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Dam methyltransferase from *Escherichia coli*: sequence of a peptide segment involved in S-adenosyl-methionine binding

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ABSTRACT

DNA adenine methyltransferase (Dam methylase) has been crosslinked with its cofactor S-adenosyl methionine (AdoMet) by UV irradiation. About 3% of the enzyme was radioactively labelled after the crosslinking reaction performed either with (methyl-^{3}H)-AdoMet or with (carboxy-^{14}C)-AdoMet. Radiolabelled peptides were purified after trypsinolysis by high performance liquid chromatography in two steps. They could not be sequenced due to radiolysis. Therefore we performed the same experiment using non-radioactive AdoMet and were able to identify the peptide modified by the crosslinking reaction by comparison of the separation profiles obtained from two analytical control experiments performed with ^{3}H-AdoMet and Dam methylase without crosslink, respectively. This approach was possible due to the high reproducibility of the chromatography profiles. In these three experiments only one radioactively labelled peptide was present in the tryptic digestions of the crosslinked enzyme. Its sequence was found to be XA-GGK, corresponding to amino acids 10–14 of Dam methylase. The non-identified amino acid in the first sequence cycle should be a tryptophan, which is presumably modified by the crosslinking reaction. The importance of this region near the N-terminus for the structure and function of the enzyme was also demonstrated by proteolysis and site-directed mutagenesis experiments.

INTRODUCTION

The Dam methylase from *Escherichia coli* catalyzes the transfer of a methyl group to the adenine in the sequence GATC using AdoMet as methyl donor. It consists of 278 amino acids (32 kD) and acts as a monomer (1, 2, 3). The natural occurring substrate is hemimethylated DNA, which is only present immediately after replication of the DNA. Several cellular processes are regulated by the methylation state of the GATC sites in the genome (for reviews see 4, 5, 6).

The GATC sites are not uniformly distributed over the *E.coli* genome (7). The highest concentrations are found in translated regions, where its principal task is to direct the mismatch repair system after replication to the newly synthesized DNA strand (8).

An unexpectedly high number of GATC sites was found in the origin of replication (oriC) of the *E.coli* chromosome. It was shown that complete methylation of oriC is necessary for the initiation of replication (9). OriC is bound to the cell membrane when hemimethylated (10) and initiation of replication is thereby inhibited (11). After replication, the membrane-bound oriC is methylated much more slowly than the rest of the genome (12). Dam methylation is thought to participate in the synchronization of replication and cell division (13).

GATC sites are also present in the promoter regions of several genes. The state of methylation of these sites contributes to the regulation of their expression (14). The production of e. g. DnaA, a protein involved in the initiation of replication (15), and Dam methylase is thereby directly coupled to the replication of the chromosome.

Although the biological role of Dam methylase is partially understood, little is known about its structure and enzymatic mechanism. Biophysical studies on the enzyme recently became possible by the construction of an overproducing strain which allows the isolation of large quantities of Dam methylase in high purity (16). In order to elucidate how the Dam methylase fulfills its different tasks in the cell, we are trying to characterize the cofactor binding site of the enzyme. In a first paper we demonstrated that the photoinduced crosslink of Dam methylase with its naturally occurring substrate AdoMet is highly specific (17). After proteolysis of the covalently-bound complex we isolated one peptide which was radioactively labelled when tritiated cofactor was used. This pepide exhibits an altered retention after crosslinking. We therefore presume that it is part of the AdoMet binding region of Dam methylase.

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EXPERIMENTAL PROCEDURES

Materials

S-adenosyl-L-(methyl-^{3}H)methionine (^{3}H-AdoMet), 80 Ci/mmole) and S-adenosyl-L-(carboxyl-^{14}C)methionine (^{14}C-AdoMet, 57 mCi/mmole) were obtained from Amersham. Modified trypsin, sequencing grade (13,000 u/mg) was from Promega, Madison. S-adenosyl-L-methionine hydrogensulfate was purchased from Boehringer, Mannheim. Solvents and buffer components used in HPLC were of HPLC gradient quality and purchased from Merck, Darmstadt. All other chemicals were of the highest purity available from commercial sources and were used without further purification.

