Aminoacyl-tRNA synthetases of rabbit reticulocytes with and without the ability to bind high-M_r RNA

A.T. Alzhanova, A.N. Fedorov and L.P. Ovchinnikov

Institute of Protein Research, USSR Academy of Sciences, 142292 Poustchino, Moscow Region, USSR

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1. INTRODUCTION

The cytoplasm of eukaryotic cells contains a special class of proteins with a strong affinity for high M_r RNA (RNA-binding proteins) [1,2] (reviews [3,4]). Some of them proved to be free excess proteins of messenger ribonucleoproteins (informosomes) [5–8]. A number of initiation factors and both elongation factors of translation were also found among RNA-binding proteins isolated by affinity chromatography on Sepharose with immobilized high M_r RNA [9,10]. It has been shown in experiments with pure preparations of elongation factors from eukaryotic and prokaryotic cells that the RNA-binding ability of the eukaryotic elongation factors can be considered as an evolutionary acquisition of eukaryotic cells whereas the prokaryotic elongation factors do not possess the affinity for high-M_r RNA [11]. It has been proposed that the RNA-binding ability is a special characteristic of many eukaryotic proteins working in the RNA-dependent processes and is necessary for their compartmentation in the large volume of the eukaryotic cell [12].

A considerable amount of aminoacyl-tRNA synthetase activity of rabbit reticulocytes was retained on high-M_r RNA immobilized on Sepharose and found in the fraction of RNA-binding proteins [13]. Aminoacyl-tRNA synthetases of *E.coli* do not bind at all to RNA–Sepharose under the same conditions [13]. Part of aminoacyl-tRNA synthetase activity of reticulocytes does not adsorb on the immobilized RNA [13]. The shape of the curves of tRNA aminoacylation by amino acids allowed us to suggest that the aminoacyl-tRNA synthetases for all or almost all amino acids are present in the preparation of RNA-binding proteins from reticulocytes. At the same time, aminoacyl-tRNA synthetases for only some amino acids are present in the reticulocyte extract deprived of RNA-binding proteins.

Here, we have determined the sets of amino acids accepted by tRNA using the fraction of RNA-binding proteins and that of proteins without an affinity to high-M_r RNA as sources of aminoacyl-tRNA synthetases, respectively. It was shown that aminoacyl-tRNA synthetases for all the 15 amino acids tested (Asp,Thr,Ser,Glu,Pro,Gly,-Ala,Val,Ile,Leu,Tyr,Phe,Lys,His,Arg) were present in the fraction of RNA-binding proteins of rabbit reticulocytes, whereas aminoacyl-tRNA synthetases for only 4–7 amino acids (Thr,Ala,Ile,Leu; and perhaps Pro,His,Arg) were found among the proteins without an affinity to high-M_r RNA.

2. MATERIALS AND METHODS

The ribosome-free extract of rabbit reticulocytes was prepared and fractionated using a column with *E.coli* ribosomal RNA immobilized on Sepharose as in [13,14]. The protein concentration was measured by the amino black staining technique on nitrocellulose filters [14,15]. The total tRNA preparation was isolated from rabbit liver as in [13].

In aminoacylation experiments each 100 μl aliquot of the reaction mixture contained 1 nmol tRNA (0.56 A_{260} units), 0.5 μCi of *Chlorella* [14C]protein hydrolysate (57 μCi/matom, Amersham) and varying amounts of the protein fractions to be tested for aminoacyl-tRNA synthetase activity. Incubation was done in 100 mM Tris–HCl (pH 7.6), 25 mM KCl, 8 mM $MgCl_2$, 4 mM 2-mercaptoethanol and 5 mM ATP for 10 min at

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37°C. This incubation time corresponds to the linear part of the kinetic curve of the reaction. The reaction was stopped by adding cold trichloroacetic acid to 5%.

To measure the total aminoacyl-tRNA synthetase activity, aliquots after adding trichloroacetic acid were filtered through GF/C filters (Whatman) to collect the acid-insoluble radioactive material (aminoacyl-tRNA). Radioactivity on filters was counted in the standard toluene–PPO–POPOP mixture using Beckman LS100C liquid scintillation spectrometer.

