# Interaction of the type I methyltransferase M.*Eco*R124I with modified DNA substrates: sequence discrimination and base flipping

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We have analysed the DNA–protein contacts made between the type I DNA methyltransferase M.*Eco*R124I and its recognition sequence. The effects of base modifications have been probed by measuring the affinity of M.*Eco*R124I for the modified sequences relative to that for the wild-type sequence by using gel-retardation competition assays. These results, along with those from methylation interference footprinting and photo-affinity cross-linking have identified the location of potential DNA contacts within the DNA recognition site. Substitution of 6-thioguanosine for each of the three specific guanines in the recognition sequence leads to a large (10–20-fold) decrease in the strength of DNA binding, indicating the importance of hydrogen-bonding interactions in the major groove of DNA. In contrast, replacement of either (or both) of the adenines at the target site for methylation by the enzyme, to produce either a base pair mismatch or loss of the base, leads to a marked increase in DNA-binding affinity. The results strongly support the proposal that type I methyltransferases employ a base-flipping mechanism to methylate their target base.

Key words: restriction-modification, base analogues, gel retardation, DNA methylation.

## INTRODUCTION

Enzymes that methylate specific DNA sequences are ubiquitous in living cells, reflecting the importance of DNA methylation in processes as varied as gene expression, cell differentiation, restriction–modification and DNA repair. Structural and mechanistic studies so far have been most successful in the analysis of type II methyltransferases (MTases); in particular the crystal structure of M.*Hha*I complexed to DNA [1] first revealed the phenomenon of base flipping that was subsequently seen in a wide range of enzymes that interact with DNA [2].

Type I MTases are large and complex enzymes, having separate subunits for DNA recognition (HsdS) and methyltransferase activity (HsdM) (reviewed in [3,4]). In contrast with the compact and symmetrical 4–6 bp sequence typically recognized by type II enzymes, type I MTases specifically recognize an asymmetric bipartite DNA sequence in which the centres of the two halves of the recognition sequence are separated by approximately one helical turn of DNA. This is accomplished by means of two discrete target recognition domains in the HsdS subunit [5,6] that bind to each half of the DNA recognition sequence. The two target recognition domains are connected by a central 'conserved domain', which is homologous to repeated regions at the N- and/or C-termini of HsdS [7–9]. The HsdS subunit interacts with two HsdM subunits to form a large multisubunit complex [10,11], probably via the conserved domains of the former [12]. Deletion mutants of HsdS can give rise to enzymes with symmetrical DNA recognition sequences, suggesting that two copies of the truncated HsdS subunits are combined in the mutant enzymes [13,14]. Internal sequence homologies suggest a novel 'circular' organization of the HsdS domains, which locates the two HsdM subunits symmetrically with respect to the target sites on the DNA [15].

Two type I methyltransferases, M.*Eco*R124I and M.*Eco*KI, have been overexpressed and purified in sufficient quantities for detailed biochemical and biophysical analysis. Both enzymes have been well characterized in terms of their subunit composition, domain structure, DNA-binding characteristics and enzyme activity [10,11,16–19].

M.*Eco*R124I consists of two copies of the HsdM subunit (each 58 kDa) and one HsdS subunit (46 kDa), forming a trimeric enzyme (162 kDa) with a subunit stoichiometry of $M_2S_1$. M.*Eco*R124I recognizes the sequence $GAAN_6RTCG$, and binds to a 30 bp DNA duplex containing this sequence with high affinity ($K_d$ 10 nM) [16]. In common with all other type I MTases, the enzyme uses the cofactor *S*-adenosyl-L-methionine to methylate individual adenines at the N-6 position on opposite strands of the DNA recognition sequence. Methylation of the target adenine on either strand of the DNA recognition sequence leads to a 30-fold reduction in DNA-binding affinity [16].

