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Characterization of the Peptidyltransferase Reaction Catalyzed by Rat Liver 60S Ribosomal Subunits†

Herbert A. Thompson and Kivie Moldave*

ABSTRACT: The formation of acetylphenylalanyl-puromycin from acetyl[^{3}H]phenylalanyl-tRNA and puromycin was catalyzed by isolated rat liver 60S subunits in the presence of 4 mM $MgCl_2$, 0.3 M KCl, and 33% alcohol, at pH 7.5. Poly(U) stimulated significantly the reaction with acetylphenylalanyl-tRNA, especially if allowed to react with 60S subunits prior to the peptidyltransferase assay, but appeared to inhibit the reaction with other acetylated aminoacyl-tRNAs; peptidyltransferase with acetylated lysyl-tRNA was stimulated slightly by preincubation of the 60S particle with poly(A). Preincubation of 60S subunits with 40S particles markedly inhibited the peptidyltransferase reaction.

The peptidyltransferase reaction represents a ribosomal activity that catalyzes the synthesis of peptide bonds between peptidyl-tRNA and aminoacyl-tRNA on adjacent ribosomal sites (see review by Lucas-Lenard and Lipmann (1971)). This activity also appears to participate in the release of the completed polypeptide chain, from peptidyl-tRNA, in the process of termination (Caskey *et al.*, 1971). Studies in both bacterial (Monro, 1967; Maden *et al.*, 1968; Monro *et al.*, 1969; Ballesta *et al.*, 1971; Nierhaus and Montejo, 1973) and mammalian (Vazquez *et al.*, 1969; Falvey and Staehelin, 1970) systems indicate that only the large subunit of the ribosome is required for transpeptidation. Using ribosomes, or the large ribosomal subunit (50 S or 60 S) and relatively high concentrations of alcohol, peptidyltransferase can be assayed by the reaction between an acylaminoacyl-tRNA or acylaminoacyl-oligonucleotide donor and puromycin (Monro and Marcker, 1967; Monro *et al.*, 1969; Pestka, 1970; Monro, 1971; Lessard and Pestka, 1972; Nierhaus and Montejo, 1973). The binding to ribosomes and activity of aminoacylated oligonucleotides of varying composition and chain length have also been reported (Hussain and Ofengand, 1972).

Previous studies from this laboratory and others described the synthesis of peptide bonds, from 80S ribosome-bound peptidyl-tRNA and exogenous aminoacyl-tRNA or puromycin (Skogerson and Moldave, 1968a, 1968b; Pestka *et al.*, 1972; Pestka, 1972; Schneider and Maxwell, 1973) and from exogenous acetylphenylalanyl-tRNA and puromycin with stripped 80S or 70S ribosomes (Siler and Moldave, 1969b; Pestka, 1970). This communication describes the characteristics of the peptidyltransferase reaction with isolated rat liver ribosomal 60S subunits and the effects of some components of the translational system on this reaction.

Materials and Methods

Rat liver ribosomes were prepared from microsomes, purified by centrifugation through discontinuous sucrose gradients containing 0.5 M NH_4Cl (Skogerson and Moldave, 1967, 1968a), stripped of endogenous peptidyl-tRNA with puromycin (Gasior and Moldave, 1972), and dissociated into subunits with 0.88 M KCl (Martin and Wool, 1968; Gasior and Moldave, 1972). The dissociated subunits were resolved by ultracentrifugation in a linear-with-radius 20–45% sucrose gradient using a Beckman Ti-15 zonal rotor; the solutions used for the gradient zonal centrifugation contained 0.88 M KCl, 0.05 M Tris-HCl (pH 7.6), 12.5 mM $MgCl_2$, 6 mM mercaptoethanol, and 1 μg/ml of poly(vinyl sulfate).

Isotopically labeled *Escherichia coli* phenylalanyl-tRNA was prepared as described (Siler and Moldave, 1969a) and acetylated with acetic anhydride (Haenni and Chapeville, 1966); the specific radioactivity of the acetylphenylalanyl-tRNA preparations used varied between 1500 and 3000 cpm/μg of tRNA, and the specific activity of the tRNA-bound [^{3}H]phenylalanine was 5350 cpm/pmol of phenylalanine. The specific radioactivity of acetyl[^{3}H]lysyl-tRNA, prepared by similar procedures, was 1600 cpm/μg of tRNA and the specific activity of the tRNA-bound [^{3}H]lysine was 5000 cpm/pmol of lysine. Acetyl[^{3}H]methionyl-tRNA was prepared by aminoacylation of rat liver $tRNA_i$ with [^{3}H]methionine and acetylation of the Met-$tRNA_i^{Met}$ resolved on BD-cellulose (Smith and Marcker, 1970); the specific radioactivity was 1400 cpm/μg of tRNA and the tRNA-bound [^{3}H]methionine had a specific activity of 1550 cpm/pmol of amino acid.

