MAMMALIAN PROTEINS WITH AFFINITY TO POLYNUCLEOTIDES: ISOLATION BY AFFINITY CHROMATOGRAPHY FROM RAT LIVER CYTOSOL AND NUCLEOSOL

A. SCHWEIGER and G. MAZUR

Max-Planck-Institut für Biochemie, Abt. Hannig, 8033 Martinsried bei München, Germany

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1. Introduction

The mechanism of nucleo-cytoplasmic transport of mRNA in eukaryotic cells is still little understood especially in details concerning the participation of proteins and their significance or function in this process. It has been suggested that nuclear 30 S-particles or components there of are involved as vehicle protein [1–4] or that mRNA and its cytoplasmic precursor are associated with proteins which are not derived from nuclear particles and appear to be changed at certain stages of the life cycle of the RNA [3,5]. In the case of mRNA–protein structures of mammalian polyribosomes a protein with a mol. wt. of 78 000 has been shown to be bound to the polyadenylate segment of the RNA [6] which fact indicates that this region may serve as a binding site for specific proteins. The idea that various of the proteins mentioned above may not exist exclusively as ribonucleoproteins but also in the RNA-free state has been promoted by the finding that the soluble fraction of nuclear or cytoplasmic extracts of eukaryotic cells contain 'RNA-binding' proteins [7–10]. Most attempts, however, to reveal such similarities or even identity were complicated by difficulties in the isolation of the binding proteins.

In the present report a procedure is described which allows the separation of soluble binding proteins from rat liver nuclei and cytoplasm on the basis of their affinity to Sepharose 4B-polyadenylate. Two fractions were obtained at KCl concentrations of 0.05 and 1.0 M. In most experiments poly(A) was used in the affinity chromatography because of several advantages such as stability and good separation properties and also because of a supposed ability to retain, among other proteins, true poly(A)-binding components.

2. Materials and methods

Livers of young Sprague–Dawley rats were homogenized with 3 ml per liver of 20 mM Tris–HCl, pH 7.6, 50 mM KCl, 5 mM $MgCl_2$, 5 mM β-mercaptoethanol, 0.25 M sucrose as described [11]. To the microsomal supernatant solid KCl was added to a final concentration of 0.15 M and it was then centrifuged for 3 hr at 50 000 rpm in the 50 Ti rotor of the Spinco centrifuge. The clear solution intermediate between the sediment and the lipid layer was carefully removed from the tubes and used as the cytosol fraction (about 1–2 ml per liver). A soluble fraction of rat liver nuclei (nucleosol) was obtained from the saline extract at pH 8.0 according to Samarina et al. [12] by centrifugation for 3 hr at 50 000 rpm.

The Sepharose–polyadenylate was prepared following the procedure described by Wagner [13]. Five g of CNBr-activated Sepharose 4B (Pharmacia, Frankfurt) was gently stirred with 10 mg potassium-polyadenylate (Boehringer, Mannheim) at 4°C for 15 hr. The amount of covalently linked poly(A) was then 0.3–0.4 mg/ml gel. Sepharose 4B-poly(U) and CM-cellulose were products of Pharmacia, Frankfurt and Serva, Heidelberg, respectively. After washing and equilibration the Sepharose–polynucleotides and the CM-cellulose were removed from the column and suspended in 30 mM Tris–HCl, pH 8.3, 0.1 mM

EDTA, 0.1 mM dithioerythrol (chromatography buffer). Usually, 2–6 ml of the supernatant fraction was slowly added to 7 ml gel in a total vol of 120 ml. After stirring in the cold for 2 hr the gel was washed in the column with chromatography buffer, and elution was accomplished in a stepwise manner with 0.05 and 1.0 M KCl in the same buffer.

The labelling of phosphoproteins in the presence of ^{32}P-γ ATP was done according to the procedures of Ljungström and Engström [14] with the cytosol and as indicated previously [15].

The in vitro RNA-binding assay, and analytical separation and identification of the proteins by SDS polyacrylamide disc electrophoresis were performed as described in earlier communications [10,11].