Although it is clear from genetic evidence that HsdS is the sole determinant of DNA sequence specificity [6,20], the HsdS subunit alone is unable to bind its DNA recognition sequence in the absence of the HsdM subunit [21,22]. This observation supports the view that HsdM, in addition to its role in the catalytic methyltransferase reaction, is required for the correct positioning of the target recognition domains of the HsdS subunit so that they can interact with the two half-sites in the DNA recognition sequence [15]. Moreover, a large number of lysine residues in both the HsdM and HsdS subunits are protected from modification when the intact MTase binds its DNA recognition sequence, suggesting that the HsdM subunit makes numerous contacts with the phosphate groups of the DNA backbone [23]. Such non-sequence-specific DNA–protein interactions are presumed to make an important contribution to the affinity of the MTase for DNA, and are likely to contribute to the large structural change in the enzyme when it binds to DNA, as observed by small-angle X-ray scattering [24].

DNAse I footprinting experiments on M.*Eco*R124I complexed to DNA containing the recognition site have shown that the DNA is protected over an unusually large region, amounting to over 20 bp on both strands of the DNA helix [25]. However,

Abbreviations used: DMS, dimethyl sulphate; HsdM, MTase subunit for methyltransferase activity; HsdS, MTase subunit for DNA recognition; MTase, methyltransferase.

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high-resolution hydroxyl radical footprinting studies revealed that within this region the target bases for modification by the MTase are not protected from cleavage; on the contrary, they are cleaved much more rapidly than in free DNA, leading to the conclusion that there is a marked change in the structure of the sugar-phosphate backbone of the DNA (in the region around the target bases for enzyme modification) when the MTase binds [25]. This structural deformation is confined to only one strand in each half-site of the recognition sequence, leading to the suggestion that the adenine might be flipped out of the helix in a similar manner to that seen for the flipping of the cytosine in type II methyltransferases such as M.*Hha*I [1].

To test this hypothesis and to further map the contacts responsible for DNA recognition by M.*Eco*R124I, we have performed a series of competition gel-shift experiments with a variety of modified DNA substrates. On the basis of these experiments, together with methylation interference footprinting and DNA–protein cross-linking studies, we are able to establish the importance of individual bases for DNA binding and sequence recognition by M.*Eco*R124I.

## EXPERIMENTAL

### Protein purification

The HsdS and HsdM subunits of M.*Eco*R124I were over-expressed in *Escherichia coli* JM109 (DE3) from the plasmid pJS4M to produce the multisubunit methyltransferase enzyme [26]. The enzyme was purified to homogeneity from crude cell extracts by ion-exchange and heparin chromatography with the use of published procedures [10]. The purity of the sample was confirmed by SDS/PAGE, exclusion chromatography and by UV spectroscopy.

### Preparation of oligonucleotide duplexes

Oligonucleotides were purchased HPLC-purified from Oswel DNA Services or Genosys. Modified oligonucleotides containing 6-thioguanine were a gift from Professor B. A. Connolly (Newcastle University, Newcastle upon Tyne, Durham, U.K.) and purified by reverse-phase HPLC. The molar absorption coefficient of each oligonucleotide was determined by digestion to completion with snake venom phosphodiesterase and summing the contributions from individual nucleotides. This value was adjusted to give the corrected value for the intact oligonucleotide, taking account of the hyperchromicity observed after digestion [16].

In total, ten 30-mer oligonucleotides were prepared: the unmodified A strand; three variants on this sequence bearing thiol groups at guanines 8, 17 and 20; and two further variants with either a uracil or an abasic site at position 10 (in place of the target adenine) on the A strand. Additionally, as well as the unmodified B strand, two variants were made with either a uracil or an abasic site in place of the adenine at position 13, along with a single thiol variant of this sequence in which guanine at the 12 position was replaced by 6-thioguanine. Various pairwise combinations of these oligonucleotides were mixed in equimolar proportions to generate 30 bp double-stranded DNA fragments containing the *Eco*R124I recognition sequence. Eleven synthetic duplexes were prepared in total (Figure 1): one unmodified duplex (AB), three containing 6-thioguanine in the A strand (A8B, A17B and A20B), one that contained thioguanine in the B strand (AB12), three containing uracils at those sites that are the targets for methylation ($A^UB$, $AB^U$ and $A^UB^U$) and also their abasic equivalents ($A^-B$, $AB^-$ and $A^-B^-$) as described below.

