EUKARYOTIC AMINOACYL-tRNA SYNTHETASES ARE RNA-BINDING PROTEINS WHEREAS PROKARYOTIC ONES ARE NOT

A. T. ALZHANOVA, A. N. FEDOROV, L. P. OVCHINNIKOV and A. S. SPIRIN
Institute of Protein Research, USSR Academy of Sciences, 142292 Poustchino, Moscow Region, USSR

Received 27 August 1980

1. Introduction

Cytoplasmic extracts of eukaryotic cells contain proteins possessing a strong non-specific affinity for high molecular weight RNA [1–4]. These RNA binding proteins are capable of interacting with any high molecular weight RNA and forming stoichiometric RNA–protein complexes of a buoyant density of 1.4 g/cm^3 in CsCl (protein:RNA ratio of ~3:1). Some of the RNA-binding proteins are identical to protein constituents of informosomes (messenger ribonucleoproteins) and represent a pool of free informosomal proteins in the cell [5,6].

The RNA-binding proteins can be isolated from the cell extract by affinity adsorption on immobilized RNA or synthetic polynucleotides [7–9]. Preparations of RNA-binding proteins of eukaryotes have been shown to contain elongation factors, both EF-1 and EF-2, as well as a number of initiation factors of translation [10,11]. A comparison of isolated elongation factors of rabbit reticulocytes with their prokaryotic analogs from *Escherichia coli* has shown that, whereas the elongation factors of rabbit reticulocytes, EF-1 and EF-2 do possess an affinity for RNA, their analogs from *E. coli*, EF-T and EF-G do not [12].

A hypothesis was proposed that RNA-binding activity is specially characteristic of many eukaryotic proteins functioning with RNA or in different RNA-dependent processes; the RNA-binding activity of such proteins can be considered as an additional evolutionary acquisition of eukaryotic cells, in comparison with prokaryotic ones, due to an essential increase of the eukaryotic cell volume and the necessity of their concentration (compartmentation) near the sites of their functioning, i.e., near RNA [13].

Here we report a study of RNA-binding properties of aminoacyl-tRNA synthetases which are a group of proteins also functioning in the process of protein biosynthesis. The experiments have shown that a considerable part of aminoacyl-tRNA synthetase activity of rabbit reticulocytes is indeed retained on high molecular weight RNA immobilized on Sepharose and can be eluted as a fraction of RNA-binding proteins. On the other hand, aminoacyl-tRNA synthetases of *E. coli* do not bind with high molecular weight RNA under the same conditions.

2. Materials and methods

The ribosome-free extract of rabbit reticulocytes was prepared and then fractionated on a column with *E. coli* ribosomal RNA immobilized on Sepharose as in [9]. The RNA-binding proteins were adsorbed on RNA at 0.01 M KCl and eluted from the column with 1 M KCl containing buffer. The ribosome-free extract of *E. coli* MRE 600 was fractionated in an analogous way.

The RNA-binding activity of the isolated fractions was measured by the technique of adsorption of RNA–protein complexes on nitrocellulose filters [1,3,9]. *E. coli* ribosomal 16 S [^{14}C]RNA was used as an exogenous RNA for the complex formation.

The protein concentration was measured by the amido black staining technique [9,14]. The tRNA preparations were isolated from rabbit liver and from *E. coli* MRE 600 by phenol–chloroform deproteinization and deacylated at pH 9, 37°C for 1 h.

In aminoacylation experiments each 100 μl aliquot of the reaction mixture contained 2 nmol tRNA (1.12 A_{260} units), 0.5 μCi *Chlorella* [^{14}C]protein hydrolysate (59 μCi/matom, Amersham) and a varying amount of the protein fraction to be tested for aminoacyl-tRNA synthetase activity. Incubation was

in 100 mM Tris–HCl (pH 7.6), 25 mM KCl, 8 mM $MgCl_2$, 4 mM 2-mercaptoethanol and 5 mM ATP for 20 min at 37°C. The reaction was stopped by adding cold 5% trichloroacetic acid. The precipitates were put onto GF/C filters (Whatman) directly or, as a control, after hydrolysis in 5% trichloroacetic acid for 20 min at 90°C. Radioactivity on filters was counted in the standard toluene–PPO–POPOP mixture using a Beckman LS100 liquid scintillation spectrometer. The control radioactivity measurements in the material non-hydrolyzable by 5% trichloroacetic acid at 90°C showed (see curve 4 in fig.3a,b) that incorporation of labelled amino acids into polypeptides was insignificant.

