DNA methyltransferases affecting the sequence 5'CCGG

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ABSTRACT

B. subtilis phage SP8 and Moraxella sp. code for DNA methyltransferases which methylate both cytosines of the sequence 5'CCGG. Experiments using a B. subtilis strain whose DNA is sensitive to HpaII and resistant to MspI degradation, indicated that methylation of the outer C of this sequence provides protection against the restriction enzyme MspI.

INTRODUCTION

The dinucleotide sequence CpG is found with exceptionally low incidence in the DNA of eukaryotes (1). Methylation of eukaryotic DNA occurs prevalently on the C contained in this rare sequence (2, 3, 4). Recently results have accumulated in various laboratories indicating that such C-methylation is involved in the regulation of transcription of the genetic message in eukaryotes (5, 6, 7, 8, 9). Portions of such DNA which are actively transcribed show very little methylation in this sequence, whereas CpG in the same regions is highly methylated when transcriptionally dormant. This observation has led to an increased interest in the methylation of DNA and analysis of the substrate character of DNA for degradation by restriction enzymes or modifiability through modification enzymes. Such studies permit precise information on the specificity and extent of methylation, provided that the restriction/modification specificities of the enzymes are known. Of particular interest here are enzymes which contain the dinucleotide CpG in their target sequence, e.g. HhaI (GCGC), HpaII (CCGG), or MspI (CCGG). The HhaI- and HpaII-specific modifications have been shown to be mediated through methylation of the central C in the respective

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target sequences (10, 11). Sneider (12), van der Ploeg et al. (13) and this laboratory (14) have suggested that methylation of C at the 5' end of the MspI target sequence was responsible for modification. However, a direct determination of MspI modification was still lacking. Here we report experiments which provide such direct proof. We also demonstrate that B. subtilis phage SPB and Moraxella sp. code for enzymes which methylate both adjacent C's in the target sequence CCGG.

MATERIALS AND METHODS

The bacteria used in this study are compiled in Table 1. Strains of B. subtilis and Moraxella sp. were grown in TY medium (15). The SPB19 lysogenic strain TB104 was induced with mitomycin as described (16). Bacterial DNA was extracted as described by Marmur (17). H. parainfluenzae DNA was a gift of Dr. R. Gramkova. Phage SPB DNA was prepared from purified phage as described for SPP1 (18). Restriction enzymes MspI, HpaII and HaeIII were purchased from Biolabs (Beverly, Ma., USA) and Boehringer (Mannheim, Germany) and used as described by the manufacturers. Analytical agarose gel electrophoresis was performed as described (19). Localization of the methylated nucleotide in the sequence CCGG was performed by the method described by Günthert et al. (20). Base sequences are always written in the direction 5' → 3'; C stands for 5-methyl-cytidine-monophosphate.

<table>
<thead>
<tr>
<th>STRAIN</th>
<th>MODIFYING ACTIVITY AFFECTING GCCC OR CCGG</th>
<th>COMMENTS/ORIGIN</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. subtilis SB1207 (SPB)</td>
<td>none</td>
<td>(16)</td>
</tr>
<tr>
<td>B. subtilis TB804 (SPB)</td>
<td>GCCC</td>
<td>(16)</td>
</tr>
<tr>
<td>B. subtilis TB104 (SPB19)</td>
<td>CCGG</td>
<td>Modifying activity manifested only after induction of prophage SPB19 (21)</td>
</tr>
<tr>
<td>B. subtilis Q</td>
<td>CCGG</td>
<td>Strain was originally obtained from B. Delaporte. The strain is not related to B. subtilis 168 strains.</td>
</tr>
<tr>
<td>Moraxella sp.</td>
<td>CCGG</td>
<td>Strain obtained from R. J. Roberts. Original source of MspI restriction enzyme.</td>
</tr>
</tbody>
</table>
RESULTS

*B. subtilis* phage SPβ and *Moraxella* sp. code for methyltransferases which cause resistance of DNA to both *MspI* and *HpaII* degradation. *B. subtilis* phage SPβ codes for a methyltransferase which methylates the internal C of the *BsuR* recognition site GGCC (16). The gene of SPβ responsible for this modifying activity was identified through the discovery of SPβ mutants which had lost the capacity for such methylation. SPβ also codes for modifying activities which affect *MspI* and *HpaII* restriction. Independent *BsuR* methyltransferase deficient mutants were isolated and found to fall into two classes depending on whether their DNA was sensitive to degradation by *MspI* and *HpaII*. The degradation patterns with restriction enzymes *MspI*, *HpaII* and *HaeIII* (an isoschizomer of *BsuR*) of DNA derived from SPβ phages is shown in Figure 1. SPβ WT DNA was resistant, while SPβ26 DNA

![Figure 1. Gel electrophoresis of SPβ DNAs after degradation with restriction enzymes *MspI*, *HpaII* and *HaeIII*.](image-url)
was sensitive to all enzymes tested. DNA from the BsuR sensitive mutant SP819 was resistant to both MspI and HpaII digestion.

