JOURNAL OF BACTERIOLOGY, Oct. 1994, p. 6238–6244
0021-9193/94/$04.00+0

Vol. 176, No. 20

Properties of a Pentapeptide Inhibitor of Peptidyltransferase That Is Essential for *cat* Gene Regulation by Translation Attenuation

ZHIPING GU, ROBERT HARROD, ELIZABETH J. ROGERS, AND PAUL S. LOVETT*

Department of Biological Sciences, University of Maryland—Baltimore County, Catonsville, Maryland 21228

Received 9 May 1994/Accepted 4 August 1994

Inducible chloramphenicol resistance genes *cat* and *cmlA* are regulated by translation attenuation. For both genes, the leader codons that must be translated to deliver a ribosome to the induction site specify a peptide that inhibits peptidyltransferase in vitro. The antipeptidyltransferase activity of the peptides is thought to select the site of ribosome stalling that is essential for induction. Using variations of the *cat-86* leader-encoded 5-mer peptide MVKTD, we demonstrate a correlation between the in vitro antipeptidyltransferase activity and the ability of the same peptide to support induction by chloramphenicol in vivo. MVKTD footprints to nucleotides 2058, 2059, and 2060 in 23S rRNA. In vivo methylation of nucleotide 2058 by the *ermC* methylase interferes neither with *cat-86* induction nor with peptide inhibition of peptidyltransferase. The methylation eliminates the competition that normally occurs in vitro between erythromycin and MVKTD. MVKTD inhibits the peptidyltransferase of several eubacteria, a representative *Archaea* species, and the eukaryote *Saccharomyces cerevisiae*. *Bacillus stearothermophilus* supports the in vivo induction of *cat-86*, and the RNA that is phenol extracted from the 50S ribosomes of this gram-positive thermophile is catalytically active in the peptidyltransferase assay and sensitive to peptide inhibition. Our results indicate that peptidyltransferase inhibition by a *cat* leader peptide is essential to induction, and this activity can be altered by minor changes in the amino acid sequence of the peptide. The broad range of organisms shown to possess peptide-inhibitable peptidyltransferase suggests that the target is a highly conserved component of the ribosome and includes 23S rRNA.

Peptide bond formation during translation is catalyzed by the ribosome-associated activity peptidyltransferase (PT) (21). PT is the target for several antibiotics including chloramphenicol and anisomycin (20). We have recently demonstrated that PT is also inhibited by the short peptides that are encoded by the leaders of attenuation regulated chloramphenicol resistance genes (10, 12). The peptide inhibitors fall into two classes according to their sizes and specific inhibition. The leaders for inducible *cat* genes specify one class of PT-inhibitor peptides; the first five codons of the leaders encode 5-mer peptides that exhibit about 1/10 of the specific inhibition of chloramphenicol (10, 12). The second class of inhibitor peptide consists of only one member, the eight-residue peptide that is encoded by the first eight codons of the *cmlA* leader (12). The *cmlA* 8-mer peptide is about one-half as inhibitory as chloramphenicol on 50S ribosomal subunits of *Bacillus subtilis* and is about fivefold-more inhibitory than the *cat* leader peptides. Members of both classes of peptides may function similarly on the basis of their common footprints on 23S rRNA and the sensitivity of the inhibitory activity to competition by erythromycin (10). The mechanism of peptide inhibition of PT is probably not identical to that of chloramphenicol since the activities of chloramphenicol and the peptides are additive (10).

The anti-PT activity of the leader peptides is thought to play a critical role in the translation attenuation regulation of *cat* (1, 8, 11, 13, 17, 26) and *cmlA* (6, 33) by selecting the site of ribosome stalling (10, 12). Thus, the in vivo synthesis of the inhibitor peptides coincides with the translation by a ribosome to the leader site at which ribosome stalling will activate downstream gene expression (1, 10). Moreover, a missense mutation in the *cat* leader that prevents induction by chloramphenicol causes an amino acid replacement in the 5-mer peptide that eliminates its anti-PT activity (12).

In the present study we demonstrate that the in vitro inhibitory activity of a leader peptide correlates with the in vivo activity of the peptide in gene regulation. Examination of the peptide sensitivity of PT from several sources suggests that the target is a sequence or structure which is conserved in prokaryotic and eukaryotic ribosomes.

MATERIALS AND METHODS

PT. The fragment reaction was used to assay PT (21). The fragment reaction measures the in vitro transfer of *N*-formylmethionine from an endonuclease T1 fragment of tRNA to puromycin. The product of the reaction is *N*-formylmethionine–puromycin which is phase extracted with ethyl acetate. PT is the catalyst for the fragment reaction, and this activity is associated with the large ribosomal subunit (20). As a source of PT activity we have used intact ribosomes or the large (50S) subunit isolated from bacterial ribosomes. For ribosome isolation, we followed standard methods as outlined by Spedding (32). In selected cases, PT activity can be recovered by phenol extraction of 50S ribosomal subunits and is associated with the partially deproteinized 23S rRNA (24). Ribosomes or rRNA (0.1 to 0.15 U of optical density at 260 nm) was added to each 75 μl of the reaction mixture. PT reaction mixtures were incubated on ice for 1 h as previously described (12). Peptide and antibiotic concentrations were calculated on the basis of the 50-μl aqueous portion of the 75-μl reaction cocktails.

