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Purification of a DNA methyltransferase from *Bacillus natto* B3364

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An S-adenosyl-L-methionine:DNA-methyltransferase, termed M·*Bna*I, was purified from *Bacillus natto* B3364 strain by successive column chromatography. The molecular weight determined by gel filtration was 37 kDa for M·*Bna*I. Analysis of methyltransferase by sodium dodecyl sulfate–polyacrylamide gel electrophoresis showed correspondence of the M·*Bna*I activity with one protein band at a molecular weight of 35 kDa. Sequencing of pUC19 DNA methylated with M·*Bna*I showed the cytosine-5 methylation in the *Bna*I recognition sequence GGAT↓CC at the position indicated by the arrow.

Restriction/modification; DNA methylation; Enzyme purification; Adenosyl-L-methionine, S-; *(Bacillus natto)*

1. INTRODUCTION

The *B. natto* strain B3364 expresses the restriction/modification system *Bna*I, an isoschizomer of *Bam*HI [1]. Biological parameters of the *Bna*I system have been described previously [2]. The restriction and the corresponding modification enzyme recognize the same symmetrical hexanucleotide sequence 5′G↓GATCC. The restriction endonuclease R·*Bna*I cleaves the recognition sequence of unmodified DNA at the position indicated by the arrow. The methyltransferase acts on unmodified DNA using S-adenosyl-L-methionine (SAM) as methyl group donor, while the restriction endonuclease requires only Mg^{2+} to produce double strand scissions within the recognition sequence of unmodified DNA. Thus, the restriction and modification enzymes of *B. natto* B3364 are defined as class II enzymes.

This paper describes the purification, molecular parameters and site specificity of *Bna*I methyltransferase.

2. MATERIALS AND METHODS

The standard methyltransferase assay measures the incorporation of [^{3}H]methyl groups from S-adenosyl-L-[methyl-^{3}H]-methionine into the appropriate DNA. The reaction mixture contained in a total volume of 100 μl: 150 mM Tris-HCl, pH 8.0, 1 mM EDTA, 1 mM dithiothreitol, 5% glycerol, 10 μg of DNA, 6.6 μM S-adenosyl-L-[methyl-^{3}H]methionine (Amersham) and 5 μl of enzyme in appropriate dilutions. After 15 min incubation at 37°C, the samples were loaded onto DE-81 paper (Whatman) disks. Then disks were washed twice in 7.5% Na_2HPO_4 and once in 70% ethyl alcohol, dried and counted for radioactivity in a toluene-based scintillator.

Electrophoretical methyltransferase assay was also used. The reaction mixture was the same as in the radioactive assay with the exception that 100 μM of nonradioactive SAM was used. After 8 h incubation at 37°C, samples were heated 10 min at 65°C and cooled. Then $MgCl_2$ and 10 units of R·*Bna*I were added to every probe. After 30 min of incubation at 37°C, samples were electrophoresed in 0.8% agarose gel. One unit of DNA methyltransferase activity is defined as that amount of enzyme which protects 1 μg of DNA from R·*Bna*I cleavage.

Protein samples in volumes of up to 5 μl were supplemented with Laemmli sample buffer [3], heated for 2 min at 100°C and loaded onto 8–25% gradient SDS-polyacrylamide gel. Electrophoresis was performed using FAST System (Pharmacia P.-L. Biochemicals). The gels were stained with $AgNO_3$.

pUC19 DNA was fully methylated in vitro by *Bna*I methylase and then cleaved with *Hin*dIII and *Bgl*I endonucleases. 202 bp *Hin*dIII-*Bgl* restriction fragment, containing the *Bna*I recognition sequence, was eluted from 5% polyacrylamide gel after electrophoresis and sequenced by the Maxam-Gilbert method [4] using [α-^{32}P]dATP.