Neither phage crosses nor mutagenesis resulted in SP8 mutants affected in either MspI or HpaII modification alone. This suggested that the simultaneous presence or absence of both modifications was caused by one and the same enzyme activity. Protection against HpaII is mediated by methylation of the central C of the MspI/HpaII target sequence CCGG (11), but this methylation does not produce MspI modification. It therefore seemed probable that MspI modification involved the outer C of the target sequence. The presence of a methyltransferase affecting this nucleotide was readily demonstrable in cell-free extracts of the induced strain TB104 which is lysogenic for SP819. Such an extract was employed to methylate BsuR modified TB804 DNA in vitro, using Ado|Me-3H|Met as the methyl donor. The subsequent determination of the distribution of radioactivity between the two C residues of the isolated dinucleotide CpC revealed that both C's had become methylated (Table 2). Such an activity was not demonstrable in cell-free extracts derived from isogenic cells which were not lysogenic for SP8 or from cells lysogenized with the MspI/HpaII restrictable phage SP826 (data not shown).

The methylation pattern with SP819 is analogous to that described for DNA isolated from Moraxella sp.. Moraxella sp. DNA is also resistant to both MspI and HpaII (J. Brooks and R. Roberts, pers. commun.), but sensitive to HaeIII (Fig. 2). Here, too, a cell free extract of Moraxella sp. will methylate either C residue in the sequence CCGG (Table 2).

Methylation of the outer C in the sequence 5'CCGG is sufficient to cause resistance of DNA to MspI degradation. Our data suggested that MspI-modification in the sequence 5'CCGG is either due to methylation of both C's or to methylation of the outer C alone. DNA from strain B. subtilis Q was found to be resistant to MspI but sensitive to HpaII (Figure 2). A cell free extract from this strain only methylated the outer C of the MspI/HpaII target sequence (Table 2). Thus methylation of this C is sufficient to produce protection against MspI restriction.
Figure 2. Gel electrophoresis of MspI, HpaII and HaeIII restricted bacterial DNAs. The methylated site(s) of the sequences CCGG and GGCC are indicated below.

TABLE 2
LOCALIZATION OF THE $^3$H-LABELLED METHYLGROUP IN d(CpC).

<table>
<thead>
<tr>
<th>CELL FREE EXTRACT</th>
<th>PHOSPHODIESTERASES$^1$</th>
<th>DISTRIBUTION OF RADIOACTIVITY BETWEEN (%)</th>
<th>TYPE OF MODIFICATION</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>dC</td>
<td>d(CpC)</td>
</tr>
<tr>
<td>B. subtilis TB104</td>
<td>SV</td>
<td>46.4</td>
<td>6.9</td>
</tr>
<tr>
<td></td>
<td>CS</td>
<td>47.8</td>
<td>4.0</td>
</tr>
<tr>
<td>Moraxella sp.</td>
<td>SV</td>
<td>49.8</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>CS</td>
<td>48.0</td>
<td>2.7</td>
</tr>
<tr>
<td>B. subtilis Q</td>
<td>SV</td>
<td>96.3</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>CS</td>
<td>9.7</td>
<td>13.0$^2$</td>
</tr>
</tbody>
</table>

$^1$SV = Snake Venon P'diesterase, CS = Calf Spleen P'diesterase

$^2$Possibly due to incomplete digestion with calf spleen P'diesterase
Simultaneous resistance of DNA to degradation by more than one restriction enzyme may be observed in regions were target sequences overlap. In the cases of SP8 and Moraxella DNA reported here, simultaneous protection against MspI and HpaII restriction is generated through methylation of both C's in the common recognition sequence 5'CCGG. Single methylation of the inner C generates HpaII resistance (11) and of the outer C generates MspI resistance (Table 2). The methyltransferase with the latter specificity was identified in B. subtilis Q and is currently being purified. We are also searching for a matching restriction enzyme which would be an isoschizomer of MspI. Are one and the same or two distinct enzymes responsible for simultaneous protection against MspI and HpaII? Purification of the enzymatic activities from Moraxella sp. and SP8 lysogenic B. subtilis are in progress to answer this question.

Irrespective of the outcome of these experiments, the finding of the activities observed raises the question about the function of such methyltransferases. To our knowledge, no restricting activity with HpaII specificity was observed in Moraxella sp.. Neither do we know of any potential host cell for SP8 which have MspI/HpaII restriction activity. As more methyltransferases are identified, which exist in the absence of matching restriction enzymes, it becomes difficult to rationalize the presence of such methyltransferases simply as enzymes which serve to protect DNA against restriction.

ACKNOWLEDGEMENTS

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REFERENCES