Does the DNA methylase *Eco dam* pair nucleotide sequences to form site-specific duplexes?

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The *Eco dam* methylase is active on denatured DNA and single-stranded synthetic oligonucleotides containing GATC sites. The results suggest that on interaction with single-stranded oligonucleotides the *Eco dam* methylase is able to form a duplex structure within the GATC site, and that this duplex site is a substrate for enzyme.

DNA methylase *Single-stranded DNA sequence* *Recognition site* *Methylatable substrate structure*

1. INTRODUCTION

The *Eco dam* DNA methylase recognizes the symmetric tetranucleotide sequence GATC in DNA and transfers two methyl groups per duplex site yielding two residues of 6-methylaminopurine [1].

A general function of bacterial DNA methylases consists of the protection of specific DNA sites against cleavage by corresponding restriction endonucleases. However, *Eco dam* methylase performs other functions in the cell. It participates in DNA strand discrimination during post-replicative mismatch repair [2,3], as well as controlling the expression of some phage [4,5] and evidently bacterial genes. The *Eco dam* methylase was found to be active on denatured DNAs [6,7].

Our purpose was to study the action of *Eco dam* methylase on denatured and unreassociated DNA as well as on some synthetic substrates.

2. MATERIALS AND METHODS

Eco dam methylase was purified from *E. coli* B834 according to [8]. Oligodeoxyribonucleotides were synthesized by the phosphotriester method [9]. A fraction of unreassociated calf thymus DNA was isolated by hydroxyapatite chromatography after reassociation of fragmented denatured DNA to Cot about 500 [10]. Enzymatic methylation of different substrates was carried out at various temperatures in 30 μl containing 40 mM potassium phosphate (pH 7.8), 7 mM 2-mercaptoethanol, 1 mM EDTA, 5×10^{-6} M *S*-adenosyl-L-[*methyl*-^{3}H]methionine (15 Ci/mmol, Amersham), 2–5 μg DNA or 30–60 pmol synthetic oligonucleotides, and about 100 units of *Eco dam*. One unit of *Eco dam* methylase is defined as that which catalyzes incorporation of 1 pmol of methyl groups into DNA in 60 min at 37°C. After incubation for 2 h the reaction mixture was spotted onto 1.5-cm squares of Whatman DE81 paper. The papers were washed 3 times with 0.02 M NH_4HCO_3, twice with ethanol, dried under a heat lamp, and tritium counted in standard toluene scintillation solution.

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3. RESULTS AND DISCUSSION

3.1. *Activity of the* Eco dam *methylase on denatured DNA and its unreassociated fraction*

The methylase *Eco dam* is able to methylate denatured calf thymus DNA and its unreassociated fraction (table 1). The extent of methylation of these DNAs reaches 30% of the duplex DNA methylation level, which is half that of denatured T7 phage DNA [7]. Therefore not every site of denatured DNA is methylated by the methylase *Eco dam*. Unlike eukaryotic DNA methylases [11], the increased salt concentration (0.2 M NaCl) did not increase *Eco dam* activity on denatured DNA. The state of methylatable sites in single-stranded DNA had not been determined. It is possible that methylatable sites of denatured DNA can exist within regions of self-complementary duplex structures. However, it is not known whether the methylase *Eco dam* can participate in the creation of such structures. To answer this question, we studied the action of *Eco dam* on synthetic substrates.

3.2. *Activity of the* Eco dam *methylase on synthetic oligonucleotides*

Activity of the *Eco dam* methylase was analyzed on the synthetic icosameric oligonucleotides CAGTTTAGGATCCATTTCAC (I), GTGAAATGGATCCTAAACTG (II), the perfect icosameric duplex A and the imperfect duplex B. Duplex A is formed by intercomplementary oligonucleotides I and II (table 2). Imperfect duplex B is formed by annealing of an equimolar mixture of oligonucleotide I with oligonucleotides TCCTAAACTG (III) and GTGAAATGG (IV).

