RAT LIVER SOLUBLE NUCLEAR AND CYTOPLASMIC PROTEINS WITH HIGH AFFINITY TO POLYNUCLEOTIDES

A. SCHWEIGER and G. MAZUR

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

Received 6 October 1975

1. Introduction

Recently, we have described the use of affinity chromatography in the isolation and fractionation of mammalian hnRNA-associated proteins [1] and free 'RNA-binding' proteins from soluble cytoplasmic and nucleoplasmic fractions [2]. In this previous study the free binding proteins having interacted with Sepharose-poly(A) or Sepharose-poly(U) were detached at increased ionic strength (up to 1.0 M KCl). The finding, however, that protein components of nuclear 30 S particles can bind so strongly to the poly(U) gel that they were released only in the presence of 50% formamide suggested that (i) also among soluble proteins species of similar high affinity to polynucleotides or RNA exist and that (ii) such proteins could be isolated using the more drastic conditions of elution.

In the present report we show that a fraction of soluble proteins still binds tightly to the Sepharose-polynucleotides applied after washing with high ionic strength buffer. This material recovered by elution with 50% formamide from the poly(A) column reproducibly contained 2 main protein components for which mol. wts of 78 000 and 52 000 were calculated.

2. Materials and methods

Nuclei from 3 rat livers were prepared as described previously [3] and disrupted by sonication [4] in 0.01 M Tris–HCl, pH 7.0, 1 mM $MgCl_2$, 0.14 M NaCl (10 sec, 50–60 W; Sonifier B 12, Branson Comp., Danbury Conn., USA). After centrifugation at 19 000 *g* for 10 min the resulting supernatant was submitted to density gradient centrifugation on 15–30% sucrose gradients (Spinco SW 25.1; 20 000 rev/min, 15 h). A 'cytosol' fraction [2] was centrifuged on identical sucrose gradients.

Affinity chromatography of soluble 'RNA-binding' proteins on Sepharose 4B-poly(A) and -poly(U) was performed exactly as indicated in the earlier communication [2]. After washing the columns with 0.05 and 1.0 M KCl in chromatography buffer, however, an additional elution at 0–4°C was done using a solution of 50% formamide in chromatography buffer plus 0.5 M KCl.

Techniques of identification of protein species by SDS gel electrophoresis and analytical procedures were those that have been described previously [1].

3. Results

Fig.1 gives the profile of optical densities after centrifugation of the nuclear sonicate on a 15–30% sucrose density gradient. The peak of 30 S particles (region N III) is clearly separated from a region which contained the soluble or supernatant components (tubes 28–33, region N I). A third fraction was collected on the 2.0 M sucrose cushion (tubes 4–10, region N IV). Material from N I–N IV as defined in fig.1 was incubated with Sepharose-poly(A) or -poly (U) in chromatography buffer to separate and detect proteins showing high affinity to the polynucleotides. A fraction of soluble cytoplasmic components (C I) corresponding to region N I of the nuclear sonicate was prepared and incubated using the same procedures.

The results of the first steps during affinity chromatography of material from N I and C I were essentially

North-Holland Publishing Company – Amsterdam

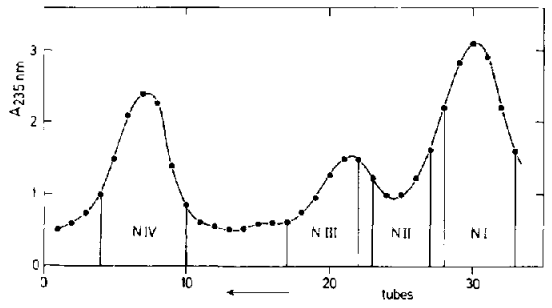

Fig.1. Density gradient centrifugation of a sonicate of rat liver nuclei. Nuclei of 3 livers were homogenized by sonic disruption and centrifuged as indicated under Materials and Methods. At the bottom of the tube a cushion was placed consisting of 5 ml of 2.0 M sucrose in buffer. Fractions were pooled for affinity chromatography according to designations N I-N IV.

identical with those concerning elutions performed with 0.05 and 1.0 M KCl in the case of the nucleosol and cytosol experiments described [2]. In fig.2 the steps are considered which involved 1.0 M KCl and 50% formamide, 0.5 M KCl as eluants. It can be clearly seen that the use of the formamide solution still removed considerable amounts of proteins from

