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Deoxyribonucleic Acid Modifications and Restriction Endonuclease Production in *Neisseria gonorrhoeae*

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Modification of gonococcal deoxyribonucleic acid (DNA) was investigated, and the relationship with endonuclease production was explored. Both chromosomal and plasmid DNA from different gonococcal strains, irrespective of their plasmid content, was poorly cleaved by the restriction endonucleases *Hae*II, *Hae*III, *Sac*II, and *Bam*HI. The fragment pattern of the Tn*3* segment present on the 7.2-kilobase gonococcal resistance plasmid, when compared to its known DNA sequence, allowed us to conclude that the *Hae*III and *Bam*HI resistance was due to modification of these sites. A comparison of the fragment pattern of the resistance plasmid, when isolated from *Escherichia coli* or *Neisseria gonorrhoeae*, revealed that the resistance of *Hae*II must also be due to modification of its recognition sequence. Isoschizomers of *Hae*II and *Hae*III can be found in isolates of *N. gonorrhoeae* (*Ngo*I and *Ngo*II, respectively). A new restriction endonuclease in gonococci, *Ngo*III, with a specificity similar to *Sac*II, is reported here. High-pressure liquid chromatography of gonococcal DNA showed the presence of 5-methylcytosine. It is suggested that the methylation of cytosine residues in the *Hae*II (*Ngo*I), *Hae*III (*Ngo*II), and *Sac*II (*Ngo*III) recognition sites is the basis for the resistance of gonococcal DNA to cleavage by these enzymes. This methylation may be part of a host restriction modification system. In two out of five gonococcal strains the sequence -GATC- was modified. One strain unable to modify this sequence was a spontaneous mutant of a strain carrying such a modifying function.

Restriction and modification systems have been extensively studied in a number of bacterial species. More than 140 restriction endonucleases have been discovered, and in many cases the corresponding methyltransferase has been described as well (28). In *Escherichia coli* K-12, 5-methylcytosine and 6-methyladenine are the only detectable methylated bases in DNA (33). However, only a minor part of these methylated bases is involved in the host restriction modification systems (19). In *E. coli*, as well as in other organisms like *Salmonella typhimurium*, *Salmonella typhi*, *Haemophilus influenzae*, and *Streptococcus pneumoniae*, the major fraction of 6-methyladenine is present in the sequence -GATC- (14, 18). In all wild-type strains of *E. coli* tested, all -GATC- sequences appear to have the adenine methylated. *E. coli dam* mutants fail to methylate the adenine of this sequence (7).

Neisseria gonorrhoeae has been shown to produce the restriction endonucleases *Ngo*I and *Ngo*II (5, 28). These were found to be isoschizomers of the *Haemophilus* endonucleases *Hae*II and *Hae*III, respectively (4). It is therefore necessary that gonococcal strains producing these enzymes should also methylate the corresponding recognition sequence.

About 83% of all gonococcal isolates harbor a 2.6×10^6-dalton (4.2-kilobase [kb]) plasmid, which has no known function (10). A detailed physical map of this plasmid was recently presented (6). It has been suggested that this small plasmid might code for *Ngo*I or *Ngo*II or both (4). A minority of *N. gonorrhoeae* isolates contain a large 24.5×10^6-dalton plasmid (39.5 kb) (10). Its presence is required in a donor cell for the conjugative transfer of a 4.4×10^6-dalton (7.2-kb) resistance plasmid (9, 27, 30). The cleavage sites on the resistance plasmid for the restriction endonucleases *Alu*I, *Bam*HI, *Hin*cII, and *Hpa*II were recently reported (31). The 7.2-kb plasmid carries approximately 40% of the transposon Tn*3* (11), which codes for a TEM-1 β-lactamase. The DNA sequence of Tn*3* and the exact location of its three genes (*tnpA*, *tnpR*, and *bla*) are known (16). By identifying the part of Tn*3* present on the 7.2-kb gonococcal plasmid it should be possible to correlate the cleavage patterns obtained with a variety of restriction endonucleases to a known DNA sequence.

