MICHAŁ M. SIKORSKI, MARINA K. KUKHANNOVA*, ALEXANDER A. KRAYEVSKY* and ANDRZEJ B. LEGOCKI

PEPTIDYLTRANSFERASE ACTIVITY OF 60S RIBOSOMAL SUBUNIT FROM WHEAT GERM**

Institute of Biochemistry, University of Agriculture, Włoska 35, 60-637 Poznań, Poland and *Institute of Molecular Biology, USSR Academy of Sciences, Vavilov 32, Moscow-334, 117894, USSR

Formation of peptide bond between various donor and acceptor substrates was studied in the presence of wheat germ ribosomes. Formation of acetylaminoacyl-puromycin from Ac-Pae-tRNA or Ac-Leu-pentanucleotide catalysed by 60S ribosomal subunits was similar to that observed for 80S ribosomes from the same source (Sikorski et al., 1977). Wheat germ ribosomes, similarly as E. coli and mammalian ribosomes, effectively catalysed a transpeptidation reaction between acetylaminoacyl-pentanucleotide (instead of puromycin) and minimal donor pA-(fMet). However, in contrast to E. coli ribosomes, they were unable to catalyse peptide bond formation using pdCpdCpra-(fMet) derivative as a peptide donor.

Peptidyltransferase catalyses synthesis of the peptide bond between the terminal carboxyl group of the peptidyl residue of peptidyl-tRNA and α-amino group of aminoacyl-tRNA on adjacent ribosomal sites (for review see Krayevsky & Kukhanova, 1979). Enzymatic activity of peptidyltransferase in transfer reaction can be assayed in the reaction between an acetylaminoacyl-tRNA or acetylaminoacyl-oligonucleotide (“fragment reaction”) as a donor and puromycin as an acceptor substrate (Monro et al., 1968; Rychlik et al., 1970; Lessard & Pestka, 1972). Another example of transpeptidation reaction is provided by a model in which aminoacyl-oligonucleotide instead of puromycin serves as an acceptor for a “minimal” peptide donor pA-(fMet)¹ (Černá et al., 1973). Since this reaction has been described for

¹ Abbreviations used: pA-(fMet), 3′(2′)-O-(N-formyl-L-methionyl)-adenosine 5′-phosphate; pC, cytidine 5′-phosphate; pC(3′NH), 3′-amino-3′-deoxycytidine 5′-phosphate; pdCpdCpra-(fMet), 5′-phosphodeoxyctydidiyl-(3′→5′)-deoxycytididiyl-(3′→5′)-[3′(2′)-O-(N-formyl-L-methionyl)]-adenosine; CACCA-(Ac-[14C]Leu), UACCA-(Ac-[14C]Ieul) and CACCA-[14C]Phe are the 3′-fragments of Ac-[14C]Leu-tRNA and [14C]Pae-tRNA, correspondingly.

** This investigation was supported by the Ministry of Science, Higher Education and Technology, Project RII 4.
E. coli and rat liver ribosomes (Černá, 1975; Kukhanova et al., 1979) it was interesting to apply the minimal substrate to wheat germ ribosomes. Peptide bond formation was described in this system using undissociated ribosomes (Gattica & Allende, 1971; Sikorski et al., 1977). This paper presents characteristics of transpeptidation reaction with isolated 60S ribosomal subunits and describes the activity of minimal donor pA-(fMet) in wheat germ system.

MATERIALS AND METHODS

Reagents. Puromycin dihydrochloride was obtained from Nutritional Biochemicals Co., [14C]leucine (125 Ci/mol) was from the Institute for Research, Production and Application of Radioisotopes (Prague, Czechoslovakia), and [14C]phenylalanine (522 Ci/mol) from Amersham Scarle, England. Ribonuclease T1 was obtained from Calbiochem (San Diego, Ca., U.S.A.), tRNA from E. coli was a product of Serva (Heidelberg, F.R.G.), tRNA from wheat germ was prepared as described by Legocki et al. (1968) and purified on BD-cellulose column according to Dudock et al., (1969). N-Acetyl-[14C]aminoacyl-tRNA was prepared according to Haenni & Chapeville (1966). 80S ribosomes were prepared from wheat germ (General Mills, Vallejo, Ca., U.S.A.) by the procedure described by Golinski & Legocki (1973). Wheat ribosomal subunits were obtained according to Sikorski et al. (1979); they were fully active after recombination in polyphenylalanine synthesis. E. coli 70S ribosomes were prepared according to Lessard & Pestka (1972).

Preparation of donor and acceptor substrates. UACCA-(Ac-[14C]Leu) and CACCA[14C]Phe were prepared from Ac-[14C]Leu-tRNA or [14C]Phe-tRNA, respectively, by digestion with RNAase T1. Usually, 20 mg of aminoacyl-tRNA was digested with 100 μg of RNAase in 1.5% sodium acetate, pH 5.4, containing 0.8 mM-EDTA for 60 min at 37°C. Terminal fragments were isolated by high voltage electrophoresis on Whatman 3MM paper in buffer composed of 0.5% pyridine and 5% acetic acid, pH 3.5, for 5 h at 50V/cm according to Monro et al. (1968). pA-(fMet) was synthesized by the imidazole method according to Azhayev et al. (1977), while pdCpdCprA-(fMet) and pC(3’NH2) were prepared as described by Azhayev et al. (1977, 1979), respectively.