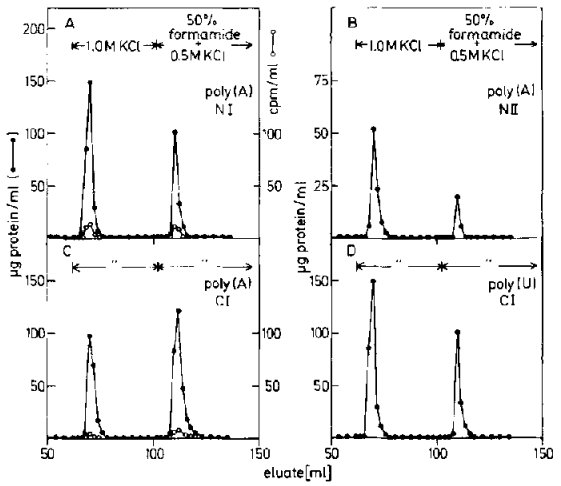

Fig.2. Affinity chromatography of density gradient fractions. A-C, chromatography on Sepharose 4B-poly(A) of nuclear fraction N I (A), N II (B) and cytosol fraction C I (C); D, chromatography of C I on Sepharose 4B-poly(U). Elution was performed as indicated. (●——●) Protein concentration. (○——○) Radioactivity (after labeling with [^{14}C] orotic acid in vivo).

the Sepharose-poly(A) gel washed with 1.0 M KCl. The yield was in the range of 100–150 μg/liver with N I (Fig.2A) and 200–250 μg/liver with C I (fig.2C). The amount of protein eluted with formamide from poly(A) when fractions N II (Fig.2B) and N III–IV (elution profiles not shown) were applied was much lower and decreased from about 20–30 μg (N II) to traces (N IV).

In some experiments the material binding to poly(A) after washing the gel with 1.0 M KCl was further fractionated: About one third of the protein was eluted using 6.0 M urea in chromatography buffer whereas the remaining amount could be obtained with 50% formamide, 0.5 M KCl but not with 50% formamide in chromatography buffer.

No labeled RNA could be detected in the material obtained (compare fig.2A and C) when preparations from animals treated with [^{14}C] orotic acid or [^{14}C] adenine in vivo for 45 min were fractionated.

As has been pointed out in the previous report [2] binding of proteins from supernatant fractions (N I and C I in this work) also occurred when Sepharose-poly(U) was used instead of the poly(A) gel and the amount of protein displaced by formamide was comparable in both cases (see fig.2D). There were, however, significant qualitative differences as revealed by gel electrophoresis (see below).

The fraction of proteins detached from Sepharose-poly(A) with the use of formamide was analysed by SDS gel electrophoresis. As is shown in fig.3 few main components were present in the nucleosol eluate (N I). For these components mol. wts of 78 000 and 52 000 (fig.3d and 5B) and, in addition, 61 000 and 23 000 (fig. 3c and 5A) were calculated. In the region N II of the density gradient which includes endogeneous 14 S poly(A)-protein particles [5] several bands with mol. wts between 38 000 and 105 000 were detected (fig.3b). The same typical band pattern has been obtained, at lower intensities, with the formamide eluates fractionating N III (fig.3a) and N IV.

The protein species with mol. wts of 78 000 and 52 000 were also reproducibly identified as main components in the formamide eluate after affinity chromatography on Sepharose-poly(A) of cytosol fraction C I (fig.3e and 5C). Two additional proteins had mol. wts of 33 000 and 23 000.

