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SPECIFICITY OF LINCOMYCIN ACTION ON PEPTIDYL TRANSFERASE ACTIVITY

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SUMMARY

The antibiotic lincomycin and twelve of its analogs were analyzed for their effects on three peptidyl transferase reactions, peptide bond formation, esterification, and hydrolysis of formylmethionyl-tRNA. Only lincomycin stimulated hydrolysis while having inhibitory effects on the other two reactions. The effects of the analogs were variable. Modifications at the carbon-7 position or loss of the carbonyl group caused dramatic alterations in lincomycin activity. Most of the analogs inhibited all three reactions indicating that interaction with the ribosome is not sufficient to elicit the unique specificity of action observed with lincomycin.

INTRODUCTION

Peptidyl transferase is the ribosomal peptide bond-forming enzyme (1). Monro and Vazquez (2) studied the activity of peptidyl transferase in the highly simplified "fragment reaction" which measures the formation of formylmethionine-puromycin from fMet-tRNA or 3'-terminal fragments of fMet-tRNA in the presence of ethanol. The reaction was independent of soluble factors, GTP, mRNA, and the small ribosomal subunit, but required the large subunit. Subsequently, similar systems were developed to study additional reactions catalyzed by peptidyl transferase. Scolnick et al. (3) showed that fMet-ethyl ester was produced from fMet-tRNA and ethanol when puromycin was replaced with uncharged tRNA. Such ribosomal-dependent ester formation has also been ob-

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served by different methods (4,5). Caskey et al. (6) replaced the ethanol with acetone and demonstrated hydrolysis of ribosomal-bound fMet-tRNA in the absence of release factors. This observation supported the concept that release factor-dependent hydrolysis of peptidyl-tRNA at termination of protein synthesis is catalyzed by peptidyl transferase (7-9).

Lincomycin is an inhibitor of bacterial protein synthesis (10) and is known to bind to the 50S ribosomal subunit (11,12). Studies of partial reactions indicated that lincomycin is an inhibitor of peptidyl transferase. Peptide bond synthesis as measured by fMet-puromycin formation (2) and release factor-mediated hydrolysis of fMet-tRNA (7,9) were inhibited by lincomycin. However, Caskey et al. (6) found that lincomycin stimulated E. coli peptidyl transferase-mediated hydrolysis of fMet-tRNA but inhibited esterification and peptide bond formation reactions. It was suggested that this differential effect may be relevant to the mechanism of peptide chain termination where release factors act as mediators of peptidyl-tRNA hydrolysis.

To investigate the specificity of lincomycin in promoting hydrolysis of fMet-tRNA, we have examined a number of analogs for their effect on the E. coli peptidyl transferase activities described above. In this report we show that lincomycin alone stimulates the hydrolysis reaction and that modifications at several sites, particularly at carbon-7, alters its effect not only on hydrolysis, but also on esterification and fMet-puromycin formation.

MATERIALS AND METHODS

[^{3}H]Methionine (6 Ci/mmol) was purchased from Amersham/Searle, and purified formylmethionine tRNA was obtained from Oak Ridge National Laboratory. Formyl-[^{3}H]methionyl-tRNA was synthesized as described by Milman et al. (13). The trinucleotide AUG was purchased from Miles Laboratories, and E. coli ribosomes were prepared by the method of Lucas-Lenard and Lipmann (14). The formation of f[^{3}H]-Met-tRNA·AUG·ribosome complexes has been described by Caskey et al. (15).

Hydrolysis of fMet-tRNA, esterification of fMet, and formation of fMet-puromycin were determined by the release of f[^{3}H]Met, f[^{3}H]Met-ethyl ester, and f[^{3}H]Met-puromycin, respectively, from f[^{3}H]Met-tRNA·AUG·ribosome substrates as described elsewhere (3,6). The buffer and ionic composition of the reaction mixtures were as follows: 0.1 M KCl, 0.03 M Mg-acetate, and 0.05 M Tris-acetate

(pH 8.0) for the hydrolysis of fMet-tRNA; 0.25 M KCl, 0.03 M Mg-acetate, and 0.06 M Tris-acetate (pH 8.0) for esterification reactions; and 0.05 M NH_4Cl, 0.03 M Mg-acetate, and 0.05 M Tris-HCl (pH 8.0) for the formation of fMet-puromycin.

