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The core element of the *Eco*RII methylase as defined by protease digestion and deletion analysis

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ABSTRACT

Binding of the *Eco*RII DNA methyltransferase to azacytosine-containing DNA protects the enzyme from digestion by proteases. The limit digest yields a product having a M_r on SDS-PAGE 20% less than the intact protein. The N terminus of the tryptic digestion product was sequenced and found to be missing the N terminal 82 amino acids. Under the conditions used unbound enzyme was digested to small peptides. Protection of the enzyme from protease digestion implies that the enzyme undergoes major conformational changes when bound to DNA. The trypsin sensitive region of the *Eco*RII methyltransferase occurs prior to the first constant region shared with other procaryotic DNA(cytosine-5)methyltransferases. To determine if this region played a role in substrate binding or specificity, N-terminal deletion mutants were studied. Deletion of 97 amino acids resulted in a decrease of enzyme activity. Further deletions caused a complete loss of activity. Enzyme deleted through amino acid 85 was purified and found to have the same specificity as wild type however there was an increase in K_m for both S-adenosylmethionine (AdoMet) and DNA of 27 and 18 fold respectively. The N-terminus of the *Eco*RII methylase, although a variable region present in many procaryotic DNA(cytosine-5)methylases, plays no role in determining enzyme specificity, although it does contribute to the interaction with both AdoMet and DNA.

INTRODUCTION

The DNA(cytosine-5)methyltransferases are a group of bacterial enzymes that recognize sequences in DNA with high specificity. They transfer a methyl group from AdoMet to the 5 position of cytosine. More than 30 such enzymes have been identified and many of these have been sequenced. Sequence comparison between these enzymes indicate there are several regions that have similar if not identical sequences (1,2). It is presumed that the constant regions are involved in catalysis, the binding of the substrate, S-adenosylmethionine (AdoMet), and sequence independent interactions of the enzyme with DNA. There is one region that is very variable in enzymes that recognize different sequences but similar in enzymes that recognize the same sequence. This region is believed to be responsible for sequence specificity.

Several enzymes have the first constant region near the amino terminus of the protein. Others have up to 100 amino acids preceding the first constant region in the protein. The *Eco*RII methylase (M.*Eco*RII), which methylates the second C in the sequence CCA/TGG, has such a sequence. Methylation by M.*Eco*RII protects the DNA from restriction by the *Eco*RII restriction endonuclease.

It has been demonstrated that DNA containing the base analog 5-azacytidine forms a tight binding complex with several DNA cytosine methylases (3, 4, & 5). In this paper we report on the analysis of those portions of the protein that are protected from protease digestion when the enzyme is bound to azacytosine-containing DNA (azaC-DNA). We have found that all but the N terminal 82 amino acids are protected from protease digestion when the enzyme is bound to azaC-DNA. No protected regions were found if azaC-DNA were not present in the reaction. The enzyme must therefore undergo major conformational changes when it binds DNA. We have also analyzed deletion mutants and find that the N terminal 97 amino acids can be deleted before complete loss of enzyme activity occurs.

MATERIALS AND METHODS

*Eco*RII endonuclease was purchased from BRL laboratories. T_4 polynucleotide kinase was a product of Promega. Other enzymes were from New England Biolabs. Enzymes were used according to the manufacturers specifications. [α-^{35}S]ATP (>1000 Ci/mmol) and [α-^{32}P]ATP (3000 Ci/mmol) were obtained from Amersham. AzaC-DNA was chromosomal DNA extracted from *E. coli* Sϕ441 grown in the presence of 20 μg/ml 5-azacytidine as previously described (6). The substitution of azacytosine for cytosine was determined by base analysis as previously described (6). Twenty seven per cent of the cytosines were replaced by azacytosine.

Escherichia coli GM271 *leu*B-6 *dcm*-6 *his*G 4 *thi*-1 *hsd*R2 *ara*-14 *lac*Y1 *gal*K2 *gal*T22 *xyl*-5 *mtl*-1 *rps*L136 *ton*A31 *tsx*-78 *sup*E44 was obtained from G.Marinus and was used as the host cell in all experiments. Bacteria were grown in LB medium (7) at 37°C. Ampicillin, 50 μg/ml was added for selection of transformants.

