SITE SPECIFICITY AND CHROMATOGRAPHIC PROPERTIES OF *E. COLI* K12 AND *Eco*RII DNA-CYTOSINE METHYLASES

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Received 6 February 1978

1. Introduction

It has been shown that DNA methylation in vitro by DNA-cytosine methylase from *E. coli* K12 provides phage λ DNA with complete resistance against RII restriction endonuclease [1]. *E. coli* C DNA-cytosine methylase and *E. coli* MRE 600 DNA-cytosine methylase II also provide λ DNA with resistance against RII restriction in transfection experiments [2].

It is likely that all these DNA-cytosine methylases can display the same or overlapping site specificity as RII DNA methylase. For *E. coli* MRE 600 DNA-cytosine methylase II [3] and *E. coli* C DNA-cytosine methylation in vivo [4] the major targets in DNA are the dinucleotide C–m^5C and trinucleotide C–m^5C–T (m^5C:5-methylcytosine). These pyrimidine fragments of DNA are identical to pyrimidine sequences (underlined) of the DNA site modified by RII DNA methylase [5]:

5′...C–m^5C–A – G–G...3′

3′...G – G–T–m^5C–C...5′

However, the pattern of DNA modification in vitro by *E. coli* K12 DNA-cytosine methylase and RII DNA methylase [6] and the only major target sequence C–m^5C–T for these two enzymes [7] do not correspond to the in vivo methylation pattern of *E. coli* K12 DNA [4] and to the mode of RII DNA methylase action.

The aim of this communication is the additional analysis of DNA sequences methylated in vitro by RII and *E. coli* K12 DNA-cytosine methylases. The results reported here show that *E. coli* K12 DNA-cytosine methylase and RII DNA methylase display the same site specificity which is identical to that of *E. coli* C and *E. coli* MRE 600 DNA-cytosine methylases. Our data on chromatographic behaviour of RII DNA methylase on phosphocellulose differ from those in [1]

2. Materials and methods

Phage λ wild-type and *E. coli* B strain 707 [8] were kindly provided by Dr W. Arber. *E. coli* B (RII) strain was constructed by conjugation transfer of RII plasmid R245 from *E. coli* 1100 (R245) [9] to *E. coli* 707. *E. coli* K12 (RII) is *E. coli* 1100 (R245) strain. *E. coli* K12 is *E. coli* C 600 strain [10]. DNA of purified phage λ was isolated by phenol extraction [11].

DNA methylases were isolated:

(1) By the method for isolation of RII restriction endonuclease in [12].

(2) According to the procedure in [3].

RII and *E. coli* K12 DNA-cytosine methylases were simultaneously isolated from *E. coli* K12 (RII) cells by method 2.

Buffer A (10 mM potassium phosphate, pH 7.0; 10 mM 2-mercaptoethanol, 1 mM EDTA, 5% glycerol, 0.2% Triton X-100) was used for chromatography. For chromatography on phosphocellulose, buffer A containing 30% glycerol was used in some experiments.

In vitro DNA modification by RII DNA methylase and *E. coli* K12 DNA-cytosine methylase in the presence of *S*-adenosyl-L-[methyl-^{3}H]methionine

(Amersham, England) was carried out as in [3].

DNA was degraded to pyrimidine sequences [13]. The released pyrimidine fragments were separated according to chain length (isopliths) and base composition by thin-layer chromatography on DEAE-cellulose (Reanal, Hungary) [14]. 5-Methylcytosine content in oligonucleotides was determined by directly counting the layers of DEAE-cellulose from relevant areas of the chromatogram in a liquid scintillation counter. For determination of the position of 5-methylcytosine in pyrimidine dinucleoside triphosphates and in trinucleoside tetraphosphates, *E. coli* phosphomonoesterase, snake venom and spleen phosphodiesterases (Worthington) and A5 exonuclease [15] were used.

3. Results and discussion

3.1. *Preparation and chromatographic behaviour of RII and* E. coli *K12 DNA-cytosine methylases*

RII DNA methylase was eluted from DEAE-cellulose (Whatman, DE-52) between 0.06 M and 0.11 M NaCl NaCl linear gradient (0–0.2 M) in buffer A. *E. coli* K12 DNA-cytosine methylase was eluted at approximately the same or sometimes at a lower NaCl concentration. Because separation of these two enzymes on DEAE-cellulose was difficult we used method 2 for their simultaneous preparation from *E. coli* K12 (RII) cells. By this method, dialyzed cell-free extract (after streptomycin sulfate and ammonium sulfate treatment) was applied to a DEAE-cellulose or DEAE–Sephadex A50 column in buffer A containing 0.15 M NaCl. Two DNA methylase activities appeared in the column flow-through. The column flow-through was applied to a column of Whatman phosphocellulose P11 equilibrated with buffer A containing 0.15 M NaCl.

RII and *E. coli* K12 DNA-cytosine methylases were separated by chromatography on phosphocellulose (fig.1).