Peptide inhibitors were typically preincubated with ribosomes for 10 min on ice before the addition of the mixture to the reaction cocktails. Antibiotic inhibitors, chloramphenicol and anisomycin, were added to the reaction mixtures with the ribosomes but without preincubation. Exceptions to this protocol are noted in the text. Peptides were synthesized by Biosynthesis, Inc. (Lewisville, Tex.) and Research Genetics

* Corresponding author. Phone: (410) 455-2249. Fax: (410) 455-3875.

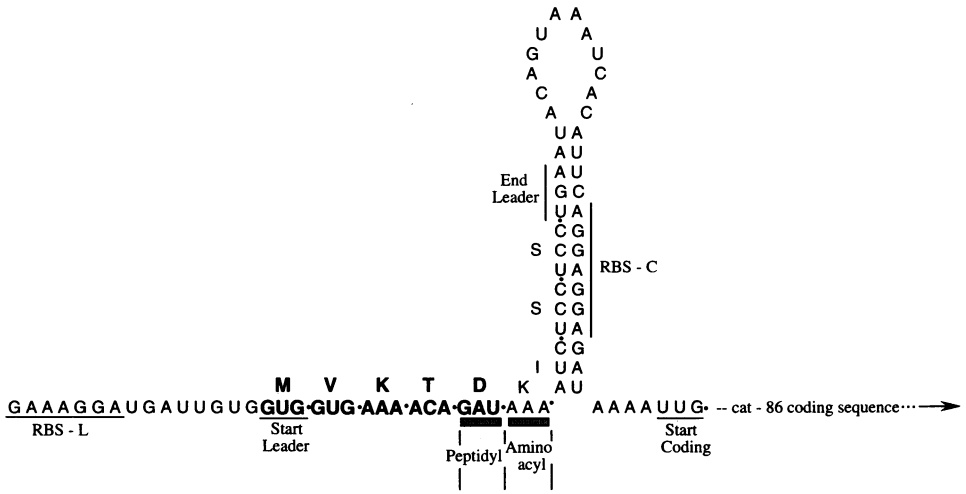

FIG. 1. *cat-86* mRNA 5′ end sequence responsible for regulation by translation attenuation. The regulatory sequence consists of two domains. The secondary structure constitutes a negatively regulating domain that blocks translation by sequestering the *cat-86* ribosome binding site (RBS-C). The positive regulator is the translated leader sequence consisting of nine codons. Destabilization of the secondary structure results from the stalling of a ribosome in the leader with its peptidyl and amino acyl sites at leader codons 5 and 6, respectively (1). The first five leader codons and the product pentapeptide are shown in bold. These five codons are translated in delivering a ribosome to the induction site and specify the MVKTD peptide that inhibits PT in vitro (1, 12). RBS-L is the ribosome binding site for the leader. The translation start codons for the leader, GUG, and the *cat-86* coding sequence, UUG, are identified.

(Huntsville, Ala.). Peptides were purified by high-pressure reverse-phase liquid chromatography as previously described (12).

Chloramphenicol induction of *cat-86*. *Bacillus subtilis* BR151 cells containing the *cat-86* gene on plasmid pPL708 (17) were grown at 37°C to mid-log phase in Penassay broth and exposed to 2 μg of chloramphenicol per ml for 2 h. Chloramphenicol acetyltransferase was assayed at 25°C by the colorimetric method described by Shaw (29). Chloramphenicol acetyltransferase specific activity is reported as micromoles of chloramphenicol acetylated per minute per milligram of protein. The amount of protein was measured by the Bradford method (2).

Peptide footprinting to 23S rRNA. Peptides and antibiotic footprinting to ribosomes were performed by using dimethyl sulfate as previously described (5, 10).

Cell growth for ribosome isolation. *Aeromonas hydrophila* RS61-6, *Pseudomonas aeruginosa* PA-3-11, *Escherichia coli* DH5αF′, and *B. subtilis* BR151 were grown in Luria broth (LB) medium (Gibco BRL) at 37°C. *Bacillus stearothermophilus* NUB 3621 was grown at 65°C in LB medium supplemented with Castenholtz salts and trace elements as described by Chen et al. (4). *Haloferax volcanii* DS2 was grown at 37°C in a yeast-tryptone-salts medium described by Nieuwlandt et al. (23). *Saccharomyces cerevisiae* 3871D was grown at 30°C in a medium consisting of 1% yeast extract, 2% peptone, and 2% glucose.

Site-directed mutagenesis. The leader of *cat-86* was cloned into M13 vectors and subjected to oligonucleotide mutagenesis (7, 34). The mutagenized fragment was sequenced (28) and used to replace the corresponding region in pPL703-P4 (17).

