Bacteriophage T4 Dam DNA-(N⁶-adenine)-methyltransferase

PROCESSIVITY AND ORIENTATION TO THE METHYLATION TARGET

We carried out steady state and pre-steady state (burst) kinetic analyses of the bacteriophage T4 Dam DNA-(N⁶-adenine)-methyltransferase (MTase)-mediated methyl group transfer from S-adenosyl-L-methionine (AdoMet) to Ade in oligonucleotide duplexes containing one or two specific GATC sites with different combinations of methylated and unmodified targets. We compared the results for ligated 40-mer duplexes with those of the mixtures of the two unliganded duplexes used to generate the 40-mers. The salient results are as follows: (i) T4 Dam MTase modifies 40-mer duplexes in a processive fashion. (ii) During processive movement, AdoMet complex is capable of undergoing a rapid reorientation to an enzymatically created GMTC site. (v) The inhibition potential of fully methylated sites 5′-GMTC/5′-GMTC is much lower for a 5′-GMTC/5′-GATC or modified 5′-GMTC site. (vi) The inhibition potential of fully methylated DNAMe site is consistent with an ordered bi-bi mechanism. (iii) T4 Dam processivity is consistent with an ordered bi-bi mechanism. (iv) Following methyl transfer at one site and linear diffusion to a hemimethylated dissociating from the DNA. However, in contrast to the steady state, here DNA Me⁴+ signifies departure from a methylated site with DNA Me⁴+ without physically dissociating from the DNA. (v) Following methyl transfer at one site and linear diffusion to a hemimethylated site, a reconstituted T4 Dam-AdoMet complex rapidly reorients itself to the (productive) unmethylated strand. T4 Dam-AdoMet cannot reorient at an enzymatically created GMTC site. (v) The inhibition potential of fully methylated sites 5′-GMTC/5′-GMTC is much lower for a long DNA molecule compared with short single-site duplexes.

Biological methylation of DNA plays an important role in the expression of genetic information. In eukaryotes, this process is essential for controlling transcription, genomic imprinting, developmental regulation, mutagenesis, DNA repair, and chromatin organization (1). In prokaryotes, Dam DNA methyltransferases (MTases)¹ regulate a number of cell functions (reviewed in Ref. 2). An essential biological role for DNA adenine methylation in determining bacterial virulence was discovered in Salmonella typhimurium, where dam mutants were found to be avirulent; such strains were effective in producing live vaccines against murine typhoid fever (3). In prokaryotes, other MTases are usually found as components of restriction-modification systems (4). Three kinds of DNA MTases are known to exist in prokaryotes, i.e. C5-cytosine (Cyt), N⁴-Cyt, and N⁶-adenine (Ade) MTases (1). The catalytic mechanism of methyl group transfer from the donor, S-adenosyl-L-methionine (AdoMet), in the case of C5-Cyt MTases, involves an intermediate in which the enzyme is covalently bound to the target Cyt (5). In contrast, N⁶-Ade and N⁴-Cyt MTases transfer a methyl group to the exocyclic amino group without forming such an intermediate. It appears, however, that all MTases access their target base by flipping it out of the DNA double helix, as first reported for HhaI MTase (6). In general, DNA MTases appear to be functional monomers, and one of their natural substrates is hemimethylated DNA, a product of semi-conservative DNA replication. Hence, one methyl group transfer is sufficient to methylate completely one specific (hemimethylated) site; this process is generally referred to as “maintenance” methylation. In this regard, productive interaction between a monomeric MTase and its hemimethylated target site is accomplished only after the enzyme is correctly oriented to the unmodified target-strand base.

