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Structure of the bacteriophage T4 DNA adenine methyltransferase

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Abstract

DNA-adenine methylation at certain GATC sites plays a pivotal role in bacterial and phage gene expression as well as bacterial virulence. We report here the crystal structures of the bacteriophage T4Dam DNA adenine methyltransferase (MTase) in a binary complex with the methyl-donor product *S*-adenosyl-L-homocysteine (AdoHcy) and in a ternary complex with a synthetic 12-bp DNA duplex and AdoHcy. T4Dam contains two domains: a seven-stranded catalytic domain that harbors the binding site for AdoHcy and a DNA binding domain consisting of a five-helix bundle and a β -hairpin that is conserved in the family of GATC-related MTase orthologs. Unexpectedly, the sequence-specific T4Dam bound to DNA in a nonspecific mode that contained two Dam monomers per synthetic duplex, even though the DNA contains a single GATC site. The ternary structure provides a rare snapshot of an enzyme poised for linear diffusion along the DNA.

DNA MTases catalyze methyl group transfer from donor *S*-adenosyl-L-methionine (AdoMet), producing *S*-adenosyl-L-homocysteine (AdoHcy) and methylated DNA. Although most prokaryote DNA MTases are components of restriction-modification systems, some MTases are not associated with cognate restriction enzymes, such as the *Escherichia coli* DNA adenine MTase (Dam). This enzyme methylates an exocyclic amino nitrogen (N6) of the adenine in GATC^{1,2}. Dam MTase gene orthologs are widespread among enteric bacteria and their bacteriophages (see Fig. 1 legend). Dam methylation is not essential for the viability of *E. coli*, unless it is combined with certain other mutations³; however, Dam is an essential gene in *Vibrio cholerae* and *Yersinia pseudotuberculosis*, at least under the tested growth conditions⁴. Dam methylation is essential for the virulence of *Salmonella serovar typhimurium* in a murine model of typhoid fever^{5–7}. These observations

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COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

raise the possibility that Dam inhibitors may have broad antimicrobial action, and *dam*⁻ mutants of these pathogens may serve as live attenuated vaccines^{6,8,9}.

E. coli Dam acts as an efficient *de novo* and maintenance MTase, methylating both unmethylated and hemimethylated GATC sites with similar efficiency¹⁰. The hemimethylated GATC sites, present immediately following DNA replication, regulate the timing and targeting of a number of cellular functions¹¹. In addition, Dam methylation is very important in the *E. coli* mismatch repair system formed by MutSL and MutH (reviewed in refs. 12,13). Dam methylation also helps to regulate chromosome replication by maintaining the methylation of the origin of chromosome replication. For example, DNA replication is controlled in part by the SeqA protein, which binds specifically to hemimethylated GATC sites near the origin of replication and delays their full methylation^{14–16}. Dam methylation regulates the expression of certain genes in *E. coli*^{17,18}, the *yopE* gene in *Yersinia pseudotuberculosis*¹⁹, and phage Mu *mom* gene (reviewed in ref. 20).

Valuable insights into the organization and function of MTases have come from the characterization of mutations in common motifs discovered by amino acid sequence alignments²¹. The solution of several MTase crystal structures, *M.HhaI*²², *M.TaqI*²³, *M.HaeIII*²⁴, *M.PvuII*²⁵, *M.DpnII*²⁶ and *M.RsrI*²⁷, has contributed essential details related to the specific protein-DNA and protein-cofactor interactions. As a preliminary step to the potential design of Dam inhibitors, we have characterized two structures containing the Dam MTase encoded by bacteriophage T4: one in a binary complex with AdoHcy, and the second in a ternary complex with both AdoHcy and a synthetic 12-bp DNA duplex.

RESULTS

Overall structure of Dam

The monomeric Dam structure contains 9 β -strands and 11 α -helices (Fig. 1). The N-terminal region (residues 1–52) and C-terminal region (residues 149–259) come together, forming the catalytic domain (green in Figs. 1 and 2a) with a seven-stranded β -sheet, a characteristic feature of the class-I AdoMet-dependent MTases²⁸. The N-terminal helices α Z and α A are located on one side of the β -sheet, and helices α C, α D1 and α D2 and α E are on the other side. Between strands β 2 and β 3 is a separate domain, which we designate as the target-recognition domain (TRD). TRD is composed of a five-helix bundle (α B1– α B5) (yellow in Figs. 1 and 2a) and a 25-residue segment containing a β -hairpin (β 8 and β 9) inserted between helices α B4 and α B5 (red). The overall dimensions of the molecule are $55 \times 34 \times 28$ Å, with an open cleft located between the catalytic domain (green) and the TRD (yellow and red).

