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Purification and characterization of DNA methyltransferases from *Neisseria gonorrhoeae*

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ABSTRACT

Three DNA methyltransferases, M·NgoAI, and M·NgoBI and M·NgoBII, free of any nuclease activities were isolated from Neisseria gonorrhoeae strains WR220 and MUG116 respectively. M·NgoAI recognizes the sequence 5′ GGCC 3′ and methylates the first 5′ cytosine on both strands. M·NgoBI and M·NgoBII recognize 5′ TCACC 3′ and 5′ GTAN$_5$CTC 3′ respectively. M·NgoBII methylates cytosine on only one strand to produce 5′ GTAN$_5$^{m}CTC 3′.

INTRODUCTION

DNA methylation is the principal form of DNA modification in bacteria but its role is still incompletely understood. In a limited number of cases, it has been clearly shown to be involved with the biological phenomenon of restriction and modification [1]. There is also a considerable body of evidence that suggests a role for the dam and dcm methyltransferases of E. coli in mismatch repair and control of gene expression [2-4]. In many cases, DNA methyltransferases are associated with specific restriction endonucleases for which no biological role has been elucidated [5]. However, several DNA methyltransferases have been described that lack the corresponding restriction endonuclease activity and the biological role of the methyltransferases is unclear [6].

The presence of 5-methylcytosine and N^6-methyladenine residues in palindromic sequences of plasmid pJD1 isolated from N. gonorrhoeae strain 82409/55 suggested that the gonococcus could possess at least eight different DNA methyltransferase activities [7, 8]. Since N. gonorrhoeae produces several restriction enzymes [9, 10], some of these predicted

methyltransferase activities could be associated with these enzymes. It is not known whether the eight DNA methyltransferases postulated by Korch et al [7, 8] are eight different enzymes or whether some of them have a double specificity, as in the case of some of the Bacillus subtilis phage encoded DNA methyltransferases [11]. No DNA methyltransferases from N. gonorrhoeae have been isolated, although their existence has been reported [12]. The aim of the present study was to determine if the predicted DNA methyltransferases exist in gonococcus and identify their sequence specificities.

MATERIALS AND METHODS

Bacterial strains and plasmids.

N. gonorrhoeae strain WR220 [13] was obtained from Dr. H. Schneider (Walter Reed Army Institute of Research). N. gonorrhoeae strain MUG116 was isolated in our laboratory as an antibiotic resistant mutant of N. gonorrhoeae strain WR302, also provided by Dr. Schneider. The plasmids used in this study were: pBR322 [14], pUC8 [15], and pC194 [16].

Media and chemicals.

Cultures were grown on GCK agar or GCP broth [17]. Phage DNA from ØX174 and lambda was purchased from New England BioLabs. [^{3}H] S-adenosylmethionine (AdoMet) (76 Ci/mMol) was purchased from Amersham. Restriction endonucleases were purchased from New England BioLabs or IBI. DEAE-Sephacell, Sephadex G-200, Heparin Sepharose CL-6B (Pharmacia), Accel QMA (Millipore), and Phosphocellulose P11 (Whatman) were used for purification of DNA methyltransferases. The chemicals for gel electrophoresis were purchased from Bio-Rad. All other chemicals were analytical grade or better and were obtained from Sigma Chemical Co..

DNA methyltransferase assay.

Incorporation of [^{3}H] methyl groups from [^{3}H] AdoMet into DNA was determined as follows. The standard reaction mixture contained in a total volume of 20 μl to 50 μl: 50 mM Tris-hydrochloride, pH 7.5, 10 mM EDTA, 7.0 mM β-mercaptoethanol, 2.0 μM [^{3}H] AdoMet, 0.3 μg to 5 μg of the appropriate DNA and 2 μl of enzyme fractions. Unincorporated label was removed from the sample by

adsorption of DNA to DE-81 filters as described by Maniatis et al [18]. For quantitative measurements of the incorporation of radioactivity into the DNA, reaction mixtures were extracted with phenol and the aqueous phase passed through a 5 ml Sephadex G-100 column. If further analysis of the DNA was necessary, the excluded DNA was subjected to digestion with restriction enzymes followed by electrophoresis and fluorography. One unit of DNA methyltransferase activity is defined as the amount of enzyme that incorporates 1 pmol of methyl groups into DNA in 1 hr at 37 ^{o}C.

