Two Alternative Conformations of S-Adenosyl-L-homocysteine Bound to Escherichia coli DNA Adenine Methyltransferase and the Implication of Conformational Changes in Regulating the Catalytic Cycle

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The crystal structure of the Escherichia coli DNA adenine methyltransferase (EcoDam) in a binary complex with the cofactor product S-adenosyl-L-homocysteine (AdoHcy) unexpectedly showed the bound AdoHcy in two alternative conformations, extended or folded. The extended conformation represents the catalytically competent conformation, identical to that of EcoDam-DNA-AdoHcy ternary complex. The folded conformation prevents catalysis, because the homocysteine moiety occupies the target Ade binding pocket. The largest difference between the binary and ternary structures is in the conformation of the N-terminal hexapeptide ("KWAGGK14"). Cofactor binding leads to a strong change in the fluorescence of Trp10, whose indole ring approaches the cofactor by 3.3 Å. Stopped-flow kinetics and AdoMet cross-linking studies indicate that the cofactor prefers binding to the enzyme after preincubation with DNA. In the presence of DNA, AdoMet binding is ~2-fold stronger than AdoHcy binding. In the binary complex the side chain of Lys9 is disordered, whereas Lys14 stabilizes the active site in the ternary complex. Fluorescence stopped-flow experiments indicate that Lys14 is important for EcoDam binding of the extrahelical target base into the active site pocket. This suggests that the hexapeptide couples specific DNA binding (Lys9), AdoMet binding (Trp10), and insertion of the flipped target base into the active site pocket (Lys14).

Although most prokaryotic DNA methyltransferases (MTases) are components of restriction-modification systems...
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Table 1
Crystallographic data and refinement statistics

| Crystal* | EcoDam-AdoHcy |
|----------|---------------|
| PDB code       | 2ORE          |
| Beamsline (wavelength Å) | APS 22-ID (0.97923) |
| Space group               | P3,21         |
| Unit cell dimensions (Å)  | 160.1, 160.1, 94.0 |
| and angles (°)             | 90, 90, 120   |
| Resolution Range (Å) (highest resolution shell) | 34.67 to 2.99 (3.1 to 2.99) |
| Measured reflections      | 129,223       |
| Unique reflections         | 28,125        |
| R(ref) (%)                 | 9.2           |
| Completeness (%)           | 99.5 (100.0)  |
| R(merge) (%)               | 0.111 (0.505) |
| R cryst (%)                | 0.202 (0.317) |
| R-free (5% data)           | 0.235 (0.330) |

Non-hydrogen atoms

| Protein DNA | 5856 (3 EcoDam) |
| Heterogen Water | 83 (3 EcoHcy) |

Root-mean-square deviation from ideality

| Bond lengths (Å) | 0.008 |
| Bond angles (°)  | 1.3   |
| Dihedral (°)     | 22.2  |
| Improper (°)     | 0.9   |

Estimated coordinate error from Luzzati plot (Å)

| from Sigma (Å) | 0.35 (0.33) |

* Crystals, acquired with a nylon loop (Hampton), were quickly transferred to mother liquor containing either 25% glycerol or ethylene glycol before being flash-frozen directly in liquid nitrogen or in a cold nitrogen gas stream at 100 K.

c(product) = B + F × (1 − exp(−k_1 t)) + k_2 t  \hspace{1cm} \text{(Eq. 1)}

where k_1 is the rate constant of the exponential phase; F is amplitude of the exponential phase; k_2 is the rate constant of the linear phase; and B is experimental background.

For error analysis the experiments were analyzed separately. Results are given as averages of the individual analyses together with their respective standard deviations. Steady-state kinetics were determined by using various concentrations of AdoMet (0.6 – 6 µM), 0.5 µM, 20-bp DNA, and 50 nM EcoDam. For each individual condition, the time course of DNA methylation was measured between 1 and 30 min. The slope of the initial part of the reaction progress curve was determined by linear regression, and the slopes were fitted to the Michaelis-Menten model to derive the K_m and k_cat values. For error analysis, by changing all other parameters K_m and k_cat were minimized and maximized separately, until the deviation of the data points from the fit was significantly worse than that of the best fit as determined by Student’s t test using p = 0.05.