Crosslinking of AdoMet to the Dam methylase

Purification of the Dam methylase from the overproducing strain HB 101 (pDOX) (16) and crosslinking, were done as previously described (17) except that the NaCl concentration was at 300 mM. The concentrations in the reaction mixture were 12 μM for AdoMet and 30 μM for Dam methylase.

Determination of incorporated radioactivity

Aliquots of photolabel reactions either performed with ^{3}H-AdoMet or with ^{14}C-AdoMet were placed on glassfiber filters (GF/C, Whatman) and the protein was precipitated with 10% trichloroacetic acid (TCA) (w/v). The filters were washed twice for 15 min. with 10% TCA, twice for 15 min. with ethanol, and airdried. For the determination of the background, reaction mixtures with equal amounts of Dam methylase and radioactive substrate were treated in the same way, but without previous UV irradiation. The radioactivity was mesured by scintillation counting in BCS-NA biodegradable scintillant (Amersham).

Proteolysis

The preparation of the crosslink for proteolysis was done essentially as described (18). To eliminate non-incorporated AdoMet, the methylase was precipitated with 10% TCA after the crosslink reaction. The pellet was washed twice with 10% TCA and once with cold acetone. After airdrying, the pellet was resuspended in 0.4 M ammonium hydrogencarbonate and 8 M urea, the pH was between 7 and 8, DTT was added to a final concentration of 4 mM and the solution was incubated at 50°C for 15 min. For modification of the cysteines iodoacetamide was added (8 mM final concentration) and the reaction was allowed to take place at room temperature. After 15 min. the urea concentration was brought to 2 M by dilution and the Dam methylase was digested overnight at 37°C after addition of 5% trypsin (w/w) with regard to Dam methylase.

Peptide purification by high performance liquid chromatography (HPLC)

The HPLC system used was a Waters 625 liquid chromatography system with a 486 tuneable detector and a Waters 746 integrator. The totality of the digest was directly injected onto a Delta Pak C_{18}-column (7.8 mm×300 mm, 300 Å, 15 μ, Millipore), kept at 40°C. The tryptic peptides were separated under the following conditions: buffer A = 10 mM ammonium acetate, pH 6.5, buffer B = 70% acetonitrile (v/v), 10 mM ammonium acetate, pH 6.5. The concentration of buffer B varied from 0 to 50% in 60 min. followed by a second step with buffer B increasing from 50 to 100% in 30 min. The flow rate was 1 ml/min. The peptides were detected by UV absorption at 214 nm. All fractions were hand collected to ensure isolation of single peaks. If radioactively marked co-substrate was used for the crosslink, small aliquots of each fraction were analyzed by scintillation counting.

Fractions containing radioactivity, or fractions eluted at the corresponding retention time when non-labelled AdoMed was used, respectively, were subjected to a second purification step. After volume reduction to about one fourth the solution was injected onto a smaller reverse phase column (2 mm×150 mm, 300 Å, 5 μ) also kept at 40°C and previously equilibrated with 1.16‰ trifluoroacetic acid (TFA) (buffer A). The peptides were eluted with a flow rate of 0.2 ml/min. under the following gradient conditions (buffer B: 50% acetonitrile, 1.1‰ TFA): concentration of buffer B was increased in a linear gradient from 0 to 30% over 30 min. and subsequently from 30 to 100% over 30 min. Detection of UV absorption and radioactivity as well as collection of the fractions were done as described above.

Peptide sequencing

Peptide sequences were determined by automated Edman degradation on an Applied Biosystems 477A pulsed liquid phase sequenator. The phenylthiohydantoin derivatives of the amino acids were identified by on line reverse phase HPLC.

