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# Substrate Requirements for ErmC′ Methyltransferase Activity

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**ErmC is a methyltransferase that confers resistance to the macrolide-lincosamide-streptogramin B group of antibiotics by catalyzing the methylation of 23S rRNA at a specific adenine residue (A-2085 in *Bacillus subtilis*; A-2058 in *Escherichia coli*). The gene for ErmC was cloned and expressed to a high level in *E. coli*, and the protein was purified to virtual homogeneity. Studies of substrate requirements of ErmC have shown that a 262-nucleotide RNA fragment within domain V of *B. subtilis* 23S rRNA can be utilized efficiently as a substrate for methylation at A-2085. Kinetic studies of the monomethylation reaction showed that the apparent $K_m$ of this 262-nucleotide RNA oligonucleotide was 26-fold greater than the value determined for full-size and domain V 23S rRNA. In addition, the $V_{max}$ for this fragment also rose sevenfold. A model of RNA-ErmC interaction involving multiple binding sites is proposed from the kinetic data presented.**

Resistance to the macrolide-lincosamide-streptogramin B group of antibiotics in bacteria is mediated through the methylation of 23S rRNA by a series of methyltransferases (methylases) designated Erm (20). In general, Erm methylases confer resistance through the mono- or dimethylation of the N-6 of a specific adenine residue in 23S rRNA (A-2085 in *Bacillus subtilis*; A-2058 in *Escherichia coli*), which results in decreased affinity of methylated ribosomes for macrolide-lincosamide-streptogramin B antibiotics (9–11, 20). Meier et al. (12) have shown that clarithromycin (an erythromycin analog)-resistant strains have undergone mutation at the A-2058-equivalent site to each of the other three nucleotides. Though the basis for macrolide-lincosamide-streptogramin B resistance has been known for more than 20 years, neither the mechanism of the methylation reaction nor the structure of the RNA segment that participates in the reaction is well understood. Kovalic et al. (8) and Vester and Douthwaite (22) have shown independently that the domain V (DV) segment of 23S rRNA, corresponding to the 23S rRNA sequences containing A-2085 from *B. subtilis* or A-2058 from *E. coli*, could serve as a substrate for in vitro methylation by ErmSF (from *Streptomyces fradiae*) and ErmE (from *Saccharopolyspora erythraea*), respectively.

One of the better studied Erm methylases is ErmC, a 29,000-Da polypeptide encoded by plasmid pE194, originally found in *Staphylococcus aureus* (6) but subsequently transferred to *B. subtilis* (19). A variant, ErmC′, encoded by the *B. subtilis* plasmid pIM13 (13) differs in 5 of the 224 total amino acids and has been shown to be more stable than ErmC (21). In this paper we have cloned and expressed *ermC′* to a high level in *E. coli* and have purified the protein to virtual homogeneity. Studies on substrate requirements of ErmC′ have shown that a 262-nucleotide (nt) RNA fragment within DV of *B. subtilis* 23S rRNA can be utilized efficiently as a substrate for methylation at A-2085. We also compared the kinetics of the monomethylation reaction using various RNAs as the substrates.

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## MATERIALS AND METHODS

**Bacterial strains, plasmids, and growth conditions.** *E. coli* DH5α (F⁻ *endA1 hsdR17 supE44 thi-1* λ⁻ *recA1 gyrA96 relA1* φ80d*lacZ*ΔM15) was obtained from Gibco BRL (Gaithersburg, Md.). *B. subtilis* BD170 (19) was obtained from D. Dubnau. Plasmid pJO200 is a derivative of pTB210 (1) that contains a multicloning site at the 3′ end of the cloned *kdsB* gene. The vector pGEM-T, which allows direct cloning of PCR-amplified fragments, was obtained from Promega (Madison, Wis.). pIM13, a *B. subtilis* vector that contains *ermC′* (13), was obtained from D. Dubnau. Superbroth medium consisted of 3 mM glucose, 3.3% (wt/vol) Bacto Tryptone (Difco), 2.0% Bacto Yeast Extract, and 0.5% NaCl. Other media and procedures for the growth and transformation of *E. coli* were from the work of Sambrook et al. (17).

