RIBOSOMES OF RAT LIVER CATALYZE 'MINIMAL' DONOR REACTION

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1. Introduction

In 1973 it was shown that 2′(3′)-*O*-(*N*-formylmethionyl) adenosine 5′-phosphate pA-(fMet) acts as donor for the residue of *N*-formylmethionine in the reaction with Phe-tRNA or CACCA-Phe catalyzed by *E. coli* ribosomes [1]. This new model system allowed a detailed investigation of the substrate specificity of the donor site of the peptidyltransferase center [2–4]. The process was shown to be catalyzed with 50 S subunits of *E. coli* ribosomes [5,6]. Cytidine 5′-phosphate (pC) effectively stimulates the reaction of pA-(fMet) with both acceptors [7]. The pC was established to bind at the donor site of the peptidyltransferase center stimulating the peptide donor activity of pA-(fMet) in terms of allosteric mechanism [8,9]. By means of an analog of the 'minimal' substrate it was found that *E. coli* ribosomes can catalyze the synthesis of the thioamide bond [10,11].

We report here on the ability of rat liver ribosomes to catalyze peptide bond formation using pA-(fMet), as a peptide donor.

2. Materials and methods

2.1. *Ribosomes*

These were prepared from a postmitochondrial supernatant of rat liver homogenate by addition of Triton X-100 and sodium deoxycholate to final concentrations of 2% and 1.3%, respectively, and centrifugation for 90 min at 105 000 × *g*. The ribosomes were resuspended in a medium of 10 mM TEA, 500 mM KCl, 1 mM $MgCl_2$, 10 mM β-mercaptoethanol (pH 7.5) to 10 mg/ml, and puromycin was added to 0.5 mM. After incubation for 20 min at 0°C and 10 min at 37°C 25 ml of the mixture was layered over a cushion of 25 ml 20% sucrose in buffer A (10 mM TEA, 500 mM KCl, 5 mM $MgCl_2$, 10 mM β-mercaptoethanol (pH 7.5)). Derived subunits were pelleted for 20 h at 25 000 rev./min in rotor SW 25.2 at 20°C. The pellets containing 60 S and 40 S particles were resuspended to 400–500 A_{260}-units/ml in 10 mM TEA, 50 mM KCl, 5 mM $MgCl_2$ (pH 7.5) and either used directly or stored with 6% sucrose and 10 mM β-mercaptoethanol in liquid nitrogen until use. Ribosomes were activated by preincubation at 40°C according to [12].

2.2. *Transfer RNA*

Total tRNA from *Escherichia coli* B was acetylated with [^{3}H]phenylalanine, spec. act. 10 Ci/mmol (Isotop, UCCR). CACCA-(Ac-[^{14}C]Leu) was obtained from [^{14}C]Leu-tRNA [1]: [^{14}C]leucine was spec. act. 342 mCi/mmol: pA-(fMet) was synthesized as in [13].

2.3. *Transfer reaction*

The reaction was carried out as in [1]. The incubation mixture contained (before methanol was added) 0.05 M Tris–HCl (pH 7.4), 0.4 M KCl, 0.02 M $Mg(OAc)_2$, ribosomes, [^{3}H]Phe-tRNA, pC and pA-(fMet) in concentrations as indicated in the figure legends. The reaction was initiated with addition of 100 μl methanol and incubated for 60 min. Termination and treatment were done as in [1].

3. Results

3.1. *Transferase activity of the rat liver ribosomes*

The peptidyl transferase activity of the preparations from rat liver ribosomes is characterized in a system with CACCA-(Ac-[^{14}C]Leu) and puromycin according to [14]. As is obvious from fig.1, the preparation of rat liver ribosomes used vigorously catalyzes the reaction, though somewhat weaker than the preparation of *E. coli* ribosomes.

