Proc. Nat. Acad. Sci. USA
Vol. 68, No. 12, pp. 3163–3167, December 1971

Hydrolysis of fMet-tRNA by Peptidyl Transferase

(reticulocytes/*E. coli*/anisomycin/R factors/lincomycin)

C. T. CASKEY, A. L. BEAUDET, E. M. SCOLNICK*, AND M. ROSMAN†

Section of Medical Genetics, Departments of Medicine and Biochemistry, Baylor College of Medicine, Houston, Texas 77025

Communicated by Marshall Nirenberg, October 12, 1971

ABSTRACT ***Escherichia coli*** **and rabbit reticulocyte (f[³H]Met-tRNA·AUG·ribosome) intermediates undergo hydrolysis, with release of f[³H]methionine, upon addition of tRNA or CpCpA in the presence of acetone. This ribosomal catalyzed reaction has similar requirements, pH optimum, and antibiotic sensitivity to those of peptidyl transferase. Two antibiotics, lincomycin with *E. coli* ribosomes and anisomycin with reticulocyte ribosomes, inhibit peptide-bond formation and transesterification activities of peptidyl transferase, but stimulate hydrolysis of f[³H]Met-tRNA. Earlier studies have suggested peptidyl transferase activity is essential for R factor-dependent hydrolysis of f(³H)Met-tRNA. These studies indicate that peptidyl transferase has the capacity for f(³H)Met-tRNA hydrolysis and, therefore, may be responsible for peptidyl-tRNA cleavage during peptide chain termination.**

Peptide chain termination can be studied as a partial reaction involving recognition of terminator codons (1) or hydrolysis of ribosomal-bound peptidyl-tRNA (2). Both intermediate events require protein release factor (*Escherichia coli*, R1 or R2; rabbit reticulocyte, R) and their corresponding ribosomes (3). This communication focuses on the mechanism of peptidyl-tRNA hydrolysis. Earlier publications have dealt with the requirements of codon recognition by R factors (1).

Release factor-dependent hydrolysis of the peptidyl-tRNA ester bond requires location of the peptidyl-tRNA in the ribosomal P-site (2), NH_4^+ or K^+ (4), and is inhibited by several antibiotics that also inhibit peptide bond formation (4–6). These characteristics are similar to those of the ribosomal enzyme peptidyl transferase. Such circumstantial information has led several investigators (4–7) to speculate on the possible involvement of peptidyl transferase in peptidyl-tRNA hydrolysis occurring at chain termination. Our interest in the mechanism of peptide chain termination led us to the study of peptidyl transferase.

Monro and Vasquez (8) have greatly simplified study of peptidyl transferase and conducted extensive studies characterizing its activity. These investigators measure the ribosomal-dependent formation of fMet-puromycin from fMet-tRNA or 3′-terminal fragments of fMet-tRNA as an index of peptidyl transferase activity. Such reactions proceed *in vitro* with fMet-tRNA, K^+ or NH_4^+, puromycin, 50S (bacterial) or 60S (eukaryotic) ribosomal subunits, and ethanol (9). Thus, peptidyl transferase activity is a function of the larger ribosomal subunit and can be studied independently of transfer factors, GTP, mRNA, or smaller ribosomal subunits (30S, bacterial; 40S, eukaryotic). We have used similar conditions in our studies of peptidyl transferase and found the ribosome to be capable of additional reactions (10).

When reactions contain fMet-tRNA, 50S (bacterial) ribosomal subunits, ethanol, and tRNA or its 3′-terminal oligonucleotide CpCpA, the reaction product is fMet-ethyl ester (10). Using different experimental approaches, ribosomal-dependent ester formation has been observed by Rich and his associates (7, 11, 12). The characteristics of both reactions indicate that transesterification is catalyzed by peptidyl transferase. This report describes the hydrolysis of ribosomal-bound fMet-tRNA by peptidyl transferase in reactions containing acetone and tRNA. The demonstration of the hydrolytic capacity of peptidyl transferase provides additional indirect evidence for its involvement in the peptide chain termination event.

