Erythromycin, lincosamides, peptidyl-tRNA dissociation, and ribosome editing

Abstract Inaccurate protein synthesis produces unstable β-galactosidase, whose activity is rapidly lost at high temperature. Erythromycin, lincomycin, clindamycin, and celesticetin were shown to counteract the error-inducing effects of streptomycin on β-galactosidase synthesized in the antibiotic-hypersensitive *Escherichia coli* strain DB-11 *Met* . Newly synthesized β-galactosidase was more easily inactivated by high temperatures when synthesized by bacteria partially starved for arginine, threonine, or methionine. Simultaneous treatment with erythromycin or lincomycin yielded β-galactosidase that was inactivated by high temperatures less easily than during starvation alone, an effect attributed to stimulation of ribosome editing. When synthesized in the presence of canavanine, β-galactosidase was inactivated by high temperature more easily but this effect could not be reversed by erythromycin. The first arginine in β-galactosidase occurs at residue 13, so the effect of erythromycin during arginine starvation is probably to stimulate dissociation of erroneous peptidyl-tRNAs of at least that length. Correction of errors induced by methionine starvation is probably due to stimulation of dissociation of erroneous peptidyl-tRNAs bearing peptides at least 92 residues in length. All the effects of erythromycin or the tested lincosamides on protein synthesis are probably the result of stimulating the dissociation from ribosomes of peptidyl-tRNAs that are erroneous or short.

Key words Protein synthesis · Translation Accuracy · Macrolide antibiotics

Introduction

Erythromycin and other macrolide antibiotics inhibit protein synthesis in susceptible cells by binding to ribosomes. These drugs are now known to stimulate the dissociation of peptidyl-transfer RNA (ptRNA) from ribosomes (Menninger 1976, 1978, 1985, 1993; Menninger and Otto 1982). Lincosamide antibiotics inhibit protein synthesis by a similar mechanism (Menninger and Coleman 1993).

Errors during protein synthesis occur: (1) when a tRNA accepts an inappropriate amino acid; or (2) when a correctly acylated aminoacyl-tRNA binds inappropriately to the ribosome A site and accepts a growing peptide. The former process has been estimated to account for errors at a frequency of $10^{-6} - 10^{-5}$ per amino acid (Fersht 1986). The latter process must be responsible for the bulk of errors, since their observed frequency in completed bacterial proteins is in the range $10^{-4} - 10^{-3}$ per amino acid (Buckingham and Grosjean 1986). The ribosome editor hypothesis (Menninger 1977) proposes that erroneous ptRNAs arising from process (2) dissociate preferentially from the ribosome, thus reducing the frequency of errors in completed proteins. After dissociation from the ribosome, peptides are hydrolyzed from ptRNA via peptidyl-tRNA hydrolase (Menninger 1976). The strongest evidence in favor of ribosome editing is the existence of mutant *Escherichia coli* cells (strain G102) that exhibit slower than normal ptRNA dissociation, reduced accuracy of β-galactosidase synthesis, and partial resistance to erythromycin (Anderson and Menninger 1987).

Erythromycin can enhance the accuracy of protein synthesis (Menninger 1985). When *E. coli* cells are grown in the presence of erythromycin and either streptomycin or ethanol, they make β-galactosidase that is inactivated at high temperature more slowly than if the cells are grown in the presence of streptomycin or ethanol alone. These results are most easily interpreted by assuming that erythromycin causes a preferential dis-
sociation of erroneous ptRNA – that is, erythromycin stimulates the ribosome editor.

Formerly, lincosamide antibiotics were thought to block peptide bond formation and/or translocation of peptidyl-tRNA from the A to the P site of the ribosome, especially in the case of short peptides (Monro et al. 1971; Cundliffe 1981; Vázquez 1985). Erythromycin was also believed to have these effects (Brisson-Noël et al. 1988; Menninger 1985). Since both macrolide and lincosamide antibiotics are now known to stimulate disso-
ciation of ptRNA from ribosomes and since some bac-
terial mutations can simultaneously confer resistance to macrolide, linosamide, and streptogramin B (MLS) anti-
tibiotics, it is possible that all three classes affect protein synthesis accuracy as erythromycin does, by stimulating the preferential dissociation of erroneous ptRNA from ribosomes.

It has been suggested that the ability of erythromycin to counteract the error-inducing effects of streptomycin could be due to competition between the two drugs (Brisson-Noël et al. 1988). It has also been suggested that erythromycin affects the synthesis of only very short peptides (Andersson and Kurland 1987; Cont-
erras and Vázquez 1977; Vázquez 1985). Translation errors induced by streptomycin or ethanol and selected against by erythromycin’s stimulating effect on ribo-
some editing seem, however, unlikely all to lie in the first few amino acid positions of a protein. These issues might be clarified if the error-prone positions were more precisely specified – for example, by inducing errors via amino acid starvation. Finally, it is possible that the influence of erythromycin on translational accuracy is the result of stimulation of degradation of erroneous proteins. If so, then this effect should be seen in the case of a temperature-sensitive enzyme produced by the incor-
poration of an amino acid analogue.