Purification of methyltransferases from N. gonorrhoeae WR220. N. gonorrhoeae WR220 (10 L) was grown overnight at 37 ^{o}C, and the cells collected by centrifugation (40 g wet weight). The cells were suspended in 100 ml of buffer A (10 mM Tris-hydrochloride, pH 8.0, 10 mM $MgCl_2$, 7 mM β-mercaptoethanol, 1 mM EDTA, 10 % glycerol) and disrupted by sonication (Sonifier Cell Disrupter, Branson Sonic Power Co., [30 sec bursts at full power for a total of 5 min]). The cell lysates were centrifuged at 48,000 x g for 60 min and the resulting supernatant made 0.2 M NaCl. Polyethylenimine (10%, pH 7.5) was added dropwise to the supernatant to a final concentration of 1%. Solid ammonium sulfate was added to 80% saturation. The precipitated proteins were collected by centrifugation, suspended in buffer B (20 mM Tris-hydrochloride, pH 8.0, 7 mM β-mercaptoethanol, 1 mM EDTA, 10 % glycerol), and dialyzed against the same buffer overnight. The dialysate was applied to a 2 x 30 cm DEAE-Sephacell column equilibrated with buffer B. After washing with 150 ml of buffer B, proteins were eluted with a 400 ml linear gradient of NaCl (0.02 to 1.0 M). Methyltransferase activity eluted between 0.15 M and 0.25 M NaCl. The active fractions were pooled, dialyzed against buffer B containing 40 mM NaCl and applied to a heparin-sepharose column (2 x 30 cm) that had previously been equilibrated with buffer B containing 40 mM NaCl. The column was washed with 1.5 column volumes of the same buffer and eluted with 400 ml of a linear NaCl gradient (0.04 to 1.0 M). The methyltransferase activity eluted between 0.1 and 0.25 M NaCl. The active fractions were concentrated to a final volume of 2 ml by dialysis against 50% Polyethylene glycol 8000 in buffer B

containing 0.05 M NaCl and applied to a Sephadex G-200 column (2 x 100 cm). The activity eluted at 0.62 column volumes and the five 2 ml fractions containing enzyme activity were pooled, dialyzed against buffer B containing 0.06 M NaCl and applied to an Accel QMA column (0.78 x 10 cm) that had been equilibrated with buffer B containing 0.06 M NaCl. The proteins were eluted with a 20 ml linear gradient of NaCl between 0.06 and 1.0 M). The methyltransferase activity eluted at 0.35 M NaCl. This methyltransferase was named M·NgoAI.

Purification of methyltransferases from N. gonorrhoeae MUG116.

Crude extracts were prepared from MUG116 by the same procedure as described for WR220. The ammonium sulfate precipitate was suspended in buffer C (0.02 M KPO_4 buffer, pH 7.2, 7 mM β-mercaptoethanol, 1 mM EDTA, 10 % glycerol) and applied to a Phosphocellulose column (1 x 30 cm) equilibrated with buffer C. After washing with 200 ml of buffer C, proteins were eluted with a 300 ml gradient of NaCl (0.02 to 1.0 M). The methyltransferase activity eluted at 0.3-0.4 M NaCl. The active fractions were pooled and dialyzed against buffer B containing 0.04 M NaCl. The methyltransferase fraction was applied to an Accel QMA column (1 x 15 cm) that had been equilibrated with buffer B containing 0.04 M NaCl. The column was washed with 50 ml of buffer B containing 0.04 M NaCl and eluted with a 60 ml linear gradient of NaCl between 0.04 and 1.0 M. Three methyltransferase activities were detected, the first, (M·NgoBI) eluted at 0.1 M NaCl, the second, (M·NgoBII) at 0.4 M NaCl, and the third activity at 0.55 M NaCl. Due to the very low level of activity in the third peak, this enzyme was not further tested. All isolated DNA methyltransferases were stable over several months when stored at -70 °C in 20 % glycerol.

Base analysis of methylated DNA.

