Balan, J. eds) pp. 65–71, Plenum, New York.
[7] Jentsch, S. (1983) J. Bacteriol. 156, 800–808.
[8] Gingeras, T.R. and Brooks, J.E. (1983) Proc. Natl. Acad. Sci. USA 80, 402–406.
[9] Shinnick, T.M., Lund, E., Smithes, O. and Blattner, F.R. (1975) Nucleic Acids Res. 2, 1911–1914.
[10] Rubin, R.A. and Modrich, P. (1977) J. Biol. Chem. 252, 7265–7272.
[11] Sato, S., Nakazava, K. and Shinomiya, T. (1980) J. Biochem. 88, 737–747.
[12] Janulaitis, A.A., Stakenas, P.S., Pjatrušyte, M.P., Bitinaite, J.B., Klimašauskas, S.J. and Butkus, V.V. (1984) Mol. Biol. (Moscow) 18, 115–129.
[13] Posfai, G., Kiss, A. and Venetianer, P. (1986) Gene 50, 63–67.
[14] Šimbochová, G., Timko, J., Zelinková, E. and Zelinka, J. (1987) Biológia (Bratislava) 42, 1129–1136.
[15] Karreman, C., Tandeau de Marsac, N. and De Waard, A. (1986) Nucleic Acids Res. 14, 5199–5205.
[16] Van der Ploeg, L.H.T. and Flavell, R.A. (1980) Cell 19, 947–958.
[17] McClelland, M. (1981) Nucleic Acids Res. 9, 6795–6804.