Puromycin reaction with N-Ac[14C]Leu-pentanucleotide. Standard incubation mixture contained in a total volume of 100 μl: 50 mM-Tris/HCl buffer, pH 7.6, 15 mM-magnesium acetate, 350 mM-KCl, 1 mM-puromycin, CACCA-(Ac-[14C]Leu), (1000 - 2000 cpm), 1 A260 unit of 60S ribosomal subunits. Reaction was started by the addition of 50 μl methanol and incubation was carried out for 2 h at 0°C. The reaction was stopped by the addition of 100 μl of 0.1M-sodium acetate, pH 5.5, in a saturated solution of MgSO4 and the reaction products were extracted with 1.5 ml ethyl acetate (Černá et al., 1970). Organic phase (1 ml) was mixed with 5 ml dioxan scintillation fluid and the radioactivity was measured in a Packard scintillation spectrometer. The amount of Ac[14C]Leu residues transferred to the puromycin was estimated as the difference between the radioactivity extracted into the organic phase after incubation with and without puromycin.
Puromycin reaction with N-Ac\textsuperscript{[14]C}Phe-tRNA. Standard incubation mixture contained in a total volume of 100 µl: 50 mM-Tris/HCl buffer, pH 7.6, 15-20 mM-magnesium acetate, 150 mM-KCl, 10 µg of poly(U), 1 mM-puromycin, 20-30 pmol of N-Ac\textsuperscript{[14]C}Phe-tRNA (10000 - 15000 cpm), 2.4 A\textsubscript{260} units of 60S ribosomal subunit. The incubation was carried out for 2 h at 45°C. Reaction products were extracted and measured as described above.

Transfer reaction between the minimal donor pA-(fMet) and CACCA-[\textsuperscript{14}C]Phe as an acceptor. Reaction mixture contained in a total volume of 100 µl: 50 mM-Tris/acetate buffer, pH 7.6, 15 mM-magnesium acetate, 350 mM-KCl, 5 A\textsubscript{260} units of ribosomes and 30 pmol of CACCA-[\textsuperscript{14}C]Phe (30000 cpm) and 2 mM-pA-(fMet). The reaction was initiated by the addition of an equal volume of methanol and the incubation was carried out at 30°C for 90 min. The reaction was terminated by addition of 50 µl of 3 M-NaOH. After incubation for another 40 min at 37°C to hydrolyse ester bonds, 200 µl 2.5 M-H\textsubscript{2}SO\textsubscript{4} was added and the reaction products were extracted with 3 ml of ethyl acetate. The organic layer was washed with 0.5 ml 1% H\textsubscript{2}SO\textsubscript{4} and 0.5 ml H\textsubscript{2}O, then was dried with anhydrous Na\textsubscript{2}SO\textsubscript{4}. The radioactivity was determined in 1.5 ml aliquots and 15 ml of dioxan scintillation fluid. The reaction was carried out in the presence or absence of 3 mM-pC.

RESULTS AND DISCUSSION

As it was shown earlier, in eukaryotes, similarly as in bacterial systems, puromycin reaction does not require the participation of 40S ribosomal subunits when the incubation is carried out in the presence of methanol (Monro et al., 1968; Thompson & Moldave, 1974). Figure 1 shows time kinetics of the reaction catalysed by 60S subunits from wheat germ with fragment (Ac-Leu-pentanucleotide) and complete (Ac-Phe-tRNA) donor substrates. With the fragment donor the reaction is completed after 2 h of incubation while with the complete tRNA derivative trans-
peptidation proceeds linearly for 3 h, similarly as with 80S ribosomes (Sikorski et al., 1977). The control level of radioactivity extracted into organic solvent observed in the absence of puromycin was 4 - 6 times lower than in the presence of puromycin. It is worth noting that the reaction with the complete donor is very efficient

![Graph](Image)

Fig. 2. Formation of Ac-Leu-puromycin (○) and Ac-Phe-puromycin (□) catalysed by 60S ribosomal subunits from wheat germ. A, Effect of donor substrates concentration. B, Effect of magnesium acetate concentration. C, Effect of 60S subunit concentration. For experimental details see Materials and Methods. Controls represent values obtained in the absence of puromycin (●, ■). Standard incubation mixture contained 20 pmol of Ac[14C]Phe-tRNA or 4 pmol of CACCA-(Ac-[14C]Leu).

at the relatively high temperature of 45°C, although at 50°C the system becomes inactivated. As shown in Fig. 2, optimal conditions for the reaction catalysed by 60S subunits are similar for both donor substrates, except that for a complete donor the optimum magnesium acetate concentration was higher (Fig. 2B). The initial rate of acetylleucyl-puromycin formation was proportional to the substrate concentration up to 25 pmol of acetylleucyl-pentanucleotide or 40 pmol of Ac-Phe-tRNA per sample (i.e 1 A260 units of 60S subunit), (Fig. 2A). The addition of 40S subunits before or after the reaction inhibited in about 30% or 40%, respectively, the formation of puromycin derivative (data not shown) which was in agreement with data reported for liver peptidyltransferase (Thompson & Moldave, 1974).