Deletion mutagenesis of the *Eco*RII methylase gene

The plasmids pUC19 (8) and pSS69A (9) were purified by the alkaline lysis method followed by CsCl centrifugation (10). Plasmid pSS69A was digested with *Pst*I and deletions made with *Bal*31 for differing time intervals as described (10). The shortened DNA was end filled with the Klenow fragment of DNA polymerase I and *Pst*I linkers attached. After digestion with *Bam*HI and *Pst*I endonucleases the shortened fragments were isolated by agarose gel electrophoresis, electroeluted, and purified with Elutip columns (Schleicher and Schuell, Keene, NH). The DNA fragments were subcloned into *Bam*HI/*Pst*I digested pUC19. Ligations were performed at 16°C overnight and used to transform *E.coli* GM271.

Plasmid DNA from single colonies was isolated and the presence of the *Bam*HI/*Pst*I fragment and the extent of the deletion determined by appropiate restriction digestion. The sensitivity of the plasmid DNA to *Eco*RII endonuclease was then analyzed. In several cases the methylase activity of crude extracts was measured (6).

Protein identification by immunoblotting

Single colonies were incubated in 2 ml LB medium containing ampicillin (50 μg/ml) at 37°C, with shaking overnight. A 1 ml aliquot was centrifuged and the pellet suspended in 1.5 ml of 10 mM Tris-HCl, pH 7.5, 5 mM 2-mercaptoethanol, centrifuged again and finally suspended in 150 μl of the same buffer. Five μl of the cell suspension was lysed by adding SDS (final concentration 2%) and heating at 100°C for 5 min. Proteins were resolved by SDS-polyacrylamide gel electrophoresis on 7.5% acrylamide gels (11). The proteins were electroblotted onto nitrocellulose paper. Blots were washed in PBS (0.15 M NaCl, 0.01 M sodium phosphate pH 7.5) containing 3% bovine serum albumin and treated with polyclonal rabbit antibody against M.*Eco*RII diluted 1:2500. The blots were then stained with Vectastain ABC-AP as described by the manufacturer (Vector Laboratories Inc. Burlingame, CA).

Sequencing of DNA deletions

The deleted derivatives of the plasmid pSS69A were sequenced by the dideoxy chain termination method (12). The plasmids were sequenced directly with the United States Biochemical sequencing kit using the 17 bp reverse oligonucleotide primer.

Protease digestion of the M.*Eco*RII-azaC-DNA adduct

M.*Eco*RII, 450 μg, and azaC-DNA, 470 μg, were incubated in a volume of 1 ml containing 40 mM NaCl, 40 mM Tris-HCl, pH 8.0, 8 mM DTT, 2 mM EDTA (buffer A) and 20 mM AdoMet at 30°C for 30 min. Trypsin, 90 μg was added, and the incubation continued for 15 h. The reaction was terminated by addition of TLCK to 0.1 mM. The protein was precipitated by addition of 4 volumes of acetone. The precipitate was dissolved in SDS loading buffer and resolved by SDS-PAGE (11). The proteins were electroblotted on a polyvinylidene difluoride membrane and sequenced by Edman degradation as previously described (13).

Enzyme assays measured the incorporation of tritium into *E. coli* B DNA from [methyl-^{3}H]AdoMet. Determination of K_m was performed as previously described (3). The concentration of the non-variable substrate was 20 μM for AdoMet and 50 μg/ml for DNA. Calculations were performed by a weighted least squares analysis with Enzfitter (Elsevier-Biosoft).

RESULTS

Protease digestion of the M.*Eco*RII-azaC-DNA adducts

The M.*Eco*RII forms a tight binding complex with azaC-DNA which inhibits enzyme activity (3). The complex dissociates when heated in 1% SDS allowing analysis of the enzyme and its proteolytic digestion products by SDS polyacrylamide gel electrophoresis (SDS-PAGE).