UV Cross-linking AdoMet Binding Studies—AdoMet binding to EcoDam was studied by UV cross-linking as described (22). Briefly, enzyme (4 µM) and AdoMet (7.2 µM) were incubated in the absence and presence of the specific oligonucleotide (2 µM) for 10 min on ice. The mixture was then subjected to UV cross-linking and AdoMet binding to the enzyme analyzed by SDS-PAGE. To avoid DNA methylation in the experiments, a catalytically inactive EcoDam variant (D181A) was used, which was purified as described (12).

2-Aminopurine Fluorescence Studies—The kinetics of base flipping were investigated by stopped-flow experiments performed in an SF-3 stopped flow device (Biologic, Claix, France) as described (12) using enzyme and DNA at equal concentra-

Experiment Results Procedures

Protein Purification and Mutagenesis—N-terminally His-tagged EcoDam used for crystallization was expressed in HMS174(DE3) cells, by the method of autoinduction (17), and purified utilizing Ni^{2+} affinity, UnoS, and S75-Sepharose sizing columns. Site-directed mutagenesis was performed as described (18). EcoDam wild type and its variants used for the biochemical experiments were purified as described (4, 9). Because we observe that residues close to the N terminus of EcoDam are involved in AdoMet binding (Trp10 in this work) and recognition of Guai (Lys2 in Ref. 4), we cloned EcoDam with C-terminal His-tag and purified the protein. Comparison of the K_m values for AdoMet and the relative methylation of substrates, which carry a nucleotide exchange at position Guai, showed that the N-terminal His tag did not interfere with AdoMet binding and Guai recognition (data not shown).

Crystallography—Concentrated binary complexes were mixed with a 12-bp oligonucleotide duplex (synthesized by the New England Biolabs, Inc) at a protein to DNA ratio of ~2:1 and allowed to stand on ice for at least two h before crystallization; final protein concentration for crystallization trials was ~15 mg/ml. Small hexagonal crystals (~50 µm) were observed under high salt conditions of ~1.2–1.6 M NH_4/Li(SO_4)_2, 50 mM MgSO_4, 100 mM buffer (MES or HEPES) (pH 6.8–7.2). Attempts to obtain crystals of the EcoDam-cofactor binary complex in the absence of DNA resulted in small particles unsuitable for x-ray crystallographic study.

The structure was determined by molecular replacement with the program REPLACE (19), using an EcoDam protomer from the refined ternary complex structure (PDB 2G1P) as a search model. Although the oligonucleotides were present, the high salt crystal form only contains three binary complexes of EcoDam-AdoHcy. During the refinement, using the program CNS (20), the 3-fold noncrystallographic restraints were imposed at the earlier stage of refinement and were released at the later cycles to account for different interaction environments of crystal packing with each molecule (Table 1). The two AdoHcy conformations in molecules β and γ were refined with equal occupancy (50%), resulting in similar crystallographic temperature factors (in the range of ~30 Å^2). The folded EcoDam conformation in molecule α was refined in 90% occupancy.

DNA Methylation Studies—Oligonucleotide substrates were purchased from Thermo Electron (Dreieich, Germany) in the purified form. Methylation experiments were performed in 50 mM HEPES (pH 7.5), 50 mM NaCl, 1 mM EDTA, 0.5 mM dithiothreitol, 0.2 µg/µl bovine serum albumin containing 3.6 µM [methyl-^3H]AdoMet (PerkinElmer Life Sciences) at 37 °C as described (4, 9, 21) using 1 µM 20-bp oligonucleotide and 0.25 µM enzyme. The 20-bp oligonucleotide containing one GATC target site was a duplex of 5’-GC GAC AGT GATC GGC CTG TC-3’ and 5’-GA CAG GCC GATC ACT GTC GC-3’, where M is N^6-methyl-Ade. Methylation experiments were repeated at least three times. The data shown are an average of all experiments. For analysis, the data were fitted to a curve describing a single exponential followed by a linear phase as shown in Equation 1,
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Conformations of AdoHcy—We can model two different conformations of AdoHcy in molecules β and γ, with an estimated 50% occupancy for each conformation (Fig. 1C). The extended conformation (green sticks in Fig. 1C) is the same one described for the ternary structure, as well as for many other DNA MTases (13, 14). In molecule α, the folded conformation (yellow sticks in Fig. 1B) is the predominant conformation. However, residual and broken electron density exists for the extended conformation in molecule α, with an estimated occupancy less than 10%.