In the experiment in which 6.0 M urea has been used as eluant prior to the 50% formamide, 0.5 M KCl

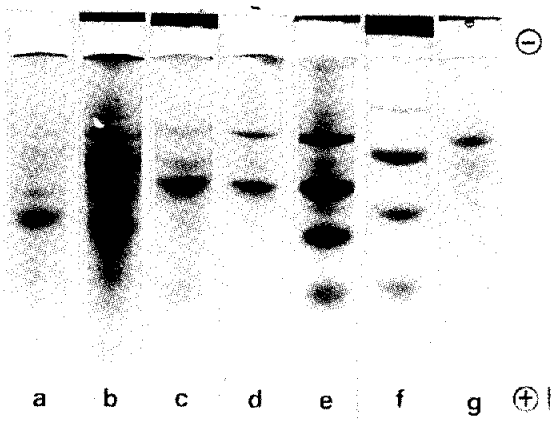

Fig.3. Sodium dodecylsulfate gel electrophoresis of proteins isolated by affinity chromatography on Sepharose 4B-poly(A) using 50% formamide, 0.5 M KCl. a–e, protein of N III (a), N II (b), N I (c and d) and C I (e); (f), mixture of marker proteins consisting of 10 μg each of chymotrypsinogen, ovalbumin, bovine serum albumin and β-galactosidase; (g) mRNA-protein particles isolated by affinity chromatography on Sepharose 4B-poly(U) [6].

only the two main species with higher mol. wts, together with 4–5 minor components, could be detected in the final eluate (fig.4e).

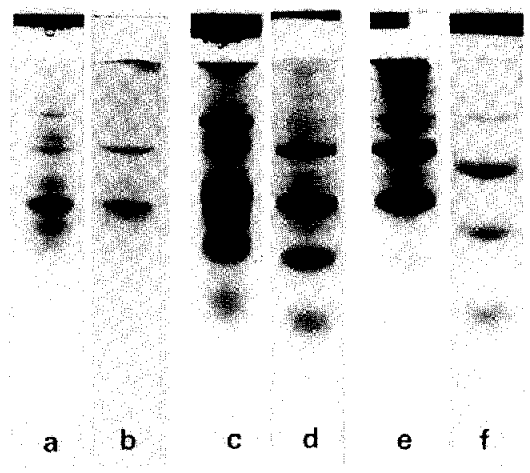

Fig.4. Comparison by sodium dodecylsulfate gel electrophoresis of proteins eluted from Sepharose-poly(A) and -poly(U) using 50% formamide, 0.5 M KCl. (a,b) protein of N I eluted from Sepharose-poly(U) (a) and -poly(A) (b); (c,d) protein of C I eluted from Sepharose-poly(U) (c) and -poly(A) (d); (e) protein of C I eluted from Sepharose-poly(A) washed with 6.0 M urea; (f), marker proteins.

The gel depicted in fig.3g gives as comparison the protein composition of a mRNA–protein complex derived from rat liver polyribosomes and isolated under conditions of base pairing by poly(U) affinity chromatography [6]. The main component on this gel is a protein with the mol. wt of 78 000.

In fig.4a–d proteins of N I and CI eluted by 50% formamide, 0.5 M KCl from Sepharose-poly(U) and -poly(A) are compared by SDS gel electrophoresis. Obviously, the band pattern in case of the poly(A)-isolated components is much clearer while species of identical molecular weight may be identified with both preparations.

4. Discussion

In this report evidence is presented that the soluble fraction of mammalian cells contains proteins which interact very strongly with Sepharose-bound polynucleotides. So far, these proteins have been detached from the affinity reagent only with the use of formamide at high ionic strength and they may be considered, from their binding properties, as a third group of 'RNA-binding' factors in addition to the two groups eluted previously from the poly(A) column with 0.05 and 1.0 M KCl [2].

The presence of significant amounts of protein species with mol. wts of 78 000 and 52 000 in the poly(A) formamide eluates of N I and C I appeared to be particularly interesting as these data may indicate that the two components are identical with or at least closely related to proteins described recently as main constituents of mammalian cytoplasmic mRNA-protein complexes [7–9]. The finding that the two mRNA proteins are most tightly bound and were not removed from the RNA by 0.5 M KCl [7,8] is also in accordance with the fact that the proteins eluted with formamide in this work remain bound to Sepharose-poly(A) after washing with 1.0 M KCl. Assuming that one or more of the proteins described here are indeed identical with mRNA-associated species one could infer that these components exist as free precursor in the soluble fraction of the cell where they are included in the pool of 'RNA-binding' proteins. A similar relationship between free and particle-bound proteins has been reported in the case of nuclear 30 S-particles [10].