3. Results

The binding proteins were separated into components of low and relatively high affinity to the polynucleotides by stepwise elution with 0.05 and 1.0 M KCl in chromatography buffer. This technique proved to be more practicable than ionic strength gradient elution which led to dilute fractions with no or little increase in separation. Fig. 1A gives the profile obtained on Sepharose–poly(A) with the cytoplasmic supernatant. Both eluates at 0.05 and 1.0 M KCl concentration which will be referred to as peak 1 and 2 respectively contained sizeable amounts of protein. The distribution of binding proteins from nucleosol (fig. 1B) was essentially the same as with those from the cytoplasmic supernatant except that the yield in peak 1 was much lower.

Fig. 1A and B also illustrate that proteins in peak 1 and peak 2 were active in the in vitro RNA-binding assay described previously [10]. When the supernatant proteins were incubated with ^{32}P-ATP prior to affinity chromatography we have found in peak 2 recovered from the nucleosol fraction considerable amounts of the label (fig. 1B) which was stable against hot TCA in the procedure described by Eil and Wool [16] and thus may be contained in phosphoproteins. Chromatography of the cytosol on Sepharose–poly(U) under the same conditions gave a similar pattern as in the poly(A) experiment depicted in fig. 1A. Control chromatography on CM-cellulose which is also polyanionic led to small amounts of protein only eluting at 0.05 M

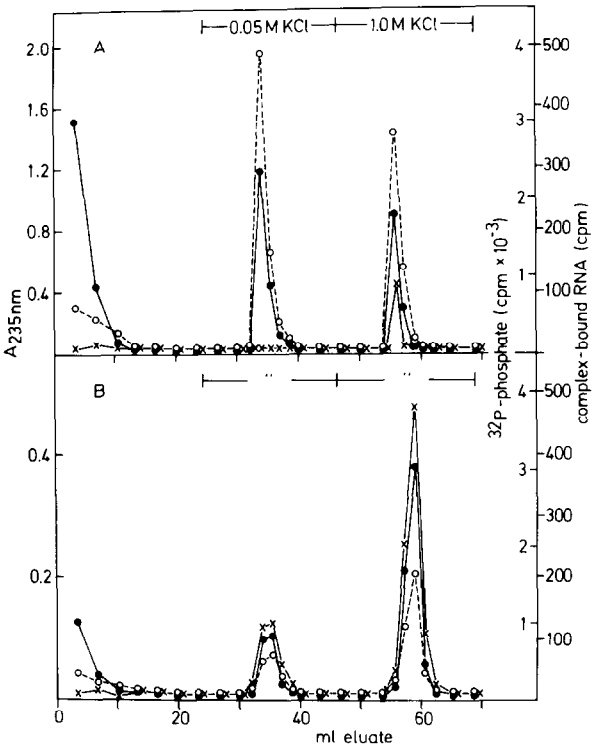


Fig. 1. Affinity chromatography on Sepharose 4B–polyadenylate of proteins of rat liver cytosol (A) and nucleosol (B). Binding proteins were eluted from a 0.9 × 12 cm column (7.5 ml gel volume) by stepwise elution at 0.05 M and 1.0 M KCl concentration. 0.25 ml aliquots of the fractions were diluted to 0.1 M KCl and assayed for RNA-binding activity (o---o). Protein-bound ^{32}P-phosphate (X——X) was determined according to Eil and Wool [16] in 1.0 ml aliquots. •——•, A_{235} nm.

KCl; negligible traces could be detected when CNBr-Sepharose inactivated by ethanolamine was applied.

Proteins from the various fractions obtained by affinity chromatography were analysed by polyacrylamide disc electrophoresis in the presence of SDS. Fig. 2b and the corresponding densitogram (fig. 3B) demonstrate the typical composition of material from cytosol peak 1 isolated by Sepharose–poly(A). The clear pattern shows one heavy double band and one less intensely stained band with relative mol. wt. of 43 000, 45 000 and 37 000 respectively. Similarly, 2 components with low mol. wt (44 000 and 51 000) could be detected in the case of protein from peak 1 of the nucleosol chromatography (fig. 2a and fig. 3A). When proteins of the high affinity fractions (peak 2)