In this paper we demonstrate that gonococcal

DNA is modified in the recognition sequence for *Hae*II (*Ngo*I), *Hae*III (*Ngo*II), *Sac*II, and *Bam*HI. We suggest that resistance to these restriction endonucleases is due to host restriction modification in *N. gonorrhoeae*. The identification of a new gonococcal restriction endonuclease (*Ngo*III) with an activity similar to that of *Sac*II supports this hypothesis. In addition, we demonstrate that modification of the sequence -GATC- occurs in some but not all gonococcal strains. This modification may be due to methylation of the adenine residue.

MATERIALS AND METHODS

Bacterial strains. The *N. gonorrhoeae* strains used in this study are listed in Table 1. The strains were maintained on a daily basis by restreaking single colonies on GC plates (22) or were stored at −80°C in 20% (vol/vol) glycerol in the same medium. Only nonpiliated variants were used in this study.

Media and growth conditions. The solid medium used for *N. gonorrhoeae* was GC medium base (Difco) supplemented with 1% (vol/vol) Kellogg supplement (22). The penicillin-resistant strain was maintained on GC plates containing 1 μg of benzylpenicillin per ml. Plates were incubated at 37°C in 6% CO_2. The liquid medium was identical in composition to the solid, except that agar was omitted and 10 mM $NaHCO_3$ was added. The medium used for growth of *E. coli* was LB (1) supplemented with medium E (34) and 0.2% glucose. Growth was followed in a Klett-Summerson photoelectric colorimeter (red filter).

DNA preparations. Plasmids were prepared from cesium chloride-ethidium bromide density gradients as described earlier (6). The chromosomal DNA band was collected from the same gradients. The *E. coli* chromosomal DNA was prepared as described elsewhere (8).

Analysis of DNA. The restriction endonuclease digestions were done in 10 mM Tris (pH 7.5)–6 mM $MgCl_2$–1 mM dithiothreitol–100 μg of bovine serum albumin per ml (buffer I), or buffer I with 50 mM NaCl added (buffer II). Buffer I was used for digests with *Bgl*II, *Bst*N1, *Fnu*DII, *Hae*II, *Hae*III, *Hpa*I, *Hpa*II, *Msp*I, *Sac*I, *Sac*II, *Sma*I (+ 5 mM Tris base), *Taq*I, and *Pvu*II. Buffer II was used for *Alu*I, *Ava*I, *Ava*II, *Bam*HI (+ 100 mM NaCl), *Bcl*I, *Hin*cII (+ 5 mM Tris base), *Hin*dIII, *Hin*fI, *Hha*I (+ 100 mM Tris, pH 7.5), *Mbo*I, *Sal*I (+ 100 mM NaCl + 5 mM Tris base), *Sau*3A, and *Xho*I (+ 100 mM NaCl). Two units of the enzyme was used for each microgram of DNA. The reactions were carried out for 3 h at 37°C for all enzymes except *Pst*I (30°C), *Bcl*I (50°C), *Bst*NI, and *Taq*I (60°C) and were terminated by the addition of 0.2 volumes of a loading buffer containing 0.25% (wt/vol) orange G, 0.25% (wt/vol) bromophenol blue, and 0.25% (wt/vol) xylenecyanol in 25 mM EDTA and 25% (wt/vol) Ficoll.

Analysis of digested DNA was performed by electrophoresis in vertical 0.7% agarose slab gels (20 by 20 by 0.4 cm) with buffer E (20 mM sodium acetate–2 mM EDTA in 33 mM Tris-hydroxyacetate, pH 7.8). Gels were run for 2 h at 200 V, stained in an ethidium bromide solution (5 μg/ml), and photographed under short-wave UV light. The molecular weight standard used was prepared as described earlier (6).

High-pressure liquid chromatography analysis. DNA was precipitated with 99% ethanol, washed with 77% ethanol and ether, and hydrolyzed in 88% formic acid at 180°C for 30 min. The hydrolysate was evaporated to dryness and dissolved in 4% formic acid. The analysis was performed essentially as described by others (12), using a Micro-Bondapak C18 column (4 mm by 6 mm), and absorbance detector model 440 (Waters Associates Inc.), and a 3380 integrator (Hewlett-Packard). The temperature was kept at 35.5°C. For elution a step gradient, with 6 and 15% methanol in 0.01 M $NH_4H_2PO_4$, pH 5.1, was used. The flow rate was 0.8 ml/min. The bases were identified by their retention times and their ratio of absorbancy at 254 nm to that at 280 nm.