Complex formation protects the enzyme from protease digestion It both decreases the rate of protease digestion of the enzyme and results in the appearance of a partial protease digestion product. Protection occurs to trypsin, chymotrypsin and Staphylococcal protease V8 digestion (Figure 1). The product of digestion of the complex with these enzymes has an M_r of 50,000 on SDS-PAGE. The intact protein has an M_r of 59,000.

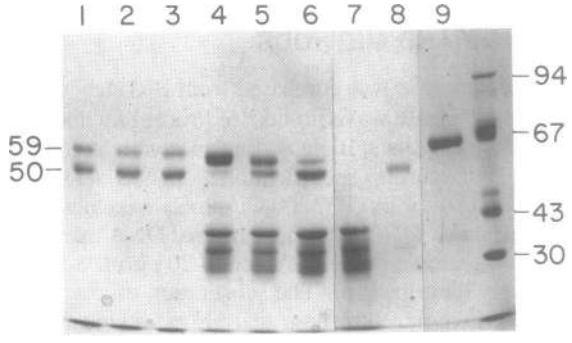

Figure 1. Digestion of M.*Eco*RII-azaC-DNA complex with proteases. M.*Eco*RII, 30 μg, was mixed with 16 μg azaC-DNA in buffer A containing 21 μM AdoMet in a volume of 30 μl and incubated at 30°C. After 30 min protease was added. Aliquots, 8 μl, were removed at the indicated times for analysis by SDS-PAGE. The gel was stained with coomassie blue. Lanes 1–3, chymotrypsin, 18 μg; lanes 4–7, Staphylococcal protease, 18 μg; lane 8, trypsin 3 μg; lane 9, M.*Eco*RII, 3.8 μg. Incubation times were: Lanes 1 & 4, 30 min; lane 2, 1 h; lanes 3 & 5, 2 h; lanes 6–8, 16 h; lane 7, control *E.coli* B DNA instead of azaC-DNA.

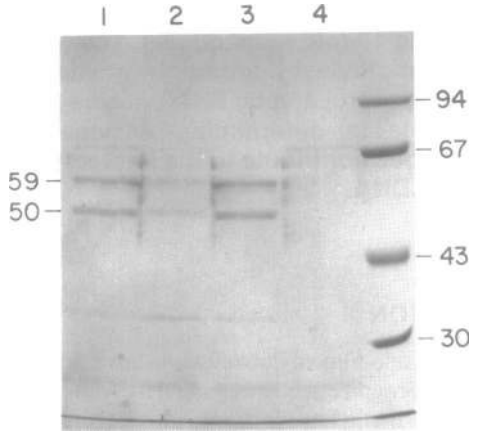

Figure 2. Effect of *Hin*fI or *Bst*NI digestion of azaC-DNA on susceptibility of the *Eco*RII-azaC-DNA complex to trypsin digestion. AzaC-DNA, 6.5 μg, was digested with 10 units of either *Bst*NI or *Hin*fI for 12 h. in a volume of 10 μl. Buffer A, 6 μl, AdoMet, final concentration 21 μM, and M.*Eco*RII, 7.5 μg, were then added, and the incubation continued for 1h at 30°C . Trypsin, 1.5 μg was added and the incubation continued for 2h at 37°C before an aliquot was analyzed by SDS-PAGE. Lane 1, *Hin*fI; lane 2, *Bst*NI; lane 3, undigested azaC-DNA; lane 4, *E. coli* B DNA.

Protection from trypsin digestion is dependent on the presence of the sequence CCA/TGG in the azaC-DNA since digestion of the DNA with *Bst*NI endonuclease, an isoschizomer of *Eco*RII that digests DNA whether or not the CCA/TGG sequence is methylated at the second C residue from the 5′ end, increases enzyme susceptibility to trypsin, whereas digestion of the DNA with HinfI (recognition sequence GANTC) does not (Figure 2). The amount of M.*Eco*RII and its digestion product remaining when the azaC-DNA is first digested with *Bst*NI, determined by densitometric analysis of the gel shown in figure 2, was 11% of the undigested control. This could be due either to a fraction of the azaC-DNA that is relatively resistant to *Bst*NI digestion or to residual activity of the digested azaC-DNA due to non specific binding to the enzyme. Since we have not been able to completely destroy the ability of azaC-DNA to inhibit M.*Eco*RII activity by *Bst*NI digestion we favor the the latter explanation. *E.coli* B DNA does not protect the enzyme from protease digestion under the conditions used.