RESULTS

Yield of the crosslinking

Crosslinking experiments performed with AdoMet radioactively labelled at either the methyl group (^{3}H-AdoMet) or at the carboxy group (^{14}C-AdoMet) led to comparable yields of labelled enzyme: 3% (^{3}H-AdoMet) or 4% (^{14}C-AdoMet) of Dam methylase, respectively, were covalently bound to the co-substrate after UV irradiation under the conditions described in the experimental procedures section. In contrast to these results, *Eco*RII methylase crosslinked with ^{3}H-AdoMet or ^{35}S-AdoMet yielded 3% or 0.5% of labelled enzyme, respectively (19). These authors also showed that radioactive labelling was the result of methylation of cysteine 186 by the tritiated methyl group. As we could find no difference between methyl group label and carboxyl group label experiments, we conclude that in the case of Dam methylase the whole AdoMet molecule is attached to the protein.

Isolation of peptides photolabelled with ^{3}H-AdoMet

1.1 mg of Dam methylase was irradiated in the presence of ^{3}H-AdoMet in order to obtain a radioactively labelled complex. After trypsinolysis the generated peptides were separated by reverse phase HPLC as described in Materials and Methods. The profiles of UV absorption as well as of measured radioactivity of the first purification step are shown in Fig. 1. Radioactivity was eluted mainly in one peak, named region R1 (Fig.1). However, two other regions, named R2 and R3, also showed radioactive labelling that was significantly higher than the background. The pattern of radioactive incorporation in the first HPLC purification was reproducible in several independently performed experiments with Dam methylase obtained from different preparations. None of the retention times of the regions corresponded to that of ^{3}H-AdoMet.

In order to prove that the presence of radioactivity in some fractions was due to covalent binding of ^{3}H-AdoMet to the enzyme induced by UV irradiation, Dam methylase was incubated