3.2. *Dependence on concentration of pA-(fMet)*

Figure 2 shows dependence of the reaction of pA-(fMet) and [^{3}H]Phe-tRNA on the pA-(fMet) concentration. One can see the reaction run is twice as effective in the presence of pC than without it. At maximum pA-(fMet) concentration, 19% of [^{3}H]Phe-tRNA (without pC) was utilized and 33% in the presence of pC. The reaction is effectively inhibited by the antibiotic puromycin (1 mM), but is not affected by chloramphenicol (1 mM) and lincomycin (1 mM) (table 1).

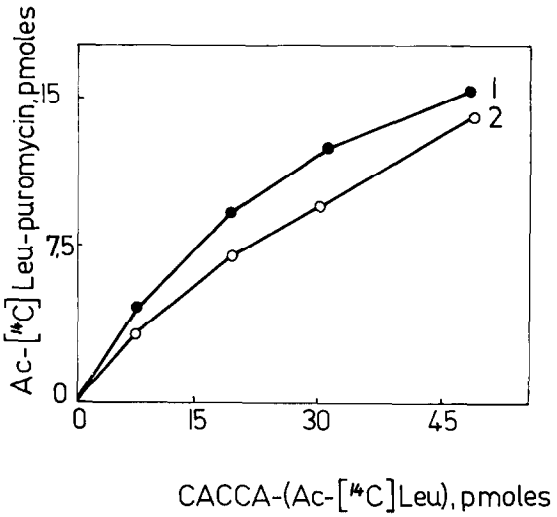

Fig.1. Dependence of Ac-[^{14}C]Leu-puromycin formation on the concentration of CACCA-(Ac-[^{14}C]Leu) for rat liver ribosomes (curve 1) and for *E. coli* (curve 2). The incubation mixture contained 75 pmol *E. coli* ribosomes or 96 pmol rat liver ribosomes and 1 mM puromycin. Incubation was for 40 min at 30°C.

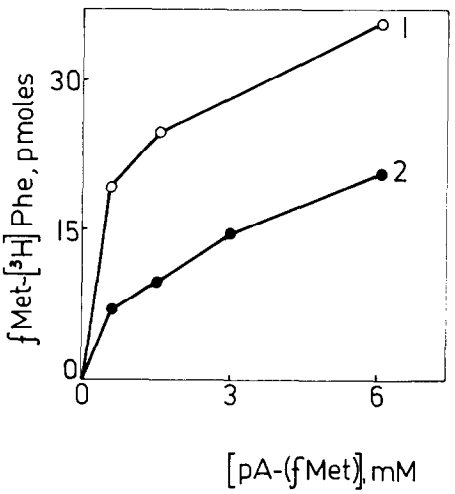

Fig.2. fMet-[^{3}H]Phe-tRNA formation at different pA-(fMet) concentrations in the presence of 2.5 mM pC (curve 1) and in its absence (curve 2). Incubation mixture contained 95 pmol rat liver ribosomes, [^{3}H]Phe-tRNA (72 × 10^{3} cpm). Incubation was for 60 min at 30°C.

3.3. *Temperature dependence of reaction of pA-(fMet) with [^{14}C]Phe-tRNA*

The data given in fig.3 show that reaction of pA-(fMet) with Phe-tRNA in the presence of 1 mM pC is about twice as effective at 30°C than at 0°C.

Table 1
Effect of some antibiotics on the reaction of pA-(fMet) with [^{3}H]Phe-tRNA in the presence of 1 mM pC

pA-(fMet) [mM]	Formation of fMet-[^{3}H]Phe-tRNA							
	–		+ puromycin		+ chloramphenicol		+ lincomycin	
	(cpm)	(%)	(cpm)	(%)	(cpm)	(%)	(cpm)	(%)
2	10 000	100	3060	31	9210	92	9340	93
4	18 120	100	6200	34	16 670	94	17 030	92

