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The *oriC* unwinding by *dam* methylation in *Escherichia coli*

Hiroshi Yamaki*, Eiichi Ohtsubo, Kazuo Nagai[1] and Yoshimi Maeda

Institute of Applied Microbiology, University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, 113 Tokyo and [1]Department of Agricultural Chemistry, University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, 113 Tokyo, Japan

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ABSTRACT

It has been shown that dam methylation is important in the regulation of initiation of DNA replication in E.coli. The question then arises as to whether dam methylation in the oriC region mediates any structural changes in DNA involved in the regulation of initiation of DNA replication. We demonstrate that the thermal melting temperature of the oriC region is lowered by adenine methylation at GATC sites. The regulation of initiation of DNA replication by dam methylation may be attributed to the ease of unwinding at GATC sites in oriC.

INTRODUCTION

The regulation of gene expression by methylation of nucleotides, particularly cytosine in eukaryotic cells, has been supported by many persuasive arguments concerning differentiation or malignancy. In prokaryotic cells, the biological significances of GATC sequences and dam methylation have been described concerning oriC function, mismatch repair mechanism, etc. We have a particular interest in how dam methylation is involved in oriC function. The replication origin of bacteria including E.coli contains 9-14 GATC target sites for dam methylation (1). Our concern is why such GATC sequences are localized so abundantly in the oriC region, and how these sequences are functioning in initiation of DNA replication in E.coli. Recent evidence has shown a reduced frequency of transformation of oriC plasmids in dam mutants (2,3), and poor functioning of oriC plasmids derived from a dam mutant in an in vitro DNA replication system (4). In an attempt to understand the molecular mechanism of regulation of DNA replication by dam methylation, we tried to search for any physical changes of DNA strands mediated by dam methylation.

Our results revealed that the ability of the oriC region to unwind is facilitated by dam methylation. The relationship between the regulation of DNA replicaton and the unwinding of oriC by dam methylation is discussed.

MATERIALS AND METHODS

Thermal melting of d(GATC)$_5$ and adenine methylated d(GATC)$_5$

d(GATC)$_5$ was synthesized with the Beckman DNA Synthesizer System 1 Plus according to the phosphoamidite method (5). The d(GATC)$_5$ was annealed at a concentration of 1 μg/ml in 0.1 x SSC (NaCl 0.015 M - Na citrate 0.0015 M). Adenine residues of double stranded d(GATC)$_5$ were methylated with MClaI (6), and the methylated d(GATC)$_5$ was extracted with phenol and precipitated with ethanol. 1 μg of DNA was dissolved in 0.25 ml of 0.1 x SSC and introduced into a quartz cuvette of 10 mm light path, and the temperature was raised at the heating rate of 0.5° C per min. The absorbance at 260 nm was recorded by Spectrophotometer 250 of Gilford Instrument Laboratories.

Thermal melting of plasmid DNA with differential scanning calorimetry (DSC)

Methylated pTSO125 DNA was obtained from an NT101 (7) transformant, and unmethylated pTSO125 was obtained from a GM33 (8) transformant. The dam methylated and unmethylated pTSO125 DNA were linearized with EcoRI, and their thermal melting profiles were investigated. DSC curves of pTSO125 plasmid DNA both unmethylated and methylated at GATC sites were obtained at the heating rate of 1° C per min using the Privalov type of adiabatic differential scanning microcalorimeter, DASM-4 (Academy of Science of the USSR, Moscow)(9). The concentration of the samples was 0.043%. The theoretical melting curves and the thermal stability maps of unmethylated and methylated pTSO125 plasmids were constructed by calculating according to the algorithms and the parameters previously reported (10,11,12).

RESULTS

We attempted to transform the dam mutant GM33 with the oriC pTSO118 plasmid (13), and found no transformants, but pTSO125 (13) carrying both oriC and pBR322 ori transformed the strain (3.14 X 10^3 transformants / μg DNA). These results indicate that dam methylation is of basic importance for oriC function, but not for that of pBR322 ori.

To gain a better understanding of these events at the molecular level, we examined them biophysically. First, in order to analyze the effect of adenine methylation on the

thermal melting of DNA, a d(GATC)$_5$ was synthesized, annealed to form a double stranded molecule and methylated with ClaI methylase (MClaI). As illustrated in Fig. 1, the thermal melting temperature of the methylated double stranded d(GATC)$_5$ is lower than that of the unmethylated molecule. This result is reasonable since a hydrogen bond between the 4-keto group of thymine and the 6-amino group of adenine may be weakened, when the 6-amino group of adenine is methylated to form an aminomethyl group (14,15).

