DNA bending induced by DNA (cytosine-5) methyltransferases

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ABSTRACT

DNA bending induced by six DNA (cytosine-5) methyltransferases was studied using circular permutation gel mobility shift assay. The following bend angles were obtained: M.BspRI (GGm5CC), 46–50°; M.HaeIII (GGm5CC), 40–43°; M.SinI (GGWm5CC), 34–37°; M.Sau96I (Gm5NCm5CC), 52–57°; M.HpaII (Cm5CC), 30°; and M.Hhal (Gm5CCG), 13°. M.HaeIII was also tested with fragments carrying a methylated binding site, and it was found to induce a 32° bend. A phase-sensitive gel mobility shift assay, using a set of DNA fragments with a sequence-directed bend and a single methyltransferase binding site, indicated that M.HaeIII and M.BspRI bend DNA toward the minor groove. The DNA curvature induced by M.HaeIII contrasts with the lack of DNA bend observed for a covalent M.HaeIII–DNA complex in an earlier X-ray study. Our results and data from other laboratories show a correlation between the bending properties and the recognition specificities of (cytosine-5) methyltransferases: enzymes recognizing a cytosine 3′ to the target cytosine tend to induce greater bends than enzymes with guanine in this position. We suggest that the observed differences indicate different mechanisms employed by (cytosine-5) methyltransferases to stabilize the helix after the target base has flipped out.

INTRODUCTION

DNA (cytosine-5) methyltransferases (C5-MTases) catalyze the transfer of a methyl group from S-adenosyl-L-methionine (AdoMet) to the C5 carbon of cytosine in specific sequences. These enzymes play an important role in several biological processes, e.g., restriction-modification in bacteria, differentiation, regulation of gene expression in eukaryotes and carcinogenesis (1–4).

C5-MTases act as monomers. The catalytic mechanism of C5-methylation involves the formation of a transient covalent intermediate between the C6 carbon of the cytosine and the sulfur of a cysteine, which is conserved in all C5-MTases (5–7).

Most of our knowledge about C5-MTases is based on studies with bacterial enzymes. Eukaryotic C5-MTases are larger proteins but the sequence homology they share with bacterial C5-MTases and the available experimental data suggest that they act using the same catalytic mechanism (8,9). Bacterial C5-MTases share a common architecture, they contain 10 conserved sequence motifs and a so-called variable region located between conserved motifs VIII and IX (7). The variable region is responsible for sequence-specific DNA recognition (10,11).

There are two C5-MTases, M.Hhal (recognition sequence GCCG, target cytosine underlined) and M.HaeIII (GGCC), whose complex with cognate DNA has been studied by X-ray crystallography. The structure of the M.Hhal complex revealed that the enzyme employs base flipping, a previously unknown mechanism, to access the target cytosine (12). The structure of the M.HaeIII–DNA complex showed that this enzyme also flips the target base (13). A major difference between the M.Hhal and M.HaeIII structures was that whereas M.Hhal did not, beyond base flipping, distort the recognition site, the HaeIII methyltransferase caused a base pair rearrangement. This rearrangement probably serves to stabilize the double-stranded structure after the target base has flipped out (13).

DNA bending is used by several DNA binding proteins as part of the recognition mechanism (14). Neither the M.Hhal nor the M.HaeIII complex showed any protein-induced bend (12,13). Later solution studies, using two different methods, also indicated that M.Hhal does not induce a DNA bend in the recognition complex (15). In contrast, circular permutation gel shift analysis of the MspI methyltransferase (CGGG) detected a very strong protein-induced curvature (16).

Here we report studies that characterize DNA bending induced by six C5-MTases. The enzymes used in these studies recognize the following sequences: M.BspRI (GCCC; 17), M.HaeIII (GCCC; 18), M.HpaII (GGGG; 19,20), M.SinI (GGGCC; 21,22), M.Sau96I (GGGCC; 23) and M.Hhal (GCCC; 24,25) was used as control. Our data indicate that C5-MTases are diverse with respect to the magnitude of methyltransferase-induced bend in the recognition complex. These results and data from other studies (15,16) suggest a correlation between the recognition specificities and bending properties of C5-MTases: enzymes recognizing a sequence in which the target cytosine is followed by a 3′ cytosine tend to induce

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greater curvature, whereas enzymes with guanine in this position induce smaller bends when bound in specific complex with substrate DNA.