Incubations for peptidyltransferase activity were, unless otherwise specified, carried out with 60 or 120 pmol of 60S subunits, acetylphenylalanyl-tRNA containing 25–30 pmol of tritium-labeled phenylalanine, 33% methanol, and 0.8 mM puromycin in buffered salts–dithiothreitol solution (40 mM Tris-HCl (pH 7.5), 4 mM $MgCl_2$, 0.3 M KCl, and 1.4 mM dithiothreitol). Some incubations, with 60 pmol of 60S sub-

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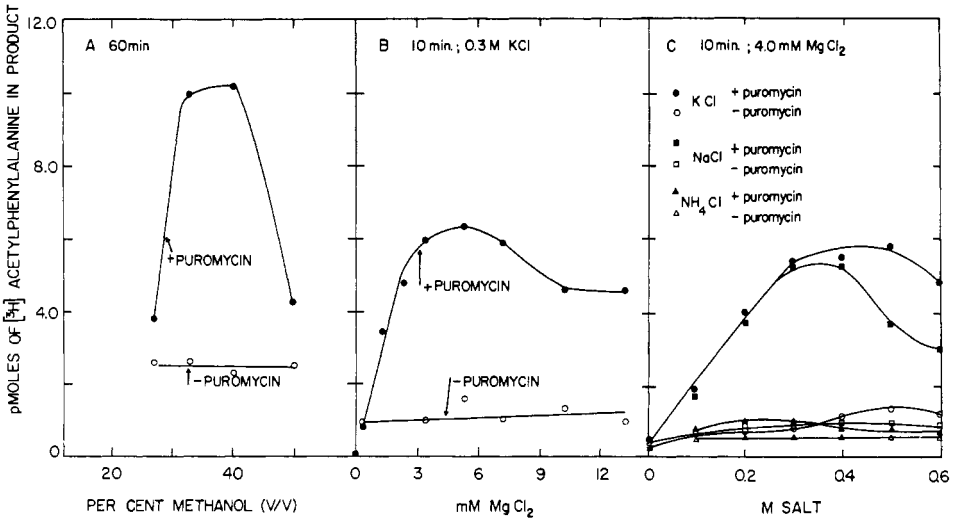

FIGURE 1: (A) The effect of methanol concentration on the peptidyltransferase reaction. Approximately 120 pmol of 60S subunits and 14 pmol of acetyl[^{3}H]phenylalanyl-tRNA were incubated with buffered salts–dithiothreitol and varying concentrations of methanol, for 60 min. Other conditions and analyses as described in the text. (B) Effect of magnesium ion concentration on peptidyltransferase. Approximately 120 pmol of 60S subunits were incubated for 10 min with acetyl[^{3}H]phenylalanyl-tRNA, methanol and buffered salts–dithiothreitol solutions containing varying concentrations of $MgCl_2$, as described in the text. (C) Effect of various salt concentrations on peptidyltransferase. Approximately 120 pmol of 60S subunits were incubated with acetyl[^{3}H]phenylalanyl-tRNA, methanol and buffered salts–dithiothreitol solutions containing varying concentrations of KCl, NaCl, or NH_4Cl, as described in the text.

units, also contained 60 pmol of 40S subunits and/or 5–50 μg of poly(U). Incubations at 20° were for 10 min in a total volume of 0.15 ml. At the end of that period, 0.1 ml of 0.1 M $BeCl_2$ in 0.3 M sodium acetate (pH 5.0) saturated with $MgSO_4$ was added (Maden and Monro, 1968) and the puromycin derivatives were extracted into 1.5 ml of ethyl acetate as described (Leder and Bursztyn, 1966; Maden and Monro, 1968). The amount of radioactive puromycin product formed was determined by counting aliquots of the organic phase from the extraction procedure with a scintillation counter. In all cases, control incubations were carried out in the absence of puromycin or subunits. The results are expressed as pmoles of [^{3}H]amino acid, from acetyl[^{3}H]aminoacyl-tRNA, extracted with ethyl acetate.

