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Sequence and Substrate Specificity of Isolated DNA Methylases from *Escherichia coli* C

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Two DNA methylase activities of *Escherichia coli* C, the *mec* (designates DNA-cytosine-methylase gene, which is also designated *dcm*) and *dam* gene products, were physically separated by DEAE-cellulose column chromatography. The sequence and substrate specificity of the two enzymes were studied in vitro. The experiments revealed that both enzymes show their expected sequence specificity under in vitro conditions, methylating symmetrically on both DNA strands. The *mec* enzyme methylates exclusively the internal cytosine residue of CC$\overset{A}{\underset{T}{}}$GG sequences, and the *dam* enzyme methylates adenine residues at GATC sites. Substrate specificity experiments revealed that both enzymes methylate in vitro unmethylated duplex DNA as efficiently as hemimethylated DNA. The results of these experiments suggest that the methylation at a specific site takes place by two independent events. A methyl group in a site on one strand of the DNA does not facilitate the methylation of the same site on the opposite strand. With the *dam* methylase it was found that the enzyme is incapable of methylating GATC sites located at the ends of DNA molecules.

Escherichia coli strains possess several sequence-specific DNA methylases (10, 19). One enzyme, the *dam* methylase, methylates adenine residues in the sequence GATC to N^6-methyladenine (m^6Ade) (3, 11, 13); a second enzyme, the *mec* gene product, is responsible for the methylation of cytosine residues in the sequence CC$\overset{A}{\underset{T}{}}$GG to 5-methylcytosine ($m^5$Cyt) (21, 23). In addition to these methylating activities, the strains *E. coli* B and *E. coli* K-12 are known to contain host-specific modification enzymes (8, 12). Two methylases analogous to those coded by the *dam* and *mec* genes in *E. coli* have been found in *Salmonella typhi* and *Salmonella typhimurium* (4), but not in *Bacillus subtilis* or *Staphylococcus aureus* (2). In the present study the substrate and sequence specificity of the isolated *dam* and *mec methylases of E. coli* C were studied. This *E. coli* strain is devoid of a type I restriction-modification system (20). Studies in vivo on the mode of methylation of DNA in this *E. coli* strain revealed that at least the *dam* methylase is capable of acting in *trans* by methylating de novo unmethylated DNA (21). It was therefore of interest to investigate the substrate specificity of these methylases. In the present study the *mec* and *dam* methylases were isolated from *E. coli* cells and physically separated from each other. The sequence and substrate specificity of each of the isolated enzymes was studied in vitro. Both enzymes methylated their respective recognition sites and seemed to methylate unmethylated duplex DNA as efficiently as hemimethylated DNA. This last characteristic is similar to what was found with type II modification enzymes (17, 22) but distinguishes these enzymes from type I modification enzymes, which modify hemimethylated DNA severalfold more efficiently (1, 25). The *mec* and *dam* methylases are also different from the mouse DNA methylase, which methylates hemimethylated DNA at a rate 100-fold higher than that for unmethylated duplex DNA (5).

MATERIALS AND METHODS

The following products were purchased from New England Biolabs Co.: the restriction enzymes *Msp*I, *Mbo*I, and *Bst*NI; *E. coli* DNA polymerase I; *E. coli* DNA polymerase I large fragment; T4 DNA ligase; and the 13-mer primer for M13 DNA synthesis. The enzyme *Sau*3A was obtained from Bethesda Research Laboratories. The enzymes micrococcal nuclease and spleen phosphodiesterase, nucleotides, and calf thymus DNA were products of Sigma Chemical Co. α-^{32}P-labeled deoxynucleoside triphosphates were obtained from New England Nuclear Corp. *S*-adenosyl-L-[*methyl*-^{3}H]methionine (15 Ci/mmol) was from Amersham Corp. DEAE-cellulose (DE 52) and phosphocellulose were purchased from Whatman Inc., and cellulose thin-layer chromatogram sheets were from Eastman Kodak.

