



Interaction of *EcoP15I* DNA Methyltransferase with Oligonucleotides Containing the Asymmetric Sequence 5'-CAGCAG-3'

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EcoP15I DNA methyltransferase (Mtase) recognizes the asymmetric sequence CAGCAG and catalyzes the transfer of a methyl group from *S*-adenosyl-L-methionine to the second adenine residue. We have investigated the DNA binding properties of *EcoP15I* DNA Mtase using gel mobility shift assays. *EcoP15I* DNA Mtase binds approximately threefold more tightly to DNA containing its recognition sequence, CAGCAG, than to non-specific sequences in the absence or presence of cofactors. Interestingly, in the presence of ATP the discrimination between specific and non-specific sequences increases significantly. These results suggest for the first time a role for ATP in DNA recognition by type III restriction-modification enzymes. In addition, we have shown that bromodeoxyuridine-containing oligonucleotides form complexes with *EcoP15I* DNA Mtase that are crosslinked upon irradiation. More importantly, we have shown that the crosslink site is at the site of DNA binding, since it can be suppressed by an excess of unmodified oligonucleotide. *EcoP15I* DNA Mtase exhibited Michaelis-Menten kinetics with both unmodified and bromodeoxyuridine-substituted DNA, with a higher specificity constant for the latter. Furthermore, gel mobility shift assays showed that proteolyzed *EcoP15I* DNA Mtase formed a specific complex with DNA, which had similar mobility as the native protein-DNA complex. Taken together these results form the basis for a detailed structure-function analysis of *EcoP15I* DNA Mtase.

Keywords: DNA methyltransferase; type III restriction modification; *S*-adenosyl-L-methionine; DNA-protein interactions

1. Introduction

Sequence-specific recognition of DNA by proteins is involved in many of the fundamental processes that occur inside cells including replication, packaging, recombination, restriction, DNA repair and transcription. Proteins that interact with particular target sequences in DNA may show sequence selectivities ranging from stringent to fairly permissive, depending on the requirements imposed by their functions. Restriction and modification enzymes are attractive model systems for studying the mechanisms of interactions of proteins with specific DNA sequences. The restriction endonuclease and the cognate methyltransferase (Mtase†) recognize the same DNA sequence although each

catalyzes quite a different reaction (Wilson & Murray, 1991). Perhaps the best studied enzymes are the *EcoRI* and *EcoRV* endonucleases, on which a variety of studies have been carried out. Crystallographic lattices of the complexes of *EcoRI* and *EcoRV* endonucleases containing their respective recognition sequences have shown that the specific binding is facilitated by a small number of specific hydrogen bonds and van der Waals interactions between functional groups of amino acid side-chains and nucleotide bases (Rosenberg, 1991; Winkler, 1992).

In contrast to the restriction endonucleases, few comparable studies of DNA binding by DNA Mtases have been reported (Bergerat & Guschlbauer, 1990; Dubey & Roberts, 1992; Taylor *et al.*, 1993; Powell *et al.*, 1993). None of the known DNA binding motifs has been detected by inspection of the protein sequences of several Mtases and yet exquisite specificity in recognizing DNA is achieved. We have focussed on the *EcoP15I* DNA methyltransferase (*EcoP15I* DNA Mtase) as a model system to study protein-DNA interactions. *EcoP15I* DNA Mtase is part of the type III restriction-modification system (Bickle & Krüger, 1993). The type III restriction and modification

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† Abbreviations used: Mtase, methyltransferase; AdoHcy, *S*-adenosyl-L-homocysteine; AdoMet, *S*-adenosyl-L-methionine; BrdUrd, bromodeoxyuridine; DMS, dimethylsulphurimidate; K_d , dissociation constant; R-M, restriction-modification.

