DNA-CYTOSINE METHYLATION IN *E. COLI* MRE 600 CELLS

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1. Introduction

Two DNA-cytosine methyltransferases (Dcm I and II) modifying cytosine in different nucleotide sequences have been isolated from *E. coli* MRE 600 cells [1,2]. Physiological role of these methylases is still unknown but it is possible that one of them is connected with the DNA modification and restriction system. This supposition is supported by the fact that the main targets in DNA for Dcm II are the pyrimidine sequences C-m^{5}C and C-m^{5}C-T (m^{5}C: 5-methylcytosine) identical to pyrimidine fragments in DNA sequences, which are methylated by DNA methylase of resistance factor R II [2].

The work presents the data on in vivo methylation of cytosine in *E. coli* MRE 600 DNA and we describe a new method for separate determination of Dcm I and Dcm II action in the cell.

Structural peculiarities of nucleotide sequences recognized by two enzymes constitute the basis of this method. More that 90% of cytosine modified by Dcm I appears in the sequence -Pu-m^{5}C-C-Pu- [1] and about 30% of cytosine methylated by Dcm II is detected in the sequence -Pu-C-m^{5}C-Pu- [2]. So, separate determination of Dcm I and Dcm II activity in the cell is based on quantitative estimation of 5-methylcytosine content at 5′- and 3′-termini of -Pu-C-C-Pu- sequences of *E. coli* MRE 600 DNA.

2. Materials and methods

Bacteria were grown to the middle of log-phase in M9 medium [3] supplemented with 0.4% glucose, 2.7×10^{-5} [Me-^{14}C]methionine (11.89 Ci/mol, Institute of Isotopes, Hungary), 10^{-4} M adenine, 8×10^{-4} M thymidine and 10^{-2} M sodium formate. DNA was isolated by the method of Marmur [4]. DNA was hydrolyzed in 57% perchloric acid. Authentic 5-methylcytosine and 6-methyladenine markers were included in each hydrolyzate and DNA bases were separated by paper chromatography [5]. The ^{14}C in nitrogen bases was determined by directly counting the paper strips in a liquid scintillation counter using a standard toluene scintillation cocktail. DNA was degraded to pyrimidine sequences by Burton and Petersen's method [6].

The released pyrimidine fragments were separated according to chain length (isopliths) and base composition by thin-layer chromatography on DEAE-cellulose [7]. 5-Methylcytosine content in oligonucleotide was determined by directly counting the layers of DEAE-cellulose from relevant areas of the chromatogram in a liquid scintillation counter.

For the determination of the position of 5-methylcytosine, pyrimidine dinucleoside triphosphates were treated with *E. coli* phosphomonoesterase and phosphodiesterases from snake venom and spleen (Worthington) [1].

3. Results and discussion

3.1. *In vivo labelling of E. coli MRE 600 DNA by [Me-^{14}C]methionine*

Hydrolysis of 100 μg of *E. coli* MRE 600 DNA showed the following radioactivity in nitrogen bases (counts/min): guanine, 16; cytosine, 64; adenine, 3; thymine, 70; 5-methylcytosine, 1518; 6-methyladenine, 1757. The radioactivity in thymine and

cytosine is less than 10% of radioactivity of 5-methylcytosine. The almost equal quantity of 5-methylcytosine and 6-methyladenine in *E. coli* MRE 600 DNA accords with the earlier data on the base composition of *E. coli* MRE 600 DNA [8].

3.2. *DNA-cytosine methylation in vivo*

The data on the distribution of 5-methylcytosine in pyrimidine isopliths of *E. coli* MRE 600 DNA methylated in vivo are shown in table 1. About 80% of the total 5-methylcytosine is present in pyrimidine di- and trinucleotides, its content in dinucleotides being approximately 3 times higher that in trinucleotides.

In the dinucleotide fraction about 100% of 5-methylcytosine is found in the dinucleotide C_2. Data on the location of 5-methylcytosine in dinucleotide C_2 are given in table 2. In *E. coli* MRE 600 DNA among pyrimidine dinucleotides the m^5C-C sequence contains about 63% of 5-methylcytosine and the C-m^5C sequence about 37%.

3.3. *Separate determination of two DNA-cytosine methylase activities in vivo*

Since Dcm I practically methylates cytosine only in m^5C-C sequences, it is possible to determine the separate shares of Dcm I and Dcm II in methylation of *E. coli* MRE 600 DNA. Such calculations show that *E. coli* MRE 600 DNA was 37.5% methylated by Dcm I and 62.5% by Dcm II. Moreover, it is possible to calculate the distribution of 5-methylcytosine in *E. coli* MRE 600 DNA modified only by Dcm II, by taking off the share of Dcm I (table 1).

This calculation is in agreement with the pattern of heterologous DNA methylation by Dcm II [2] and with the model of action of resistance factor RII DNA-methylase [9]. Increase of 5-methylcytosine content in mononucleotides can be explained by the fact that in the initial data certain radioactivity of thymine and cytosine is not taken into account.

So, specificity of the two *E. coli* MRE 600 DNA-cytosine methylases allows one to determine quantitatively and separately the action of these enzymes on cell DNA in vivo. Besides the analysis of 5-methylcytosine at 5′- and 3′-termini of dinucleotide C_2 the activity of Dcm I and Dcm II can be determined directly from the quantitative 5-methylcytosine ratio in pyrimidine di- and trinucleotide isopliths of *E. coli* MRE 600 DNA. If Dcm I in cells is completely inactive then the ratio of 5-methylcytosine in di- and trinucleotides should be about 1. If in cells Dcm II is completely inactive the ratio of 5-methylcytosine in di- and trinucleotides should be about 100.

Under the observed activity of the two DNA-cytosine methylases this 5-methylcytosine ratio in *E. coli* MRE 600 DNA is equal to 2.7.

The second of the approaches mentioned can be used assuming that under any conditions in the cell the action of Dcm II will result the same occurrence frequency of 5-methylcytosine among pyrimidine isopliths of *E. coli* MRE 600 DNA.

It should be noted that *E. coli* MRE 600 cells possessing two specific DNA-cytosine methylases are a unique object for the separate quantitative determination of the action of these enzymes on bacterial DNA during the process of cell activity.

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Table 1
Occurrence frequency of 5-methylcytosine in pyrimidine isopliths of *E. coli* MRE 600 DNA methylated in vivo by two DNA-cytosine methylases

DNA-methylase	5-Methylcytosine of total 5-methylcytosine (in %)					
	I	II	III	IV	V	> V
Dcm I - Dcm II (experimental data)	5.54	59.12	21.64	7.34	3.05	3.31
Dcm II (theoretical calculation)	8.87	34.61	34.61	11.74	4.88	5.29

Table 2

Analysis of the location of [^{14}C]5-methylcytosine in dinucleotide C_2 isolated from *E. coli* MRE 600 DNA methylated in vivo

Distribution of radioactivity (5-methylcytosine) in two PDE digestion products of C_2 (cpm)						% of 5-methylcytosine at the 3'- and 5'-terminus							
						5'-terminus				3'-terminus			
Snake venom PDE			Spleen PDE			Snake venom PDE		Spleen PDE		Snake venom PDE		Spleen PDE	
Digestion product	Exp 1	Exp 2	Digestion product	Exp 1	Exp 2	Exp 1	Exp 2	Exp 1	Exp 2	Exp 1	Exp 2	Exp 1	Exp 2
Nucleotide	539	541	Nucleotide	884	1023	61.2	60.7	65.2	66.7	38.8	39.3	34.8	33.3
Nucleoside	850	834	Nucleoside	472	510								

PDE, phosphodiesterase

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