MATERIALS AND METHODS

Preparation of f[³H]Met-tRNA·Ribosome Intermediates. *E. coli* (f[³H]Met-tRNA·AUG·ribosomes) intermediates are prepared (13) by the use of AUG to bind purified *E. coli* f[³H]Met-tRNA to ribosomes washed with 1.0 M NH_4Cl. After formation of the complex, $Mg(OAc)_2$ is added to a final concentration of 0.03 M and the complex is stored in a liquid-nitrogen refrigerator until use. Reticulocyte (f[³H]Met-tRNA·ribosome) intermediates are prepared as described (3) by the use of 0.055 M Mg $(OAc)_2$ to bind purified *E. coli* f[³H]Met-tRNA to ribosomes washed with 0.5 M KCl. After formation, the complex is stored in a liquid-nitrogen refrigerator until use.

Hydrolysis of f[³H]Met-tRNA. A typical reaction containing bacterial ribosomes is incubated at 4°C for 15 min and contains in 0.05 ml: 3–5 pmol of (f[³H]Met-tRNA·AUG·ribosome); 0.1 M KCl; 0.03 M Mg $(OAc)_2$; 0.05 M Tris-OAc (pH 8.0); 0.1 A_{260} unit of unfractionated *E. coli* tRNA; and is initiated by the addition of 30% (v/v) acetone. Reactions will also proceed at 24°C with as little as 10% acetone. A typical reaction containing reticulocyte ribosomes is incubated at 24°C for 15 min and contains in 0.05 ml: 3–4 pmol of (f[³H]Met-tRNA·ribosome); 0.06 M KCl; 0.011 M $Mg(OAc)_2$; 0.02 M Tris-OAc (pH 7.4); 0.1 A_{260} unit of unfractionated *E. coli* tRNA; and is initiated by the addition of 30% acetone. Reactions proceed poorly at 4°C and require 30% acetone. The release of f[³H]methionine is quantitated by extraction of the product with ethyl acetate after

* Present address: Laboratory of Viruses, NCI, Bethesda, Md.
† Present address: Department of Medicine, University of California, San Francisco Medical Center, San Francisco, Calif.
Reprint requests should be addressed to Dr. Caskey.

TABLE 1. *Effect of solvents on formation of fMet and fMet-ester*

Solvent	Δ pmoles f[^{3}H]Met	Δ pmoles f[^{3}H]Met-ester
E. coli ribosomes		
Ethanol	0.00	1.65
Isopropanol	0.53	0.71
Tertiary butanol	0.96	0.02
Acetone	0.91	0.00
Reticulocyte ribosomes		
Ethanol	1.33	0.05
Acetone	0.07	1.57

Reactions containing *E. coli* ribosomes are incubated 20 min at 4°C and contain in 0.05 ml: 5.3 pmol (fMet-tRNA·AUG·ribosomes); 0.05 A_{260} unit of tRNA; 0.1 M KCl; 0.03 M Mg $(OAc)_2$; 0.05 M Tris-acetate (pH 8.0); and 30% (v/v) of the indicated solvent. Reactions containing rabbit reticulocyte ribosomes are incubated 15 min at 24°C and contain, in 0.05 ml: 3.6 pmol (fMet-tRNA·ribosome); 0.2 A_{260} unit of tRNA; 0.06 M KCl; 0.011 M Mg $(OAc)_2$; 0.02 M Tris-acetate, (pH 7.4); and 30% (v/v) of the indicated solvent. The f[^{3}H]Met (0.6 pmol) and f[^{3}H]Met-ester (0.05 pmol) formed in the absence of tRNA is subtracted from all values.

adjustment of the reaction to pH 1.0 (13). All values are reported as total f[^{3}H]methionine released.

The product of these reactions, and those containing lincomycin, was identified as f[^{3}H]methionine by thin-layer chromatography on Eastman silica-gel plates (14).

Formyl Methionine-Ethyl Ester Assay. Reaction components and conditions have been described (10). All reactions are terminated by the addition of 0.1 M imidazole buffer (pH 6.0) and f[^{3}H]Met-ethyl ester is extracted into ethyl acetate for quantitation. Since fMet-ethyl ester is extracted into ethyl acetate at both pH 1.0 and 6.0 and fMet is extracted only at pH 1.0, in reactions that contain both products the amount of ester is determined by extraction at pH 6.0 and fMet is determined by the amount extracted at pH 1.0 in excess of the fMet-ethyl ester found. All values are reported as total fMet-ethyl ester formed (10). fMet-puromycin is quantitated by the method of Leder and Brusztyn(15).