Purified methylated DNA was digested to 5' monophosphates with 10 μg of pancreatic DNAase in a 20 mM Tris-hydrochloride, pH 8.0, 10 mM $MgCl_2$ and 10 mM $CaCl_2$ buffer for 90 min at 37 °C, followed by digestion with snake venom phosphodiesterase for an additional 90 min at 37 °C. HPLC analyses were performed on a 30 cm C-18 column (Waters Corp.) using a 0.5 M NaH_2PO_4, pH 5.0 buffer at a flow rate of 1 ml per min. Using this procedure, it is possible

Table 1. Restriction and Modification enzymes present in N. gonorrhoeae.

N. gonorrhoeae strains	Protected sequences in DNA[a]	PuGGCPy	GGCC	CCGCGG	GCCGGC	GGNNCC	GATC	GCSGC	TCACC	GTAN5CTC
WR220	Predicted presence	+[e]	+[f]	+	+	+	-	+	+	-
MUG116	of DNA methyltransferases[b]	+	-	+	+	+	+	+	+	-
WR220	Purified DNA	-	+	-	-	-	-	-	-	-
MUG116	methyltransferase[c]	-	-	-	-	-	-	-	+	+
WR220	Presence of	+	-	+	-	-	-	-	-	-
MUG116	Restriction enzymes[d]	-	-	-	-	-	-	-	+	-

a) The protected sequences are the recognition sequences for the enzymes tested: PuGGCPy, HaeII; GGCC, HaeIII; CCGCGG, SacII; GCCGGC, NaeI; GGNNCC, BamHI; GATC, MboI; TCACC, HphI; GCNGC, Fnu4HI; and GTAN5CTC, not tested.
b) The presence of DNA methyltransferases was predicted based on the resistance of the strains DNA to cleavage with restriction enzymes whose activity is blocked by methylation in the recognition sequence.
c) DNA methylation Enzymes that we have been able to detect in these strains.
d) DNA restriction enzymes that we have been able to detect in these strains.
e) + indicates that the sequence was protected or enzyme was purified.
f) - indicates that the sequence is not protected from cleavage or the enzyme was not purified.

to differentiate between 5-methyl cytosine and N4-methyl-cytosine.

RESULTS

Purification of DNA methyltransferases from N. gonorrhoeae.

Digestion of chromosomal and plasmid DNA isolated from the two N. gonorrhoeae strains used for our experiments indicated that they resisted cleavage with a variety of restriction enzymes [19] (see summary of these results in Table 1). Such resistance could be attributed to the presence of methylated DNA, whose presence in DNA is known to block their cleavage. However, when we attempted to isolate these DNA methyltransferases, only one or two were found at levels sufficient for purification. One DNA methyltransferase was isolated from N. gonorrhoeae strain WR220, and three from strain MUG116. After several steps of purification (see Materials and Methods) M·NgoAI was present at very high levels, (more than 3 x 10^3 U/g of cells), while M·NgoBI and M·NgoBII were present at only 10 % of the level of M·NgoAI. The three DNA methyltransferases differed slightly in their optimal conditions for methyltransferase activity. They were in general, most active in the pH range 7.0-8.5 at temperatures between 35 - 40 °C. The presence of EDTA in the reaction mixtures stimulated the activity for all of the enzymes while Mg^{+2} (10 mM) decreased the activity of M·NgoAI. ATP did not

stimulate the activity of any of the enzymes. Since none of the isolated DNA methyltransferases had restriction activities when tested in the presence of Mg^{+2}, or Mg^{+2}, ATP and S-adenosylmethionine, this indicates that the isolated methyltransferases are not part of a more complex type of restriction/modification system as seen in type one and type three enzymes [19a, 20].

HPLC analysis of the DNAs methylated by all three DNA methyltransferases showed that these enzymes transfer the methyl groups only to the cytosine residues. Furthermore, the cytosine was methylated at the 5 position (data not shown).

Determination of enzyme specificity.

The method originally developed for the determination of type III restriction enzymes recognition sequences [20, 21] was used to establish the specificity of the isolated DNA methyltransferases. In this method DNAs of known sequence are methylated with [^{3}H]-AdoMet, digested with a variety of restriction enzymes, the fragments separated by gel electrophoresis and those containing a recognition sequence identified by fluorography. DNA sequences unique to the labeled fragments and missing from the unlabeled fragments were identified by computer analysis. The computer program used for analyzing sequences was INTREST, version 5/16/84.