Oligonucleotides containing an abasic site were prepared from synthetic oligonucleotides containing a uracil base at the desired site, after treatment with uracil DNA glycosylase (New England Biolabs). Successful removal of the uracil base was confirmed by alkaline cleavage of the abasic site on heating, by following published procedures [27].

Methylation interference footprinting required the use of a larger oligonucleotide; for this a 60 bp oligonucleotide and its complementary strand were synthesized. Strand-specific labelling was achieved by treating each of the oligonucleotides with polynucleotide kinase and [$\gamma$-$^{32}$P]ATP in their single-stranded form before mixing them with the unlabelled complementary strand and forming a duplex as described previously.

### Gel-retardation competition assays

Approx. 10 $\mu$g of duplex was labelled for use in a particular set of experiments. Duplex DNA was phosphorylated with T4 polynucleotide kinase (New England Biolabs) and [$\gamma$-$^{32}$P]ATP. Labelled nucleic acid was separated from unincorporated material by using 'Nuctrap' columns (Stratagene), precipitated with 2.5 vol. of ethanol and washed with 80 % (v/v) ethanol. The DNA pellets were resuspended in distilled water. The concentration of DNA was determined from its absorbance at 260 nm and the specific radioactivity was determined by Čerenkov counting in a liquid-scintillation counter (Packard).

In a typical experiment an equimolar mixture of methylase and labelled duplex (typically between 100 and 500 nM) was incubated with unlabelled competitor oligonucleotide duplexes at various concentration ratios so that the labelled DNA duplex could be competed off. Samples were mixed in binding buffer [10 % (v/v) glycerol/50 mM Tris/HCl (pH 8.2)/5 mM $MgCl_2$/1 mM dithiothreitol] and incubated at 4 °C for 20 min before loading on a 6 % (w/v) polyacrylamide native TAE gel [40 mM Tris/acetate (pH 7.4)/1 mM EDTA]. Gels were run at 100 V at 4 °C until the Bromophenol Blue marker dye had migrated two-thirds of the length of the gel. After electrophoresis, the gels were dried under vacuum; bands were detected by autoradiography.

The intensities of the bands were estimated initially by densitometry of the autoradiograph or, in later experiments, by using a PhosphorImager (Molecular Dynamics). From the concentration of the competitor DNA required to decrease binding by 50 %, the relative binding constants for the two duplexes was determined as described previously [16].

### UV cross-linking

A 30 $\mu$l drop of a 10 $\mu$M complex of M.*Eco*R124I bound to a $^{32}$P-labelled DNA duplex was prepared in the binding buffer used for gel-retardation experiments. This was spotted on Saran wrap and placed on top of a long-wave TL33 transilluminator. Samples were irradiated for between 5 and 20 min; the drop volume was maintained at approx. 30 $\mu$l by the addition of distilled water during the course of the irradiation and the drop temperature was maintained at all times between 18 and 23 °C. After irradiation, samples were analysed by SDS/PAGE. Protein in the cross-linked sample was revealed by staining with Coomassie Brilliant Blue and the DNA was detected by autoradiography. The efficiency of the cross-linking was estimated by comparison of the radioactivity in the cross-linked band with that in the free DNA band.