3. RESULTS

3.1. *Purification of* Bna*I methylase*

Restricting/modifying strain B3364 was the source of methyltransferase whose purification is

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Table 1

Purification of *BnaI* methyltransferase from *B. natto* B3364

Purification step	Total protein (mg)	Total activity (1000 units)	Specific activity (1000 units/mg protein)	Yield (%)
(1) Cell lysate	230	105	0.20	64
(2) Ammonium sulfate precipitation	180	165	0.45	100
(3) PII-cellulose	5	80	1.20	48
(4) Hydroxyapatite	0.1	60	3.65	36
(5) Mono S	0.08	42	3.12	25

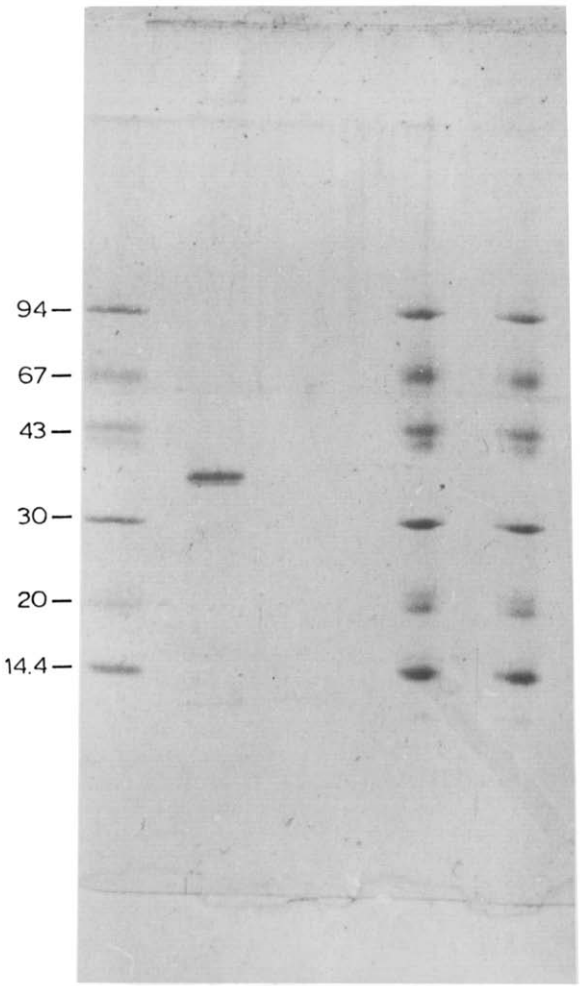

Fig.1. SDS-polyacrylamide 8–25% gradient gel electrophoresis of purified *BnaI* methylase.

described in this paper. B3364 was grown in LB medium until the end of the logarithmic phase of growth. The cells were collected by centrifugation, washed once in buffer A (20 mM KPO_4, pH 7.8, 50 mM KCl, 1 mM DTT, 0.5 mM EDTA, 5% glycerol) and suspended in the same buffer, containing 10 mg/ml of lysozyme. This and all the following steps were performed at 4°C. After 60 min of incubation cells were sonicated using an MSE sonicator. The cell lysate was clarified by centrifugation at 45 000 × *g* for 1 h and suspended in buffer A. This material was dialyzed against buffer A and used for further purification. Applying liquid chromatography, *Bna*I methylase was then purified as a homogeneous protein in the following steps: PII-cellulose, hydroxyapatite, Mono S (Pharmacia P.-L. Biochemicals). The results obtained on purification are summarized in table 1.

3.2. *Molecular weight and site specificity determination*

*Bna*I methylase was purified up to 150-fold and gave a single band on SDS-PAGE (fig.1). The molecular mass estimated from SDS-PAGE was 35 kDa. The value determined from the elution volume on gel filtration was approx. 37 kDa, indicating that the methylase is a monomer.