Reich and Mashhoon (7), measuring single turnovers in the reaction catalyzed by EcoRI MTase, showed that there was no specificity in binding orientation; i.e. the enzyme methylated only 50% of the substrate-hemimethylated duplexes. However, a different result was obtained in pre-steady state kinetics of methylation of 20-mer duplexes by the bacteriophage T4 Dam (N⁶-Ade)-MTase. Equal bursts of product formation were observed with both a symmetric site GATC/GATC and a hemimethylated, asymmetric site, GATC/GMTC (where M represents N⁶-methyl Ade) (8). This was contrary to expectation for a monomeric enzyme binding randomly to its target site with respect to an unmethylated (productive) versus methylated (nonproductive) strand. We hypothesized that the T4 Dam-AdoMet complex is capable of undergoing a rapid reorientation following binding to the nonproductive DNA strand. Analogous results were obtained for the RsrI (9), HhaI (10), and EcoDam (11) DNA MTases.

Earlier, we investigated the kinetic characteristics of relatively short (20-mer) oligonucleotide substrates containing one specific site, native 5′-GATC/5′-GATC or modified 5′-GATC/5′-GATC (hemimethylated, or with substitutions/deletions of different structural elements in one of the two strands) (8, 12). These studies permitted us to characterize the interaction of T4 Dam with one specific site as well as the influence of defined modifications in site structure on kinetic parameters. In addition, we used fluorescence titration to investigate the interaction of T4 Dam with these 20-mer oligonucleotide duplexes in which one or two target Ade residues were substituted by 2-aminopurine (N), and we showed that AdoMet plays a crucial role in the methylation process.

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‡ The abbreviations used are: MTase, methyltransferase; AdoMet, S-adenosyl-L-methionine; AdoHcy, S-adenosyl-L-homocysteine; DNA Me⁴+, methylated DNA; M, N⁶-methyl Ade; N, 2-aminopurine.

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role in T4 Dam reorientation about the DNA duplex (13). The intensity of 2-aminopurine (N) residue fluorescence is low inside the double-stranded DNA helix; however, it increases sharply if the fluorophor is out of stacking interaction, permitting one to study the base flipping process induced by enzyme binding (14). The addition of T4 Dam at a saturating concentration to an unmethylated target (N/A duplex) or its methylated derivative (N/M duplex) resulted in an up to 50-fold increase in fluorescence (13). This indicated that T4 Dam binding promotes or stabilizes base (nucleoside) flipping out of the DNA helix. However, the addition of AdoMet sharply reduced the Dam-induced fluorescence with these complexes. In contrast, AdoMet had no effect on the fluorescence increase produced with an N/N doubly substituted duplex. Because the N/M duplex cannot be methylated, the AdoMet-induced decrease in fluorescence could not be due to methylation per se. We proposed that T4 Dam randomly binds to the asymmetric N/A and N/M duplexes and that AdoMet induces an allosteric T4 Dam conformational change, allowing a rapid reorientation of the enzyme to the strand that contains the unmethylated Ade. This capability is likely to be advantageous for more efficient maintenance methylation in vivo.

Natural in vivo DNA substrates are much longer and contain many potential methylation sites. Thus, in vitro methylation of short single-site duplexes is not going to take into account possible processive behavior of the DNA MTases; i.e. movement of the enzyme along the DNA via one dimensional (or linear) diffusion and carrying out multiple turnovers on the same substrate molecule (11, 15–16). To initiate a bridge between studies using short (20-mer) one-site DNA substrates and natural long DNA substrates, we constructed two-site DNA duplexes to compare their substrate characteristics with those of their one-site constituents. While this work was in progress, Urig et al. (11) reported that the EcoDam MTase acts in a processive manner on both synthetic duplexes and polymeric phage λ DNA.