The digested fragment was purified and sequenced for 6 cycles. Two sequences were obtained from the fragment, _LPEAPA and _MLPEAP. The first cycle did not yield unique products. These sequences begin 83 and 82 amino acids from the amino terminus of the protein. Amino acids 81 and 82 are arginine and lysine respectively. These products are consistent with the known specificity of trypsin.

Molecular weights of 54,500 and 44,900 are expected from the DNA sequence for the intact protein and trypsin fragment respectively. The difference in molecular weight determined by SDS-PAGE between the digestion product and the intact protein of 9,000 is in agreement with the expected difference based on sequence analysis.

This result implies that little if any digestion occurs at the carboxy terminus of the protein. However, since the error of the molecular weight estimation is at least 10% we analyzed the size of fragments produced by hydrolysis with formic acid. Formic acid will hydrolyze the *Eco*RII methylase preferentially at the unique asp-pro bond at position 271. The trypsin fragment should yield two peptides of 23,600 and 21,300 from the carboxy and amino terminus respectively, if the carboxy terminus is intact. The intact protein yields an amino terminal fragment of 31,000. Analysis of the formic acid hydrolysis products of the complex treated with trypsin yielded only one band that had the same mobility as the carboxyterminal product from the intact protein (Figure 3). Within the limits of this analysis we conclude that the carboxy terminus has lost less than 20 amino acids by trypsin digestion of the complex.

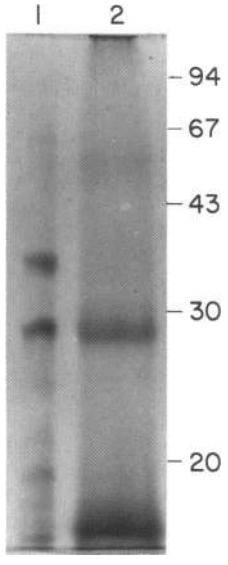

Figure 3. Formic acid hydrolysis of tryptic digestion products of M.*Eco*RII-azaC-DNA complex. AzaC-DNA, 300 μg in 600 μl buffer A was incubated with M.*Eco*RII, 240 μg, and treated with trypsin, 54 μg. The digestion products were then precipitated with 4 volumes of acetone. The precipitate was washed with acetone, dried and incubated in 0.2 ml 70% formic acid at 40°C for 42 h. The products were analyzed by SDS-PAGE on a 15% polyacrylamide gel and stained with coomassie blue. Lane 1, Acid digestion products of M.*Eco*RII; Lane 2, Acid digestion products of tryptic digest; Lane 3, standards.

Analysis of deletion mutants of M.*Eco*RII

The lack of protection of the N terminus of M.*Eco*RII by azaC-DNA from protease digestion indicates that the amino-terminus of the methylase is not directly involved in binding to DNA. We investigated the role of the amino terminus by preparing deletion mutants.

The plasmid pSS59 contains the M.*Eco*RII gene from the antibiotic resistance plasmid N3 cloned in pUC19 (9). The plasmid pSS69A contains the M.*Eco*RII gene cloned in pUC9 with 101 bp deleted downstream from the translational start site and contains a 3′ noncoding region of about 400 bp. The gene is in frame with the *lacZ* translational start site. Plasmid pSS69B has the same insert in pUC9 as pSS69A except it is out of phase with the *lacZ* translational start site. Plasmid pSPXba specifying a protein containing a carboxyterminal deletion was prepared by placing an Xba linker in the *Stu*I site, 148 bp from the terminal stop codon. This linker contains a TGA stop codon in all three reading frames.