In some experiments, the 60S subunits were preincubated for varying periods of time with polynucleotide, 40S subunits, or both, prior to the peptidyltransferase assay with puromycin and methanol. Approximately 60 pmol of 60S subunits, 60 pmol of 40S subunits, and/or 5–50 μg of poly(U) or other polynucleotides were incubated at 37°, in a total volume of 0.05 ml, with the following components: Tris-HCl (pH 7.3), 50 mM; $MgCl_2$, 4 mM; KCl, 50 mM; dithiothreitol, 1 mM. At the end of the preincubation period, acylaminoacyl-tRNA containing 17–18 pmol of tritium-labeled amino acid, methanol, and puromycin were added and the reaction components were adjusted to the concentrations used for the peptidyltransferase assay described above. Polynucleotide or 40S subunits were added to some of the samples that were preincubated without them. The solutions were then incubated at 20° for 10 min, and extractions and radioactivity determinations were performed as described.

Results

When acetyl[^{3}H]phenylalanyl-tRNA was incubated with rat liver 60S subunits, methanol, and puromycin, a significant amount of the labeled phenylalanine was converted to the puromycin derivative which was extracted with ethyl acetate. The formation of acetylphenylalanyl-puromycin was carried out by 60S subunits, did not require the participation of 40S subunits that may have been present to a small extent in the 60S preparation, and was not due to 80S particles formed as the result of contamination with 40S subunits. The 60S preparations used contained less than 5% 40S subunits as revealed by (1) analytical sucrose gradient centrifugation of the particles, (2) gel electrophoresis of the RNA isolated from the 60S preparations, (3) polyphenylalanine synthesis as compared to incubations containing 60S subunits and varying concentrations of 40S subunits (Siler and Moldave, 1969a), and (4) nonenzymatic (high magnesium ion) binding of acetylphenylalanyl-tRNA which occurs with 40S subunits only, as compared to that obtained with varying concentrations of 40S subunits (Gasior and Moldave, 1972). Also, as described below, addition of 40S subunits inhibited the peptidyltransferase reaction.

As shown in Figure 1, optimal requirements for the formation of acetylphenylalanyl-puromycin were about 30–40% methanol (A), 3–7 mM $MgCl_2$ (B), and 0.3–0.6 M KCl (C); NaCl (C, ■) was somewhat less effective than KCl at higher concentrations and NH_4Cl (▲) was inactive. The "control" levels of radioactivity extracted into ethyl acetate in the absence of puromycin (○) were 4–6 times lower than those extracted under optimal conditions in the presence of puromycin (●); varying the concentration of methanol, Mg^{2+}, or monovalent cation over a wide range had little or no effect on these "control" levels of radioactivity. Other studies not shown here indicated that with these components, the optima with respect to puromycin concentration was 0.8×10^{-3} M, and to pH was between 7.5 and 8.25. As shown in Figure 2A the optimal temperature range was between 15 and 30°; at 37°, the peptidyltransferase reaction was not detected. The reaction was linear with time for about 30 min (Figure 2B). In all of the experiments described below, the peptidyltransferase reaction was carried out for 10 min in order to measure initial rates.

The effect of varying concentrations of acetylphenylalanyl-tRNA on the initial velocity of the peptidyltransferase reaction, with two concentrations of 60S subunits, is shown in Figure 3. The initial rate was proportional to the concentration of substrate up to about 25 pmol of acetylphenylalanyl-tRNA per incubation, corresponding to about 1.67×10^{-7} M.