***ermC*-specified methylation of nucleotide 2058 in 23S rRNA.** *ermC* on plasmid pIM13 is constitutively expressed (19). Replication of pIM13 is temperature sensitive in *B. subtilis* (19), and even at the permissive temperature (32°C) the plasmid is frequently lost from host cells. To facilitate the isolation of uniformly methylated ribosomes from *ermC*-positive cells grown without erythromycin, we constructed, using PCR, an *Eco*RI fragment spanning the *ermC* gene from pIM13 and inserted it into pUB110. The fragment extends from a site between the −10 and −35 promoter sequences of *ermC* to the approximate middle of the putative transcription terminator for the gene (19). Accordingly, the gene lacks a functional promoter and terminator. Hence, transcription of the gene in pUB110 depends on read-through transcription from plasmid sequences. The sequences of the primers used to construct the *Eco*RI fragment were 5′-ATAGAATTCAGAGCTCGTGC-3′ and 5′-AAGGAATTCAGTTTATGCATCCC-3′. The resulting plasmid, pErm, was stably maintained in *B. subtilis* at temperatures of up to 45°C and conferred resistance to erythromycin.

RESULTS

Amino acid substitutions in the *cat-86* 5-mer peptide that abolish or diminish anti-PT activity have a corresponding effect on *cat-86* inducibility by chloramphenicol. The sequence of the first five residues of the *cat-86* leader-encoded peptide is MVKTD (Fig. 1) (17). 5-mer peptides having individual amino acid changes from the sequence MVKTD were tested for anti-PT activity. For selected examples, the corresponding mutation was made in the *cat-86* leader, which allowed the effect of the altered peptide on in vivo induction to be tested. In each case, general agreement was observed between the effect of an amino acid replacement on PT inhibition and its effect on inducibility by chloramphenicol (Fig. 2). For example, changing Lys-3 of MVKTD to Asn generates the sequence MVNTD, which retained about one-third of the anti-PT activity in the in vitro assay. Similarly, substitution of codons specifying MVNTD for those specifying MVKTD in the *cat-86* leader permitted chloramphenicol induction of the modified gene, but the level of induced expression was approximately one-third of that observed with the wild-type gene. These results and those of previous studies (10, 12) demonstrate a correlation between the ability of a 5-mer peptide to inhibit PT

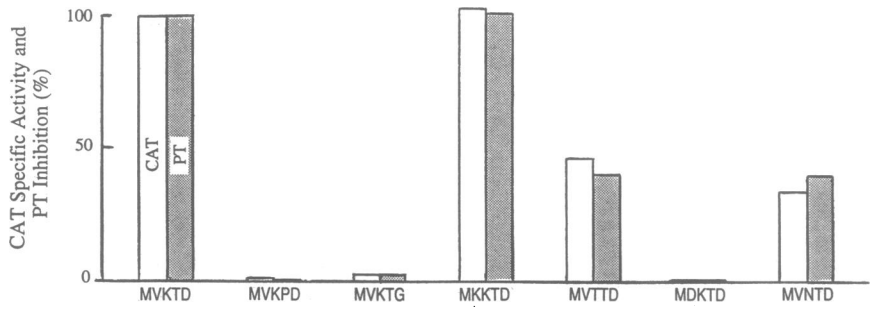

FIG. 2. Effects of variations of *cat-86* leader peptide on PT inhibition and induction by chloramphenicol. PT inhibition was done with 1 mM peptide as described in Materials and Methods. Chloramphenicol inductions were performed in Penassay broth by exposing host cells to 2 μg of the antibiotic per ml for 2 h. Values shown are expressed as the percentages of a control. The control for PT inhibition was the level of inhibition of ribosomes by 1 mM MVKTD. The control for induction was the CAT specific activity resulting from the induction of the wild-type *cat-86* gene. Control assays were performed in parallel with the assay of the modified peptide or modified leader.

and the ability of the corresponding coding sequence to support induction by chloramphenicol.

Methylation of nucleotide 2058 of 23S rRNA prevents erythromycin from competing with peptide inhibition of PT. The *ermC* gene confers erythromycin resistance to host cells by encoding an enzyme that methylates nucleotide 2058 of 23S rRNA (15, 16, 31). Nucleotide 2058 is one of three adjacent nucleotides in 23S rRNA to which the inhibitor peptides MVKTD and MSTSKNAD footprint (10) (Fig. 3). We were interested in determining if *ermC*-mediated methylation of nucleotide 2058 interfered with the induction of *cat-86* and anti-PT activity of MVKTD.

A constitutively expressed version of *ermC*, on plasmid pIM13 (19), did not interfere with induction of *cat-86* by chloramphenicol (data not shown). Since the foregoing results indicated that the anti-PT activity of the leader peptide correlates with inducibility, we suspected that methylation of nucleotide 2058 would not interfere with the in vitro activity of the peptide as a PT inhibitor. To test this proposal, ribosomes were isolated from cells expressing *ermC*, on plasmid pErm, and isogenic cells not carrying *ermC*. The corresponding 23S rRNA was used as a template for reverse transcriptase. RNA from the *ermC*-positive cells caused termination of reverse transcriptase at the nucleotide preceding 2058, whereas RNA from *ermC*-negative cells did not cause termination at this site (Fig. 4). Accordingly, the *ermC*-encoded methylase methylated nucleotide 2058.