Secondly, the effect of dam methylation on the temperature -induced helix-coil transition of oriC plasmid pTSO125 was examined by differential scanning calorimetry (DSC), a method useful for analyzing the helix-coil transition of DNA (16). pTSO125 is the plasmid which is able to replicate in both wild-type and dam mutant cells because it contains pBR322 ori. The

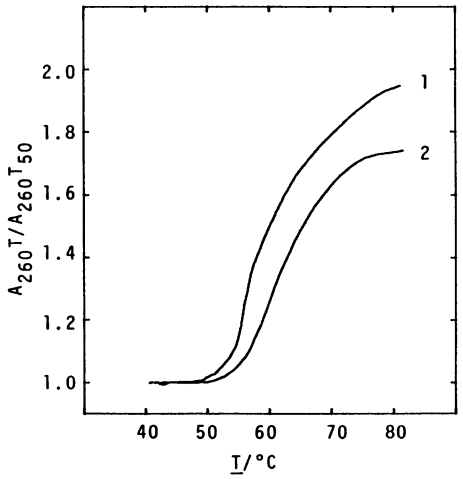

Fig. 1. The thermal denaturation of synthetic double stranded d(GATC)$_5$ and the adenine methylated molecule. The thermal denaturation curves of MClaI methylated d(GATC)$_5$ [1], and d(GATC)$_5$ [2], are presented. Absorbance of DNA at 260 nm at the temperature shown along the horizontal line is expressed by $A_{260}T$. Absorbance of DNA at 260 nm at 50° C before increase of absorbance is expressed by $A_{260}T_{50}$. The value of $A_{260}T/A_{260}T_{50}$ was scored as the degree of denaturation of DNA at T° C. The difference in Tm (midpoint of thermal melting) between unmethylated d(GATC)$_5$ (63° C) and methylated d(GATC)$_5$ (59° C) was 4° C.

dam methylated and unmethylated pTSO125 DNAs were linearized with EcoRI, and their thermal melting profiles were investigated. As shown in Fig. 2-A-1, the DSC curve of the methylated pTSO125 plasmid DNA has several peaks, demonstrating stepwise and cooperative melting of double stranded DNA. The DSC curve of the unmethylated pTSO125 plasmid DNA was totally different from the methylated one in the position, number and height of peaks, possibly due to methylation of the adenine residues at the GATC sequences, in particular peak number 2 which was shifted to a lower temperature in the methylated pTSO125 plasmid DNA. To see if the helix-coil transition of the region of oriC is responsible for the shift of peaks in DSC curves, each peak of the DSC curves was assigned to a particular region of the DNA sequence, as follows; the theoretical melting profiles were first constructed by calculations based on the helix-coil transition theory from the nucleotide sequences of the plasmid DNA, using the three different parameters for A-T, G-C, and mA-T pairs (10,11). As shown in Fig. 2, the theoretical melting curves of methylated and unmethylated plasmid DNA resemble the respective DSC curves. A thermal stability map (17) was constructed along the pTSO125 genome.

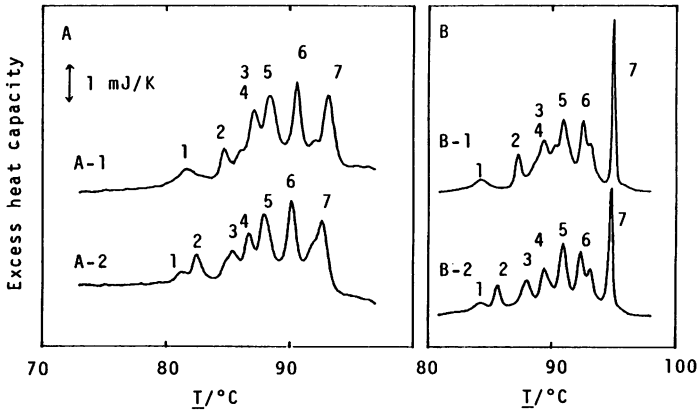

Fig. 2. Differential scanning calorimetry (DSC) curves [A], and theoretical melting curves [B] of unmethylated and dam methylated plasmid pTSO125 DNA. Those of pTSO125 plasmid DNA unmethylated [A-1, B-1] and methylated [A-2, B-2] at GATC sites are presented. The DSC peaks which correspond to those of the theoretical curves are numbered 1 to 7.