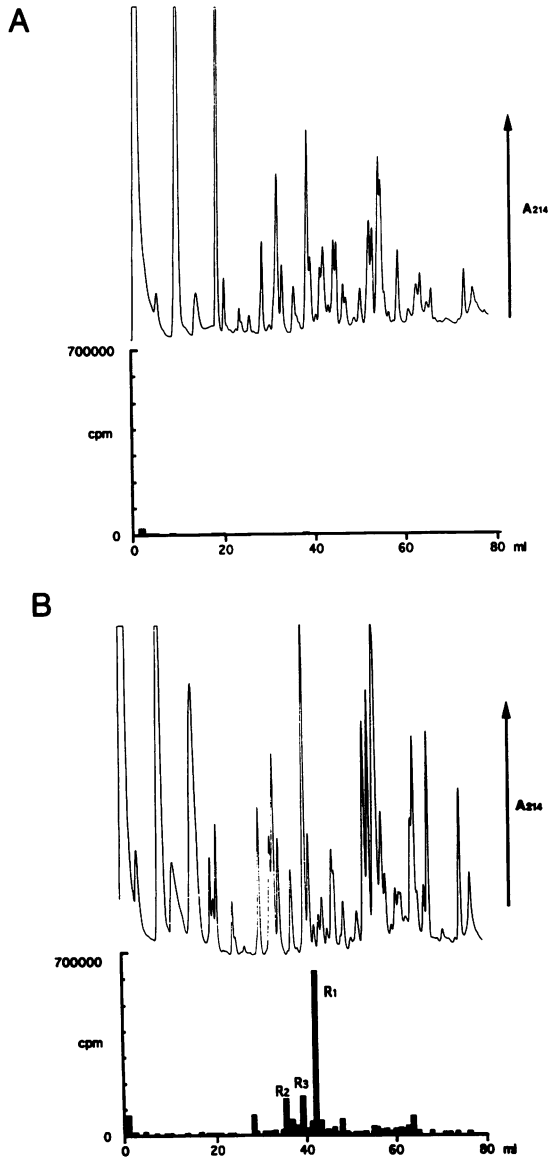

Figure 1. First HPLC purification step of the tryptic digest of Dam methylase. (**A**): Dam methylase (30 μM) was UV irradiated in the presence of 12 μM ^{3}H-AdoMet for one hour. The crosslinked protein was TCA precipitated, cysteines were modified with iodoacetamide, and the enzyme was digested with trypsin as described under Experimental Procedures. The peptides were fractionated on a Delta Pak C_{18}-column with a gradient of acetonitrile in 10 mM ammonium acetate (pH 6.5). Acetonitrile gradient: 0–35% between 10 and 70 min. and 35- 70% between 70 and 100 min. The flow rate was 1 ml/min. (**B**): Dam methylase and ^{3}H-AdoMet were preincubated under identical conditions but **not** UV irradiated. Digestion and purification were performed as described for A. The peptides were detected by UV absorption at 214 nm. Small aliquots of the hand collected fractions were analyzed for tritium content by liquid scintillation counting. The bars indicate the the total radioactivity found in each fraction. Radioactivity which eluted during the gradient was due to crosslinking of ^{3}H-AdoMet to Dam methylase as there was no radioactivity higher than the background found in B.

in the presence of ^{3}H-AdoMet under identical conditions but without UV irradiation. The UV absorption profile of the first HPLC purification step after trypsinolysis showed the same characteristics as that of the crosslinked sample. In the experiment without UV irradiation, none of the HPLC fractions contained more than background radioactivity.

To assure homogeneity of the peptides, the radioactive fractions were subjected to a second reverse phase HPLC dimension under different solvent conditions. Neither the number of peaks, nor their retention times were highly reproducible. The degree of alteration depended on the duration and conditions of storage prior to the second HPLC purification step, suggesting that peptides were being damaged by radiolysis. The peaks of the second HPLC became wider and more numerous the longer the samples had previously been stored at −20°C. Several precaution steps, e. g. the addition of 10% ethanol or avoiding a cycle of freezing and thawing of the fractions, did not prevent degradation of the peptides. Peptides resulting from this radioactive series of experiments yielded difficultly interpretable results, although the amount of material had been sufficient for sequence analysis.

The specific activity of ^{14}C-AdoMet was too low to monitor the radioactivity during the purification procedure.

Isolation and identification of non-radioactively modified peptides

Due to these difficulties, we changed our strategy and attempted the crosslinking of Dam methylase to a non-labelled substrate.

In a preparative experiment (P), 3.75 mg of Dam methylase (final concentration 30 μM) were irradiated in the presence of 12 μM AdoMet and trypsinyzed as described above. In parallel, two analytical control experiments (C1 and C2) were carried out using the same preparation of Dam methylase:

(C1) 275 μg of Dam methylase were treated as in the preparative experiment but with ^{3}H-AdoMet instead of non-labelled co-substrate. (C2) 275 μg of Dam methylase were trypsinyzed without previous crosslinking to AdoMet. For all three experiments incubation times and conditions were identical.

After proteolysis, all the three samples were submitted to the first HPLC separation. Fractions were collected by hand. In the case of ^{3}H-labelled crosslink (C1), the radioactivity of each fraction was determined. Thus, the fractions corresponding to R1, R2, and R3 were identified. In the case of the preparative (P) and non-crosslinked (C2) samples, the same regions could be identified due to the highly reproducible UV-absorption profiles.

The fractions corresponding to R1, R2, and R3 of all three experiments were subjected to the second HPLC purification step. This second chromatography was performed the next day in order to minimize radiolysis of the peptides in the control experiment (C1).

The UV absorption profiles of the rechromatographed R1 regions, as well as the radioactivity profile obtained from C1 are shown in Fig. 2. Radioactivity was eluted at the same position as a UV doublet peak, i. e. with a retention time of 32 to 34 min., respectively. The corresponding HPLC chromatogram of the preparative experiment (P) showed the same doublet peak profile, whereas in the control experiment without crosslinking (C2), only one peak instead of the doublet peak could be seen.