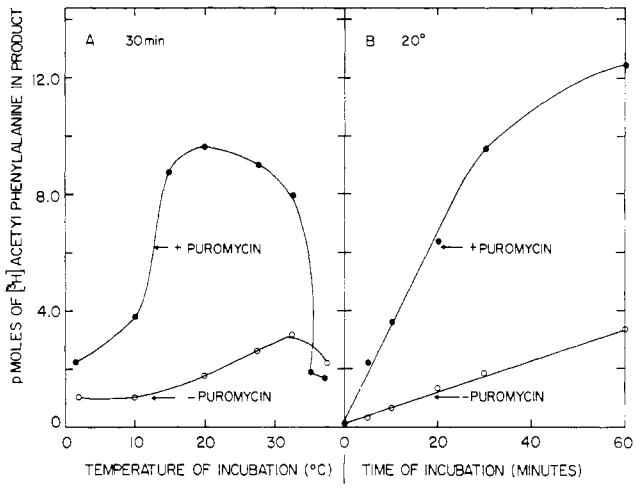

FIGURE 2: (A) The effect of temperature on peptidyltransferase. Approximately 120 pmol of 60S subunits were incubated for 30 min as described in the text, with acetyl[^{3}H]phenylalanyl-tRNA, methanol, and buffered salts–dithiothreitol, at various temperatures from 2 to 37°. (B) Time-dependent formation of acetylphenylalanyl-puromycin. Approximately 120 pmol of 60S subunits were incubated as described in Figure 2A and in the text, at 20°, for varying periods of time as indicated.

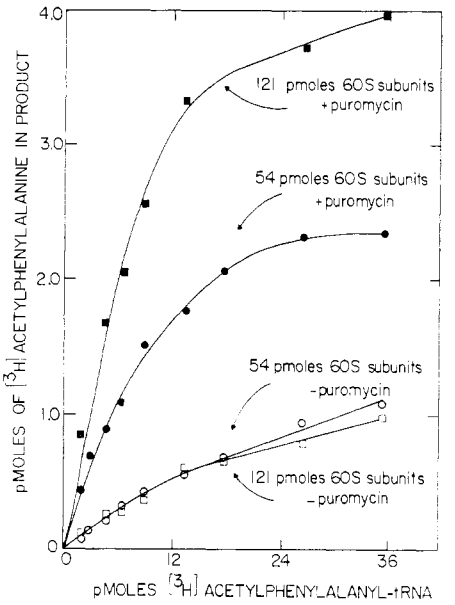

FIGURE 3: The effect of acetylphenylalanyl-tRNA and 60S subunit concentrations on peptidyltransferase. Incubations containing 121 or 54 pmol of 60S subunits were incubated as described in the text with methanol, buffered salts–dithiothreitol, with and without puromycin, and varying concentrations of acetyl[^{3}H]phenylalanyl-tRNA.

Double reciprocal plots of initial velocity *vs.* substrate concentration revealed a linear function and the apparent Michaelis constant for acetylphenylalanyl-tRNA was estimated to be about 5.2×10^{-8} M. The initial rate obtained with 121 pmol of 60S subunits (■) was two times greater than that with 54 pmol. Similar incubations with varying concentrations of 60S subunits indicated that the initial rate was directly proportional to the concentration of particles up to more than 200 pmol of 60S subunits in the incubation mixture.

Peptide bond formation with 80S ribosomes containing endogenous peptidyl-tRNA on the P site and mRNA required only the presence of relatively high concentrations of monovalent cations when aminoacyl-tRNA or puromycin was placed on the A site (Skogerson and Moldave, 1968a,b). The reaction with the isolated large ribosomal subunit (60 S or 50 S) required significant amounts of alcohol as well (Monro *et al.*, 1969; Vazquez *et al.*, 1969; Falvey and Staehelin, 1970). An explanation for this observation was that alcohol may effect a conformational change in the large subunit, similar to that obtained when it is complexed to the small subunit and mRNA (Monro *et al.*, 1969). In order to compare directly the reaction catalyzed on 60S particles with that on 80S ribosomes or 80S–mRNA complexes, experiments were carried out with combinations of poly(U), 40S and 60S subunits. Table I shows the rather unexpected finding that poly(U) stimulated significantly the transpeptidation reaction with 60S subunits and acetylphenylalanyl-tRNA. The addition of 40S subunits to the incubation containing 60S particles, acetylphenylalanyl-tRNA, methanol, and puromycin inhibited the reaction slightly, and the addition of poly(U) had no effect on the 40S induced inhibition.