MATERIALS AND METHODS

Enzymes and chemicals

M. BspRI, M. SinI and M. Sau96I were purified from over-producing Escherichia coli clones by procedures to be described elsewhere. All three enzyme preparations were at least 95% pure as judged from one-dimensional SDS–polyacrylamide gel electrophoresis. M. HhaI was a gift of S. Klimasauskas (Vilnius, Lithuania). M. HaeIII and M. HpaII were purchased from New England Biolabs (Beverly, MA) and Fermentas (Vilnius, Lithuania), respectively. Concentrations of M. HaeIII and M. HpaII were estimated by comparing band intensities after running, in SDS–polyacrylamide gels, aliquots of the commercial preparations along with known amounts of BspRI methyltransferase.

Restriction endonucleases, T4 DNA polymerase, E. coli DNA polymerase I large (Klenow) fragment, T4 polynucleotide kinase and T4 DNA ligase were from New England Biolabs and Fermentas. Taq DNA polymerase was purchased from Pharmacia (Budapest, Hungary), and 5′-[γ-32P]ATP and 5′-[γ-32P]ATP (3000 Ci/mmol) were from Izotóp Intézet Kft (Budapest, Hungary). Linkers were purchased from New England Biolabs and other deoxyoligonucleotides from Integrated DNA Technologies (Coralville, PA).

Construction of DNA fragments for bending analysis

All plasmids used in this work are derivatives of the ‘bend vector’ plasmid pBend2 (26). The EcoRI–HindIII polynucleotide fragment of pBend2, which contains a tandemly repeated array of restriction sites, was transferred into pUC18 to yield pBend6. pBend6 was later modified to obtain plasmids which contain, in the middle of their polynucleotide sequence, a single binding site for the methyltransferases used in this study. Construction of the plasmids involved insertion of oligonucleotides containing the binding sites into the single XbaI site located between the repeats and, when required, elimination of multiple sites. Nucleotide sequence of the polynucleotide region of these plasmids is shown in Figure 1.

Plasmid pBend-GGCC contains two PstI linkers (5′-GCTGCAGC) in both StuI sites of the original polynucleotide sequence. Insertion of the PstI linkers served to eliminate StuI sites, which contain the recognition site GGCC. It also carries, next to one of the PstI linker insertion sites (Fig. 1), a 2 bp deletion, which arose as a cloning artifact. To introduce a single 5′-GGCC site into the middle of the polynucleotide region, an ApaI linker (5′-GGGGCC, recognition site in bold) was ligated into the filled-in XbaI site. Because of the ApaI linker sequence, this plasmid could also be used to generate fragments with a GGSCC binding site.

Plasmid pBend-GGWCC was constructed by ligating two annealed, partially complementary oligonucleotides AK27 (5′-CTAGCA GGACC TG) and AK28 (5′-CTAGCA GGTCC TG) into the single XbaI site in the middle of the pBend6...
polylinker. Ligation of the AK27/AK28 duplex to the plasmid DNA was facilitated by the XbaI-compatible single-strand overhangs of the AK27/AK28 duplex (see also for AK11 and AK32).

Plasmid pBend-GGCC was constructed by ligating a short duplex formed by the partially self-complementary oligonucleotide AK11 (5′-CTAGCAGCCGGTG) into the XbaI site of pBend6.

To construct pBend-CGGG, the Smal sites in each of the polylinker halves were destroyed by inserting PstI linkers. Subsequently, a short duplex formed by the partially self-complementary oligonucleotide AK32 (5′-CTAGCAGCCGGTG) was cloned into the single XbaI site of the polylinker. Structure of the plasmids was verified by DNA sequencing.