EXPERIMENTAL PROCEDURES

Enzymes and Chemicals—[3H-CH3]AdoMet (15 Ci/mmol; 1 mCi/ml) was purchased from Amersham. Unlabeled AdoMet (Sigma) was purified further by chromatography on a C18 reversed phase column as described previously (17). Oligonucleotides (Table I) were synthesized by Integrated DNA Technologies, Inc. (Coralville, IA); their concentrations were determined spectrophotometrically. The duplexes were obtained by heating and annealing complementary oligonucleotide chains from 90 to 20 °C over 7–12 h. The 40-mer duplexes were constructed from corresponding short component duplexes using T4 polynucleotide kinase and T4 DNA ligase (SibEnzyme). T4 Dam MTase was purified to homogeneity as described previously (17). The protein concentration was determined by the Bradford method (18), which yielded values in close agreement with those determined spectrophotometrically at 260 nm from the known composition and molar extinction coefficients of individual aromatic amino acid residues in 6.0 M guanidinium hydrochloride and 0.02 M phosphate buffer, pH 6.5 (19).

DNA MTase Assay—DNA MTase assays were similar to those reported previously (20). T4 Dam reactions were carried out at 25 °C in buffer containing 100 mM Tris-HCl, pH 8.0, 1 mM EDTA, 1 mM dithiothreitol, 5% glycerol, and 0.2 mg/ml bovine serum albumin (17). The reactions were initiated by the addition of prewarmed T4 Dam to preincubated mixtures of [3H-CH3]AdoMet and substrate DNA. At appropriate intervals, aliquots (15 μl) were withdrawn from the mixture and spotted on a DE51 anion-exchange filter disc (Whatman, 1.5 cm). Filters were washed three times with 0.02 M NH2HCO3, twice with water, once with 75% ethanol, and then dried. They were counted in a liquid scintillator. The molar concentrations of [3H-CH3]groups incorporated into DNA were quantified as described previously (21). The validity of the quantification procedure was confirmed under control conditions (1 h at a 1:2 enzyme/substrate ratio) where the calculated number of [3H-CH3] groups incorporated into DNA coincided with the number of methylatable Ade residues. All experiments were done at least twice. Kinetic data were analyzed using the program Scientist 2.01 (MicroMath®) for regression analysis. The burst values (B) and steady state rate constants (kcat) were determined using the equation [3H-DNA]/[enzyme] = B + kcat/[S].

RESULTS AND DISCUSSION

Rationale for Determining T4 Dam Processivity—Investigation of the kinetics and processivity of methylation of oligomeric and concatemeric DNAs containing multiple GATC and (hemi) methylated GMTC sites can extend our understanding of the mechanism of site/strand selectivity. To approach these issues, we constructed substrates by ligating short, defined synthetic duplexes containing complementary single-stranded overhangs (Table II) and generated a series of 40-mer duplexes with different combinations of GATC and GMTC sites (Table III). For example, ligation of duplexes 1a/a and 2a/a (each containing a single symmetric unmodified site GATC/GATC) created the control prototype duplex A as illustrated below in Structure 1,

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5' -CATCCGAAATGGATCCTAAACTG-3'
3' -GATGCATAAGGATCCCTGGGT-5'
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Ligated 40-mer duplex A

with further information provided in Tables I and II. Consider the methylation of the 40-mer duplex E (1m/m*2m/a), which has only one GATC site available for methylation. At high [DNA]/[enzyme] ratios, a T4 Dam monomer will bind the duplex, as seen in Structure 2,

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5' -CATCCGAAATGGATCCTAAACTG-3'
3' -GATGCATAAGGATCCCTGGGT-5'
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structure 2

asymmetrically at one of four possible positions (denoted “1”–“4”). If the enzyme is unable to reorient on the duplex and does not act processively, then we would expect a maximum “burst” value of 0.25 (number of [3H-CH3] groups transferred per bound T4 Dam in the pre-steady state phase) because “4” is the only productive site, and appropriate orientation to it is necessary for methylation. However, if the enzyme is capable of reorienting at position “3” to position “4,” then the burst could be as high as 0.5. Moreover, T4 Dam bound at position “1” may be capable of moving (by linear diffusion) to “3” and reorient to “4”; perhaps it might even reorient from “2” to “1” and diffuse to “3,”
where it reorients. If some combination of these events occurs, then even higher burst values could be registered (with 1.0 as the theoretical maximum). Hence, by determining the burst value we can ascertain whether T4 Dam was able to adapt to a substrate in which a productive target site-strand represented only one-quarter of the initial binding orientations. This type of experiment was carried out with several 40-mer duplexes containing differing combinations of variant sites.

