RNA-BINDING PROTEIN FROM RABBIT RETICULOCYTE EXTRACT

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1. Introduction

RNA-binding proteins of rat liver cytoplasmic [1–4] and nuclear [5] extracts have been described in previous communications. RNA-binding proteins were also found in HeLa-cell cytoplasmic extracts [6]. The common property of these proteins is their ability to interact with RNA forming stoichoimetric complexes with a buoyant density in CsCl of about 1.4 g/cm^3 (informosome-like particles). An assumption was made that the RNA-binding proteins in vivo are responsible for the formation of informosomes, characteristic ribonucleoprotein particles of animal cells [7,8]. A comparison of the RNA-binding proteins with protein components of informosomes (nuclear, free cytoplasmic and polyribosome-bound) is at present very timely. The first step in these studies can be the electrophoretic comparison of polypeptide chains of the proteins after their isolation in a pure form.

The present communication describes RNA-binding protein of rabbit reticulocyte extract. A method for isolation of this protein in a pure state has been developed and the molecular weight of its polypeptide chains determined. An electrophoretic comparison has been made of the mobility of the reticulocyte RNA-binding protein polypeptide chains and the polypeptide chains of the protein component (informofer) of rat liver nuclear informosomes.

2. Materials and methods

The preparation and lysis of reticulocytes was done by the method of Adamson et al. [9]. To obtain ribosome-free extract the lysates were centrifuged at 30 000 *g* for 15 min and then at 105 000 *g* for 90 min. Assay of RNA-binding activity, centrifugation in a sucrose gradient, chromatography on ion-exchange celluloses, formaldehyde fixation of particles and centrifugation in a CsCl density gradient have been described in a previous communication [2]. Protein labeling in vitro with ^{125}I was performed according to Helmkamp et al. [10]. Preparation of proteins and electrophoresis in polyacrylamide gel with SDS was done according to Weber and Osborn [11] with the only difference that a 5% concentration of polyacrylamide gel was used. Preparation of rat liver nuclear 30 S particle protein (informofer) and polyacrylamide gel electrophoresis with 6 M urea at pH 4.5 were carried out as described by Samarina et al. [12].

3. Results

The rabbit reticulocyte ribosome-free extract was fractionated by centrifugation in a sucrose gradient. As seen in fig. 1, the RNA-binding activity is mainly displayed as one component with a sedimentation

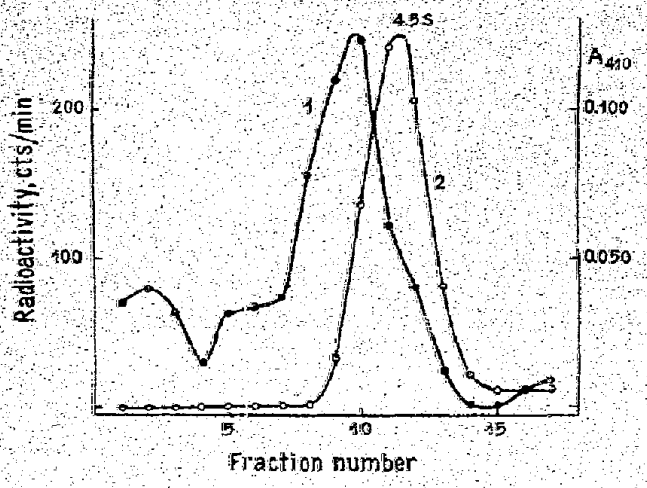

Fig. 1. Sedimentation distribution in a sucrose gradient of RNA-binding activity of the reticulocyte ribosomefree extract. A 10–20% sucrose gradient was prepared on a buffer solution containing 0.01 M triethanol-amine, 0.01 M KCl, 0.001 M $MgCl_2$, 0.001 M mercaptoethanol, pH 7.8 (standard buffer). Centrifugation in a SW-39 rotor at 36 000 rpm for 20.5 hr. 1 – RNA-binding activity; 2 – absorption at 410 nm (distribution of hemoglobin used as a marker).

informosome-like particles of a buoyant density in CsCl of about 1.4 g/cm^3 and a sedimentation coeffi-