The adenine and ribose rings of AdoHcy in both conformations occupy the same positions (Fig. 1C). One side of the adenine ring forms face-to-edge van der Waals contacts with the phenyl ring of Phe35 (Fig. 1, B and C); this interaction is highly conserved among DNA MTases via the FJGXXG motif I (23). On the other side of the adenine ring lies the side chain of Ile58 of motif II. A strongly conserved acidic residue (Asp54 of motif II) forms two hydrogen bonds with both the ribose 2’ and 3’ hydroxyls (Fig. 1, B and C); this is nearly universal to class I MTases (13). These interactions have been experimentally shown to be essential for AdoMet binding for many DNA MTases, including the bacterial M.EcoRV (24) and M.HhaI enzymes (25) and the murine Dnmt3a enzymes (22). A unique interaction of EcoDam involves the indole group of Trp10, whose ring nitrogen forms a hydrogen bond with one of the ribose hydroxyls (Fig. 1, B and C). This residue had been shown before to be in close proximity to the cofactor by UV cross-linking reaction (26).

The dihedral angle, C-4’–C-5’–S6–Cγ, begins to define whether the AdoHcy is extended (~160°) or folded (~80°). In the extended conformation (green sticks in Fig. 1, C–E), the peptide backbone atoms of GXG of motif I lie underneath the homocysteine moiety, whereas Asp181 interacts with the amino group (NH2). In the folded conformation (yellow sticks in Fig. 1, B–D), the amino group of AdoHcy makes a cation–π interaction with the aromatic ring of Tyr184 and forms a salt bridge with the carboxyl group of Asp181. In addition, the AdoHcy carboxyl oxygen atoms (COO−) interact with the backbone amide of Tyr184 (Fig. 1, B and C). The S6–Cy bond occupies a position corresponding to the S8–CH3 bond in AdoMet as observed in M.RsrI (14) (Fig. 2A). Thus, in the folded conformation the homocysteine moiety of the AdoHcy occupies the active site pocket, where the target Ade would bind to after being flipped out from the double helix. Previously, two distinct binding conformations (one for AdoMet and the other for AdoHcy) were observed in M.RsrI (14) and M.TaqI in the absence of DNA (27). Superimposition indicates that the extended conformation of AdoHcy in EcoDam represents the AdoMet conformation in M.RsrI, whereas the folded AdoHcy conformation in EcoDam corresponds to the AdoHcy conformation in M.RsrI (Fig. 2A) and M.TaqI (data not shown).

Solution Studies on AdoMet and AdoHcy Binding—We suspected that the close proximity of cofactor and Trp10 might induce changes in the fluorescence of this tryptophan upon binding of cofactor or its analog. As shown in Fig. 2B, the fluorescence of tryptophan in EcoDam was reduced by about 10% after rapid mixing of AdoMet and EcoDam-DNA complex. To test if this change indeed is because of the interaction of AdoMet with EcoDam-DNA complex, a 340 nm cut-off filter. The dead time of the experiments was 3.1 ms. Typically, 15–20 injections were accumulated, averaged, and analyzed. All stopped-flow experiments were carried out at least in triplicate. The data represent examples of individual experiments. Error margins are reported as standard deviations based on the comparison of the results of the individual repeats of the experiments.

Trpophan Fluorescence Stopped-flow Studies—Changes of tryptophan fluorescence upon AdoMet binding were determined by stopped-flow experiments performed essentially as described above. In these experiments 20 bp of DNA and enzyme (0.35 μm each) were premixed in buffer containing 50 mM HEPES (pH 7.5) and 50 mM NaCl and rapidly mixed with AdoMet in the same buffer. In alternative setups, the experiments were carried out in the absence of DNA. The tryptophan fluorescence was excited at 295 nm, and emission was observed using a 320 nm cut-off filter. The dead time of the experiments was 3.1 ms. Typically, 15–20 shots were accumulated, averaged, and analyzed. All stopped-flow experiments were carried out at least in triplicate.

