Lincosamide Antibiotics Stimulate Dissociation of Peptidyl-tRNA from Ribosomes

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Received 15 January 1993/Returned for modification 15 March 1993/Accepted 24 June 1993

At nonpermissive temperatures the peptidyl-tRNA hydrolase of pth(Ts) bacterial mutants is inactivated, and cells accumulate peptidyl-tRNA and die. Doses of erythromycin, lincomycin, or clindamycin that inhibited the growth of antibiotic-hypersensitive DB-11 pth+ cells accelerated the killing of DB-11 pth(Ts) cells at nonpermissive temperatures. Erythromycin and lincomycin also stimulated the accumulation of peptidyl-tRNA. Lincomycin and clindamycin stimulated peptidyl-tRNA dissociation from ribosomes.

Lincosamide antibiotics inhibit protein synthesis by binding to prokaryotic ribosomes. This binding was reported to block peptide bond formation and/or peptidyl-tRNA translocation from the A to the P site of the ribosome (8, 18, 22). Formerly, erythromycin and other macrolide antibiotics were also believed to have this mechanism (3, 14). Since some mutations can confer resistance simultaneously to macrolides and lincosamides, it is possible that both classes inhibit protein synthesis as macrolides are known to do, by stimulating peptidyl-tRNA dissociation from ribosomes (16).

Peptidyl-tRNA dissociates from ribosomes normally during protein synthesis (11, 12). The effects are usually masked by peptidyl-tRNA hydrolase, an enzyme that catalyzes hydrolysis of intact peptidyl-tRNA. Cells with the pth(Ts) mutation grow normally at 30°C, but their hydrolase is inactivated if the temperature is raised to ≥40°C (2, 17), leading to peptidyl-tRNA accumulation, protein synthesis inhibition, and ultimately, cell death (13). If lincosamide antibiotics stimulate peptidyl-tRNA dissociation in a pth(Ts) mutant at high temperatures, they should impair cell survival and lead to a higher rate of peptidyl-tRNA accumulation.

Cell survival. The Escherichia coli DB-11 met mutant was obtained from B. Weissblum, University of Wisconsin. DB-11 pth(Ts) was isolated from a DB-11 met trp mutant by cotransducing pth(Ts) with trp+ by using P1 bacteriophage from AA7852 or from DB-11 by cotransducing pth(Ts) with Tetr (0.5 min apart [1]) from Tn10 (Tet+) transposon-containing strain TGM8031. Peptidyl-tRNA hydrolase activity in DB-11 pth(Ts) was demonstrated to be temperature sensitive (data not shown).

Bacteria were grown to saturation overnight at 30°C in LP8 medium (61 mM Na2HPO4, 9.6 mM KH2PO4, 43 mM NaCl, 5.6 mM d-glucose, 5 g of Difco yeast extract per liter, 10 g of Difco tryptone per liter, adjusted to pH 8.0 with NaOH). The method was essentially that described previously (13). The fraction surviving was determined by dividing the density of CFU present after various times at the inactivation temperature by the density at time zero.

For comparison with previous results (16), we report the effects of chloramphenicol and erythromycin, as well as lincosamide antibiotics. At elevated temperatures, growth of wildtype pth+ cells was arrested by the doses used (Fig. 1). Mutant pth(Ts) cells showed the expected loss of colony-forming ability at the high temperature because of accumulation of peptidyl-tRNA in the absence of peptidyl-tRNA hydrolase activity (13). Loss of survival slowed if cells were first treated with chloramphenicol (Fig. 2) but accelerated if cells were first treated with erythromycin (Fig. 1), consistent with previous results. Accelerated killing was observed if cells were treated with lincomycin (Fig. 1) or clindamycin (Fig. 2) before experiencing the high temperature. The doses of antibiotics used did not kill cells at 30°C (Fig. 2; data for erythromycin and lincomycin not shown).