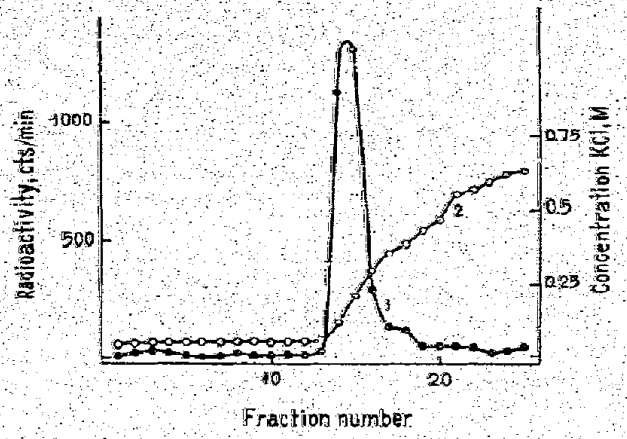

Fig. 2. Chromatography of reticulocyte ribosome-free extract on DEAE-cellulose (DE-32). The extract was layered on the column equilibrated with the standard buffer containing 0.03 M KCl. Elution was done with a KCl gradient in the standard buffer. 1 – RNA-binding activity; 2 – KCl concentration.

coefficient of 6–7S. Such a sedimentation coefficient corresponds to a molecular weight of about 100 000–120 000 for globular proteins.

In order to obtain data on the charge of the reticulocyte RNA-binding protein molecules chromatography of the extract on ion-exchange cellulose columns was done. It was found that the RNA-binding protein was not retained on a carboxymethyl-cellulose column equilibrated with the standard buffer (see legend to fig. 1). On a diethylaminoethyl (DEAE)-cellulose column (fig. 2) the RNA-binding protein was completely adsorbed and was then eluted with a rise in the KCl concentration (up to 0.1–0.2 M) as a single rather homogeneous component. Thus, the rabbit reticulocyte RNA-binding protein macromolecules are homogeneous in their charge and have a weakly acidic character.

The common property of RNA-binding proteins is their ability to form informosome-like particles with exogenous RNA [1–7]. With the addition of *E. coli* 23 S rRNA to the rabbit reticulocyte ribosome-free extract a complexing of RNA-binding protein and the RNA takes place with the formation of

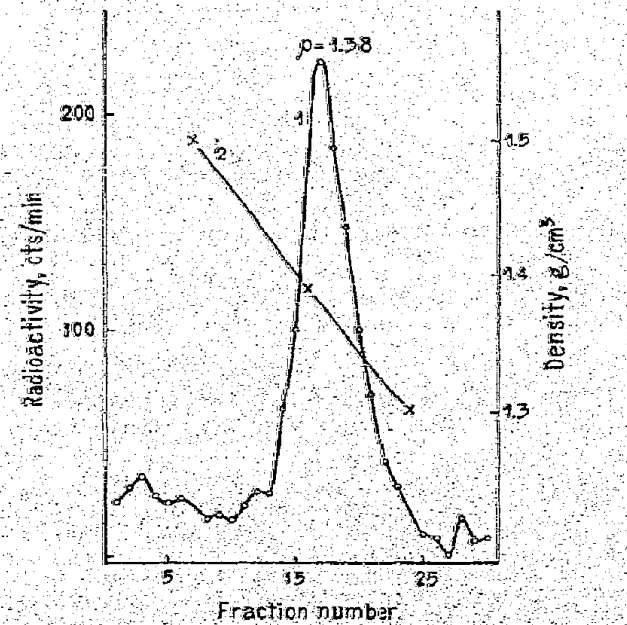

Fig. 3. Density distribution in a CsCl gradient of artificial ribonucleoprotein particles obtained upon addition of *E. coli* 23 S [^{14}C]rRNA to the material eluting from DEAE-cellulose between 0.1 and 0.3 M KCl. 1 – Radioactivity; 2 – CsCl density.

cient of about 60 S [13]. Informosome-like particles are also formed if after fractionation of the reticulocyte ribosome-free extract on DEAE-cellulose the *E. coli* rRNA is added to the fractions with RNA-binding activity (fig. 3).