Table 1

Methylation of calf thymus DNA of different secondary structure by *Eco dam* methylase

DNA	Percentage of native DNA methylation
Native	100
Denatured	33
Unreassociated	25

The *Eco dam* methylase incorporated 10 pmol of CH_3 groups into 1 μg native calf thymus DNA. Reaction was carried out at 37°C

Table 2

Activity of *Eco dam* methylase on synthetic substrates

Substrate	Percentage of duplex A methylation at 37°C
Oligonucleotide I	
CAGTTTAGGATCCATTTCAC	
Reaction at 20°C	100
30°C	87
37°C	11
Oligonucleotide II	
GTGAAATGGATCCTAAACTG	
Reaction at 20°C	100
30°C	83
37°C	5
Duplex A	
CAGTTTAGGATCCATTTCAC (I)	
GTCAAATCCTAGGTAAAGTG (II)	
Reaction at 20°C	73
30°C	93
37°C	100
Duplex B	
CAGTTTAGGATCCATTTCAC (I)	
GTCAAATCCT GGTAAAGTG	
(III) (IV)	
Reaction at 20°C	0
30°C	3
37°C	4

Icosameric oligonucleotides I and II consist of the central symmetric hexanucleotide (*Bam*HI site) containing the GATC sequence (*Eco dam* site) flanked with uncomplementary heptanucleotides from 5′- and 3′-sides. Due to the destabilizing influence of its uncomplementary flanked heptanucleotides, none of such icosameric oligonucleotides is able alone to form the duplex structure within the central *Bam*HI site (at any rate, we failed to detect such a structure at temperatures above 5°C).

Oligonucleotide III (decamer) is complementary to the 5′-moiety and oligonucleotide IV (nonamer) complementary to the 3′-moiety of oligonucleotide I. This permits their combination with oligonucleotide I to yield the duplex structure lacking one adenine nucleotide in the second chain (duplex B).

Data on *Eco dam* methylase activity on separate

oligonucleotides I and II as well as duplex A and B are given in table 2.

At 20°C, the *Eco dam* methylates single-stranded oligonucleotides I and II to the same level as perfect duplex A. These results might be interpreted as indicating the ability of *Eco dam* methylase to act on the true single-stranded sites. However, the complete absence of *Eco dam* methylase activity on imperfect duplex B at 20°C contradicts this supposition. These results suggest that on interaction with the only one sort of single-stranded oligonucleotides I or II the *Eco dam* methylase is able to form the duplex structure within the GATC site, and that this duplex site is a substrate for enzyme.

At the same time, *Eco dam* methylase is inactive on imperfect duplex structure B in which one of two chains has a gap due to a loss of adenine nucleotide in the *Eco dam* site.

It should be noted that this situation differs from that of restriction endonucleases which are able to perform cleavage in only one strand in the case of incorrect duplex site structure [12,13].

At 37°C, the *Eco dam* enzyme methylates single-stranded icosamer by only about 10% of its level at 20 and 30°C. The temperature optimum for *Eco dam* methylase on native duplex DNAs, denatured DNAs and perfect icosameric duplex A is 37°C. Decrease of the temperature optimum for *Eco dam* methylase activity on single-stranded oligonucleotides evidently results from the destabilizing effect of elevated temperature on the site-specific methylatable complex formed with participation of *Eco dam* methylase. It should be noted that DNA methylases of some *Haemophilus* strains are also able to methylate denatured and single-stranded DNAs [14].

Of all the *E. coli* methylases, the *Eco dam* enzyme displays this ability to the greatest degree [15].

The ability of *Eco dam* methylase to pair single-stranded DNA sequences giving the duplex structure within the GATC site may reflect a functional role of this enzyme in the cell. There is evidence of the participation of *Eco dam* methylase in the DNA-replicative complex [16] as well as of the role of DNA enzymic methylation in the initiation of DNA replication in *E. coli* cells [17,18]. Of much interest in this connection is the enrichment of chromosome origin replication of *E. coli* and the other enterobacteria as well as their plasmids [19–22] in GATC sites. The gene for initiation of DNA replication in the *E. coli dna* A is also enriched in these sites [23].

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