**DNA manipulations.** Procedures for plasmid isolation (minipreparations), restriction digestion, ligation, agarose gel electrophoresis, recovery of DNA from agarose gels, and DNA hybridization were from the work of Sambrook et al. (17). PCR amplifications were performed in a GeneAMP PCR System 9600 (Perkin-Elmer Cetus) with reagents and conditions described previously (16). All DNA sequencing employed double-stranded templates with Sequenase 2 (U.S. Biochemicals, Cleveland, Ohio) by procedures previously described (18, 23).

**In vitro RNA preparations.** The 23S rRNA gene was amplified by PCR from a *B. subtilis* BD170 chromosomal preparation and cloned into *E. coli* DH5α by using the pGEM-T vector (Promega). A minipreparation of plasmid carrying the 23S rRNA gene was purified and linearized completely by *Nde*I restriction enzyme digestion prior to being used as a template for in vitro transcription employing the Promega RiboMAX Kit under the conditions described by the supplier with the following modification: an additional half amount of RNA polymerase was added 1.5 h after the incubation was started. With this modification, 10 μg of template DNA could produce up to 1 mg of RNA during a 3-h transcription reaction. In general, this modification not only increased the yield of RNA but also circumvented premature transcription termination, thereby giving rise to a better purity of RNA.

DV of the 23S rRNA gene corresponding to the sequence from nt 2065 to 2687 (Fig. 1) was obtained by PCR using primers oligo-5 and oligo-6 (Table 1). Similarly, PCR was used to generate a number of mutations in the 23S rRNA gene, as described in Table 1. The wild-type and mutated DV sequences were then used as templates in second PCRs using oligo-14 and oligo-6 as primers (Table 1) to insert the T7 promoter upstream of the coding sequences, and the purified DNAs from the second PCRs were used directly as templates for in vitro transcription reactions as described above. Truncated DV ($A^-$) containing the sequence nt 2065 to 2618 was obtained by PCR using oligo-5 and oligo-12 as primers and transcribed in vitro by T7 RNA polymerase.

The DV ($B^-$) segment was made by ligation of sequences from nt 2065 to 2097 (fragment 1) and nt 2464 to 2687 (fragment 2). Fragment 1 was made by allowing sense oligo-15 and antisense oligo-16 (Table 1) to hybridize in Tris-EDTA buffer. Fragment 2 was obtained by PCR using the 23S rRNA gene-containing plasmid as the template and primers oligo-6 and oligo-13 (containing a *Bam*HI cloning site at the 5′ end). The purified PCR product was cut by *Bam*HI digestion and then ligated to fragment 1. The ligation product was purified by agarose gel electrophoresis and then PCR amplified with oligo-14 and oligo-6 as primers. All fragments produced were confirmed by nucleotide sequencing.

**In vitro methylation of RNA.** Methylation of RNA in vitro catalyzed by ErmC′ methylase was done by following previously described procedures (3) with the following modifications. Each reaction mixture contained 5 pmol of RNA, 0.08 pmol of [$^3$H]*S*-adenosylmethionine ([$^3$H]SAM) (84 Ci/mmol; Amersham, Arling-