Both parts of the doublet peak of experiment P as well as the corresponding single peak from experiment C2 were subjected to N-terminal peptide sequencing.

Analysis of that half of the doublet peak that was eluted first led to the amino-terminal sequence XAGGK. The second part of the doublet contained a peptide whose sequence was LHVVK, and was also contaminated with the peptide of the first part.

These sequences correspond to the tryptic peptides of Dam methylase comprising amino acids 10–14 and 242–246,

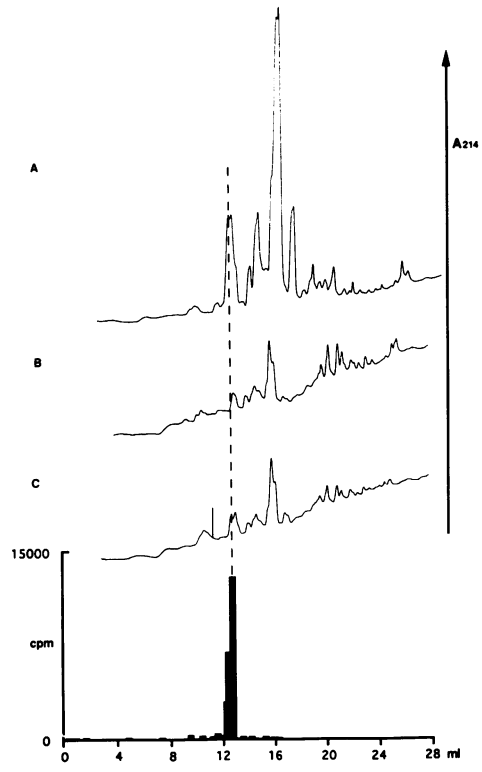

Figure 2. Rechromatography of peak R1 from fig. 1. (**A**): preparative experiment P (crosslink with non-labelled AdoMet); (**B**): control experiment C1 (Dam methylase without crosslink); (**C**): control experiment C2 (crosslink with ^{3}H-AdoMet). The peaks corresponding to R1 from each of these experiments were rechromatographed with a flow rate of 0.2 ml/min. on a narrowbore Delta Pak C_{18}-column with a gradient of acetonitrile in 1.1__ TFA. Acetonitrile gradient: 0–15% between 5 and 35 min. and 15–50% between 35 and 65 min. The radioactivity of the fractions of C2 was measured and indicated in the radioactive profile diagram. The first part of the double peak (indicated as a dotted line) was only present in experiments P and C2.

respectively. Analysis of the single peak detected in the control experiment resulted in only the peptide sequence LHVVK.

From these findings we conclude that the peptide 10–14 WAGGK near the N-terminus of the Dam methylase is modified in the crosslinking experiments by covalent binding of the co-substrate. All amino acids with the exception of tryptophan could be identified unambiguously by peptide sequencing (see Table 1). There were no peaks with retention times not corresponding to the PTH-derivatives of one of the amino acids. We therefore assume that the first amino acid of the peptide, a tryptophan, has been modified by covalent binding to AdoMet. This assumption cannot be proven directly because identification of tryptophan in automatic peptide sequencing is always very difficult. To exclude the possibility that the change in the retention time of this peptide after the crosslink reaction is due to photolysis of the tryptophan, Dam methylase was UV irradiated in the absence of AdoMet. After proteolysis, the peptides were subjected to the same purification procedure as described above. The rechromatography of peak R1 led to the same result as that of control experiment C1. The first part of the double peak was also absent. Tryptophan does not seem to be affected by UV-irradiation.

