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Procaryotic and Eucaryotic Traits of DNA Methylation in Spiroplasmas (Mycoplasmas)

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Differences in the type of base methylated (cytosine or adenine) and in the extent of methylation were detected by high-pressure liquid chromatography in the DNAs of five spiroplasmas. Nearest neighbor analysis and digestion by restriction enzyme isoschizomers also revealed differences in methylation sequence specificity. Whereas in *Spiroplasma floricola* and *Spiroplasma* sp. strain PPS-1 5-methylcytosine was found on the 5′ side of each of the four major bases, the cytosine in *Spiroplasma apis* DNA was methylated only when its 3′ neighboring base was adenine or thymine. In *Spiroplasma* sp. strain MQ-1 over 95% of the methylated cytosine was in C-G sequences. Essentially all of the C-G sequences in the MQ-1 DNA were methylated. Partially purified extracts of *S. apis* and *Spiroplasma* sp. strain MQ-1 were used to study substrate and sequence specificity of the methylase activity. Methylation by the MQ-1 enzyme was exclusively at C-G sequences, resembling in this respect eucaryotic DNA methylases. However, the MQ-1 methylase differed from eucaryotic methylases by showing high activity on nonmethylated DNA duplexes, low activity with hemimethylated DNA duplexes, and no activity on single-stranded DNA.

Whereas procaryotic DNA may be methylated at the adenine (6-methyladenine; m^6Ade) or cytosine (5-methylcytosine; m^5Cyt) residues, higher eucaryotes have been shown to contain m^5Cyt exclusively. Methylated bases are known to reside at specific sequences in DNAs from all sources studied so far. In many procaryotes the methylated sequence is species specific and is composed of four or more nucleotides. In *Escherichia coli* C, for example, all methylated cytosines are located at the $CC^{A}_{T}GG$ sequence, whereas methylated adenines reside at the GATC sites. In eucaryotes, on the other hand, the methylated cytosines appear mainly at the dinucleotide C-G (for a review, see reference 18). C-G methylation is a known trait of higher eucaryotes and is believed to play a role in the process of differentiation and control of differential gene expression (20, 21). Many procaryotic sequence-specific methylations have been shown to play a role in the restriction modification phenomenon (29), whereas other sequence-specific methylations, such as the adenine methylation at GATC sites in *E. coli*, have been shown to be associated with postreplicative mismatch repair (17). However, the biological significance of most DNA modifications and the evolution of the extensive diversity of DNA methylation is essentially unknown.

In the present investigation we have studied the base and sequence specificity of DNA methylation in the spiroplasma branch of the mycoplasma group (taxonomically forming the class *Mollicutes*, the wall-less bacteria). Mycoplasma DNA methylation is of special interest in the light of the marked genotypic and phenotypic variability exhibited by organisms included in the mycoplasma group. The phylogeny and evolution of mycoplasmas have been a subject of controversy. Some workers claim that mycoplasmas are the closest existing relatives of the primitive organisms from which procaryotes and eucaryotes evolved (13, 14), whereas others regard mycoplasmas as descendants of gram-positive bacteria, more specifically of some clostridia with a low DNA G + C content (11, 23, 28). Methylated bases were detected in the DNA of several *Mycoplasma* and *Acholeplasma* species (6, 9, 19). Like the DNAs of many other procaryotes, all of the five species tested contained m^6Ade. One species, *Mycoplasma hyorhinis*, in addition contained m^5Cyt.

In the present study we show that spiroplasmas differ widely in their DNA methylation patterns, as expressed in the type of the base methylated, the extent of methylation, and the sequence specificity of the methylation reactions. Surprisingly, one of the spiroplasmas, *Spiroplasma* sp. strain MQ-1, contained methylated cytosine in the dinucleotide sequence C-G, the typical methylation trait in eucaryotes.