Conserved residues and the effects of mutations

Based on the linear arrangement of conserved motifs in DNA MTases, T4Dam is classified in the α group, which has a characteristic motif order of motif I (Phe-X-Gly) followed by TRD and motif IV (Asp-Pro-Pro-Tyr)²¹. Our structural determination of T4Dam has allowed us to map conserved residues of Dam orthologs and several related restriction-

modification MTases onto the structure (Fig. 1). Conserved residues in the primary sequence, including 16 invariant positions, appear scattered throughout the catalytic domain and TRD. The folded structure, however, shows that these invariant residues are clustered on two surface patches (Fig. 2b). We suggest that the conserved surface patches have functional importance, being involved in one of three steps in the methylation reaction: AdoMet binding (Phe32 and Gly34 of motif I, Asp50 of motif II), DNA binding and sequence-specific recognition (Arg91 of α B3, Arg116 and Asn118 of the β -hairpin), and catalysis of methyl transfer (Asp-Pro-Pro-Tyr of motif IV, Lys11 of motif X and Glu254 of β 7). The N-terminal Gly9 (motif X) is involved in making a sharp turn that positions the nearby conserved residue Lys11 into the active site (see below).

The structure of the Dam MTase offers a rationale for its conservation. For example, Pro126 (after the β -hairpin) is exposed on the surface together with Arg91, Arg116 and Asn118 (Fig. 2b), invariant residues that have been implicated in specific *EcoRV*-DNA interactions²⁹. A P126S mutation generates the so-called Dam^h form of T4Dam and T2Dam³⁰; which is prone to methylate noncognate sites such as the internal adenine in AGACC resulting in resistance to cleavage by the *R.EcoP1* restriction endonuclease². *In vivo* Dam^h causes virion DNA hypermethylation³¹. *In vitro* T2Dam^h exhibited a two- to four-fold higher k_{cat} than the wild-type MTase on DNA containing various noncanonical sites; however, the two forms had the same k_{cat} on DNA containing the canonical GATC³². The altered activity of Dam^h may be due to the involvement of the surface serine hydroxyl in extra hydrogen bonding with DNA at noncognate sites.

Dam-AdoHcy interactions

In the binary Dam-AdoHcy complex, the methyl-donor product AdoHcy (added during purification) is observed at the carboxyl ends of the parallel strands β 1, β 2 and β 4 (Fig. 2a). It is surrounded by the conserved residues from motifs X (Tyr7), I (Phe32-Ser33-Gly34), II (Asp50), VI (Asp171-Pro172-Pro173) and α D1 (Tyr181, Phe184, Trp185) (Fig. 2c). Mutation of Pro172 to alanine or threonine seemed to abolish enzyme activity *in vitro*, owing to an increase in the K_m for AdoMet of 5- or 20-fold, respectively³³. Thus, when the AdoMet concentration was raised well above the K_m , the overall k_{cat} of these variants was reduced by only two-fold. Pro172 lies next to AdoHcy and the backbone carbonyl oxygen between Asp171 and Pro172 makes van der Waals contact with the homocysteine carbon C γ (Fig. 2c). The two mutations of Pro172 are likely to change the backbone conformation, thus substantially lowering the affinity for AdoMet and rate of exchange with bound AdoHcy, thereby lowering the k_{cat} .

Dam-AdoHcy interactions can be grouped according to the three moieties of AdoHcy. (i) One side of the adenine ring forms edge-to-face van der Waals contacts with the phenyl ring of Phe32 after strand β 1; this interaction is highly conserved among DNA MTases³⁴. On the other side of the adenine ring lies the side chain of Ile51 (after strand β 2). The exocyclic amino group (N6) of adenine makes water-mediated hydrogen bonds to the main chain carbonyl oxygen of Phe184. The ring nitrogen N1 of adenine hydrogen bonds to the backbone amide NH of Phe157. (ii) A strongly conserved acidic residue (Asp50) at the carboxyl end of strand β 2 forms bifurcated hydrogen bonds with the ribose hydroxyls; this is

nearly universal to class-I MTases²⁸. In addition, Tyr7 and Gln52 each form a hydrogen bond with one of the two ribose hydroxyls. (iii) The conserved Gly34 lies underneath the homocysteine moiety, while Asp171 interacts with the amino group (NH_3^+). The AdoHcy carboxyl oxygens (COO^-) interact with the backbone amide NH of Lys11 and the side chain hydroxyl of Ser37.