To determine the sets of aminoacyl-tRNA synthetases in the protein fractions, the acid-isoluble material was pelleted (instead of filtration) at 4000 rev./min for 15 min at 4°C, and the pellet was washed 4 times in 5% trichloroacetic acid, then once in 70% aqueous ethanol and was dissolved in 1 ml water. For deacylation of aminoacyl-tRNA, the pH of the solution was adjusted to 9 by adding 25% NH_4OH and the mixture was incubated for 1 h at 37°C. The reaction of deacylation was stopped by adding cold 96% ethanol and 20% $CH_3CO_2NH_4$ (pH 5.0) to final concentrations of 70% and 2%, respectively. After 12 h the precipitate was removed by centrifugation and washed once by the same ethanol–acetate mixture. Supernatants containing the radioactive amino acids were lyophilized. The radioactive amino acids were dissolved in 20 μl sample buffer (pH 2.2), containing 0.2 M sodium citrate, 2% thiodiglycol, 0.1% phenol, which is commonly used for amino acid analysis and 10 nmol of the mixture of non-radioactive amino acids (Amino Acid Standard H, Pierce) were added. The amino acids were separated in the Durrum D-500 amino acid analyzer with disconnected ninhydrin reactor. The fractions of the radioactive material from the analyzer were collected on GF/C filters (Whatman). Radioactivity on filters was counted as described above.

3. RESULTS

Fig.1 presents the results of affinity fractionation of the ribosome-free extract of rabbit reticulocytes on the RNA–Sepharose column. Under the conditions used, up to 90% of the RNA-binding activity was adsorbed on the column. This represents nearly 1% of the total protein of the extract. About 50% of the aminoacyl-tRNA synthetase activity is adsorbed under these conditions (fig.1, table 1); the other half of the aminoacyl-tRNA synthetase activity is not retained in the RNA–Sepharose column even after the second passing of the fraction through the column, whereas the remaining 10% of the RNA-binding activity is completely adsorbed (not shown).

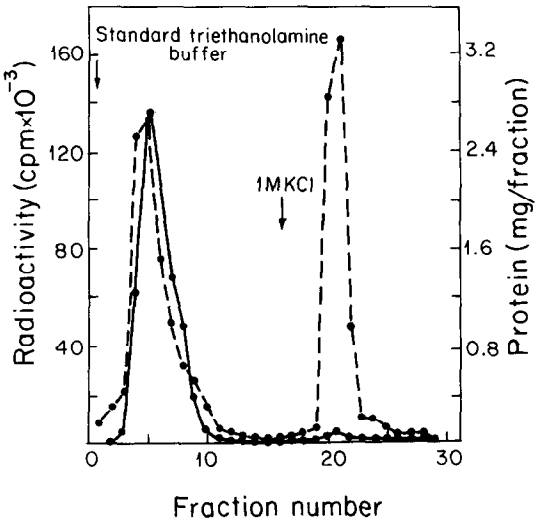

Fig.1. Fractionation of the ribosome-free extract of rabbit reticulocytes on the RNA–Sepharose column. Rabbit reticulocyte extract, 0.25 ml, was applied onto a 1.7 ml column (7 mg immobilized RNA). The column was pre-equilibrated with a low ionic strength buffer (0.01 M KCl). The column was washed with the same buffer to remove the unadsorbed proteins. The RNA-binding proteins were eluted with a buffer containing 1 M KCl: (•—•) protein; (•- - -•) aminoacyl-tRNA synthetase activity (radioactivity on filters, cpm).

The ratio between the aminoacyl-tRNA synthetase activity in the fraction of RNA-binding proteins and in that of proteins without the affinity for RNA was well reproducible and did not change significantly after preincubation of the extract at 37°C with or without exogenous ATP (5 mM) (Table 1).

Fig.2 demonstrates the results of determination of the amino acid sets accepted by tRNA in the presence of the non-fractionated ribosome-free extract and of each of its 2 fractions obtained by chromatography on RNA–Sepharose (see fig.1) which were used as a source of aminoacyl-tRNA synthetases.