Special control experiments showed that aminoacylation systems strongly depended on exogenous tRNA (incorporation in endogenous tRNA of the extracts was ⩽3% for reticulocytes and 8% for the *E. coli* system).

3. Results and discussion

Fig.1 represents the results of affinity fractionation of the ribosome-free extracts of rabbit reticulocytes (fig.1a) and *E. coli* (fig.1b) using RNA–Sepharose columns. Two fractions were collected from each column: the main bulk of proteins not retained on the column (extract deprived of RNA-binding proteins) and the proteins retained in the column and eluted by 1 M KCl (RNA-binding proteins). Fig.2 shows the results of determination of the RNA-binding activity of the fractions from the RNA–Sepharose columns.

The RNA–Sepharose fractionation of the ribosome-free extract of rabbit reticulocytes has resulted in adsorption in the column of only ~1% of the total protein and at the same time of ⩾99% of the RNA-binding activity (see fig.1a,2a). In analogous experiments with ribosome-free extracts of *E. coli*, ~3% of the protein and ⩾99% of the RNA-binding activity are retained in the column (fig.1b,2b). Electrophoretic analyses in the presence of sodium dodecyl sulfate of the fractions of RNA-binding proteins eluted from the column with 1 M KCl were done in [9,11,12] and revealed several main and a large number of minor polypeptide components.

The fractions obtained and the original (non-fractionated) ribosome-free extracts were assayed for aminoacyl-tRNA synthetase activity. Fig.3a shows aminoacylation of rabbit liver tRNA when the non-fractionated extract (curve 1), or the fraction not retained on the RNA–Sepharose column (curve 2), or the RNA-binding proteins (curve 3) of rabbit reticulocytes were used as the source of the enzymes.

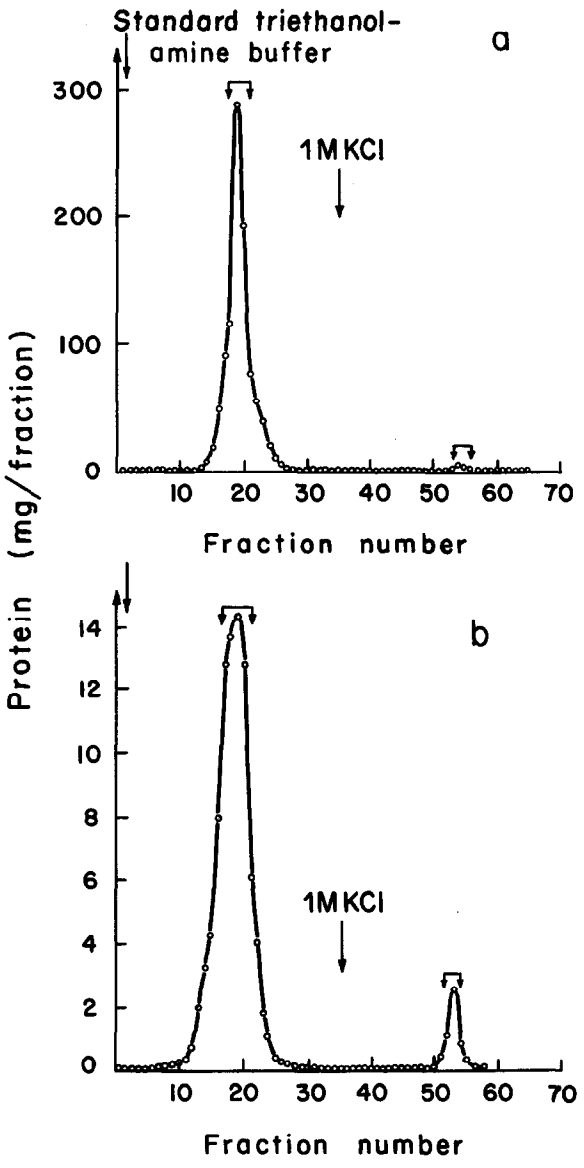

Fig.1. Fractionation of ribosome-free extracts of rabbit reticulocytes (a) and *E. coli* (b) on the RNA–Sepharose column. Rabbit reticulocyte extract, 20 ml (1000 mg protein) or *E. coli* extract, 4 ml (140 mg protein) were applied onto a 65 ml column (0.4 g immobilized RNA). The column was pre-equilibrated with the 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 the buffer containing 1 M KCl.