(R-M) systems *EcoPI*, *EcoP151* and *HinfIII* and recently isolated *StyLTI* (De Backer & Colson, 1991) form a small family of closely related enzymes that nevertheless recognize different DNA sequences. Type III R-M systems are multifunctional proteins that exert both methylation and restriction activities. They are composed of two different polypeptides, the products of *res* and *mod* genes, and require ATP and Mg^{2+} as cofactors for restriction (Bickle & Krüger, 1993). It has been shown that only one of the two subunits of these enzymes is involved in DNA sequence recognition in both the restriction and modification reactions; namely, that coded by the *mod* gene (Hadi *et al.*, 1983). The *mod* gene product is also a modification methylase. In the case of *EcoP151* DNA Mtase it methylates the second adenine nucleotide in the canonical site 5'-CAGCAG-3' with *S*-adenosyl-L-methionine (AdoMet) as the cofactor (Meisel *et al.*, 1991). We have recently expressed the *EcoP151 mod* gene at high levels under the control of the P_L promoter and have purified the *EcoP151* DNA Mtase to near homogeneity (Rao *et al.*, 1989). With the availability of large amounts of purified *EcoP151* DNA Mtase, we are interested in understanding how the canonical DNA sequence is recognized and modified by *EcoP151* DNA Mtase. Here, we report the results of studies of the interaction of *EcoP151* DNA Mtase with its duplex DNA recognition sequence CAGCAG, using electrophoretic mobility shift assays and UV crosslinking studies.

2. Materials and Methods

(a) Bacterial strains and plasmids

Escherichia coli WA3782 (*recA*) was used as a transformation host for the expression construct pDN8 (Rao *et al.*, 1989).

(b) Chemicals

Adenosine triphosphate (ATP), dimethylsuberimidate (DMS), *S*-adenosyl-L-methionine (chloride salt), *S*-adenosyl-L-homocysteine (AdoHcy), sinefungin and *Staphylococcus* protease V8 were obtained from Sigma Chemical Co., U.S.A. [*Methyl*- 3H]AdoMet (80 Ci/mmol) was purchased from Amersham, England. Sources for all other chemicals and bacteria used in this study have been described earlier (Rao *et al.*, 1989). The calibration kit for molecular masses of proteins for SDS/polyacrylamide gels was purchased from Pharmacia, Sweden. Centricon 30 microconcentrator units were purchased from Amicon, U.S.A. [α - ^{32}P]dATP (3000 Ci/mmol) and [γ - ^{32}P]ATP (3500 Ci/mmol) were purchased from Bhabha Atomic Research Center, Bombay, India. Deoxynucleoside triphosphates were from Pharmacia, Sweden. Bacteriophage T4 polynucleotide kinase, and Klenow DNA polymerase were obtained from Boehringer Mannheim, Germany. All other chemicals used were of the highest purity reagent grade.

(c) Purification and assay of *EcoP151* DNA methyltransferase

EcoP151 DNA Mtase was purified from *E. coli* strain WA3782 carrying plasmid pDN8 as described (Rao *et al.*,

1989). All Mtase assays monitored incorporation of tritiated methyl groups into pUC19 DNA and the specific activity of the enzyme was measured as described (Rao *et al.*, 1989). Mtase assays with oligonucleotides as DNA substrates monitored incorporation of tritiated methyl groups into DNA using a modified ion-exchange filter assay (Rubin & Modrich, 1977). Briefly, reactions contained 100 mM Hepes (pH 8.0), 0.25 mM EDTA, 6.4 mM $MgCl_2$, 11 mM 2-mercaptoethanol, 1 μM [*methyl*- 3H]AdoMet and DNA (as indicated). After incubation at 30°C, reactions were stopped by transferring aliquots to small Whatman DE 81 filter-paper discs that had been soaked in 50 μM AdoMet and dried. Filters were washed five times (five minutes each) with 0.2 M ammonium bicarbonate solution equilibrated at 4°C, thrice with 95% (v/v) ethanol and once with diethyl ether. Filters were air-dried and the tritium content was determined in 7 ml of scintillation fluid (0.5%, 2,5-diphenyloxazole and 0.05% 1,4-bis[2-(5-phenyloxazolyl)]benzene in 1:1 (v/v) 2-methoxyethanol and toluene) using an LKB RackBETA model II liquid scintillation counter.

(d) Chemical crosslinking of *EcoP151* DNA Mtase

Stock solutions of dimethyl suberimidate (25 mg/ml) were made in 1 M triethanolamine (titrated to pH 8.5 with HCl) immediately before use. Crosslinking reactions were carried out as follows. Increasing amounts of *EcoP151* DNA Mtase was preincubated on ice for 15 minutes. DMS was then added to the protein solution to a final concentration of 5 $\mu g/\mu l$ and the solution was incubated at room temperature (approximately 25°C) for one hour. During this period, 1 μl of DMS stock solution was added to the reaction every 15 minutes. The reaction products were separated by electrophoresis on a denaturing polyacrylamide gel (0.1% (w/v) SDS, 7% (w/v) polyacrylamide) and visualized by silver staining.