MATERIALS AND METHODS

Organisms and growth conditions. The spiroplasma strains (Table 1) were obtained from R. F. Whitcomb (U.S. Department of Agriculture, Beltsville, Md.). The spiroplasmas were grown at 32°C in M1 medium (27) to the late logarithmic phase and were then washed twice in cold 0.25 M NaCl containing 0.01 M EDTA.

Preparation of DNA. Spiroplasma DNA was prepared by the Marmur procedure (12). M13 phage single-stranded DNA was prepared as previously described (7). Hemimethylated and nonmethylated M13 double-stranded DNA was synthesized in vitro by primed repair synthesis (7) with DNA polymerase I and a synthetic primer (New England Biolabs, Inc., Beverly, Mass.).

High-pressure liquid chromatography. High-pressure liquid chromatographic analysis of DNA base composition was carried out on acid hydrolysates as described previously (19).

Analysis of nearest neighbor to m^5Cyt in spiroplasmal DNA (8). DNA (5 μg) was randomly nicked by pancreatic DNase I (Sigma Chemical Co., St. Louis, Mo.) in the presence of 5 mM $CaCl_2$. The nicked DNA was nick translated with *E. coli* polymerase I (New England Biolabs) in the presence of a single α-^{32}P-deoxynucleoside triphosphate (3,000 Ci/mmol; Amersham Corp., Arlington Heights, Ill.). The labeled DNA was digested to deoxynucleoside 3′-monophosphates, which

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TABLE 1. Base composition of DNA of spiroplasmas and other mollicutes determined by high-pressure liquid chromatography[a]

Organism	Serogroup	Composition (mol%) of:					
		G	C	A	T	m^5Cyt	m^6Ade
S. citri Maroc R8A2, B	I	13.7	12.5	36.0	37.9	ND	0.10
S. floricola BNR-1	III	13.7	12.6	37.7	35.8	0.29	0.004
S. apis B-31	IV	15.4	14.0	34.4	36.0	0.37	0.007
Spiroplasma sp. strain PPS-1	IV	15.4	13.5	34.4	35.8	0.95	0.004
Spiroplasma sp. strain MQ-1	VII	17.0	14.8	33.9	33.9	0.43	ND

[a] Values represent an average of at least three determinations with independent DNA preparations. ND, Not detected.

were separated by thin-layer chromatography (16), and the chromatographs were autoradiographed on X-ray RP2 film (Agfa-Gevaert). The relative amount of radioactivity in the spots was assessed by densitometry, using a Quick Scan R&D densitometer (Helena Laboratories).

Cleavage by restriction endonucleases. Restriction enzyme *Msp*I was from New England Biolabs, and *Hpa*II was obtained from Boehringer Mannheim Biochemicals, Indianapolis, Ind. Digestions were performed at 37°C for 2 h in buffer mixtures recommended by the manufacturers. The digested DNAs were analyzed by electrophoresis in 0.8% agarose (Seakem) at 30 V for 18 h. Gels were stained with ethidium bromide (1 μg/ml) and photographed by a Polaroid MP4 land camera with 667 type film.

Isolation of DNA methylases from spiroplasma cells. All steps were carried out at 0 to 4°C. Washed packed cells from 2-liter cultures were suspended in 50 mM Tris hydrochloride (pH 8.0) containing 10 mM EDTA and 10 mM β-mercaptoethanol. Cells were ruptured by sonication in ice for six periods of 15 s each, with intervals of 15 s, in a sonicator (Heat-Systems Ultrasonics, Plainview, N.Y.) at an output of 40 W. The resulting suspension was centrifuged at 140,000 × *g* for 1 h in a Beckman L2 ultracentrifuge. The supernatant fluid was dialyzed for 18 h against the above buffer supplemented with 50% glycerol. The methylase activity of this preparation, referred to as fraction 1, remained stable for at least 4 months at −20°C.