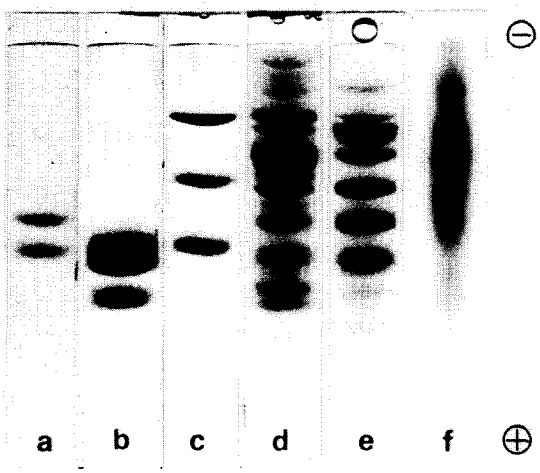


Fig. 2. Sodium dodecylsulfate polyacrylamide disc electrophoresis of supernatant proteins isolated by affinity chromatography on Sepharose–poly(A). a,b, protein from peak 1 of the nucleosol and cytosol respectively; d, protein from peak 2 of the cytosol; e, protein from peak 2 of the nucleosol; c, mixture of 10 μg each of ovalbumin, bovine serum albumin and β-galactosidase. Gels contained 10% acrylamide and half the normal amount of cross-linker as described by Weber and Osborn [17]; f, identification by autoradiography of ^{32}P-labeled proteins on gel 2e: the gel was sliced longitudinally, dried on a filter paper and exposed to X-ray film (Ilford, Industrial G) for 5 days.

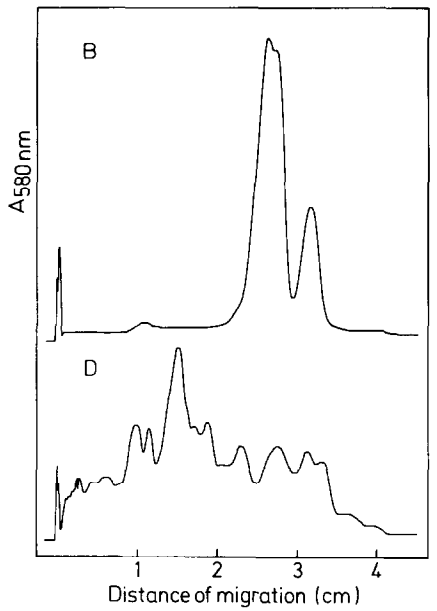
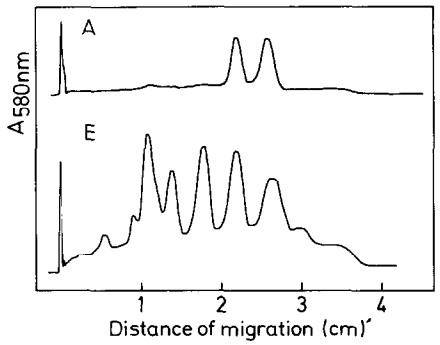


Fig. 3. Absorbance profiles of sodium dodecylsulfate gels shown in fig. 2. Profiles 3A,B,D,E correspond to gels 2 a,b,d,e.

were resolved little material eluting at 0.05 M KCl could be found; species of higher molecular weights predominated. As can be seen from fig. 2d and 3D there was one main band with a mol. wt. of 77 000 after electrophoresis of the cytosol sample. Several minor bands were distributed on the gel in the mol. wt. range from 34 000 to 130 000. A similar pattern was revealed with proteins from peak 2 of the



