RNA-BINDING PROTEIN FACTOR IN THE NUCLEAR EXTRACT OF RAT LIVER CELLS

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1. Introduction

RNA-binding proteins present in the cytoplasmic extract of rat liver cells have been described in previous communications [1–4]. These proteins specifically react with RNA, forming stoichiometric complexes with a buoyant density in CsCl of about 1.4 g/cm^3 (informosome-like particles). These proteins are homogeneous in size and have a sedimentation coefficient of 8–10 S. Cytoplasmic RNA-binding proteins can be divided according to the charge into two fractions, the acidic and the slightly basic [2,3].

RNA-binding proteins in the nuclear extract of rat liver cells are reported in the present paper. It is shown that in contrast to the cytoplasmic RNA-binding protein, the nuclear protein is heterogeneous in size, displaying two predominating components with sedimentation coefficients of 30 S and 4 S and homogeneous in charge, exhibiting only the acidic fraction.

2. Materials and methods

The nuclear extract was obtained by the method of Georgiev and collaborators [5]. The rat liver was homogenized in 1–2 vol of sucrose solution with a density of 1.27 g/cm^3 and containing 0.5% glycerophosphate. The homogenate was layered on a cushion of the same sucrose solution and centrifuged at 20000 *g* for 1 hr. The pellet of nuclei was washed with a solution containing 0.01 M triethanolamine (TEA), pH 7, 0.14 M KCl, 0.001 M $MgCl_2$. A following 4-fold extraction with a small volume of the same solution at pH 8 was then done.

Assay of RNA-binding activity, centrifugation in the sucrose gradient, chromatography on columns of CM and DEAE cellulose and centrifugation in the CsCl density gradient are described in the previous communication [2].

3. Results

The nuclear extract was fractionated by centrifugation in the sucrose gradient. As seen in fig. 1, the RNA-binding activity is displayed as two main components: the first has a sedimentation coefficient of about 35 S and the second a sedimentation coefficient of about 4 S (the sedimentation coefficients were estimated relatively to subunits of *E. coli* ribosomes and hemoglobin sedimenting in parallel tubes). It should be noted that the RNA-binding activity in the 4 S zone significantly exceeds the same activity in the 35 S zone. The sedimentation distribution does not change with the increase of the KCl concentration to 0.15 M.

The fractions corresponding to the peaks of the RNA-binding activity, i.e., the "35 S"-protein and the "4 S"-protein, were pooled from the sucrose gradient. *E. coli* 23 S [^{14}C]RNA was added to each preparation and the mixture was fixed with formaldehyde for analysis of the obtained particles in the CsCl density gradient. Fig. 2 represents the particles obtained in this manner. It is seen that both the "35 S"-protein and the "4 S"-protein are capable of forming particles with a density of 1.4 g/cm^3, i.e., informosome-like complexes.

Analysis of the "35 S"-protein and "4 S"-protein on a column of CM-cellulose has shown that both proteins are acidic and are eluted before the salt gradient. The basic component (eluted by salt) is not observed (fig. 3). This conclusion is confirmed by chromatography of the nuclear extract on DEAE-cellulose

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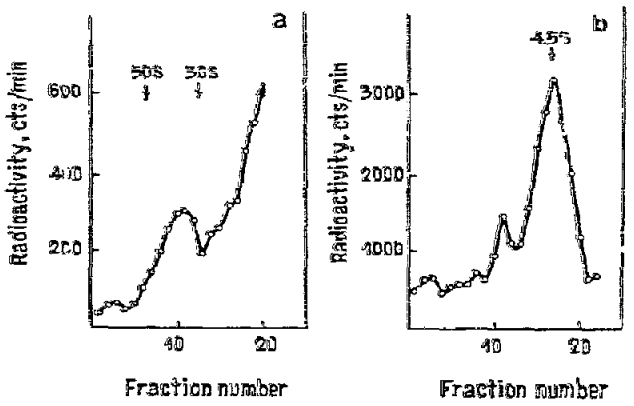

Fig. 1. Sedimentation distribution in the sucrose gradient of RNA-binding activity of the nuclear extract. A 10–20% sucrose gradient was prepared on a buffer solution containing 0.01 M TEA, pH 7.8, 0.01 M KCl, 0.001 M $MgCl_2$, 0.001 M mercaptoethanol. Centrifugation in a SW-39 rotor at 36000 rpm for: a) 5 hr, b) 20 hr.