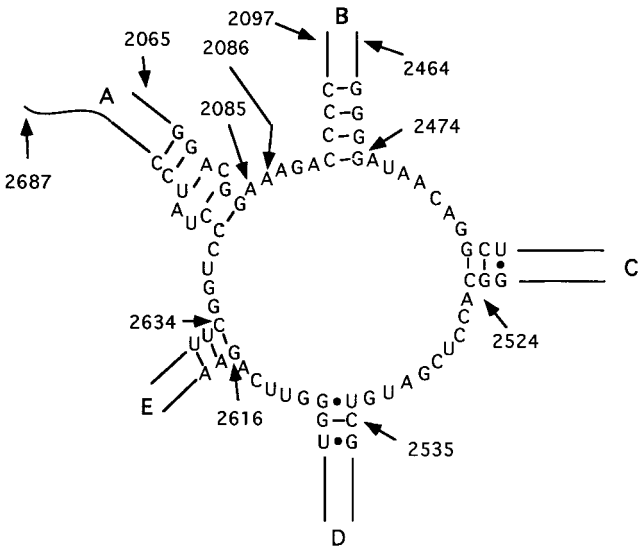


FIG. 1. Proposed secondary structure of the peptidyltransferase region in DV of *B. subtilis* 23S rRNA adapted from that proposed for *E. coli* (5, 14). A-2085 is the site of methylation. Various other residues are indicated. Segments A to E correspond the defined helices 73, 74, 89, 90, and 93 after the work of Brimacombe et al. (2).

ton Heights, Ill.), 50 mM Tris-HCl (pH 7.5), 40 mM KCl, 4 mM $MgCl_2$, 10 mM dithiothreitol, 50 μg of bovine serum albumin, 10 U of RNase inhibitor (5 Prime to 3 Prime, Inc., Boulder, Colo.) per ml, and 0.25 μg of ErmC′ in a total volume of 50 μl. All additions were performed at 0°C, and the reaction tube was then transferred to 37°C for 30 min. For kinetic experiments, 25-μl reaction mixtures containing various concentrations of RNA were preincubated at 37°C for 10 min prior to the addition of ErmC′. To determine initial velocities of methylation, samples were taken every 3 min for up to 15 min. Reactions were stopped by adding 1 vol of 12% ice-cold trichloroacetic acid. The samples were centrifuged in a microcentrifuge, and the pellets were washed once with 5% trichloroacetic acid, dissolved in 1 ml of scintillation fluid (INSTA-GEL FX from Packard), and counted. The extent of RNA methylation was determined from the incorporation $^3H$. The reaction was linear for up to 60 min, and the RNA-free and enzyme-free blanks yielded 50 to 80 cpm under these conditions. At least two independent measurements were made for every experiment.

**TLC.** Products from in vitro methylation reactions (50 μl) were precipitated by the addition of 5 μl of 3 M sodium acetate and 130 μl of cold ethyl alcohol. The pellets were washed once with 70% ethyl alcohol and then resuspended in 10 μl of $H_2O$ and 10 μl hot 2 N HCl. Samples were boiled in a sealed plastic pipette tip for 1 h, and 5 μl of acid hydrolysates was separated on cellulose thin-layer chromatography (TLC) sheets (Kodak no. 13254) by employing a solution of isopropanol-water-concentrated $NH_4OH$ (79:20:1) (4). Standard compounds, guanine, adenine, $N^6$-monomethyladenine, and $N^6$, $N^6$-dimethyladenine were purchased from Sigma.

**Purification of recombinant ErmC methylase.** *E. coli* pERM1 was grown overnight at 30°C in Luria-Bertani medium supplemented with 3 mM glucose and containing 100 μg of ampicillin per ml. The culture was diluted 100-fold into 10 liters of fresh medium and grown in a Bioflo III fermentor at 25°C maintaining the pH at 7.0. Methylase synthesis was induced by the addition of 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG) when an $A_{600}$ of 0.8 to 1.0 was reached, and growth was continued for an additional 14 to 15 h. The cells were harvested, and ErmC′ was purified essentially as described previously (19), with two modifications. The phosphocellulose chromatographic step was replaced by S-Sepharose with *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES), pH 7.6, as the column buffer, and the DEAE-Sephadex step was omitted. The final preparation was essentially pure as judged by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The modified purification method described here was simpler and faster compared with the previously reported one and yielded material of equal or greater purity.