Preparation and assay of restriction endonuclease extracts. The various strains were grown in GC medium to a density of about 3×10^8 cells per ml.

TABLE 1. *Bacterial strains*

Strain	Plasmid content (kb)	Comments	Source
N. gonorrhoeae 82409/55	4.2 (pJD1)		A. Reyn, Copenhagen
N. gonorrhoeae Um06	4.2	Spontaneous fast-growing derivative from 82409/55	This laboratory
N. gonorrhoeae KH7764-45	39.5		L. Mayer
N. gonorrhoeae KH4318			S. Falkow
N. gonorrhoeae Um07	7.2 (pMR0360)	Constructed by conjugation[a]	This laboratory
N. gonorrhoeae CDC67	4.2, 7.2, 39.5		S. Falkow (27)
N. gonorrhoeae Um08	4.2, 39.5	Spontaneous revertant from CDC67	This laboratory
E. coli K-12 LA51			(24)
E. coli SN01	7.2 (pMR0360)	Constructed by conjugation[b]	This laboratory (24)
E. coli SN01	18.5 (pNU1)		This laboratory (8)

[a] Strain Um07 was constructed by using the procedure described elsewhere (23) in a conjugation between CDC67 and KH4318.

[b] The *E. coli* strain SN01 obtained the 7.2-kb plasmid pMR0360 from strain CDC67 by conjugation (23).

Cells were harvested by centrifugation (13,000 × *g* for 15 min) and washed once with 100 ml of 10 mM Tris-hydrochloride (pH 7.5). The pellet was suspended in 25 ml of a buffer containing 20 mM Tris (pH 7.5), 10 mM $MgCl_2$, and 7 mM 2-mercaptoethanol, and the preparation of restriction endonuclease extracts was performed as described by Bickle et al. (2). Their method B was chosen for the polyethyleneimine treatment. We used heparin-Sepharose CL-6B from Pharmacia Fine Chemicals in the column. The presence of restriction endonuclease activity was assayed by using the *E. coli* plasmid pNU1 (8). The reaction mixture contained 1 μg of DNA in a mixture of 60 mM Tris (pH 7.4), 50 mM NaCl, 60 mM $MgCl_2$, 10 mM dithiothreitol, 2 μg of bovine serum albumin, and 5 μl of sample in a final volume of 20 μl. After 1 h at 37°C the reaction was terminated by the addition of 0.2 volume of the loading buffer, and the samples were analyzed on a 0.7% agarose gel. Fractions containing endonuclease activities were pooled and stored at −20°C in the presence of 50% (vol/vol) glycerol.

Chemicals and enzymes. Restriction endonucleases were obtained from either Bethesda Research Laboratories, Inc., Rockville, Md., or New England Biolabs, Beverly, Mass. Agarose was from Bio-Rad, Richmond, Calif. Nucleotides were from Sigma Chemicals, St. Louis, Mo. Benzylpenicillin was kindly provided by Astra AB, Södertäje, Sweden.

RESULTS

Gonococcal DNA is resistant to cleavage by restriction endonucleases *Hae*II, *Hae*III, *Sac*II, and *Bam*HI. The restriction endonucleases *Hae*II and *Hae*III (isochizomers of *Ngo*I and *Ngo*II, respectively) failed to cleave the 4.2-kb gonococcal plasmid prepared from strain 82409/55 (Table 2). The *E. coli* plasmid pBR322 has approximately the same size and is known from sequence data to contain 11 *Hae*II and 22 *Hae*III sites (32). Most of these sites on pBR322 have also been identified by their susceptibility to cleavage (3). Chromosomal DNA prepared from strain 82409/55 was also resistant to cleavage by these two enzymes (Table 2). To identify possible resistance to other restriction endonucleases, chromosomal gonococcal DNA was digested with 26 restriction enzymes. It was found that chromosomal DNA of strain 82409/55 was poorly cleaved by *Sac*II and *Bam*HI (Table 2). Chromosomal DNA from *E. coli* K-12 was cleaved by *Hae*II, *Hae*III, *Sac*II, and *Bam*HI (Table 2) into fragment sizes expected from the sizes of their respective recognition sequences. Since *Sac*II and *Bam*HI both recognize a 6-base pairs (bp) sequence, cleavage by these enzymes results in a bulk of large fragments.