(e) Oligonucleotides and radiolabeling

Oligonucleotides were synthesized on a Pharmacia automated DNA synthesizer Gene Assembler plus, employing B-cyanoethyl phosphoramidite chemistry. The oligonucleotides were purified on a 20% polyacrylamide gel containing 8 M urea. The gel was UV shadowed and the desired oligonucleotide eluted with TE buffer (10 mM Tris-HCl (pH 8.0), 1 mM EDTA). The following oligonucleotides were used:

Duplex I:

5' -TAGGTCAGAATTCAGCAGACCCTAAGTAGCC-3'
3' -ATCCAGTCTTAAGTCGTCGGGATTCATCGG-5'

Duplex II:

5' -TAGGTCAGAATTCAGCAGACCCTAAGTAGCC-3'
3' (ATCCAGTCTTAA)GBCGTCGGGATTCATCGG-5'

where B stands for bromodeoxyuridine and the sequence in parentheses was generated by completing the strand using Klenow DNA polymerase.

Duplex III:

5' -TAGGTCAGAATTCAGCTGACCCTAAGTAGCC-3'
3' -ATCCAGTCTTAAGTCGACTGGGATTCATCGG-5'

The underlined regions of the oligonucleotides represent the *EcoP15I* DNA Mtase recognition site. Duplex I was used for specific DNA binding assay. Duplex II was also used for specific DNA binding assay as well as for UV crosslinking studies. In the bottom strand of duplex II, one of the thymine bases in the *EcoP15I* recognition sequence is replaced by a bromouracil (B). Duplex III used to detect non-specific binding contained the sequence CAGCTG which differs from the recognition sequence CAGCAG.

Basic procedures for purification and labeling of oligonucleotides were from Sambrook *et al.* (1989). Briefly, following synthesis, each oligonucleotide was gel-purified as described in the earlier section and recovered by extraction with butanol and precipitation with ethanol. Duplex II was annealed and labeled with [α - 32 P]dATP by completing the strand using the Klenow DNA polymerase to give a radiolabeled, fully double-stranded oligonucleotide of 31 base-pairs. Duplex I and III were labeled with [γ - 32 P]ATP using T4 polynucleotide kinase (Sambrook *et al.*, 1989). Unincorporated ATP was removed by precipitating the mixture in ethanol. The specific activity of the radiolabeled duplexes varied from experiment to experiment but the variation was not significant. These labeled oligonucleotides were then gel-purified on 20% polyacrylamide gels containing 8 M urea, run in TBE buffer (89 mM Tris, 89 mM boric acid, 1 mM EDTA, pH 8.0), eluted in TE buffer (10 mM Tris-HCl (pH 8.0), 1 mM EDTA), recovered by precipitation in ethanol and stored at -70°C .

(f) Electrophoresis

Non-denaturing polyacrylamide gels were used for the electrophoretic mobility shift assays (Fried, 1989). Oligonucleotide duplexes labeled with [α - 32 P]dATP or [γ - 32 P]ATP were incubated for ten minutes on ice in binding buffer (50 mM Tris-HCl (pH 7.5), 20 mM NaCl, 10 mM MgCl₂, 7 mM 2-mercaptoethanol, 10% (v/v) glycerol, 1 mM EDTA) and protein. Reaction volumes were typically 10 μ l. These were electrophoresed on 6% polyacrylamide gels in 0.5 \times TBE buffer. Electrophoresis was done at 6°C and at 90 V for three to five hours depending upon the separation required. Protein-DNA complexes formed were visualized by exposing the dried gels to X-ray film (24 to 72 hours) at -70°C using film cassettes containing intensifying screens.