Plasmid pSS69A was used to prepare a series of *Bal*31 deletions that were cloned into pUC19. The resulting transformants were screened for resistance to *Eco*RII endonuclease. Plasmids encoding active *Eco*RII methyltransferase would be resistant to digestion by the *Eco*RII endonuclease. All of 15 plasmids containing inserts longer than 1.6kb were resistant to *Eco*RII

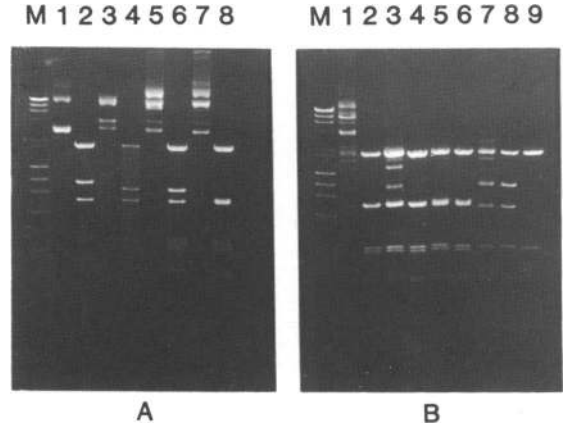

Figure 4. Restricton analysis of deletion mutants. Plasmids were isolated from an overnight culture as described in the Methods, digested with either *Bst*NI or *Eco*RII, and the products analyzed by electrophoresis. A: lanes 1 & 2, pSS59; lanes 3 & 4, pSS69A; lanes 5 & 6, pSS69B; lanes 7 & 8, pLY15-11. B: lanes 1 & 2 pLY14-122; lanes 3 & 4 pLY14-121; lanes 5 & 6, pLY14-3; lanes 7 & 8, pSPXba; lane 9, pUC19. Lanes 1, 3, 5, 7, & 9, *Eco*RII; Lanes 2, 4, 6, & 8, *Bst*NI.

restriction (data not shown). Some isolates containing inserts of 1.57−1.60 kb were partially resistant to the enzyme and all inserts shorter than 1.57 kb were completely sensitive to digestion.

Selected clones were tested for the presence of a protein that reacted with a polyclonal antibody to the *Eco*RII methylase by western blot. All clones that yielded plasmids that were either totally or partially resistant to *Eco*RII digestion made an immunoreactive protein of 46,000 or greater, whereas all plasmids coding for proteins smaller than 46,000 were digested to completion by *Eco*RII (Figure 4 & 5B).

Inserts in pUC19 were sequenced. The DNA and derived protein sequences are presented in Figure 6. Since the M.*Eco*RII gene in plasmid pSS69B is out of phase with the *lacZ* translational start site the protein made in strains carrying pSS69B presumably starts at an internal translational start codon 247 bp from the normal initiation site. Nineteen base pairs upstream from this site is a Shine−Delgarno AGAGG sequence. All inserts longer than 1.6kb would contain this initiation site. Bacteria containing pSS69B had less enzyme activity than bacteria containing pSS69A (Table I).