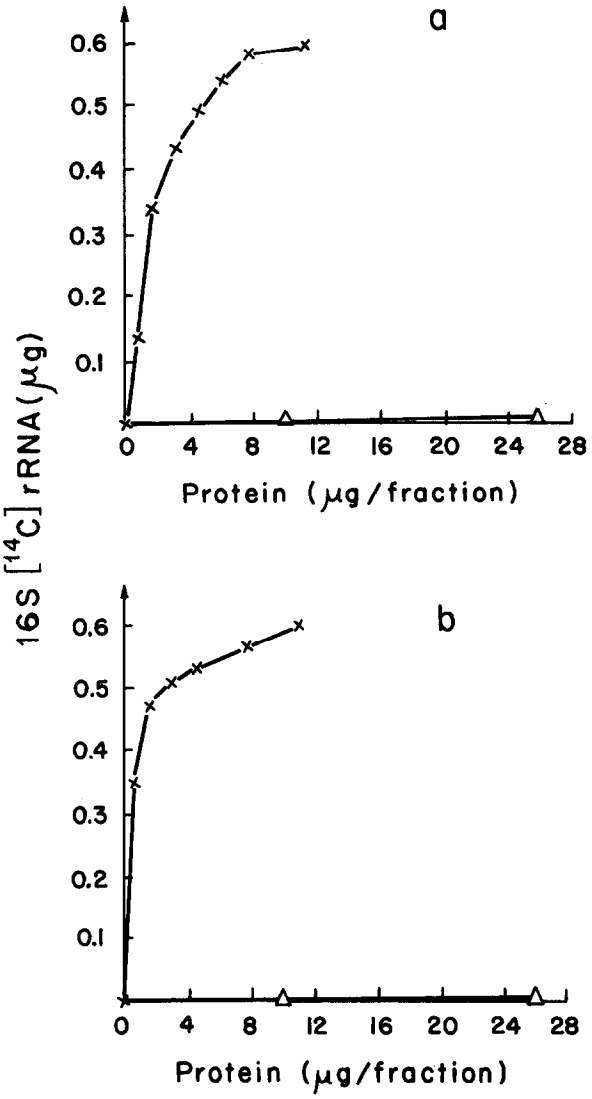

Fig.2. RNA-binding activity of the fractions of the ribosome-free rabbit reticulocyte extract (a) and that of *E. coli* (b) obtained by separation on the RNA–Sepharose column (fig.1). 0.7 μg ribosomal *E. coli* 16 S [^{14}C]RNA were added to each fraction aliquot and the samples were filtered through nitrocellulose filters with an average pore diameter of 0.5 μm. (–△–) The ribosome-free extract deprived of RNA-binding proteins; (–X–) RNA-binding proteins.