(g) Limited proteolysis

Partial proteolysis of *EcoP15I* DNA Mtase was done as described (Ahmad & Rao, 1994). Briefly, *EcoP15I* DNA Mtase (1 μ g/ μ l) in buffer A (20 mM Tris-HCl (pH 8.0), 7 mM 2-mercaptoethanol, 40 mM NaCl, 0.2 mM EDTA) was incubated with 1% (w/w) *Staphylococcus* V8 protease (in 0.5 M sodium phosphate buffer, pH 7.5) at 30°C for 15 minutes. The digestion was stopped by adding SDS-sample buffer containing SDS followed by boiling at 100°C for three minutes. The samples were analyzed on 10% polyacrylamide gels containing 0.1% SDS, run in Tris-glycine buffer (Laemmli, 1970). The gels were stained with Coomassie blue and destained. In another set of experiments, *EcoP15I* DNA Mtase was digested with V8 protease as described above. One half of the reaction mixture was used directly in subsequent gel mobility shift assays and the other half of the reaction mixture was analyzed on a 6% polyacrylamide gel under non-denaturing conditions. These gels were stained with Coomassie blue and destained.

(h) Photochemical crosslinking of bromodeoxyuridine-containing oligonucleotide and *EcoP15I* DNA Mtase

Labeled duplex II and *EcoP15I* DNA Mtase in up to 20-fold molar excess were irradiated at 4°C in the binding buffer as described earlier, except that 2-mercaptoethanol and glycerol were omitted. For this purpose, 10 μ l samples were pipeted into a microtiter plate and then irradiated for five minutes in the dark with a hand-held UV lamp (254 nm, 4 W) placed on top of the plate. Polyacrylamide gel electrophoresis (10%) with gels containing 0.1% SDS was carried out (Laemmli, 1970) for analysis of the reaction products. After electrophoresis, gels were dried and exposed at -80°C for 24 hours to X-ray films using an intensifying screen.

(i) Miscellaneous procedures

AdoMet was purified using a Biorex 70 cation-exchange column and stored at -20°C in 0.1 M HCl (Reich & Mashhoon, 1990). Protein estimation was done by using bovine serum albumin as standard (Bradford, 1976).

3. Results

(a) *EcoP15I* DNA Mtase exists as a dimer

EcoP15I DNA Mtase (1 mg/ml) eluted from a FPLC Superose 6 column in a symmetric peak at a position corresponding to a globular protein with a relative molecular mass (M_r) of approximately 150,000 (data not shown). The subunit M_r of the *EcoP15I* DNA Mtase as determined by SDS-PAGE is 75,000 (Humbelin *et al.*, 1988) and, therefore, these results are consistent with the fact that the enzyme exists as a dimer under native conditions. The enzyme eluted as a dimer in the concentration range 0.25 to 2 mg/ml (data not shown). The dimeric structure of *EcoP15I* DNA Mtase was confirmed by chemical crosslinking of the subunits by treatment with dimethyl suberimidate. We observed that the DMS-treated *EcoP15I* DNA Mtase (Figure 1) migrated as a diffuse band with a relative molecular mass of 180,000. It has been reported that DMS-treated proteins migrate as a smear in an SDS-PAGE experiment due to non-uniform modification of the protein population by DMS, which results in non-specific binding of SDS (Davies & Stark, 1970). It is evident also that increasing concentrations of *EcoP15I* DNA Mtase in the crosslinking reaction mixture resulted in an increase in the DMS-crosslinked *EcoP15I* DNA Mtase (Figure 1). This increase is further enhanced when crosslinking of *EcoP15I* DNA Mtase is done in the presence of duplex I and sinefungin (data not shown). No tetramer or other greater molecular mass species could be obtained by crosslinking with DMS and the overall efficiency of the reaction is low.

(b) Interaction of *EcoP15I* DNA Mtase with oligonucleotides containing *EcoP15I* recognition sequence

We have monitored the extent of binding of *EcoP15I* DNA Mtase to DNA by using a fixed