DNA fragments for bending analysis were generated by PCR amplification of the polylinker segment of plasmids pBend-GGCC, pBend-GGWCC, pBend-GGCC and pBend-CCGG. Oligonucleotides bend1 (5′-GAATTCAAGCGTAG) and bend2 (5′-AAGCTTGGATCCCTCGATT) and AK30 (5′-AAGCTTGGATCCCTCGATT), that hybridize to sequences flanking the polylinker segment were used as primers. PCR reactions contained, in a 50 µl volume, ~50 ng plasmid DNA, 100 pmol primer oligonucleotides, 2 mM MgCl2, 50 mM KCl, 10 mM Tris–HCl pH 8.0, 0.1% Triton-X100, 6 pmol 5′-[α-32P]dATP and 2.5 U Taq DNA polymerase. Fragments were amplified in 30 cycles with the following profile: 30 s denaturation at 94 °C, 30 s annealing at 55 °C, 30 s extension at 72 °C. The amplified product was purified from an 8% non-denaturing polyacrylamide gel and cleaved with different restriction enzymes to yield a circularly permuted set of DNA fragments.

DNA fragments with a methylated BspRI/HaeIII site were prepared by in vitro methylation of the PCR product synthesized from the pBend-GGCC template. Methylation was performed in reactions containing 50 mM Tris–HCl pH 8.0, 10 mM EDTA, 7 mM 2-mercaptoethanol, 5 µM AdoMet, 0.3 µM M.HaeIII and the gel-purified fragment. After the reaction, the DNA fragments were phenol-extracted and ethanol-precipitated, then methylated twice more to ensure complete methylation of the binding site. Completeness of methylation was assessed by testing resistance of the methylated fragments to digestion by BspRI endonuclease.

Circular permutation gel shift assay

DNA binding reactions contained, in addition to the PCR-amplified radioactively labeled DNA fragments (2000–3000 c.p.m.), the following components: M.Sau96I reactions. 0.3 µM M.Sau96I, 50 mM Tris–HCl pH 8.5, 2 µM AdoHcy, 100 µg/ml polydIdC, 50 mM NaCl, 10 mM DTT. M.HpaII reactions. 0.25 µM M.HpaII, 25 mM Tris–HCl pH 8.0, 2 µM sinefungin, 50 µg/ml polydIdC, 1 mM DTT, 20 mM NaCl.

Binding reactions were performed in 5–10 µl volumes at room temperature for 5 min (M.BspRI, M.HaeIII, M.HhaI and M.Sau96I), at 32 °C for 5 min (M.SinI), or at 37 °C for 15 min (M.HpaII).

Bound and unbound DNA fragments were separated by electrophoresis in 6% non-denaturing polyacrylamide gels at 4 °C. The gel buffer contained 50 mM Tris–borate pH 8.3, 1 mM EDTA and 2 µM AdoHcy. AdoHcy was used for all enzymes except M.HpaII to increase complex stability during electrophoresis. Methytransferase–DNA complexes were detected by conventional autoradiography and by a Phosphor Image Analyzer (Molecular Dynamics 445SI). Mobilities were derived from positions of the maximum radioactivity detected.

Relative mobilities were calculated by dividing mobility of the complex with that of free DNA and were plotted as a function of the fractional displacement. Fractional displacement was defined as the distance of the center of the binding site from the 5′-end of the fragment divided by the total length of the fragment (considering always the top strand as shown in Fig. 1). In cases of pentanucleotide binding sites (GGGCC and GGGCC) in which the center of the binding site falls on a nucleotide, the internucleotide position used to calculate the fractional displacement was arbitrarily chosen as indicated (GGGACC and GGCGC, considering the strand shown in Fig. 1). Bend angles (α) were calculated by the method described by Ferrari et al. (27). In this method data are fitted to the parabolic function y = ax2 + bx + c, and values for a, b and c are used to derive the bend angle (27,28).