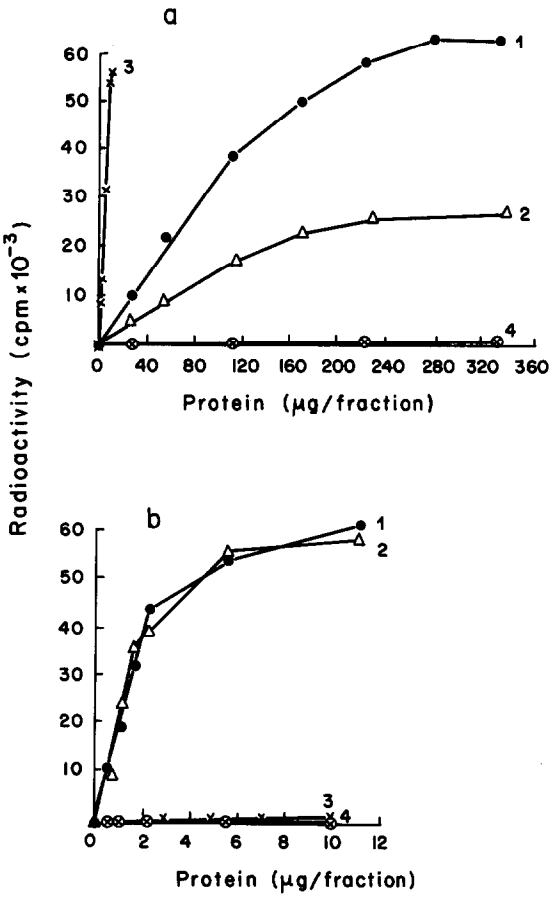

Fig.3. Aminoacylation of tRNA by *Chlorella* [^{14}C]protein hydrolysate. The following fractions of rabbit reticulocytes (a) and *E. coli* (b) served as a source of aminoacyl-tRNA synthetases: (1) non-fractionated ribosome-free extracts (–●–); (2) ribosome-free extracts deprived of RNA-binding proteins (–△–); (3) RNA-binding proteins (–X–); (4) as (1) but radioactivity was counted after hydrolysis of the samples in 5% trichloroacetic acid at 90°C (–⊗–).

It is seen that the passing of the ribosome-free extract of reticulocytes through the RNA–Sepharose column retaining the RNA-binding activity (and only ~1% of the total protein of the extract) results in a significant decrease of the aminoacyl-tRNA synthetase activity of the extract. The upper level of the aminoacylation of the total tRNA at the excess of the extract deprived of RNA-binding proteins is also 2–3-times lower than that of the aminoacylation by the original (non-fractionated) extract. This suggests that some of the amino acid-specific aminoacyl-tRNA synthetase activities have been lost from the extracts as a result of the passing through RNA column. On the contrary, a high aminoacyl-tRNA synthetase activity is found in the fraction of RNA-binding proteins eluted from the column with 1 M KCl (fig.3a, curve). The specific aminoacylating activity of the RNA-binding proteins of rabbit reticulocytes is ~30-times greater than that

of the original extract. The upper level of the aminoacylation of the total tRNA by the fraction of RNA-binding proteins approaches the level reached in the case of the original extract.

The latter indicates that the RNA-binding protein fraction of a rabbit reticulocyte extract contains aminoacyl-tRNA synthetases for all or almost all amino acids. However, the extract deprived of RNA-binding proteins is likely to contain aminoacyl-tRNA synthetases only for some amino acids. Thus it seems that some of the amino acid-specific aminoacyl-tRNA synthetases in rabbit reticulocyte extracts can exist in two forms, just one of which is RNA-binding.

In any case, the experiments demonstrate that >50% of the aminoacyl-tRNA-synthetase activity of rabbit reticulocytes can be selectively adsorbed on RNA–Sepharose. In other words, a significant part of aminoacyl-tRNA synthetases of reticulocytes behave as RNA-binding proteins.

Fig.3b shows aminoacylation of *E. coli* tRNA by the non-fractionated *E. coli* ribosome-free extract (curve 1), by the *E. coli* extract deprived of RNA-binding activity (curve 2) and by the fraction of *E. coli* RNA-binding proteins (curve 3). Contrary to the case of the rabbit reticulocyte extract, all aminoacyl-tRNA synthetase activity of the *E. coli* ribosome-free extract passes through the RNA–Sepharose column without retention. That is, *E. coli* aminoacyl-tRNA-synthetases do not posses any RNA-binding activity.

The results obtained here give a good support to the hypothesis proposed [13] that the RNA-binding activity is characteristic of many eukaryotic proteins having something to do with protein biosynthesis and other RNA-dependent processes and that the non-specific RNA-binding activity of such proteins is an evolutionary acquisition of eukaryotic organisms as compared with prokaryotes.

Eukaryotic aminoacyl-tRNA synthetases, unlike prokaryotic ones, are found in extracts as multi-enzyme complexes with high sedimentation coefficients [15–28] and can be bound with ribosomal particles and polyribosomes [16,19,29–34]. It is not unlikely that these features of eukaryotic aminoacyl-tRNA synthetases are directly related to the fact that they possess RNA-binding activity. The non-specific RNA-binding activity of eukaryotic aminoacyl-tRNA synthetases could serve for their concentration and compartmentation near the sites of their functioning (e.g., within polyribosomes).