### Methylation interference footprinting

A 60 bp oligonucleotide containing the M.*Eco*R124I cognate site was uniquely labelled on one strand. The DNA was modified


**Figure 1 Synthetic oligonucleotide duplexes used in this study**

DNA strands are arbitrarily assigned A and B and are numbered 1–30 from 5′ to 3′. The positions of the 6-thioguanine-modified base (S), the uracil mismatch (U) and the abasic site (—) are indicated. The 60 bp DNA duplex was used for the DMS interference experiments. The base numbering system follows that for the 30-mer oligonucleotides, with the recognition sequence ($GAAN_6RTCG$) corresponding to positions 8–20 on strand A, and its complement corresponding to positions 11–23 on strand B. The two specific half-sites are shown in bold.

with dimethyl sulphate (DMS; Aldrich) for 1 min at room temperature; the reaction was quenched by the addition of 1/10 vol. of 250 mM dithiothreitol by following published procedures [17]. The modified DNA was then precipitated with ethanol and resuspended in distilled water to approx. 100 nM. An equimolar concentration of M.*Eco*R124I was added to the modified DNA; the sample was loaded on a 6% (w/v) non-denaturing gel to separate free and bound DNA. The bound DNA band was eluted from the preparative native gel by using the crush–soak method [28] and precipitated with ethanol. After cleavage with 10% (v/v) piperidine for 30 min at 90 °C, the samples were run on a 12% (w/v) denaturing gel, fixed in a solution of 10% (v/v) acetic acid/10% (v/v) methanol and detected by autoradiography. For the control lane, an identical reaction was performed in the absence of the MTase. This procedure was performed in independent experiments with either the A or B strand of the 60 bp DNA duplex labelled (see Figure 1).

## RESULTS

### Methylation interference footprinting

Methylation interference with DMS is a useful probe of interactions of a protein with the N-7 of guanine in the major groove [29] and has been used to probe DNA–protein contacts in the related methyltransferase M.*Eco*KI [17]. The results obtained from DMS interference footprinting of M.*Eco*R124I are shown in Figure 2. For the A strand, sequence analysis of DNA recovered from the bound fraction clearly showed that a number of bands were missing compared with the free DNA lane, indicating sites at which modifications interfered strongly with DNA binding. These sites included all three guanines located within the two specific binding regions (half-sites) of the DNA recognition sequence. In contrast, guanines located outside the two half-sites did not seem to interfere with DNA binding. For example, of the two adjacent guanines on the A strand at positions A16 and A17, only the modification at position A17 interfered with complex formation; modification of the preceding guanine (A16) located in the spacer region had no significant effect on DNA binding.

A similar set of results was obtained by footprinting with the B strand labelled. DNA from the bound fraction again showed absent bands corresponding to the single guanine located within the DNA recognition sequence. Modification of the guanine located within the spacer region of the DNA did not interfere with the binding when modified, as expected. Surprisingly, however, modification at position B10 just outside the recognition sequence did seem to prevent M.*Eco*R124I from binding to DNA [16]. This was the only base contact detected outside the two half-sites within the DNA recognition sequence.

### Effect of 6-thioguanosine modifications on DNA binding

The effects of modification of adenines within the recognition sequence of M.*Eco*R124I have previously been studied using