TABLE 2. *Peptidyl transferase hydrolysis of fMet-tRNA*

Components	Δpmole f[^{3}H]Met with ribosomes from *E. coli*	Δpmole f[^{3}H]Met with ribosomes from Reticulocytes
Complete	1.34	1.31
− tRNA	0.00	0.10
− tRNA + CpCpA	1.42	0.30
− Acetone	0.00	0.07
− Ribosomes	0.00	0.00

Complete reactions containing *E. coli* ribosomes are incubated 15 min at 4°C and contain, in 0.05 ml: 3.8 pmol *E. coli* (f[^{3}H]Met-tRNA·AUG·ribosome), other components as in Table 1, and, where indicated, 0.1 A_{260} unit of CpCpA. Complete reactions containing rabbit reticulocyte ribosomes are incubated 10 min at 24°C and contain, in 0.05 ml: 3.8 pmol (f[^{3}H]Met-tRNA·ribosome), other components as in Table 1, and, when indicated, 0.1 A_{260} unit of CpCpA. A zero-time value (0.43 pmol) is subtracted from all data.

TABLE 3. *Ribonucleotide stimulation of fMet-tRNA hydrolysis by peptidyl transferase*

Ribonucleotide	f[^{3}H]Met, Δpmole
tRNA	1.34
CpCpA	1.38
CpA	0.05
A	0.01
ApApC	0.02
CpApC	0.01
None	(0.10)

All reactions are as indicated for *E. coli* in Table 2.

Materials. The oligonucleotides were prepared and identified as described (10). *E. coli* (16) and reticulocyte R (17) preparations were purified. The L-[^{3}H-methyl]methionine (7.0 Ci/mmol) was supplied by Amersham/Searle. Antibiotics were described (2, 17).

RESULTS

Peptidyl transferase of mammalian (3) and bacterial ribosomes (2) is readily investigated with f[^{3}H]Met-tRNA·ribosome intermediates as substrate. The f[^{3}H]Met-tRNA is bound to *E. coli* ribosomes in response to AUG, and to rabbit reticulocyte ribosomes at 55 mM Mg^{++} without added mRNA template. These intermediates yield f[^{3}H]Met-puromycin upon addition of puromycin. When these intermediates are incubated with tRNA and an organic solvent, additional reactions catalyzed by peptidyl transferase are observed (Table 1). Reaction products vary with the organic solvent chosen for study. In the presence of ethanol or methanol (not shown), both bacterial and mammalian ribosomal intermediates yield f[^{3}H]Met-ethyl (or methyl) esters, as with bacterial ribosomes (10). Acetone or tertiary butanol added to these reactions gives f[^{3}H]methionine. Isopropanol, a secondary alcohol, gives a mixed product, f[^{3}H]methionine and f[^{3}H]methionine-ester. There is no conversion of f[^{3}H]Met-ester to f[^{3}H]methionine in these reactions. Variation of the organic solvent and, presumably, the predominant nucleophilic acceptor gives different products. The requirements for f[^{3}H]Met-tRNA hydrolysis are given in Table 2. Hydrolysis of fMet-tRNA with either *E. coli* or rabbit reticulocyte ribosome requires acetone, ribosomes, and tRNA. The trinucleotide CpCpA, which corresponds to the 3′-nucleotide sequence of tRNA, can substitute for tRNA. Reticulocyte ribosome preparations are less sensitive to the trinucleotide CpCpA, and have been reported not to participate in *in vitro* termination of peptide chains directed by the trinucleotides; UAA, UAG, and UAA (17). The reasons for the lowered sensitivity of these mammalian ribosomes to trinucleotides is not apparent.

Both tRNA and CpCpA stimulate hydrolysis of fMet-tRNA by *E. coli* ribosomes, while the sequence isomers ApApC and CpApC, the doublet CpA, and adenosine have no effect (Table 3). CpCpA identically stimulates the formation of fMet-ethyl ester (10) from reactions containing (fMet-tRNA··AUG·ribosome) intermediates and ethanol. This oligonucleo-

tide specificity suggests that fMet-tRNA hydrolysis is catalyzed by the same enzyme responsible for ribosomal transesterification, peptidyl transferase. Vasquez (9), and Rychlik (18) have used, respectively, ribonuclease fragments of aminoacyl-tRNA and 2′(3′)-*O*-aminoacyl nucleosides as small as A-Phe for the study of peptide bond formation. The nucleotide specificity of these acceptor molecules also indicates a recognition of the 3′-tRNA terminus CpCpA by a ribosomal component, presumably peptidyl transferase.