Using these same ribosomes, we footprinted erythromycin and MVKTD, employing dimethyl sulfate as the chemical probe. Ribosomes from *ermC*-positive cells failed to show the characteristic erythromycin footprint, which is a reduction of dimethyl sulfate methylation of nucleotides 2058 and 2059 (18). The erythromycin footprint was present in the rRNA from the cells not carrying the *ermC* gene (data not shown, but

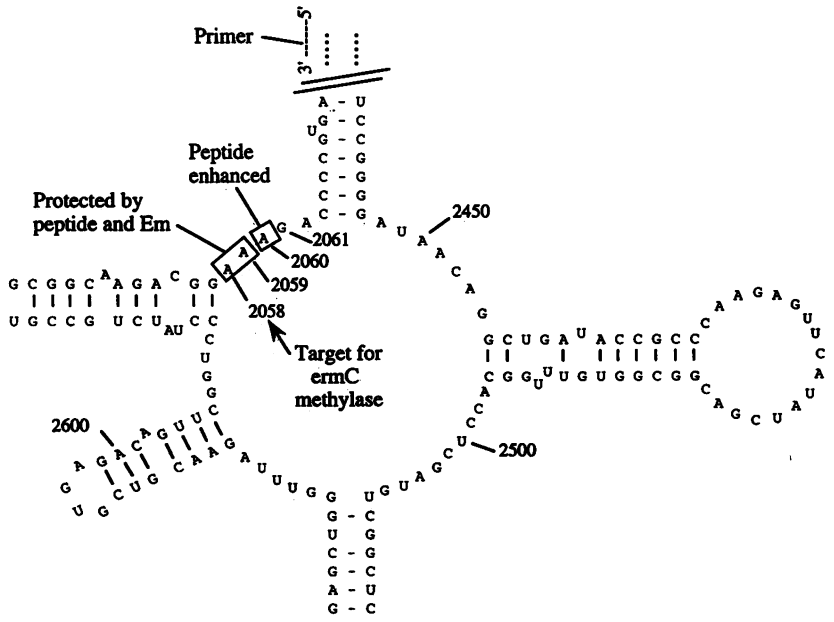

FIG. 3. Sequence of PT center of 23S rRNA. The numbering of nucleotides is done in accordance with the *E. coli* system, although the ribosomes used for this study were from *B. subtilis*, whose 23S rRNA differs slightly in size from that of *E. coli* (9). The primer used for reverse transcriptase mapping and the relationship between the nucleotides footprinted by erythromycin, peptide, and the nucleotide methylated by the *ermC* methylase are shown.

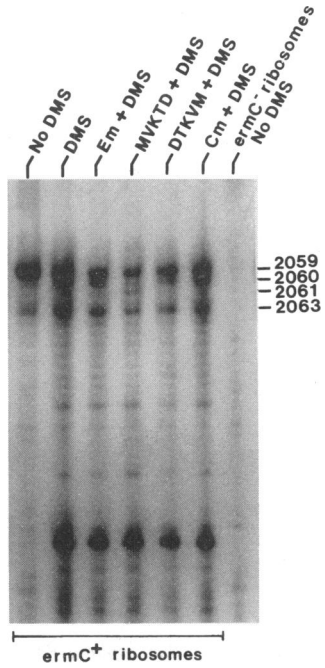

FIG. 4. Autoradiogram of reverse transcriptase mapping of sites of methylation in 23S rRNA of *B. subtilis*. Ribosomes from cells carrying the constitutively expressed *ermC* gene on pErm and from cells otherwise isogenic but lacking pErm were used for reverse transcriptase mapping. In each case, the RNA was phenol extracted from the ribosomes and served as a template for reverse transcriptase by using as primer a DNA oligonucleotide complementary to nucleotides 2142 to 2161 in 23S rRNA. Footprinting was done by incubating MVKTD (500 μM) or erythromycin (Em; 200 μM) with ribosomes for 10 min on ice and then by exposure to dimethyl sulfate (DMS) as described previously (5, 10).

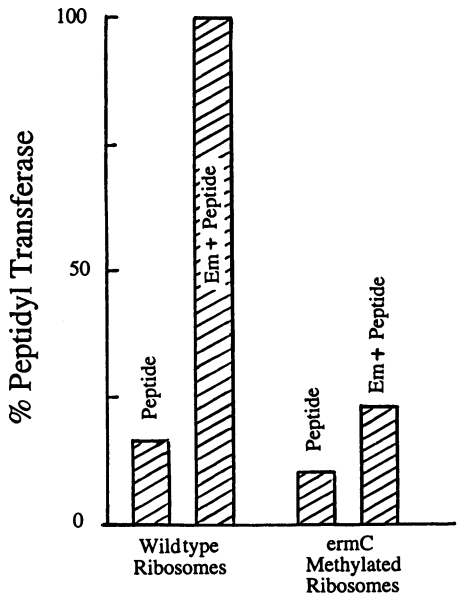

FIG. 5. MVKTD inhibition of PT of 50S ribosomes from *ermC*-positive and *ermC*-negative *B. subtilis* cells and ability of erythromycin (Em) to compete with inhibition. 50S subunits of *ermC*-positive and *ermC*-negative cells were preincubated with 1 mM MVKTD for 10 min and assayed for PT activity. The same ribosomes were preincubated for 5 min with 200 μM erythromycin and were then preincubated with peptide for an additional 10 min before PT was assayed.

see reference 10). The MVKTD footprint is characterized by a reduction of dimethyl sulfate methylation of nucleotides 2058 and 2059 and an enhancement of methylation of nucleotide 2060 (Fig. 3) (10). The MVKTD footprint on the rRNA from the *ermC*-positive cells was very similar to that on rRNA from *ermC*-negative cells (Fig. 4) (10), suggesting that the methylation of nucleotide 2058 did not alter the interaction of the peptide with the rRNA. This was confirmed by testing the response of the PT activity of the ribosomes to inhibition by MVKTD. Ribosomes from the *ermC*-positive or *ermC*-negative cells were about equally sensitive to peptide inhibition (Fig. 5).