Fraction 1 was applied to a DEAE-cellulose column (5 by 0.5 cm) which was preequilibrated with 10 mM Tris hydrochloride buffer (pH 8.0) containing 10 mM EDTA, 10 mM β-mercaptoethanol, and 20 mM NaCl. Elution was carried out with the same buffer. A 30-ml stepwise gradient of 20 to 300 mM NaCl was applied in 3-ml samples. Samples (5 μl) of each eluted fraction were assayed for methylase activity as described below. Fractions with peak activity were combined and dialyzed for 18 h as described above for fraction 1. The column chromatography step resulted in a five- to sixfold concentration of the methylase activity, which remained stable for at least 2 months at −20°C. This enzyme preparation was used in all of the enzymatic reactions reported in this paper.

Eucaryotic methylase with a specificity for C-G sequences was prepared from Friend murine erythroleukemia cells by 0.3 M NaCl extraction of purified nuclei (1).

Assay of methylase activity. The methylase activity was assayed in a standard reaction mixture (25 μl) containing 50 mM Tris hydrochloride (pH 7.0), 10 mM EDTA, 10 mM β-mercaptoethanol, 25% glycerol, 4.5 μM [*methyl*-^{3}H] *S*-adenosylmethionine (15 Ci/mmole; Amersham), 0.2 mM phenylmethylsulfonyl fluoride, 5 μg of *Micrococcus lysodeikticus* DNA (Sigma), and the tested enzyme preparation (1 to 5 μg of protein). After 1 h of incubation at 32°C the reaction was terminated by the addition of 0.2 ml of 1% (wt/vol) sodium dodecyl sulfate and transfer to 65°C for 10 min. To isolate the DNA, 0.2 ml of 0.1 M NaOH was added, and incubation was continued for 10 min at 65°C. The mixture was then extracted with 1 volume of chloroform. The upper phase was separated, and the DNA in it was precipitated by incubation for 10 min in ice with 5 ml of 10% (wt/vol) trichloroacetic acid containing 250 μg of calf thymus DNA (Sigma) as a carrier. The precipitated DNA was collected on Whatman GF/C filters, and its radioactivity was measured by liquid scintillation counting.

RESULTS

Base composition and methylated bases in spiroplasmal DNA. Most of the studies on the G + C content of spiroplasmas applied indirect methods such as CsCl density centrifugation or thermal denaturation curves of DNA, methods which are unsuitable for detection of methylated bases (4). Therefore, we employed high-pressure liquid chromatography for direct quantitative analysis of the free and methylated bases. Five spiroplasma strains were tested (Table 1). The G + C content of the organisms tested by high-pressure liquid chromatography was generally in good agreement with the values obtained by the indirect methods cited in the literature (4, 22). The data in Table 1 reveal that the DNAs of *Spiroplasma floricola* (serogroup III), *S. apis*, *Spiroplasma* sp. strain PPS-1 (serogroup IV), and *Spiroplasma* sp. strain MQ-1 (serogroup VII) have m^5Cyt, and only a small amount or none at all of m^6Ade, whereas the *S. citri* strain tested contains small amounts of m^6Ade and no m^5Cyt.

The extent of methylation was apparently influenced by the age of the culture. Thus, in DNA of *S. floricola* BNR-1, harvested at the early logarithmic phase, 2.3% of the cytosine residues were methylated. The value for m^5Cyt increased to 4.4% in a sample taken 22 h after that (late logarithmic phase) and remained at about the same level in a sample taken 46 h after the first one (stationary phase).

Sequence analysis of m^5Cyt in spiroplasmal DNA. To obtain a more detailed picture of the species specificity of DNA methylation in spiroplasmas, we have utilized two previously described methodologies to study the sequence specificity of m^5Cyt in the four spiroplasma strains containing this base. The degree of methylation of C-X-containing sequences was determined by two methods. (i) By an extension of the standard nearest neighbor analysis (8), the state of methylation of the C-G, C-C, C-T, and C-A dinucleotide sequences was estimated. (ii) The second method used pairs of restriction enzymes (isoschizomers) in which one of each pair cleaves the DNA when cytosine is methylated, whereas the other, which has a similar recognition site, does not act when cytosine is methylated. By nearest neighbor analysis *S. floricola* and *Spiroplasma* sp. strain PPS-1 showed a similar methylation pattern; m^5Cyt in both orga-