RESULTS

The variety of compounds tested are analogs of either lincomycin, which has the structure shown in Fig. 1, or clindamycin, an analog of lincomycin chlorinated at carbon-7, denoted by the asterisk. The descriptions of these analogs are given in Table I. As shown in Table II, the esterification reac-

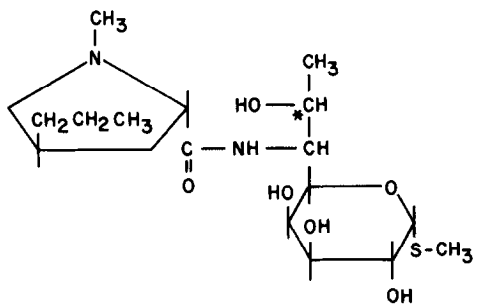

Fig. 1. Chemical structure of lincomycin (16). Clindamycin = 7-chlorolincomycin.

Table 1

Chemical Names and Code Designations for Lincomycin Analogs

Code	Chemical Name
U-51,544E	8-norlincomycin
U-19,665A	7-deoxylincomycin
U-25,585	deoxolincomycin
U-20,244E	7-epilincomycin
U-36,337A	methyl(7S)-N-(2-aminolauroyl)-7-chloro-7-deoxythio-lincosaminide
U-34,728E	1'-demethyl-1'-(2-hydroxyethyl)-clindamycin
U-50,501E	(7S)-7-deoxy-7-[[2-(methylthio)ethyl]thiol]-lincomycin
U-48,853E	(7S)-7-deoxy-7-[[[(methoxymethyl)thio]ethyl]thio]-lincomycin
U-35,854E	(7S)-7-(cyclohexylthio)-7-deoxylincomycin
U-35,411E	7-deoxy-7(S)-[(2-hydroxyethyl)thio]-lincomycin
U-24,729A	1'demethyl-4'-depropyl-4'-pentyl-clindamycin

Table II

Effect of Lincomycin Analogs on Peptidyl Transferase Activity

	Product (pmol)		
Analog	f[^{3}H]Met	f[^{3}H]Met-ethyl ester	f[^{3}H]Met-puromycin
None	0.76	2.42	1.98
Lincomycin	0.96	0.13	0.49
U-51,544E	0.75	1.29	1.97
U-19,665A	0.54	0.37	1.11
U-25,585	0.47	0.61	2.03
U-20,244E	0.43	0.49	0.96
U-36,337A	0.35	0.29	1.66
U-34,728E	0.32	0.06	0.01
Clindamycin	0.20	0.04	0.07
U-50,501E	0.07	0.06	0.03
U-48,853E	0.06	0.06	0
U-35,854E	0.03	0.10	0
U-35,411E	0.03	0.06	0.02
U-24,729A	0	0.02	0.02

Release of the indicated product from 5.07 pmol of f[^{3}H]Met-tRNA·AUG·ribosome complex was determined in 0.05 ml reactions which were initiated and incubated as follows: (a) release of f[^{3}H]Met - addition of 0.1 A_{260} units of unfractionated E. coli B tRNA and acetone to give 30% (v/v), 10-minute incubation at 0°C; (b) formation of f[^{3}H]Met-ethyl ester - addition of 0.06 A_{260} units of tRNA and ethanol to give 20% (v/v), 5-minute incubation at 0°C; (c) formation of f[^{3}H]Met-puromycin - addition of puromycin to yield a final concentration of 10^{-5} M, incubation for 20 minutes at 24°C. All lincomycin analogs were present at 10^{-4} M. Production of f[^{3}H]Met (0.18 pmol), f[^{3}H]Met-ethy ester (0.08 pmol), and f[^{3}H]Met-puromycin (0.05 pmol) occurring in the absence of tRNA or puromycin was subtracted from all values.

tion was inhibited by all of the compounds. However, the lincomycin analogs possess variable ability to inhibit the release of f[^{3}H]Met or the formation of f[^{3}H]Met-puromycin. Lincomycin was the only compound that stimulated hydrolysis but severely inhibited ester and peptide bond formation.

