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Methylated DNA in *Borrelia* Species

CARRIE A. NORTON HUGHES* AND RUSSELL C. JOHNSON

Department of Microbiology, School of Medicine, University of Minnesota, Minneapolis, Minnesota 55455

Received 16 April 1990/Accepted 27 August 1990

The DNA of *Borrelia* species was examined for the presence of methylated GATC sequences. The relapsing-fever *Borrelia* sp., *B. coriaceae*, and only 3 of 22 strains of *B. burgdorferi* contained adenine methylation systems. *B. anserina* lacked an adenine methylation system. Fundamental differences in DNA methylation exist among members of the genus *Borrelia*.

DNA methylation occurs in some eucaryotic and procaryotic organisms. Generally, a type 1 restriction-modification system contains a restriction enzyme that cleaves a specific DNA sequence and a methylase that modifies the same sequence by transferring methyl groups from *S*-adenosylmethionine to either adenine (N-6 position) or cytidine (C-5 position). The restriction-modification system enables the cell to selectively destroy unmethylated foreign DNA. DNA methylation may also play a role in DNA replication and recombination, gene expression, and development (17).

Among the bacteria, DNA methylation has been found to occur in some gram-negative and gram-positive organisms. In *Escherichia coli*, all GATC sequences in the DNA are methylated at position 6 of the adenine residue (*dam* methylation) (17). One hypothesis for the role of *dam* methylation is that the DNA methylation allows mismatch repair enzymes to discriminate between old and new DNA strands at the replication fork. This hypothesis is supported by the preferential removal of mismatched bases from the unmethylated strand of the heteroduplex DNA (13, 16). The DNA of *E. coli* also contains minor amounts of 5-methylcytosine (*dcm* methylation). Complete Dam^+ phenotypes have been found in bacteria of nine genera related to the families *Enterobacteriaceae*, *Parvobacteriaceae*, and *Vibrionaceae* and in cyanobacteria (1). Certain archaebacteria also have Dam methylation (12). In contrast, three genera of archaebacteria and nine other genera of eubacteria have tested Dam^- (1). Although methylated adenine and cytosine residues have been detected in the DNA of *Bacillus subtilis* (4) and *Staphylococcus aureus* (19), the DNA of these gram-positive organisms is not methylated by *dam*- and *dcm*-like enzymes (5). Another gram-positive organism, *Streptococcus pneumoniae*, contains *dam* methylation (11). Dam^- bacteria appear to be more common than Dam^+ bacteria.

Spirochetes have not been thoroughly examined for the presence of methylated DNA. The presence of an adenine methylation system in *Borrelia hermsii*, a relapsing-fever *Borrelia* species, has been suggested (15). The possibility of DNA modifications in *B. burgdorferi* tick isolate CT-1 has also been reported (9). We have explored this possibility by examining members of the genus *Borrelia* for the presence of methylated DNA sequences.

The sources of organisms used in this study, isolated from humans, animals, and ticks from North America, Africa, Asia, and Europe, are shown in Table 1. The spirochetes were cultured in Barbour-Stoenner-Kelly medium (2) at 30°C.

Total (genomic) DNA was isolated by a modification of the procedures of Hansen et al. (7) and Hayes et al. (8). Restriction endonuclease digestion was performed according to the manufacturer's specifications (Boehringer Mannheim, Indianapolis, Ind.). Following digestion with selected restriction endonucleases (*Dpn*I, *Sau*3AI, and *Mbo*I), the DNA fragments were separated by electrophoresis in 1.2% agarose gels (10 by 14 cm) at 30 V for 12.5 h. Lambda DNA digested with *Hin*dIII served as size markers. The gels were stained with ethidium bromide, illuminated with UV light, and photographed.

The DNA of *B. anserina*, the type species of the genus, was examined for the presence of methylated adenine residues at the DNA sequences of GATC (Dam phenotype). The DNA was not digested with *Dpn*I, which recognizes GATC sequences methylated on both strands of DNA, suggesting that this organism lacks an adenine methylation system (Fig. 1). This observation was verified by the digestion of the DNA with *Mbo*I, which is active on unmethylated GATC sequences (Fig. 1).

In contrast, the relapsing-fever borreliae *B. hermsii* (Fig. 1), *B. duttonii*, *B. turicatae*, and *B. parkeri*, were found to possess adenine methylation systems (Table 2). The DNA of these borreliae was digested by *Dpn*I but was resistant to the action of *Mbo*I. In addition to the relapsing-fever borreliae, *B. coriaceae*, the putative agent of epizootic bovine abortion, also contained methylated DNA (Table 2).