Further support for peptidyl transferase catalysis of these reactions is found in Fig. 1, where formation of fMet-puromycin by *E. coli* ribosomes is compared with the fMet-tRNA transesterfication and hydrolysis reaction. All reactions contain 30% acetone and, as indicated, puromycin, (fMet-puromycin), tRNA (fMethionine), and both tRNA and 4% ethanol (fMet-ethyl ester). All values are rate determinations. The similar effect of pH on these reactions and their similar pH optima suggest that all three reactions are catalyzed by a single enzyme, peptidyl transferase. Maden and Monro (19)

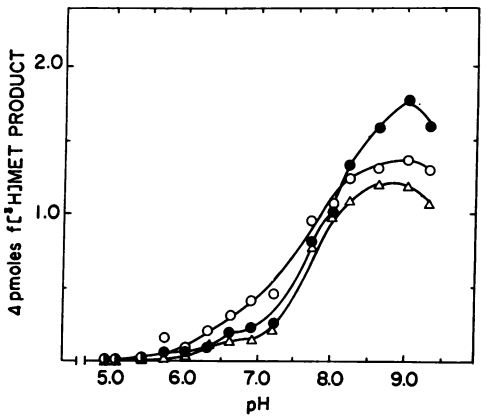

FIG. 1. All reactions are at 4°C and run either 5 min (fMet-puromycin) or 15 min (fMet-ethyl ester and fMethionine) and contain, in 0.05 ml: 3.4 pmol *E. coli* (f[³H]Met-tRNA·AUG·ribosome); 0.1 M KCl; 0.03 M Mg $(OAc)_2$; 0.05 M cacodylate (pH 4.8–6.0); imidazole (pH 6.3–7.4) or Tris-Acetate (pH 7.6–9.3); 30% acetone; 0.02 A_{260} unit tRNA (fMet-ester and fMethionine) or 0.1 mM puromycin (fMet-puromycin); and 2% ethanol (fMet-ethyl ester). A zero-time value (0.25 pmol) is subtracted from all data. ○, fMet-ethyl ester; ●, fMet; △, fMet-puro.

TABLE 4. *Antibiotic inhibitor*

Antibiotic added	Peptidyl transferase activity, Δpmoles		
	fMet-ethyl ester	fMet-puro	fMet
	E. coli ribosomes		
None	0.93	1.44	1.00
Sparsomycin	−0.10	0.03	−0.12
Gougerotin	0.21	0.12	0.00
Amicetin	0.55	0.40	0.24
Chloramphenicol	0.38	0.41	0.57
Lincomycin	0.03	0.15	1.45
Anisomycin	0.95	1.70	1.00
Fusidic acid	0.86	1.34	0.99
Streptomycin	0.97	1.00	1.15
	Reticulocyte ribosomes		
None	1.59	1.29	1.39
Sparsomycin	0.00	0.03	0.02
Gougerotin	1.17	1.42	1.09
Anisomycin	0.00	0.09	3.50
Amicetin	1.60	1.47	1.47
Lincomycin	1.60	1.52	1.49

All *E. coli* peptidyl transferase reactions are incubated 20 min at 4°C and contain in 0.05 ml: 0.05 M Tris-acetate (pH 8.0), 0.05 M NH_4OAc; 0.03 M Mg $(OAc)_2$, 4.5 pmol of (f[³H]Met-tRNA·AUG·ribosome) intermediates, 0.05 A_{260} unit of unfractionated *E. coli* B tRNA, where indicated, 10 μM puromycin, 30% acetone, and 2% ethanol in the case of fMet-ethyl ester reactions. The formation of f[³H]Methionine, 0.42 pmol, f[³H]Met-ethyl ester, 0.16 pmol, and f[³H]Met-puro, 0.14 pmol, occurring in the absence of added tRNA or puromycin, is subtracted from the indicated values. All antibiotics were added to a final concentration of 0.1 mM. All reticulocyte peptidyl transferase reactions are incubated at 24°C for 5 min (fMet-puromycin) or 15 min (fMet- and fMet-ethyl ester) and contain, in 0.05 ml, the components indicated in Table 2, either 50 μM puromycin or 0.2 A_{260} unit of tRNA, 30% acetone (fMet- and fMet-puro) or 20% ethanol (fMet-ethyl ester), and, where, indicated 50 μM sparsomycin, 1 mM gougerotin, 1 mM anisomycin, 0.1 mM lincomycin, or 1 mM amicetin. Formyl-methionine formation in the absence of added tRNA is 0.54 pmol, f[³H]Met-ethyl ester, 0.16 pmol, and f[³H]Met-puro, 0.01 pmol; these values are subtracted from the indicated values.