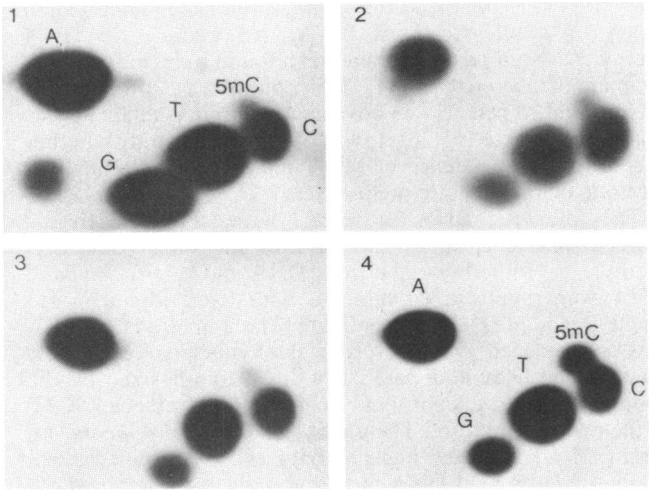

FIG. 1. Analysis of m^{5}Cyt at C-X sequences of *Spiroplasma* sp. strain PPS-1 DNA. Analysis was performed as described in Materials and Methods with α-^{32}P-labeled (1) dGTP, (2) dCTP, (3) dATP, and (4) dTTP. The letters G, C, A, T, and 5mC correspond to dGMP, dCMP, dAMP, dTMP, and 5-methyl dCMP, respectively.

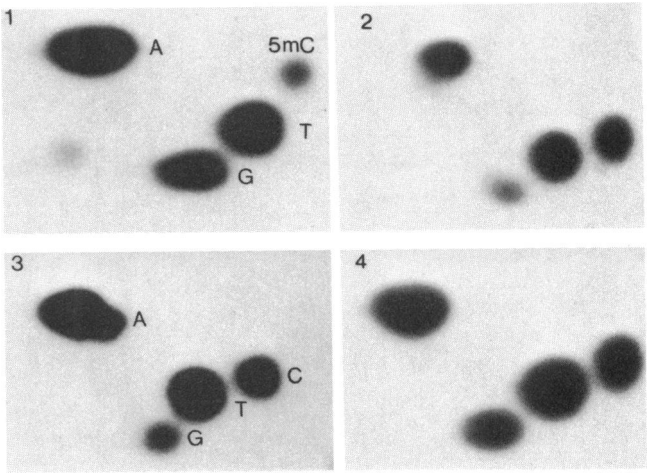

FIG. 2. Analysis of m^{5}Cyt at C-X sequences of *Spiroplasma* sp. strain MQ-1 DNA. Panels are as in Fig. 1. Essentially all the cytosine in the C-G sequence is methylated, whereas none of the cytosines in the other sequences is methylated.

nisms was found to be on the 5′ side of each of the four major bases (Fig. 1), but the degree of methylation varied with each neighboring base (Table 2). The cytosine in *S. apis* DNA was methylated only when its neighboring base was adenine or thymine, whereas *Spiroplasma* sp. strain MQ-1 presented a special case in which 95 to 100% of the methylated cytosine was associated with guanine (Fig. 2 and Table 2).

Further support for these results was obtained by using the pair of restriction enzyme isoschizomers *Hpa*II and *Msp*I to probe for methylation at the CCGG sequence. When the DNAs of the four spiroplasma strains containing m^{5}Cyt were subjected to these restriction enzymes, the DNA of *Spiroplasma* sp. strain MQ-1 resisted cleavage by *Hpa*II, but was readily cleaved by *Msp*I. *S. floricola* BNR-1 DNA is partially cleaved by *Hpa*II, as expected by its extent of methylation (Table 2). The other DNAs were cleaved by both enzymes (Fig. 3). These results indicate that most, if not all, of the cytosine residues adjacent to guanine in the CCGG sites in *Spiroplasma* sp. strain MQ-1 are methylated.