Peptidyl-tRNA accumulation. To isolate tRNA, 1,320 ml of LP8 was inoculated with an overnight-saturated culture to give an apparent A650 of 0.01 and then grown at 30°C with

\[ \text{DB-11} \]

\[ \text{Ery} \]

\[ \text{Linco} \]

\[ \text{No Drug} \]

\[ \text{DB-11} \text{ pth} \]

\[ \text{Ery} \]

\[ \text{Linco} \]

\[ \text{No Drug} \]

\[ \text{Time (min) at 43°C} \]

FIG. 1. Time course of survival of pth(Ts) mutant and pth+ wild-type cells at 43°C in the presence and absence of erythromycin and lincomycin. Strains DB-11 pth(Ts) (filled symbols and solid lines) and DB-11 pth+ (hollow symbols and dashed lines) were grown at 30°C to mid-exponential phase (apparent A650 0.04). One-third of each culture was treated with erythromycin (3.0 μg/ml [squares]), one-third was treated with lincomycin (150 μg/ml [triangles]), and one-third remained untreated (circles) for 15 min before the temperature was raised to 43°C.

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shaking aeration to an apparent $A_{450}$ of $\sim 0.10$. Antibiotic was added for 5 min before the culture was split into six 220-ml aliquots, 50 ml of 95°C medium was added, and aliquots were incubated at the inactivation temperature. Further treatment, tRNA isolation, and leucine-accepting activity assays (including [I] or not including [N] active peptidyl-tRNA hydrolase) were essentially as described previously (11, 12, 16). The percent peptidyl-tRNA\textsuperscript{Leu} graphed is 100 (I – N)/I.

When tRNA was isolated from wild-type pth\textsuperscript{*} cells, the peptidyl-tRNA fraction was low and unaffected by being placed at high temperatures (Fig. 3). Peptidyl-tRNA in pth(Ts) cells increased during exposure to high temperatures, rising from approximately 0 to 50% in 40 min. Treatment with chloramphenicol inhibited and treatment with erythromycin increased the rate and extent of the peptidyl-tRNA accumulation, consistent with previous observations on other pth(Ts) strains. Acceleration of peptidyl-tRNA accumulation was also elicited by treatment with lincomycin, direct chemical evidence for an effect on peptidyl-tRNA dissociation.

Discussion. Like erythromycin, lincosamides inhibit the formation of fMet-puromycin in the fragment reaction (19). This was interpreted as being due to inhibiting peptide transfer, but lincomycin does not inhibit transfer of peptidyl-tRNA peptides to puromycin on isolated native polyribosomes (20). Lincomycin does, however, inhibit ribosome binding of the pentanucleotide 3′ termini of $N$-acetyl-Leu tRNA\textsubscript{Leu}, Leu-tRNA\textsubscript{Leu}, and Phe-tRNA\textsubscript{Phe} (9). Conversion of polyribosomes into 70S ribosomes is stimulated in vivo by lincomycin (6) and chloramycin (7), but drugs like chloramphenicol that block peptide transfer elicit stable distributions of polyribosome size, a response also expected from inhibiting translocation of peptidyl-tRNA. Also arguing against inhibiting translocation is the report that lincomycin does not inhibit GTP hydrolysis that is dependent on EF-G and ribosomes (21). To summarize, Gale et al. (8) suggested that lincomycin acts only on ribosomes bearing short peptidyl-tRNAs, inhibiting the peptide transfer reaction perhaps via impairing substrate recognition.

This last view is consistent with results presented above, that lincosamides stimulated dissociation of peptidyl-tRNA. Our data cannot rule out all inhibition of peptide transfer or translocation by lincosamides, but general inhibition seems unlikely because to accumulate high fractions of peptidyl-tRNA, ribosomes must continue protein synthesis by reinitiating multiple times (tRNAs substantially outnumber ribosomes), with all but the first dissociation involving short peptidyl-tRNAs. We have also shown that viomycin, which inhibits translocation, slows accumulation of peptidyl-tRNA in pth(Ts) cells (15). The simplest conclusion is that stimulating peptidyl-tRNA dissociation is a primary effect of lincosamide antibiotics.

Peptidyl-tRNA dissociation could lead to ribosome detachment from mRNA. This detachment does not occur when the ermC leader peptide is translated (10). Instead, ribosomes pause, allowing downstream tRNA methylase expression via translational deattenuation. Our experiments have not measured effects of macrolides or lincosamides on translating ermC-like leaders.

We thank the University of Iowa Graduate College and Department of Biological Sciences for support, Lee-Na Tsai for assisting.
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