have reported a similar pH curve for the ability of peptidyl transferase to use CpCpA-Leu as an acceptor in peptidyl transferase reactions. Additional evidence for catalysis of the three reactions by peptidyl transferase is found in Table 4. Since eukaryotic and prokaryotic ribosomes differ in their sensitivity to antibiotic inhibitors of peptidyl transferase, we have examined the effect of several antibiotics on both ribosomal types for their formation of fMet-ethyl ester, fMet-puromycin, and fMethionine. Two antibiotics, sparsomycin and gougerotin, are inhibitors of the three reactions by *E. coli* and reticulocyte ribosomes. Both antibiotics were reported (20, 21) to be inhibitors of eukaryotic and prokaryotic peptidyl transferase. Amicetin and chloramphenicol inhibit formation of fMet-ethyl ester, fMet-puromycin, and hydrolysis of fMet-tRNA by *E. coli* ribosomes. Amicetin (Table 4) and chloramphenicol have little or no effect on eukaryotic peptidyl transferase. Lincomycin has a striking differential effect on the three reactions with *E. coli* ribosomes. Lincomycin inhibits formation of fMet-puromycin and fMet-ethyl ester (90%), stimulates hydrolysis of fMet-tRNA 1.5-fold, and has no effect on reticulocyte ribosomes. Anisomycin affects these three reactions by reticulocyte ribosomes in a manner similar to lincomycin. Anisomycin is a potent inhibitor of fMet-puromycin and fMet-ethyl ester (90%) formation, stimulates hydrolysis of fMet-tRNA (2.5-fold), and has no effect on *E. coli* ribosomes. The rate of fMet-tRNA hydrolysis by bacterial and reticulocyte ribosomes is shown in Fig. 2, together with the effect of lincomycin and anisomycin. At short time periods, *E. coli* ribosomes are stimulated 1.5-fold by lincomycin, while reticulocyte ribosomes are stimulated 10-fold by anisomycin. The requirements for this stimulatory effect are given in Table 5. Both ribosomes and acetone are required. The low level of fMet-tRNA hydrolysis occurring without added tRNA may be due to tRNA that contaminates the ribosomal preparations. Sparsomycin, a potent inhibitor of reticulocyte (21) and *E. coli* (20) peptidyl transferase, inhibits fMet-tRNA hydrolysis in the presence

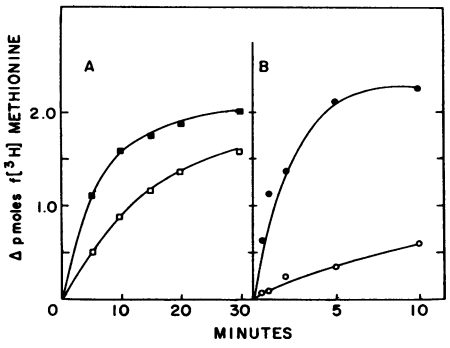

FIG. 2. fMet-tRNA hydrolysis. Reactions containing bacterial ribosomes are at 4°C for the indicated time and contain the components described in Table 2 except 0.1 A_{260} unit of tRNA, 30% acetone, and, where indicated, 0.1 mM lincomycin. A zero-time value (0.25 pmol) is subtracted from all data. Reactions containing reticulocyte ribosomes are incubated as in Table 2. 30% acetone, and, where indicated, 1 mM anisomycin are added and CpCpA is omitted. A zero-time value (0.25 pmol) is subtracted from all data. (*A*) *E. coli:* ■, +lincomycin; □, −lincomycin. (*B*) Reticulocyte: ●, +anisomycin; ○, −anisomycin.

of lincomycin (*E. coli* ribosomes) or anisomycin (reticulocyte ribosomes) (data not shown). Thus, with the exception of lincomycin (*E. coli*) and anisomycin (reticulocyte), the antibiotic sensitivity of ribosomal catalyzed fMet-puromycin formation, fMet-ethyl ester formation, and fMet-tRNA hydrolysis appear similar. These data suggest that the three reactions are catalyzed by peptidyl transferase. Conversely, the inhibition by lincomycin and anisomycin of peptide bond formation and transesterification, and stimulation of fMet-tRNA hydrolysis, could be argued as evidence for at least two ribosomal enzymes for the three reactions. If such is the case, both are sensitive to sparsomycin.