The 7.2-kb β-lactamase plasmid pMR0360, when prepared from *E. coli*, exhibits one site for *Hae*II, five sites for *Hae*III (only three bands are seen in Fig. 1), and two *Bam*HI sites (Fig. 1) (L. Mayer, personal communication). *Sac*II did not cleave pMR0360. When the same plasmid was isolated from *N. gonorrhoeae* Um07, it was totally resistant to cleavage by *Hae*II, *Hae*III, and *Bam*HI (Fig. 1A). Sequencing data have revealed one *Sac*II restriction site on the 4.2-kb gonococcal plasmid, which was not cleaved by *Sac*II (C. Korch, personal communication).

Since Tn*3* has been sequenced it was possible to elucidate which segment of this transposon is present on pMR0360. The plasmid was cleaved by the restriction endonucleases *Pst*I, *Hin*cII, *Hin*fI, *Taq*I, and *Bcl*I. The sizes of the fragments from single and double digests are listed in Table 3, and the deduced map is given in Fig. 2. By comparing the location of restriction endonuclease sites on pMR0360 with those obtained from the sequence of Tn*3* (16), we estimated that at least 28% of Tn*3* was present on pMR0360 (from the *Taq*I site at 3,400 bp to the end of the *bla* gene). Besides the *bla* gene, part of the *tnpR* gene is also present (Fig. 3). Sequence data reveal the presence of seven *Hae*III sites and one *Bam*HI site on this segment of DNA (16). Some of the *Hae*III sites are very close to each other. This explains why only five sites have been identified by us as well as by others (L. Mayer, personal communication). As neither the *Hae*III sites nor the *Bam*HI site were susceptible to cleavage, we conclude that they are pro-

TABLE 2. *Sensitivity of different DNAs to cleavage by HaeII, HaeIII, SacII, and BamHI*

Restriction enzyme	Recognition sequence	Cleavage sites[a] of different DNAs for:					
		pJD1 in *N. gonorrhoeae*	pBR322 in *E. coli*[b]	pMR0360 in *N. gonorrhoeae*	pMR0360 in *E. coli*	Gonococcal chromosomal DNA[c]	*E. coli* chromosal DNA
*Hae*II	PuGCGC↓Py	No cleavage	11	No cleavage	1	Poor cleavage	Cleavage
*Hae*III	GG↓CC	No cleavage	22	No cleavage	5	No cleavage	Cleavage
*Sac*II	CCGC↓GG	No cleavage	No cleavage	No cleavage	No cleavage	Poor cleavage	Cleavage
*Bam*HI	G↓GATCC	No cleavage	1	No cleavage	2	Poor cleavage	Cleavage

[a] Number of restriction endonuclease fragments.

[b] Data taken from Sutcliffe (32).

[c] This DNA, which was isolated from strain Um06, was cleaved by *Alu*I, *Ava*I, *Ava*II, *Bcl*I, *Bgl*II, *Bst*NI, *Eco*RI, *Fnu*DII, *Hha*I, *Hin*cII, *Hin*dIII, *Hin*fI, *Hpa*I, *Hpa*II, *Mbo*I, *Mps*I, *Pst*I, *Pvu*II, *Sac*I, *Sal*I, *Sau*3A, *Sma*I, *Taq*I, and *Xho*I.

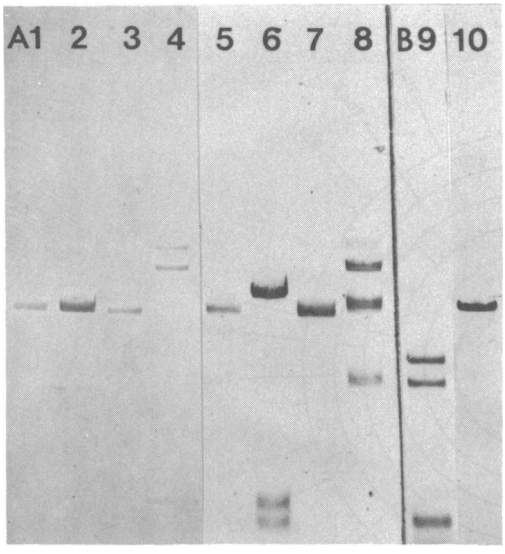

FIG. 1. *Susceptibility of plasmid pMR0360 to cleavage by HaeII, HaeIII, BamHI (A), and BclI (B). Lanes 1 and 2 show undigested DNA of plasmid pMR0360 isolated from N. gonorrhoeae 82409/55 and E. coli SN01, respectively. pMR0360 in N. gonorrhoeae and E. coli was digested by HaeII (lane 3 and 4), HaeIII (lane 5 and 6), BamHI (lane 7 and 8), and BclI (lane 9 and 10).*