The etiological agent of Lyme disease, *B. burgdorferi*, was examined for the presence of methylated DNA. Heterogeneity in adenine methylation was observed among the 22 strains of this spirochete that were assayed. The DNA of the type strain *B. burgdorferi* B31 and 18 additional strains had the same pattern of sensitivity to *Dpn*I and *Mbo*I as the type species, *B. anserina*. These results indicate the absence of methylated adenine residues (Table 2). Virulent and avirulent variants of strain 297 exhibited the same methylation patterns. Three strains of *B. burgdorferi*, P/Gau, MMI, and IPF, were sensitive to *Dpn*I digestion, suggesting the presence of methylated adenine residues at the GATC sequences. Variation in sensitivity to the action of *Dpn*I was observed among the three strains. Some intact, unsheared high-molecular-weight DNA (chromosomal and plasmid) from strains MMI (Fig. 1) and IPF (not shown) was not digested by *Dpn*I. Increasing the enzyme concentration from 3 to 6 U did not significantly alter this pattern (data not shown). As shown in Fig. 1, it appears that strain MMI contained a plasmid (approx. 5 kb) that was not digested by *Dpn*I but was digested by *Mbo*I, suggesting that this plasmid is not methylated. In contrast, intact, unsheared high-molec-

* Corresponding author.

TABLE 1. Strains used in this study

Borrelia strain or isolate	Source	Location
B. coriaceae Co53 (ATCC 43381)	*Ornithodoros coriaceus*	California
B. duttonii	*Ornithodoros moubata*	Africa
B. hermsii M1001 (HSI, ATCC 35209)	*Ornithodoros hermsi*	Washington
B. turicatae M2007	*Ornithodoros turicata*	Western United States
B. anserina Es	Poultry	California
B. parkeri M3001	*Ornithodoros parkeri*	Western United States
B. burgdorferi		
B31 (IDS, ATCC 35210)	*Ixodes dammini*	New York
Virulent 297 (hamster passaged)	Human spinal fluid	Connecticut
Avirulent 297	Human spinal fluid	Connecticut
P/Gau	Human skin	Federal Republic of Germany
P/Bi	Human cerebrospinal fluid	Federal Republic of Germany
P/STO	Human skin	Federal Republic of Germany
TXGW	Human	Texas
NCH-1	Human skin	Wisconsin
$20047P_2$	*Ixodes ricinus*	France
MMTI	*Ixodes dammini*	Minnesota
MMI	*Peromyscus leucopus*	Minnesota
K48	*Ixodes ricinus*	Czechoslovakia
G25	*Ixodes ricinus*	Sweden
CRT	*Ixodes dammini*	Minnesota
IPS	*Ixodes pacificus*	California
ALA-4-10-88	*Ixodes pacificus*	California
HUM-7-8-14	*Ixodes pacificus*	California
SM-1-6-88	*Ixodes pacificus*	California
NEV-5-4-88	*Ixodes pacificus*	California
SON-3-1-89	*Ixodes pacificus*	California
BUCO-2-10-89	*Ixodes pacificus*	California
IPF	*Ixodes persulcatus*	Japan

ular-weight DNA from strain P/Gau was digested by *Dpn*I (data not shown).

The DNA from all of the borreliae examined was digested by the restriction endonuclease *Sau*3AI, which recognizes GATC sequences regardless of the methylation state.

DNA methylation has been studied in *E. coli* (14) and other bacteria (1, 5, 6). However, members of the *Spirochaetaceae* have not been included in these surveys. In this study, members of the genus *Borrelia* were surveyed for the presence of methylated adenine residues. Our results reveal that heterogeneity in DNA methylation exists among the *Borrelia* species. The adenine methylation observed in species of *Borrelia* appears to be similar to that found in other gram-negative bacteria in that the DNA of the borreliae was digested by restriction enzymes specific for GATC sequences (Dam phenotype).

All of the relapsing-fever borreliae examined in this study were found to possess methylated DNA (Dam^+). However, only a limited number of these borreliae are available for study. The possibility remains that some relapsing-fever borreliae are Dam^-.

It is known that GATC and hexanucleotide sequences containing GATC are among the most frequently recognized restriction endonuclease target sequences (18). The restriction endonucleases that recognize these sequences are primarily present in bacteria with the Dam^- phenotype whose GATC sequences are methylated on the cytosine residue. Therefore, *dam* methylation may protect GATC-rich regions in the DNA of Dam^+ species against nucleases.