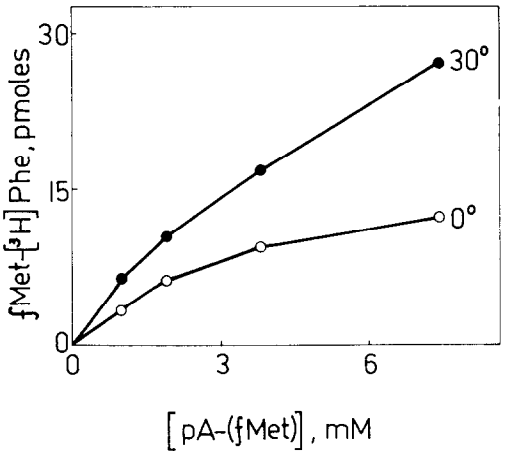

Fig.3. Reaction of pA-(fMet) with [^{3}H]Phe-tRNA in the pC presence at 0°C (curve 1) and at 30°C (curve 2). The incubation mixture contained 95 pmol rat liver ribosomes and 7×10^4 cpm [^{3}H]Phe-tRNA.

3.4. *Dependence of reaction of pA-(fMet) with [^{3}H]-Phe-tRNA on ribosome concentration*

Figure 4 shows that the yield of formyldipeptidyl-tRNA is proportional to the ribosome concentration. This also points to the ribosomal nature of the reaction. Similar dependence for *E. coli* ribosomes is given for comparison.

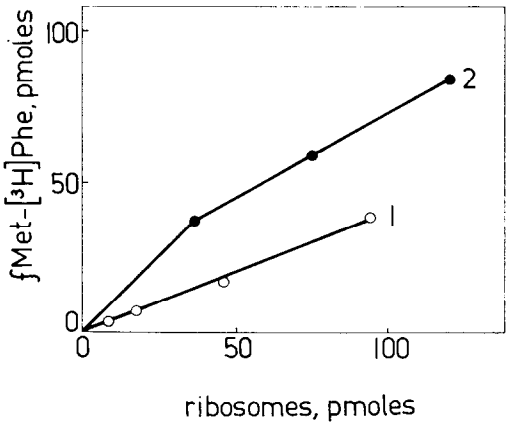

Fig.4. fMet-[^{3}H]Phe-tRNA formation at different concentrations of rat liver ribosomes (curve 1) and *E. coli* ribosomes (curve 2). Each sample contained 10^5 cpm [^{3}H]Phe-tRNA and was incubated for 60 min at 30°C.

4. Discussion

It has been reported that ribosomes of human tonsils [14], mouse and rat liver [15], yeast [16], rabbit reticulocytes [17] and wheat germs [18] catalyze fragment reaction, i.e., the reaction between CACCA-(AcLeu) and puromycin. However, nothing has been reported so far as to the ability of ribosomes of higher organisms to catalyze the reaction between pA-(fMet) and Phe-tRNA.

The quality of ribosomes in the peptidyltransferase reaction was controlled by the Monro system [14]. Figure 1 shows that rat liver ribosomes effectively catalyze the transfer of the AcLeu residue from CACCA-(AcLeu) onto puromycin. The feature of the reaction is that it was run at 30°C whereas the Monro reaction is normally done at 0°C. In the described case CACCA-(AcLeu) was utilized to 30–50% whereas at 0°C 70–80% were reached (data are not given).

From the data obtained one can see that effectiveness of the fragment reaction decreases with rising temperature. The reaction of pA-(fMet) and [^{3}H]Phe-tRNA in the presence of pC depends on the concentration of pA-(fMet) (fig.2,3) and on the concentration of ribosomes (fig.4). These data prove the ribosomal nature of the reaction. Figure 3 also represents the dependence of the yield of peptide on temperature. Similarly, as shown [19] for *E. coli* ribosomes the temperature range from 0–30°C increased the effectiveness of transfer of the fMet residue of Phe-tRNA. Comparison of these data with the results presented in fig.1 points to the opposite effect of temperature on the reaction run with the pentanucleotide fragment of peptidyl-tRNA and the 'minimal' donor. We believe that decrease in the yield of products in the Monro system accompanied by temperature rise results from destabilization of the secondary structure of the fragment and therefore from decrease of its binding to the ribosome. This temperature range affects neither the conformation of the 'minimal' donor nor Phe-tRNA as noted [19].

pC vigorously stimulated the reaction of pA-(fMet) with Phe-tRNA as reported [7–9] for *E. coli* ribosomes; puromycin inhibited the transfer (table 1) [6], but chloramphenicol and lincomycin had no effect [14–18].