Materials and methods

Bacterial cells

E. coli strain CP79 (arg his leu thr) was obtained from Dr. B.J. Bachmann, Yale University. E. coli strain DB-11, Met− Lac+, hypersensitive to antibiotics, was obtained through the courtesy of Dr. B. Weisblum, University of Wisconsin. It was derived from strain K802 by nitrosoguanidine mutagenesis and screening for hypersensitivity to antibiotics, and performed exper-
iments by adding to each 10-ml culture 200 µl of tolune, followed by vigorous shaking, and 50 µl of 5% N-lauryl sarcosine, folled by swiriling so as to mix thoroughly but avoid foaming.

Antibiotics

Erythromycin base was a gift from Abbot Laboratories. Lin-
comycin, clindamycin, and celesticetin were obtained either from Dr. B. Weisblum or from commercial sources.

Cell growth, enzyme induction, thermal inactivation, and β-galactosidase assay

DB-11 cells were grown to early exponential phase with a dou-
bling time of approximately 85 min by shaking aeration at 30°C in M9 minimal medium (Menninger et al. 1973), buffered at pH 8.0 and supplemented with glucose (1.5%; these cells do not grow well in glycerol-based media), thiamine (5 µg/ml), biotin (0.1 µg/ml), and 20 amino acids (40 µg/ml). Aliquots (10 ml) were distributed to pre-warmed shaking flasks containing various drugs. After 15 min at 30°C, β-galactosidase synthesis was in-
duced by adding 1.0 mM isopropyl-β-D-thiogalactopyranoside and 2.0 mM 3’,5’-cyclic AMP. After 30 min at 30°C, cells were permeabilized by adding to each 10 ml culture 200 µl of tolune, followed by vigorous shaking, and 50 µl of 5% N-lauryl sarcosine, folled by swiriling so as to mix thoroughly but avoid foaming.

Thermal inactivation and β-galactosidase assay

The method of Hall and Gallant (1972) was implemented by incu-
bating 0.5-ml aliquots of permeabilized cells in Wasser
tman tubes for various times at the inactivation temperature, then placing the tubes at 30°C. Enzyme activity was assayed by adding to the cells 1.0 ml of 1.0 mg/ml α-nitrophenyl-β-D-galactosidase, incu-
bating at 30°C until color development was appropriate, adding 0.5 ml of 1.0 M Na2CO3, and assaying absorbance at 420 nm. The increase in 420 nm absorbance per min, suitably corrected for un-
catalyzed controls, measured enzyme activity. Fraction Gz Sur-
viving is the β-galactosidase activity observed after the indicated heating time divided by enzyme activity in the absence of heating.

Results

Wild-type E. coli cells are not very sensitive to lin-
cosamides so we used strain DB-11, which was selected for hypersensitivity to antibiotics, and performed exper-
iments at a pH (8.0) where macrolides and lincosamides are more effective than under neutral conditions. We measured accuracy of protein synthesis by assaying the resistance of β-galactosidase to thermal inactivation (Hall and Gallant 1972; Menninger 1985). Enzymes must be able to withstand small structural alterations under physiological conditions in order to bind sub-
strate, catalyze reactions, and release product. Balance between conformational stability and flexibility is the result of interactions among amino acid side chains in the normal protein. At elevated temperatures, structural stability is overwhelmed and inactivation occurs. Miss-
sense errors during protein synthesis impair normal in-
teractions and lead generally to lower structural stabil-
ity. An erroneous protein, therefore, is more likely to be inactivated in a given time at high temperature. Mis-
sense errors are expected to occur randomly and only some will affect the thermal stability of an enzyme. The kinetics of inactivation of a population of enzyme molecules containing errors is therefore expected to be complex. Since synthesis of β-galactosidase can be in-
duced quickly to high levels, > 102-fold, effects of treat-
ments on protein synthesis can be assessed even if the cells cannot survive. Enzymes synthesized by E. coli are normally so accurate that it is difficult to detect im-
provement in thermal stability. To assay accuracy-enh-
ancing effects, protein synthetic errors are first induced by some perturbation.

Aminoglycoside antibiotics like streptomycin en-
hance errors during bacterial protein synthesis (Gorini 1974). Figure 1A shows the effect of treating DB-11 cells with streptomycin during synthesis of β-galactosidase