Preparation of DNA. *E. coli* DNA was prepared by the Marmur procedure (16). M13 phage (mp7) single-stranded DNA was prepared as previously described

(6). Hemimethylated mp7 DNA was synthesized in vitro by using primed repair synthesis (6). The reaction mixture contained 50 μM of each of the four deoxynucleoside triphosphates, 10 mM dithiothreitol, 66 mM Tris-hydrochloride (pH 7.4), 6.6 mM $MgCl_2$, 100 μM ATP, 1 μg of single-stranded mp7 DNA, 0.15 μg of 13-mer primer, 15 U of *E. coli* DNA polymerase I, and 10 U of T4 DNA ligase. After incubation at 30°C for 1 h, the DNA was extracted with phenol and precipitated with ethanol. In this DNA the methylated bases m^5Cyt and m^6Ade were present only in the template DNA strand.

Analysis of the nearest neighbor to m^5Cyt in DNA. DNA (5 μg) was randomly nicked by pancreatic DNase I by using 5 mM $CaCl_2$ instead of $MgCl_2$ in the reaction buffer. The nicked DNA was nick translated with *E. coli* DNA polymerase I in the presence of a single α-^{32}P-labeled deoxynucleoside triphosphate (600 Ci/mmol). The labeled DNA was digested to deoxynucleoside-3'-monophosphates, which were separated by thin-layer chromatography and the chromatographs autoradiographed as previously described (7). The radioactive spots corresponding to cytosine and m^5Cyt were scraped from the plastic sheets, and the amount of radioactivity in each spot was determined by liquid scintillation counting.

Reconstitution of GATC sites by end filling and ligation. A 2-μg sample of *E. coli* DNA was digested with 4 U of *Sau*3A for 2 h at 37°C. After phenol extraction and alcohol precipitation, the GATC sticky ends of the *Sau*3A fragment were filled by using *E. coli* DNA polymerase I (large fragment) in a reaction mixture (20 μl) containing 50 μM of each of the four deoxynucleoside triphosphates, 66 μM ATP, 10 mM dithiothreitol, 66 mM Tris-hydrochloride (pH 7.4), 6.6 mM $MgCl_2$, the *Sau*3A fragments, and 4 U of *E. coli* DNA polymerase I (large fragment). After incubation at 37°C for 10 min, 1,600 U of T4 DNA ligase were added and the incubation continued for another 24 h at 25°C. In some experiments we used labeled 50 μM [α-^{32}P]dTTP (20 Ci/mmol) instead of the unlabeled dTTP.

Preparation of DNA methylases from *E. coli* C cells. All steps were carried out at 0 to 4°C. Frozen *E. coli* C cells were ground with twice their wet weight of alumina 305 (Sigma), and the paste was suspended in 5 volumes of buffer A (10% glycerol [vol/vol], 1 mM dithiothreitol, 1 mM EDTA, 40 mM Tris-hydrochloride [pH 8.0]). The homogenous suspension was spun at 12,000 × *g* for 10 min. Centrifugation was repeated until a clear supernatant was obtained. This crude preparation was fully active after storage at −70°C for more than 2 years and will be designated here as fraction I. The second step of purification was streptomycin sulfate precipitation followed by ammonium sulfate fractionation. To 30 ml of fraction I, 6 ml of 20% (wt/vol) streptomycin sulfate was slowly added with continuous stirring over a period of 30 min. After 10 min of centrifugation at 12,000 × *g* the precipitate was discarded and 15.3 g of solid ammonium sulfate was slowly added with continuous stirring over a period of 30 min. The precipitate obtained by this ammonium sulfate concentration (70% saturation) after centrifugation for 10 min at 12,000 × *g* was dissolved in 8 ml of buffer A. This enzyme preparation, which will be called fraction II, was either dialyzed against buffer A for 18 h with three buffer changes or desalted through Sephadex G-50 before further purification. The last step in our partial purification procedure was chromatography on a DEAE-cellulose column. Fraction II, after dilution (1:4 vol/vol) in 2 mM potassium phosphate buffer (pH 7.8)–0.1 mM EDTA, was applied to a DEAE-cellulose column (14 cm by 2 cm^2) which was preequilibrated with 50 mM phosphate buffer (pH 7.8)–1 mM EDTA and washed with the dilution buffer. A 300-ml linear gradient of KCl from 0 to 0.7 M in the dilution buffer was applied, and 3-ml fractions were collected. Portions (10 μl) of each fraction were assayed for methylase activity as described below. The activity peak fractions were combined and dialyzed for 24 h against buffer A containing 50% glycerol. This treatment resulted in a five- to sixfold concentration of the enzymatic activity, which remains completely stable at −20°C for at least 1 year.