## RESULTS

**Cloning and expression of *ermC* in *E. coli*.** PCR primers were designed with *Sal*I and *Eco*RI restriction sites for subcloning the *ermC′* sequence from pIM13 into plasmid pJO200. To provide translational coupling of *ermC′* to *kdsB*, the sense PCR primer contained a ribosome binding site for the *ermC′* cistron and a stop codon for the upstream *kdsB* gene 5′ to the start codon for *ermC′* (Table 1). The upstream cistron encodes the first 14 amino acids of the *kdsB* gene followed by a 3-amino-acid linker. The dicistronic vector carrying *ermC′* was designated pERM-1. The integrity of *ermC′* was confirmed by nucleotide sequencing. Expression of the gene was examined in *E. coli* XL-1 Blue. As can be seen in Fig. 2, growth at 37°C yielded high-level expression but most of the ErmC′ produced was found as an insoluble aggregate in the cellular pellet (lanes 5 and 6). Proportionately more soluble material was found when the cells were grown at 30°C, and virtually all of the ErmC′ produced at 25°C was soluble, although the total amount of ErmC′ produced was somewhat reduced. Purification of ErmC′ (see Materials and Methods) from a 10-liter culture grown at 25°C in a fermentor resulted in the recovery of >200 mg of highly purified, soluble enzyme.

**Kinetics of ErmC methylation of in vitro-produced 23S rRNA.** Although *B. subtilis* 23S rRNA has been shown to be the optimum substrate for ErmC and ErmC′ (3), a commercially available, unpurified preparation of rRNA extracted from *E. coli* has generally been used as a substrate for ErmC′ and many other Erm methylases. We have found that the RNA produced from the in vitro transcription of the cloned *B. subtilis* 23S rRNA gene could serve as a methylation substrate for ErmC′ at least as well as the commercial *E. coli* rRNA preparation (data not shown). A very low concentration of SAM (0.08 pmol/50 μl) relative to that of RNA (5 pmol/50 μl) was employed so that most of the reacted RNA was found in the monomethylated form (Fig. 3). In all experiments reported here, therefore, the ErmC′ concentration was chosen from a lower point within the linear range to give a high degree of reproducibility of the reaction rate. Under our assay conditions, the reaction was linear for up to 1 h (data not shown). The relative kinetics of the various RNA substrates used in the reactions shown here did not change when the SAM concentration was raised to allow dimethylation to occur. In methylation studies, in vitro-produced RNAs treated at 75°C for 10 min in 1× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) buffer and then slowly cooled to room temperature showed as substrates no difference from those without the hybridization treatment, indicating that the proper secondary and tertiary structures had been formed during the in vitro RNA synthesis reactions.

**Substrate specificity.** The DV segment of 23S rRNA (nt 2065 to 2687) was predicted to include a central loop and five regions, A to E, containing secondary structure (Fig. 1). This 623-nt segment was produced by in vitro transcription with T7 RNA polymerase by employing a DV DNA fragment generated by PCR that used a 5′ primer containing the T7 promoter. When this RNA was used in kinetic experiments with ErmC′, it was found that the apparent $K_m$ and $V_{max}$ matched the values determined for the full-length substrate (Table 2 and Fig. 4A). In addition, the site of methylation on DV RNA was shown by TLC to have adenine as the only base to have been methylated (Fig. 3). The specificity of methylation on A-2085 was confirmed by site-directed mutagenesis. All three mutated DV fragments, A-2085 to T (A2085T), A2085G, and A2085C, were methylated to less than 10% of the level observed with the correct DV RNA fragment (Table 3). In addition, the fragment designated DV ($A^-$), designed to have the central loop structure of DV broken (Fig. 1), was also found to have very low substrate activity toward ErmC′ (Table 3).

To test whether the primary sequence of DV is essential for enzyme recognition, mutations at the nucleotides neighboring A-2085 were generated and tested for their activities as sub-