Peptide bond formation can also be studied in a system in which aminoacyl-pentanucleotide instead of puromycin acts as an acceptor and pA-(fMet) as a minimal donor (Černá et al., 1973; Černá, 1975; Kukhanova et al., 1979). We found that wheat germ ribosomes similarly as E. coli and rat liver ribosomes effectively catalyse a transpeptidation reaction between these substrates. Figure 3A presents a comparison of time kinetics of minimal donor reaction catalysed by wheat germ and E. coli ribosomes. The amount of the reaction product formed was in the case of wheat germ ribosomes much lower (2 times for 60S and 3 times for 80S) than in the case of 70S ribosomes. Cytidine 5’-phosphate stimulated very effectively the reaction (Fig. 3B). At 3 mM concentration pC stimulated about 4 times the peptidyltransferase from wheat and about 5 times that from E. coli ribosomes. We have also
measured the effect of pC and its aminoderivative pC(3'NH₂) on the catalytic activity of 60S and 80S ribosomes. As shown in Fig. 4, the latter stimulated much more effectively the minimal donor reaction, which confirms earlier findings with bacterial ribosomes (Krayevsky & Kukhanova, 1979). A possible mechanism of pC and its amino-derivatives stimulatory activities on peptidyltransferase may involve its allosteric binding to the enzyme center (Krayevsky et al., 1976).

Fig. 3. Reaction between minimal donor pA-(fMet) and CACCA-[14C]Phe as an acceptor. A. Time-course of the reaction catalysed by 80S (○) and 60S (●) ribosomes from wheat germ, and 70S ribosomes from E. coli (△) in the presence of 2 mM pA-(fMet). B. Effect of donor concentration on transfer reaction catalysed by 60S ribosomal subunits from wheat germ (○, ●) and 70S ribosomes from E. coli (△, △) in the absence (○, △) and presence (○, △) of 3 mM-pC. For details see Materials and Methods.

Fig. 4. Effect of pC and pC(3'NH₂) on “fragment reaction” catalysed by wheat germ ribosomes. Reaction was carried out in the presence of 60S ribosomal subunits (○, ●) or 80S ribosomes (△, △) and varying amounts of pC (○, △) or pC(3'NH₂), (●, △). For details see Materials and Methods.
A striking difference in the reaction of the pA-(fMet) fragment between plant and bacterial ribosomes was observed when ester pdCpdCprA-(fMet) was used as a donor substrate. Figure 5 shows that neither 80S nor 60S ribosomes were active while 70S ribosomes effectively catalysed transpeptidation between these substrates.

![Graph showing the reaction between pdCpdCprA-(fMet) donor and CACCA-[¹⁴C]Phe acceptor.](image)

**Fig. 5.** Reaction between pdCpdCprA-(fMet) donor and CACCA-[¹⁴C]Phe acceptor. Effect of donor concentration on fMet-Phe product formation in the presence of 60S (○) and 80S (●) ribosomes from wheat germ, and 70S ribosomes from *E. coli* (△). For details see Materials and Methods.

However, as seen from the data presented above, a number of similarities are apparent between the transpeptidation reaction catalysed by wheat germ ribosomes and *E. coli* or rat liver ribosomes. This provides a rationale for functional and perhaps structural similarity of peptidyltransferase center in both types of ribosomes.

**REFERENCES**


**AKTYWNOŚĆ TRANSFERASY PEPTIDYLowej PODJEDNOSTEK RYBOSOMOWYCH 60S Z ZARODKÓW PSZENICY**

**Streszczenie**

Badano tworzenie się wiązania peptydowego między różnymi modelowymi substratami donorowymi i akceptorowymi w obecności rybosomów zarodków pszenicy. Synteza acetyloaminoacyl-puromycin z Ac-tRNA lub Ac-Leu-pentanukleotyd katalizowana przez podjednostki rybosomowe 60S była podobna jak w przypadku rybosomów 80S tego samego pochodzenia (Sikorski et al., 1977). Rybosomy z zarodków pszenicy, podobnie jak rybosomy z *E. coli* i ssaków efektywnie katalizowały reakcję transsptrydacji pomiędzy acetyloaminoacyl-pentanukleotydem i puromycin z minimalnym donorem P-A-(fMet). Jednakże, w przeciwieństwie do *E. coli*, rybosomy z zarodków pszenicy nie wykazywały zdolności katalizowania syntezy wiązania peptydowego przy użyciu pdCpdCp-A-(fMet) jako donoru peptydowego.

Received 15 May, 1981