The hydrolysis of fMet-tRNA that occurs with R at peptide chain termination is somewhat similar to that occurring with tRNA and acetone (Fig. 3). In these studies, the pH optima for fMet-tRNA hydrolysis with *E. coli* R1 and R2 are determined in reactions containing 20% ethanol. At 4°C under

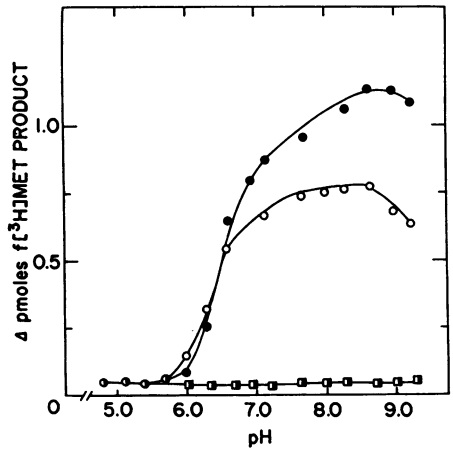

FIG. 3. R-dependent fMet-tRNA hydrolysis. All reactions are at 4°C for 5 min as in Fig. 1, except 20% ethanol, no tRNA or antibiotics, and either 2.5 μg of R1 or 3.1 μg of R2, as indicated, are added. Formylmethionine released in the absence of R (0.25–0.4 pmol) is subtracted from all values. 0.16 pmol is subtracted from all fMet-ethyl ester values. ○, fMet, R1; ●, fMet, R2; ▯, fMet-ester, R1 or R2.

TABLE 5. *Requirement for antibiotic-stimulated f[³H]Met-tRNA hydrolysis*

Components	Δpmoles f(³H)Methionine	
	Reticulocytes	*E. coli*
Complete	3.19	1.42
−Ribosomes	0.00	0.01
− tRNA	0.43	0.08
− Acetone	0.07	−0.01
− Lincomycin	—	1.02
− Lincomycin + anisomycin	—	1.02
− Anisomycin	0.28	—
− Anisomycin + lincomycin	0.28	—

A complete *E. coli* reaction is incubated as described in Table 2, except 0.1 A_{260} unit of tRNA, 0.1 mM lincomycin or, where indicated, 1 mM anisomycin and 30% acetone are added. A complete reticulocyte reaction is incubated 2 min at 24°C and contains in the components in Table 2, except 0.2 A_{260} unit of tRNA, 1 mM anisomycin or, where indicated, 0.1 mM lincomycin and 30% acetone (CpCpA is omitted). Formyl-methionine found at zero time (0.25 pmol) is subtracted from all values.

such conditions, R binds to (f[³H]Met-tRNA·AUG·ribosome) intermediates independently of terminator codons, with resultant fMet-tRNA hydrolysis (2). Hydrolysis of fMet-tRNA with R (Fig. 3) or tRNA (Fig. 1) and ribosomes is initially observed at pH 4.8, with optimal pH values of 8.8–9.0 for R1, 8.3–8.5 for R2, and 9.0 for tRNA. The pH optimum for R1 was 9.0 when reactions contained 30% acetone, rather than 20% ethanol. Under similar conditions, R2 had a pH optimum of 8.6 (not shown). Similarly, when the pH optima for R1 and R2 were determined in reactions containing terminator codons, rather than organic solvents, the R1 pH optimum was 9.0 and that for R2 was 8.8. Thus, it appears that under various *in vitro* conditions, the hydrolysis of fMet-tRNA with R and tRNA have similar pH sensitivity. These studies suggest, but do not prove, that fMet-tRNA hydrolysis occurring with R or tRNA and acetone are catalyzed by a common mechanism or require a common intermediate event. R Factor, however, will neither stimulate formation of fMet-ethyl ester (bottom of Fig. 3) or degrade fMet-ethyl ester (not shown). Thus, reactions that contain R are somewhat similar to reactions containing tRNA, ethanol, ribosomes, and either lincomycin (*E. coli* ribosomes) or anisomycin (reticulocyte ribosomes) (data not shown). The expected product, fMet-ethyl ester, is not found; rather, fMet-tRNA hydrolysis occurs. We have examined these antibiotics for their effect on R-mediated fMet-tRNA hydrolysis in reactions containing 30% acetone. As reported, lincomycin inhibits fMet-tRNA hydrolysis by *E. coli* R and ribosomes