TABLE 1. Deoxyoligonucleotides employed

| Deoxyoligonucleotide | Description | Sequence |
|---|---|---|
| oligo-1 | Sense PCR primer for *ermC′* cloning | ATATAAGTCGACTAAGGAGGTATAATATGAACGAGAAAAATATAAAACACA<br>*Sal*I RBS[a] |
| oligo-2 | Antisense PCR primer for *ermC′* cloning | GGTGGAATTCGGCTAGTTATTACTTATTAAATAATTTATAGCTATTGAA<br>*Eco*RI |
| oligo-3 | Sense PCR primer for 23S rRNA cloning | GGTTAAGTTAGAAAGGGCGC |
| oligo-4 | Antisense PCR primer for 23S rRNA cloning | ATGGTTAAGTCCTCGATCGA |
| oligo-5 | Sense PCR primer for DV | GGTTACCCGCGACAGGACGGAAAGAC<br>2085 |
| oligo-6 | Antisense PCR primer for DV | CGTACTAAGGACAGCTCCTCTCA |
| oligo-7 | Sense PCR primer for A2085T | GGTTACCCGCGACAGGACGGTAAGAC<br>2085 |
| oligo-8 | Sense PCR primer for A2085C | GGTTACCCGCGACAGGACGGCAAGAC<br>2085 |
| oligo-9 | Sense PCR primer for A2085G | GGTTACCCGCGACAGGACGGGAAGAC<br>2085 |
| oligo-10 | Sense PCR primer for A2086T | GGTTACCCGCGACAGGACGGATAGAC<br>2086 |
| oligo-11 | Sense PCR primer for G2084A | GGTTACCCGCGACAGGACGAAAAGAC<br>2084 |
| oligo-12 | Antisense PCR primer for DV ($A^-$′) | TTCTGAACCCAGCTCGCGTA |
| oligo-13 | Sense PCR primer for DV ($B^-$) fragment 2 | CAAGGGATCCTACCCCGGGGATAACA<br>*Bam*HI |
| oligo-14 | Sense T7 promoter-containing PCR primer | TAATACGACTCACTATAGGTTACCCGCGACAGGA<br>T7 promoter |
| oligo-15 | Sense oligonucleotide for DV ($B^-$) fragment 1 | TAATACGACTCACTATAGGTTACCCGCGACAGGACGGAAAGACCCGTGGT<br>T7 promoter |
| oligo-16 | Antisense oligonucleotide for DV ($B^-$) fragment 1 | GATCTACCACGGGTCTTTCCGTCCTGTCGCGGGTAACCTATAGTGAGTCGTATTA |

[a] RBS, ribosome binding site.

strates of ErmC′. The G2084A change reduced the methylation of the resultant DV fragment to ca. 12% of the level of the wild-type DV fragment under the same assay conditions (Table 3). In a preliminary test, DV RNA carrying the A2086T change was methylated to ca. 50% of the level of wild-type DV RNA (Table 3). Kinetic studies with this substrate indicated very little change in the $V_{max}$ but an about fivefold increase in the $K_m$ (Table 2; Fig. 4A). Thus, a change in the sequence surrounding A-2085 can affect the binding of the RNA to the enzyme but may not change the overall rate of methylation.

A small fragment of DV was constructed by combining sequences from nt 2065 to 2097 and nt 2464 to 2687 of the 23S rRNA gene. RNA transcribed from this fragment was expected to form a central loop-like structure on the basis of the computer model shown in Fig. 1 but would be missing the 466-nt segment (nt 2098 to 2463) that was predicted to contain a great amount of secondary structure of segment B. The apparent $K_m$ of this DV ($B^-$) 262-nt RNA oligonucleotide was 26-fold greater than the value determined for DV RNA. In addition, the $V_{max}$ for this fragment also increased sevenfold (Fig. 4B; Table 2).

We have also tested a number of RNA oligonucleotides ranging in size from 10 to 50 nt. The oligonucleotides were designed as either a simple single strand with A-2085 in the center or with the segment A-2085AAGA either on the end of segment A or preceding segment B. None of the oligonucleotides made displayed detectable substrate activity. Thus, we have not yet determined the minimal size of RNA required for methylation by ErmC′.