When DNA methylase was prepared from *E. coli* B cells the same procedure was used, and the *dam* methylase was purified up to the fraction II step. Since the *E. coli* B cells do not contain the *mec* enzyme and the *dam* enzyme of *E. coli* B is identical to the *E. coli* C *dam* enzyme by all tested criteria (data not shown), the *dam* enzymes from the two strains were used interchangeably.

Assay of methylase activity. The methylase activity was assayed in a standard reaction mixture (50 μl) containing 50 mM Tris-hydrochloride (pH 8), 10 mM EDTA, 5 mM dithiothreitol, 8 μM *S*-adenosyl-L-[*methyl*-^{3}H]methionine (15 Ci/mmol), 6 μg of DNA, and the methylase (60 μg of protein). After 1 h of incubation at 37°C the reaction was terminated by the addition of 0.2 ml of sodium lauryl sulfate (25% wt/vol). Calf thymus DNA (250 μg) was added as carrier and the volume brought to 2 ml with water. The mixture was incubated for 10 min at 60°C, extracted with chloroform:isoamyl alcohol (24:1), and the DNA precipitated with perchloric acid. The pellet was treated with 0.5 N NaOH for 10 min at 60°C to remove RNA, and the labeled DNA was finally precipitated with trichloroacetic acid, collected on Whatman GF/C filters, and quantitated by liquid scintillation counting.

RESULTS

Physical separation of the *E. coli* C *mec* and *dam* methylases. To study separately the sequence and substrate specificity of the *dam* and *mec* enzymes, DEAE-cellulose column chromatography was used to separate the two methylases. Two peaks of DNA methylase activity eluted from the column by a salt concentration gradient at 0.15 M and 0.25 M, respectively (Fig. 1, peak I and peak II). In the course of this chromatography both methylases were entirely purified from residual RNA, which remained in the preparation after streptomycin sulfate precipitation (Fig. 1). This purification turned out to be important since the *dam* enzyme was found to be unstable and could be stabilized in the presence of nucleic acids. Incubation of the partially purified *dam* methylase caused irreversible inactivation of the enzyme. The inactivation of the enzyme caused by incubation at 37°C could be prevented if DNA or RNA were present in the incubation mixture. The mode of

