High Correlation of the Presence of Methyladenine in *Neisseria gonorrhoeae* DNA with the AHU Auxotype

AMY B. KOLODKIN, VIRGINIA L. CLARK,† FRED C. TENOVER,† AND FRANK E. YOUNG

*Department of Microbiology, University of Rochester, Rochester, New York 14642*

Received 24 September 1981/Accepted 23 December 1981

Strains of *Neisseria gonorrhoeae* were tested for the presence of methyladenine in the DNA sequence GATC by using the site-specific restriction endonucleases *MboI* and *DpnI*. It was found that 43 of 83 strains tested contained methylated DNA. When methylation was compared with the auxotype of the organism, 35 of 35 strains with the AHU (arginine-, hypoxanthine-, and uracil-requiring) auxotype and 8 of 48 strains with other auxotypes contained methyladenine. When the incidence of methylation in strains isolated from patients suffering from disseminated gonococcal infection was compared with that in strains isolated from patients suffering from uncomplicated gonococcal infection, no correlation with methylation and disseminated gonococcal infection was observed.

The existence of methylated DNA in both procaryotic and eucaryotic systems has been well documented. In some procaryotes, methylated DNA serves in the recognition of foreign DNA which may enter the cell during bacteriophage infection (7). It is thought that another possible function for the presence of modified DNA might be in the regulation of protein-DNA interactions (9). It has been observed in *Escherichia coli* that mutants which lack the ability to methylate the adenine residue of the GATC sequence (*dam* mutants) display an increased rate of mutation, probably due to a defective mismatch-repair system (8).

In preliminary experiments performed in our laboratory by Tenover et al. (11), a possible correlation was noted between the appearance of methyladenine in the DNA of *N. gonorrhoeae* strains and a requirement for arginine, hypoxanthine, and uracil (AHU) for growth. The AHU auxotype is of particular interest in this regard because of the association of strains of this auxotype with disseminated gonococcal infection (3). We report here an expanded study that supports our earlier observations that there is an association between strains of *N. gonorrhoeae* displaying the AHU auxotype and the appearance of methyladenine in the DNA. However, this correlation is not absolute; although all AHU strains methylate, some non-AHU strains also methylate adenine residues in the DNA.

**MATERIALS AND METHODS**

*Bacterial strains*. Strains of *N. gonorrhoeae* used in this study are given in Table 1. All of the strains were grown on GC medium base (Difco Laboratories) with 1% IsoVitaleX supplements (BBL Microbiology Systems). Liquid medium was GCP plus 1% IsoVitaleX (6).

The Centers for Disease Control (CDC) 500 series is a collection of *N. gonorrhoeae* strains obtained from venereal disease centers in Boston, Mass.; Miami, Fla.; Denver, Colo; Des Moines, Iowa; Dayton, Ohio; Norfolk, Va; Oakland, Calif.; Seattle, Wash.; and Newark, N.J. The collection consists of 50 strains from each city and 50 strains that were resubmitted as controls (4). The study presented here contains strains from all nine cities.

**DNA extraction.** Chromosomal DNA was extracted either by the method of Marmur (5) or by a rapid isolation method previously described (1). Briefly, with the latter technique, colonies were picked and bacteria were suspended in 0.5 ml of a solution containing 50 mM Tris-hydrochloride (pH 8.5), 50 mM Na₂EDTA, 15% sucrose, and 1 mg of lysozyme per ml. After a 10-min incubation at room temperature, 20 µl of 10% sodium dodecyl sulfate was added, and the tubes were inverted to mix the contents. The tubes were placed in a 70°C water bath for 5 min to obtain chromosomal DNA, and 100 µl of 5 M potassium acetate was added. The protein was allowed to precipitate by incubating the tubes on ice for at least 30 min, after which the tubes were centrifuged (Brinkman centrifuge, 3,200 × *g*) for 15 min. At least 2 volumes of ethanol were added to each supernatant, and the tubes were centrifuged in the microfuge for 5 min. The supernatant was decanted, the tube was inverted and drained, and the dried precipitate was dissolved in 10 mM Tris-hydrochloride buffer (pH 7.5) containing 1 mM Na₃EDTA and 10 µg of RNase per ml. DNA concentrations were approximated from the ratio of absorbance at 260 and 280 nm, assuming that an absorbance of 1.0 is equivalent to 50 µg of DNA per ml (1).