Measurement of T4 Dam Processivity—The pre-steady state burst values (B) and steady state rate constants ($k_{cat}$) were determined for both the 40-mers and the stoichiometric mixtures of the unligated component duplexes used to generate each 40-mer (Fig. 1 and Table III). The $k_{cat}$ values for 40-mer duplexes A–D (with two potential sites for methylation) and the corresponding mixtures of constituents were all comparable; they were close to the value of $k_{cat} = 0.015$ s$^{-1}$ observed for a single-site 20-mer duplex (20). Hence, it appears that T4 Dam steady state methylation of 40-mer duplexes is similar to the methylation of a shorter 20-mer duplex, and both have the same rate-limiting step in the overall reaction. In this regard, we recently showed that methylation of a 20-mer duplex is consistent with a steady state-ordered bi-bi mechanism in which the order of substrate binding and product release (methylated DNA, DNA$^{Me}$, and the S-adenosyl-L-homocysteine, AdoHcy) is AdoMet $\rightarrow$ DNA $\rightarrow$ DNA$^{Me}$ $\rightarrow$ AdoHcy $\uparrow$ (21). The related EcoDam MTase has a steady state-ordered bi-bi mechanism where the order of substrate binding is DNA $\rightarrow$ AdoMet $\downarrow$ (11). After the chemical step (methyl group transfer from AdoMet to DNA), product DNA$^{Me}$ dissociates relatively rapidly ($k_{off} = 1.7$ s$^{-1}$) from the complex. In contrast, dissociation of product AdoHcy proceeds relatively slowly ($k_{off} = 0.018$ s$^{-1}$), indicating that its release is the rate-limiting step, consistent with the $k_{cat} = 0.015$ s$^{-1}$ (20).

In contrast to the results for steady state methylation, the burst values differed for the 40-mer duplexes versus the stoichiometric mixtures of their component-short duplexes. For example, 40-mer duplex A (1a/a$^2$/2a/a) had a burst of 1.86 compared with 1.01 for the corresponding mixture of short constituents “1a/a + 2a/a”. The latter burst value was also observed with a single-site 20-mer duplex (8). It follows that, after methylation of one Ade in the palindromic GATC/GATC site, T4 Dam dissociates from a short duplex prior to its exchanging product AdoHcy for substrate AdoMet. In contrast, the burst value of 1.8 with 40-mer duplex A suggests that, after methylation of one site, T4 Dam is capable of linear diffusion, release of product AdoHcy without dissociating from DNA, binding another AdoMet, and methylation of a second site. This appears to contradict the sequence of events observed during steady state cycles, viz. AdoMet $\downarrow$ DNA $\downarrow$ DNA$^{Me}$ $\uparrow$ AdoHcy $\downarrow$. To reconcile this apparent discrepancy, we propose the following series of events during the pre-steady state methylation phase of the two-site 40-mer duplex A.

First, T4 Dam binds substrate AdoMet (AdoMet $\downarrow$), this complex binds randomly to the 40-mer duplex (DNA $\downarrow$), and methylation of a target GATC ensues (GATC $\rightarrow$ GMTC). Next, T4 Dam bound with product AdoHcy leaves the methylated site (GMTC $\uparrow$); at a nonspecific DNA sequence, AdoHcy rapidly dissociates from the T4 Dam-DNA-AdoHcy complex (AdoHcy $\downarrow$) and is exchanged with AdoMet (AdoMet $\downarrow$). Only this enzymic form is capable of reorientation (8, 13) at a hemimethylated site (see below). This sequence of the events does not contradict the scheme AdoMet $\downarrow$ DNA $\downarrow$ DNA$^{Me}$ $\uparrow$ AdoHcy $\downarrow$, but here the event DNA$^{Me}$ $\uparrow$ signifies departure from the GMTC site without physically dissociating from the DNA duplex.