tected by modification in *N. gonorrhoeae*. The single *Hae*II site identified by us and by L. Mayer (personal communication) was not present within the Tn*3* portion of pMR0360, but, as mentioned above, was resistant to cleavage when pMR0360 was isolated from *N. gonorrhoeae*.

Study of modification of the sequence -GATC- in *N. gonorrhoeae*. The restriction endonuclease *Sau*3A is known to cleave at the sequence -GATC- irrespective of adenine methylation. The isoschizomer *Mbo*I, on the other hand, is unable to cleave this sequence if the adenine is methylated (7, 13). Chromosomal DNA prepared from a *dam*$^+$ *E. coli* strain is cleaved by *Sau*3A but not by *Mbo*I (26). In contrast, chromosomal DNA prepared from *N. gonorrhoeae* Um06 was susceptible to cleavage by both enzymes (Fig. 4). Strain Um06 is a fast-growing spontaneous derivative of strain 82409/55. Chromosomal DNA prepared from the parental strain was found to be resistant to cleavage by *Mbo*I but sensitive to *Sau*3A (Fig. 4). Thus, strain Um06 is apparently a modification-deficient derivative of strain 82409/55. Strain Um07 is not related to strain 82409/55. Chromosomal DNA as well as pMR0360 DNA prepared from this strain was sensitive to cleavage by *Mbo*I. In contrast, another wild-type strain, KH7764-45, was not cleaved by *Mbo*I (data not shown).

The physical map of pMR0360 when isolated from *N. gonorrhoeae* Um07 (Fig. 2) reveals the presence of three *Bcl*I recognition sites. No cleavage was observed with this enzyme when pMR0360 DNA was prepared from *E. coli* (Fig. 1B). *Bcl*I recognizes the sequence -TGATCA- (28). This sequence is known to be modified in *E. coli* (*dam*$^+$) due to methylation of adenine. It seems therefore likely that the *Bcl*I sites of pMR0360 are methylated when it is harbored in *E. coli*, but not so when present in *N. gonorrhoeae* Um07.

The base composition of hydrolyzed chromosomal DNA from *N. gonorrhoeae* strains 82409/55 and Um06 was analyzed after high-pressure liquid chromatography of hydrolyzed DNA. Peaks corresponding to 5-methylcytosine were present in DNA prepared from these gonococcal strains. The resolution was, however, not high enough to determine whether or not 6-methyladenine was present in DNA from these two strains (data not shown).

Susceptibility to cleavage of DNA prepared from strains lacking the 4.2-kb plasmid. Strain 82409/55 contains an indigenous 4.2-kb plasmid. Since this plasmid has been proposed to carry information for restriction endo-

TABLE 3. *Restriction endonuclease fragments of pMR0360*

Enzyme	Fragments[a] (bp)	Sum of fragments (bp)
*Bcl*I	4,200; 2,400; 660	7,260
*Hin*fI	3,000; 1,950; 1,200; 1,100	7,250
*Hin*cII	7,200	7,200
*Pst*I	7,200	7,200
*Taq*I	1,600; 1,300; 1,160; 1,050; 670; 510; 350; 325; 270	7,235
*Bcl*I/*Hin*fI	2,700; 1,950; 1,100; 660; 425; 325	7,160
*Bcl*I/*Hin*cII	3,250; 2,400; 950; 660	7,260
*Bcl*I/*Pst*I	2,950; 2,400; 1,250; 660	7,260
*Pst*I/*Hin*fI	3,000; 1,950; 1,200; 880; 260	7,290
*Taq*I/*Bcl*I	1,600; 1,300; 1,160; 670; 660; 510; 325; 290; 270; 200	6,985
*Taq*I/*Hin*fI	1,600; 1,080; 870; 640; 530; 510; 460; 350; 325; 270; 225; 210	7,070
*Taq*I/*Pst*I	1,600; 1,300; 1,160; 780; 670; 510; 380; 350; 325; 270	7,345

[a] The average of several experiments. The size of fragments larger than 1,000 bp was determined from 0.7% agarose gels, whereas fragments of smaller size were calculated from 2% agarose gels. Fragments less than 200 bp were not detected on these gels.