To find the sequence recognized by the DNA methyltransferase M·NgoAI, plasmid pBR322 DNA was methylated and subjected to the type of analysis indicated above. Figure 1 shows an example of one such experiment. It was found that at least six regions in pBR322, with the following coordinates: 170-1484, 1812-2031, 2375-3159, 3325-3506, 3728-3865 and 4291-199 are methylated. Of the eight hypothetical DNA methyltransferases of N. gonorrhoeae postulated by Korch et al [7,8], only M·NgoII, recognizing the sequence GGCC, has recognition sites in all of the regions that are methylated and none in the regions that are not methylated. In order to verify that M·NgoAI recognizes the above sequence several other experiments were performed. Plasmid pC194 has only one HaeIII (NgoII) recognition site [16] and when this DNA was methylated and digested with TaqI, only the fragment containing the HaeIII site was methylated (Fig. 1, panel D). Furthermore,

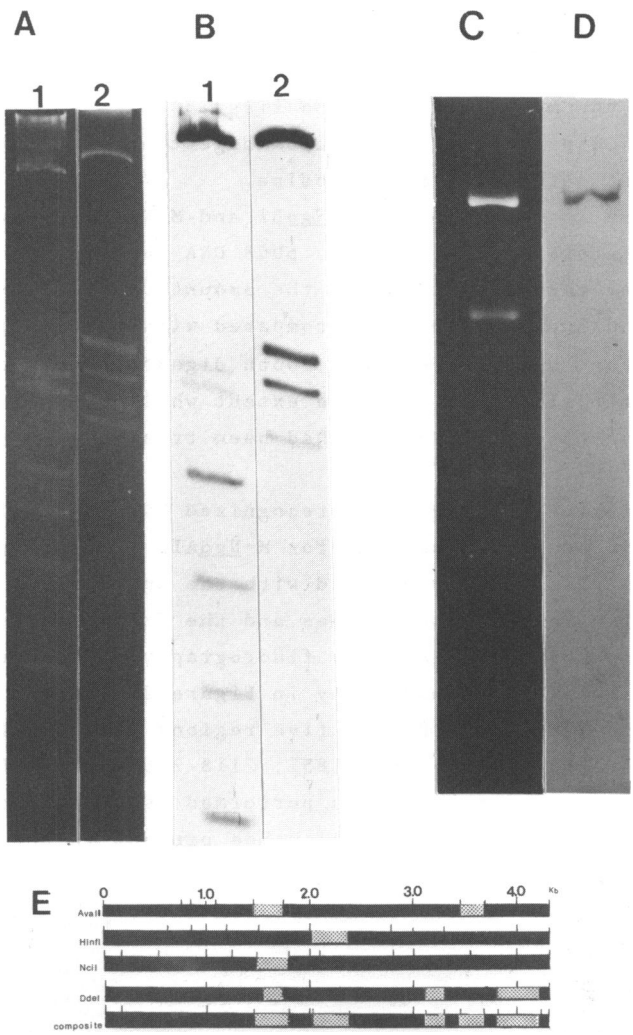
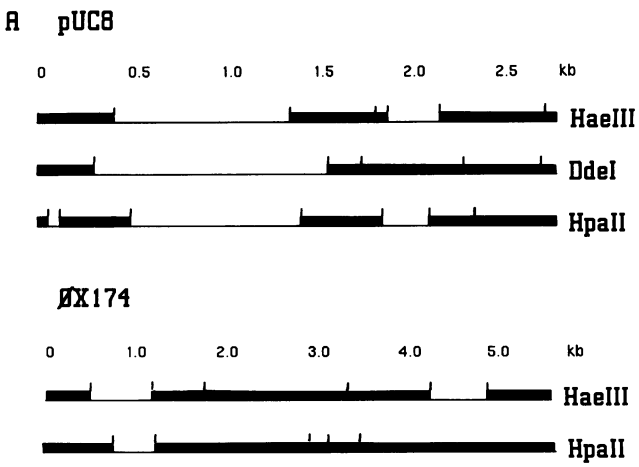


