ISOLATION OF RNA-BINDING PROTEINS FROM RAT LIVER NUCLEAR 30 S-PARTICLES

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1. Introduction

In order to detect the significance of the relatively large proportion of protein of nuclear 30 S-particles which accounts for about 80% of the particle weight [1] several investigations have been performed [2–5]. Recently, it has been shown that the protein moiety comprises enzyme components involved in the processing of DNA-like RNA [6]. Another inherent property of 30 S-particles is their ability to bind extra RNA added in vitro [7]. This reaction possibly reflects a function of the particles in vivo and studies on it further offer an approach to certain aspects of their structural organisation. The binding may be explained from two principal modes of interaction: (i) The affinity to DNA-like RNA depends on the complex quaternary structure [8] of the whole intact particle or (ii) is conferred to the particle by some of its subunits or components, most likely proteins [9].

The results presented in this report are in favour of the second alternative. They show that the RNA-binding property of the particles can be attributed to extractable proteins which are highly active in the RNA-binding assay described previously [10]. The isolated proteins had molecular weights of 25 000 to 42 000. From studies on the in vitro incubation of the particles in the presence of [^{32}P] ATP it appeared that the extractable material also contained one or more phosphoprotein components.

2. Materials and methods

30 S-particles from rat liver nuclei were isolated as described in a previous report [2]. Fractions corresponding to the 30 S region of the gradients were pooled and the particles sedimented by centrifugation at 50 000 rpm for 3 hr in the rotor 50 Ti of the Spinco centrifuge. The pellets thus obtained from the nuclear extract of 5 livers were gently resuspended with 1 ml of the suspension buffer (2°C) containing 0.045 M Tris–HCl, pH 8.3, 0.1 mM EDTA, 0.1 mM dithioerythritol (DTE), 0.4 M KCl, and after 60 min homogenized in a small Dounce homogenizer using pestle B. The slightly turbid suspension was kept at ice temperture for about 15 hr and then 1 ml of the same solution was added. Unsoluble material was removed by centrifugation at 15 000 rpm for 10 min. The supernatant was layered on 5–20% sucrose gradients prepared with suspension buffer, and centrifuged at 36 000 rpm for 22 hr in the Spinco SW 40 rotor. Aliquots of the gradient fractions were diluted with suspension buffer lacking the KCl to reduce the concentration to 0.1 M KCl. The RNA-binding assay was then performed as described previously [10].

The labeling of phosphoproteins of the 30 S-particles in vitro and the preliminary identification of protein-bound ^{32}P-phosphate were done according to the procedures described by Eil and Wool with ribosomes [11]. Pellets of nuclear particles were suspended in a total volume of 1 ml of a solution containing 0.04 M Tris–HCl, pH 7.7, 6 mM $MgCl_2$, 4 mM β-mercaptoethanol, 5% glycerol, and incubated for 15 min at 37°C after the addition of 50–100 μCi [γ-^{32}P]ATP (1320 mCi/mM; Amersham-Buchler, Braunschweig). Then, 1 ml of this solution plus 0.8 M KCl was added. Further treatment and centrifugation of the suspension were as given above. Polyacrylamide disc electro-

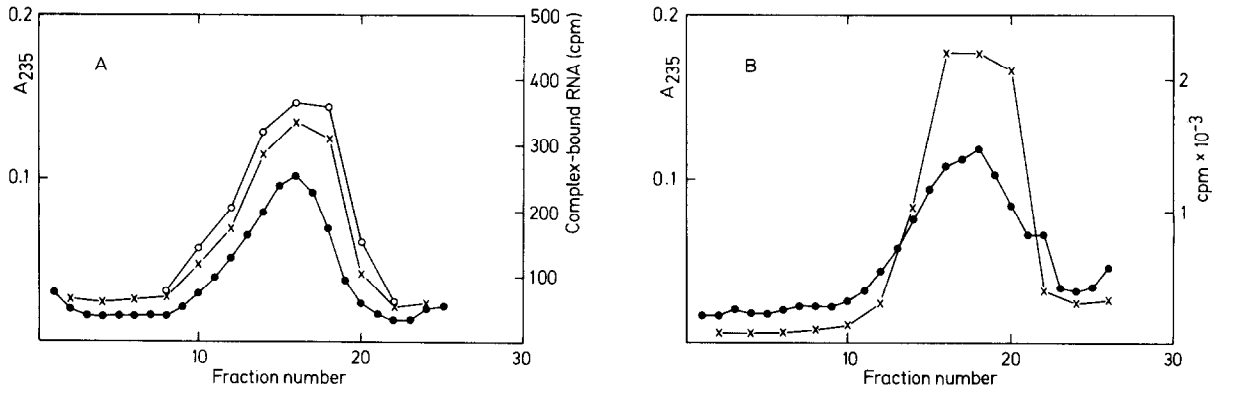

Fig. 1. Density gradient centrifugation of proteins dissociated from 30 S-particles. 5–20% Sucrose gradients in suspension buffer were centrifuged for 22 hr at 36 000 rpm. (A), extract from unlabeled particles: 0.25 ml aliquots of the 1 ml fractions were diluted and assayed for RNA-binding activity without (o——o) or with previous treatment by CM-cellulose (X——X). 2 mg CM-cellulose (dry-weight) in buffer plus 0.1 M KCl was added to the samples and removed by centrifugation after 15 min (2°C). •——•, A_{235}. (B), extract from particles labeled with [^{32}P]ATP in vitro: Protein-bound ^{32}P-phosphate (X——X) was determined according to Eil and Wool [11] in 0.25 ml of the 1 ml fractions.

phoresis in the presence of sodium dodecylsulfate, calculation of relative molecular weights of the peptides, and the scanning of stained gels were performed as indicated in a previous communication [12].