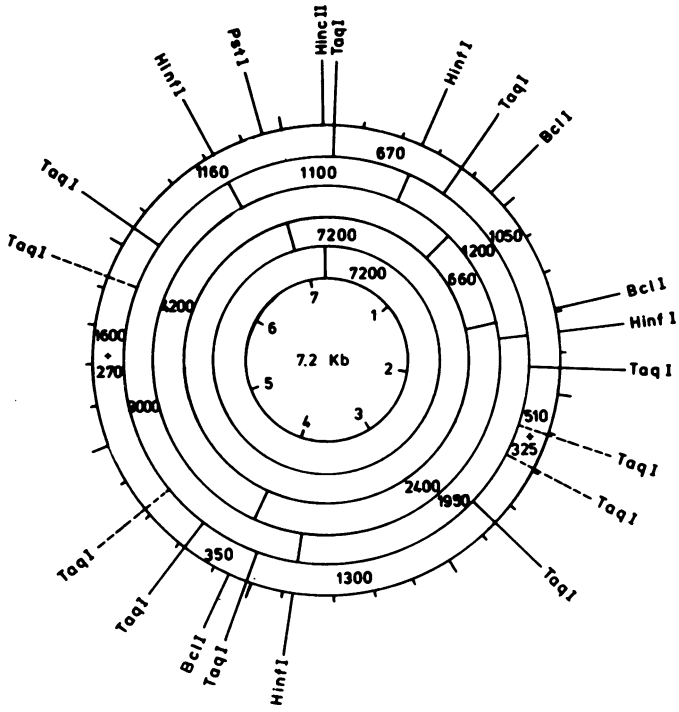

FIG. 2. *Physical map of the 7.2-kb β-lactamase plasmid pMR0360 isolated from strain Um07. This map was constructed from the data given in Table 3. Neither the 270-bp and 1,600-bp TaqI fragments nor the 325-bp and 510-bp TaqI fragments could be orientated with respect to each other. The dotted lines mark the alternative positions for these TaqI sites.*

nucleases (4), we wanted to determine whether or not the presence of this plasmid affected the modification of *Hae*II, *Hae*III, *Bam*HI, and *Sac*II recognition sites. For that purpose chromosomal DNA from the plasmid-free strain KH4318 was digested with the four enzymes. No or very poor cleavage was observed. Chromosomal DNA from strain KH7764-45, which carries only the 39.5-kb plasmid, also exhibited this resistance to cleavage by these four restriction endonucleases (data not shown). This suggests that the corresponding modification system is chromosomally encoded in *N. gonorrhoeae*.

Restriction endonuclease production: endo R·*Ngo*III, a new restriction enzyme in *N. gonorrhoeae*. We were unable to detect any restriction endonuclease activity from the plasmid-free strain KH4318 or from the strain 82409/55 which carries the indigenous 4.2-kb plasmid. An endonuclease extract from strain KH7764-45, which harbors the 39.5-kb plasmid, produced a distinct fragment pattern from plasmid pNU1 (Fig. 5, lane 7). This pattern was clearly different from the digestion pattern produced by an extract from strain Um08, which contained *Ngo*II activity. The enzymatic activity found in strain KH7764-45 seems to be similar to that of *Sac*II (Fig. 5, lane 5). A mixture of *Sac*II and endonuclease extract from KH7764-45 gave the same banding pattern as that produced by *Sac*II alone. The activity present in KH7764-45 has been denoted endo R·*Ngo*III (29). Neither *Ngo*II nor *Ngo*III extracts cleaved chromosomal DNA from the gonococcal strains 82409/55, KH7764-45, and Um08.