RESULTS

We used an autoinduction procedure (17) that yielded ~7 mg of purified EcoDam from a 0.5-liter bacterial culture. In the last purification step and during concentration (see “Experimental Procedures”), AdoHcy was added to the protein. During the trials for obtaining crystals of ternary complexes, concentrated binary complexes were mixed with oligonucleotide duplex. Hexagonal crystals were observed under high salt conditions. Although the oligonucleotides were present in solution, the high salt crystal form contains only the binary complexes of EcoDam-AdoHcy.

Overall Structure of EcoDam—The crystallographic asymmetric unit contains three molecules of EcoDam (Fig. 1A), named α, β, and γ, that are highly similar to each other with a pairwise root-mean-square deviation of less than 0.3 Å comparing 242 pairs of Cα atoms. The binary structure is also highly similar to the ternary structure, with a root-mean-square deviation of 0.5 Å between molecule α (binary) and the protein component from the ternary structure of EcoDam-DNA

AdoHcy (4) (see Fig. 1D for an extract of the superposition). The largest difference in protein conformation between the binary and the ternary structures lies in the hexapeptide (“KWAGGK14” ending with Lys14 whose side chain is disordered in the binary structure (Fig. 1D), with the Cα atoms of Gly12 and Gly13 moved more than 2.0 Å. This hexapeptide sequence motif is highly conserved with two invariant residues of GXK among the GATC-related DNA-(adenine-Ν6)-MTase orthologs (see Fig. 1 of Ref. 8), and one residue of that stretch (Lys8) forms the only sequence-specific contact of EcoDam to the Gua of the GATC sequence (4).
AdoMet with Trp^{10}, EcoDam variants were prepared in which Trp^{10} or Trp^{236} (the only other tryptophan in this protein) was mutated to tyrosine (W10Y) or leucine (W236L). Both variants exhibited wild type-like catalytic activity (data not shown). However, the W10Y variant did not show a change in fluorescence upon AdoMet addition, whereas the W236L variant behaved like the wild type enzyme. This indicates that it is the close approximation of the cofactor and Trp^{10} that causes the change of fluorescence in EcoDam.

The fluorescence stopped-flow signal was employed to study the kinetics of AdoMet/AdoHcy binding to EcoDam and EcoDam-DNA complexes. As shown in Fig. 2C, neither AdoMet nor AdoHcy binding to the free enzyme was detectable. Although it cannot be ruled out completely that AdoMet or AdoHcy bind to the free enzyme as well but do not cause a change in fluorescence at Trp^{10}, this is very unlikely because the close contact between cofactor and Trp^{10} is seen in the binary (this work) and ternary complexes (4). In this context it is noteworthy that DNA was present in the initial mixture used for crystallization; the high salt conditions probably disrupted the protein-DNA interaction, but the cofactor stayed bound to the enzyme after the DNA disassociation. AdoMet binding was detectable when the enzyme was preincubated with the 20-bp oligonucleotide substrate. Preferential binding of AdoMet to EcoDam-DNA complexes was confirmed by UV cross-linking cofactor binding studies using the catalytically inactive D181A variant (Fig. 2E).

Given these results our data suggest that in the catalytic cycle of EcoDam, AdoMet usually associates with enzyme bound to DNA (either at a specific site or at nonspecific sites). This agrees with the observation that the catalytic efficiency of the enzyme preincubated with DNA and then mixed with AdoMet is higher than if the enzyme is preincubated with AdoMet (28).

On the basis of the total fluorescence changes observed in the stopped-flow cofactor binding experiments, in the presence of DNA, binding to AdoMet was 1.8 ± 0.2-fold stronger than binding to AdoHcy, if the enzyme was bound to DNA.
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result was confirmed by stopped-flow AdoMet/AdoHcy binding experiments conducted in the presence of specific DNA at different concentrations of AdoMet or AdoHcy (supplemental Fig. 2). The fluorescence traces were fitted to mono-exponential curves, and the apparent rate constants were re-plotted against the cofactor concentrations to determine the rate constants of cofactor binding. Protein and DNA were used at 350 nM, and AdoMet was at 10 μM, and AdoHcy was at 10 μM, and AdoMet was at 10 μM, and AdoHcy was at 10 μM, and AdoMet was at 10 μM, and AdoHcy was at 10 μM. One caveat of these experiments is that DNA methylation takes place in the presence of specific DNA and AdoMet. Then the AdoMet is turned over to AdoHcy during the experiment, indicating that the preference for AdoMet might be even higher than determined here.