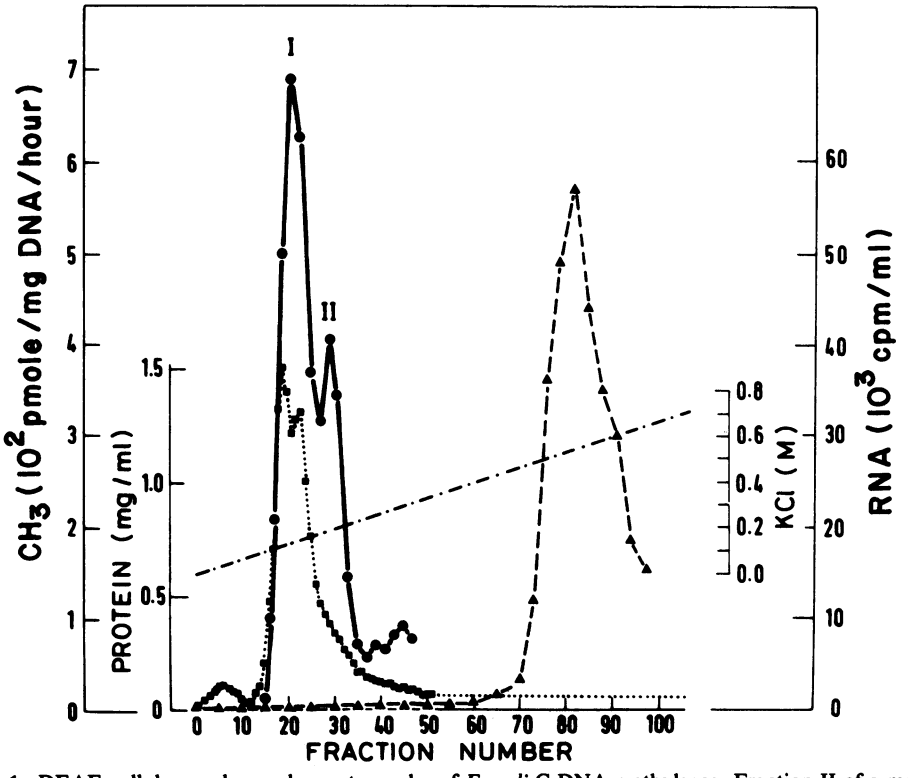


FIG. 1. DEAE-cellulose column chromatography of *E. coli* C DNA methylases. Fraction II of a methylase preparation obtained from cells labeled with [^{3}H]uracil was applied to a DEAE-cellulose column and eluted by a linear salt gradient (0 to 0.7 M KCl) as described in the text. Symbols: ..., protein; ———, methylase activity; – – – –, [^{3}H]RNA; —·—·—, salt concentration. Protein was determined by the procedure of Lowry et al. (14); methylase activity was assayed as described in the text; [^{3}H]RNA was determined by trichloroacetic acid precipitation; and salt concentration was determined by conductometry.

inactivation was temperature dependent and of a first-order type with a half-life of 10 min at 37°C. The *mec* methylase was entirely stable under the same incubation conditions, even in the absence of DNA or RNA.

Fractions of each of the two peaks of methylase activity which eluted from the DEAE-cellulose column (see Fig. 1) were combined and used to methylate calf thymus DNA as described above. The methylated DNA was hydrolyzed by formic acid and the free bases were chromatographed and autoradiographed (20). The radioactive spots of m^6Ade and m^5Cyt were scraped off and counted in a liquid scintillation counter. The results indicate that peak I fractions represent a *mec* methylase which is slightly contaminated by *dam* methylase activity (14%) and that peak II fractions represent the *dam* methylase (8% *mec* contamination).

Sequence and substrate specificity of the *mec* methylase. The sequence specificity of the *E. coli* C *mec* methylase was studied by analyzing the sequences which were methylated in vitro by the isolated enzyme. One approach was to subject calf thymus DNA which was methylated in vitro to the modified nearest neighbor analysis as described above. m^5Cyt was detected by this analysis in CpA and CpT dinucleotides but not in CpC sequences (data not shown). Methylation in CpG sequences was not attempted since the calf thymus DNA is already methylated in this sequence. These results and the amount of incorporated methyl groups (6 pmol of CH_3 per μg of DNA), which was consistent with the frequency of CC^A_TGG in calf thymus DNA (0.2%), suggested that this is the only sequence methylated in vitro and that only the internal cytosine of the sequence is methylated. This conclusion was further supported by another experimental approach. *E. coli* B DNA, being devoid of m^5Cyt, serves as an efficient substrate for the *mec* methylase. However, when this DNA was digested with *Bst*NI, a restriction enzyme which cleaves at CC^A_TGG, and used as substrate for the *mec* methylase, no methylation could be detected (Table 1). Digestion of *E. coli* B DNA with the restriction enzyme *Msp*I (recognition site CCGG) did not cause a drastic decrease in the

TABLE 1. Substrate and sequence specificity of the *E. coli* C *mec* methylase[a]

DNA substrate	Treatment	*mec* methylase activity (% of control)
E. coli B	None	100
E. coli B	Denatured and reannealed	70
E. coli B and *E. coli* C	Denatured and reannealed	60
E. coli B	*Bst*NI digested	≤1
E. coli B	*Msp*I digested	60

[a] Restriction of the DNA was under conditions recommended by the manufacturers of the enzymes. A 2-μg portion of DNA was incubated for 10 min with the *E. coli* C *mec* methylase under the standard assay conditions (see the text). The reaction carried out with the various substrates was linear for at least 60 min. Activity obtained with *E. coli* B DNA served as 100% activity control. *E. coli* B DNA (Mec$^-$) and *E. coli* C DNA (Mec$^+$) were mixed and denatured for 10 min at 100°C. After adding 0.4 M NaCl the mixture was reannealed at 67°C for 20 h.

activity of the *mec* enzyme. A direct method was used to quantitate the methylation at the CC$^{A}_{T}$GG sequence. Calf thymus DNA was methylated in vitro by the isolated *mec* methylase, and the methylated DNA was digested by the restriction enzyme *Bst*NI. The sticky ends of the *Bst*NI fragments ($^{CC}_{GGT}$ and $^{AGG}_{CC}$) were filled by using *E. coli* polymerase I with either [α-^{32}P]dATP or [α-^{32}P]dTTP. The end-labeled fragments were digested to deoxynucleoside-3′-monophosphates, thus labeling the internal cytosine residue of the CC$^{A}_{T}$GG sequences. The nucleotides were separated by two-dimensional chromatography and autoradiographed (Fig. 2). The radioactive spots were scraped off and counted in a liquid scintillation counter. The results showed that over 90% of the internal cytosine residues in the CC$^{A}_{T}$GG sequences were methylated in vitro by the *mec* enzyme. These results indicate that the *mec* methylase has the same sequence specificity in vitro as was shown previously by in vivo experiments (21, 23).