Erythromycin competes with the activity of MVKTD as an inhibitor of PT. Because of the overlapping footprints of erythromycin and peptide on rRNA, we have presumed that the competition results from the interference of erythromycin with the binding of the peptide. Since ribosomes from the *ermC*-positive cells should not bind erythromycin, we suspected that on these ribosomes erythromycin would not compete with the anti-PT activity of MVKTD. The data shown in Fig. 5 support this interpretation. Peptide inhibition of PT of ribosomes from *ermC*-negative cells was very sensitive to competition by erythromycin, and peptide inhibition of PT associated with ribosomes from *ermC*-positive cells was virtually insensitive to competition by erythromycin.

Inhibition of PT of ribosomes from various microorganisms. The PT activity of intact ribosomes from several microorganisms was tested for sensitivity to MVKTD. 70S ribosomes from the eubacteria *B. subtilis*, *E. coli*, *P. aeruginosa*, and *A. hydrophia* were about equally sensitive to MVKTD (Table 1). The PT activity of intact ribosomes from a representative of the *Archaea*, *H. volcanii*, was also sensitive to inhibition by MVKTD and insensitive to chloramphenicol (Table 1).

Eukaryotic ribosomes are insensitive to many antibiotic inhibitors that act on bacterial ribosomes, such as chloramphenicol. Since the *Archaea* ribosomes were insensitive to chloramphenicol but sensitive to inhibition by MVKTD, we examined the 80S ribosomes isolated from the yeast *S. cerevisiae*. We observed that the PT activity of yeast ribosomes was inhibited by MVKTD and anisomycin but was insensitive to chloramphenicol (Fig. 6). Thus, the PT activity of ribosomes from representatives of the three kingdoms was sensitive to MVKTD inhibition.

The *cmlA* 8-mer leader peptide is about fivefold-more inhibitory for PT of prokaryotic ribosomes than is MVKTD

TABLE 1. Effects of MVKTD and chloramphenicol on PT activity of ribosomes from several microorganisms[a]

Source of ribosomes	% PT inhibition with:	
	MVKTD	CM
B. subtilis	92	54
E. coli	93	56
P. aeruginosa	78	64
A. hydrophila	91	59
H. volcanii	80	3

[a] Intact ribosomes were preincubated with 1 mM MVKTD or 200 μM chloramphenicol (CM) for 10 min, and each was then assayed for PT activity for 1 h. Inhibition by the reverse 5-mer DTKVM, at 1 mM, was less than 8% for each ribosome preparation analyzed. Activities of the uninhibited reaction mixtures ranged from 350,000 to 420,000 cpm.

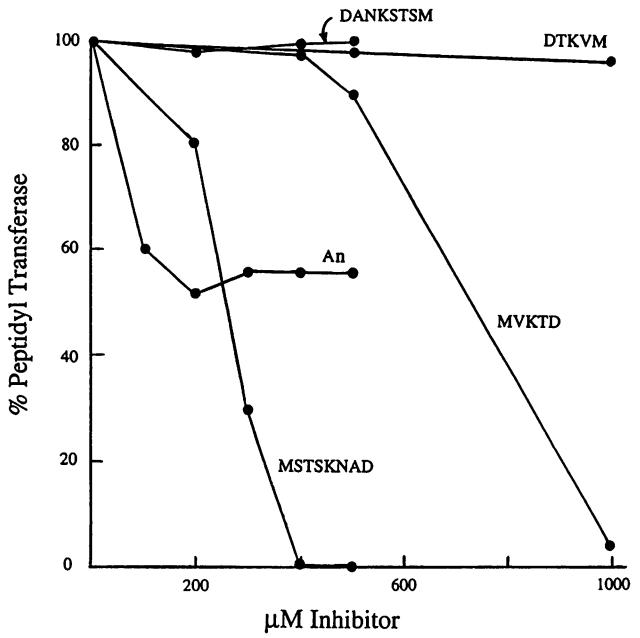

FIG. 6. Inhibition of PT of yeast ribosomes by MVKTD and MSTSKNAD. Yeast 80S ribosomes were exposed to various concentrations of the inhibitor peptides, the reverse 5-mers, chloramphenicol (data not shown) or anisomycin (An). The peptide was preincubated with the ribosomes for 10 min, and antibiotics were added to reactions mixes with the ribosomes but without preincubation. Chloramphenicol was not detectably inhibitory for the PT activity.