DISCUSSION

N. gonorrhoeae DNA is protected against cleavage by *Hae*II and *Hae*III. These enzymes are isoschizomers of the *N. gonorrhoeae* endonucleases *Ngo*I and *Ngo*II. Resistance to cleavage by these enzymes seems to be the result of modification of bases in their respective recognition sequence. Gonococcal DNA from different strains was also found to be poorly cleaved by *Sac*II. One of these strains, KH7764-45, was found to produce a restriction endonuclease different from *Ngo*I and *Ngo*II. This new restriction endonuclease in *N. gonorrhoeae* is hereby designated endo R·NgoIII. These studies indicate that *Ngo*III recognizes the same palindromic sequence as *Sac*II (-CCGCGG-), a restriction endonuclease isolated from *Streptomyces achromogenes* (28). It seems therefore

probable that resistance to *Sac*II (and *Ngo*III) is also due to modification of the relevant recognition sequence. Modification of *Ngo*I, *Ngo*II, and *Ngo*III recognition sites apparently also occurs in gonococcal strains that do not produce detectable amounts of one or more of these three restriction endonucleases. It is thought that the poor cleavage of gonococcal DNA from *Bam*HI also reflects modification. It is therefore possible that isoschizomers to *Bam*HI may be present in some gonococcal strains.

It is known that methylation of the internal cytosine in -GGCC- protects against cleavage by *Hae*III (20). Recently, Korch et al. (17) found direct evidence for the presence of a methylated cytosine in the third position of the *Hae*III sequence by the reaction of this nucleotide during sequencing of gonococcal DNA by the Maxam-Gilbert procedure. Resistance of gonococcal DNA to *Hae*III is therefore most likely due to such a cytosine modification. No corresponding methyltransferase has been reported for *Hae*II or *Sac*II (29). These enzymes recognize the sequences -PuGCGCPy- and -CCGCGG-, respectively. Modification of one of the cytosine residues in these sequences might render the gonococcal DNA resistant to cleavage. *Bam*HI recognizes the sequence -GGATCC-. It is known that methylation of the cytosine in the fifth position protects the sequence from cleavage by this enzyme (15). We therefore think that in *N. gonorrhoeae* there is also a cytosine modification of *Bam*HI recognition sequences.

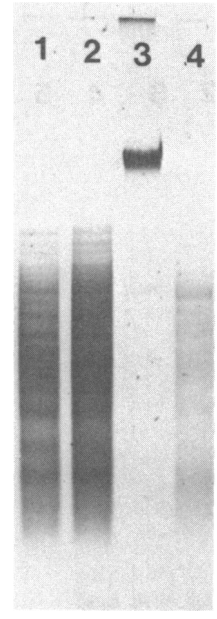

FIG. 4. *Agarose gel with MboI (lanes 1 and 3) and Sau3A (lanes 2 and 4) digests of chromosomal DNA from N. gonorrhoeae strains Um06 (lanes 1 and 2) and 82409/55 (lanes 3 and 4).*

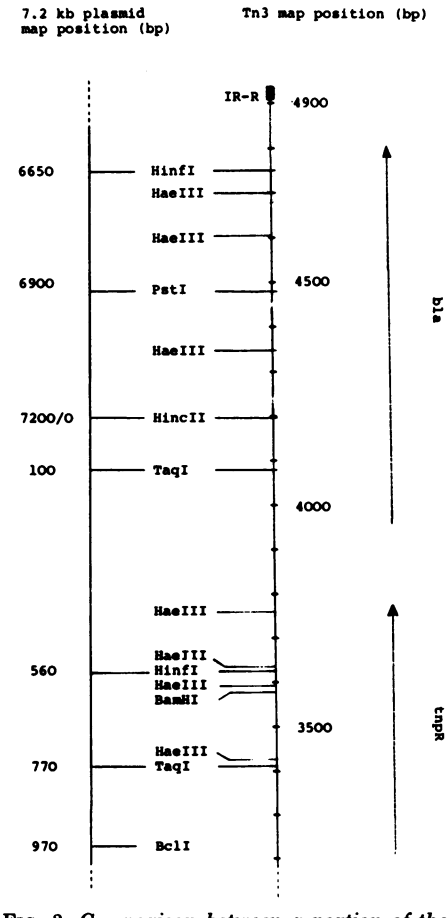

FIG. 3. *Comparison between a portion of the 7.2-kb plasmid pMR0360 of N. gonorrhoeae and the bla region of Tn3, as deduced from the reported DNA sequence (16).*