TABLE 6. *Peptidyl transferase catalyzed reactions*

Substrate	Additions	Product
A. f[³H]Met-tRNA ribosome	Puromycin	f[³H]Met-puromycin
B. f[³H]Met-tRNA ribosome	Ethanol, tRNA, or CCA	f[³H]Met-ethyl ester
C. f[³H]Met-tRNA ribosome	Acetone, tRNA, or CCA	f[³H]Met

(2) and anisomycin inhibits fMet-tRNA hydrolysis by reticulocyte R and ribosomes (17).

DISCUSSION

Hydrolysis of fMet-tRNA with prokaryotic and eukaryotic ribosomes can occur with protein release factor (2, 17) or, as shown in this report, in reactions that contain acetone and tRNA. The hydrolysis reaction can be induced by the oligonucleotide CpCpA in place of tRNA, requires NH_4^+ or K^+ (data not shown), has a pH optimum similar to that of ribosome-catalyzed peptide bond formation, and is inhibited by most antibiotic inhibitors of peptidyl transferase. These studies suggest that peptidyl transferase can catalyze hydrolysis of fMet-tRNA, in addition to its known capacity to catalyze peptide bond formation and transesterification. Peptidyl transferase can therefore be described as an enzyme that has the general catalytic capacity of facilitating nucleophilic attack on the ester bond of peptidyl-tRNA. The *in vitro* requirements of each reaction are given in Table 6. In reaction *A*, where the attacking nucleophile is the amino group of puromycin or an amino acid (not shown), the product is a peptide bond. When the nucleophile is ethanol or methanol (not shown), as in reaction *B*, the product is fMet-ethyl (or methyl) ester. In reaction *C*, where no amino or alcohol acceptor is present, the attacking nucleophile is OH^- or H_2O, and fMet-tRNA hydrolysis occurs.

Two antibiotics, lincomycin and anisomycin, can differentially inhibit the ability of peptidyl transferase to catalyze the three reactions outlined in Table 6. Hydrolysis of fMet-tRNA by *E. coli* peptidyl transferase is stimulated by lincomycin, while the reticulocyte ribosomal hydrolysis is stimulated by anisomycin. The antibiotics inhibit peptide bond formation and transesterification by the appropriate ribosomes. The mechanism of these effects is not known. Two possible mechanisms are suggested, however. The differential effect may be a function of limitation of the nucleophile available to the peptidyl-transferase catalytic site. If lincomycin or anisomycin allow OH^-, but restrict ethanol or puromycin, at the catalytic site, the result would be active fMet-tRNA hydrolysis and inhibited peptide bond formation and transesterification. Alternatively, lincomycin and anisomycin may uncouple intermediate steps that occur during peptide bond formation. Antibiotics are known that specifically inhibit one ribosomal function involved in protein biosynthesis without affecting others (22, 23). Antibiotics may therefore be useful to identify intermediate events that occur in peptide bond formation by differentially inhibiting the intermediate reactions. The lincomycin and anisomycin effects on peptidyl transferase may be the first evidence in support of the concept of intermediate reactions occurring in peptide bond formation. For example, cleavage of the peptidyl-tRNA ester linkage is requisite for peptide bond formation, transesterification, and peptidyl-tRNA hydrolysis. Additional transient intermediate steps involving ribosomal components, or CpCpA of the acceptor tRNA located in the ribosomal A-site, may be required for peptide bond formation. The stimulation of fMet-tRNA hydrolysis and inhibition of peptide bond formation and transesterification may reflect inhibition of later intermediate reactions normally operative in peptide bond formation.