A unique interaction of T4Dam involves the short helix αD1 (after the Asp-Pro-Pro-Tyr motif), which covers the AdoHcy molecule, nearly burying it in a closed acidic pocket (Fig. 2d). The buried AdoHcy suggests that the exchange between the methyl donor AdoMet and reaction product AdoHcy requires the movement of the 3_{10} -helix and the loops flanking it, which may explain the observation that AdoMet is partially co-purified with the enzyme (data not shown). This helix includes Trp185, which is likely to be involved in the quenching of intrinsic tryptophan fluorescence that results from T4Dam binding of either AdoMet or AdoHcy (K_d 10 $\times$ higher for AdoHcy)³⁵. This suggests that when T4Dam binds its cofactor or methylation product, it undergoes a conformational change that alters the environment of Trp185 (the other two tryptophans, Trp67 and Trp226, are located >20 Å away from the AdoHcy). The same closed-flap AdoHcy binding site is observed in the nonspecific ternary structure (see below); in both cases, the 3_{10} -helix and the flanking loops are not involved in any crystal packing contacts. An open conformation was observed in the binary *M.DpnII*-AdoMet structure²⁶, where the AdoMet is largely visible and the cover (a five-residue longer loop after strand $\beta 4$) is opened up (Fig. 2d).

Active site

The active site is likely to be situated in a narrow surface pocket next to AdoHcy. The AdoHcy sulfur atom lies in the bottom of the pocket and is visible (Fig. 2d). The loop after strand $\beta 4$ contributes most of the residues (Asp-Pro-Pro-Tyr of motif IV and Tyr181) in the active site pocket (Fig. 2e). In the absence of the target adenine, the active site is occupied by two ordered water molecules, one of which bridges the side chains of Asp171 and the backbone carbonyl oxygen between Pro172 and Pro173, while the other one interacts with Tyr181. Remarkably, the invariant Asp-Pro-Pro-Tyr of T4Dam is superimposable onto the corresponding motif in the ternary complex of *M.TaqI*²³ (Fig. 2e), as well as the binary complex of *M.DpnII*²⁶ and *M.RsrI*²⁷ in the absence of DNA, suggesting that the conformation of Asp-Pro-Pro-Tyr is quite stable and highly conserved. In addition, an invariant Lys11 (motif X) interacts with Asp171 (via charge-charge interaction) and Tyr174 (via hydrogen bond) (Fig. 2e). The Asp171-Lys11-Tyr174 interactions confer additional stability to the active site. Interestingly, the mutant of the corresponding lysine in *M.EcoRV* (K16R) showed an altered specificity toward the target base³⁶.

The structure of a ternary complex of *M.TaqI* with DNA and a nonreactive AdoMet analog revealed that the target adenine is swung completely out of the helix and into the active site pocket by torsional rotation of its flanking sugar-phosphate backbone bonds²³, so called 'base flipping'²². Indirect evidence for flipping of the target adenine by T4Dam has been obtained by fluorescence analysis with substrates containing the base analog 2-aminopurine³⁷. Encouraged by the similar conformations of the Asp-Pro-Pro-Tyr motif in all N6-adenine MTase structures, we superimposed the target adenine of *M.TaqI* onto

T4Dam (Fig. 2e). This superimposition placed the flipped-out adenine with the target amino nitrogen atom occupying the position of a water molecule in the active site. It has been suggested²⁵ that the spinning of this amino group is impeded and its pK_a altered by the formation of a pair of hydrogen bonds, one of which is to a MTase backbone carbonyl oxygen. In the case of T4Dam, this would be between Pro172 and Pro173 in the conserved Asp-Pro-Pro-Tyr of motif IV. This particular carbonyl is expected to be fairly rigid as it is between two prolines. The second proton of the target amino group makes a hydrogen bond with the side chain of Asp171. Together these hydrogen bonds may increase the nitrogen electron density and facilitate a nucleophilic attack on the AdoMet methylsulfonium group. In addition, the hydroxyl group of Tyr181 is between the target amino group and the AdoHcy sulfur (Fig. 2e), where the transferable methyl group will be attached in the AdoMet, potentially also facilitating the reaction.