Side Chain of Lys<sup>14</sup> Communicates between DNA Binding, Cofactor Binding, and Base Flipping—In the ternary EcoDam-DNA-AdoHcy complex (4), the side chain of Lys<sup>14</sup> interacts with side chains of Asp<sup>181</sup> and Tyr<sup>184</sup> of the DPPY motif (Fig. 1E). The same DKY interactions were observed in the structures of T4 Dam (8), where the corresponding lysine residue Lys<sup>11</sup> is close to the ring N-1 atom of the target Ade (9). Substitution of arginine for the corresponding lysine in M.EcoRV (K16R) altered the specificity toward the target base suggesting that the lysine residue is close to the active site in this enzyme as well (29). Taken together, the conserved DKY interaction is likely to be critical for normal function of EcoDam by providing additional stability to the active site. In the binary structure presented here, the DKY interaction is disrupted, and the side chain of Lys<sup>14</sup> in all three molecules (α, β, and γ) is disordered. Comparison of the ternary and binary structures of EcoDam suggests that in the presence of specific DNA, AdoHcy prefers the extended conformation, and Lys<sup>14</sup> helps to stabilize the binding pocket for the flipped base.

We studied the kinetic properties of a K14A variant to define the role of Lys<sup>14</sup> in base flipping. At a ratio 1:4 of enzyme (0.25 μM) to substrate (1 μM), DNA methylation by EcoDam showed a burst phase (first turnover) followed by a linear phase (following turnovers in steady state) (Fig. 3A). During the burst, the K14A variant methylated DNA at about a 2-fold reduced rate compared with wild type EcoDam; in addition, the magnitude of the burst was reduced 3-fold indicating some partitioning of the complex into nonproductive conformations. Under steady-state conditions (Fig. 3B), the $k_{cat}$ value for AdoMet of the K14A variant was similar to wild type (wild type, 2.8 ± 0.3 μM; K14A, 2.3 ± 0.1 μM), which is in agreement with the structural data indicating no role of Lys<sup>14</sup> in AdoMet binding. However, the $k_{cat}$ value of the mutant was reduced by 8-fold, from 3.3 ± 0.4 min<sup>-1</sup> for the wild type to 0.4 ± 0.02 min<sup>-1</sup>. This ratio is very similar to the combined effect observed under single turnover conditions, the 2-fold reduced single turnover rate and the 3-fold reduced size of the burst (Fig. 3A). We conclude that the K14A variant has difficulty reaching the transition state of the methylation reaction that is manifested in a reduced single turnover rate and a reduced size of the burst in single turnover kinetics.

Based on the observation that Lys<sup>14</sup> stabilizes the base binding active site pocket in the ternary structure, we investigated if this residue has any role in the insertion of the flipped base into the pocket. Base flipping of DNA MTases can be studied using the modified base analog 2AP, which fluoresces after rotating (flipping) out of the DNA helix (30, 31). Previously, we have performed fluorescence stopped-flow studies using a 20-bp DNA substrate that has 2AP in place of the target Ade residue (4, 12). A biphasic fluorescence change was observed in the
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At first glance, this result appears to be inconsistent with the moderately reduced enzymatic activity of the K14A variant. However, we note that 2AP is a base analog that lacks the exocyclic 6-amino group, the target for methylation, that interacts with the active site DPPY tetrapeptide of adenine-\(N^6\) MTases (9, 32). The absence of this interaction between the target amino group and the DPPY as well as the presence of the additional amino group at position 2 is expected to make binding of 2AP into the active site pocket less favorable. In this regard, it should be noticed that the time scale of insertion of the flipped 2-aminopurine into the binding pocket is not in agreement with the single turnover rate constant, which is also the case for the wild type enzyme, where the rate constant of binding 2AP to the active site is smaller than 0.1 s\(^{-1}\) (12), whereas the single turnover rate constant is in the order of 0.15 s\(^{-1}\) (4). These observations point toward a limitation in using 2AP for base flipping studies as far as analyzing binding of the modified base into the active site pocket is concerned. 2AP is well suited to probe the initial flipping of the base into the extrahelical state, but the rates of insertion of the flipped base into the active site pocket might not be the same as the rates for a flipped adenine. Moreover, depending on the specificity of the active site pocket for the target base, binding of 2-aminopurine could be delayed; and the magnitude of this difference may vary among MTases.