Fig. 3 shows that peak number 2, which shifted most markedly to lower temperatures in methylated pTSO125, can be assigned to the transition of the oriC region in pTSO125. This result indicates that dam methylation of the target GATC sites in the oriC region makes melting of this region much easier.

DISCUSSION

Our results as well as previous papers (2,3,18) indicate that dam methylation is of importance in the regulation of DNA replication in E.coil. If dam methylation is essential for oriC function, an interesting question arises as to how the mutant GM33 which is defective in dam methylation is able to replicate

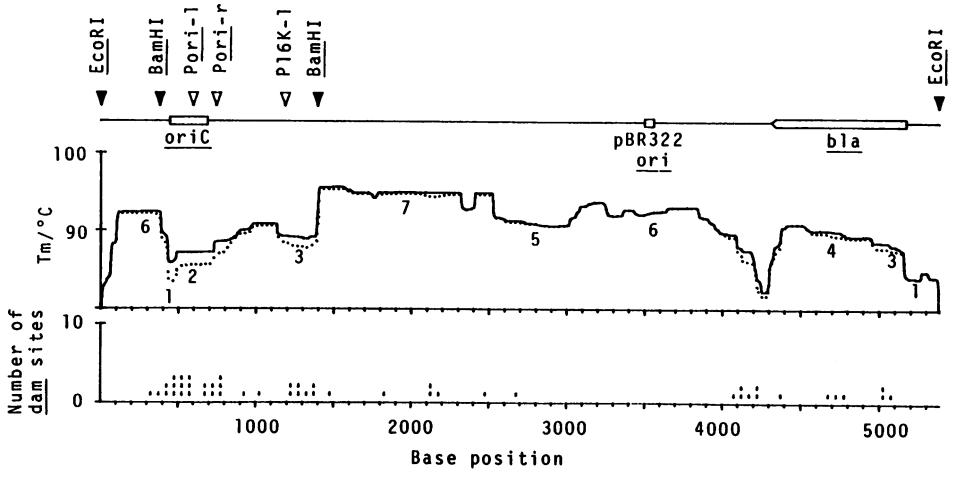

Fig. 3. The thermal stability maps of unmethylated and dam methylated pTSO125 plasmid DNA. The thermal stability map of unmethylated (solid line) and methylated (dotted line) pTSO125 DNAs were constructed according to the methods described in MATERIALS AND METHODS. The numbers under the map show the cooperatively melting regions responsible for the respective DSC peaks shown in Fig.2. The number of GATC sites in the segments at intervals of 50 base pairs are indicated at the bottom. In the genetic map of pTSO125 shown at the top, oriC, pBR322 ori and ampicillin resistance (bla) (24,25) are boxed . Pori-l, Pori-r and a P16k-l indicate promoters for leftward and rightward transcriptions around oriC and for another leftward transcription encoding a 16K protein, respectively (26,27). BamHI and EcoRI sites of pTSO125 are marked by solid arrowheads.

its chromosome and to grow. It is assumed that oriC independent initiation of DNA replication (19) is working in GM33.

It is possibile that the dam methylated DNA is recognized by a certain initiator protein in DNA replication, as previously discussed (2). However, it has been clearly demonstrated that the local stability of the helix-ciol transition along the DNA chain is closely related to the functional regions, such as transcriptional promoters, terminators, and the protein coding regions of genes (16,17,20). Our observation of facilitation in melting of the oriC region caused by dam methylation at GATC may thus have a significant role in initiation of DNA replication. It is likely that dam methylation might result in more efficient transcription at promoters near oriC which may stimulate initiation of DNA replication at oriC. It has been reported that the site of the transition from primer RNA synthesis to DNA synthesis exists within the oriC region (21, 22). It is then likely that methylation of the adenine residues of GATC in oriC to give rise to a termination signal for synthesis of primer RNA, to generate 3'-OH ends which can be used to prime DNA synthesis. Although how the methylation of adenine residues at GATC sites in oriC functions in the regulation of DNA replication remaines obscure at present, our experimental procedures may provide a clue to understanding the role of methylation in connection with the physical structure of DNA.

After submission of this manuscript, Bramhill and Kornberg (23) have reported that the DnaA protein recognizes thirteen base pair repeat sequences, each containing GATC site, which are responsible for opening the oriC duplex DNA. The opening of these sequences by the DnaA protein could be facilitated by unwinding at the dam-methylated GATC sites, as discussed above.

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*To whom correspondence should be addressed

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