Characterization of spiroplasmal methylases. The sequence and substrate specificities of a DNA methylase are important features of DNA methylation which can be studied (7). To study sequence specificity of spiroplasma methylases, DNA substrate was synthesized as follows. Bacteriophage M13 DNA was used as a template to synthesize the labeled complementary strand with a synthetic primer, *E. coli* DNA polymerase I and a mixture of all four deoxynucleoside triphosphates (one of which was α-^{32}P labeled). The enzymatically synthesized substrates were subjected to methylation by the methylases and were then analyzed for the nearest neighbor of the labeled nucleotide incorporated. The results of the in vitro methylation of M13 double-stranded DNA by the *S. apis* methylase are shown in Fig. 4. Methylated cytosine was detected in the dinucleotide sequence C-A and C-T, but not in C-C and C-G (Fig. 4). Thus, the in vitro methylation of M13 DNA by *S. spis* methylase resembles the in vivo methylation of *S. apis* DNA both in specificity and in

TABLE 2. Nearest neighbor analysis of m^{5}Cyt in spiroplasmal DNA

Organism	% Methylation of cytosine in the following sequences[a]:			
	C-G	C-A	C-C	C-T
S. floricola BNR-1	15.0	10.5	3.1	6.3
Spiroplasma sp. strain PPS-1	3.1	8.5	22.0	36.2
S. apis B-31	0	5.0	0	5.7
Spiroplasma sp. strain MQ-1	>95.0	0	0	0

[a] Determined by densitometry of spots revealed by autoradiography of the thin-layer plates, as described in Materials and Methods.

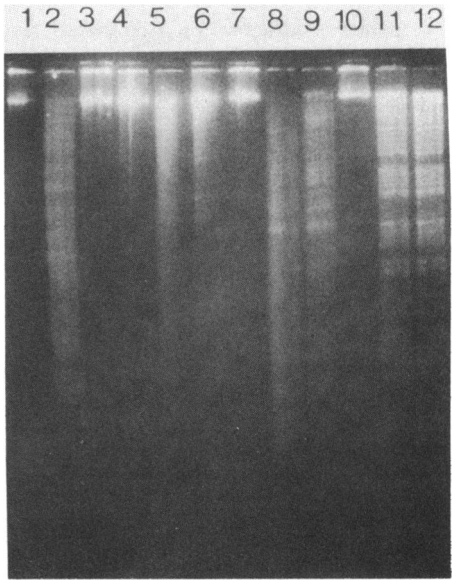

FIG. 3. Detection of sequence specificity of methylation by restriction enzyme analysis. Spiroplasmal DNAs were digested by the isoschizomers *Hpa*II and *Msp*I. Lanes: 1, MQ-1 undigested DNA; 2, MQ-1 DNA digested by *Msp*I; 3, MQ-1 DNA digested by *Hpa*II; 4, BNR-1 undigested DNA; 5, BNR-1 DNA digested by *Msp*I; 6, BNR-1 DNA digested by *Hpa*II; 7, PPS-1 undigested DNA; 8, PPS-1 DNA digested by *Msp*I; 9, PPS-1 DNA digested by *Hpa*II; 10, *S. apis* undigested DNA; 11, *S. apis* DNA digested by *Msp*I; 12, *S. apis* DNA digested by *Hpa*II.

