PEPTIDE BOND SYNTHESIS IN THE FRAGMENT REACTION CATALYZED BY MAMMALIAN LIVER RIBOSOMES*

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SUMMARY. Canine and rat liver 70S ribosomes catalyze transfer of N-formylmethionine from N-formylmethionyl-tRNA of E. coli to peptide linkage with the amino group of puromycin in the ethanol stimulated fragment reaction. The peptide bond forming step of mammalian as well as bacterial ribosomes may thus be studied by the fragment reaction. Up to four fold activation of ribosomal fragment reaction activity was found with some ribosomal preparations by preincubation at 40°, suggesting conformational rearrangement to more active configuration.

Peptide bond formation in protein synthesis occurs by transfer of the acyl group of polypeptidyl-tRNA from ester linkage with the terminal adenosine of tRNA to peptide linkage with the alpha amino group of an incoming molecule of aminoacyl-tRNA (Bretscher, 1965). The amino group of puromycin will substitute for the alpha amino group of aminoacyl-tRNA in ribosome catalyzed peptide bond formation (Zamir, et al., 1966). The apparently enzymatic activity for the acyl transfer from ester to amide linkage resides on the 50S ribosomal subunit (Monro, 1967) and has been termed peptidyl transferase (Maden, et al., 1968).

Peptide bond formation catalyzed by E. coli 70S and 50S ribosomes has been studied free of requirement for supernatant factors and GTP in the presence of ethanol, puromycin and

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monovalent and divalent cations (fragment reaction) (Monro and Marker, 1967; Monro, 1967). The acyl donor in this system may be a number of N-substituted aminoacyl-tRNA's or any such molecule retaining at least the 3'terminal CpCpA portion of the tRNA (Monro, et al., 1968). Ethanol as well as other organic oxygen containing compounds are not absolute requirements for the fragment reaction with 70S E. coli ribosomes, but greatly accelerate the rate at which it occurs in purely aqueous environment (Silverstein, 1969). NH_4^+ (Miskin, et al., 1968; Silverstein, 1969) or other monovalent cation (K^+ or Cs^+) (Miskin, et al., 1968; Zamir, et al., 1969) is required for fragment reaction activity, while elevated concentrations of NH_4^+ moderately activate the reaction (Maden and Monro, 1968; Silverstein, 1969).

Since the fragment reaction had been demonstrated only with 70S bacterial ribosomes, it was of interest to see whether the peptide bond forming step in protein synthesis of higher organisms may be similarly studied. This report shows that canine and rat liver ribosomes are active in the fragment reaction.

Salt-washed ribosomes were prepared from canine and rat liver (Moldave and Skogerson, 1967) and E. coli MRE 600 (Nishizuka and Lipmann, 1966; Cammack and Wade, 1965). Transfer RNA was obtained from E. coli MRE 600 (Zubay, 1966) and charged with N-formylmethionine (Vogel, et al., 1968) using either ^{14}C-L-methionine (233 mc/mmol) or ^{35}S-L-methionine (1.2 C/mmol). N-formylmethionyl-tRNA hexanucleotide fragment, CAACCA-met-F, was prepared with T_1 ribonuclease (Monro and Marcker, 1967). The fragment reaction was assayed as previously described (Silverstein, 1969).

The ability of mammalian liver ribosomes to catalyze peptide bond formation by transfer of the N-formylmethionyl

TABLE 1

FORMATION OF N'-FORMYL-METHIONYL-PUROMYCIN IN THE FRAGMENT REACTION CATALYZED BY MAMMALIAN AND BACTERIAL RIBOSOMES

RIBOSOMES		N'-formylmethionyl-puromycin formed,
Source	volume used, µl	counts/minute
canine liver	2	104
	5	262
	10	538
rat liver	2	63
	5	169
	10	342
E. coli MRE 600	2	210
	5	526
	10	1034

The reaction mixtures contained 38 mM Tris-Cl, pH 8.2, 238 mM KCl, 119 mM Mg acetate, 480 µM puromycin, 4,500 counts/minute ^{35}S-N-formyl-methionyl-tRNA, 38% ethanol and 0.61 (E. coli MRE 600), 0.43 (canine liver) and 0.31 (rat liver) mg/ml of ribosomes. Counts of duplicate reaction mixtures lacking puromycin have been subtracted from all the counts listed. Reaction was for 10 minutes at 0°.

moiety from ester linkage with tRNA to peptide linkage with the amino group of puromycin is illustrated in Table 1. The activity of mammalian ribosomes was slightly less than that of ribosomes of E. coli MRE 600. The N-formylmethionyl-tRNA hexanucleotide was also active. Ribosomes prepared from frozen canine liver were completely inactive.