Figure 1. Mapping of DNA methyltransferase M·NgoAI recognition sites on pBR322 and pC194 DNAs. Plasmid DNAs were methylated in vitro with M·NgoAI and [^{3}H]AdoMet, digested with restriction enzymes and the resulting fragments separated by electrophoresis on a 3% polyacrylamide gels. The gels were stained with ethidium bromide, photographed, dried and fluorographed. Panel A represents the stained gels and panel B the fluorograms for pBR322. DNA was digested with HinfI (lane 1) or DdeI (lane 2). Panel C represents the stained gel and panel D the flourogram for pC194. DNA was digested with TaqI. Schematic representation of the regions of pBR322 not methylated by M·NgoAI is shown in panel E of the figure. The dark region [■], indicates those fragments that were methylated, while the hatched region [▒] indicates regions that were not methylated.

plasmid pBR322 was completely protected from digestion by HaeIII after methylation with the isolated enzyme (data not shown). Since only the methylation of the first 5' cytosine in the sequence GGCC protects the DNA from digestion with HaeIII [22] M·NgoAI must methylate this cytosine.

In order to determine if M·NgoBI and M·NgoBII recognize a sequence different from M·NgoAI, pUC8 DNA was digested with HaeIII prior to methylation and the amount of ^{3}H-methyl groups incorporated into this DNA was compared with the undigested control. For these two enzymes, both digested and undigested DNAs were methylated to the same extent while M·NgoAI was unable to methylate the pUC8 DNA that had been treated with HaeIII prior to methylation.

To determine the sequences recognized by M·NgoBI and M·NgoBII the same procedure was used as for M·NgoAI. Plasmid pUC8 and phage ØX174 DNAs were methylated with the appropriate enzymes, digested with restriction enzymes and the fragments separated by gel electrophoresis followed by fluorography. These experimental data are presented schematically in Figure 2. It was found that M·NgoBI methylates pUC8 DNA at five regions with the following coordinates: 1520-1696; 1771-1851; 2118-2263; 2263-2653; and 2653-55. Computer analysis was performed as described above and the data showed that the only sequence present in the methylated



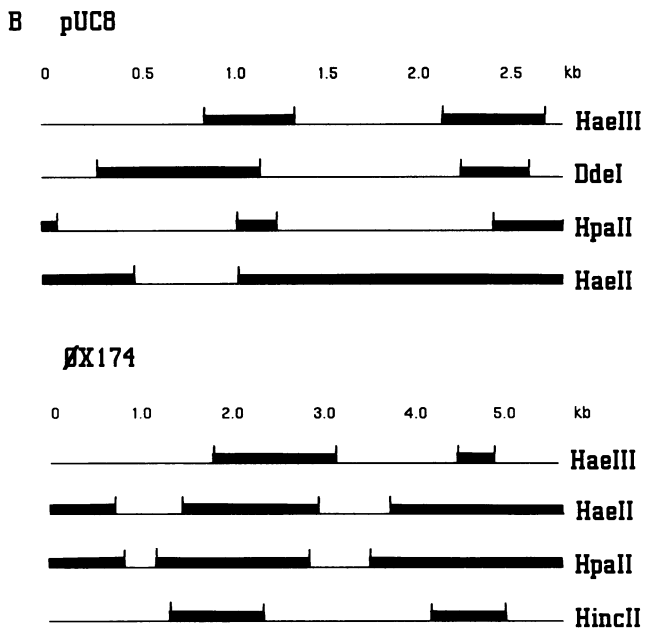


Figure 2. Location of Methyltransferases M·NgoBI and M·NgoBII recognition sites in pUC8 and Ø174. Panel A is the data for M·NgoI and panel B is the data for M·NgoBII. The regions that are methylated are represented by the darkened line (▬). The numbers represent the size of the DNA is kb. The enzymes listed are those that were used to digest the various DNAs. The hatchmarks on the linear maps represent the location of the various restriction sites on the DNA.

bands but missing in the unlabelled bands is 5′ TCACC 3′. An additional proof that this sequence is recognized by M·NgoBI was obtained from the analysis of the methylated sites on ØX174 DNA. Only those fragments which have the sequence 5′ TCACC 3′ were methylated. The sequence 5′ TCACC 3′ was predicted for the hypothetical M·NgoVIII methyltransferase [8]. This is the same sequence recognized by HphI [24]. Korch et al [8] postulated that M·NgoVIII methylates the first 5′ cytosine in this sequence, while Nelson et al [24] have shown that methylation of this cytosine inhibits the cleavage by HphI. The results presented in Figure 3 show that pUC8 DNA methylated with M·NgoBI is protected from digestion by HphI enzyme. This result strongly suggest that M·NgoBI methylates the first 5′ cytosine in the recognition