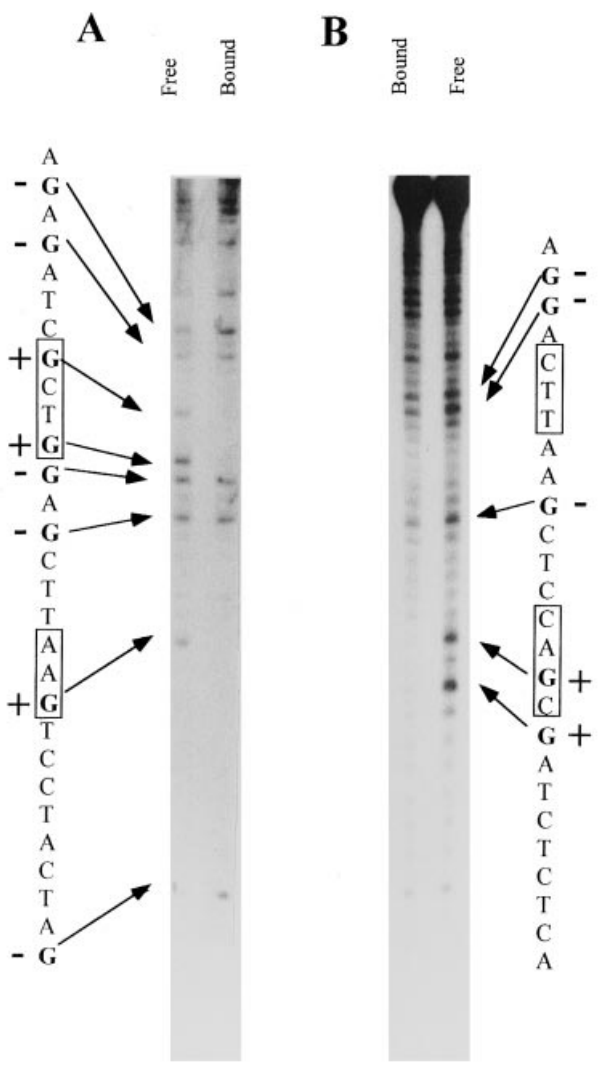


**Figure 2 DMS interference footprinting of M.*Eco*R124I**

The M.*Eco*R124I binding sites are boxed. Those sites that interfere with protein binding are indicated with (+); those that do not interfere are indicated by (−).

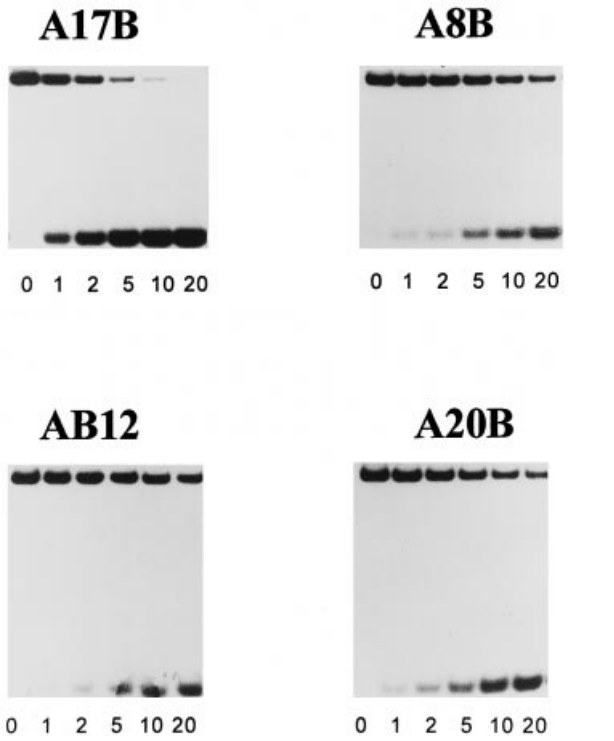


**Figure 3 Gel-retardation competition assay with 6-thioguanine modified DNA**

Competition gel shifts for the labelled oligonucleotide substrates AB in the presence of increasing amounts of the modified (unlabelled) DNA substrates A17B, A8B, AB12 and A20B. The molar concentration ratios of unlabelled to labelled DNA are shown under the appropriate lane on each gel.

**Table 1 Relative affinity of *Eco*R124I methylase for recognition sequences containing 6-thiodeoxyguanosine**

Base modifications were incorporated at specific locations within the enzyme recognition sequence (Figure 1) and the relative binding strengths of these modified substrates were estimated from the results shown in Figure 3. The relative affinity of the methylase for each substrate is expressed as the ratio of the equilibrium binding constant for the modified duplexes ($K_2$) to that for the unmodified duplex ($K_1$). Large values of $K_1/K_2$ reflect a decreased affinity for the modified DNA substrate.

| Duplex | $K_1/K_2$ |
|---|---|
| AB | 1 |
| A8B | 19 |
| AB12 | 21 |
| A17B | 2 |
| A20B | 11 |

gel-retardation competition assays [16]. We have applied the same methodology to investigating the binding of duplexes containing the 6-thioguanine modification, to elucidate the role of each guanine base within the DNA recognition sequence.