Incubation of ribosomes at 40° prior to fragment reaction assay at 0° sometimes resulted in up to 400% activation of the reaction. (Table 2) This finding suggests temperature related rearrangement of ribosmal structure to more active conformation of all ribosomes or of a portion of the total population of ribosomes which are inactive or partially active. Conformational rearrangement of ribosomal structure has been suggested for the heat re-

TABLE 2

ACTIVATION OF RIBOSOMES IN THE FRAGMENT REACTION BY PRE-INCUBATION AT 40°

Ribosomal Pre-treatment	N'-formylmethionyl-puromycin formed, counts/minute
None	232
Pre-incubation at 40°	680

E. coli MRE 600 ribosomes were pre-incubated at 40° in 80 mM Tris-Cl, pH 8.2, 0.5 M KCl, 0.25 M Mg acetate for 5 minutes. Fragment reaction was assayed at 0° as in Table 1 with 0.3 mg/ml ribosomes.

activation of inactive ribosomes (Miskin et al., 1968; Zamir, et al., 1969) and for the heat requirement for formation of active 30S subunits by reassembly of individual components. (Traub and Nomura, 1968).

The reaction with mammalian ribosomes did not require homologous tRNA and occurred with N-formylmethionyl-tRNA hexanucleotide fragment. This suggests that the requirement for reactivity in peptide bond formation of the peptidyl donor tRNA may be the terminal CpCpA portion of the tRNA with mammalian liver ribosomes, as has been found for E. coli ribosomes (Monro, et al., 1968).

These results indicate that the peptide bond forming step catalyzed by mammalian as well as bacterial ribosomes may be studied by the fragment reaction. This conclusion is supported by recent independent findings of activity in the fragment reaction of ribosomes from yeast and human tonsil and from rat liver.*

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REFERENCES

Bretscher, M. S., J. Mol. Biol. 12, 913 (1965).
Cammack, K. A., and Wade, H. E., Biochm. J. 96, 671 (1965).
Maden, B. E. H., Traut, R. R., and Monro, R. E., J. Mol. Biol. 35, 333 (1968).
Maden, B. E. H., and Monro, R. E., Eur. J. Biochem. 6, 309 (1968).
Miskin, R., Zamir, A., and Elson, D., Biochem. Biophys. Res. Comm. 33, 551 (1968).
Moldave, K., and Skogerson, L., Methods in Enzymology 12A, 478 (1967).
Monro, R. E., and Marcker, K. A., J. Mol. Biol. 25, 347 (1967).
Monro, R. E., J. Mol. Biol. 26, 147 (1967).
Monro, R. E., Cerna, J., and Marcker, K. A., Proc. U. S. Nat. Acad. Sci. 61, 1042 (1968).
Nishizuka, Y., and Lipmann, F., Proc. U. S. Nat. Acad. Sci. 55, 212, (1966).
Silverstein, E., Biochem. Biophys. Acta, in press.
Traub, P., and Nomura, M., Proc. U. S. Nat. Acad. Sci. 59, 777 (1968).
Vogel, Z., Zamir, A., and Elson, D., Proc. U. S. Nat. Acad. Sci. 61, 701 (1968).
Zamir, A., Leder, P., and Elson, D., Proc. U. S. Nat. Acad. Sci. 56, 1794 (1966).
Zamir, A., Miskin, R., and Elson, D., FEBS Letters 3, 85 (1969).
Zubay, G., in "Procedures in Nucleic Acid Research" (G. L. Cantoni, and D. R. Davies, eds.), p. 455. Harper and Row, New York (1966).