DISCUSSION

In enzymatic catalysis binding of substrates, release of products, and conformational changes of the enzyme and substrate must be coordinated to avoid population of inactive dead-end complexes. The catalytic cycle of DNA MTases includes several steps as follows. DNA has to be bound and contacted sequence specifically, and AdoMet has to enter the active site. The target base has to be flipped out of the DNA helix into an extrahelical position and then bound into the active site pocket of the enzyme as well. The latter step should only occur if the correct DNA sequence is bound and if the active site contains AdoMet, because the base would block access of AdoMet.

We have previously shown that in EcoDam cofactor binding, DNA binding and binding of the flipped target base into the active site pocket are coupled (4, 12). Here, on the basis of a new binary structure of EcoDam in complex with AdoHcy and bio-

 presence of AdoMet, in which a fast phase of fluorescence increase (corresponding to flipping of the target base) was followed by a slower phase of fluorescence decrease. The slow phase represents the insertion of the flipped base into the active site pocket, where it regains some stacking interaction with Tyr\(^{184}\). Interestingly, although the initial rate of flipping by the K14A variant was not altered, the second slow phase of fluorescence decrease was not observed (Fig. 3C), even in experiments lasting for up to 70 s (data not shown). We conclude that loss of Lys\(^{14}\) side chain in K14A prevents or at least strongly slows down the insertion of the flipped 2-aminopurine into the active site pocket.

FIGURE 3. DNA methylation and base flipping by wild type EcoDam and K14A. A, methylation experiments using wild type EcoDam (gray squares) and K14A (black diamonds) were carried out using 1 \(\mu\)M 20-bp DNA, 0.25 \(\mu\)M enzyme, and 3.6 \(\mu\)M AdoMet. The line shows the best fit to a single exponential followed by a linear phase using the following values for the parameters: \(k_1 = 0.56 \pm 0.02\) min\(^{-1}\), \(F = 4040 \pm 240\) cpm, and \(k_2 = 62 \pm 17\) cpm/min for EcoDam; and \(k_1 = 0.31 \pm 0.04\) min\(^{-1}\), \(F = 1380 \pm 60\) cpm, and \(k_2 = 11 \pm 1\) cpm/min for K14A. B, steady-state methylation experiments using wild type EcoDam (gray squares) and K14A (black diamonds) were carried out using 0.5 \(\mu\)M 20-bp DNA, 50 nM enzyme, and variable amounts of AdoMet (0.7 to 5.5 \(\mu\)M). At each condition a time course of DNA methylation was measured, and the rate of the reaction was determined by linear regression of the initial phase. The rates were fitted to the Michaelis-Menten model to determine the \(K_m\) and \(k_{cat}\) values. C, base flipping by wild type EcoDam and K14A. Stopped-flow experiment detected the 2-aminopurine fluorescence of EcoDam (gray line) and K14A (black line). In this experiment 0.35 \(\mu\)M enzyme was pre-equilibrated with 10 \(\mu\)M AdoMet in buffer and rapidly mixed with 0.35 \(\mu\)M DNA dissolved in buffer containing AdoMet. Fluorescence was excited at 313 nm and detected >340 nm.

TABLE 3

| Condition | AdoMet (\(\mu\)M) | Rate (pmol CH3/mol/min) | 2AP (\(\mu\)M) | 2AP Rate (pmol CH3/mol/min) |
|-----------|------------------|------------------------|---------------|-----------------------------|
| 0.05       | 1.0              | 0.02                   | 0.01          | 0.00                        |
| 0.075      | 1.0              | 0.03                   | 0.01          | 0.00                        |
| 0.1        | 1.0              | 0.04                   | 0.01          | 0.00                        |