Reorientation of T4 Dam to the Methylation Target—We proposed earlier that AdoMet induces an allosteric T4 Dam conformational change, allowing a rapid reorientation of the enzyme to the DNA strand that contains the unmethylated GATC (8, 13). Thus, when comparing bursts for different duplexes, it is important to keep in mind that, according to our model, only T4 Dam-AdoHcy is capable of reorientation at a hemimethylated site. It follows that when a hemimethylated site is generated enzymatically, the T4 Dam-product AdoHcy cannot reorient to the other strand. Rather, it diffuses away from the newly created GMTC.

| No. | Combination of oligonucleotides | Structure |
|-----|---------------------------------|-----------|
| 1a/a | I + II | 5'-CAGTTTAGATTCATTTCCG 3'-GTCAAATTCCTAGGTAAGGCTAC |
| 1m | I + II | 5'-CAGTTTAGATTCATTTCCG 3'-GTCAAATTCCTAGGTAAGGCTAC |
| 1a/m | I + IIm | 5'-CAGTTTAGATTCATTTCCG 3'-GTCAAATTCCTAGGTAAGGCTAC |
| 1m/m | I + IIm | 5'-CAGTTTAGATTCATTTCCG 3'-GTCAAATTCCTAGGTAAGGCTAC |
| 2a | III + IV | 5'-CAGTGCAATAGGATGCTCTGTT 3'-GTATTCCTAGGGACCCA |
| 2m/a | IIIm + IV | 5'-CAGTGCAATAGGATGCTCTGTT 3'-GTATTCCTAGGGACCCA |

![Table II](image-url)
T4 Dam Methyltransferase Processivity

Pre-steady state and steady state kinetic analyses of T4 Dam methylation were carried out on 40-mer duplexes with stoichiometric amounts of their corresponding unmethylated component duplexes. Kinetic data were analyzed using the program Scientist 2.01 (MicroMath®) for regression analysis. The burst values (B, the number of \(^{3}H\)-CH\(_3\)) groups incorporated per T4 Dam during the pre-steady state phase) and \(k_{cat}\) values were determined using the equation \[^{3}H\text{-DNA}] / [enzyme] = B + \(k_{cat}\).

### TABLE III

**Kinetic characteristics of duplexes methylation**

| Duplexes | Specific site position and methylation status | Burst values \((k_{cat} \text{ sec}^{-1}) \times 10^2\) |
|----------|-----------------------------------------------|-----------------------------------------------|
| A = 1a/a + 2a/a | -A- -A- -A- -A- | 1.86 ± 0.13 1.92 ± 0.10 |
| 1a/a + 2a/a | -A- -A- -A- -A- | 1.01 ± 0.09 1.57 ± 0.09 |
| B = 1a/a + 2m/a | -M- /-M- /-M- /-M- | 1.7 ± 0.11 1.89 ± 0.12 |
| 1a/a + 2m/a | -M- /-M- /-M- /-M- | 1.05 ± 0.12 1.93 ± 0.10 |
| C = 1m/a + 2m/a | -M- -M- -M- -M- | 1.86 ± 0.09 1.45 ± 0.07 |
| 1m/a + 2m/a | -M- /-M- /-M- /-M- | 1.12 ± 0.11 1.63 ± 0.16 |
| D = 1a/m + 2m/a | -M- -M- -M- -M- | Not Done Not Done |
| 1a/m + 2m/a | -M- /-M- /-M- /-M- | 1.74 ± 0.08 1.37 ± 0.07 |
| E = 1m/m + 2m/a | -M- /-M- /-M- /-M- | 1.18 ± 0.09 1.33 ± 0.09 |
| 1m/m + 2m/a | -M- /-M- /-M- /-M- | 0.69 ± 0.05 0.67 ± 0.04 |
| F = 1m/m + 2a/a | -M- /-M- /-M- /-M- | 0.97 ± 0.09 1.20 ± 0.07 |
| 1m/m + 2a/a | -M- /-M- /-M- /-M- | 0.52 ± 0.05 0.73 ± 0.05 |