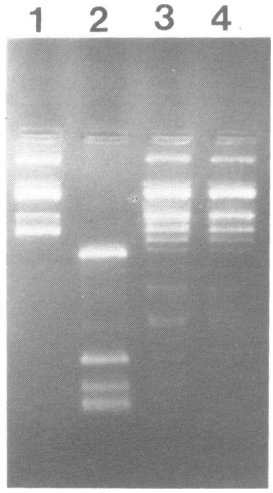


Figure 3. Protection of pUC8 DNA from digestion by HphI restriction enzyme after in vitro methylation by M·NgoBI. The reaction mixture contained in a total volume of 50 ul: 50 mM tris-hydrochloride, pH 7.2, 10 mM EDTA, 7.0 mM β-mercaptoethanol, 80 uM AdoMet and 5 ug of pUC8 DNA. At the indicated reaction times, the reaction mixtures were extracted with phenol and the aqueous phase passed through a Sephadex G-100 column. The DNA (1 μg) was digested for 3 h with 5 units of HphI restriction enzyme and the resulting fragments separated on 1.4 % agarose gel. Lane (1), undigested methylated pUC8 DNA; lane (2), unmethylated DNA cleaved with HphI; lane (3), DNA methylated for 1 h before digestion with HphI; lane (4), DNA methylated for 3 h before digestion with HphI.

sequence. An unexpected finding was that DNA digested with HphI can no longer be a substrate for the methyltransferase M·NgoBI. Since HphI cleaves a short distance from the recognition site [23] it suggests that M·NgoBI methyltransferase needs not only the presence of the recognition site but also some of the neighboring sequences.

The enzyme M·NgoBII methylates ØX174 DNA and pUC8 DNAs at only two regions with the coordinates; 1777-2352 and 4739-4760; and 1083-1100 and 2263-2653 respectively. Computer analysis of the above data showed that the only sequence consistent with these results was 5′ GTAN$_5$CTC 3′. A computer search on lambda DNA indicated that there are 17 such sequences. When lambda DNA was methylated and cleaved with HinDIII, PvuII, AvaI, and ClaI,

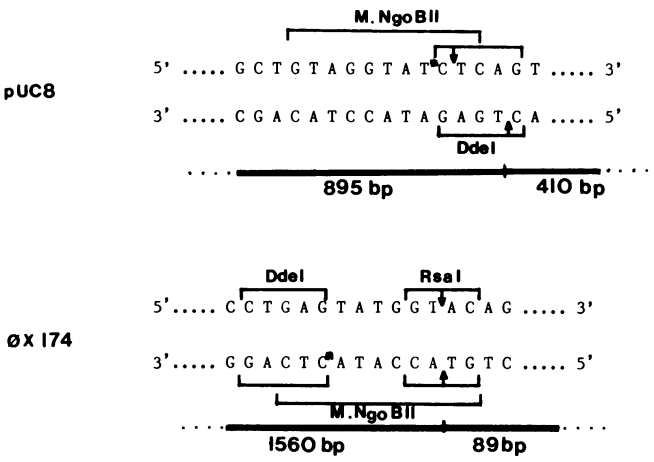


Figure 4. Overlap of the proposed M·NgoBII recognition sequence with those of DdeI and RsaI on pUC8 and ØX174 DNAs. pUC8 and ØX174 DNA sequences containing one of the M·NgoBII sites are presented. The numbers refer to the coordinates of the pUC8 sequence [15] and ØX174 DNA sequence [31]. The horizontal lines indicate the sequences recognized by M·NgoBI, DdeI or RsaI. Arrows indicate cleavage sites. The bold horizontal lines shows the fragments generated by the DdeI and RsaI restriction enzymes. The * indicates the cytosine residue that is methylated.

respectively it was found that all methylated fragments, except one, have the predicted sequence (data not shown). The one exception, a 1602 bp AvaI fragment (coordinates 19398-21000) contains the sequence 5' GTAN$_5$CTG 3'. This disagreement between our data and the published sequence of lambda DNA [25] may be due to the presence of cytosine in the position 20009 in the lambda DNA used in this experiment. The computer analysis of the pUC8 and ØX174 DNA sequences showed that in pUC8, one of the two predicted sites overlaps the sequence recognized by DdeI (coordinates 1101-1117) while in ØX174, one of the two predicted sites recognized by M·NgoBII overlaps sequences recognized by DdeI and RsaI (coordinates 4739-4750) (see Figure 4). If the predicted sequence for M·NgoBII is correct then the cleavage of pUC8 DNA by DdeI before methylation should destroy one of the M·NgoBII sequences and only one fragment should be methylated. The inability of M·NgoBII to methylate the one fragment is shown