Phasing analysis

Four plasmids (pBSuf1, pBSuf11, pBSuf12 and pBSuf13), which contain an intrinsically bent polyA tract placed at different distances from the GGCC binding site, were constructed. Plasmid pBend28 (a generous gift of I. Boros, Biological Research Center, Szeged, Hungary) carries an insert consisting of one half of the pBend2 multiple cloning site (EcoRI–SalI fragment) (26) and a triplicated polyA tract. This fragment was ligated into pBluescript SKII+ (between the EcoRI and SalI sites) to yield pBSuf1 (Fig. 3). Plasmid pBSuf11 was constructed by cleaving pBSuf1 at the unique Acc65I site, filling-in the ends using Klenow polymerase and religating the ends. Plasmid pBSuf12 was constructed from pBSuf11 by BamHI cleavage, filling-in and religation. pBSuf13 was generated from pBSuf12 by the same method but using XbaI cleavage. Plasmids pBSuf1, pBSuf11, pBSuf12 and pBSuf13 differ in the distance between the polyA tract and the methytransferase binding site (Fig. 3).

Cultures of E.coli XL1-Blue MRF' Kan host cells (Stratagene, Cambridge, UK) harboring pBSuf1, pBSuf11, pBSuf12 or pBSuf13 were infected with the helper phage R176 (29), then single-strand phagemid DNA was prepared using polyethylene glycol precipitation and phenol extraction. Hemimethylated DNA fragments were prepared by in vitro synthesis of the complementary strand. A methylated 16mer oligonucleotide AK12 (5′-TCGCGAAGGm5CCTCCCG),

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spanning the methyltransferase binding site was terminally labeled using 5′-[γ-32P]ATP and T4 polynucleotide kinase (30). The phosphorylated AK12 and the ~20 M13/pUC sequencing primer 5′-GTAAACAGCAGGACG-3′ (20 pmol each) were annealed to ~1 μg single-stranded template in 20 μl by heating to 95°C for 5 min then slowly cooling to room temperature. The complementary strand was synthesized in a reaction that contained the annealed template/primers, 40 mM Tris–HCl pH 7.5, 50 mM NaCl, 10 mM MgCl2, 1 mM DTT, 0.1 mg/ml bovine serum albumin, 0.5 mM dNTPs, 1 mM ATP, 5 U T4 DNA polymerase and 5 U T4 DNA ligase. After incubation at room temperature for 30 min the DNA was purified by gel filtration on a 1 ml Sephadex G50 column, phenol/chloroform-extraction and ethanol-precipitation. The precipitated DNA was dissolved and digested with EcoRI and SalI to generate the fragments for phasing analysis. Binding reactions and electrophoresis were performed as described above for gel shift analysis.

DNA sequencing

DNA sequence was determined either manually using a T7 sequencing kit (Pharmacia) or by an automated sequencer (ABI).

RESULTS

Footprinting studies with the C5-MTase M.BspRI suggested that the enzyme might induce a distortion, perhaps a bend in substrate DNA (31). To test this hypothesis, we performed mobility shift assays using a circularly permuted set of DNA fragments. This method relates electrophoretic mobility of substrate DNA to the helical phasing between the protein-induced bend and a sequence-directed intrinsic bend on the same DNA fragment (34). In these experiments we used a set of DNA fragments which carried three helically phased (A/T)5-tracts and a single GGCC site. The fragments differed from each other in the distance between the GGCC site and the (A/T)5-tracts (Fig. 3). It is known that (A/T)5-tracts are characterized by a bend toward the minor groove, thus the direction of a protein-induced bend can be derived from the dependence of electrophoretic mobility on the helical phasing between the two bends (34). In designing this experiment, we wanted to avoid potential problems arising from the fact that an unmethylated GGCC site can bind methyltransferase molecules in either of two orientations and the two resulting complexes may not be equivalent with regard to the induced bend. Therefore, to allow just one binding orientation, hemimethylated DNA fragments were prepared as described in Materials and Methods. (Later, gel shift experiments with fragments carrying methylated GGCC sites revealed that M.HaeIII can also form specific complex with methylated sites, see below.) Autoradiography of the gels with complexes formed between M.HaeIII and the labeled fragments showed that the relative mobility was a function of the distance between the (A/T)5-tracts and the GGCC site (Fig. 4). The complex migrating with the lowest relative mobility was formed with the fragment, in which the distance between the center of the intrinsic bend and the GGCC site was 73 bp, which corresponds to approximately seven helical turns of B DNA. The complex with the fragment in which this distance was 69 bp (close to 6.5 helical turns) migrated with the highest relative mobility (Fig. 4). In these experiments we assumed that the center of the protein-induced curvature falls on the center of the recognition sequence. This assumption was based on the observation that in permutation gel shift assays with unmethylated DNA fragments we did not detect any heterogeneity of the shifted bands, suggesting that the bend centers were very close or identical. The pattern of the relation between mobility and the distance of the bend centers suggests that the direction of the protein-induced bend is toward the minor groove. Similar results were obtained with M.BspRI (not shown).