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] Baltimore, D. and Huang, A. S. (1970) J. Mol. Biol. 47, 263–273.
[3] Stepanov, A. S., Voronina, A. S., Ovchinnikov, L. P. and Spirin, A. S. (1971) FEBS Lett. 18, 13–18.
[4] Ajtkhozhin, M. A. and Kim, T. N. (1975) FEBS Lett. 53, 102–104.
[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] Blanchard, J. M., Brissac, C. and Jeanteur, P. (1974) Proc. Natl. Acad. Sci. USA 71, 1882–1886.
[8] Schweiger, A. and Mazur, G. (1974) FEBS Lett. 46, 255–259.
[9] 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.
[10] Vlasik, T. N., Ovchinnikov, L. P., Radjabov, Kh. M. and Spirin, A. S. (1978) FEBS Lett. 88, 18–20.
[11] Ovchinnikov, L. P., Spirin, A. S., Erni, B. and Staehelin, T. (1978) FEBS Lett. 88, 21–26.
[12] Domogatsky, S. P., Vlasik, T. N., Seryakova, T. A., Ovchinnikov, L. P. and Spirin, A. S. (1978) FEBS Lett. 96, 207–210.
[13] Spirin, A. S. (1978) FEBS Lett. 88, 15–17.
[14] Schaffner, W. and Weissmann, C. (1973) Anal. Biochem. 56, 502–514.
[15] Vennegoor, C. J. G. M., Stols, A. L. H. and Bloemendal, H. (1972) J. Mol. Biol. 65, 375–378.
[16] Hampel, A. and Enger, M. D.(1973) J. Mol. Biol. 79, 285–293.
[17] Smulson, M., Lin, C. S. and Chirikjian, J. G. (1975) Arch. Biochem. Biophys. 167, 458–468.
[18] Som, K. and Hardesty, B. (1975) Arch. Biochem. Biophys. 166, 507–517.
[19] Ussery, M. A., Tanaka, W. K. and Hardesty, B. (1977) Eur. J. Biochem. 72, 491–500.
[20] Deeney, R. M. (1977) Arch. Biochem. Biophys. 183, 156–167.
[21] Hele, P. and Herbert, L. (1977) Biochim. Biophys. Acta 479, 311–321.
[22] Dang, C. V. and Yang, D. C. H. (1978) Biochem. Biophys. Res. Commun. 80, 709–714.
[23] Kellermann, O., Brevet, A., Tonetti, H. and Waller, J.-P. (1979) Eur. J. Biochem. 99, 541–550.
[24] Brevet, A., Kellerman, O., Tonetti, H. and Waller, J.-P. (1979) Eur. J. Biochem. 99, 551–558.
[25] Dang, C. V. and Yang, D. C. H. (1979) J. Biol. Chem. 254, 5350–5356.
[26] Saxholm, H. J. K. and Pitot, H. C. (1979) Biochim. Biophys. Acta 562, 386–399.
[27] Ritter, P., Enger, M. D. and Hampel, A. E. (1979) Biochim. Biophys. Acta 562, 377–385.
[28] Arbeeny, C. M., Briden, K. L. and Stirewalt, W. S. (1979) Biochim. Biophys. Acta 564, 191–201.

[29] Irwin, J. D. and Hardesty, B. (1972) Biochemistry 11, 1915–1920.
[30] Roberts, W. K. and Coleman, W. H. (1972) Biochem. Biophys. Res. Commun. 46, 206–214.
[31] Agris, P. F. and Woolverton, D. K. (1976) Proc. Natl. Acad. Sci. USA 73, 3857–3861.
[32] Tanaka, W. K., Som, K. and Hardesty, B. A. (1976) Arch. Biochem. Biophys. 172, 252–260.
[33] Quintard, B., Mouricout, M., Carias, J. R. and Julien, R. (1978) Biochem. Biophys. Res. Commun. 85, 999–1006.
[34] Smith, D. W. E., Silbert, P. E. and McNamara, A. L. (1979) Biochim. Biophys. Acta 562, 453–461.