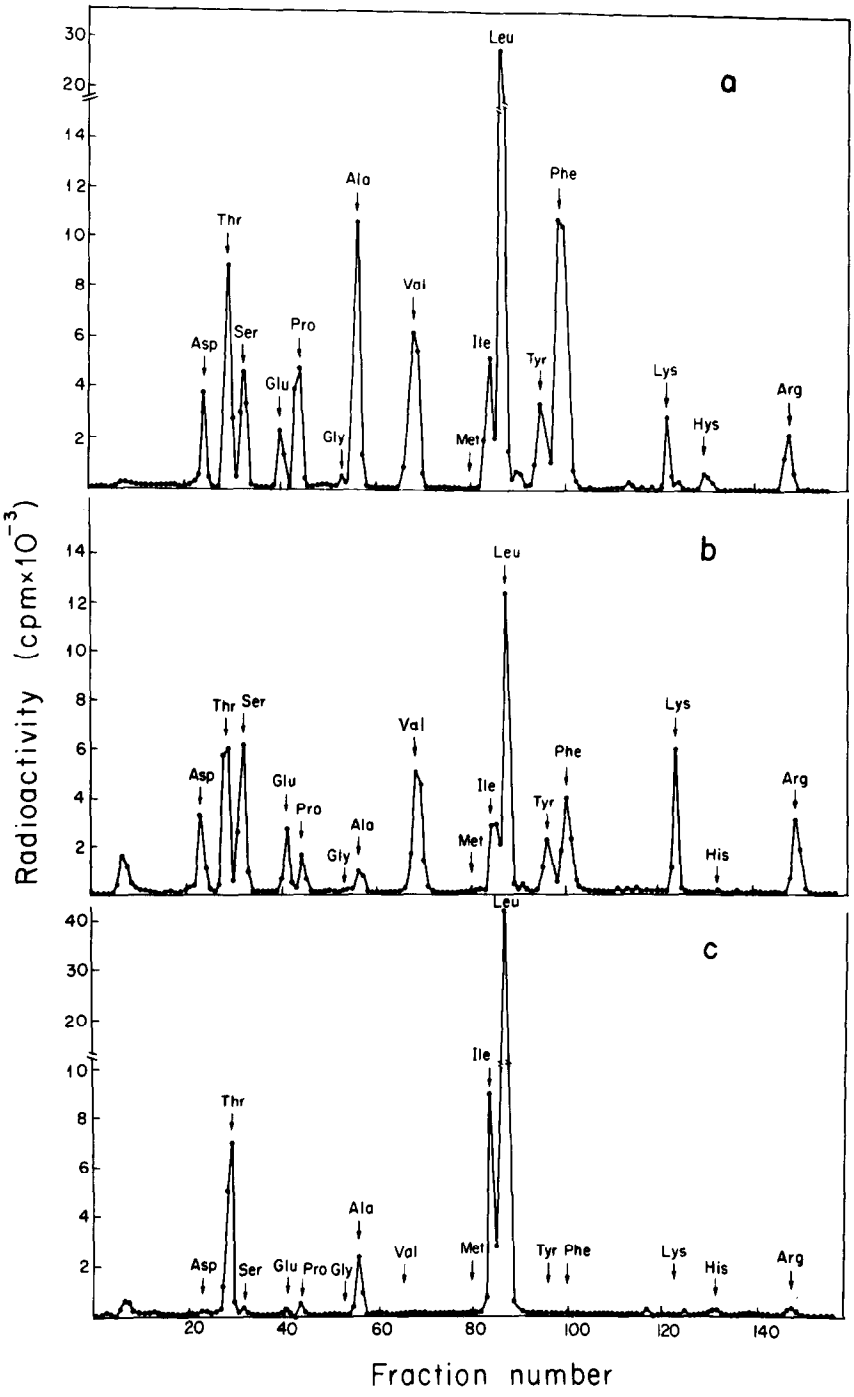

Fig.2. The sets of [^{14}C]amino acids accepted by tRNA in the reactions of aminoacylation using as enzymes preparations: (a) rabbit reticulocyte ribosome-free extract; (b) RNA-binding proteins; and (c) ribosome-free extract deprived of RNA-binding proteins. The tRNAs were aminoacylated with the mixture of [^{14}C]amino-acids of *Chlorella* protein hydrolysate. Amino acids were separated in a Durrum D-500 amino acid analyzer. The amount of radioactive amino acids applied onto the analyzer column was: (a) 5×10^5 cpm; (b, c) 2.5×10^5 cpm.

Table1

Amount of protein and aminoacyl-tRNA synthetase activity in the fractions of ribosome-free rabbit reticulocyte extracts

Ribosome-free extract treatment	Protein (%)[a]		Aminoacyl-tRNA synthetase activity (%)[a]	
	Proteins without RNA affinity	RNA-binding proteins	Proteins without RNA affinity	RNA-binding proteins
Control	98.7	1.3	55	45
Incubation 90 min, 37°C	99.1	0.9	48	52
Incubation with ATP, 90 min, 37°C	99.2	0.8	59	41

[a] 100% is the amount of protein and aminoacyl-tRNA synthetase activity in both the fractions

Fig.2a shows that the activities of aminoacyl-tRNA synthetases specific for 15 amino acids contained in the *Chlorella* [^{14}C]protein hydrolysate are present in the non-fractionated extract of rabbit reticulocytes. (We failed to reveal methionine, amino acid 16 of the *Chlorella* hydrolysate.) The fraction of RNA-binding proteins, just as the non-fractionated extract, contains the aminoacyl-tRNA synthetase activities for the same 15 amino acids (fig.2b). In the extract deprived of RNA-binding proteins, aminoacyl-tRNA synthetase activities were revealed for 7 of the 15 tested amino acids: Thr, Pro, Ala, Ile, Leu, His and Arg (fig.2c), 4 of them (Thr, Ala, Ile and Leu) being predominant.

It is noteworthy that the set of aminoacyl-tRNA synthetases in each of the two fractions of the rabbit reticulocyte extract is quite stable and well reproducible from one extract to another.