The 40-mer duplex B (Table III) is distinguished from duplex A by having one hemimethylated target; however, the two bursts were almost identical. This can be explained according to the following scenario. T4 Dam bound oriented to the lower strand can methylate one or two A residues (positions “2” and “4”), assuming for simplicity that diffusion is unidirectional (e.g., only 5’ → 3’). (In contrast, EcoDam appears to diffuse bi-directionally; i.e., does a random walk on the DNA; Ref. 11). Some T4 Dam molecules may bind duplex B oriented to the top strand and methylate “position 1.” These then diffuse away from the product GMTC site, and exchange AdoHcy for substrate AdoMet. Upon reaching methylated site “3,” the T4 Dam-AdoMet complex reorients to “4” and is proficient for methyl transfer. This route allows for a successive transfer of up to 3 methyl groups, at positions “1,” “2,” and “3,” respectively. Finally, some T4 Dam molecules may bind at “3,” where they can reorient to “4” and then transfer up to two methyl groups to the bottom strand. Thus, with duplex B, the theoretical maximum burst is somewhere between 2.0 and 3.0. However, some T4 Dam molecules may catalyze a methyl transfer and diffuse off the duplex before exchanging product AdoHcy for AdoMet. The observed burst of 1.0 for both unligated A and B component 20-mer duplexes (Table III) is consistent with this notion. It should be noted that our assumption about unidirectional movement is not implausible. The transfer of the methyl group from AdoMet to DNA can be considered irreversible for the T4 Dam MTase (22), because the reverse reaction was estimated to be at least 500-fold slower than the forward one. Hence, it follows, that DNA methylation is accompanied by the liberation of significant energy (\(\Delta G_{0} = -3.7 \text{ kcal/mole}\)). This energy can be used in part for T4 Dam isomerization (22) and for unidirectional 5’ → 3’ movement of T4 Dam along the DNA.

We measured burst values for duplexes in which two methylated positions are located in cis on one strand (duplex C) or in trans on the complementary strands (duplex D). As shown in Table III, their burst values were about 1.8. If no reorientation could occur, then we would expect a maximum burst of 0.75 with duplex C, because only one or two methyl transfers would be catalyzed by T4 Dam productively bound on the lower strand. T4 Dam bound unproductively oriented on the top strand would not register any methyl transfers. Furthermore, with duplex D, we’d expect a maximum of one methyl group transferred to each strand. Thus, the observed bursts can best be explained by T4 Dam being capable of rapid reorientation at hemimethylated sites. In this regard, formation of T4 Dam dimers cannot explain these results, because the calculated burst values would have to be reduced 2-fold to account for the binding of two enzyme molecules. Moreover, under the conditions similar to those used in these experiments, we found no evidence for the formation of appreciable amounts of T4 Dam dimers (13).