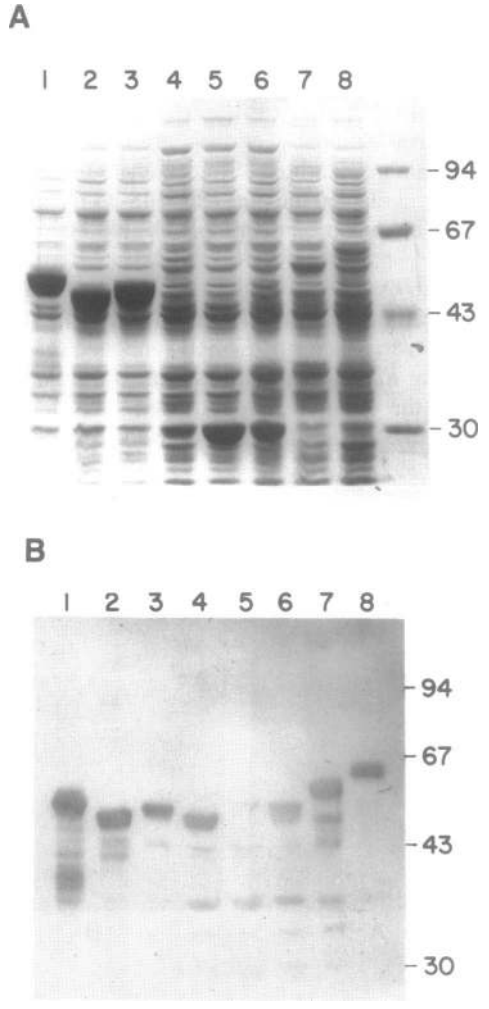

Figure 5. Analysis of proteins synthesized by deletion mutants. A. SDS-PAGE analysis of samples stained with coomassie blue. B. Transblot of gel shown in panel A analyzed with M.*Eco*RII antibody. Lane 1, pSPXba; lane 2, pLY14-3; lane 3, pLY14-121; lane 4, pLY14-122; lane 5, pLY15-11; lane 6, pSS69B; lane 7, pSS69A; lane 8, pSS59.

Deletions between 247 and 281 bp from the normal initiation codon caused a decrease in enzyme activity. Removal of 7 more bp caused a marked reduction in activity although partial protection of the plasmid from *Eco*RII digestion could still be detected. Further deletions caused complete loss of enzyme activity (Figure 4, Table I).

The relative size of the proteins synthesized from these deletions was consistent with their predicted molecular weights. Some deletions that were out of phase with the *lacZ* translational start site in pUC19 (pLY15-11, pLY14-122) synthesized active enzyme, presumably from a translational start site in the *Sph*I site of the polylinker. Deletions that removed the internal M.*Eco*RII start codon noted above, and were fused out of phase with either the *lacZ* or *Sph*I translational start site, did not synthesize an immunoreactive protein (data not shown).

The carboxyterminal truncated protein synthesized in cells containing the plasmid pSPXba may have some activity in vivo since complete digestion with *Eco*RII endonuclease of plasmids isolated from a *dcm*⁻ strain could not be achieved (Figure 4).

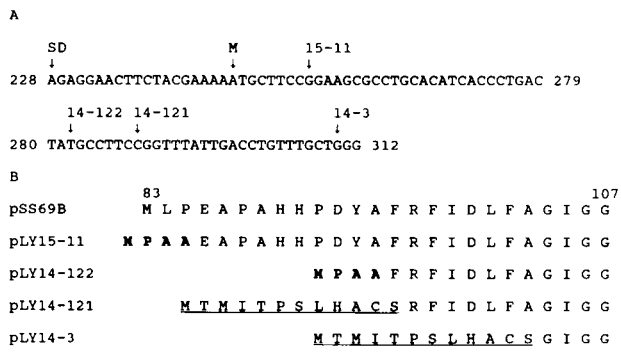

Figure 6. Sequence of deletion mutants. A. DNA sequence of part of the *Eco*RII methylase with a putative internal Shine−Delgarno, SD; methionine start codon, M; and start of sequence in plasmids pLY15-11, pLY14-122, pLY14-121 and pLY14-3. Numbers correspond to distances from the translational start site of the M.*Eco*RII gene. B. Derived amino acid sequence of N-terminus of protein made by these deletions. These sequences are initiated either at the *lacZ* initiation codon (pLY14-121, pLY14-3), or the *Sph*I site in the multiple cloning region of pUC19 (pLY15-11, pLY14-122). Additional amino acids from the *Sph*I site are in bold type; those from *lacZ* are underlined.

Table I. Properties of deletion mutants[a]

Plasmid	M_r	Derived MW	Enzyme activity[b]	Digestion by *Eco*RII[c]
pSS59	60,000	54,500	1	R
pSS69A	54,000	51,600	0.04	R
pSS69B	50,000	45,100	0.005	R
pLy15-11	51,000	45,200	0.004	R
pLY14-122	46,000	38,800	0.001	P
pLY14-121	48,000	44,800	n.d[d]	P
pLY14-3	46,000	38,800	n.d.	S
pSPXba	51,000	48,900	n.d.	P

[a] The relative molecular weights of the M.*Eco*RII proteins made by deletion mutants were determined from Western blots as in Figure 5.
[b] Enzyme activity was determined as described in METHODS from overnight cultures. The activity is expressed relative to that determined for pSS59 which was 57 pmol/min/mg protein.
[c] R, resistant; P, partial digestion; S, complete digestion.
[d] n.d. none detected

