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A Gratuitous Inducer of *cat-86*, Amicetin, Inhibits Bacterial Peptidyl Transferase

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Expression of the chloramphenicol resistance gene *cat-86* is regulated by translation attenuation. Among the three ribosomally targeted antibiotics that can induce the gene, only amicetin has an unknown mode of action. Here we demonstrate that the nucleoside antibiotic amicetin is an inhibitor of bacterial peptidyl transferase. Thus, the three inducers of *cat-86*, chloramphenicol, erythromycin, and amicetin, interact with the peptidyl transferase region of bacterial ribosomes.

Inducible antibiotic resistance genes *cat* and *erm*, which occur in gram-positive bacteria, and *cmlA*, which is detected in gram-negative organisms, are regulated by translation attenuation (4, 12, 20). For each gene, the inducer is an antibiotic to which the gene confers resistance. Thus, *cat* and *cmlA* specify resistance to chloramphenicol and chloramphenicol is the inducer of gene expression (3, 18). The chloramphenicol resistance gene *cat-86* is highly unusual because it can additionally be induced by two antibiotics to which it does not confer resistance, amicetin and erythromycin (5, 16). Induction by amicetin is of particular interest because missense mutations in the *cat-86* leader that abolish inducibility by amicetin and have no effect on induction by chloramphenicol have been identified (10).

The mode of action of amicetin as an antibiotic is not known, but the antibiotic activities of the other two inducers of *cat-86* have been examined. Chloramphenicol is an inhibitor of bacterial peptidyl transferase (PT), and erythromycin inhibits translocation (14, 15, 19). Both chloramphenicol and erythromycin footprint to specific nucleotides in domain V of 23S rRNA when dimethylsulfate or kethoxal is used as a probe (13). Using similar methods, we were unable to footprint amicetin to rRNA of amicetin-sensitive bacteria.

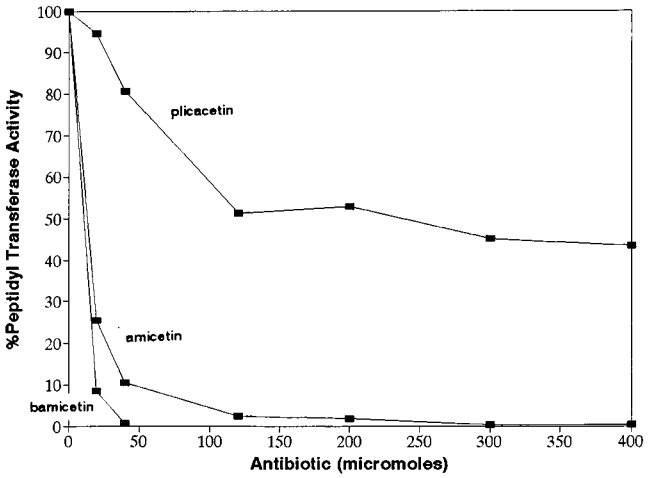

FIG. 1. Effects of members of the amicetin family of antibiotics on the PT activity of *B. subtilis* 70S ribosomes. Various concentrations of the antibiotics were added to 0.55 μM 70S ribosomes purified from *B. subtilis* BR151 cells as described elsewhere (17), and the mixture was used to initiate the PT assay as previously described (7, 8, 15).

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The amicetin family of nucleoside antibiotics consists of three members: amicetin, bamicetin, and plicacetin (19). Amicetin and bamicetin are inducers of *cat-86*, and plicacetin is not (1). The structures of the three antibiotics offer no obvious basis for the difference in inducing activities (1). We observed that amicetin and bamicetin were highly inhibitory for PT of *Bacillus subtilis* ribosomes and plicacetin was only marginally active as an inhibitor (Fig. 1). Amicetin and bamicetin also inhibited the PT of *Escherichia coli* 70S ribosomes, but plicacetin showed little inhibition (Table 1). At concentrations sufficient to inhibit bacterial PT by more than 85%, amicetin and bamicetin inhibited the PT of ribosomes isolated from the archaeon *Haloferax volcanii* and that of 80S ribosomes from *Saccharomyces cerevisiae* by less than 30% (Table 1).

