RNA-BINDING PROTEIN FACTOR OF ANIMAL CELL EXTRACTS

A.S. STEPANOV, A.S. VORONINA, L.P. OVCHINNIKOV and A.S. SPIRIN
A.N. Bakh Institute of Biochemistry, Academy of Sciences of the USSR, Moscow, USSR

and

Institute of Protein Research, Academy of Sciences of the USSR, Puostchino, Moscow Region, USSR

Received 23 August 1971

1. Introduction

In a previous communication [1] data were reported indicating the presence in a rat liver cell extract of a special factor complexing with exogenous RNA added to the extract. Preliminary experiments evidenced size homogeneity and high molecular weight of the RNA-binding factor [1, 2]. A claim was made about the RNA-binding factor being of a protein nature, and participating in the formation of "informosome-like particles" [1]. Later, an RNA-binding factor of a protein nature was found by other authors in HeLa cell extracts as well [3].

In this paper we have shown that the RNA-binding factor is a special fraction of high molecular-weight proteins of rat liver cells. The factor can be divided into two discrete subfractions: acidic (component I) and slightly basic (component II). Both the components have a sedimentation coefficient of about 8 to 10 S (molecular weight no less than 200,000) and are capable of complexing with RNA. It is also shown that it is the RNA-binding protein factor that is responsible for the formation of informosome-like particles with a buoyant density of about 1.4 g/cm^3. Both the acidic and basic components of the RNA-binding factor are required for the formation of such ribonucleoprotein (RNP) complexes.

It should be noted that an analogous RNA-binding protein factor of a high molecular weight was found in cytoplasmic extracts of loach embryo cells (Bystrova and Voronina, in preparation) and rabbit reticulocytes (Preobrazhensky and Ovchinnikov, in preparation) as well.

2. Materials and methods

2.1. *Preparation of cell and ribosome-free extracts of rat liver cells*

Liver of white rats starved for over 24 hr was used. The technique of homogenization of the liver was described in detail previously [1]. The obtained homogenate was centrifuged at 20,000 rpm for 15–20 min. The supernatant (cell extract) was further purified: it was layered onto a 0.5–1 ml of a 10% sucrose solution in a buffer containing 0.01 M triethanolamine-HCl, 0.01 M KCl, 0.001 M $MgCl_2$, 0.001 M β-mercaptoethanol, pH 7.8 (standard buffer No. 1) and centrifuged at 36,000 rpm for 90 min. After the centrifugation the upper $\frac{3}{4}$ of the layered material was collected. The collected fraction was used in the experiments as a ribosome-free extract.

2.2. *Assay of RNA-binding activity in fractions of the extract*

RNA-binding activity is designated as the capability of some extract fractions to form complexes with the added RNA which are retained (adsorbed) on nitrocellulose filters. A definite amount of ^{14}C-RNA (radioactive *E. coli* ribosomal RNA with a specific activity of 3×10^6 or 8×10^6 counts/min per mg) was added in the cold to an assayed fraction which

was preliminarily diluted to 0.5–1 ml with standard buffer No. 1. After a few minutes the mixture was diluted with cold buffer containing 0.01 M triethanolamine-HCl, 0.01 M KCl and 0.005 M $MgCl_2$, pH 7.8 (buffer No. 2) and filtered through a nitrocellulose filter (average pore diameter 0.5 μm). The filter was washed with 20–30 ml of the same buffer, dried and the radioactivity retained on the filter was counted. The relative RNA-binding activities of the fractions were judged from the radioactivity of the filters. Free RNA was not retained on the filters in the conditions of the experiment.

2.3. *Fractionation of the extract in gel and ion-exchange cellulose columns*

Sephadex G-200 (Pharmacia, Sweden), carboxymethylcellulose (Reanal, Hungary) and diethylaminoethylcellulose (Reanal, Hungary) were used for fractionating the extract. Preliminary treatment, column packing and buffer equilibration were performed by standard techniques in accordance with Pharmacia (Sweden) and Whatman (England) manuals. All eluting solutions were prepared with standard buffer No. 1. In the case of CM- and DEAE-cellulose columns elution was performed with the buffer containing KCl gradient. Samples for measuring optical absorbance and RNA-binding activity were taken from the elution fractions.

2.4. *Sedimentation analysis in the sucrose concentration gradient*

The material to be analyzed was layered on a 10–20% sucrose gradient made with standard buffer No. 1. Centrifugation was carried out at 36,000 rpm for 20 hr in the SW 39 rotor of the Spinco L preparative ultracentrifuge. The sedimentation coefficients of the fractions were estimated from their position relative to the hemoglobin reference (4.5 S).