Enzyme activity

The relative methylase activity determined in vivo by restriction digestion of the isolated plasmids correlated with the enzyme activity determined in broken cell extracts. Several constructs that demonstrated minimal activity in vivo were inactive in vitro. We therefore determined if these strains were deficient in enzyme protein. As shown in Figure 5 the major protein synthesized in cells containing the partially active plasmids pSPXba3 and pLY14-121 and the inactive plasmid pLY14-3 was the M.*Eco*RII protein. The lack of activity cannot therefore be ascribed to lack of enzyme protein but must be due to the inactivity of the protein synthesized.

Relative amounts of the M.*Eco*RII proteins were measured in enzyme extracts to determine whether protein levels were responsible for the differences in methylase activity measured *in vitro*. Relative abundance of the M.*Eco*RII was determined by immunoblotting of SDS-PAGE separations of the enzyme present in supernatants of cell extracts prepared for activity determinations. The relationship between enzyme activity and enzyme protein was similar in extracts from cells containing pSS59 and pSS69A. Enzyme activity of extracts from cells containing pLY15-11 was 30% of that expected on the basis of enzyme protein present (data not shown). The decrease in enzyme activity of extracts from cells containing pSS69A and pLY15-11 was primarily due to a decrease in enzyme content of the extract.

Characteristics of pLY15-11

The *Hind*III/*Bam*HI fragment from pLY15-11 was cloned into plasmid pT7-6 placing the gene under control of a phage T7 promoter and transformed into WP2(pGP1-2), a *lon*$^-$ strain of *E.coli* B containing plasmid pGP1-2, which has the T7 RNA polymerase under control of the lambda P_L promoter (14). The plasmid also contains the heat sensitive lambda repressor. The enzyme was purified from this strain.

The specificity of the enzyme was determined by analyzing the *Hin*fI restriction fragments of pBR322 methylated with [methyl-^{3}H]AdoMet. The fragments contained radioactivity in the same proportion as when the plasmid is methylated with the intact enzyme (data not shown).

The K_m for AdoMet of the truncated protein was 7.6 ± 3.1 uM as compared to 0.28 ± 0.03 μM for wild type enzyme. The K_m for DNA of the truncated protein was 4.2 ± 0.6 μg/ml as compared to 0.23 ± 0.05 μg/ml. Deletion of the amino terminus has a similar effect on the K_m for both AdoMet and for DNA.

DISCUSSION

Ten blocks of sequences are conserved among the procaryotic DNA(cytosine-5)methyltransferases and are distributed in the C terminal 350 amino acids of the *Eco*RII methylase. The N terminal 97 amino acids of M.*Eco*RII have no sequence similarity to other cytosine methylases (1,2). Since these methylases recognize different sequences in DNA the conserved structural features must be important for functions other than sequence recognition. In this study we have found that all but the N terminal 82 amino acids are protected from protease digestion when the enzyme is bound to azaC-DNA. The size of the protected fragment was similar whether chymotrypsin, trypsin or Staphylococcal V8 protease was used to digest the complex. Enough protease was used in these experiments to digest the free enzyme, or enzyme in the presence of *E.coli* B DNA, within one hour. With trypsin, up to 30% of the methylase in the azaC-DNA complex yielded a partial digestion product after 16 h of incubation. The complex dissociates on heating in 1% SDS allowing for the identification of the digestion products by SDS-PAGE.

Acid hydrolysis of the intact protein has been shown to occur at the unique asp-pro bond yielding a carboxy terminal fragment of 206 amino acids (13). The acid hydrolysis product of the trypsin digested protein has the same mobility as the carboxyterminal peptide released from the intact protein. Therefore little if any proteolytic digestion occurs at the carboxy terminus of the protein when it is bound to azaC-DNA. The amino terminal fragment is expected to contain 189 amino acids. It does not appear as a separate band on SDS-PAGE (figure 3).