All three reactions were inhibited by seven of the analogs, U-34,728E; clindamycin; U-50,501E; U-48,853E; U-35,854E; U-35,411E; and U-24,729A. When the structures of these were examined it was noted that the common feature was

the replacement of the C-7 hydroxyl group of lincomycin by a more bulky constituent. Compound U-36,337A also has a bulky C-7 group (chlorine) but showed little inhibition of peptide bond formation and about 50% inhibition of f[^{3}H]-Met release. However, this analog differs from those listed above since it does not possess the substituted proline moiety present in all others.

More subtle modifications at the C-7 position resulted in partial loss of inhibitory capacity. U-19,665A has no C-7 hydroxyl group, and U-20,244E has the hydroxyl in the "S" configuration, rather than the "R" configuration of lincomycin. These compounds showed approximately 60-70% and 50% inhibition of hydrolysis and f[^{3}H]Met-puromycin formation, respectively. U-51,544E has no C-7 methyl group (lacks carbon-8) and did not affect hydrolysis or peptide bond formation, and inhibited esterification by only 50%. It should be noted that compound U-25,585, which is identical to lincomycin at carbon-7 but lacks the carbonyl group, also did not affect f[^{3}H]Met-puromycin formation and showed moderate (40%) inhibition of hydrolysis. Therefore, it appears that the precise structure of lincomycin around at least two carbon sites must be preserved to elicit its unique specificity of action. Certainly, carbon-7 is sensitive to even the slightest modification.

DISCUSSION

The mode of action of lincomycin in intact cells is not clear since it does not inhibit peptide bond formation on endogenous polysomes (17). However, the effect of the antibiotic and its analogs on peptidyl transferase activity can be studied in model systems, such as those described in this report, which employ washed ribosomes and simple substrates.

The apparent difference in the ability of any single lincomycin analog to inhibit the three reactions studied is probably due to their variable affinity for ribosomes in the different solvent systems used. For example, it has been shown that the binding of lincomycin to E. coli ribosomes is considerably increased in the presence of ethanol (12). This is consistent with our observation that esterification of f[^{3}H]Met (performed in 20% ethanol)

was the most inhibited reaction. Substitution of the C-7 hydroxyl group of lincomycin by a more bulky residue resulted in dramatic inhibition of all three reactions. Small modifications at carbon-7, loss of the carbonyl group, or loss of the substituted proline moiety resulted in a decrease in the inhibitory capacity. These effects also may be a reflection of altered binding of the antibiotics to the ribosome. This is supported by the fact that changes in the effect on one reaction were accompanied by similar changes for the other reactions. Only in the case of lincomycin itself was the hydrolysis of f[^{3}H]-Met-tRNA stimulated while ester and peptide bond formation were inhibited. This stimulation was very reproducible and has been reported previously (6). Clearly, lincomycin must interact with the f[^{3}H]Met-tRNA·AUG·ribosome substrate to produce this effect. In addition to binding, therefore, other features of the molecule must be important in promoting the hydrolysis reaction.

Peptidyl transferase catalyzes a general nucleophilic attack on the ester bond of peptidyl-tRNA. Peptide bond formation results when the nucleophile is an amino group; ester bonds are produced when the nucleophilic agent is an alcohol; and hydrolysis occurs via attack by water. Since lincomycin exhibits a striking differential effect on these reactions, it may be capable of restricting the availability of nucleophiles at the peptidyl transferase catalytic site, resulting in hydrolysis only. On the other hand, if normal peptide bond formation involved discrete intermediate events, such as cleavage of the peptidyl-tRNA bond and subsequent attack by aminoacyl-tRNA, lincomycin could uncouple these events by inhibiting only the latter reaction. Thus far no laboratories have succeeded in the identification of intermediate events involving covalent chemical bonds. It is tempting to suggest that the release factors, which have no esterase activity alone but promote the hydrolysis of peptidyl-tRNA at peptide chain termination, may function by altering the specificity of the peptidyl transferase.

Another compound, anisomycin, has been shown to differentially affect peptidyl transferase reactions in eukaryotic systems in a manner similar to that

of lincomycin in E. coli (6,18). Since the structure of anisomycin bears considerable resemblance to that of lincomycin, it may be that the ribosomal sites involved and the mechanism of preferential stimulation of hydrolysis are very similar between prokaryotes and eukaryotes. No analogs of anisomycin are presently available for study. However, Chinese hamster lung cells with in vivo resistance to anisomycin have been isolated (19) which offer an opportunity for examining the eukaryotic peptidyl transferase.

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