2.5. *Fixation of the preparations with formaldehyde and their analysis in the CsCl density gradient*

Preparations for the CsCl gradient analysis were preliminarly fixed by adding 40% formaldehyde (pH 5.5–6.5) to a final concentration of 8%. The samples were dialyzed for over 24 hr against a buffer containing 0.01 M triethanolamine-HCl, pH 7.8, 0.01 M KCl, 0.001 M $MgCl_2$ and 8% CH_2O, and then for a few hours against a buffer with 0.01 M triethanolamine-HCl, pH 7.8, 0.001 M $MgCl_2$ and 4% CH_2O. After dialysis the samples were centrifuged at 20,000 rpm for 20 min the SW 39 rotor to remove possible aggregates and then analyzed by running in the preformed CsCl gradient according to the technique described previously [4].

3. Results

The results of filtering the cell extract and ^{14}C-RNA mixture through nitrocellulose filters testify to the presence in the extract of a factor retaining RNA on the filters. After the microsome fraction is pelleted, the major part of the RNA-binding factor remains in the ribosome-free extract. The sedimentation distribution of RNA-binding activity during fractionation of the ribosome-free extract by sucrose gradient centrifugation is seen in fig. 1a. The sedimentation coefficient value coincides with preliminary evaluations [1, 2] of 8 to 10 S. However, the prolonged centrifugation in the given case reveals some heterogeneity of the RNA-binding factor (fig. 1a).

Pronase treatment of the extract results in the disappearance of the 8–10 S peak of the RNA-binding activity (fig. 1b). The latter evidences in favour of the protein nature of the RNA-binding factor.

The 8–10 S sedimentation coefficient value corresponds to a molecular weight of no less than 200,000 daltons for globular proteins. This estimation can be compared with the results of Sephadex G-200 gel chromatography of the ribosome-free extract: the RNA-binding factor was eluted in the first fractions of the column (fig. 2). The distribution of RNA-binding activity during elution from Sephadex G-200 (fig. 2), as well as the results of sedimentation analysis (fig. 1a) witness to a large size of the RNA-binding proteins.

The question of why RNA-binding components of a lesser size occasionally appear (figs. 1a and 2) was not specially studied. It is not excluded that these components are subunits of the 8–10 S factor possessing RNA-binding activity as well.

Chromatography of the ribosome-free extract in ion-exchange cellulose columns reveals a charge heterogeneity of the RNA-binding proteins. Fig. 3 represents the RNA-binding factor elution profile on CM cellulose. Component I, which is eluted from the CM-cellulose by the standard buffer, has been shown to be retained on DEAE-cellulose and thus represents an

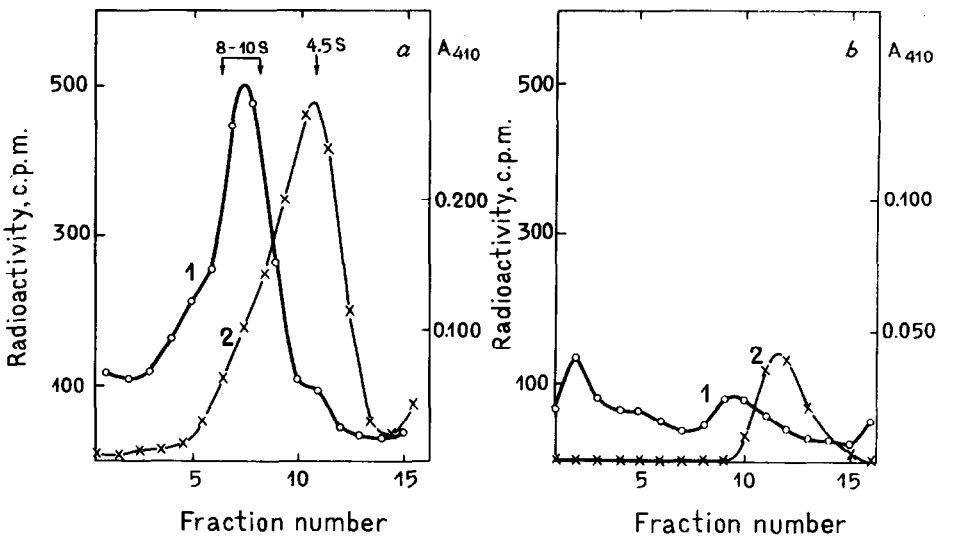

Fig. 1. Sedimentation distribution of RNA-binding activity upon centrifugation of ribosome-free extract in the sucrose concentration gradient. (a) 0.2 ml of the extract containing no more than 10 optical density units (measured at 260 nm) was layered onto 10–20% sucrose gradient. Centrifugation was done at 36,000 rpm for 20 hr in a SW 39 rotor of the Spinco L ultracentrifuge. (b) Pronase was added to the extract (up to 2.5 mg/ml; the pronase was pre-incubated for 1 hr at 35°) and after 30 min incubation a 0.2 ml portion was layered onto 10–20% sucrose gradient. Centrifugation conditions were the same as in (a). 1 (o——o) – RNA-binding activity; 2 (x——x) – absorption at 410 nm (distribution of hemoglobin taken as reference).