A possible explanation of the contradiction between our data (M.HaeIII-induced bend) and the crystal structure (unbent DNA in the M.HaeIII complex) could be that the gel shift experiments characterize the initial recognition complex, whereas the X-ray structure was that of a post-methyl transfer intermediate in which the enzyme and the DNA substrate were covalently bound (13). We considered a model in which, at the beginning of the methyltransfer reaction, the enzyme induces a curvature in the DNA to facilitate sequence-specific recognition and base flipping, then after the methyl transfer has occurred, the DNA again assumes the undistorted B form. To test this hypothesis, circular permutation gel shift assays were performed with PCR-generated fragments that had been methylated in vitro. These experiments yielded a protein-induced bend of 32–33° (Fig. 2C).
Because of the contradiction between the \textit{M. Hae} III structure and our results we were concerned that the observed electrochemical behavior of the \textit{M. Hae} III and \textit{M. Bsp} RI complexes might be due to some DNA conformational effect other than bending. Therefore, as a control, we tested DNA bending by the \textit{Hha} I methyltransferase. No protein-induced bend was detected in the \textit{M. Hha} I–DNA complex (12), which was further confirmed by studies using circular permutation gel shift assay and scanning force microscopy (15). We constructed the plasmid pBend-GCGC and used it to synthesize permutated fragments with a single \textit{Hha} I recognition site (Fig. 1). In the gel shift assay the \textit{M. Hha} I complexes had essentially the same mobility indicating that \textit{M. Hha} I induced only a very slight bend (Fig. 2D). This finding increases the reliability of our data with \textit{M. Hae} III and \textit{M. Bsp} RI.

We extended these studies to three other C5-MTases: \textit{M. Sin} I (GGWC C), \textit{M. Sau} 96I (GGNC C) and \textit{M. Hpa} II (CC GG). We chose \textit{M. Sin} I and \textit{M. Sau} 96I because the recognition sites of these enzymes are related to the recognition site of \textit{M. Bsp} RI/\textit{M. Hae} III and represent different degrees of degeneracy. \textit{M. Hpa} II was chosen because it shares recognition specificity with \textit{M.Msp} I (C CGG), an enzyme which had previously been shown to cause a very sharp DNA bend (Table 1) (16).

Because, already at a relatively early stage of this work, the available data suggested a connection between the magnitude of the methyltransferase-induced DNA bend and the base
recognized 3′ to the target cytosine (see Discussion), we thought that it would be interesting to test M.HpaII which differs from M.MspI in the methylated base. To generate permuted sets of fragments with single binding sites for M.SinI and M.HpaII, the plasmids pBend-GGWCC and pBend-CCGG (Fig. 1) were constructed. M.SinI has two recognition sites: GGG/CCC and GGWCC. For the GGWCC site, fragments amplified from the pBend-GGCC template were used because the GGCC site in the plasmid overlaps with a GGSCC sequence (Fig. 1).