The methylation results with duplexes E and F are distinct from those obtained with the other 40-mer duplexes (Table III). First, duplexes E and F have only one methylatable site (M/A and A/A, respectively) plus one fully methylated site (M/M). Both 40-mers gave burst values of about 1.0 (almost 2-fold lower compared with duplexes A-D). In the case of duplex F, this indicates that T4 Dam was not able to reorient following methylation at the A/A site, essentially what would be predicted if T4 Dam-AdoHcy diffused away from an enzymatically created GMTC site and could not reorient. Thus, the interaction of T4 Dam with duplex F is functionally equivalent to that observed for a 20-mer duplex with a single unmethylated site, because both give bursts of 1.0. The unligated duplex F component 20-mers, 1m/m and 2a/a, gave a burst of 0.5 as expected, because one-half of the T4 Dam molecules bound unproductively to the fully methylated 1m/m duplex. For duplex E, T4 Dam binding oriented to the lower strand would result in the
transfer of zero or one methyl group, depending on the location of the initial binding and assuming unidirectional diffusion. In contrast, T4 Dam binding oriented to the upper strand would not result in any methyl group transfer to that strand. However, the enzyme could reorient at the hemimethylated M/A site and catalyze methyl transfer. It follows that a burst of one with duplex E is possible only if T4 Dam were able to reorient at the hemimethylated site.

The mixtures of unligated, component 20-mers for duplexes E and F contained stoichiometric amounts of molecules with a fully methylated M/M site and a methylatable A/A or M/A site. These mixtures are similar to those for duplexes A and C, except that the latter do not contain any M/M duplexes. Because half of the 20-mer duplex E and F components can not contribute to a burst, we would predict that the burst values should be ~50% of those obtained with the duplex A and duplex C mixtures. As seen in Table III, this was indeed the case, i.e. 1.01 versus 0.52 and 1.12 versus 0.69. Taken together, these results lend strong support to the model for pre-steady state methylation described above.

Finally, it should be noted that the stoichiometric mixtures of the E and F unligated component-short duplexes had about 2-fold lower $k_{cat}$ and burst values compared with those for duplexes A-D; this might be due to “product inhibition” by the fully methylated, short duplex “1m/m.” In contrast, judging from the higher $k_{cat}$ values for the 40-mer duplexes E and F, it would appear that a fully methylated site exerted little inhibition. This suggests that T4 Dam is better able to move from one site to another on longer 40-mer duplexes compared with dissociation/reassociation with the shorter one-site duplexes.

CONCLUSIONS

Based on the results presented, we draw the following conclusions. (i) T4 Dam MTase modifies the 40-mer two-site duplexes in a processive fashion. (ii) During processive movement on DNA from one site to the next, T4 Dam is capable of rapidly exchanging product AdoHcy (in the ternary complex) for substrate AdoMet without dissociating from the DNA duplex. (iii) The processive steps of T4 Dam action are consistent with an ordered bi-bi mechanism AdoMet $\downarrow$ DNA $\downarrow$ DNA$^{3m}$ $\downarrow$ AdoHcy $\uparrow$. However, in contrast to the steady state, here DNA$^{3m}$ $\downarrow$ signifies departure from a methylated site, GMTC $\uparrow$, without physically dissociating from the DNA molecule. (iv) Following methyl transfer at one site and linear diffusion to a hemimethylated site, T4 Dam-AdoMet is capable of rapidly reorienting itself to the (productive) unmethylated strand. In contrast, T4 Dam-AdoHcy is not capable of reorientation at an enzymatically created GMTC site. (v) The inhibition potential of fully methylated sites, 5'-GMTC/5'-GMTC, is much lower in a long DNA molecule compared with short single-site duplexes.

The results of the present work are important for refining our understanding of T4 Dam methylation and its relation to the mechanisms of other processive DNA MTases. First, the ability of T4 Dam to reorient to the methylation target, deduced earlier by us (8, 13) and directly demonstrated in this work, may be a general property of processive DNA MTases. Second, in long DNA molecules the inhibition potential of fully methylated sites must be low for other DNA MTases. If a processive DNA MTase performs a random walk on the DNA molecule, as recently suggested for EcoDam (11), it may signify that the enzyme passes over fully methylated sites without any kinetic retardation.

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Bacteriophage T4 Dam DNA-(N\textsuperscript{6}-adenine)-methyltransferase: PROCESSIVITY AND ORIENTATION TO THE METHYLATION TARGET

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