The competition assays for the 6-thioguanine substitutions are shown in Figure 3 and the results are summarized in Table 1. These results show that guanines modified at A8 or B12 greatly decrease the DNA binding affinity ($\approx$ 20-fold). Modification at A20 also decreased the affinity significantly (11-fold). In contrast, modification of the guanine at position A17 had very little effect on the DNA-binding affinity. Thus the degree of sequence discrimination at this site was less than at other sites in the recognition sequence.

### Binding to abasic and uracil mismatched DNA substrates

Results obtained previously from hydroxyl radical footprinting revealed an unusual hypersensitivity to cleavage by the hydroxyl radical footprinting probe at the two adenines (one per strand) that are the sites for methylation [25]; one possible explanation was that the two target adenine bases could flip out of the DNA helix. It has been reported that for the cytosine methyltransferase, M.*Hha*I, the enzyme binds with an increased affinity to mismatched bases at the target cytosine [30]. We wished to investigate the effects of base substitution at the target adenine base (or complete removal of the base) on the DNA-binding affinity of M.*Eco*R124I. We therefore separately replaced each of the two target adenines in the M.*Eco*R124I recognition site by uracil (forming U·T mismatches at each site). Additional DNA

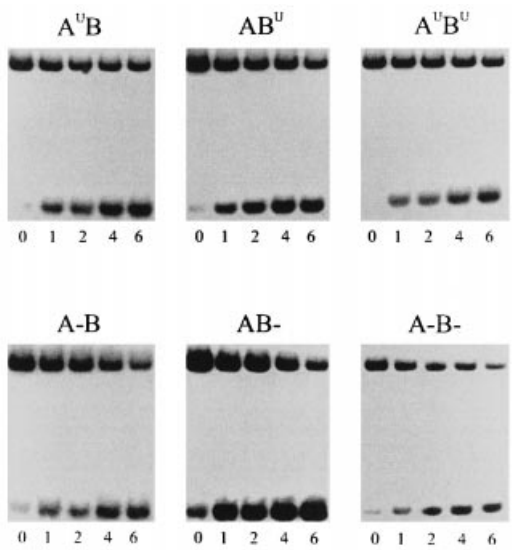


**Figure 4 Competition gel-retardation assay with labelled abasic substrates (bottom panels) and labelled uracil mismatched substrates (top panels)**

Complexes were formed with M.*Eco*R124I and the modified substrates at a 1:1 ratio of protein to DNA. Increasing concentrations of unlabelled wild-type duplex were added as competitor. The molar concentration ratios of unlabelled to labelled DNA are shown under the appropriate lane on each gel.

**Table 2 Relative affinity of *Eco*R124I methylase for recognition sequences containing abasic or mismatched sites**

Uracils or abasic sites were incorporated at specific locations within the enzyme recognition sequence (Figure 1) and the relative binding strengths of these modified substrates were estimated from the results shown in Figure 4. Analysis was carried out as described in Table 1 except that in this case the modified DNA was labelled and the wild-type duplex (AB) was used as competitor. The relative affinity of the methylase for each substrate is expressed as the ratio of the equilibrium binding constant for the modified duplexes ($K_2$) to that for the unmodified duplex ($K_1$). Large values of $K_2/K_1$ reflect an increased affinity for the modified DNA substrate.

| Duplex | $K_2/K_1$ |
|---|---|
| AB | 1 |
| $A^UB$ | 10 |
| $AB^U$ | 5 |
| $A^UB^U$ | 16 |
| $A^-B$ | 15 |
| $AB^-$ | 7 |
| $A^-B^-$ | 23 |

substrates were prepared in which the uracil base was removed by treatment with uracil DNA glycosylase (see the Experimental section).