All three enzymes produced mobility shift patterns which are characteristic for protein-induced bending (Fig. 5). The bend angles were estimated to be 30°, 34–37° and 52–57° for M.HpaII, M.SinI and M.MspI, respectively (Table 1). The M.MspI methyltransferase bent fragments containing either recognition site to a similar degree. Perhaps the most interesting observation in these experiments was that M.HpaII induced only a moderate curve in substrate DNA. This contrasts drastically with the bend characterizing the M.MspI complex (Table 1) (16).

**DISCUSSION**

DNA bending is a widely used mechanism to establish specific interface between proteins and DNA. From among DNA methyltransferases, two DNA (adenine-6)-DNA methyltransferases (M.EcoRV and M.EcoRII) and two C5-MTases (M.HhaI and M.MspI) have been studied in this respect. M.EcoRII and M.EcoRV were found to bend DNA in the specific recognition complex by 52 and 60°, respectively (15,35). Phasing experiments indicated that the direction of the M.EcoRV-induced bend was toward the major groove (35). M.HhaI was found not to bend DNA (15), whereas M.MspI induced a very sharp bend (Table 1) (16).

In this paper we report experiments analyzing the bending properties of five other C5-MTases. Our results indicate that different C5-MTases bend DNA to different extents. To verify our findings, we tested M.HhaI, which had previously been shown by two independent methods to induce only a 2° bend (15). We obtained a bend value of 13° which, at first glance, seems to contradict observations by the other group. We believe that 13° is the correct value and there is no contradiction between the two sets of data. Garcia et al. (15) used two methods, circular permutation assay and scanning force microscopy, to assess bending by M.HhaI. In their gel shift experiments only two fragments were used whereas our data are based on the use of six fragments, which should allow a better assessment of the bend angle. More importantly, their data obtained by scanning force microscopy (figure 6B in ref. 15) seem to be more consistent with a bend angle of 10–15°, than with an angle of 2°. Thus, the experiments with M.HhaI encourage us to believe that results obtained in this work are reliable.

The M.HaeII-induced bending derived from the electrophoretic behavior of the recognition complex contrasts with the cocrystal structure in which the DNA was straight (13). The reason for this discrepancy is not clear. It is important to note, however, that neither of the two complexes whose electrophoretic behavior we studied (M.HaeII with unmethylated and with fully methylated DNA) was identical with the complex described in the crystal structure, because the latter one was a covalent intermediate. We are aware of studies showing that certain DNA binding proteins can produce anomalous electrophoretic behavior, which can be incorrectly interpreted as protein-induced bending. Such behavior was observed for some members of the leucine zipper family and the anomalous electrophoretic mobility was attributed to the effect of rigid protein motifs (36) or extended shapes of the protein (37). However, neither the shape of M.HaeII (13), nor the specific conditions of the phasing experiment [the distance between the binding site and the A-tract (37)] would explain an
anomalous electrophoretic behavior. Moreover, in control experiments, \textit{M.HhaI} which has the same overall structure as \textit{M.HaeIII} (7,12,13), caused only a very small bend under similar conditions. We suggest that the discrepancy between our data and the crystal structure reflects a genuine difference in the DNA conformation characterizing the covalent intermediate and the two complexes we studied by the gel shift method. Alternatively, it is possible that the DNA was distorted (straightened) during the crystallization process either due to the high concentration of cations used in the crystallization method or because of crystal packing forces favoring straight DNA (38).

What function could DNA bending have in the action of a DNA methyltransferase? In addition to a possible role in sequence-specific DNA recognition, it may play a role in base flipping. During base flipping the target base is rotated out from the helix by 180°. A connection between bending and base pair opening was indicated by molecular modeling. It was shown that DNA bending facilitates base pair opening and that, conversely, once a base pair is disrupted, DNA can bend very easily (39). This leads to the question whether bending is a cause or a consequence of base flipping. One of the alternatives is that DNA bending is a mechanism by which the enzyme overcomes the forces holding the target base in the helix. Alternatively, bending may follow base flipping in time and its role may be to stabilize the DNA structure after the base has flipped out. The second model seems to be more likely. First, base stacking energy values do not support the first model. Using