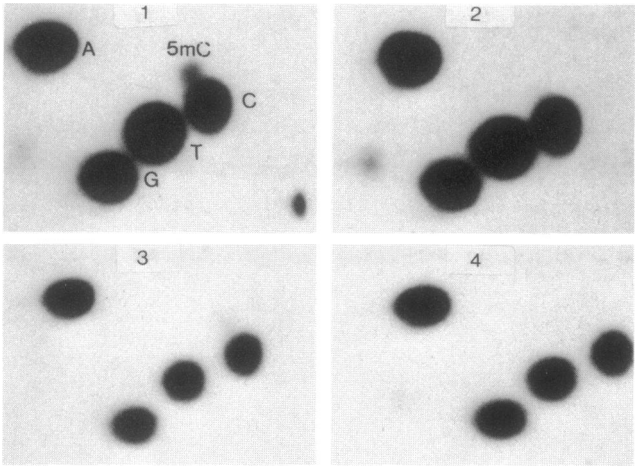

FIG. 4. In vitro methylation of cytosine of bacteriophage M13 DNA by the DNA methylase preparation from *S. apis*. M13 replicative-form DNA (0.1 μg) was synthesized in vitro in the presence of α-^{32}P-labeled (1) dATP, (2) dCTP, (3) dTTP, and (4) dGTP by primed repair synthesis and incubated for 24 h at 32°C with the enzyme preparation (1 μg of protein) with the standard reaction mixture for DNA methylation described in Materials and Methods. The treated M13 DNA was extracted with 1 volume of phenol followed by a second extraction with 1 volume of chloroform-isoamyl alcohol (24:1, vol/vol). The upper phase was filtered through Sephadex G-50, and the resulting purified DNA was subjected to nearest neighbor analysis as described in the text.

the relative amounts of C-A and C-T methylated (Table 2). *Spiroplasma* sp. strain MQ-1 methylase showed in vitro methylation of cytosine residues of M13 DNA only at the dinucleotide sequence C-G (data not shown), resembling the specificity of the methylase in vivo (Fig. 2). However, the rate of methylation of the M13 DNA was low, since only 4 to 11% of the cytosine residues were methylated (Fig. 5) as compared to almost 100% methylation found under in vivo conditions (Fig. 2).

Comparison of substrate specificity of the C-G methylase from *Spiroplasma* sp. strain MQ-1 and a eucaryotic C-G methylase. Since the methylated sequences in the DNAs of procaryotes and eucaryotes usually constitute palindromes, two modes of methylation have been proposed: (i) methylation by a semiconservative mechanism based on methylating hemimethylated sequences by a maintenance enzyme, as postulated previously to explain the inheritance of the methylation pattern in DNA, and (ii) do novo methylation of specific sequences, showing preference for nonmethylated DNA (for a recent review, see reference 21). Maintenance enzymes will, on the other hand, prefer hemimethylated DNA or single-stranded DNA. All eucaryotic DNA methylases studied so far have been found to be more active as maintenance enzymes (1, 7); in contrast, *E. coli* enzymes were found to perform de novo methylation as efficiently as maintenance activity (26). It was of interest to study whether the substrate specificity of MQ-1 methylase resembles eucaryotic or procaryotic methylases.

The following DNA substrates were subjected to the spiroplasmal C-G methylase and to the eucaryotic C-G methylase. Unmethylated duplex DNA and hemimethylated DNA were synthesized in vitro with bacteriophage M13 single-stranded DNA as a template. The unmethylated DNA was synthesized using all four deoxynucleoside triphosphates. The hemimethylated DNA was synthesized by the same procedure, except that 5-methyl dCTP replaced dCTP in the reaction mixture. The methylase from *Spiroplasma* sp. strain MQ-1 showed high activity with double-stranded nonmethylated M13 DNA, a low activity with hemimethylated double stranded DNA, and no activity with single-stranded M13 DNA as the substrate (Fig. 6), whereas the eucaryotic methylase showed a marked preference for hemimethylated and single-stranded M13 DNA at low concentrations of DNA (Fig. 6). High concentrations of DNA inhibited the eucaryotic methylase activity.