Among the most important characteristics of DNA methyltransferases is the nature of the modified base and the localization of the methyl group introduced into the heterocyclic nucleus. We have determined (not shown) that *Bam*HI and *Bna*I endonucleases did not cleave DNA's from *B. natto* B3364 and *Bacillus amyloliquifaciens* 42 (the last is the strain producing enzymes of the *Bam*HI restriction/modification system). The methylation of adenine in the sequence GGATCC does not prevent the cleavage of DNA with *Bam*HI, but the methylation of internal and external cytosines resulting in m4C and m5C, respectively, does [5]. This allowed us to suppose that one of the cytosines in the GGATCC *Bna*I recognition sequence is a target for *Bna*I methylase. In the case of m5C to be the product of *Bna*I methylation, the Maxam-

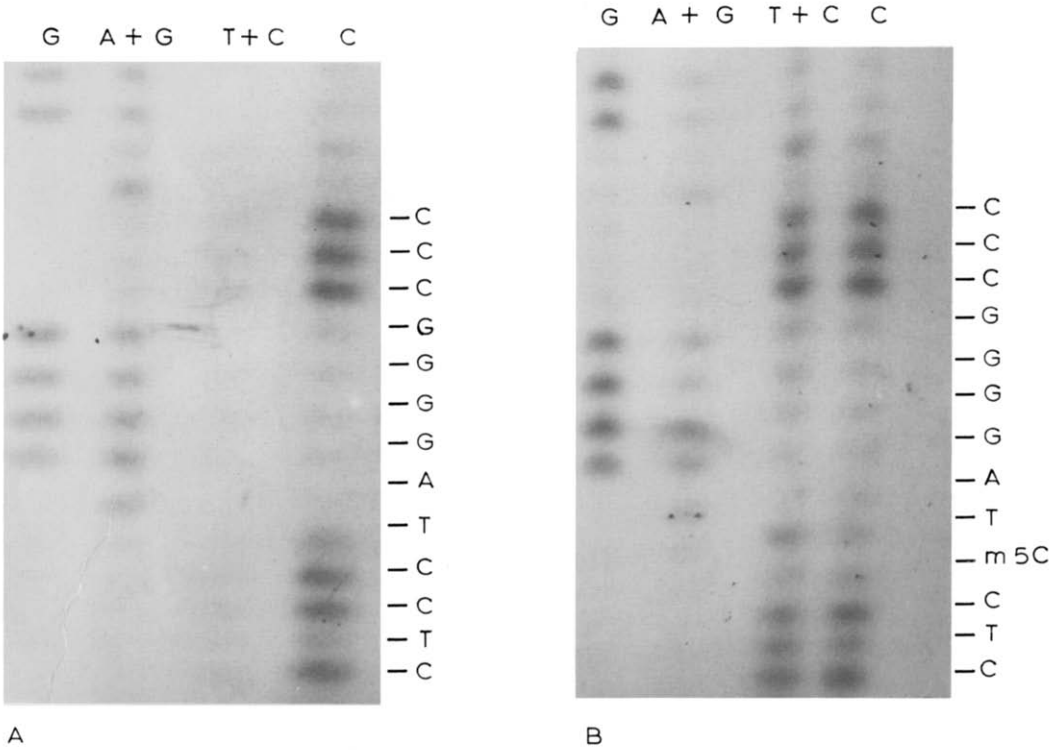

Fig.2. Sequencing of pUC19 202 bp fragment (positions 447–245 [7]), methylated with *Bna*I. (A) nonmethylated pUC19; (B) *Bna*I methylated pUC19.

Gilbert DNA sequencing procedure allows direct determination of the position and character of the methylated base. The sequencing of pUC19 fragment containing *Bna*I methylated site showed the presence of an internal m5C in the GGATCC sequence (fig.2B).

4. DISCUSSION

*Bna*I methyltransferase from *B. natto* B3364 strain was purified to homogeneity. In spite of having the same with *Bam*HI recognition sequence, the product of *Bna*I methylation is m5C, whereas *Bam*HI methylation results in m4C [5]. Thus, both m5C and m4C methylation of an internal cytosine in GGATCC sequence protect DNA from *Bam*HI and *Bna*I cleavage. *Bna*I and *Bam*HI methylases are the enzymes with different molecular parameters: 35 kDa (this paper) and 56 kDa [6]. Recognition of the same specific sequence in double-stranded DNA by two distinct and biochemically different enzymes is one of the most interesting aspects of the restriction/modification phenomenon.

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