Dam-DNA nonspecific interactions

We were also able to crystallize T4Dam in a ternary complex with AdoHcy and a GATC-containing synthetic DNA duplex. The oligonucleotide, a self-complementary 12-bp palindrome, had the sequence 5'-ACAGGATCCTGT-3', where the target GATC sequence is centrally located. In the crystal, the DNA duplexes (primarily in B-form) were stacked head-to-end along the crystallographic *a* axis with an $\sim 15^\circ$ twist at the joint, forming a pseudo-continuous DNA duplex (Fig. 3a). The identity of the DNA sequence was confirmed by replacing the 5'-end cytosine with 5-iodocytosine. Unexpectedly, a nonspecific loose binding mode³⁸ was observed in the crystal with two Dam monomers (molecules A and B) bound to one DNA duplex (Fig. 3a). Molecule A binds to a single DNA duplex spanning seven base pairs, whereas molecule B binds in the joint of two DNA molecules spanning six base pairs. The buried solvent-accessible surface area³⁹ between protein and DNA is $474 \text{ \AA}^2$ and $542 \text{ \AA}^2$ for molecules A and B, respectively. This area is $\sim 3\text{--}4\times$ lower than those observed in the specific complexes of *M.HhaI*-DNA ($1,529 \text{ \AA}^2$) and *M.TaqI*-DNA ($1,886 \text{ \AA}^2$) and $\sim 2\times$ lower than those observed in the non-sequence-specific protein-DNA complex HMG-D ($905 \text{ \AA}^2$) and for chromosomal protein Sac7d ($865 \text{ \AA}^2$) (ref. 38). The ratio of polar to nonpolar contacts of the buried surface, calculated by using the CONTACT program in CCP4 to determine the contacting atoms and then adding up the polar and nonpolar ones, is ~ 0.8 for molecule A and 0.6 for molecule B. These ratios are within the range observed for non-sequence-specific DNA recognition (0.5 for HMG and Sac7d and 1.1 for the nucleosome)³⁸.

The two Dam monomer structures in the nonspecific ternary complex are quite similar to each other and to the binary structure, with an r.m.s. deviation of $0.55 \text{ \AA}$ over all C α atoms. There are no substantial conformational changes evident after nonspecific binding in the ternary complex. The only obvious differences involve side chain rotamers of several residues (Asn133, Lys134 and Asn135) involved in phosphate interactions. However, the orientations of the two molecules relative to the DNA helical axis are different: molecule A is rotated by $\sim 20^\circ$ relative to molecule B, resulting in a larger cavity⁴⁰ for molecule A ($170 \text{ \AA}^3$) vs. molecule B ($30 \text{ \AA}^3$) at the interface of protein and DNA. The smaller cavity and the larger buried surface area suggest that the binding mode of molecule B might be closer to a specific binding mode than that of molecule A.

Protein-phosphate interactions are primarily mediated via the TRD, that is, a stretch of ~20 amino acids from the β -hairpin to the N-terminal end of helix α B5 (Fig. 3b). Both Dam monomers approach the DNA from the minor groove side. In molecule A, Arg130, Asn133, Lys134 and Asn135 interact with the three phosphates (T10, G11 and T12) near the 3' end of one strand and Lys129 and Lys81 of helix α B3 interact with the two phosphates neighboring the C5 of the target GATC in the complementary strand (Fig. 3c). Molecule B interacts symmetrically with the joint between two DNA molecules by contacting two phosphates flanking the A3 of one DNA molecule and the two phosphates flanking the A10 from the complementary strand of the next DNA duplex (Fig. 3c). Although electrostatic interactions are dominant, Phe111 of the β -hairpin makes van der Waals contacts with the phosphate backbone carbons (C4' and C5') of A10. The interactions occur primarily in the minor groove side, the protein structure is maintained and virtually no sequence-specific hydrogen bonds are formed—all features that are also shared in other examples of non-sequence-specific DNA binding³⁸. In addition, the locations of the DNA-interacting residues are in agreement with the solution studies for probing the DNA interface in *M.EcoRV*²⁹, where DNA interaction residues (specific and nonspecific) have been mapped to the corresponding N-terminal loop, the hairpin and helix α D1.