Substrate specificity of the *mec* enzyme was assayed by comparing its activity with hemimethylated DNA and nonmethylated DNA as substrates. Hemimethylated DNA was obtained by mixing *E. coli* B and *E. coli* C DNA and denaturing and reannealing as described in Table 1. The renatured DNA was expected to be partially heteroduplex and therefore be hemimethylated at CC$^{A}_{T}$GG sites. The *mec* methylase showed the same activity with homologous and heterologous reannealed DNAs as substrates. The *mec* methylase, therefore, seems to have no preference for hemimethylated sites. This is in contrast to the preference shown by type I modification enzymes (1, 25) or a mammalian methylase (5) toward hemimethylated DNA, but in accord with substrate specificity shown by type II modification enzymes (17, 22).

Sequence and substrate specificity of the *dam* methylase. Previously reported in vivo experiments indicate that the *dam* methylase is specific to GATC sequences (3, 13). In the present study the sequence specificity of the isolated *dam* methylase was studied in vitro. *E. coli* Dam$^-$ DNA was prepared from *E. coli* strain F42/GM48 (15) and digested by *Sau*3A (a restriction enzyme that cleaves at GATC sites, irrespective of the presence of m^{6}Ade in the site), and the fragments were used as substrate for the *dam* methylase. No methylation was observed with the *Sau*3A fragments, whereas *Msp*I fragments of the same DNA served as an effective substrate (see Table 2). Since *Msp*I digestion of the Dam$^-$ DNA produces fragments of the same

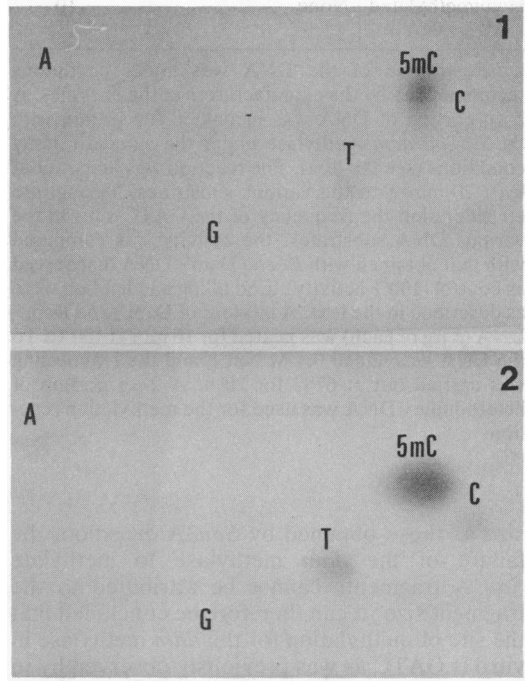


FIG. 2. Direct analysis of m^{5}Cyt at CC$^{A}_{T}$GG sequences. Calf thymus DNA (5 μg), after methylation in vitro with the *mec* enzyme as described in the text, was digested with 10 U of *Bst*NI for 1 h at 60°C. The DNA fragments were labeled at their 3′ ends by filling the sticky ends by using 2 U of *E. coli* DNA polymerase I (large fragment) with 600 Ci/mmol of either [α-^{32}P]dATP (panel 1) or [α-^{32}P]dTTP (panel 2) for 10 min at 37°C. The labeled fragments were purified and digested by micrococcal nuclease and spleen phosphodiesterase, and the deoxynucleoside-3′-monophosphates were chromatographed and autoradiographed as previously described (7).