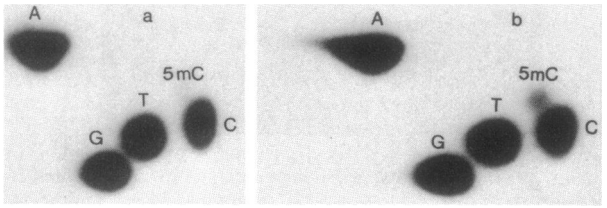

FIG. 5. In vitro methylation of cytosine of bacteriophage M13 DNA by the DNA methylase preparation from *Spiroplasma* sp. strain MQ-1. The reaction mixture and experimental procedure were as described in the legend to Fig. 4, but the M13 DNA was labeled only with [α-^{32}]dGTP. The amounts of protein in the enzyme preparation were 1.1 μg (a) and 2.2 μg (b).

DISCUSSION

The spiroplasmas included in our study, apart from *S. citri*, differ from *E. coli* and the mycoplasmas tested so far in having m^{5}Cyt as the only methylated base in their DNA, a feature characteristic of higher eucaryotic DNA (18) and of mouse mitochondrial DNA (16). Moreover, the extent of cytosine methylation also resembles that reported for eucaryotic DNA; in the case of *Spiroplasma* sp. strain MQ-1, methylation is exclusive to the dinucleotide sequence C-G, the most common sequence methylated in eucaryotic DNA

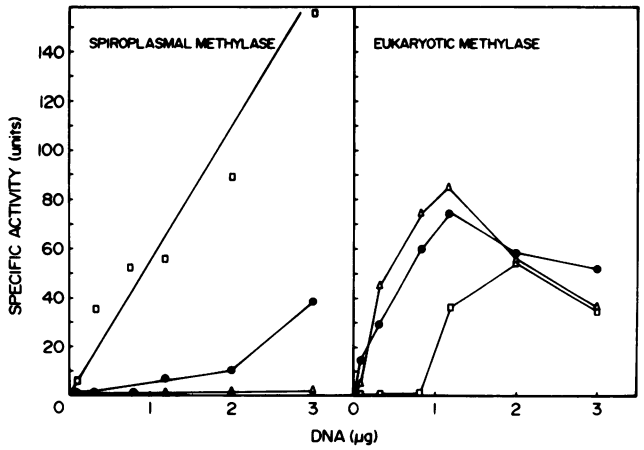

FIG. 6. Substrate specificity of the C-G methylases from *Spiroplasma* sp. strain MQ-1 and from Friend murine erythroleukemia cells. Methylation of bacteriophage M13 single-stranded DNA (△), hemimethylated M13 double-stranded DNA (●), and nonmethylated M13 double-stranded DNA (□) was tested in the standard reaction mixture described in Materials and Methods with the spiroplasmal methylase preparation (1.1 μg of protein) or the eucaryotic enzyme preparation (20 μg of protein). Specific activity units are defined as picomoles of CH_3 incorporated per hour per milligram of protein.

(18). Whether methylation patterns can serve as genetic markers to characterize and differentiate species of the class *Mollicutes* cannot be decided on the basis of the available data. It appears that the type of base methylated, whether it is cytosine, adenine, or both, may be of relevance in this respect. Yet, in light of our findings with *S. floricola* tested at various culture ages the extent of methylation represents a more variable parameter than the type of base methylated.

The methylation sequence specificity probably constitutes a more meaningful criterion for differentiating mycoplasmas, since it must reflect the nucleotide sequence recognized by the organism's DNA methylase(s). Our data reveal significant differences in the sequence in which cytosine is methylated in the various spiroplasmas. Thus, the two spiroplasmas belonging to serogroup IV, *S. apis* and *Spiroplasma* sp. strain PPS-1, differ in the sequence in which cytosine is methylated (Table 2). These two spiroplasmas show about 80% homology by DNA-DNA hybridization tests and considerable relatedness of their cell protein patterns (3, 15), enabling their inclusion in one species according to currently accepted criteria (10).