The results of the competition assays with oligonucleotide duplexes bearing either abasic or uracil-substituted sites are shown in Figure 4. The relative binding affinities derived from these results are given in Table 2. Initial experiments were performed with modified duplexes as competitor; however, these experiments showed that competition was occurring with competitor concentrations less than that of the labelled DNA, indicating that the affinity of the MTase was increased by the modification. Subsequent experiments were therefore done with the modified duplex labelled, and competition was observed by the addition of unlabelled duplex AB. The results show quite clearly that (1) both U·T mismatch and abasic sites enhance the binding to the MTase (by a factor of 5–15 in comparison with the natural A·T base pair), (2) modification on the A strand has a more pronounced effect than that on the B strand, (3) abasic sites are bound slightly more strongly than mismatches, and (4) the modification of both strands leads to further enhancement of binding.

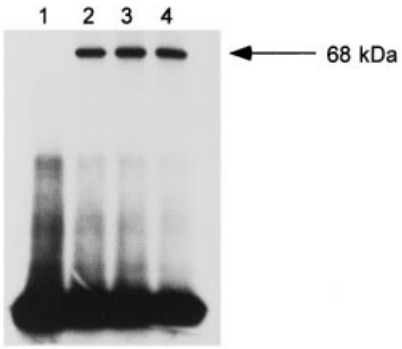


**Figure 5 SDS/PAGE analysis of the complex of M.*Eco*R124I with the oligonucleotide duplex A8B after cross-linking with UV**

Complexes were preformed at a concentration of 5 $\mu$M and irradiated for 0, 10, 20 and 30 min respectively (lanes 1–4). The molecular mass of the major product (68 kDa) was estimated by comparison with stained protein molecular mass markers.

### Cross-linking to 6-thioguanine

The modified base 6-thioguanine is photoreactive when irradiated with long-wave UV (340–360 nm) and can form covalent cross-links between DNA-binding enzymes and their cognate DNA sequences [31]. We used the thiol-containing sequences shown in Figure 1 as substrates in a photochemical cross-linking experiment with *Eco*R124I methylase. Complexes of M.*Eco*R124I with each duplex (1:1 molar ratio) were irradiated with long-wave UV for between 10 and 30 min. The products of the cross-linking reaction were analysed by SDS/PAGE. The proteins were revealed by staining the gel with Coomassie Blue and the nucleic acid was detected by autoradiography. All duplexes containing 6-thioguanine modifications gave rise to covalent DNA protein adducts, with 5–10% of the DNA being present in the cross-linked products. Of the various cross-linked DNA duplexes, only A8B produced a major well-defined band on the SDS gel (Figure 5); the other duplexes gave rise to a broad distribution of smeared bands (results not shown). The size of the covalently linked complex was estimated (with respect to molecular mass protein markers) as 68 kDa, corresponding to the size expected for an HsdS subunit (46 kDa) bound to the 20 kDa DNA duplex.

## DISCUSSION

Figure 6 shows a summary of the results of the methylation interference and competition binding experiments with 6-thioguanine-modified substrates. The competition gel-retardation assays show that modifying three of the four guanines in the DNA recognition site greatly perturbs binding. Therefore one can deduce that, at these sites, M.*Eco*R124I makes close contacts with the O6 of guanine in the major groove. In contrast, after modification at position A17 (the 5′ base of the tetranucleotide half of the recognition sequence), there is little if any decrease in binding affinity. This result is consistent with the

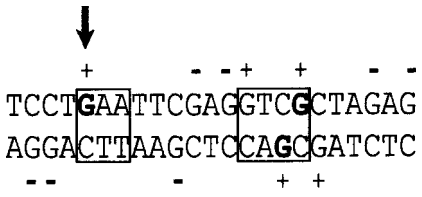


**Figure 6 Summary of results**

Those sites that result in a decrease in the binding affinity when modified to 6-thioguanine are indicated in bold. Those sites that interfere with protein binding after modification with DMS are indicated with (+); those that do not interfere are indicated by (−). The position of the cross-link formed on irradiation is indicated by an arrow.

weak discrimination shown by the enzyme [the requirement is only for a purine (R) at this position] because the *Eco*R124I R–M system can tolerate both A and G at this site (bearing amino and oxo groups respectively at the C-6 position of the purine base).