Therefore, the occurence of a new peak in crosslinked samples

Table 1. Data derived from sequencing the crosslinked peptide[a]

Cycle	1	2	3	4	5
amino acid	^{10}X	Ala	Gly	Gly	Lys^{14}
pmol		77	56	77	20

[a] The peptide was obtained from the preparative experiment P (first part of the double peak, see fig. 2). The sequence corresponds to aa 10–14 from Dam methylase where X is presumed to be a tryptophan covalently bound to AdoMet.

can be judged as an indication of a modification of the peptide by the crosslinking to AdoMet.

The modified peptides eluted in regions R2 and R3 were purified and identified in the same way as described for R1. Analysis of R2 yielded the same peptide as R1. In R3, the amount of peptide was insufficient to permit sequence determination.

DISCUSSION

The amino acid sequence of the segment of Dam methylase involved in AdoMet binding has been identified by UV crosslinking, proteolysis of the complex and purification of the peptide that had changed its retention time in reverse phase HPLC. The new peak found after crosslinking was identified as the pentapeptide XAGGK. The N-terminal amino acid is denoted as X because in the first sequencing cycle no amino acid could be identified. As AGGK is a unique motif in Dam methylase preceded by a tryptophan (W), we assume that the unidentified amino acid was a tryptophan that had been modified by the crosslinking reaction.

Both the carboxyl group labelled (^{14}C-AdoMet) and the methyl group labelled (^{3}H-AdoMet) co-substrates gave essentially the same crosslinking yields. Therefore, we conclude that the entire AdoMet molecule rather than the methyl group is attached to the enzyme. The specificity and reproducibility of the crosslinking has been demonstrated in a previous article (17).

Data from limited proteolysis and site-directed mutagenesis experiments further support the importance of the N-terminal region of the Dam methylase for its structure and function. Partial digestion of the Dam methylase with nonspecific proteases like e.g. elastase or chymotrypsin generated two fragments of 20 kD and a 12 kD, respectively, with the 20 kD fragment corresponding to the N-terminus of the enzyme. Unlike the smaller C-terminal fragment, the N-terminal fragment showed substantial resistance to further proteolysis which indicates that it is folded in a compact structure. The presence or absence of AdoMet did not change the rate at which the Dam methylase was digested by proteases nor the resulting fragment sizes (Wenzel, C., unpublished results).

Site-directed mutagenesis experiments corroborated these findings: a truncated Dam methylase mutant shortened by ten N-terminal amino acids could not be expressed at all or only as insoluble inclusion bodies (20).

AdoMet probably binds to Dam methylase at two different sites, as has already been published (21, 22). It is assumed to act as an allosteric effector when bound to the allosteric site and as a methyl donor when bound to the catalytic site. The reaction conditions used for the crosslinking experiments described in this article exclude the possibility of AdoMet binding to the allosteric site, because at salt concentrations exceeding 200 mM NaCl the allosteric effect of AdoMet is lost (21). In the crosslinking experiment, radioactively labelled AdoMet could be replaced in

the complex by the competitive inhibitors AdoHcy and sinefungine (17). The fact that these inhibitors can compete with AdoMet for binding to the Dam methylase shows that only the catalytic site is involved in the crosslinking, since the allosteric site seems to have no affinity for the inhibitors (22).

Up to now, the AdoMet binding sites of four other methylases have been identified: *Eco*RI methylase (23), *Eco*RII methylase (19), CheR methylase (24), and rat guanidinoacetate methylase (25). In contrast to the nucleotide binding sites of various enzyme families (26), no sequence similarities were detected for the AdoMet binding sites amongst the different methylases.

UV crosslinking experiments with *Eco*RII methylase and AdoMet showed that the tritiated methyl group of AdoMet is transferred to a cysteine residue that is part of the PC sequence motif highly conserved in all C5-methylcytosine (m^5C)-methylases (19). The importance of this cysteine for the catalytic function of m^5C-methylases had already been postulated by Wu and Santi (27). According to their results, the C6 of the cytosine in the specific DNA sequence is attacked by a nucleophilic amino acid residue of the methylase and thus a covalent bond between enzyme and DNA is formed. Subsequently, the methyl group of AdoMet is transferred to the C5 of the same cytosine with a configurational change of the methyl-C consistent with an S_N2 mechanism (28).