**Figure 5.** (A, B, C and D) Gel electrophoretic mobility of methyltransferase–DNA complexes as a function of the position of the methyltransferase binding site. Restriction enzymes used to generate the permuted fragments are indicated above the lanes. C, methyltransferase–DNA complex; F, free DNA fragment.
energy levels derived from quantum-chemical calculations for B DNA (40), the total (intrastrand plus interstrand) stacking energies between a cytosine base and its neighboring base pairs in an NCCG context are –6.82, –5.52, –7.81 and –5.75 kcal/mol for sequences with N = A, C, G and T bases, respectively. The stacking energy for cytosine in a CCG context is –6.17 kcal/mol. These values do not explain for example, why M.MspI induces a much greater bend than M. HpaII (Table 1). Secondly, structural models suggest that C5-MTases flip the cytosine through the minor groove (12,13,41). Molecular modeling indicates that base rotation toward the minor groove is energetically much less favorable than toward the major groove, and leads to steric crowding of the atoms involved in the base pair hydrogen bonding (39). Therefore, if C5-MTase-induced bending is toward the minor groove, as our phasing experiments with M. HaeIII seem to indicate, bending should inhibit rather than facilitate base rotation. We suggest that bending follows base flipping and its role would be to stabilize the DNA helix in the recognition complex. All three enzymes so far investigated with hydroxyl radical footprinting yielded footprints, which were asymmetrically located relative to the recognition sequence, however, the direction of the shift was opposite for M. BspRI and for two enzymes (M. HhaI and M. SssI) from the other subgroup. Given the common architecture shared by C5-MTases (7), these data are surprising and may indicate substantial differences in the mechanisms by which C5-MTases interact with substrate DNA. Recognition of different sequences should obviously be reflected in the structure of the complex, but the variance seen in the available crystal structures, the magnitude of protein-induced bending and footprint phenotypes suggests greater differences.

Future crystallographic studies will reveal whether the suggested classification is correct and reflects the molecular details of the recognition complex. We predict that monospecific C5-MTases recognizing a guanine 3′ to the target cytosine do not cause, beyond base flipping, significant distortion of the recognition sequence, whereas enzymes with cytosine in this position, induce a greater distortion. The predicted distortion is likely to be the same as what was seen in the M. HaeIII–DNA complex (13). Obvious candidates for such studies are M. HpaII and M. MspI.

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REFERENCES


Table 1. Selected features characterizing the C5-MTase–DNA interaction

<table>
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<tr>
<th>MTase</th>
<th>Recognition site</th>
<th>X-ray structure</th>
<th>Bend</th>
<th>-OH footprint shifted to</th>
<th>Reference</th>
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<tr>
<td>HhaI</td>
<td>GCGC</td>
<td>base flipping</td>
<td>2°, 13°</td>
<td>5°</td>
<td>(12,15); this work</td>
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<td></td>
<td></td>
<td>5°</td>
<td>(45)</td>
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<td>30°</td>
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<td>HaeIII</td>
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<td>40–43°</td>
<td>3°</td>
<td>(13); this work</td>
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<tr>
<td>BspRI</td>
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<td></td>
<td>46–50°</td>
<td>3°</td>
<td>(31); this work</td>
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<tr>
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<tr>
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<td>Sau96I</td>
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<td>130–140°</td>
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The DNA stretch, which is covered by the enzyme in the recognition complex. All three enzymes so far investigated with hydroxyl radical footprinting yielded footprints, which were asymmetrically located relative to the recognition sequence, however, the direction of the shift was opposite for M. BspRI and for two enzymes (M. HhaI and M. SssI) from the other subgroup. Given the common architecture shared by C5-MTases (7), these data are surprising and may indicate substantial differences in the mechanisms by which C5-MTases interact with substrate DNA. Recognition of different sequences should obviously be reflected in the structure of the complex, but the variance seen in the available crystal structures, the magnitude of protein-induced bending and footprint phenotypes suggests greater differences.

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