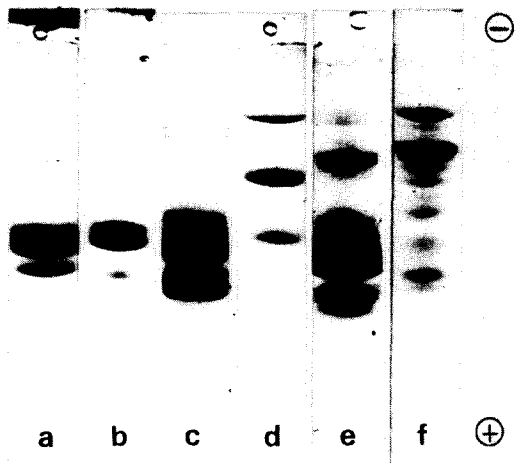


Fig. 4. Sodium dodecylsulfate polyacrylamide disc electrophoresis of cytosol proteins eluted from poly(U)-Sepharose and CM-cellulose, a–c, protein eluted at 0.05 M KCl from CM-cellulose (a), poly(A)-Sepharose (b), poly(U)-Sepharose (c); e,f protein eluted at 1.0 M KCl from poly(U)-Sepharose (e) and poly(A)-Sepharose (f); d, marker proteins. Same conditions of electrophoresis as given in legend to fig. 2.

nucleosol sample as depicted in fig. 2e (see also fig. 3E). Obviously, there were 5 main bands for which mol. wt. of 42 000, 51 000, 63 000, 79 000, and 103 000 were calculated. The pattern of cytosol proteins eluted from the poly(U) column and from CM-cellulose (0.05 M KCl) is shown in fig. 4.

It should be noted that the high speed supernatant of the nuclear extract at pH 7.0 according to Samarina [12] was less suitable as the nucleosol preparation because it showed cytoplasmic components eluting in peak 1 of the chromatography on Sepharose–poly (A). Nucleosol proteins which were labeled with ^{32}P-ATP in vitro are identified by autoradiography in fig. 2f.

4. Discussion

It has been shown in this report that a small fraction of the soluble proteins of eukaryotic cells becomes associated with polynucleotides in vitro and thus may be isolated by affinity chromatography on Sepharose–poly(A) or –poly(U) columns. Several aspects of our findings indicate that the specificity of at least some of the isolated proteins is not strictly directed to one of the polynucleotides used but includes other homopolynucleotides and presumably also ribonucleic acids. Of the two groups of binding proteins that have been separated on the basis of different affinities to poly(A) the high affinity proteins would be expected, however, to comprise, among other components, factor(s) which bind specifically to the poly(A) segment of mRNA.

As the binding proteins have been characterized so far only by their molecular weights in SDS disc electrophoresis it is difficult to evaluate whether they are related to proteins that have been described earlier, associated with nonribosomal RNA in some cases. Of the cytosol proteins isolated in peak 1 of the affinity chromatography on Sepharose–poly(A) the component with a mol. wt of 43 000 (fig. 2b) is identical with the 'basic RNA-binding factor' characterized earlier [11]. The other component (mol. wt 45 000) of the double band is more acidic and may be obtained in a pure state by chromatography on phospho-cellulose (unpublished results). The observation that these two proteins with closely related molecular weights have also very similar amino acid compositions could mean that certain binding proteins exist in different forms which are also revealed as double bands on SDS disc electrophoresis.

Considering the high affinity proteins (peak 2) from the nucleosol preparation, it appears that the component with a mol. wt of 42 000 was identical with one of the main protein species of 30 S-particles. Further, the pattern formed by the other bands on the gel in fig. 2e resembled those that have been described for 'minor proteins' of the rat liver nuclear particles by several authors [18,19]. Among cytosol proteins of peak 2, a component with a mol. wt of 77 000 was prominent in all cases. Its molecular weight and the fact that it was eluted from the Sepharose–poly(A) column with the high affinity fraction suggested that this component was closely related to or identical with a protein specifically binding to the poly(A) segment of mammalian polysomal mRNA [6]. When the disc electrophoretic patterns of peak 2 proteins of the poly(A) chromatography from the nucleosol and cytosol are compared striking similarities may be observed. These findings could mean: (1) that cross contamination occurs i.e. high affinity proteins of cytoplasmic origin are present as contaminants in the nucleosol fraction and vice versa, or (2) that the presence of these proteins in the two compartments of the cell is of physiological significance and indicative of their possible role in the mechanism of nucleo-cytoplasmic transport of RNA.

Very recently, affinity chromatography has been applied in the isolation and characterization of proteins of cytoplasmic ribonucleoprotein particles from plasma tumor cells [20]. Using a method which was also based on the affinity of protein to RNA a binding protein has been isolated from rabbit reticulocyte extract [21].

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