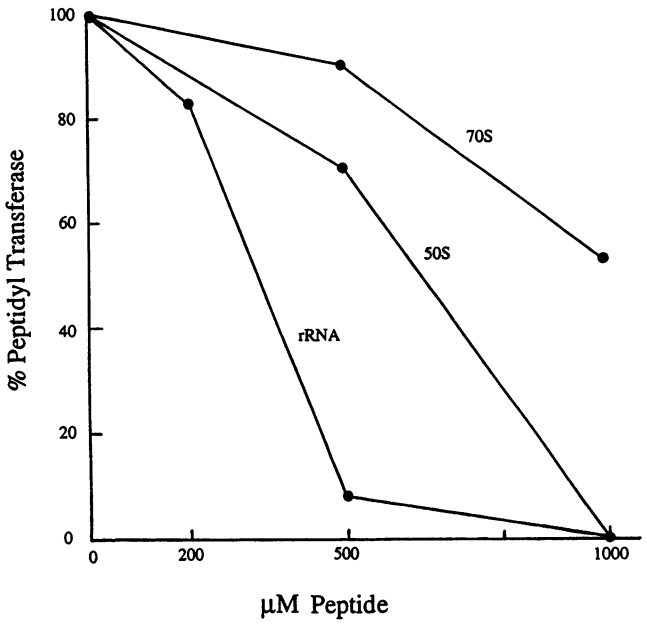

FIG. 7. Inhibition of *B. stearothermophilus* PT activity by MVKTD. *B. stearothermophilus* 70S ribosomes, 50S subunits, and rRNA that was phenol extracted from the 50S subunits were tested for sensitivity to MVKTD and the reverse 5-mer DTKVM.

(10). Similarly, the 8-mer peptide MSTSKNAD was significantly more inhibitory for yeast 80S ribosomes than was MVKTD (Fig. 6). These data suggest that the 5- and 8-mer peptides have similar modes of action on the PT of prokarotic and eukaryotic ribosomes.

PT activity can be phenol extracted from *B. stearothermophilus* ribosomes and is sensitive to peptide inhibition. *cat* genes are induced poorly or not at all in *E. coli*, while various gram-positive hosts, including the thermophile *B. stearothermophilus*, support induction levels of 10- to 50-fold following the addition of low levels of chloramphenicol. Noller et al. (24) demonstrated that the rRNA phenol extracted from the ribosomes of a gram-negative thermophile, *Thermus aquaticus*, is catalytically active in the PT reaction, and we have shown that this activity is sensitive to inhibition by MVKTD and MSTSKNAD (10). To determine if the resistance of PT to phenol treatment might be a characteristic of thermophiles, we examined the PT activity of *B. stearothermophilus*; this organism was of particular interest because it supports *cat-86* induction in vivo. We observed that phenol extraction of 50S ribosomes of *B. stearothermophilus* generated an RNA product that retained 50 to 80% of the PT activity of the intact 50S subunits. This activity was inhibited by MVKTD (Fig. 7). Similar phenol treatment of 50S ribosomal subunits of the mesophile *B. subtilis* eliminated all PT activity. It is of interest that the susceptibility of *B. stearothermophilus* PT activity to the inhibitor peptide followed a hierarchy; 70S ribosomes were the most resistant to inhibition, and the extracted rRNA was the form of PT that was most sensitive to inhibition by MVKTD (Fig. 7).

An inhibitor peptide lacks a regular structure. The PT inhibitor peptide MVKTD was examined by nuclear magnetic resonance in the absence and presence of 500 μM chloramphenicol to determine if the peptide had detectable structure in solution. No regular structure could be detected. Thus, if the peptide does assume a regular folding pattern, it must do so under conditions other than those tested, such as during the binding to its ribosomal target.

DISCUSSION

A search for a ribosome-pausing function of the *cat* leader was initiated to resolve two obviously contradictory features of translation attenuation regulation of *cat* genes: *cat* genes are induced by chloramphenicol, an antibiotic that blocks translation at random sites in mRNA, yet *cat* expression depends on the precise stalling of a ribosome with its amino acyl site at leader codon 6 (1). We initially observed that the codons which precede the leader stall site participate in selecting the site of stalling (22, 25, 26). This leader function now appears to be a direct result of the antiribosome activity of the product pentapeptide (12).

Expression of the 5-mer peptide MVKTD within cells does not slow growth (25), indicating that the anti-PT activity of the leader peptide does not interfere with overall protein synthesis in vivo. We have further shown that the 5-mer peptide communicates specifically with its translating ribosome in vivo and alters the function of that ribosome (27). Our model therefore proposes that the 5-mer peptide acts exclusively in *cis* to pause elongation of its translating ribosome by inhibiting PT.

To critically examine the apparent correlation between the role of the 5-mer peptide as an inhibitor of PT and its role in the regulation of *cat-86*, we constructed several variations of the amino acid sequence of the 5-mer that either eliminated or diminished its activity as a PT inhibitor or had no effect on the inhibition. In each case, when the corresponding mutation was made in the *cat-86* leader, the level of induction by chloramphenicol paralleled the level of PT inhibition observed in vitro.

These data therefore support the proposal that the antiribosome activity of the peptides is necessary for regulation by translation attenuation.