4. DISCUSSION

Fractionation of the rabbit reticulocyte extracts on the immobilized high-M_r RNA separates aminoacyl-tRNA synthetases into 2 fractions:

(i) About 1/2 of the activity is found in the fraction of RNA-binding proteins representing ~ 1% of the extract proteins;

(ii) The rest is revealed among the proteins without the RNA-binding ability (99% of the total protein of the extract).

A study of the set of aminoacyl-tRNA synthetases in the 2 protein fractions have shown that some activities, such as Asp-, Ser-, Glu-, Gly-, Val-, Tyr-, Phe- and Lys-tRNA synthetases, are encountered only among the RNA-binding proteins. The others, such as Thr-, Ala-, Pro- and His-tRNA synthetases are detected both among the RNA-binding proteins and the proteins without affinity to RNA.

The distribution of aminoacyl-tRNA synthetases specific for different amino acids among the 2 protein fractions suggests the existence of 2 forms of eukaryotic aminoacyl-tRNA synthetases, the RNA-binding one and that without the affinity to high-M_r RNA.

At present the structural difference between the 2 forms of aminoacyl-tRNA synthetases with the same amino acid specificity is not known. Neither is the biological significance of the existence of RNA-binding and RNA-non-binding synthetases clear. They may be the products of different genes. Another explanation is that the same protein undergoes some post-translational modification or interacts with some other component of the cell leading to the loss or, on the contrary, to the acquisition of non-specific affinity for high-M_r RNA. In particular, there is an indication that phosphorylation of proteins may change their affinity for RNA [16].

Assuming that the RNA-binding activity of a number of eukaryotic proteins functioning in the RNA-dependent processes serves for their compartmentation and concentration in the sites of their

functioning [12], it is tempting to speculate that the loss of the affinity for RNA by such proteins may cause their decompartmentation and so affect the rate of the process itself. The reversible loss or acquisition of the RNA-binding ability by such proteins and, in particular, by aminoacyl-tRNA synthetases might have an important functional significance and could regulate the rate of translation (elongation) in the cell.

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REFERENCES

[1] Ovchinnikov, L.P., Voronina, A.S., Stepanov, A.S., Belitsina, N.V. and Spirin, A.S. (1968) Molekul. Biol. 2, 752–764.
[2] Stepanov, A.S., Voronina, A.S., Ovchinnikov, L.P. and Spirin, A.S. (1971) FEBS Lett. 18, 13–18.
[3] Preobrazhensky, A.A. and Spirin, A.S. (1978) Prog. Nucleic. Acid. Res. Mol. Biol. (Cohn, W. ed) vol. 21, pp. 1–38, Academic Press, New York.
[4] Spirin, A.S. and Ovchinnikov, L.P. (1980) in: Soviet Scientific Reviews, Section D, Biology Reviews, Physico-Chemical Aspects (Skulachev, V.P., ed) vol. 1, pp. 157–213, Gordon and Breach, New York.
[5] Elizarov, S.M., Stepanov, A.S., Felgenhauer, P.E. and Chulitskaya, E.V. (1978) FEBS Lett. 93, 219–224.
[6] Alzhanova, A.T., Bezlepkina, T.A. and Ovchinnikov, L.P. (1979) Dokl. Akad. Nauk SSSR 246, 486–489.
[7] Mazur, G. and Schweiger, A. (1978) Biochem. Biophys. Res. Commun. 80, 39–45.
[8] Setyono, B. and Greenberg, J.R. (1981) Cell 24, 775–783.
[9] Vlasik, T.N., Ovchinnikov, L.P., Rajabov, Kh.M. and Spirin, A.S. (1978) FEBS Lett. 88, 18–20.
[10] Ovchinnikov, L.P., Spirin, A.S., Erni, B. and Staehelin, T. (1978) FEBS Lett. 88, 21–26.
[11] Domogatsky, S.P., Vlasik, T.N., Seryakova, T.A., Ovchinnikov, L.P. and Spirin, A.S. (1978) FEBS Lett. 96, 207–210.
[12] Spirin, A.S. (1978) FEBS Lett. 88, 15–17.
[13] Alzhanova, A.T., Fedorov, A.N., Ovchinnikov, L.P. and Spirin, A.S. (1980) FEBS Lett. 120, 225–229.
[14] Ovchinnikov, L.P., Seriakova, T.A., Avanesov, A.Ts., Alzhanova, A.T., Radzhabov, H.M. and Spirin, A.S. (1978) Eur. J. Biochem. 90, 517–525.
[15] Schaffner, W. and Weissmann, C. (1973) Anal. Biochem. 56, 502–514.
[16] Gallinaro-Matringe, H., Stevenin, J. and Jacob, M. (1975) Biochemistry 14, 2547–2554.