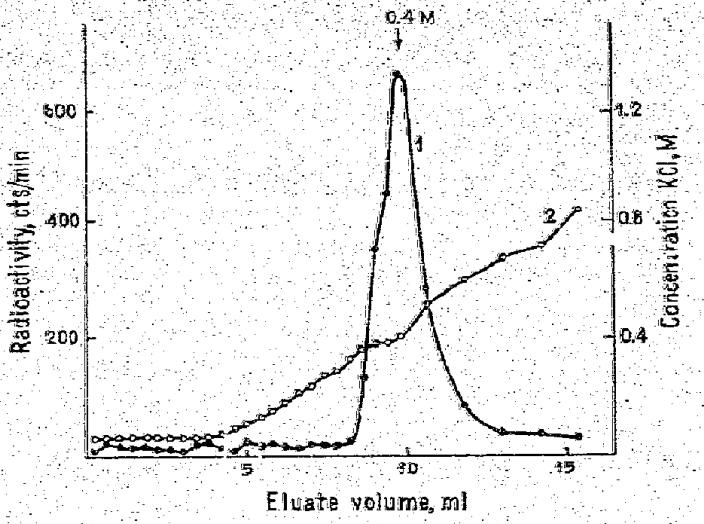

Fig. 4. Chromatography on DEAE-cellulose of informosome-like particles formed upon addition of *E. coli* 23 S [^{14}C]rRNA to the reticulocyte ribosome-free extract. Elution was with a KCl gradient in the standard buffer. 1 — Radioactivity; 2 — KCl concentration.

Fig. 4 shows the results of fractionating on DEAE-cellulose of the informosome-like particles formed in the reticulocyte ribosome-free extract with *E. coli* rRNA. The particles are eluted from the column at higher KCl concentrations (about 0.4 M) than free RNA-binding protein. It is known that at such concentrations of monovalent ions part of the protein dissociates from informosome-like particles [14]. Indeed, CsCl-density gradient centrifugation of the particles after the column shows a heterogeneous distribution with a maximum of about 1.6 g/cm^3 (fig. 5). Calculations (see [15] or [7] for the formula) show that these ribonucleoprotein particles eluted at 0.4 M KCl from DEAE-cellulose retain about one quarter of the total original protein contained in the informosome-like particles with a density of 1.4 g/cm^3 [15].

The difference in the chromatographic behaviour

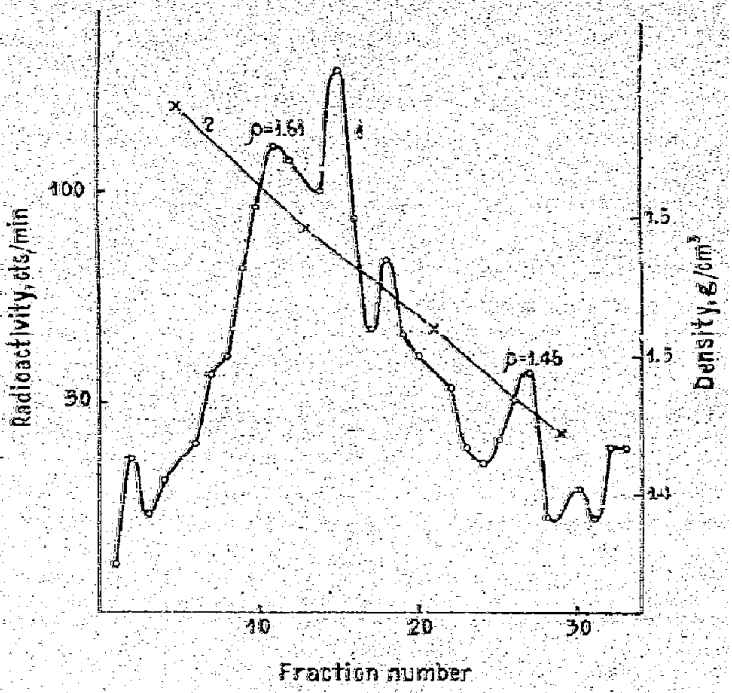

Fig. 5. Density distribution in a CsCl gradient of the artificial (informosome-like) ribonucleoprotein particles after chromatography on DEAE-cellulose as indicated in the legend to fig. 4. 1 — Radioactivity; 2 — CsCl density.