3. Results

Fig. 1A illustrates the separation by density gradient centrifugation of material removed from the 30 S-particles at the ionic strength of 0.4 There is one slowly sedimenting broad peak for which an S value of 3–6 has been calculated using hemoglobin and bovine serum albumin as references. Fractions of the peak region were highly active in the RNA-binding assay and it appears that the activity coincides with the distribution of protein as indicated by the profile of optical densities at 235 nm.

In this experiment the possibility has been checked that fractions of the density gradient contain traces of histones, mainly F1, extracted by the suspension buffer [13] from small amounts of a chromatin contaminant eventually present in preparations of the 30 S-particles and which would also bind to added RNA. To exclude histones, aliquots of the diluted fractions were treated with CM-cellulose prior to the assay as described in the legend to fig. 1. The two curves of RNA-binding activity in fig. 1A represent values obtained with untreated (upper curve) and treated aliquots (lower curve). As can be seen there is little difference which fact evidences that the binding activity may not be ascribed to the presence of histones.

The centrifugation of proteins dissociated from 30 S-particles that had been labeled previously in the presence of [^{32}P] ATP in vitro is shown in fig. 1B. In this case, a radioactivity peak has been detected together with protein at roughly the same position as the peak of RNA-binding activity in fig. 1A. It seems to be reasonable to assume that the labeled material was phosphoprotein as the label was (1) insoluble in 10% TCA at 90–95°C, (2) hydrolyzed by 1.0 N NaOH at 100°C, and (3) could be identified by autoradiography with protein bands after disc electrophoresis.

Fractions of the protein peak after density gradient centrifugation were analysed by polyacrylamide disc electrophoresis in the presence of SDS. A typical separation of protein isolated from unlabeled particles is shown in fig. 2a and the corresponding densitogram (fig. 3A). For the single heavy band on this gel a peptide molecular weight of 39 000 has been determined. The slightly asymmetrical shape of the band was due to the presence of material of somewhat lower respectively higher molecular weight. A second very faintly staining component had a molecular weight of 72 000. This band pattern could be revealed reproducibly with all fractions of the protein peak of gradients as in fig.

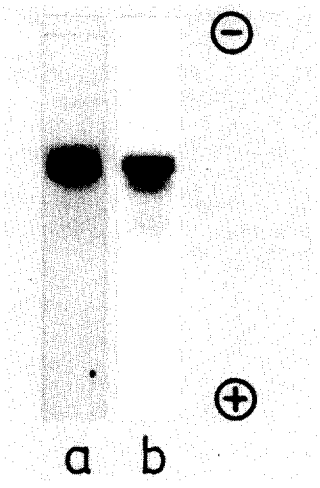

Fig. 2. Sodium dodecyl sulfate polyacrylamide disc electrophoresis of particle proteins isolated by density gradient centrifugation. (a), protein from unlabeled particles (peak, fig. 1A) and (b), from particles after labeling with [^{32}P]ATP (peak, fig. 1B).

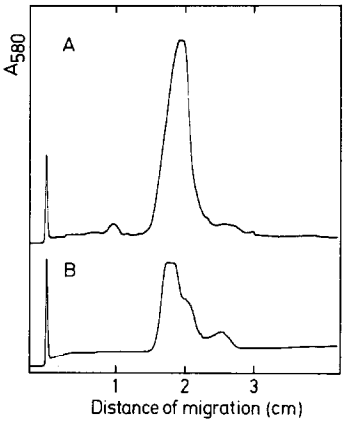

Fig. 3. Absorbance profiles of sodium dodecylsulfate gels shown in fig. 2. Profile 3A corresponds to gel 2a, 3B to gel 2b.

1A. With protein removed from the labeled 30 S-particles 3 distinct bands could be detected with molecular weights of 25 000, 36 000 and 42 000 (fig. 2b and 3B). It appeared that the two components of higher molecular weight had identical mobilities as the proteins migrating close to the main band in fig. 2a.

4. Discussion

Previously, preparations of proteins of nuclear 30 S-particles have been isolated and stabilized using urea-containing solutions. In this report it is shown that a fraction of these proteins may also be obtained in a buffer-soluble not denatured state. The components thus isolated belong to the group of low molecular weight species which are considered as the main proteins of the particles. From the high RNA-binding capacity and S-values of 3–6 respectively 4.5 it could be suggested that the material described in this communication and a nucleosol factor reported by Voronina [14] are related proteins, possibly after having been integrated into the particle structure at the one hand and in a free state.

The labeling experiments confirm the findings of Gallinaro-Matringe and Jacob [15] that the 30 S-particles also contain phosphorylated proteins. They further show that the particles possess proteinphosphokinase activity which may be demonstrated in the in vitro assay. It appears that soluble RNA-binding proteins of the nuclear particles could be used in studies on the question whether and to what extent phosphorylation of the binding proteins and their affinity to RNA are correlated phenomena.

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