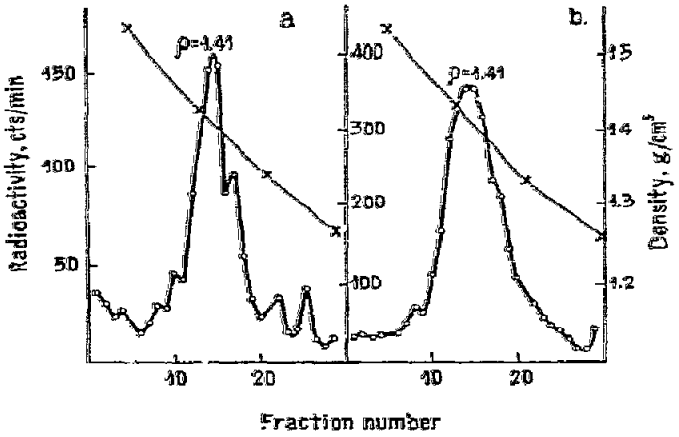

Fig. 2. Density distribution in the CsCl gradient of artificial particles obtained upon addition of *E. coli* 23 S [^{14}C]RNA to: a) "35 S"-protein, and b) "4 S"-protein. Centrifugation in a SW-39 rotor at 36000 rpm for 20 hr.

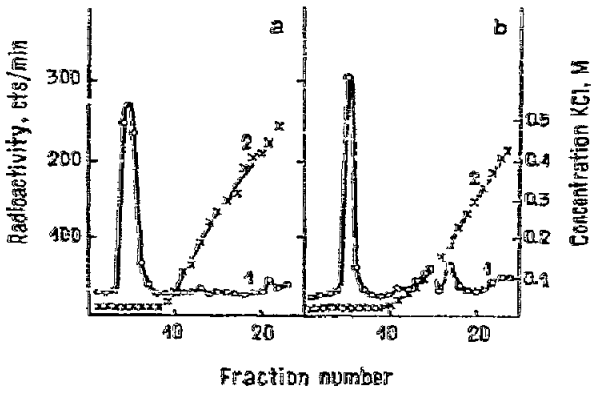

Fig. 3. Chromatography of the "35 S"-protein (a) and "4 S"-protein (b) on carboxymethylcellulose. The preparations for chromatography were obtained by centrifugation of the nuclear extract in the sucrose gradient (see fig. 1). Elution was done with the KCl gradient on a buffer containing 0.01 M TEA, pH 7.8, 0.001 M $MgCl_2$, 0.001 M mercaptoethanol. 1: radioactivity; 2: KCl concentration.

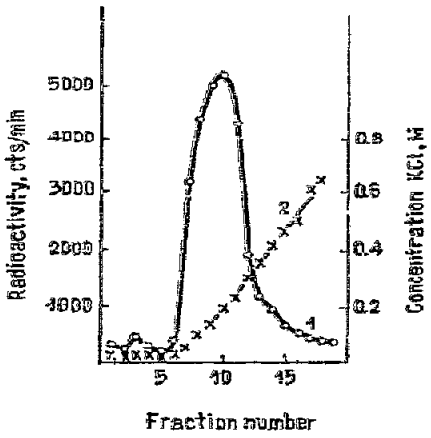

Fig. 4. Chromatography of the nuclear extract on diethylaminoethylcellulose. Prior to chromatography the nuclear extract was dialyzed against a buffer containing 0.01 M TEA, pH 7.8, 0.01 M KCl, 0.001 M $MgCl_2$, 0.031 M mercaptoethanol. Elution with the KCl gradient was done in the same buffer. 1: radioactivity; 2: KCl concentration.

where the component eluted before the salt gradient is not observed (fig. 4). It is seen that all the RNA-binding activity of the extract appears as one peak, eluting from the column at a 0.15 M KCl concentration.

4. Conclusion

Just as in cytoplasmic extracts [1–4], free RNA-binding proteins are present in the nuclear extract of rat liver cells. These proteins are shown up by their ability to retain RNA on nitrocellulose filters (RNA-binding activity). The common property of nuclear and cytoplasmic RNA-binding proteins is their ability to form stoichiometric complexes with RNA in the excess protein with a buoyant density of 1.4 g/cm^3 (informosome-like particles). However, unlike cytoplasmic proteins with a sedimentation coefficient of

8–10 S, nuclear proteins have a sedimentation coefficient of 35 S and 4 S. Moreover, the nuclear RNA-binding proteins are homogeneous according to their charge and do not contain a basic fraction, in contrast to the cytoplasmic ones.

It is interesting that the protein moiety of nuclear informosomes (the so-called "informofer" according to Georgiev and co-workers) has a sedimentation coefficient of about 30 S [6], which coincides with the sedimentation coefficient of one of the components of the nuclear RNA-binding protein.

The significance of the differences demonstrated between cytoplasmic and nuclear RNA-binding protein factors is not known. Nuclear and cytoplasmic RNA-binding proteins can differ in primary structure, i.e., be different proteins. However, it can also be assumed that the difference in the properties of nuclear and cytoplasmic proteins is stipulated only by different combinations or degree of aggregation of similar subunits. For example, different ionic conditions in the nucleus and cytoplasm and also the presence (or absence) of some co-factors or additional protein subunits can lead to a different degree of association of similar RNA-binding protein subunits.

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