of the free RNA-binding protein and the complexes of this protein with RNA was used to isolate the RNA-binding protein in a pure state. To this end the ribosome-free extract was dialyzed against the standard buffer with 0.28 M KCl. The extract was then passed through a DEAE-cellulose column equilibrated with the same buffer. After this the RNA-binding protein fraction was dialyzed against the standard buffer and *E. coli* rRNA was added to obtain informosome-like particles. The KCl concentration was then again adjusted to 0.28 M and the mixture passed through a DEAE-cellulose column equilibrated with the standard buffer with 0.28 M KCl. In these conditions only the informosome-like particles formed by RNA-binding protein with the added RNA were absorbed on the column. The column was thoroughly washed with the standard buffer containing 0.28 M KCl and the particles were eluted by the standard buffer with 1 M KCl. The preparation obtained was dialyzed against a borate buffer for labeling with ^{125}I [10]. Purification from

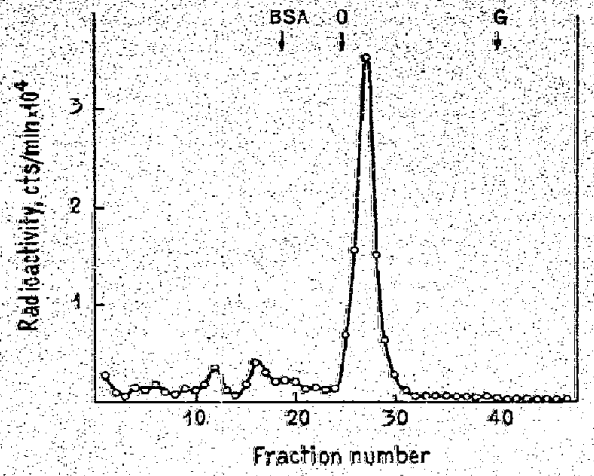

Fig. 6. Electrophoresis of purified reticulocyte RNA-binding protein labeled with ^{125}I in polyacrylamide gel in the presence of SDS. Arrows indicate the positions of the protein-marker bands, bovine serum albumin (BSA), ovalbumin (O) and globin (G), migrating in the same gel.

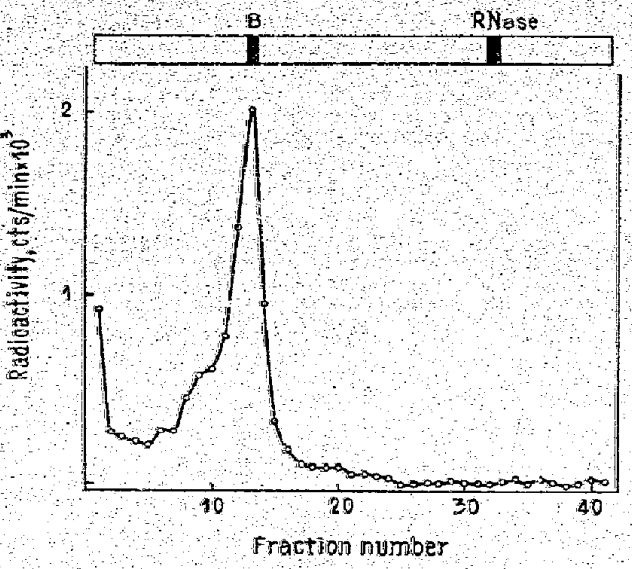

Fig. 7. Electrophoresis of purified reticulocyte RNA-binding protein labeled with ^{125}I and unlabeled rat liver nuclear 30 S particle protein in polyacrylamide gel in the presence of urea. The position of the main band of nuclear 30 S particle protein (B) and ribonuclease (RNase) after gel staining is pictured schematically above; below is the radioactivity distribution profile obtained after fractionation of this gel.

non-incorporated ^{125}I was performed on a Sephadex G-15 column after which the preparation was incubated with pancreatic ribonuclease (100 mg/ml) at 20°C for 30 min. In a separate control experiment it was shown that the RNA preparation used to obtain informosome-like particles did not contain admixtures which in our conditions would be labeled with radioactive iodine.