The above data show the reaction of pA-(fMet) with Phe-tRNA to be effectively catalyzed by rat liver

ribosomes. In addition, this stimulation proves general similarity of the structures of the peptidyltransferase donor site of procaryotic and eucaryotic ribosomes.

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References

[1] Cerna, J., Rychlik, I., Krayevsky, A. A. and Gottikh, B. P. (1973) FEBS Lett. 37, 188–191.
[2] Cerna, J., Rychlik, I., Krayevsky, A. A. and Gottikh, B. P. (1974) Acta Biol. Med. Germ. 33, 877–883.
[3] Krayevsky, A. A., Kukhanova, M. K. and Gottikh, B. P. (1975) Nucl. Acids. Res. 2, 2223–2236.
[4] Kukhanova, M. K., Krayevsky, A. A. and Gottikh, B. P. (1977) Molekul. Biol. 11, 1357–1376.
[5] Kotusov, V. V., Kukhanova, M. K., Krayevsky, A. A. and Gottikh, B. P. (1976) Mol. Biol. Rep. 3, 151–156.
[6] Kotusov, V. V., Kukhanova, M. K., Salnikova, N. S., Nikolayeva, L. V., Krayevsky, A. A. and Gottikh, B. P. (1977) Molekul. Biol. 11, 651–676.
[7] Cerna, J. (1975) FEBS Lett. 58, 94–98.
[8] Krayevsky, A. A., Victorova, L. S., Kotusov, V. V., Kukhanova, M. K., Treboganov, A. D., Tarussova, N. B. and Gottikh, B. P. (1976) FEBS Lett. 62, 101–104.
[9] Kotusov, V. V., Kukhanova, M. K., Victorova, L. S., Krayevsky, A. A., Treboganov, A. D. and Gottikh, B. P. (1976) Molekul. Biol. 10, 1394–1401.
[10] Victorova, L. S., Kotusov, V. V., Azhayev, A. V., Krayevsky, A. A., Kukhanova, M. K. and Gottikh, B. P. (1976) FEBS Lett. 68, 215–218.
[11] Azhayev, A. V., Kotusov, V. V., Ozols, A. M., Victorova, L. S., Kukhanova, M. K., Krayevsky, A. A. and Gottikh, B. P. (1977) Bioorgan. Khim. 3, 739–745.
[12] Miskin, R., Zamir, A. and Elson, D. (1968) Biochem. Biophys. Res. Commun. 33, 551–558.
[13] Azhayev, A. V., Popovkina, S. V., Tarussova, N. B., Florentiev, V. L., Krayevsky, A. A., Kukhanova, M. K. and Gottikh, B. P. (1977) Nucl. Acids Res. 4, 2223–2234.
[14] Neth, R., Monro, R. E., Heller, G., Battaner, E. and Vazquez, D. (1970) FEBS Lett. 6, 198–202.
[15] Falvey, A. K. and Staehelin, T. (1970) J. Mol. Biol. 53, 1–19.
[16] Battaner, E. and Vazquez, D. (1971) Biochim. Biophys. Acta 254, 316–330.
[17] Carrasco, L. and Vazquez, D. (1975) Eur. J. Biochem. 50, 317–323.
[18] Sikorsky, M. M., Cerna, J., Rychlik, I. and Legocki, A. B. (1977) Biochim. Biophys. Acta 475, 123–130.
[19] Kotusov, V. V., Nikolayeva, L. V., Kukhanova, M. K., Krayevsky, A. A. and Gottikh, B. P. (1978) Molekul. Biol. 12, 628–639.