Erythromycin is not a PT inhibitor, but erythromycin competes with chloramphenicol for binding to its ribosomal target (19). To test the effect of erythromycin on amicetin inhibition of PT, ribosomes were exposed to a fourfold molar excess of erythromycin 5 min prior to exposure to amicetin. As shown in Fig. 2, erythromycin did not diminish PT inhibition by amicetin or bamicetin. Therefore, erythromycin is not a competitor of these antibiotics.

Amicetin has been reported to compete with chloramphenicol for binding to ribosomes (2). This observation was confirmed in the present study, since preincubation of ribosomes with amicetin diminished the ability of chloramphenicol to inhibit PT (Fig. 3). In contrast, preincubation of ribosomes with chloramphenicol did not reduce the ability of subse-

TABLE 1. Effects of the amicetin family of antibiotics on PT of ribosomes from *E. coli*, *S. cerevisiae*, and *H. volcanii*[a]

Antibiotic	PT activity (%) of ribosomes from:		
	E. coli	*S. cerevisiae*	*H. volcanii*
Amicetin	9	72	74
Bamicetin	13	87	78
Plicacetin	88	88	83

[a] Amicetin was used at 100 μM, and plicacetin and bamicetin were used at 50 μM. PT assays were performed as previously described (6, 8, 14, 15). The inhibition of PT of ribosomes from *B. subtilis* was comparable to PT inhibition shown for *E. coli* ribosomes.

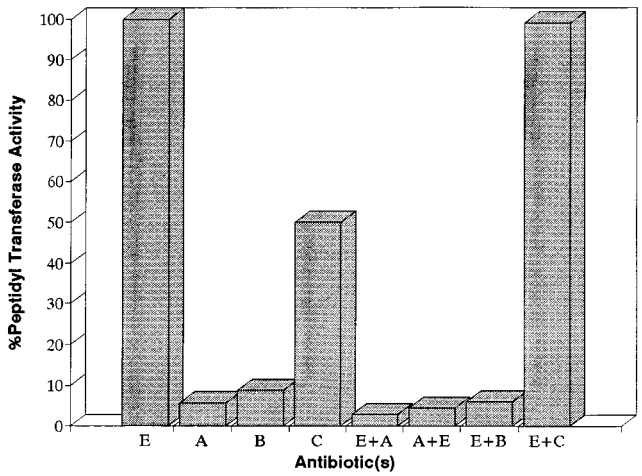

FIG. 2. Effect of erythromycin on PT inhibition by amicetin and chloramphenicol. *B. subtilis* 70S ribosomes (0.76 μM) were exposed to the various antibiotics indicated and immediately assayed for PT activity. Pairs of antibiotics were assayed by preincubating the ribosomes with the first antibiotic for 5 min, at which time the second antibiotic was added and the ribosomes were then assayed for PT. Throughout, erythromycin (E) was used at 400 μM, amicetin (A) was used at 100 μM, bamicetin (B) was used at 25 μM, and chloramphenicol (C) was used at 50 μM.

quently added amicetin to inhibit PT, as evidenced by the additivity of the two antibiotics. These results suggest that amicetin and chloramphenicol probably bind to different sites on the ribosome and that the binding of amicetin to its ribosomal target interferes with the subsequent binding of chloramphenicol, possibly by altering the conformation of the chloramphenicol target.