RII DNA methylase and *E. coli* K12 DNA-cytosine methylase were eluted from phosphocellulose between 0.32 M and 0.37 M NaCl and 0.43 M and 0.52 M NaCl, respectively, with a linear gradient of NaCl (0.15–0.80 M) in buffer A. Our data on the sequence of RII and *E. coli* K12 DNA-cytosine methylases elution from phosphocellulose are in contrast to

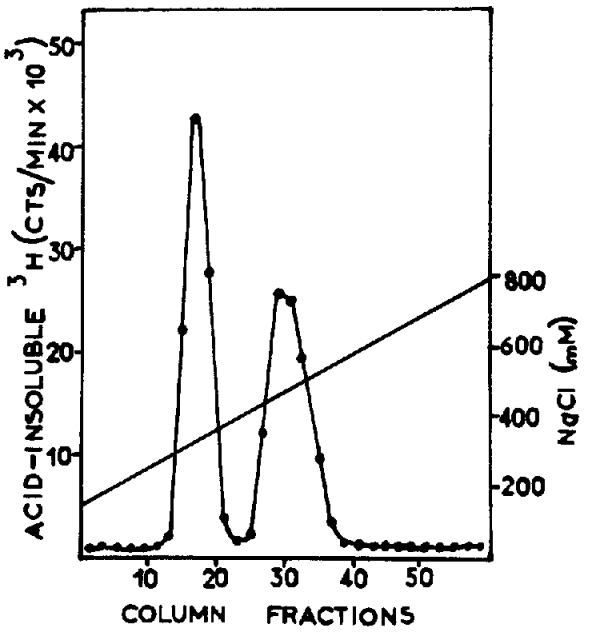

Fig.1. Elution of *Eco*RII (peak 1) and *E. coli* K12 (peak 2) DNA-cytosine methylases from phosphocellulose. DEAE-cellulose column flow-through (40 mg protein) was applied to a column (1.6 × 15 cm) of Whatman P11 phosphocellulose as described in section 2. Linear gradient of 0.15–0.80 M NaCl, total vol. 500 ml. Collected fractions (8 ml) were assayed for DNA methylase activity on *E. coli* B DNA.

others [1]. The chromatographic behaviour of these enzymes on phosphocellulose did not change when they were isolated separately from *E. coli* B (RII) and *E. coli* K12 cells.

Co-rechromatography of the mixture of these purified enzymes on phosphocellulose again gave the two peaks of DNA-cytosine methylase activity at the same NaCl concentration. Two enzymes were purified about 100-fold and contained 35–40% DNA-cytosine methylase activity of the starting material (ammonium sulfate fraction). It should be noted that *E. coli* K12 DNA-cytosine methylase contained some DNA-adenine methylase activity, whereas RII DNA methylase was always without any DNA-adenine methylase.

3.2. *In vitro DNA modification by RII and* E. coli *K12 DNA-cytosine methylases*

More than 75% total 5-methylcytosine is contained

Table 1
Occurrence frequency of 5-methylcytosine in pyrimidine isopliths of λ·B DNA methylated in vitro by RII and *E. coli* K12 DNA-cytosine methylases

Isoplith	5-Methylcytosine total DNA 5-methylcytosine (%)	
	RII DNA methylase	*E. coli* K12 DNA-cytosine methylase
I	1.03	0.73
II	35.41	31.97
III	41.95	43.31
IV	10.36	11.66
V	4.89	5.72
> V	6.36	6.61

λ·B is phage λ propagated on *E. coli* B

in di- and tripyrimidine sequences of λ DNA methylated in vitro by RII and *E. coli* K12 DNA-cytosine methylases (table 1). No enzyme specificity differences with respect to the amount of 5-methylcytosine in the pyrimidine isopliths of λ DNA were observed. In contrast to analogous data [6] we found approximately equal 5-methylcytosine contents in di- and trinucleotides of the λ DNA. The similar pattern of 5-methylcytosine distribution among pyrimidine isoplith components in the λ DNA was also found out for these two enzymes (table 2). Thus, for RII and *E. coli* K12 DNA-cytosine methylases there are two major targets in λ DNA: dinucleotide C_2 with sequence 5′...C–m^5C...3′ and trinucleotide C_2 T with sequence 5′...C–m^5C–T...3′.

The patterns of λ·K DNA modification in vitro by RII and *E. coli* K12 DNA-cytosine methylases are similar to analogous patterns for λ·B DNA modification (data not shown). This indicates that the specificity of in vivo and in vitro modification of λ DNA sites by RII and *E. coli* K12 DNA-cytosine methylases is the same despite λ·K DNA being incompletely modified in vivo [6].

Thus, DNA-cytosine methylases of RII-type from different *E. coli* strains (including *E. coli* K12) display the same specificity of in vitro DNA methylation, as that of RII DNA methylase.

Acknowledgements

We are very grateful to Dr W. Arber (Basel) for kindly sending us bacterial and phage strains and to Dr R. I. Tatarskaya for providing the A5-exonuclease.

Table 2
Distribution of 5-methylcytosine among pyrimidine isoplith components of λ·B DNA methylated in vitro by RII and *E. coli* K12 DNA-cytosine methylases

Isoplith	Component	5-Methylcytosine of total isoplith 5-methylcytosine (%)	
		RII DNA methylase	*E. coli* K12 DNA-cytosine methylase
I	C	Not analyzed	Not analyzed
	T	Not analyzed	Not analyzed
II	C_2	98.73	96.62
	CT	1.27	3.38
	T_2	0	0
III	C_3	22.24	21.86
	C_2T	76.25	76.38
	CT_2	1.51	1.76
	T_3	0	0
IV	C_4	6.07	6.34
	C_3T	42.13	47.03
	C_2T_2	50.26	45.23
	CT_3	1.54	1.40
	T_4	0	0

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