## DISCUSSION

Translational coupling in a dicistronic vector that employed *kdsB* as the upstream gene was used previously for the high-level expression of human 12-kDa FK506 binding protein in *E. coli* (15). We have shown here that this expression system is also useful for obtaining high levels of ErmC′. The finding that the production of soluble, enzymatically active protein required cultivation of the cells at 25°C was unexpected, however. Previous work has shown that active ErmE methylase could be obtained from *E. coli* grown at 37°C or higher (7, 22) but that the enzyme is found associated with ribosomes. We

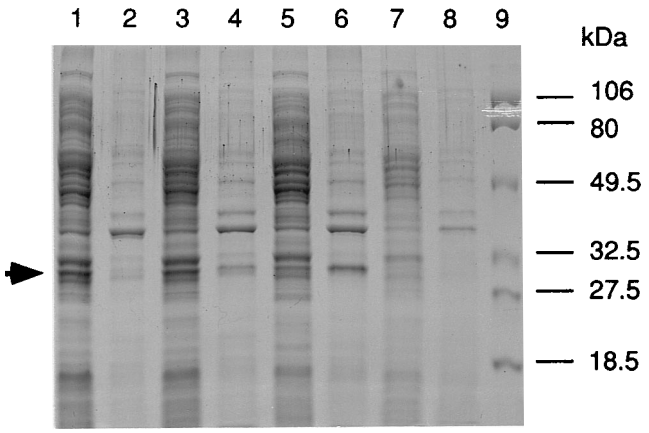


FIG. 2. Expression of *ermC′* at various temperatures: SDS–10% PAGE of *E. coli* XL-1 Blue expressing *ermC′* from pERM-1. Cells were grown in shaker flasks in Superbroth medium containing 50 μg of ampicillin per ml and 15 μg of tetracycline per ml. The cultures were induced with 1 mM IPTG upon reaching an optical density at 600 nm of 2.0 and grown for a further 18 h before being harvested. The cells were then resuspended in 50 mM Tris (pH 8.0)–50 mM NaCl–1 mM EDTA and lysed by sonication. All lanes contain the supernatant or pellet from centrifugation at 10,000 × *g* for 1 min. Lanes 1 and 2, supernatant and pellet, respectively, from 25°C expression; lanes 3 and 4, supernatant and pellet, respectively, from 30°C expression; lanes 5 and 6, supernatant and pellet, respectively, from 37°C expression; lanes 7 and 8, supernatant and pellet, respectively, of XL-1 Blue (no plasmid) grown at 30°C; lane 9, Bio-Rad prestained low-molecular-mass markers. The arrow indicates ErmC′.

have not examined whether all of the active ErmC′ recovered from *E. coli* grown at either 25 or 37°C was also ribosome associated. In addition, we have not attempted to resolubilize the insoluble ErmC′ aggregates produced at the higher temperatures.

We have shown here that in vitro-generated RNA containing the sequence of the 23S rRNA from *B. subtilis* can be used as a substrate for methylation by the ErmC′ methylase and that methylation takes place very likely at A-2085 (according to our site-directed mutagenesis data), the naturally occurring site of

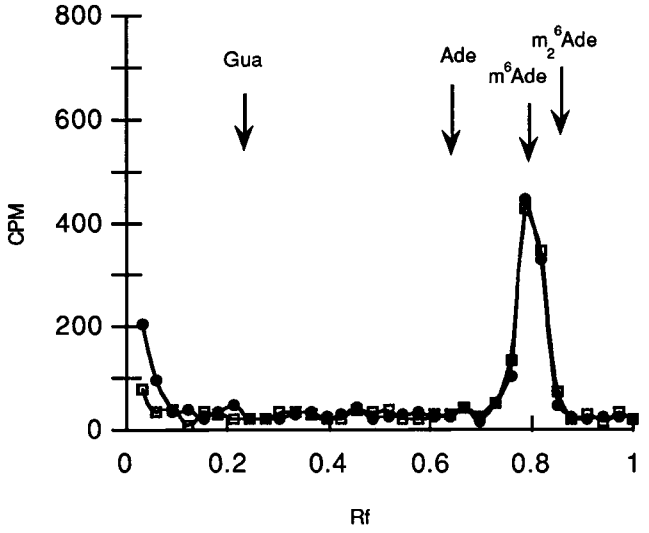


FIG. 3. TLC of hydrolysates from in vitro-methylated *B. subtilis* 23S rRNA (●) and DV ($B^-$) (□). Methylated RNA labeled with $^3H$ was hydrolyzed with 1 N HCl, and the resulting hydrolysates were separated by cellulose TLC (see Materials and Methods). DV gave the same TLC pattern.