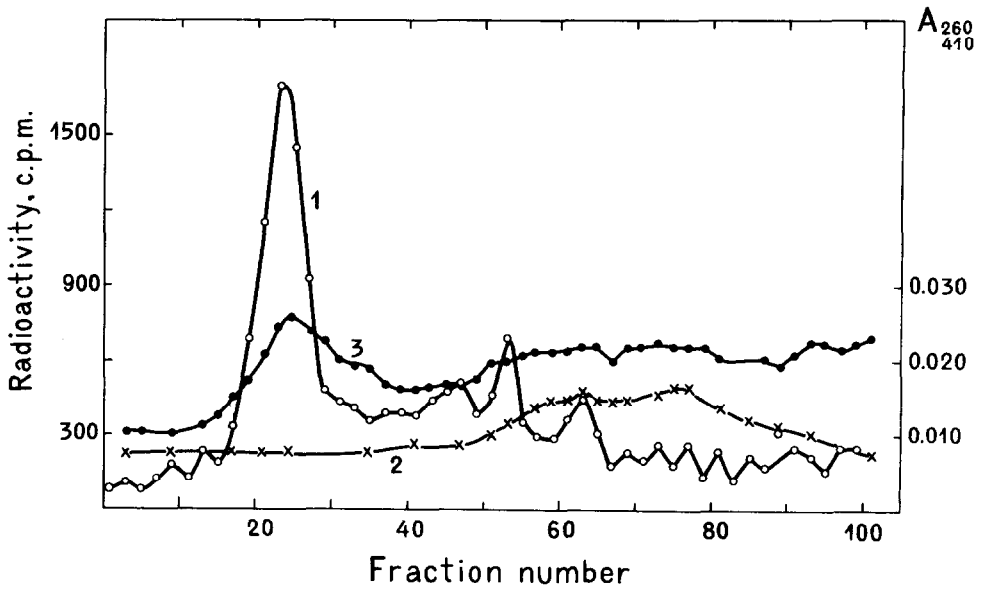

Fig. 2. Distribution of RNA-binding activity upon Sephadex G-200 gel chromatography of ribosome-free extract. The extract was layered on the Sephadex column equilibrated with standard buffer No. 1 and eluted with the standard buffer. Void volume of the column comprised about 20 first fractions. 1 (o——o) – RNA-binding activity; 2 (x——x) – absorption at 410 nm; 3 (•——•) – absorption at 260 nm.

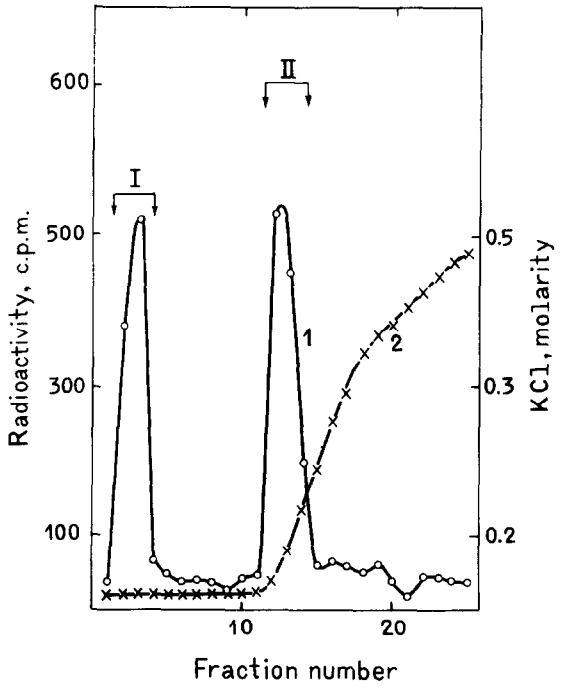

Fig. 3. Distribution of RNA-binding activity upon carboxymethyl (CM) cellulose chromatography of ribosome-free extract. The extract in an amount not exceeding the ion-exchange volume (up to 7 optical density units measured at 280 nm per g of CM-cellulose dry weight), was layered on CM-cellulose column equilibrated with standard buffer No. 1. Elution was done with the standard buffer containing a gradually incerasing KCl concentration. 1 (o—o) – RNA-binding activity; 2 (x—x) – KCl molarity.