**Restriction enzyme analysis.** The chromosomal DNA (approximately 10 µg) was incubated with either 1 U of the restriction endonuclease *DpnI* (Bethesda Research
Laboratories, Bethesda, Md.) of 2 U of the restriction endonuclease MboI (New England Biolabs, Inc., Beverly, Mass.). In a few cases, the endonuclease Sau3AI (Bethesda Research Laboratories) was used in place of DpnI. Incubation was carried out in silanized glass tubes (10- by 75-mm) in 20 µl of buffer containing 6 mM Tris-hydrochloride (pH 7.5), 6 mM MgCl₂, and 6 mM β-mercaptoethanol. After 2 to 3 h at 37°C, 2.5 µl of a solution containing 7.5 mM Tris-hydrochloride (pH 8), 45% sucrose (wt/vol), 75 mM Na₂EDTA, and 0.2% bromphenol blue was added; the sample was applied to a 1% agarose gel containing 1 µg of ethidium bromide per ml. The gel was suspended in 89 mM Tris-borate buffer (pH 8.2) containing 2.5 mM Na₂EDTA. Gels were run overnight at 30 V.

RESULTS

In this study, three different site-specific restriction endonucleases were used to determine the presence or absence of methyladenine in the DNA of various N. gonorrhoeae strains. In most cases, both MboI, which only recognizes and cleaves the DNA sequence GATC if the adenine residue is not methylated, and DpnI, which only recognizes and cleaves the sequence GATC if the adenine residue is methylated, were used. In a few instances, Sau3AI, which cleaves the GATC sequence regardless of methylation, was used in place of DpnI. Figure 1 shows the results of a representative experiment in which the MboI and DpnI digests were separated by agarose gel electrophoresis as described above. In the experiments utilizing the enzymes MboI and DpnI, digestion with MboI indicated the presence of unmethylated DNA, whereas digestion with DpnI indicated the presence of methyladenine in the DNA sequence GATC. Digestion of DNA from a single gonococcal strain with both endonucleases was not seen. In the few cases in which Sau3AI was substituted for DpnI, the DNA was considered unmethylated if it was digested with MboI, and methylated if it was not. As a control in this case, all samples were digested with Sau3AI.

All of the strains used in this study, except strains FA534 and KH45, contained the 2.6-Mdal gonococcal cryptic plasmid (2), as is apparent in Fig. 1. This plasmid contains a single GATC sequence, and cleavage of the plasmid therefore converts it from the closed or open circular form to the linear form (see Fig. 1). This serves as an internal control to detect the presence of exonucleases in the digestion, as under this condition the linear plasmid band would not be present. (In Fig. 1, the 2.6-Mdal plasmid of strains 020 and 021 were only partially cleaved with DpnI. Complete digestion was observed in other experiments.)

![Figure 1](http://iai.asm.org/)
In our preliminary study (11), which considered only 16 strains of *N. gonorrhoeae*, we found that all of the strains that required AHU methylated the adenine residue of the GATC sequence, whereas none of the non-AHU strains contained methyladenine. We therefore expanded our survey of gonococcal strains (Table 1) to determine if there was an absolute correlation between the AHU auxotype and the presence of methyladenine. In Table 2, the auxotypes of the various strains tested are given along with the methylation results. We found that 35 of 35 AHU strains and 8 of 48 non-AHU strains were digested with *DpnI*, indicating the presence of methyladenine. Although the correlation between the AHU auxotype and methylation is not absolute, it is very high (*P < 0.001*).