DISCUSSION

We do not understand why T4Dam failed to bind specifically with GATC sites (which are separated in the crystal by 12 base pairs from center to center). One explanation for the loose binding between T4Dam and DNA in the ternary structure derives from the fact that T4Dam, like *E. coli* Dam⁴¹, methylates DNA with multiple GATC sites in a processive manner; in other words, more than one methyl group may be transferred per bound Dam monomer⁴². Thus, under single-turnover conditions (Dam:DNA ratio > 1.0), a catalytically competent complex was formed that contained two Dam monomers per synthetic duplex⁴³. Since the rate-limiting step in the overall T4Dam methylation process is the release of product AdoHcy from the enzyme⁴⁴, it follows that after methyl transfer the Dam–AdoHcy complex must release from the target site, diffuse along the DNA, and AdoMet must exchange for AdoHcy before the next methyl transfer can occur. We suggest that in the ternary crystal structure, the T4Dam–AdoHcy complex may be positioned on the duplex in a fashion that corresponds to the stage following methyl transfer. That is, it is not in contact with the GATC target site; rather it contacts the phosphodiester backbone and is primed for diffusion and/or exchange of AdoHcy with AdoMet. The cofactor cover (the 3_{10} -helix α D1 and the flanking loops) is not involved in the nonspecific phosphate backbone interactions. Therefore, it is possible that the cover will be opened up (as observed in the *M.DpnII*–AdoMet structure) during the cofactor exchange while the enzyme stays bound to the DNA. Notably, restriction-associated MTases are distributive⁴¹, because processive methylation of DNA would interfere with the biological function of restriction-modification systems. However, the structural basis for T4Dam processivity is not obvious in comparison with *M.DpnII*; these two proteins have quite similar structures but differ substantially in their processivity. Breyer and Matthews⁴⁵ suggested that processive enzymes tend to enclose their substrates to a greater extent. More structural work will be required to settle this point.

METHODS

Protein purification and crystallization

T4Dam was overexpressed and purified to near homogeneity essentially as described previously⁴⁶. Bacterial cells were lysed and the cell debris was removed by centrifugation. The resulting supernatant (in 20 mM potassium phosphate, pH 7.4, 1 mM EDTA, 0.05% (v/v) β -mercaptoethanol, 400 mM NaCl) was loaded onto a 5-ml HiTrap Q column (Amersham Pharmacia Biotech) and the flowthrough was collected and then loaded onto a 5-ml HiTrap SP column. The protein was eluted from the SP column at ~420–470 mM NaCl. The peak activity fractions were pooled, concentrated and loaded onto a sizing column (equilibrated with 20 mM HEPES, pH 7.4, 1 mM EDTA, 0.05% (v/v) β -mercaptoethanol, 400 mM NaCl); T4Dam migrated as a ~30-kDa globular protein in Superdex S75.

For preparation of the Dam–AdoHcy binary complex, AdoHcy was added to the protein to a final concentration of 0.2 mM and the mixture was incubated on ice for 2 h before it was loaded onto the sizing column. The resulting Dam–AdoHcy complex was concentrated to ~26 mg ml⁻¹ and used for crystallization. Crystals were grown by vapor diffusion at 16 °C as hanging drops using reservoir solution of 2.4 M ammonium sulfate and 100 mM MES pH 6.0.

For preparation of the ternary complex, the Dam–AdoHcy solution was mixed at an ~1:1 molar ratio with annealed DNA oligonucleotide. The crystals with a 12-mer DNA with blunt ends, formed with a reservoir solution of 20–25% (w/v) PEG 8000, 100 mM HEPES, pH 7.5, and 10 mM ammonium sulfate.

Structure determination

We encountered several problems during the course of structural determination of the ternary complex. First, the crystals suffered severe radiation damage, often resulting in incomplete data sets. Second, the cell dimensions changed from crystal to crystal, with variation of up to ~8 Å in the crystallographic *b* axis (Table 1), making crystals nonisomorphous and different data sets impossible to merge. After screening >100 crystals, we used the following data to solve the structure: two-wavelength anomalous diffraction data at Hg absorption edge (Hg-soaked crystal I) and a single-wavelength (at 1.0 Å) data set from the Hg-soaked crystal II and from crystal III with iodinated oligonucleotide (Table 1).