Chen *et al.* (29) isolated and characterized a covalently-bound complex between *Hae*III methylase and an oligonucleotide containing a 5-fluoro-2'-deoxycytidine in the recognition sequence of the methylase: the modified nucleoside was attached to the cysteine residue of the PC motif.

In contrast to m^5C-methylases, the structure-function studies of N6-methyladenosine (m^6A)-methylases are not as far advanced. Concerning their reaction mechanism, the only detail known so far concerns the direct transfer of the methyl group to the N6 of adenine: there is no evidence for a Dimroth-rearrangement after transfer of the methyl group to N1 (30). During its transfer, the methyl group undergoes an inversion of configuration (28).

Crosslinking experiments with the m^6A-methylase *Eco*RI demonstrated that the segment including aa 206–221 is involved in AdoMet binding (23). The results of limited proteolysis of the complex led to the conclusion that this part of the enzyme is located in a flexible connecting region between two more compact domains (31). In the free enzyme, peptide bonds of lysine 14 and 16 are readily cleaved by trypsin, whereas in the binary complex with AdoMet, these peptide bonds are protected against trypsinolysis. This could be hinting at a participation of the N-terminus of *Eco*RI methylase in AdoMet binding. In any case, the binding of AdoMet causes some kind of conformational rearrangement of the enzyme because in the presence of AdoMet its proteolysis is much slower.

In all DNA methylases whose sequences have been analyzed so far, two more or less highly conserved sequence motifs, FXGXG and PPY (32, 33, 34) have been found. The role of these sequences is not yet known. The peptide segment XAGGK of Dam methylase identified as being involved in AdoMet binding corresponds to neither of these sequence motifs. However, this does not exclude the possibility of their participation in AdoMet binding, especially since mutations in the latter conserved region of Dam methylase did indeed have a dramatic influence on its function (20).

Recently, crystals of the m^5C-methylase *Hha*I in complex with AdoMet have been obtained (35) as well as crystals of the m^6A-methylase *Taq* (36). Preliminary evaluation of the X-ray data confirmed the contribution of the FXGXG motif to AdoMet binding by the *Hha*I methylase, whereas in the *Taq* methylase it apparently makes no contacts to AdoMet (communicated in 37).

Other groups of methylases however, which also make use of AdoMet as a methyl donor but methylate substrates other than DNA, do not share these sequence motifs. In CheR methylase, an enzyme that methylates membrane bound chemoreceptors in bacteria, there is also a cysteine residue that functions as an intermediate methyl group receptor, as could be shown by crosslinking experiments. This methylase, however, contains none of the sequence motifs conserved in DNA methylases.

Although our knowledge of the biological role of methylation has seen a substantial progress in the last years, little is known about their structure and function. Therefore the forthcoming publication of the X-ray structure analysis of several DNA methylases is eagerly anticipated.