TABLE 2. Sequence and substrate specificity of the *E. coli* C *dam* methylase[a]

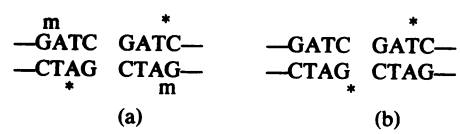
DNA substrate	Treatment	*dam* methylase activity (% of control)
E. coli (Dam$^-$)	None	100
E. coli (Dam$^+$)		≤2
E. coli (Dam$^-$)	Denatured and reannealed	75
E. coli (Dam$^-$) + (Dam$^+$)		75
E. coli (Dam$^-$)	*Sau*3A digested	≤1
E. coli (Dam$^+$)		≤1
E. coli (Dam$^-$)	*Sau*3A digested and end filled	≤1
E. coli (Dam$^+$)		≤1
E. coli (Dam$^-$)	*Sau*3A digested, end filled, and ligated	89
E. coli (Dam$^+$)		90
E. coli (Dam$^-$)	*Msp*I digested	80
Hemimethylated M13 DNA	None	110

[a] Restriction of the DNA was under conditions recommended by the manufacturers of the enzymes. A 2-μg portion of DNA was incubated for 10 min with the *E. coli dam* methylase under the standard assay conditions (see the text). The reaction was linear for at least 20 min with the various substrates. Taking into consideration the frequency of the GATC sites in the various DNA substrates, the activity was compared with that obtained with *E. coli* Dam$^-$ DNA that served as control (100% activity). End filling and ligation were as described in the text. A mixture of Dam$^+$ and Dam$^-$ DNA (5 μg of each) was heated for 10 min at 100°C. To the DNA was added 0.4 M NaCl, and the reannealing was carried out at 67°C for 20 h. A 2-μg portion of heteroduplex DNA was used for the methylation reaction.

size as those obtained by *Sau*3A digestion, the failure of the *dam* methylase to methylate *Sau*3A fragments cannot be attributed to the fragment size. It can therefore be concluded that the site of methylation for the *dam* methylase in vitro is GATC as was previously observed by in vivo experiments. This conclusion gains further support by the following experiment. The GATC tails of *Sau*3A fragments were filled with *E. coli* DNA polymerase I (large fragment), thereby restoring the GATC sites. Although the restored sites were expected to be unmethylated, these fragments were still inert as substrate for the *dam* methylase, suggesting that the methylase is inactive at the ends of DNA molecules. Thus, when the blunt ends of the filled *Sau*3A fragments were ligated for 24 h at 25°C by using an excess of T4 polynucleotide ligase and the ligated fragments used as substrate, 100% restoration of activity was observed (Table 2).

Substrate specificity of the *E. coli dam* methylase was studied by three different experimental approaches. The results of these experiments are summarized in Table 2. Denaturing and reannealing of a Dam$^+$ and Dam$^-$ DNA mixture as described in Table 2 was expected to produce hemimethylated DNA that was methylated on one strand of the DNA at GATC sequences. The efficiency of this hemimethylated DNA as substrate for the *E. coli dam* methylase was identical to that observed with Dam$^-$ DNA. Another approach for analyzing substrate specificity of the *dam* methylase was to determine its activity by using hemimethylated DNA synthesized in vitro as substrate. Hemimethylated DNA was synthesized by primed repair synthesis, using the single-stranded bacteriophage M13 DNA as template (6). This DNA contains eight GATC sites which are unmethylated on the in vitro-synthesized complementary strand. The sites on the viral strand are fully methylated as judged by the failure of the restriction enzyme *Mbo*I to digest the hemimethylated DNA (data not shown). The *dam* methylase activity observed with this hemimethylated DNA as substrate was similar to the activity obtained with Dam$^-$ DNA as substrate (Table 2). In a third type of experiment, *Sau*3A fragments of Dam$^-$ and Dam$^+$ were filled at the GATC sticky ends, and the fragments were ligated and used as substrates for the isolated *dam* methylase. Again, as can be seen in Table 2, enzyme activity (initial rate) with Dam$^+$ DNA (Fig. 3a) was identical to the activity obtained with the Dam$^-$ DNA (Fig. 3b). These results indicate that the *dam* methylase does methylate nonmethylated and hemimethylated sites with the same efficiency. Moreover, the essentially identical activity obtained with the two substrates, despite the fact that substrate a in Fig. 3 has twice as many unmethylated sites as substrate b, suggests that each binding of the enzyme to a site results in a single methylation event on one strand of the DNA. Methylation of the same site on the opposite strand requires another binding event. To confirm this conclusion, the *Sau*3A fragments of Dam$^+$ and Dam$^-$ DNA were end filled with [α-

FIG. 3. Substrates for the *dam* methylase prepared by end filling of *Sau*3A fragments. (a) *Sau*3A fragments of Dam$^+$ DNA, end filled and ligated. (b) *Sau*3A fragments of Dam$^-$ DNA, end filled and ligated. Symbols: ^{m}A, N^6-methyl Ade; *, ^{32}P label.