The initial phases were obtained from analysis of the two-wavelength Hg-MAD data of crystal I. SOLVE⁴⁷ determined three mercury sites whose positions were confirmed by visual examination of the anomalous-difference Patterson map. The phases were improved by maximum-likelihood density modification using RESOLVE⁴⁸, showing a clear solvent boundary. In parallel, the phases calculated by PHASES⁴⁹ using the three identical mercury sites were improved by carrying out many rounds of solvent flipping using the program SOLOMON⁵⁰, which enabled us to identify two protein molecules within an asymmetrical unit bound to a single DNA duplex. The phases were greatly improved by noncrystallographic symmetry averaging along with histogram matching implemented in the program DM⁵¹. A partial model was then built and refined by simulated annealing

implemented by CNS⁵². The resulting model was then used to search the molecular replacement solution and the corresponding three mercury sites in crystal II (which provided the cross-averaging matrix between the two crystals) using the program AMoRe⁵³. Using these three mercury sites, a SAD phased map was calculated using SOLVE⁴⁷. The cross-crystal averaging between MAD (crystal I) and SAD (crystal II) was accomplished using the program DMMULTI⁵¹; phases were dramatically improved and a nearly complete model could be built into the density.

The model was refined against the data from crystal III, which contains a 5-iodocytosine at position 2 from the 5' end. The single electron density peak (6σ above noise level) provided unambiguous determination of the DNA sequence. Numerous cycles of refining using CNS⁵² and manual rebuilding were conducted. A series of simulated annealing omit maps were used to correct the model. The noncrystallographic restraints were imposed on the two protein molecules throughout the refinement and were only released from protein side chains at the last cycle to account for a different interaction environment between the interfaces of DNA with each molecule.

The structure of the binary complex was readily solved by molecular replacement, using the protein coordinates from the ternary structure. The crystal contains one binary complex in the asymmetric unit.

Solvent-accessible surface areas were calculated using AREAIMOL³⁹ of CCP4 (ref. 54). Contacts of buried surface were calculated using CONTACT⁵⁴. Cavity sizes were calculated using VOIDOO of RAVE⁴⁰.

Coordinates

The coordinates of the binary and ternary structures of T4Dam have been deposited in the Protein Data Bank (accession codes 1Q0S and 1Q0T, respectively).

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Figure 1.

Structure-based sequence alignment of the selected Dam MTase orthologs. SWISSPROT database accession numbers are listed in parentheses: bacteriophage T4 (P04392) (T4Dam), *Escherichia coli* (P00475) (EcoDam), and restriction-modification MTases *EcoRV* (P04393) and *DpnIIA* (p04043). In addition, the following sequences are used to derive the invariant (white characters highlighted in black) and the conserved residues (light blue): *Salmonella typhimurium* (P55893), *Serratia marcescens* (P45454), *Yersinia pestis* (Q9F7U9), *Vibrio cholerae* (Q08318), *Neisseria meningitidis* (Q9X3Y7), *M.HindIV* (P44431), *M.MboIA* (p34720), *M.CviBI* (Q01511) and *M.MjaIII* (Q58015). The secondary structure of T4Dam is indicated above the sequence with cylinders for helices and arrows for strands. The MTase catalytic domain is green, the TRD yellow and the β -hairpin red. The conserved sequence motifs characteristic of DNA-amino MTases are labeled with roman numerals according to Malone *et al*²¹. Dashed lines show the unstructured regions: the loop between strands β 6 and β 7 is disordered in both the binary and ternary structures of T4Dam, as well as in the *M.DpnII*-AdoMet structure²⁶. T4Dam was susceptible to proteolysis near residue 250 within this loop (data not shown).

Figure 2.

The binary structure of T4Dam-AdoHcy. (a) Ribbon⁵⁵ representation uses the same color scheme and labels of helices and strands as in Figure 1; the AdoHcy is shown in stick model. (b) Two views of GRASP⁵⁶ surface representation. The invariant residues (see Fig. 1) are green (catalytic domain), red (β -hairpin) and yellow (helix bundle). T2 and T4Dam differ at three residues⁵⁷: residues 20 (T4 serine, T2 proline) and 188 (T4 aspartate, T2 glutamate) are colored in blue, residue 26 (T4 asparagine, T2 aspartate) is on a different surface in both views. Single or pair-wise mutations (T4 S20P, T4 N26D or both) converted T4Dam to a T2Damlike enzyme with a higher k_{cat} , whereas T4 D188E showed no change in enzyme activity⁵⁸. (c) A stereo view of the AdoHcy binding site. The ball-and-stick representation of AdoHcy is superimposed with a simulated-annealing omit map ($F_o - F_c$) that contoured at 2.5σ . The hydrogen bonds are shown as dashed lines. (d) Closed pocket