Two of the high affinity binding proteins, with mol. wts of 78 000 and 52 000, could be reproducibly identified in the formamide eluates from poly(A) of both the nucleosol (N I) and cytosol (C I). The possibility that proteins isolated from the nucleosol were contaminants and for example of cytoplasmic origin had to be taken into consideration. This explanation, however, appeared to be unlikely because of the fact that the formamide eluate of N I contained relatively large amounts of protein whereas the component with a mol. wt of 33 000 which could always be observed in the corresponding cytosol fraction was completely absent (see fig.5) Further, binding proteins from regions N II and N III of a gradient have been excluded as sources of major contamination in N I also on the basis of quantitative calculations. Therefore, provided that components of the high affinity group of soluble binding proteins described here participate in the mechanism of nucleo-cytoplasmic transport of informational RNA in eukaryotes we suggest that the two proteins mentioned above, in particular the protein with a mol. wt of 52 000, would be probable candidates for this role. The significance of a third protein (mol. wt 23 000) which had also been detected both in nuclei and cytoplasm was not clear.

Obviously, a somewhat different set of proteins (fig.4) was obtained in the 50% formamide eluates when N I or C I were submitted to chromatography on Sepharose-poly(U). The clear separation on poly(A) of molecular weight species that are considered to be poly(A)-associated in endogenous complexes (78 000 in polyribosomal mRNP [8] and 60 000 in rat liver nuclear 14 S-particles [5]) possibly reflects more specific interaction of proteins with this Sepharose-bound polynucleotide. One could further speculate from the distribution between N I and C I of the protein with a mol. wt of 61 000 and its relatively high concentration in N I that this component is a specific species confined to the nucleus.

Our results show that proteins of identical mol. wt may be recovered at different conditions of elution. A protein with a mol. wt of 52 000 for example when eluted from Sepharose-poly(A) was present in the two KCl fractions of the nucleosol [2] and in the formamide fraction. Whether this different binding behaviour means that 'RNA-binding' proteins exist in states of different affinity to the RNA remains to be investigated. Recent experiments on hnRNA-associated proteins in rat brain indicated that phosphorylated species were less easily removed from the RNA at increased ionic strength than non-phosphorylated ones [11].

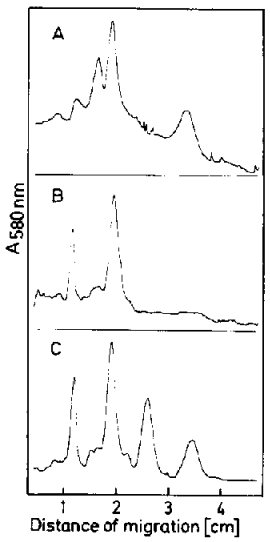

Fig.5. Absorbance profiles of sodium dodecylsulfate gels shown in fig.3. Profile 5A corresponds to gel 3c, 5B to 3d and 5C to 3e.

Acknowledgements

We wish to thank Professor K. Hannig for continuous interest in this work. The valuable technical assistance of Mrs E. Koch and Mrs E. Fiegert is greatly appreciated.

References

[1] Schweiger, A. and Mazur, G. (1975) FEBS Lett. 54, 39–43.
[2] Schweiger, A. and Mazur, G. (1974) FEBS Lett. 46, 255–259.
[3] Schweiger, A. and Hannig, K. (1968) Z. Physiol. Chem. 349, 943–944.
[4] Firtel, R. A. and Pederson, T. (1975) Proc. Nat. Acad. Sci. USA 72, 301–305.
[5] Molnar, J. and Samarina, O. P. (1975) Mol. Biol. Rep. 2, 1–10.

[6] Mazur, G. (1975) Inaugural Dissertation, Universität München.
[7] Blobel, G. (1972) Biochem. Biophys. Res. Comm. 47, 88–95.
[8] Blobel, G. (1973) Proc. Nat. Acad. Sci. USA 70, 924–928.
[9] Barrieux, A., Ingraham, H. A., David, D. N. and Rosenfeld, M. G. (1975) Biochem. 14, 1815–1821.
[10] Schweiger, A. and Schmidt, D. (1974) FEBS Lett. 41, 17–20.
[11] Gallinaro-Matringe, H., Stevenin, J. and Jacob, M. (1975) Biochem. 14, 2547–2554.