It is most likely that the modified cytosine residues in the recognition sequences for *Hae*II (*Ngo*I), *Hae*III (*Ngo*II), *Sac*II (*Ngo*III), and *Bam*HI are 5-methylcytosines, as this modified base was found to be present in gonococcal DNA. DNA from the plasmid-free strain showed the same resistance towards *Hae*II, *Hae*III, and *Sac*II as DNA from strains carrying either the 4.2-kb or the 39.5-kb plasmid. This implies that the methylating system(s) is chromosomally encoded in *N. gonorrhoeae*. Apparently, this modification may operate in host restriction modification since at least some gonococcal strains produce corresponding restriction endonucleases.

It has been suggested that *Ngo*I and *Ngo*II

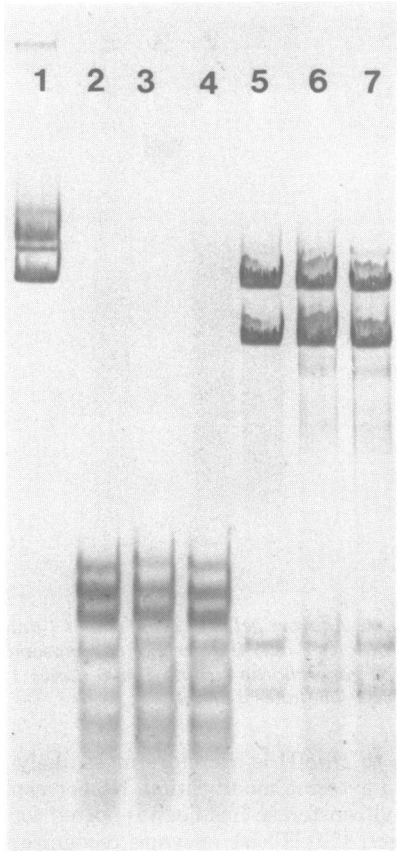

FIG. 5. *Cleavage of pNU1 with the restriction endonuclease activities isolated from strains KH7764-45 and Um08. Lane 1, Undigested pNU1. Lanes 2 to 7, pNU1 cleaved by: the Um08 extract (lane 2), the Um08 extract plus HaeIII (lane 3), HaeIII (lane 4), SacII (lane 5), the KH7764-45 extract plus SacII (lane 6), and the KH7764-45 extract (lane 7).*

might be plasmid encoded (4). As the single plasmid-free strain studied lacked detectable endonuclease activity, this hypothesis cannot be excluded. Strains carrying either or both of the two above gonococcal plasmids differ in their production of endonuclease activity. Thus, there exists no apparent correlation between plasmid content and endonuclease production.

DNA from gonococcal strain KH4318 and Um06 was sensitive to *Mbo*I, which recognizes the sequence -GATC-. Strain Um06 was derived from strain 82409/55. The latter strain was resistant to this restriction endonuclease. These strains, which varied in sensitivity to *Mbo*I, were all resistant to *Bam*HI. This argues that resistance to *Mbo*I is not caused by modification of the cytosine residue in the sequence -GATC- but by modification of the adenine residue, as is the case in *E. coli* (7). The methylation of adenine residues in *E. coli* K-12 occurs primarily in the sequence -GATC- and is carried out by a methylase coded for by the chromosomal gene *dam* (21). It therefore appears that a *dam*-like function may exist in *N. gonorrhoeae*. The presence of such a modification function in gonococci has recently also been reported by others (35). This putative adenine methyltransferase does not seem to be plasmid encoded in *N. gonorrhoeae*, as no correlation between plasmid content and adenine methylation at the sequence -GATC- has been observed.

E. coli dam mutants have been shown to be spontaneous mutators, due to a defective mismatch repair function (25). Thus, methylation of -GATC- enables the mismatch repair system to discriminate between old and newly replicated DNA strands. The gonococcal strains lacking modification at the sequence -GATC- might therefore be deficient in this DNA repair function. However, preliminary data show no difference in reversion frequencies for auxotrophic markers between modifying and nonmodifying strains. It is nevertheless tempting to suggest that variations in this modification may have implications for physiology and pathogenicity of *N. gonorrhoeae*.

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