The rate of methylation is given as the initial rate determined by linear regression of the initial phase. The rate of base flipping is given as the initial rate determined by linear regression of the initial phase.
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chemical studies, we describe some of the structural and mechanistic details of this process. Evidently, the N-terminal loop of EcoDam comprising Lys9–Lys14 is involved in all three of these binding events; it contacts the DNA by a Lys9–Gua1 base-specific H-bond and Gly12–phosphate interaction as shown previously (4), and it contacts the cofactor by Trp10 (as shown here) and supports binding of the flipped target base into the active site pocket by Lys14 (as shown here). Superposition of the protein conformation in the binary structure reported here and the ternary complex with specific DNA (4) shows a small movement in the positions (≈2 Å) of the hexapeptide (Lys9 to Lys14) in response to specific DNA binding. In addition, the side chain of Lys14 is disordered in the binary structure and AdoHcy occupies two conformations, one extended (similarly as seen in the ternary structure) and one folded. Our data indicate that these slight conformational changes couple the three binding events of cofactor binding, DNA binding, and binding of the flipped target base into the active site pocket of EcoDam, which are essential for enzymatic activity of EcoDam.

The conformation of the N-terminal loop seen in the binary structure is not compatible with specific DNA binding, because the phosphate group between Gua and Ade of GATC would clash with Gly12 in the binary conformation. In the ternary complex, the peptide chain at Gly12 and Gly13 is moved by 2 Å and the side chain of Lys14 is moved toward the active site and interacts with Asp181 and Tyr184 (4). Therefore, the conformational change is a direct consequence of the close approach of the DNA to the enzyme in the specific complex. Unfortunately, we do not know about the structure of the hexapeptide in complex to nonspecific DNA and in free enzyme. The observation that DNA binding stimulates coenzyme binding, although the interaction between Trp10 and the cofactor is the same in the ternary complex with specific DNA shows a small movement in the positions (≈2 Å) of the hexapeptide (Lys9 to Lys14) in response to specific DNA binding. In addition, the side chain of Lys14 is disordered in the binary structure and AdoHcy occupies two conformations, one extended (similarly as seen in the ternary structure) and one folded. Our data indicate that these slight conformational changes couple the three binding events of cofactor binding, DNA binding, and binding of the flipped target base into the active site pocket of EcoDam, which are essential for enzymatic activity of EcoDam.

Modeling an AdoMet (by adding a methyl group onto the sulfur atom of the AdoHcy in correct stereochemistry) in the extended conformation indicates that in the ternary complex the methyl group would be positioned such that its hydrogen atoms are in contact with the backbone carbonyl oxygen atoms of Ala11 and Gly12 (C=H-O bonds). Because the AdoMet S+–CH3 hydrogen atoms are positively polarized, the dipolar interaction between the carbonyl oxygen and the CH3 hydrogens will stabilize the extended conformation. In contrast, in the folded conformation there are no interaction partners available for the AdoMet methyl group in the ternary conformation. Such preferential interaction with AdoMet is in agreement with the preferred binding of AdoMet as compared with AdoHcy shown here. In agreement with these considerations, in all DNA MTases AdoMet adopts the extended conformation, and in the only examples where AdoMet and AdoHcy binding can be directly compared (M.TaqI and M.RsrI) AdoMet is extended whereas AdoHcy is folded (14, 33). Therefore, the extended conformation most likely is the preferred conformation for AdoMet, whereas AdoHcy can adopt both the extended and the folded conformations in the binary complex. Modeling an AdoHcy (or AdoMet) in the folded conformation into the ternary complex conformation of EcoDam would bring the amino group of the homocysteine (or methionine) moiety into steric conflict with the -amino group of Lys14. This makes the extended conformation favorable if the protein is in specific complex with DNA. We have shown that the side chain of Lys14 is important for the insertion of the flipped target base. On the basis of the structure this effect might be caused by the stabilization of the base binding pocket by Lys14 via the bridging interaction to Tyr184 and Asp181 or by forcing the cofactor into the extended conformation or both. Irrespective of the exact mechanism, the conformation of Lys14 is an important trigger that couples DNA recognition and AdoMet binding to target base insertion.

Our observation of alternative AdoHcy conformations in the binary complex might explain the earlier observation that AdoMet could bind to EcoDam in two different “environments” (11) and having different roles (10). In addition to serving as a methyl group donor, AdoMet was found to play the role of an allosteric effector, which increases the affinity of the enzyme for the DNA. This result can be interpreted on the basis of our data, because AdoMet binding will pre-structure the N-terminal loop and thereby improve DNA binding, which is thermodynamically linked to our finding that DNA binding improves AdoMet binding.

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