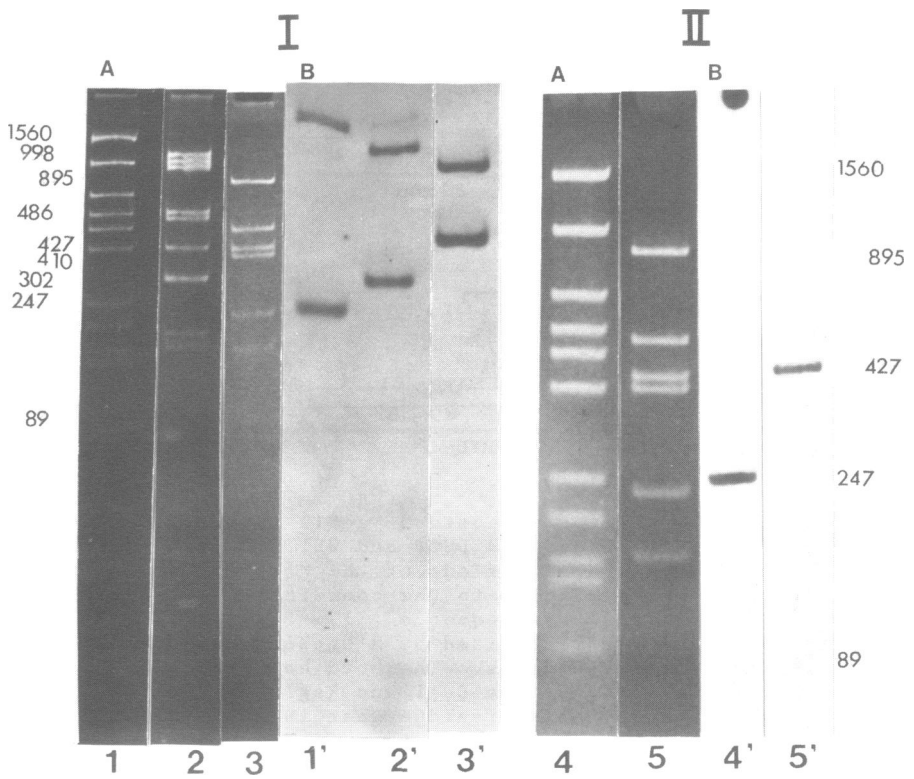


Figure 5. Confirmation of the recognition sequence of M·NgoBII. pUC8 and ØX174 DNAs were methylated by M·NgoBII before (I) or after (II) cleavage with various restriction enzymes, and the resulting fragments separated by electrophoresis on a 3% polyacrylamide gel stained with ethidium bromide, photographed, dried and flourographed. Panel A are the stained gels and panel B are the fluorograms. ØX174 DNA cleaved with RsaI (lanes 1, 1', 4, 4'); ØX174 DNA cleaved with DdeI (lanes 2, 2') and pUC8 DNA cleaved with DdeI (lanes 3, 3', 5, 5'). Numbers to the left and right of the lanes are the sizes of the DNA fragments (in bp).

in figure 5, lanes 3' and lanes 5'. The same was expected to occur for ØX174 DNA cleaved with RsaI prior to methylation by M·NgoBII (compare lane 1' with lane 4'). These results are consistent with the predicted recognition sequence for M·NgoBII.

The overlap of the DdeI and RsaI with the sequence recognized by M·NgoBII allowed identification of the cytosine which is methylated by the enzyme. The fact that among the DdeI fragments of pUC8, the 895 bp but not the 410 bp fragment is methylated

(Figure 5, lane 3') indicates that the last 5' cytosine in the upper strand is not methylated (See figure 4 for sequence). This was also confirmed by the methylation of the 998 bp DdeI fragment but not the 486 bp fragment of ØX174 DNA (Figure 5, lane 2'). On the other hand when methylated ØX174 DNA was cleaved with RsaI the 1560 bp fragment was methylated but not the adjacent 89 bp fragment, showing that the cytosine in the lower strand was not methylated (Figure 5, lane 1'). In conclusion, M·NgoBII methylates the first 5' cytosine on only one strand as shown in Figure 4.