Methylation interference footprinting provides further evidence that recognition and binding of M.*Eco*R124I to its cognate DNA sequence occur in the major groove (where DMS methylates the N-7 of guanine). However, in contrast with the competition experiments with 6-thioguanosine, methylation interference experiments suggest that the guanine at position A17 is critical for binding. This implies that important contacts are made to the N-7 of the purine at this site; taken together with the results above, this provides an explanation of why only G or A is allowed at this position in the recognition sequence because there is no equivalent hydrogen-bond acceptor at this position in the pyrimidine bases.

The identification by DMS interference of the guanine at position B10 as an important contact was unexpected, because this base does not form part of the recognition sequence. Although there is no requirement for N-7 at this position in the DNA (i.e. pyrimidines, as well as purines, are allowed), space in the major groove in this region must be limited, such that the addition of a methyl group to the N-7 abolishes binding.

Photo-affinity cross-linking reveals a stable cross-link between the protein and the first guanine in the trinucleotide half site of the recognition sequence (A8). The duplex that contains the modification at A8B forms a well-defined covalent complex, with approx. 80% of the cross-linked product appearing as a single species. From the mobility of the labelled complex on the SDS/PAGE gel it seems likely that this cross-linked species is formed with the HsdS polypeptide (DNA recognition subunit) of the methylase and the double-stranded duplex (Figure 5). Similar experiments with a BrdU cross-linking agent identified a stable cross-link between the equivalent site within the recognition sequence of the related enzyme M.*Eco*KI to an amino acid residue in the N-terminal variable region of the DNA recognition subunit HsdS [32]. It is this region of HsdS that has been shown to be responsible for binding to the trinucleotide half of the DNA recognition sequence [6].

The phenomenon of base flipping has been widely reported for cytosine methyltransferases [1,33]. Results obtained from the binding of M.*Eco*R124I to mismatched or abasic DNA substrates strongly support the notion that this enzyme flips the target base out of the DNA helix and into the active site of the protein before methylation as suggested by hydroxyl radical footprinting experiments [25]. It has been reported that the type II cytosine methyltransferase, M.*Hha*I, binds more strongly to DNA substrates in which the target base is mismatched or the base has been removed [30]. Base analogue experiments with the type II adenine methyltransferase M.*Eco*RV produced similar results [34]. Our observations that M.*Eco*R124I binds more strongly to the U·T mismatched target site or an abasic site than to the wild-type A·T base pair strengthen the idea that base flipping might be a general mechanism employed by a wide variety of methyltransferases.

It has been proposed that the increase in binding affinity for non-Watson–Crick base pairs can be explained by the decrease in energy to break the base pair and flip the target base [30,34]. Any situation that weakens the stability of the base pair will result in the free energy gained being channelled into binding. It seems that for substrate binding, this effect predominates over base recognition by the enzyme, because replacement of the native purine (adenine) by a pyrimidine (uracil), which lacks all the functional groups of the former, is highly favourable. The requirement for an adenine at this site must therefore occur at the catalytic step.

It is interesting to compare these results with those in which the target adenines were replaced by 6-methyladenine [16]. Modification (on either strand) in this case leads to a 30-fold reduction in binding affinity, indicating that the N-6 methyl group cannot be accommodated into the active site of the enzyme (as indeed is required, because it is the product of the reaction and must be released). However, the same modification leads to an approx. 100-fold increase in the rate of methylation (at the other site). Thus, whereas the replacement of the target adenine by uracil leads to an increase in affinity and a decrease in enzyme activity, replacement by 6-methyladenine has precisely the opposite effect for both binding and activity.

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