The anti-PT activity of MVKTD can be demonstrated by using ribosomes from organisms representing the three kingdoms. Moreover, the PT activity that is phenol extracted from 50S ribosomes of gram-negative and gram-positive thermophiles is also subject to peptide inhibition. Thus, the target for the action of the peptides appears to be a highly conserved sequence or structure that probably resides in 23S or 23S-like rRNA.

MVKTD alters the ability of dimethyl sulfate to methylate three nucleotides in 23S rRNA, nucleotides 2058, 2059 and 2060. In vivo methylation of 2058 by the *ermC*-encoded methylase did not interfere with the inducibility of *cat-86* or the anti-PT activity of the peptide. Thus, the interaction of peptide with nucleotide 2058 and the immediately adjacent nucleotides might be secondary to a site of primary binding elsewhere in the rRNA. Anti-PT antibiotics footprint to nucleotides in the region of 2451 (18) (Fig. 3). We are currently determining if MVKTD footprints to nucleotides in that region of rRNA.

The *cmlA* 8-mer peptide MSTSKNAD is more inhibitory than the *cat* leader pentapeptides MVKTD, MKKTD, and MKKSE, although they function in qualitatively similar manners. A sequence similarity among the four peptides can be seen in the four C-terminal amino acids, suggesting that the C-terminal end of the molecules may be essential to a function that is common to all the peptides, such as PT inhibition. Consistent with this idea is the fact that the N-terminal amino acid of the *cat-86* 5-mer can be switched between two very unlike amino acids, Met and Ser, while full inhibitory activity is retained (10). This also suggests that the inhibitory domain of the *cat* peptides and perhaps the *cmlA* peptide may not be the N terminus. This suggestion is compatible with our view of the mechanism by which the peptides regulate translation in vivo: inhibition of PT must not occur until the final amino acid of the 5- or 8-mer has been incorporated into the nascent peptide. Precisely why the 8-mer is more inhibitory in vitro than the *cat* 5-mers is not known. Efforts are under way to increase the activity of the *cat-86* 5-mer by modifications of its sequence and by moderate changes in the size of the peptide.

ACKNOWLEDGMENTS

We thank Chuck Daniels, Jim Nataro, Jim Kaper, Neil Welker and Phil Farabaugh for strains and information related to the growth of various microorganisms. Donald Creighton is thanked for his continuing advice on peptide chemistry and the use of his facilities. We acknowledge Michael Summers of the Howard Hughes Medical Institutes and the Department of Chemistry and Biochemistry, University of Maryland Baltimore County, for the analysis of peptide structure by NMR.

This investigation was supported by Public Health Service grant GM42925.