In order to facilitate the formation of an 80S ribosome or an 80S–template complex prior to assaying for peptide bond formation, the 60S subunits were preincubated for a short period of time with poly(U), 40S subunits, or both before the addition of acetylphenylalanyl-tRNA and puromycin (Table II). The results indicated that poly(U) stimulated the reaction, but the presence of 40S subunits in the preincubation markedly inhibited acetylphenylalanyl-puromycin synthesis. The amount of product obtained in the ethyl acetate extract in the presence of 40S subunits was only 15% of that obtained in the absence of 40S particles. The addition of poly(U) to the 40S containing preincubation stimulated the reaction 2–3-fold as compared to the inhibited levels obtained by adding 40S subunits only, but these values were considerably lower than those obtained in control incubations without poly(U) or 40S subunits. It should be noted that in all these experiments methanol was present during the reaction with puromycin; in the absence of alcohol, peptidyltransferase was not detected even in the presence of poly(U), 40S subunits, or both. Although not shown here, inhibition of the transpeptidation reaction by 40S subunits was also observed when the preincubation phase of the reaction was carried out at 4° (as compared to 37°) or with relatively high concentrations of KCl (0.3 M as compared to 50 mM); however, the inhibition was

TABLE I: The Effect of Poly(U) and 40S Subunits on the Peptidyltransferase Reaction with Rat Liver 60S Subunits.[a]

Incubation Additions	pmol of Ac-[^{3}H]Phe-puromycin Formed
None	1.84
Poly(U)	2.55
40S subunits	1.28
Poly(U) and 40S subunits	1.25

[a] Approximately 60 pmol of 60S subunits were incubated with acetyl[^{3}H]phenylalanyl-tRNA and methanol, with and without puromycin; some incubations also received 5 μg of poly(U), 60 pmol of 40S subunits, or both. Other incubation conditions and analyses as described in the text. The results are calculated from the amount of radioactive phenylalanine extracted into ethyl acetate from incubations in the presence of puromycin, after subtraction of the values obtained from similar incubations in the absence of puromycin.

TABLE II: The Effect of Preincubation with Poly(U) and 40S Subunits on the Peptidyltransferase Reaction with Rat Liver 60S Subunits.[a]

First Incubation Additions	Second Incubation: pmol of Ac-[^{3}H]Phe-puromycin Formed
None	1.17
Poly(U)	2.51
40S subunits	0.18
Poly(U) and 40S subunits	0.52

[a] Ribosomal 60S subunits were preincubated as such, with 5 μg of poly(U), 40S subunits, or both poly(U) and 40S subunits as described in the text, and assayed for peptidyltransferase with acetyl[^{3}H]phenylalanyl-tRNA and methanol, plus and minus puromycin. The results represent the amount of radioactive phenylalanine extracted, after subtraction of the values obtained from similar incubations in the absence of puromycin.

markedly diminished when 33% methanol was present. Further, gradient centrifugation of mixtures containing 60S plus 40S subunits and 50 mM KCl revealed the formation of significant amounts of 80S particles.

The template specificity for the polynucleotide-stimulated phase of the reaction was examined by carrying out the preincubation of 60S subunits with poly(U), poly(A), and poly(C). The results summarized in Table III indicated that only poly(U) enhanced the reaction between acetylphenylalanyl-tRNA and puromycin, and that the effect was considerably greater with 50 μg of the polynucleotide as compared to 5 μg; the other polynucleotides inhibited the reaction slightly. The polynucleotide effect on peptidyltransferase was also examined with other acetylated aminoacyl-tRNAs (Table IV). Polyuridylate, which stimulated the reaction with acetylphenylalanyl-tRNA, markedly inhibited the conversion of acetylated lysyl-tRNA and methionyl-tRNA to the corresponding puromycin derivatives. Polyadenylate stimulated the formation of acetyllysyl-puromycin and inhibited slightly the formation of acetylphenylalanyl-puromycin, but did not appear to affect the reaction with acetylmethionyl-tRNA. Results with other polynucleotides such as copoly(G,U,A), ApUpG(pU)$_{40-50}$, or ApUpG were inconclusive; they either inhibited slightly or had no effect on the various peptidyltransferase assays.

TABLE III: The Effect of Preincubation with Various Polynucleotides on the Peptidyltransferase Reaction with Rat Liver 60S Subunits.[a]

Polynucleotide	pmol of Ac[^{3}H]-Phe-puromycin Formed
None	1.15
Poly(U), 5 μg	1.72
Poly(U), 50 μg	4.07
Poly(A), 5 μg	0.76
Poly(A), 50 μg	1.06
Poly(C), 5 μg	0.69
Poly(C), 50 μg	0.82

[a] Ribosomal 60S subunits were preincubated for 2 min as described in the text, with 5 or 50 μg as noted of poly(U), poly(A), or poly(C), then assayed for peptidyltransferase with acetyl[^{3}H]phenylalanyl-tRNA and methanol, plus and minus puromycin. The results represent the amount of radioactive phenylalanine extracted, after subtraction of the values obtained from similar incubations in the absence of puromycin.