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REFERENCES

1. Hattman, S., Brooks, J. E. and Masurekar, M. (1978) J. Mol. Biol. **126**, 367–380
2. Geier, G. E. and Modrich, P. (1979) J. Biol. Chem. **254**, 1408–1413
3. Herrman, G. E. and Modrich, P. (1982) J. Biol. Chem. **257**, 2605–2612
4. Marinus, M. G. (1987) Ann. Rev. Genet. **21**, 113–131
5. Messer, W. and Noyer-Weidner, M. (1988) Cell **54**, 735–737
6. Barras, F. and Marinus, M. G. (1989) Trends Genet. **5**, 139–143
7. Barras, F. and Marinus, M. G. (1988) Nucleic Acids Res. **16**, 9821–9838
8. Lahue, R. S. and Modrich, P. (1988) Mutat. Res. **198**, 37–43
9. Landoulski, A., Hughes, P., Kern, R. and Kohiyama, M. (1989) Mol. Gen. Genet. **216**, 217–223
10. Ogden, G. B., Pratt, M. J. and Schaechter, M. (1988) Cell **54**, 127–135
11. Landoulski, A., Malki, A., Kern, R., Kohiyama, M. and Hughes, P. (1990) Cell **63**, 1053–1060
12. Campbell, J. L. and Kleckner, N. (1990) Cell **62**, 967–979
13. Boye, E. (1991) Trends Cell. Biol. **1**, 107–109
14. Sternberg, N. (1985) J. Bacteriol. **164**, 490–493
15. Braun, R. E. and Wright, A. (1986) Mol. Gen. Genet. **202**, 246–250
16. Hülsmann, K.-H., Quaas, R., Georgalis, Y., Saenger, W. and Hahn, U. (1991) Gene **98**, 83–88
17. Wenzel, C., Moulard, M., Lbner-Olesen, A. and Guschlbauer, W. (1991) FEBS Lett. **280**, 147–151
18. Stone, K. L., LoPresti, M. B., Crawford, J. M., DeAngelis, R. and Williams, K. R. (1989) in *A Practical Guide to Protein and Peptide Purification for Sequencing* (Matsudaira, P. T., Ed.) pp 31–47, Academic Press, San Diego
19. Som, S. and Friedman, S. (1991) J. Biol. Chem. **266**, 2937–2945
20. Guyot, J.-B., Grassi, J., Hahn, U. and Guschlbauer, W. (1993) Nucleic Acids Res. **21**, 3183–3190
21. Bergerat, A. and Guschlbauer, W. (1990) Nucleic Acids Res. **18**, 4369–4375
22. Bergerat, A., Guschlbauer, W. and Fazakerley, G. V. (1991) Proc. Natl. Acad. Sci. USA **88**, 6394–6397
23. Reich, N. O. and Everett, E. A. (1990) J. Biol. Chem. **265**, 8929–8934
24. Subbaramaiah, K. and Simms, S. A. (1992) J. Biol. Chem. **267**, 8636–8642
25. Takata, Y. and Fujioka, M. (1992) Biochemistry **31**, 4369–4374
26. Moller, W. and Amons, R. (1985) FEBS Lett. **186**, 1–7
27. Wu. J. C. and Santi, D. V. (1987) J. Biol. Chem. **262**, 4778–4786
28. Ho, D. K., Wu, J. C., Santi, D. V. and Floss, H. G. (1991) Arch. Biochim. Biophys. **284**, 264–269

29. Chen, L., MacMillan, M., Chang, W., Ezaz-Nikpay, K., Lane, W. S. and Verdine, G. L. (1991) Biochemistry **30**, 11018–11025
30. Pogolotti, A. L., Ono, A., Subramaniam, R. and Santi, D. V. (1988) J. Biol. Chem. **263**, 7461–7464
31. Reich, N. O., Maegley, K. A., Shoemaker, D. D. and Everett, E. (1991) Biochemistry **30**, 2940–2946
32. Lauster, R., Kriebardis, A. and Guschlbauer, W. (1987) FEBS Lett. **220**, 167–176
33. Klimasauskas, S., Timinskas, A., Menkevicius, S., Butkienè, D., Butkus, V. and Janulaitis, A. (1989) Nucleic Acids Res. **17**, 9823–9832
34. Lauster, R. (1989) J. Mol. Biol. 206, 313–321
35. Kumar, S., Cheng, X., Pflugrath, J. W. and Roberts, R. J. (1992) Biochemistry **31**, 8648–8653
36. Schildkraut, I., personal communication
37. Anderson, J. E. (1993) Curr. Op. Struct. Biol. **3**, 24–30