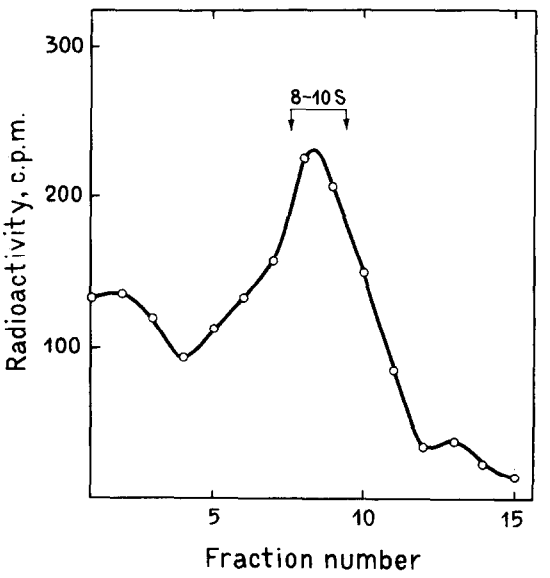

Fig. 4. Sucrose gradient sedimentation distribution of the acidic component of the RNA-binding factor. 0.2 ml were taken from the acidic component fraction (I), marked in fig. 3, and layered onto 10–20% sucrose gradient. Centrifugation was done at 36,000 rpm for 20 hr in the SW 39 rotor of the Spinco L ultracentrifuge.

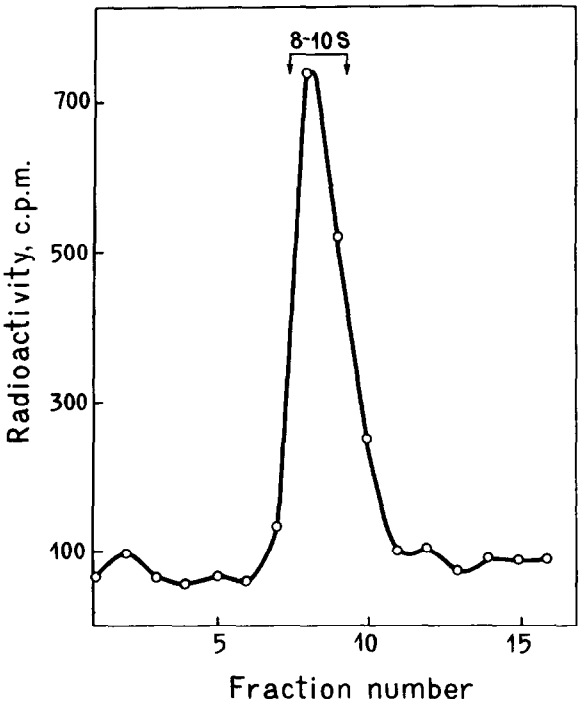

Fig. 5. Sucrose gradient sedimentation distribution of the slightly basic component of the RNA-binding factor. 0.2 ml were taken from the basic component fraction (II), marked in fig. 3, and centrifuged as indicated in fig. 4. Sedimentation coefficients in figs. 4 and 5 were estimated from the position of hemoglobin sedimenting in a parallel tube.