TABLE IV: The Effect of Preincubation with Different Polynucleotides on the Peptidyltransferase Reaction with Rat Liver 60S Subunits and Various Acetylated Aminoacyl-tRNAs.[a]

Polynucleotide Added	pmol of Acyl[^{3}H]aminoacyl-puromycin Formed: Acetyl-phenyl-tRNA	Acetyllysyl-tRNA	Acetyl-methionyl-tRNA$_i$
None	1.20	0.23	5.70
Poly(U)	3.44	0.11	2.92
Poly(A)	1.02	0.36	5.60
Poly r(G,U,A)	0.75	0.22	3.20

[a] Ribosomal 60S subunits were preincubated as such, or with 50 μg of poly(U), poly(A), or poly(U,G,A) as described in the text; the reaction mixtures were then assayed for peptidyltransferase with methanol, acetylated [^{3}H]phenylalanyl-tRNA, [^{3}H]lysyl-tRNA, or [^{3}H]methionyl-tRNA$_i$, plus and minus puromycin. The results represent the amount of radioactive amino acid extracted, after subtraction of the values obtained from similar incubations in the absence of puromycin.

The effect of varying concentrations of poly(U) during the preincubation part of the reaction, on the subsequent peptidyltransferase activity, is demonstrated in Figure 4. The activity of 60S particles was stimulated 80–100% when 10–40 μg of poly(U) was added directly to the peptidyltransferase reaction (■). Preincubation for 2 min with increasing concentrations of poly(U) stimulated markedly the activity of the 60S particles (●); the stimulation was dependent on the concentration of poly(U) and 30 μg of polyuridylate resulted in an increase in activity of about 250%. Varying the time of preincubation of 60S subunits with poly(U), before the peptidyltransferase assay, indicated that maximal stimulation was obtained within 2–3 min.

The effect of varying concentrations of 40S subunits during the preincubation part of the reaction, on the subsequent peptidyltransferase activity, is shown in Figure 5. The inhibition of peptidyltransferase was proportional to the concentration of 40S subunits in the first phase of the two-part incubation. With 60 pmol of 60S subunits, 50% inhibition was obtained with about 20 pmol of 40S subunits. The time required to exhibit the maximum inhibitory effect, at any given concentration of 40S subunits, at 37°, was less than 30 sec. When 40S subunits were present during the second phase of the reaction only, with methanol, acetyl[^{3}H]phenylalanyl-tRNA, and puromycin, the inhibition observed was less pronounced. Under these conditions, addition of 60 pmol of 40S subunits inhibited the reaction only 20–25%.

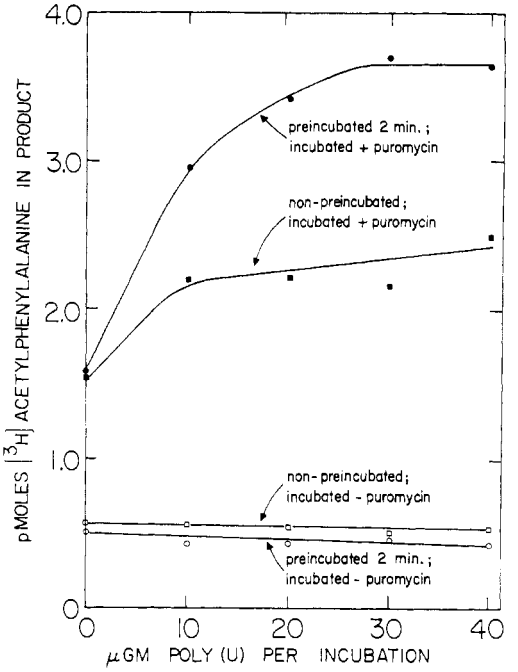

FIGURE 4: The effect of poly(U) concentration on peptidyltransferase. Ribosomal 60S subunits were preincubated at 37° for 2 min as such (squares) or with varying concentrations of poly(uridylic acid) (circles), as described in the text. At the end of the first incubation period, the samples that did not have poly(uridylic acid) received varying concentrations of poly(U) as noted (squares), and the reaction mixtures were assayed for peptidyltransferase with (closed symbols) and without (open symbols) puromycin.