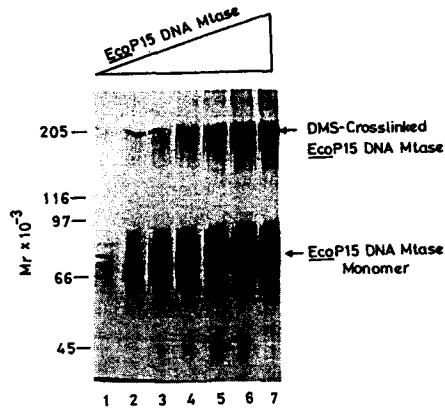


Figure 1. Crosslinking of *EcoP15I* DNA Mtase using DMS. Increasing concentrations of *EcoP15I* DNA Mtase were incubated in 0.5 M triethanolamine HCl (pH 8.5) with DMS at final concentration of 5 $\mu\text{g}/\mu\text{l}$ for one hour at ambient temperature. The reaction volume was 20 μl . At 15 min intervals 1 μl of DMS stock solution was added and incubation continued. The reaction products were analyzed on a 7% polyacrylamide gel containing 0.1% SDS. Proteins were visualized by silver staining. Lane 1, 100 $\mu\text{g}/\text{ml}$; lane 2, 200 $\mu\text{g}/\text{ml}$; lane 3, 300 $\mu\text{g}/\text{ml}$; lane 4, 400 $\mu\text{g}/\text{ml}$; lane 5, 500 $\mu\text{g}/\text{ml}$; lane 6, 750 $\mu\text{g}/\text{ml}$; lane 7, 1 mg/ml.

concentration of 31-mer oligonucleotide containing the specific recognition sequence 5'-CAGCAG-3' (duplex I) and varying concentrations of *EcoP15I* DNA Mtase. The reaction was carried out in the presence of 100 μM sinefungin and 10 mM MgCl_2 . Increasing amounts of protein increased binding of *EcoP15I* DNA Mtase to duplex I with a corresponding decrease in the amount of free

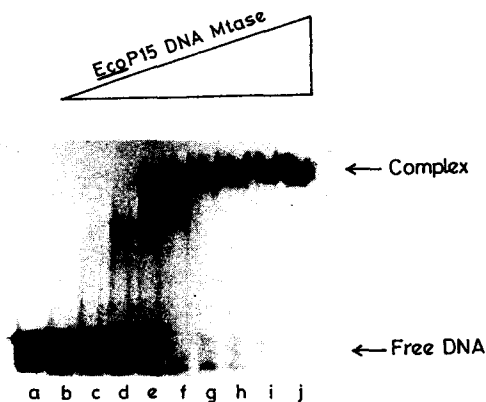


Figure 2. Binding of *EcoP15I* DNA Mtase to duplex I. The 5' end-labeled duplex I (100 nM) and increasing amounts of *EcoP15I* DNA Mtase (as indicated) were incubated in binding buffer (50 mM Tris-HCl (pH 7.5), 20 mM NaCl, 10 mM MgCl_2 , 7 mM 2-mercaptoethanol, 10% glycerol and 1 mM EDTA) containing 100 μM sinefungin for 10 min on ice. Samples were analyzed on 6% polyacrylamide gel as described in Materials and Methods. Lane a, no protein; lane b, 0.16 μM ; lane c, 0.33 μM ; lane d, 0.66 μM ; lane e, 1.0 μM ; lane f, 1.33 μM ; lane g, 2.0 μM ; lane h, 2.67 μM ; lane i, 3.33 μM and lane j, 4.0 μM .

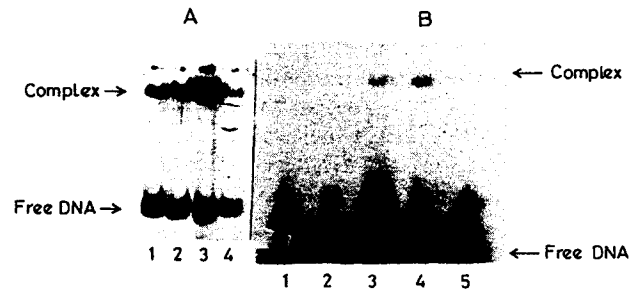


Figure 3. Effect of divalent metal ions on *EcoP15I* DNA Mtase binding to DNA. A, *EcoP15I* DNA Mtase binding to duplex I. The 5' end-labeled duplex I DNA (approximately 100 nM) was incubated with *EcoP15I* DNA Mtase (1.33 μM) in binding buffer containing 10 μM sinefungin (without MgCl_2) and analyzed as described in Materials and Methods. Lane 1, no metal ion; lane 2, 10 mM MgCl_2 ; lane 3, 10 mM MnCl_2 ; lane 4, 10 mM CaCl_2 . B, *EcoP15I* DNA Mtase binding to duplex II. *EcoP15I* DNA Mtase (0.66 μM) and the 31-mer ($\alpha\text{-}^{32}\text{P}$)-labeled duplex II (approximately 100 nM) were incubated in binding buffer containing 10 μM sinefungin and analyzed as described for A. Lane 1, no protein; lane 2, no metal ion; lane 3, 10 mM MgCl_2 ; lane 4, 10 mM MnCl_2 ; lane 5, 10 mM CaCl_2 .