If dam methylation in *Borrelia* spp. signals which DNA strand should be corrected in mismatch repair, either Dam^- bacteria cannot perform mismatch repair, or they use some other method to guide strand selection. The latter possibility seems more likely, since gram-negative eubacteria (with the exception of cyanobacteria and bacteria of *E. coli* lineage)

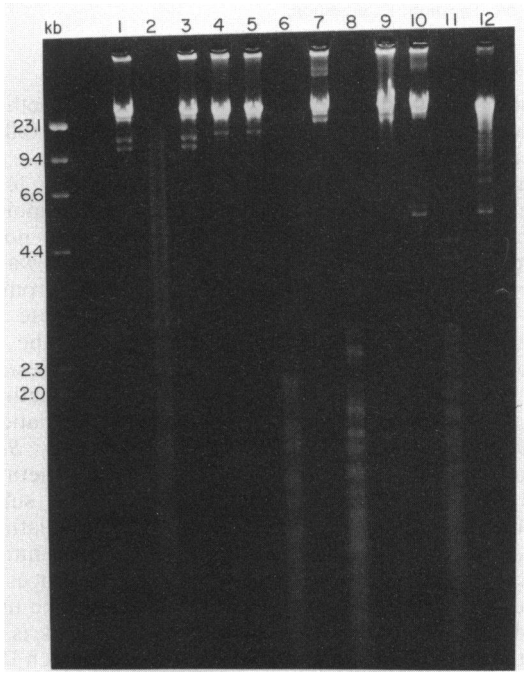

FIG. 1. Restriction endonuclease digests of *Borrelia* species DNA. Lanes: *B. anserina* DNA, undigested (lane 1) and digested with *Mbo*I (lane 2) and *Dpn*I (lane 3); *B. hermsii* DNA, undigested (lane 4) and digested with *Mbo*I (lane 5) and *Dpn*I (lane 6); *B. burgdorferi* B31, undigested (lane 7) and digested with *Mbo*I (lane 8) and *Dpn*I (lane 9); *B. burgdorferi* MMI, undigested (lane 10) and digested with *Mbo*I (lane 11) and *Dpn*I (lane 12). Digests and gel were run as described in the text with 3.5 U of *Mbo*I and 3.0 U of *Dpn*I.

TABLE 2. Digestion of genomic DNA from various *Borrelia* species by *Mbo*I, *Sau*3AI, and *Dpn*I

Strain	Genomic DNA digestion[a]		
	*Mbo*I	*Sau*3AI	*Dpn*I
B. anserina	+	+	−
B. coriaceae	−	+	+
B. duttonii	−	+	+
B. hermsii	−	+	+
B. turicatae	−	+	+
B. parkeri	−	+	+
B. burgdorferi			
ATCC 35210	+	+	−
Virulent 297	+	+	−
Avirulent 297	+	+	−
P/Gau	+	+	+
P/Bi	+	+	−
P/STO	+	+	−
TXGW	+	+	−
NCH-1	+	+	−
$20047P_2$	+	+	−
MMTI	+	+	−
MMI	+	+	+
K48	+	+	−
G25	+	+	−
CRT	+	+	−
IPS	+	+	−
ALA-4-10-88	+	+	−
HUM-7-8-14	+	+	−
SM-1-6-88	+	+	−
NEV-5-4-88	+	+	−
SON-3-1-89	+	+	−
BUCO-2-10-89	+	+	−
IPF	+	+	+

[a] −, No digestion; +, digestion.

and some archaebacteria are Dam^- (1). GATC methylation may also be involved in gene regulation, or it may reflect the presence of a dispensable plasmid or transposon.

Three of the 22 strains of *B. burgdorferi* were found to possess methylated adenine residues. High-molecular-weight DNA from strains MMI and IPF was not fully digested by *Dpn*I, while DNA from strain P/Gau was more susceptible to complete digestion. The DNA from these three strains was also digested by *Mbo*I. These results suggest that in these three strains, either all the GATC sequences are methylated but not at the adenine residue, or some but not all of the GATC sequences are methylated. *Sau*3AI digestion is inhibited by cytosine methylation, but the *B. burgdorferi* DNA was still restricted by *Sau*3AI, indicating that these residues are probably not methylated. More likely, the partial methylation involves a subset of GATC sequences that would correspond to methylation only at GATC sequences within a particular recognition sequence, such as GGATCC (10). The latter sequences would be methylated but the rest of the GATC would be unmethylated. Because the frequency of tetramer sites is higher than that of hexamer sites, *Dpn*I would cleave such DNA to large fragments, while *Mbo*I would cleave it to small fragments. This pattern was observed with *B. burgdorferi* strains MMI, IPF, and P/Gau. A partial Dam^+ phenotype has also been observed in the archaebacterium *Halobacterium saccharovorum* and *Methanobacterium* strain Ivanov (1). The three strains of *B. burgdorferi* which possessed *dam* methylation, P/Gau, MMI, and IPF, were isolated from diverse geographic locations and were carried by different *Ixodes* species. The implications of methylation in these isolates are not known at this time.

It is important to recognize potential problems that may occur when attempting the cloning and restriction of *Borrelia* DNA into an *E. coli* system. The initial amplification of libraries that may contain methylated DNA should be carried out in an *E. coli* host that lacks methylation-dependent restriction systems (3). In addition, the presence of methylated DNA should be considered in future experiments that involve the uptake and transformation of *Borrelia* DNA.

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