TABLE 2. Monomethylation of various rRNA fragments by ErmC′ in vitro

| RNA fragment (nt) | $K_m$ (nM) | $V_{max}$ (pmol/min/mg of ErmC′) |
|---|---|---|
| 23S rRNA (2,927) | 37.5 | 6.7 |
| DV (623) | 34.4 | 6.3 |
| DV A2086T (623) | 144 | 8.9 |
| DV ($B^-$) (262) | 910 | 48 |

methylation in vivo. We have also shown that in vitro-generated RNA corresponding to DV of *B. subtilis* 23S rRNA can be methylated at A-2085 by ErmC′ equally as well as the full-length rRNA sequence. This finding is in agreement with the recent results of Kovalic et al. (8) and Vester and Douthwaite (22), who employed ErmSF and ErmE, respectively, to methylate A-2085 of in vitro-produced DV from *B. subtilis* 23S rRNA and A-2058 from *E. coli* 23S rRNA. Both the $K_m$ and $V_{max}$ of the methylation reactions do not change when the DV fragment is used in place of the full-length RNA, suggesting that the local structure of the RNA that binds to ErmC′ and undergoes methylation is the same in the two different-size substrates. It should be noted that the RNA substrates used in our kinetic measurements were produced by in vitro transcription and thus were totally free of posttranscription modification, whereas the RNA used by Denoya and Dubnau was mature 23S rRNA isolated from *B. subtilis* (4). The differences in RNA sources may explain the discrepancy of $K_m$ values between the two reports. In addition, the measurement of apparent $K_m$ of RNA reported here was achieved under extremely low concentrations of SAM, well below the reported $K_m$. Since only monomethylation was observed in our assays, the kinetic data observed here can reflect a simple one-step reaction instead of a mixture of two consecutive reactions as discussed by Denoya and Dabnau (4). Under these extreme conditions, the apparent $K_m$ could differ from true $K_m$ values.

The proposed structure of DV has the methylation site A-2085 in a single-stranded region that is bordered immediately upstream and 5 nt downstream by regions containing secondary structure, segments A and B, respectively (Fig. 1). Chemical footprinting analysis has shown that ErmC′ methylase covers an extensive region on 23S rRNA surrounding A-2085 including regions in segment A and B (21). Kinetic experiments presented here are consistent with the notion that both segments are involved in the binding of the substrate to the enzyme. The near total loss of substrate activity in both the DV fragment missing the 3′ region of segment A ($A^-$) and in the mutated substrate carrying the G2084A change suggests that both G-2084 and the region of segment A containing the G-2084–C-2639 base pair make important contacts with the enzyme to position A-2085 correctly at the active site for efficient methylation. Because of the proximity of segment A to the methylation site, the removal of the segment or a change in its structure would be expected to reduce the overall rate of methylation. The large increase in the $K_m$ of methylation for the DV ($B^-$) RNA fragment, which is missing most of segment B, implicates this segment, as well, in the binding of the substrate to the enzyme, but the sevenfold increase in the $V_{max}$ of the DV ($B^-$) RNA fragment indicates higher turnover of the substrate. Thus, although the presence of segment B in the RNA enhances its binding to ErmC′, it is apparent that the binding of segment B has an unfavorable effect on the rate at which the enzyme produces methylated RNA. Because of its potentially large size—up to almost 400 nt—and its 5-nt dis-