(T4Dam) and open pocket (*M.DpnII*) of the cofactor binding site. The bound AdoHcy and AdoMet are in ball-and-stick model with the sulfur atom in green. The region forms the cover in T4Dam, which includes residues 175–203 (green) after motif IV. The corresponding region in *M.DpnII* (amino acids 195–212 in red) has a five-residue insertion that is unstructured. (e) A stereo view of the superposition of the active site of T4Dam-AdoHcy (yellow) and *M.TaqIDNA* (blue) complexes. Two water molecules (red balls) form hydrogenbonds with Asp171 and the backbone carbonyl of Pro172 (wat1) and Tyr181 (wat2). The target adenine-N6 amino group occupies the site of wat1.

Figure 3.

The loose ternary structure of T4Dam–DNA–AdoHcy. (a) The 12-bp DNA (one strand in blue and the other in red) is stacked head-to-end forming a pseudo long duplex along the crystal *a* axis with the length of ~40 Å. Molecule A binds to a single DNA molecule, whereas molecule B binds at the joint of two DNA molecules. (b) T4Dam-phosphate interactions. The same regions (residues 129–134) of molecules A and B are shown relative to a single DNA molecule. (c) Schematic diagram of the protein-phosphate interactions. Residues making interactions are black for molecule A and red for molecule B. The asterisks indicate residues from a neighboring symmetry-related molecule.

Table 1

Crystallographic data and refinement statistics

	T4Dam-AdoHcy-DNA			T4Dam-AdoHcy	
	Crystal I Mercury (Hg)	Crystal II Hg	Crystal III Iodine	Native	
Data Collection					
Wavelength (Å)	1.0107	0.971	1.1	1.1	1.1
Energy (eV)	12,266	12,768	11,271	11,271	11,271
Space group	$P2_1$	$P2_1$	$P2_1$	$P2_1$	$P2_1,2_1,2_1$
Unit cell dimensions					
a (Å)	40.1	40.2	39.8	39.7	58.6
b (Å)	116.2	116.4	108.5	109.7	63.1
c (Å)	74.9	75.0	73.4	73.6	72.7
β (°)	106.9	107.0	103.9	104.5	–
Resolution range (Å)	20.0–3.5	20.0–3.5	20–3.2	20–3.1	20–2.3
Completeness (%)	94.0	92.3	79.7	98.6	97.6
R_{merge}	7.7	8.5	11.1	9.6	5.6
$\langle I/\sigma \rangle$	10.3	8.4	7.5	13.9	16.5
Observed reflections	20,689	19,529	20,713	43,112	56,092
Unique reflections	8,015	8,198	8,123	11,038	12,701
Anomalous sites	3	3	3	2	–
Highest resolution shell					
Resolution range (Å)	3.62–3.5	3.63–3.5	3.31–3.2	3.21–3.1	2.35–2.37
Completeness (%)	89.3	90.6	75.0	97.3	99.6
R_{merge}	0.219	0.256	0.227	0.152	0.229
$\langle I/\sigma \rangle$	3.1	2.6	3.8	8.6	3.9
Refinement					
Number of atoms					
Protein				1,991 × 2	1,991
DNA				486	–
AdoHcy				26 × 2	26
Water				–	128

	T4Dam-AdoHcy-DNA			T4Dam-AdoHcy
	Crystal I Mercury (Hg)	Crystal II Hg	Crystal III Iodine	Native
R_{factor}		0.238		0.222
R_{free} (5% of the data)		0.293		0.266
R.m.s. deviation from ideal				
Bond lengths (Å)		0.008		0.007
Bond angles (°)		1.4		1.3
Dihedrals (°)		23.1		22.9
Improper (°)		1.2		0.81

$R_{\text{merge}} = \sum |I - \langle I \rangle| / \sum I$ where I is the observed intensity and $\langle I \rangle$ is the average intensity of multiple symmetry-related observations of the reflection. $R_{\text{factor}} = \sum |F_o - F_c| / \sum |F_o|$.