Amicetin-resistant mutants of *B. subtilis* are readily isolated (5). The corresponding mutation maps to the ribosomal gene cluster in *B. subtilis* and causes an alteration of the electrophoretic mobility of a single large-subunit protein, BL12a (5). *B. subtilis* cells carrying the *ami-1* mutation do not support *cat-86* induction by amicetin, but in these mutant cells the gene can still be induced by chloramphenicol (5). The data were interpreted to indicate that bacterial mutants whose ribosomes

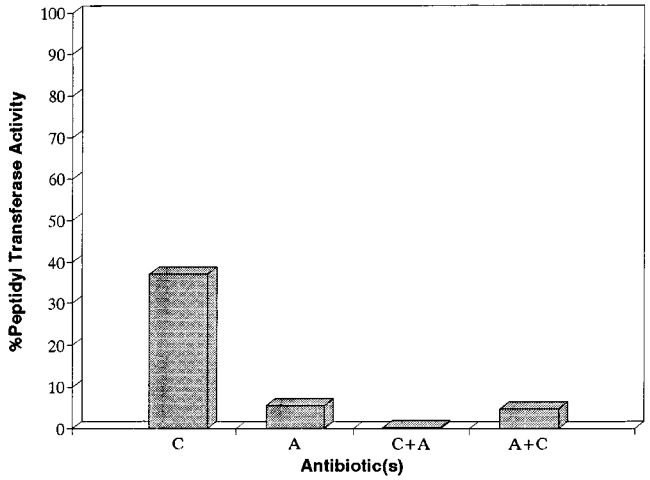

FIG. 3. Combined effects of chloramphenicol and amicetin on PT of *B. subtilis* 70S ribosomes. The experiment was performed as described in the legend to Fig. 2. Chloramphenicol (C) was used at 400 μM, and amicetin (A) was used at 100 μM. Results of replicate assays of PT varied by less than ±6%.

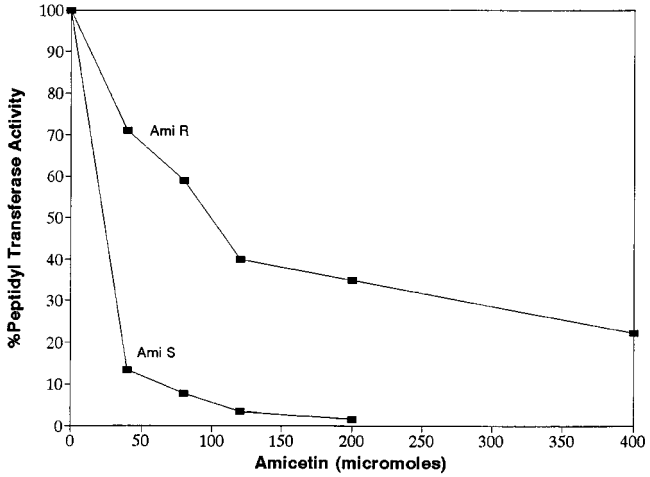

FIG. 4. Inhibition of PT by amicetin with ribosomes isolated from an amicetin-resistant mutant of *B. subtilis*. The PTs of ribosomes from *B. subtilis* BR151 (Ami S) and BR151 *ami-1* (Ami R) were assayed for susceptibility to inhibition by amicetin.

are insensitive to the inducer fail to support induction by that antibiotic. To determine if the PT of ribosomes from an *ami-1*-containing *B. subtilis* cell was resistant to amicetin, the corresponding ribosomes, and ribosomes from a nonmutant but otherwise isogenic cell, were assayed for PT response to amicetin. The PT activity of ribosomes from the *ami-1*-containing cells was much less sensitive to inhibition by amicetin than was PT of wild-type cells (Fig. 4). The PTs of ribosomes from both cell types were equally sensitive to chloramphenicol (data not shown).

Our results argue that amicetin interacts with the PT center of bacterial ribosomes. This notion is also supported by the observation that an amicetin resistance mutation in the rRNA gene of *Halobacterium halobium* is in the region encoding the PT center (11).

We have proposed that the role of the inducers in translation attenuation regulation is to stall a ribosome at a site in the leader of regulated transcripts that is selected by the anti-PT activity of the leader-encoded peptide (6–9). Since specific missense mutations in the *cat-86* leader selectively block the ability of amicetin and bamicetin to induce the gene (10), we suggest that the antibiotic and the leader peptide must exert a cooperative effect on a ribosome for induction. Efforts to determine the nature of that cooperativity are under way.

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