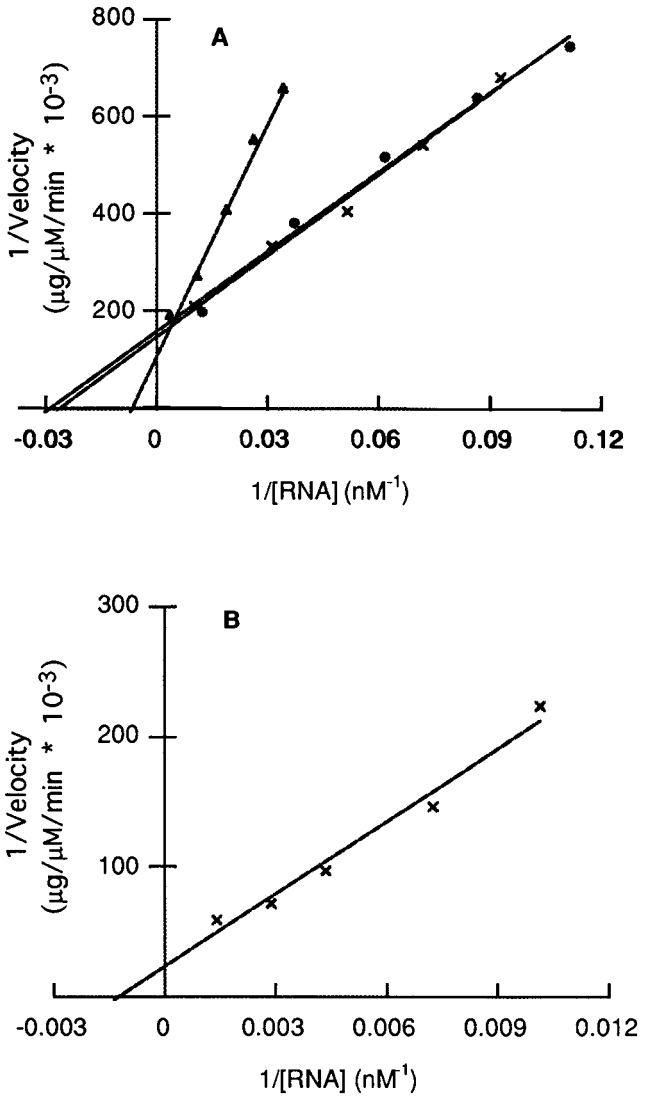


FIG. 4. Initial velocity studies of the ErmC′-catalyzed monomethylation reaction. (A) Plots of the reciprocal of initial velocity versus the reciprocal of concentrations of 23S rRNA (×), DV (●), and A2086T mutated DV (▲); (B) plots of the reciprocal of initial velocity versus the reciprocal of concentrations of DV ($B^-$). Lines were fitted by the method of least squares.

tance from the site of methylation, we propose that segment B binds at one or more sites on the enzyme away from the active site and that such binding has little effect either on the orientation of A-2085 in the active site or on the chemistry of the methylation event. Rather, the effect of the binding of segment B is a decreased rate at which the RNA is released from the enzyme after methylation occurs. Thus, though the substrate lacking segment B may bind less tightly to the enzyme, its more rapid release from the enzyme after methylation results in higher net turnover of methylated RNA.

The possibility that the increase in $V_{max}$ through the loss of segment B of DV is the result of a change at the reaction center cannot be ruled out. For example, the binding of segment B may cause an allosteric effect on the enzyme resulting in either destabilization of the enzyme-substrate reaction center (although the overall enzyme-substrate complex is stabilized) or a change in the chemistry of the reaction, possibly through changes in enzyme-substrate linkages at the reaction center. Though possible, it is difficult to imagine that the enzyme, which has been selected to confer upon the host the ability to survive, would also be selected to be underutilized by its natural substrate. It is more likely that the substrate, the mass of which exceeds that of the enzyme about 30-fold, binds the enzyme independently at several sites and that the rate of breakage of one of these ErmC′-RNA interactions is the rate-determining step of the reaction.

TABLE 3. Methylation of various rRNA fragments by ErmC′ methylase in vitro

| RNA fragment | RNA size (nt) | % Substrate activity[a] |
|---|---|---|
| Full-size 23S rRNA | 2,927 | 100 |
| DV ($A^-$) | 554 | <10 |
| DV A2085T | 623 | <10 |
| DV A2085C | 623 | <10 |
| DV A2085G | 623 | <10 |
| DV A2086T | 623 | 53 |
| DV G2084A | 623 | 12 |

[a] Determined by measuring $^3H$ incorporation under standard assay conditions (see Materials and Methods) and comparing counts per minute with the value from full-size 23S RNA. The absolute numerical value that corresponds to 100% is 4,934 ± 432 cpm.


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