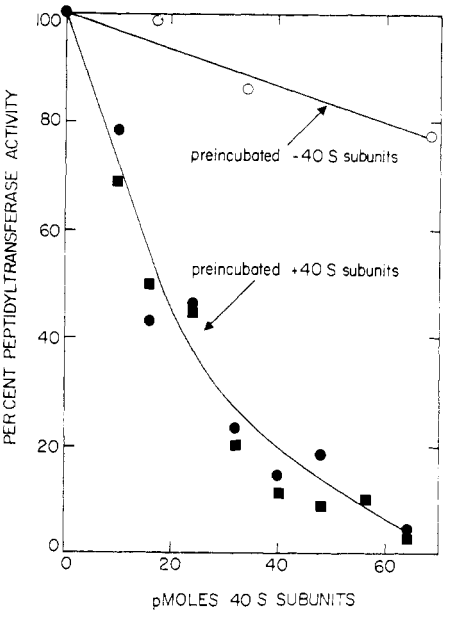

FIGURE 5: The effect of 40S subunit concentration on peptidyltransferase. Ribosomal 60S subunits were preincubated at 37° for 2 min as such or with varying concentrations of 40S subunits as described in the text. At the end of the preincubation period, varying concentrations of 40S subunits as noted were added to the samples that did not contain 40S subunits, and the incubations were then assayed for peptidyltransferase activity. The results are expressed as per cent activity, compared to control incubations without 40S subunits. The closed circles and squares represent two individual experiments, carried out independently.

Discussion

In protein synthesis, peptidyltransferase catalyzes the formation of peptide bonds on 80S ribosomes in the process of chain elongation. Nascent peptidyl-tRNA on the P site of ribosomes reacts with a new incoming aminoacyl-tRNA, placed at the A site in a codon-specific interaction requiring elongation factor EF-1 and GTP. The peptide chain is transferred to the amino group of the aminoacyl-tRNA, making a new peptidyl-tRNA, one amino acid longer, at the A site. Before another peptide bond can be formed, at least two additional steps must occur; one is the translocation of the newly synthesized peptidyl-tRNA and the corresponding codon triplet from the A to the P site, which requires elongation factor EF-2 and GTP; the other step is the decoding of the next triplet at the A site through the interaction of the corresponding aminoacyl-tRNA, which requires EF-1 and GTP.

In the series of reactions described above, repeated in the process of chain elongation as the internal codons in messenger RNA are translated, one or both of the two ribosomal tRNA-binding sites is always occupied. In the post-translocated ribosome, the P site is occupied by peptidyl-tRNA and the A site is unoccupied. After the addition of aminoacyl-tRNA, the P site has peptidyl-tRNA and the A site has aminoacyl-tRNA. After transpeptidation, the A site is occupied by peptidyl-tRNA and the P site is occupied by stripped tRNA. In the peptidyltransferase assay described here, the isolated 60S subunit, free of the small ribosomal subunit and mRNA, does not have its tRNA-binding "sites" occupied with endogenous intermediates. This *in vitro* system thus requires an additional step, not normally a part of the chain elongation process. This step is the binding of an analog peptidyl-tRNA (such as acetylated aminoacyl-tRNA) to the P site which is usually filled by translocation. Also, although puromycin is presumed to react in the peptidyltransferase reaction by accepting the peptide chain (or an acylaminoacyl moiety) at the A site, it does not participate in all of the interactions required for the binding of aminoacyl-tRNA to the A site.

A number of similarities are apparent between the reactions catalyzed by an 80S ribosome containing endogenous mRNA and peptidyl-tRNA, and those catalyzed by an isolated 60S particle with exogenous acylaminoacyl-tRNA. For example, the requirement for monovalent cation is extremely high (at least 0.3 M) in both systems. Some marked differences, however, are also apparent. Ammonium is an effective monovalent cation in promoting peptide bond formation with 80S ribosomes–mRNA and endogenous peptidyl-tRNA (Skogerson and Moldave, 1968a, 1968b) or with 80 S–poly(U) and acetylphenylalanyl-tRNA (Siler and Moldave, 1969b). The isolated 60S subunit–exogenous acylaminoacyl-tRNA system described here is active in the presence of potassium ions and to some extent with sodium ions, but not with ammonium ions. The isolated 50S subunit–exogenous aminoacyl-oligonucleotide system described by Maden and Monro (1968) also revealed that ammonium ions were less effective than potassium ions in promoting peptide bond formation. Also, in contrast to the 80S ribosome catalyzed reaction, the isolated 60S subunit reaction requires alcohol.