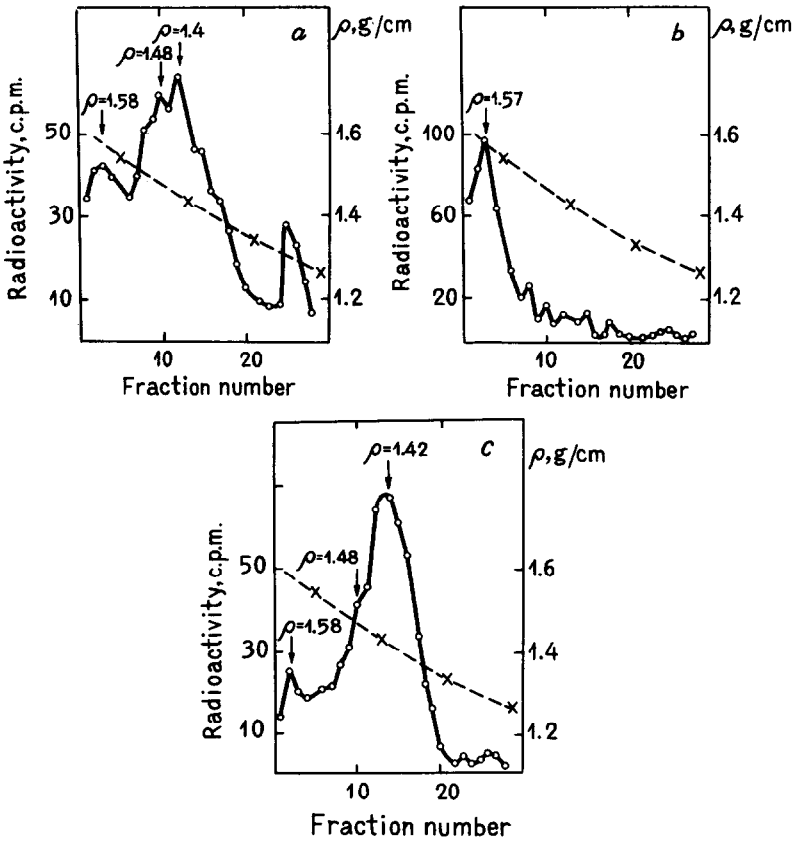

Fig. 6. Density distribution in the CsCl gradient of particles, formed by the RNA-binding protein fractions, obtained upon CM-cellulose chromatography. 23 S ^{14}C-RNA (in an amount twenty-fold less than the RNA-binding activity of a preparation) was added to preparations of the protein I (acidic component), or protein II (slightly basic component), or to a mixture of both. The mixtures were incubated for 15 min in the cold and fixed with formaldehyde as described in section 2.5. Centrifugation was done at 36,000 rpm for 24 hr in the SW 39 rotor of the Spinco L ultracentrifuge. (a) mixture of component I and ^{14}C-RNA; (b) mixture of component II and ^{14}C-RNA; (c) mixture of component I, II and ^{14}C-RNA. (o—o) – Radioactivity; (x—x) – CsCl density.

acidic fraction; component II, eluted from CM-cellulose in 0.05 M KCl, is not retained on DEAE-cellulose and apparently is a slightly basic fraction of the RNA-binding proteins.

The separation into acidic and basic components by chromatography of the RNA-binding factor on CM-cellulose (fig. 3) reflects the real heterogeneity and not the dissociation of the 8–10 S factor into subunits: subjecting the corresponding fractions, eluted from CM-cellulose, to sucrose gradient centrifugation, we have shown that the sedimentation coefficient value of both the acidic and slightly basic fractions of the RNA-binding factor is of 8 to 10 S (figs. 4 and 5). A narrow, homogeneous sedimentation distribution is characteristic of component II (slightly basic protein, fig. 5), while component I (acidic) sediments in a wider zone (fig. 4). It is probable that some sedimentation heterogeneity of the RNA-binding factor mentioned earlier (fig. 1a) may be explained by a tendency of the component I to aggregation.

As earlier shown [1, 2], in conditions of great excess of cell extract, an exogenous RNA is capable of complexing with some cell component resulting in stoichiometric complexes denoted as "informosome-like particles". The buoyant density value of the informosome-like particles in CsCl was about 1.4 g/cm^3.

Both the components of the RNA-binding factor were tested for their capability to form informosome-like particles. 23 S ^{14}C-RNA was added to preparations of component I and component II. After 15 min incubation in the cold the mixture was fixed with formaldehyde and run in the CsCl gradient. The results of the density analysis (figs. 6a and 6b) show that complete informosome-like particles are formed neither by the acidic nor by the basic fraction taken separately, though it is known that they can independently bind to RNA. The formed complexes have a buoyant density higher than 1.4 g/cm^3, i.e., are not completed with protein. A requisite and adequate provision for the formation of particles with a buoyant density of about 1.4 g/cm^3 is the presence of both the components (I and II together) of the RNA-binding factor in the reaction mixture (fig. 6c). Thus, both the subfractions of the RNA-binding factor seem to be functionally complementary: stoichiometric ribonucleoprotein complexes (informosome-like particles) are formed only with the simultaneous participation of both.

4. Conclusion

It follows that special 8–10 S proteins, capable of complexing with RNA, are present in animal cell extracts. These proteins are rather complicated, possessing a high molecular weight, displaying heterogeneity and being functionally complementary in forming stoichiometric complexes with RNA of a buoyant density of about 1.4 g/cm^3 (in CsCl). It may be that, *in vivo*, such proteins are responsible for the formation of informosomes, characteristic ribonucleoprotein particles of animal cells [2, 5].

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