The question of a correlation between the AHU auxotype and methyladenine is of particular interest because of the association of this auxotype with disseminated gonococcal infection (DGI). The strains obtained from A. R. Ronald were isolated from patients with DGI (W numbers 100 or above) and from patients with uncomplicated gonococcal infection (W numbers less than 100). Of the DGI strains, 5 of 12 contained methyladenine (3 were AHU strains), whereas 5 of 12 of the non-DGI strains contained methylated DNA (3 were AHU strains). Thus, of these 10 methylating strains, 6 were of the AHU auxotype. Therefore, in this group of strains it does not appear that there is a correlation between methylation of the GATC sequence and incidence of DGI.

**TABLE 1. N. gonorrhoeae strains used**

<table>
<thead>
<tr>
<th>Strain designation</th>
<th>Source</th>
<th>Adenine methylation&lt;sup&gt;a&lt;/sup&gt;</th>
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<td>001, 004, 007, 018, 019, 032, 033, 034, 144, 154, 155, 157, 158, 159, 160, 162, 189, 225, 244, 398, 439</td>
<td>Centers for Disease Control, 500 series</td>
<td>−</td>
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<td>002, 005, 006, 014, 020, 021, 028, 030, 044, 048, 052, 055, 064, 067, 068, 080, 090, 103, 118, 122, 125, 129, 139, 150, 180, 208, 432, 441, 443, 447, 450, 469, 470</td>
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<tr>
<td>RUG38</td>
<td>ATCC 19424</td>
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<tr>
<td>KH45</td>
<td>K. K. Holmes</td>
<td>+</td>
</tr>
<tr>
<td>F62</td>
<td>D. S. Kellogg</td>
<td>−</td>
</tr>
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<td>FA534</td>
<td>P. F. Sparling</td>
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</tr>
<tr>
<td>H4</td>
<td>F. Tyerar</td>
<td>−</td>
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<td>Y10</td>
<td>Centers for Disease Control, Atlanta series</td>
<td>−</td>
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<sup>a</sup> +, Presence and −, absence of methyladenine.

**DISCUSSION**

The purpose of this study was to expand the earlier observation made by Tenover et al. (11) that the presence of methyladenine in the DNA of some strains of *N. gonorrhoeae* appears to correlate with the AHU auxotype. Although we were able to show a high degree of statistical significance, the correlation was not absolute: whereas all strains with the AHU auxotype were methylated, not all non-AHU strains were non-methylated. In addition, because of the more frequent appearance of the AHU auxotype among strains causing disseminated gonococcal infection, our studies also included a preliminary attempt to correlate the appearance of methyladenine with strains isolated from patients with disseminated gonococcal infection. No such correlation was found with the data available.

Since partial hydrolysis by both *MboI* and *DpnI* was never seen, we assume that methylation at the GATC sequence is an all or none phenomenon. The lack of methylation in many strains could be due to the absence of a methylase or to lack of expression of a methylase gene. Since our studies utilized only restriction endonucleases which cleave specifically at the DNA sequence GATC, it must be recognized that some or all of the “nonmethylated” strains may actually be methylated at other sites.

Since all of the strains with the AHU auxotype were found to methylate the adenine of the GATC sequence, an interesting possibility is that methylation actually contributes to the sta-
bility of this auxotype by preventing back mutation. It is known that methylase-deficient E. coli strains display increased sensitivity to penicillin (12), correlation with disseminated infection (3), and now, methylation of the adenine residue of the GATC sequence. Thus, these strains may actually represent a subspecies of N. gonorrhoeae.

ACKNOWLEDGMENTS

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LITERATURE CITED


<table>
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<th>Methylating strains</th>
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<tr>
<td>Wild type (zero auxotype)</td>
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</tr>
<tr>
<td>Arg-</td>
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<tr>
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</tr>
<tr>
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<td>1</td>
<td>1</td>
</tr>
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<td>AHU His- Ile- Val-</td>
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<td>1</td>
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<tr>
<td>AHU Ile- Leu- Val-</td>
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