oligonucleotide (Figure 2). The apparent dissociation constant, K_d , for binding of *EcoP15I* DNA Mtase to duplex I was estimated by determination of the Mtase concentration needed to complex half of the DNA under conditions where the Mtase was in excess over the DNA (Fried, 1989). The K_d from such an experiment was estimated to be 0.8 to 1 μM .

(c) *Binding of EcoP15I DNA Mtase to DNA in the absence and presence of divalent cations*

DNA-protein interactions are in many instances highly dependent on the concentration of monovalent and divalent cations. We were therefore interested in determining if any divalent metal cation was necessary for DNA binding. It is clear from Figure 3B that when duplex II was used in mobility shift assays, there was no complex formed in the absence of divalent cations (Figure 3B, lane 2). In the presence of 10 mM MgCl_2 , complex formation was seen (Figure 3B, lane 3). Increasing the concentration of Mg^{2+} in the binding buffer showed that as low as 1 mM Mg^{2+} was adequate for the enzyme to bind to duplex II (results not shown). In the presence of 10 mM MnCl_2 , the intensity of the band as a result of complex formation is at least two- to threefold more than that seen when Mg^{2+} was used as a divalent cation (Figure 3B, lane 4). In the presence of Ca^{2+} , weak binding was observed (Figure 3B, lane 5). Although we observed binding of *EcoP15I* DNA Mtase to duplex I in the absence of any divalent metal ions (Figure 3A, lane 1), MgCl_2 (10 mM) in the binding buffer increased the extent of binding (Figure 3A, lane 2). MnCl_2 (10 mM) in the binding buffer further increased the extent of binding of *EcoP15I* DNA Mtase to duplex I (Figure 3A, lane 3). The extent of binding in the presence of

CaCl₂ (10 mM) was about the same as without any metal ion (Figure 3A, compare lanes 2 and 4).

We monitored the methylation activity of the enzyme in the absence and presence of divalent cations. *EcoP15I* DNA methyltransferase catalyzed the methylation of DNA containing CAGCAG sequence (duplex I or II) using AdoMet as the cofactor only in the presence of Mg²⁺. In the presence of Mn²⁺ there was only <2% of the original activity (Table 1). It is possible that even the small amount of activity that was seen in the presence of Mn²⁺ could be due to methylation at non-cognate site(s). pUC19 has a unique *Pst*I site (CTGCAG), which differs from *EcoP15I* site by one base-pair change. If methylation occurred at this site, pUC19 should become resistant to cleavage by *Pst*I restriction endonuclease. However, this was not the case (results not shown).

(d) Kinetics of DNA methylation

It was essential to investigate the dependence of initial enzyme velocity on the concentration of DNA (duplex I and II) since *EcoP15I* DNA Mtase binds to duplex I and II in the absence and presence of divalent metal ions, respectively. We have previously determined the kinetic parameters for *EcoP15I* DNA Mtase with pUC19 as DNA substrate (Rao *et al.*, 1989). With increasing concentrations of duplex I or duplex II, *EcoP15I* DNA Mtase exhibited a rectangular hyperbola type of saturation with increasing concentrations of duplex I or duplex II (data not shown). The points were fit to a Michaelis-Menten curve using a non-linear, least-squares procedure. Table 1 shows the K_{cat} and K_m values obtained when duplex I and duplex II were used as DNA substrates. The fivefold increase in specificity constant (K_{cat}/K_m) with duplex II (Table 2) is due to a threefold increase in K_{cat} and a slight decrease in K_m . We could not detect any methylation activity when duplex III was used in these assays.

Table 1
Effect of divalent metal ions on *EcoP15I* DNA Mtase activity

Metal ion	Specific activity† (pmol/min per mg protein)
None	0
Mg ²⁺	53.3
Mn ²⁺	0.8

pUC19 DNA was used as the substrate.