REFERENCES

1. **Alexieva, Z., E. J. Duvall, N. P. Ambulos, Jr., U. J. Kim, and P. S. Lovett.** 1988. Chloramphenicol induction of *cat-86* requires ribosome stalling at a specific site in the leader. Proc. Natl. Acad. Sci. USA **85**:3057–3061.
2. **Bradford, M. M.** 1976. A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **167**:842–849.
3. **Bruckner, R., and H. Matzura.** 1985. Regulation of the inducible chloramphenicol acetyltransferase gene of the *Staphylococcus aureus* plasmid pUB112. EMBO J. **4**:2295–2300.
4. **Chen, Z., S. F. Wojcik, and N. E. Welker.** 1986. Genetic analysis of *Bacillus stearothermophilus* by protoplast fusion. J. Bacteriol. **165**: 994–1001.
5. **Christiansen, J., J. Egebjerg, N. Larsen, and R. A. Garrett.** 1990. Analysis of ribosomal RNA structure: experimental and theoretical considerations, p. 229–251. *In* G. Spedding (ed.), Ribosomes and protein synthesis: a practical approach. IRC Press, New York.
6. **Dorman, C. J., and T. J. Foster.** 1985. Posttranscriptional regulation of the inducible nonenzymatic chloramphenicol resistance determinant of IncP plasmid R26. J. Bacteriol. **161**:147–152.
7. **Duvall, E. J., N. P. Ambulos, Jr., and P. S. Lovett.** 1987. Drug-free induction of a chloramphenicol acetyltransferase gene in *Bacillus subtilis* by stalling ribosomes in a regulatory leader. J. Bacteriol. **169**:4235–4241.
8. **Duvall, E. J., and P. S. Lovett.** 1986. Chloramphenicol induces translation of the mRNA for a chloramphenicol-resistance gene in *Bacillus subtilis*. Proc. Natl. Acad. Sci. USA **83**:3939–3943.
9. **Green, C. J., G. C. Stewart, M. A. Hollis, B. S. Vold, and K. F. Bott.** 1985. Nucleotide sequence of the *Bacillus subtilis* ribosomal RNA operon, *rrnB*. Gene **37**:261–266.
10. **Gu, Z., R. Harrod, E. J. Rogers, and P. S. Lovett.** 1994. Antipeptidyl transferase leader peptides of attenuation regulated chloramphenicol resistance genes. Proc. Natl. Acad. Sci. USA **91**:5612–5616.
11. **Gu, Z., and P. S. Lovett.** 1992. Perturbing highly conserved spatial relationships in the domain that regulates inducible *cat* translation. Mol. Microbiol. **6**:2769–2776.
12. **Gu, Z., E. J. Rogers, and P. S. Lovett.** 1993. Peptidyl transferase inhibition by the nascent leader peptide of an inducible *cat* gene. J. Bacteriol. **175**:5309–5313.
13. **Harrod, R., Z. Gu, and P. S. Lovett.** 1994. Analysis of the secondary structure that negatively regulates inducible *cat* translation by use of chemical probing and mutagenesis. Gene **140**:79–83.
14. **Horinouchi, S., and B. Weisblum.** 1982. Nucleotide sequence and functional map of pC194, a plasmid that specifies inducible chloramphenicol resistance. J. Bacteriol. **170**:815–825.
15. **Lai, C. J., and B. Weisblum.** 1971. Altered methylation of ribosomal RNA in an erythromycin resistant strain of *Staphylococcus aureus*. Proc. Natl. Acad. Sci. USA **68**:856–860.
16. **Lai, C. J., B. Weisblum, S. Fahnestock, and M. Nomura.** 1973. Alteration of 23S ribosomal RNA and erythromycin-induced resistance to lincomycin and spiramycin in *Staphylococcus aureus*. J. Mol. Biol. **74**:67–72.
17. **Lovett, P. S.** 1990. Translation attenuation as the regulator of inducible *cat* translation. J. Bacteriol. **172**:1–6.
18. **Moazed, D., and H. F. Noller.** 1987. Chloramphenicol, erythromycin, carbomycin and vernamycin B protect overlapping sites in the peptidyl transferase region of 23S ribosomal RNA. Biochemie **69**:879–884.
19. **Monod, M., C. Denoya, and D. Dubnau.** 1986. Sequence and properties of pIM13, a macrolide-lincosamide-streptogramin B resistance plasmid from *Bacillus subtilis*. J. Bacteriol. **167**:138–147.
20. **Monro, R. E.** 1967. Ribosome-catalysed peptidyl transferase: effects of some inhibitors of protein synthesis. J. Mol. Biol. **28**:161–165.
21. **Monro, R. E., and K. A. Marcker.** 1967. Ribosome-catalysed reaction of puromycin with a formylmethionine-containing oligonucleotide. J. Mol. Biol. **25**:347–350.
22. **Mulbry, W. W., N. P. Ambulos, Jr., and P. S. Lovett.** 1989. *Bacillus subtilis* mutant allele *sup-3* causes lysine insertion at ochre codons: use of *sup-3* in studies of translational attenuation. J. Bacteriol. **171**:5322–5324.
23. **Nieuwlandt, D. T., E. S. Haas, and C. J. Daniels.** 1991. The RNA component of RNase P from the archaebacterium *Haloferax volcanii*. J. Biol. Chem. **266**:5689–5695.
24. **Noller, H. F., V. Hoffarth, and L. Zimnick.** 1992. Unusual resistance of peptidyl transferase to protein extraction procedures. Science **256**:347–350.
25. **Rogers, E. J., N. P. Ambulos, Jr., and P. S. Lovett.** 1990. Complementarity of *Bacillus subtilis* 16S rRNA with sites of antibiotic-dependent ribosome stalling in *cat* and *erm* leaders. J. Bacteriol. **172**:6282–6290.
26. **Rogers, E. J., U. J. Kim, N. P. Ambulos, Jr., and P. S. Lovett.** 1990. Four codons in the *cat-86* leader define a chloramphenicol sensitive ribosome stall sequence. J. Bacteriol. **172**:110–115.

27. **Rogers, E. J., and P. S. Lovett.** 1994. *cis* effect of a nascent peptide on its translating ribosome: influence of the *cat-86* leader pentapeptide on translation termination at leader codon 6. Mol. Microbiol. **12:**181–186.
28. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:**5463–5467.
29. **Shaw, W. V.** 1975. Chloramphenicol acetyltransferase from chloramphenicol resistant bacteria. Methods Enzymol. **43:**737–755.
30. **Shaw, W. V., D. G. Brenner, S. F. J. LeGrice, S. E. Skinner, and A. R. Hawkins.** 1985. Chloramphenicol acetyltransferase of staphylococcal plasmid pC221. FEBS Lett. **179:**101–106.
31. **Skinner, R., E. Cundliffe, and F. J. Schmidt.** 1983. Site of action of a ribosomal RNA methylase responsible for resistance to erythromycin and other antibiotics. J. Biol. Chem. **258:**12702–12706.
32. **Spedding, G.** 1990. Isolation and analysis of ribosomes from prokaryotes, eukaryotes and organelles, p. 1–27. *In* G. Spedding (ed.), Ribosomes and protein synthesis: a practical approach. IRC Press, New York.
33. **Stokes, H. W., and R. M. Hall.** 1991. Sequence analysis of the inducible chloramphenicol resistance determinant in the Tn*1696* integron suggests regulation by translational attenuation. Plasmid **26:**10–19.
34. **Taylor, J. W., J. Ott, and F. Eckstein.** 1985. The generation of oligonucleotide-directed mutations at high-frequency using phosphorothioate-modified DNA. Nucleic Acids Res. **13:**8765–8785.