The data of nearest neighbor analysis of methylated cytosine in the DNAs of *S. apis* and *Spiroplasma* sp. strain MQ-1 resembled those obtained with bacteriophage M13 DNA exposed to methylase preparations of these spiroplasmas. Thus, the patterns of in vivo and in vitro methylation of cytosine correspond very well. The *Spiroplasma* sp. strain MQ-1 methylase acted on cytosine only in the dinucleotide C-G sequences. This finding is important in two respects. (i) It is the first report of such a methylation pattern in procaryotes; procaryotic DNAs are methylated at recognition sites containing at least four nucleotides. (ii) In eucaryotes the extent of cytosine methylation at C-G sequences reaches a maximum of 85 to 90%, whereas in stationary-phase *Spiroplasma* sp. strain MQ-1 DNA essentially all C-G sites were methylated. Interestingly, in murine mitochondrial DNA the methylated cytosine also appears in the dinucleotide sequence C-G, but only 3 to 5% of the cytosine residues in this sequence are methylated (16).

The finding in a spiroplasma of a methylation pattern, which so far has been regarded as unique for eucaryotes, may be of interest to researchers of mycoplasma phylogeny. The marked genotypic and phenotypic diversity shown by members of the class *Mollicutes* could be a result of this group being in a state of rapid evolution due to high mutation rates (23). The unique and somewhat bizzare properties of the mycoplasmas can be explained in this way. Accordingly, the presence of a C-G methylase in a member of *Mollicutes* may be another of the unexpected properties of these procaryotes and may have little weight in considerations on their phylogenetic status.

Our finding that all of the C-G sequences in *Spiroplasma* sp. strain MQ-1 are methylated could be taken to suggest that DNA methylation in this organism operates as part of a restriction-modification system. However, all of our attempts to isolate by conventional procedures a restriction enzyme with C-G specificity from lysates of this mycoplasma have been unsuccessful. Despite the fact that our enzyme preparations were rather crude, the data enable us to draw some preliminary conclusions as to the preference of the *Spiroplasma* sp. strain MQ-1 methylase for substrates. Thus, the spiroplasmal C-G methylase is capable of de novo methylation, as shown by its preference for nonmethylated double-stranded DNA, resembling methylases of other procaryotes (26). The eucaryotic methylases, on the other hand, act mostly as maintenance methylases, preferring hemimethylated double- or single-stranded DNA as substrates (21) (Fig. 6).

Our finding that in *Spiroplasma* sp. strain MQ-1 DNA the only methylated cytosine is located in C-G sequences, and that 100% of these sequences are methylated, can be used to assess the doublet frequency of C-G in this DNA. Calculations based on the expected random frequency of the C-G doublet, taking into account the G + C content of the DNA, yield a value of 2.25%. However, calculations based on the amount of m^5Cyt in the *Spiroplasma* sp. strain MQ-1 DNA (Table 1) yield a value of 0.45% for C-G sequences, indicating a considerable underrepresentation of this sequence in the spiroplasmal DNA. This supports the conclusions of Russel et al. (24), pointing to the underrepresentation of the C-G sequence in the DNAs of a variety of bacteria, including a few animal mycoplasmas. Moreover, underrepresentation of C-G sequences is a common feature in vertebrate DNA (2).

The discovery of >95% C-G methylation in a procaryote raises some interesting questions as to the mechanism and role of C-G methylation. It has been recently suggested that C-G methylation is a late development in the evolution of eucaryotic organisms aimed at modulating differential gene expression (2, 20, 21). As the procaryotic spiroplasmas do not differentiate, this may suggest that C-G methylation may have other biological roles. In vitro methylation of C-G sequences has been shown to silence a variety of eucaryotic genes (5, 25). Inactivation of genes by C-G methylation seems not to occur in strain MQ-1. This may suggest that C-G methylation by itself does not in general impose a physical stress on DNA sufficient to prevent its transcription. Other factors may exist in eucaryotic cells to differentiate between methylated and unmethylated sequences. The methylase of *Spiroplasma* sp. strain MQ-1 may therefore serve as an interesting system to study the biological role of C-G methylation. Moreover, the discovery of a de novo methylase may be of great practical contribution to the DNA methylation research, enabling C-G-specific in vitro methylation of DNA.

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