†*EcoP15I* DNA Mtase activity was performed as described earlier (Rao *et al.*, 1989). pUC 19 DNA (6.6 µg) was incubated with 0.5 µM [*methyl*-³H]AdoMet and enzyme in the assay buffer as described earlier (Rao *et al.*, 1989) without metal ions or with MgCl₂ (6.6 mM) or MnCl₂ (10 mM) at 37°C for 30 min. Reaction was stopped by adding an equal volume of phenol (equilibrated with TE buffer) and the aqueous phase passed through a Sephadex G25 column.

Table 2
Kinetic constants for *EcoP15I* DNA Mtase

Substrate	K_{cat} (s ⁻¹)	K_m (µM)	K_{cat}/K_m (s ⁻¹ /µM)
Unmodified DNA (duplex I)	5.3×10^{-3}	0.8	6.6×10^{-3}
Bromodeoxyuridine substituted DNA (duplex II)	1.38×10^{-2}	0.5	2.76×10^{-2}

Duplex I or duplex II (0.1 to 4.0 µM) were incubated with [*methyl*-³H]AdoMet (1.0 µM) and enzyme in the assay buffer as described earlier (Rao *et al.*, 1989) for 30 min at 30°C. Samples were analyzed as described in Materials and Methods.

(e) Interaction of *EcoP15I* DNA Mtase with oligonucleotides containing the *EcoP15I* recognition sequence in the presence of AdoMet and its analogs

We examined DNA binding in the presence of AdoMet, AdoHcy, the end product of the reaction and sinefungin, an analog of AdoMet that is a known inhibitor of DNA methyltransferases. AdoHcy was earlier shown to competitively inhibit *EcoP15I* DNA Mtase (Rao *et al.*, 1989). In these experiments, the specific 31-mer oligonucleotide (duplex I) was incubated with increasing concentrations of AdoMet, AdoHcy or sinefungin. It is clear that the presence of increasing concentrations of AdoMet or its analogs, AdoHcy and sinefungin (0.1 µM to 10 µM) significantly increased the binding of the enzyme to duplex I (Figure 4). In the presence of sinefungin *EcoP15I* DNA Mtase showed highest binding affinity to duplex I (Figure 4, lanes 9 to 11).

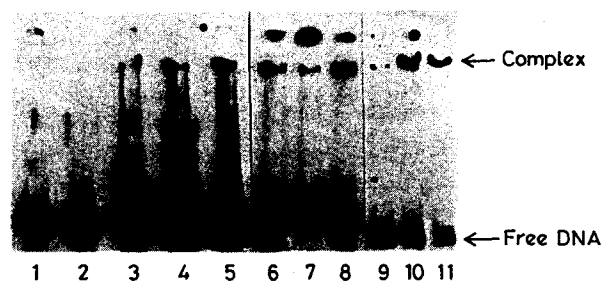


Figure 4. Effect of AdoMet, AdoHcy and sinefungin on binding of *EcoP15I* DNA Mtase to duplex I. Increasing amounts of AdoMet (as indicated) and its analogs were added during the binding reaction and analyzed as described in Materials and Methods. The concentration of 5' end-labeled duplex I was around 100 nM. Approximately 1.5 µM of *EcoP15I* DNA Mtase was used in all binding reactions. Lanes 1 to 5, effect of increasing concentrations of AdoMet on the binding of *EcoP15I* DNA Mtase to duplex I. Lane 1, no protein; lane 2, no AdoMet; lane 3, 0.1 µM AdoMet; lane 4, 1.0 µM AdoMet; lane 5, 10.0 µM AdoMet. Lanes 6 to 8, effect of increasing concentrations of AdoHcy on binding of *EcoP15I* DNA Mtase to duplex I. Lane 6, 0.1 µM AdoHcy; lane 7, 1.0 µM AdoHcy; lane 8, 10.0 µM AdoHcy. Lanes 9 to 11, effect of increasing concentrations of sinefungin on binding of *EcoP15I* DNA Mtase to duplex I. Lane 9, 0.1 µM sinefungin; lane 10, 1.0 µM sinefungin; lane 11, 10.0 µM sinefungin. The data shown are obtained from three different sets of experiments.