The ability of the small ribosomal subunit to carry out the binding of specific aminoacyl-tRNA in the presence of the appropriate codon (Kaji *et al.*, 1966; Matthaei *et al.*, 1966; Pestka and Nirenberg, 1966) has suggested that codon–anticodon recognition in the translation of mRNA is primarily a property of the small subunit. The observations presented here, that poly(U) stimulates the peptidyltransferase reaction with 60S subunits and acetylphenylalanyl-tRNA, is therefore not easily interpretable in terms of the model proposed for the decoding of mRNA. The findings that some polynucleotides appear to stimulate the reaction of tRNA derivatives

with a complimentary anticodon triplet, and that some polynucleotides inhibit transpeptidation with other acetylated aminoacyl-tRNAs, could suggest that this effect is template specific. Jonak and Rychlik (1970) reported the poly(A) specific enhancement of oligolysyl-tRNA binding to the 50S subunit of *E. coli* ribosomes. These observations suggest that to some extent, the large ribosomal subunit is also capable of codon–anticodon recognition, although it does not necessarily imply a physiological role for free 60S subunits within the cell. The results on the effect of polynucleotides described here do not appear to be due to the formation of an 80S–mRNA complex with preformed 80S ribosomes, which then carries out peptidyltransferase with acylaminoacyl-tRNA and puromycin as described previously (Siler and Moldave, 1969b). The 60S subunit preparations do not contain significant amounts of 40S subunits, and the addition of 40S particles inhibits the peptidyltransferase reaction markedly. When both 40S subunits and polynucleotides are added to the reaction with 60S subunits, the values obtained are 50% lower than those obtained in their absence. Also, the formation of acetylphenylalanyl-puromycin with 60S subunits in the presence of 40S particles and poly(U) requires alcohol, whereas the equivalent reaction with 80S ribosomes–poly(U) and acetylphenylalanyl-tRNA does not (Siler and Moldave, 1969b); indeed, 33% alcohol inhibits the reaction between 80S bound acylaminoacyl-tRNA and puromycin.

The polynucleotide-dependent increase in the initial rate of the peptidyltransferase reaction could be due to an enhanced 60S subunit–substrate interaction, or to a direct effect on the peptidyltransferase "active center;" thus poly(uridylic acid) could exert its effect by increasing the amount of acetylphenylalanyl-tRNA bound to the P site of the 60S subunit, or the stabilization or proper alignment of the bound substrate. Experiments in progress, to be described subsequently, are designed to examine the effect of polynucleotide on binding and on the subsequent conversion of the bound acylaminoacyl-tRNA to the puromycin derivative.

A possible explanation of the results obtained with 40S subunits is that two types of aggregates may be formed when 60S and 40S subunits are allowed to interact in the absence of methanol. (1) A functional 80S ribosome can be formed; 80S particles are rapidly and spontaneously formed, even at 4°, and polyphenylalanine is synthesized when poly(U), phenylalanyl-tRNA, and 40S plus 60S subunits are incubated with the appropriate factors and cofactors. However, at low magnesium ion concentrations (4 mM) in the absence of methanol, 80S–poly(U) complex does not bind acetylphenylalanyl-tRNA (Siler and Moldave, 1969b) and in high magnesium chloride containing solutions (20 mM), acetylphenylalanyl-tRNA is bound to the A site only and is unreactive toward puromycin. Thus, any 80S–poly(U) formed would not be expected to show peptidyltransferase activity under the assay conditions used above. (2) The inhibitory effect of 40S subunits could also be due to their interaction with the 60S particles at other positions, in such a way as to interfere with the appropriate binding of acetylphenylalanyl-tRNA; this would result in the formation of an inactive aggregate or one with reduced peptidyltransferase activity. Experiments designed to examine the manner in which 40S subunits exert their effect on the transpeptidation reaction are also in progress.

Acknowledgments

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