In order to determine if the protected fragment was sufficient for activity we constructed deletion mutations of the enzyme. We expected one-third of the isolates to be in phase with the *lac*Z translational start site. However all of 15 isolates containing inserts longer than 1.6 kb had methylase activity. This is consistent with a start site at an internal ATG 83 amino acids from the N terminus of the enzyme. This methionine is also the N terminal amino acid of the fragment protected from protease digestion by azaC-DNA. Fragments smaller than this only had activity when fused in phase with either the *lac*Z translational start site or an ATG in the polylinker. Proteins that had deletions through amino acid 96 had little activity. This amino acid is two amino acids prior to the first constant region. It is also the equivalent of the first amino acid of the *Dde*I methylase, the cytosine methylase with the shortest amino terminal sequence prior to the first constant region (1,2). The SPR methylase has been shown to be inactivated by removal of the amino terminal 15 amino acids (15). In this case the deletion removes part of the first constant region.

One carboxy terminal deletion was constructed missing 49 amino acids by inserting stop codons in each reading frame in an internal StuI site. This construction should delete the carboxy terminal constant region. Suprisingly, plasmid DNA isolated from this construction could not be completely digested with *Eco*RII endonuclease, even though control plasmids were digested to completion (figure 4). There may have been suppression of the stop codon which could explain the remaining activity. Placement of an in frame insertion at this site does not destroy enzyme activity (data not shown). Wilke et al (16) have found that deletion of seven amino acids from the carboxy terminus of the SPR methylase markedly reduces activity.

Protein synthesized by the deletion mutation encoded by pLY15−11 was purified. This enzyme had the same specificity as the *Eco*RII methylase although the K_m for both AdoMet and DNA were increased about 27 and 18 fold respectively. The amino terminus of the protein, although a variable region in these methylases, therefore plays no part in determining sequence specificity.

The protection of 80% of the protein from protease digestion by azaC-DNA indicates that the protein undergoes a marked structural alteration when it binds to DNA. The results of the deletion analysis indicate that the amino terminal portion of the *M.Eco*RII, consisting of 20% of the protein, which is part of a variable region present in a fraction of the DNA cytosine methylases, is not critical for substrate binding or the sequence specificity of this protein.

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REFERENCES

1. Lauster, R., Trautner, T. A. and Noyer-Weidner, M. (1989) *J.Mol. Biol.* **206,** 305–312.
2. Posfai, J., Bhagwat, A. S., Posfai, G., and Roberts, R. J. (1989) *Nucleic Acids Res.* **17,** 2421–2435.
3. Friedman, S. (1985) *J. Biol. Chem.* **260,** 5698–5705.
4. Friedman, S. (1986) *Nucleic Acids Res.* **14,** 4543–4556.
5. Santi, D. V., Norment, A. and Garrett, C. E.(1984) *Proc. Nat'l. Acad. Sci. USA.* **81,** 6993–6997.
6. Friedman, S. (1981) *Mol. Pharmacol.* **19,** 314–320.
7. Miller, J. H. (1972) Experiments in Molecular Genetics pp. 432–433, Cold Spring Harbor Laboratory, Cold Spring Harbor, N. Y.
8. Celeste, Y.-P., Vieira, J., and Messing, J. (1985) *Gene* **33,** 103–119.
9. Som, S., Bhagwat, A. S., and Friedman, S. (1987) *Nucleic Acids Res.* **15,** 313–332.
10. Maniatis, T., Fritsch, E. F. and Sambrook, J. (1982) in Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, N. Y.
11. Laemmli, U. K. (1970) *Nature* **227,** 680–685.
12. Sanger, F., Nicklen, S. and Coulson, A. R. (1977) *Proc. Nat'l. Acad. Sci. USA.* **74,** 5463–5467 .
13. Som, S., and Friedman, S. (1991) *J. Biol. Chem.* **266,** 2937- 2945.
14. Tabor, S., and Richardson, C. C. (1985) *Proc. Nat'l. Acad. Sci. USA.* **82,** 1074–1078 .
15. Lauster, R. (1987) Doctoral Thesis, Freie Universitat Berlin.
16. Wilke, K., Rauhut, E., Noyer-Weidner, M., Lauster, R., Pawlek, B., Behrens, B., and Trautner, T. A. (1988) *EMBO J.* **7,** 2601–2609.