^{32}P]dTTP in the deoxynucleoside triphosphate mixture. The labeled DNA was ligated and used as substrate for the *dam* methylase. The extent of methylation in this experiment was estimated by digestion of the DNA with micrococcal nuclease and phosphodiesterase; the resulting nucleoside-3'-monophosphates were separated by chromatography and the chromatographs autoradiographed (18). The radioactive nucleotides were scraped off, and the radioactivity was measured by liquid scintillation counting. The amount of [α-^{32}P]N^6-methyl dAMP was twofold higher with the DAM$^+$ DNA than with the DAM$^-$ DNA (substrates a and b, respectively, in Fig. 3). This result is consistent with the conclusion that the methylase does not methylate the site on both strands in one binding event. This conclusion can be drawn since 50% of the methylation events with substrate b occur on nonlabeled GATC sequences and go, therefore, undetected.

DISCUSSION

Achieving physical separation of the *mec* and *dam* methylases in a partially purified preparation of *E. coli* C enabled us to study in vitro the sequence and substrate specificity of the individual enzymes. The *E. coli* C *mec* methylase was found to methylate CC$\overset{A}{T}$GG sequences as is the case for the *E. coli* K-12 methylase (9). We also showed that it methylates exclusively the internal cytosine of the CC$\overset{A}{T}$GG sequence. This finding left unexplained the discrepancy between the frequency of m^{5}Cyt residues and that expected for CC$\overset{A}{T}$GG sites in *E. coli* C DNA (21). However, in an independent study, the sequence CC$\overset{A}{T}$GG was found to be overrepresented to an extent that accounts for the relatively high m^{5}Cyt content (to be published elsewhere). The in vitro studies with the *dam* methylase indicate that the enzyme methylates only adenine residues in GATC sites as was previously shown by in vivo experiments (3).

Restriction digestion of DNA at GATC sites followed by restoration of the site by filling the sticky ends provided suitable substrates to study methylation at the ends of DNA molecules. The *dam* enzyme was found to be inactive at sites positioned at the ends of DNA molecules.

Substrate specificity of both the *mec* and *dam* enzymes was analyzed using hemimethylated and nonmethylated DNAs as substrate. Both enzymes showed the same activity (as judged by initial rate) with the two substrates. This feature of the *E. coli* enzymes might shed some light both on the mechanism by which these enzymes methylate the *E. coli* DNA and the possible biological function of the methylation process. The first implication of this result is that the enzyme is insensitive to the state of methylation of the specific site on the opposite strand as was reported before for type II modification enzymes (17, 22). This is in contrast, however, to the specificity of the modification enzymes type I, M. *Eco*B (25), and M. *Eco*K (1), which were found to be severalfold more active on hemimethylated DNA than on nonmethylated sequences. Also, the methylase from mouse cells methylates preferentially hemimethylated DNA (5). This in vitro performance of the enzymes is consistent with their in vivo mode of methylation. The *E. coli* C *dam* methylase has previously been shown to methylate in vivo unmethylated foreign DNA by a de novo methylation mechanism (21). The mouse methylase was found to perform in vivo semiconservative methylation, whereas de novo methylation of unmethylated DNA was a rare or nonexistent event (24). To fulfill their biological function, the modification type I enzymes are expected to operate within the cell with higher efficiency on the host hemimethylated DNA than on foreign nonmethylated DNA. The observed difference in substrate specificity displayed by the *dam* or *mec* methylases as compared with the mammalian methylase might reflect a different mode of enzyme-substrate interaction and different biological functions. It is feasible that the mammalian methylase binds to both strands or at least makes contact with the opposite strand and performs the methylation only when the opposite strand is methylated, whereas the *E. coli* enzymes might rather bind to one strand and methylate only this strand. Methylation of the same site on the opposite strand is an independent event. These different methylation mechanisms probably allow the bacterial methylase to work in *trans*, whereas the mammalian methylase can act only in *cis*.

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