The mechanism of peptidyl-tRNA hydrolysis occurring with R factor at peptide chain termination is believed to be relevant to the hydrolysis reaction detailed in this paper. Our earlier studies (2), and those of others (4, 6), have indicated that R-dependent fMet-tRNA hydrolysis requires an active ribosomal peptidyl transferase. The studies in this report suggest two possible mechanisms for R-factor participation in fMet-tRNA hydrolysis. Since peptidyl transferase facilitates nucleophilic attack on the peptidyl-tRNA ester bond, a nucleophilic group on the protein molecule, R may participate in the attack on and cleavage of the peptidyl-tRNA ester bond. Precedent for this type of hydrolysis reaction is established in the case of the serine residue of trypsin (24). Alternatively, R may promote fMet-tRNA hydrolysis in a manner similar to lincomycin and anisomycin without directly participating in the hydrolysis reaction. Ribosomal-bound R would interact with peptidyl transferase and modify peptidyl transferase specifically for the hydrolysis of fMet-tRNA.

C. T. C. is a Howard Hughes Investigator. A. L. B. was supported by NIH Special fellowship GM 51598-01. This study was supported PHS grant GM-18682-01.

1. Scolnick, E., and C. T. Caskey, *Proc. Nat. Acad. Sci. USA*, **64**, 1235 (1969).
2. Tompkins, R. K., E. M. Scolnick, and C. T. Caskey, *Proc. Nat. Acad. Sci. USA*, **65**, 702 (1970).
3. Goldstein, J. L., A. L. Beaudet, and C. T. Caskey, *Proc. Nat. Acad. Sci. USA*, **67**, 99 (1970).
4. Vogel, Z., A. Zamir, and D. Elson, *Biochemistry*, **8**, 5161 (1969).
5. Caskey, C. T., and A. L. Beaudet, *Molecular Mechanisms of Antibiotic Action on Protein Biosynthesis and Membranes* (Springer-Verlag, Berlin, 1971), in press.
6. Capecchi, M. R., and H. A. Klein, *Cold Spring Harbor Symp. Quant. Biol.*, **34**, 469 (1969).
7. Fahnestock, S., H. Neumann, V. Sharbona, and A. Rich, *Biochemistry*, **9**, 2477 (1970).
8. Monro, R. E., and D. Vasquez, *J. Mol. Biol.*, **28**, 161 (1967).
9. Monro, R. E., T. Staehelin, M. L. Celma, and D. Vazquez, *Cold Spring Harbor Symp. Quant. Biol.*, **34**, 357 (1969).
10. Scolnick, E., G. Milman, M. Rosman, and T. Caskey, *Nature*, **225**, 152 (1970).
11. Fahnestock, S., and A. Rich, *Nature New Biol.*, **229**, 8 (1971).
12. Fahnestock, S., and A. Rich, *Science*, **173**, 340 (1971).
13. Caskey, C. T., E. Scolnick, R. Tompkins, G. Milman, and J. Goldstein, *Methods Enzymol.*, **20**, 367 (1971).
14. Caskey, C. T., B. Redfield, and H. Weissbach, *Arch. Biochem. Biophys.*, **120**, 119 (1967).
15. Leder, P., and H. Bursztyn, *Biochem. Biophys. Res. Commun.*, **25**, 233 (1966).
16. Milman, G., J. Goldstein, E. Scolnick, and T. Caskey, *Proc. Nat. Acad. Sci. USA*, **63**, 183 (1969).
17. Beaudet, A. L., and C. T. Caskey, *Proc. Nat. Acad. Sci. USA*, **68**, 619 (1971).
18. Rychlik, I., J. Cerna, S. Chladek, J. Zemlicka, and Z. Haladova, *J. Mol. Biol.*, **43**, 13 (1969).
19. Maden, B. E. H., and R. E. Monro, *Eur. J. Biochem.*, **6**, 309 (1968).
20. Monro, R. E., M. L. Celma, and D. Vazquez, *Nature*, **222**, 356 (1969).
21. Vazquez, D., E. Battaner, R. Neth, G. Heller, and R. E. Monro, *Cold Spring Harbor Symp. Quant. Biol.*, **34**, 369 (1969).
22. Modolell, J., B. Cabrer, A. Parmeggiani, and D. Vazquez, *Proc. Nat. Acad. Sci. USA*, **68**, 1796 (1971).
23. *Molecular Mechanisms of Antibiotic Action on Protein Biosynthesis and Membranes* (Springer-Verlag, Berlin, 1971), in press.
24. Vallee, B. L., and J. F. Riordan, *Annu. Rev. Biochem.*, **38**, 733 (1969).