DISCUSSION

The protection of chromosomal and plasmid DNA isolated from the two N. gonorrhoeae strains against cleavage by the restriction enzymes led to the prediction of eight DNA methyltransferases [19]. However, only two of the predicted enzymes and one new one were present in the crude extracts at levels that allowed for their purification.

The first of the isolated DNA methyltransferases, M·NgoAI recognizes the same sequence as NgoSI (an isoschizomer of HaeIII) and is part of the restriction-modification system that acts in vivo against foreign DNA during transformation of Neisseria [26]. This DNA methyltransferase recognizes a DNA sequence characteristic for type II restriction enzymes and has properties characteristic of their corresponding DNA methyltransferases [27].

M·NgoBI belongs to a rare group of DNA methyltransferases which do not recognize palindromic sequences and methylate only one strand within the recognition sequence [22]. M·MboII and M.HphI have the corresponding restriction enzymes which cleave the DNA at a short distance from the recognition sequence [23]. Similarly, M·NgoBI also has the corresponding restriction enzyme (an isoschizomer of HphI) [Unpublished observations] and contrary to the prediction by Korch and coworkers [8], both DNA methyltransferase and restriction enzyme have properties characteristic for type II enzymes.

The third isolated DNA methyltransferase is a novel one, and so far no restriction enzyme or DNA methyltransferase recognizing

the sequence 5' GTAN$_5$CTC 3' has been described. This is a distinctive DNA methyltransferase which recognizes a longer non-palindromic sequence with an internal (N_5) sequence and methylates the cytosine residue on only one strand of the recognition sequence.

Our analysis of several other N. gonorrhoeae strains has shown the presence of at least four additional DNA methyltransferases which, like those described in this paper, recognize unique sequences [Piekarowicz-unpublished results]. This brings the total number of DNA sequences methylated by the gonococcus to thirteen, five of which have the corresponding restriction enzymes [9, 10, John Davies, Personal Communication, unpublished observations]. By using different methods for the purification of DNA methyltransferases, we have always been able to demonstrate the presence of one or two enzymes at levels which allowed for their purification. The enzyme detected was independent of the purification scheme used. This indicates that in a given strain, the DNA methyltransferases are synthesized at different levels. The analysis of the level of the restriction enzymes in N. gonorrhoeae strains has shown that these enzymes are also produced at different levels. Moreover, the high level of a DNA methyltransferase does not always correspond with a high level of the corresponding restriction enzyme. For example, strain WR220 produces more than 10^6 U/g of cells of NgoAIII (an isoschizomer of SacII) [Piekarowicz-unpublished results] but we were unable to isolate the corresponding DNA methyltransferase. The different levels of expression of particular DNA methyltransferase genes could be explained by the differences in their regulatory sequences, or in the different sensitivity of these sequences to the changes in the environmental conditions, like pH, ion concentration, or the presence of iron. Alternatively, these enzymes might exhibit differential stabilities whose activity is lost during purification.

Since the gonococcus produces so many methylation enzymes, but fails to produce the corresponding restriction enzymes, the role that these enzymes play in the cell remain unclear. Since DNA methylation has been implicated in the control of phase variation in Bordetella pertussis [32], it is likely that these

enzymes are also involved in the control of antigenic variation in N. gonorrhoeae. We have cloned some of these DNA methyltransferases, and have been able to isolate the enzymes from E. coli. Since these genes can be readily detected in E. coli, our failure to detect them in extracts in N. gonorrhoeae indicates that they are not produced in significant quantities under the growth conditions that we have employed.

Table 1 summarizes our findings with regards to restriction and modification enzymes present in the two strains analyzed. These data indicates that there is no correlation between the restriction enzyme present and the methyltransferase isolated from the strain. Furthermore, we were unable to purify most of the predicted enzymes, but were able to isolate enzymes with novel specificities. Our failure to isolate all enzymes with the growth conditions employed suggests these enzymes may fall under some kind of environmental control. Studies of the effect of environmental changes on the expression of these genes are currently underway in our laboratory.

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