Electrophoresis of the isolated RNA-binding protein in polyacrylamide gel with SDS displays one main peak of ^{125}I-radioactivity (fig. 6). The molecular weight of RNA-binding protein polypeptide chains determined by this method is 37 000. This molecular weight is close to that of the main class of polypeptide chains of rat liver nuclear ribonucleoprotein particles containing pre-mRNA (nuclear informosomes) which is 40 000–45 000 according to Krichevskaya and Georgiev [16] and 36 000 according to Niessing and Sekeris [17].

In a subsequent experiment we compared the polypeptide chain electrophoretic mobility of the rat liver nuclear informosome protein and the reticulocyte RNA-binding protein in the presence of 6 M urea at pH 4.5. As seen in fig. 7, during electrophoresis in such conditions the mobilities of the polypeptide chains of both proteins coincide.

4. Conclusion

The demonstration of RNA-binding protein in nuclei-free cells such as rabbit reticulocytes is an important point of principle confirming the true cytoplasmic nature of RNA-binding proteins which were found in cytoplasmic extracts of other cells.

The method developed for isolating the reticulocyte RNA-binding protein based on the specific affinity of this protein for RNA allows isolation of only part of the protein content of informosome-like particles with a buoyant density of about 1.4 g/cm^3. When the protein of the informosome-like particles is heterogeneous, the method suggested permits isolation of only the RNA-binding protein fraction bound most firmly with RNA.

Polypeptide chains of the RNA-binding protein isolated from rabbit reticulocytes are similar in size to the main class of polypeptide chains of nuclear informosomes and do not greatly differ from them in charge. At the same time it cannot yet be said whether these proteins are identical, closely-

related or different in respect to their primary structure.

Acknowledgements

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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, 752–763.
[2] Stepanov, A. S., Voronina, A. S., Ovchinnikov, L. P. and Spirin, A. S. (1971) FEBS Letters 18, 13–18.
[3] Stepanov, A. S., Voronina, A. S., Ovchinnikov, L. P. and Spirin, A. S. (1972) Biokhimiya 37, 3–9.
[4] Voronina, A. S., Stepanov, A. S. and Ovchinnikov, L. P. (1972) Biokhimiya 37, 10–15.
[5] Voronina, A. S. (1973) FEBS Letters 32, 310–312.
[6] Baltimore, D. and Huang, A. S. (1970) J. Mol. Biol. 47, 263–273.
[7] Spirin, A. S. (1969) Eur. J. Biochem. 10, 20–35.
[8] Ovchinnikov, L. P. and Spirin, A. S. (1970) Naturwissenschaften 57, 514–521.
[9] Adamson, S. D., Herbert, E. and Godchaux, W. (1968) Arch. Biochem. Biophys. 125, 671–683.
[10] Helmkamp, R. W., Contreras, M. A. and Bale, W. F. (1967) Int. J. Appl. Rad. and Isotopes 18, 737–746.
[11] Weber, K. and Osborn, M. (1969) J. Biol. Chem. 244, 4406–4412.
[12] Samarina, O. P., Lukanidin, E. M., Molnar, J. and Georgiev, G. P. (1968) J. Mol. Biol. 33, 251–263.
[13] Preobrazhensky, A. A. and Ovchinnikov, L. P. (1974) Dokl. Akad. Nauk SSSR 214 (in press).
[14] Voronina, A. S., Stepanov, A. S., Preobrazhensky, A. A. and Ovchinnikov, L. P. (1972) Biokhimiya 37, 430–436.
[15] Ovchinnikov, L. P., Ajtkhozhin, A. A., Bystrova, T. F. and Spirin, A. S. (1969) Molekul. Biol. 3, 449–464.
[16] Krichevskaya, A. A. and Georgiev, G. P. (1969) Biochim. Biophys. Acta 194, 619–621.
[17] Niessing, J. and Sekeris, C. E. (1971) FEBS Letters 18, 39–42.