FRACTIONATION BY AFFINITY CHROMATOGRAPHY OF PROTEINS OF RAT LIVER NUCLEAR 30S-PARTICLES

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1. Introduction

Studies on the protein moiety of nuclear 30S-particles (dRNP-particles) from various eukaryotic tissues led to different and in some respects quite controversial results [1–8]. Especially, data concerning number and molecular weights of protein species appear to depend much on the source and mode of isolation of the particles and on the method of analytical protein separation [9]. In a recent report [10] we have presented evidence that individual particle proteins are bound to dRNA in a complex structure from which they became extractable at 0.4 M KCl. The isolated components with peptide molecular weights between 25 000 and 42 000 were characterized by the high activity in an in vitro RNA-binding assay. These findings suggested that, in a novel approach, proteins of 30S-particles could be fractionated on the basis of their RNA-binding properties i.e. affinity to RNA. Moreover, such experiments might contribute to the question if there exist in dRNP-particles either specific RNA-binding proteins or the proteins in general bind to RNA, possibly at different affinities. Using Sepharose-polyuridylate and -polyadenylate as affinity reagents the techniques applied in this work were similar to those described recently for the isolation of soluble RNA-binding factors from rat liver [11].

The results show that more than 95% of the protein of the 30S range from a gradient bind to Sepharose-poly(U) at 0.2 M NaCl.

Protein species with average mol. wt of 42 000 and 47 000 and different amino acid composition were eluted at 25% and 50% formamide respectively. The amount of high mol. wt components as detected by sodium dodecylsulfate polyacrylamide gel electrophoresis was significantly reduced in these experiments.

2. Materials and methods

30S-particles from rat liver nuclei were prepared as described earlier [2] except that the period of extraction of the nuclei at pH 8.0 was extended to 4 hr. In all steps of the isolation, reagents of lowest content of heavy metal ions available were used. Prior to affinity chromatography, fractions corresponding to the 30S region of the gradients were pooled and the sodium chloride concentration adjusted to 0.2 M at a pH of 7.8. Techniques of affinity chromatography on Sepharose-poly(U) and -poly(A) have been described [11]. The 0.9 × 12 cm column (7.5 ml gel) was equilibrated with 0.05 M Tris–HCl, pH 7.8, 0.2 M NaCl, 5 mM EDTA, 0.1 mM dithioerythrol (equilibration buffer) and 30S-particles from nuclei of 5 livers (about 3 mg protein) were slowly passed through the gel. After washing with equilibration buffer, proteins were eluted using a gradient of 0–50% formamide in equilibration buffer or in a stepwise manner with 25% and 50% formamide in the same buffer.

When dRNA was labeled, 25–50 μCi of 6-[^{14}C]orotic acid (60.8 mCi/mmol) or 8-[^{14}C]adenine (54.2 mCi/mmol, Amersham–Buchler) per animal was injected intraperitoneally 30 min prior to death.

Analytical separation of proteins by SDS gel electrophoresis and determination of amino acid composition were performed as indicated previously [11,12]. Protein was precipitated in 15% TCA and

washed with 80% ethanol. For amino acid analysis the material was further treated with pancreas ribonuclease (5 μg/ml) and reprecipitated. Acid precipitable radioactivity was collected on cellulosenitrate membranes and determined by liquid scintillation counting. Protein was estimated according to the method of Lowry et al. [13], using bovine serum albumin as a standard.

3. Results

When 30S-particles were applied to the Sepharose-poly(U) column about 97% of the total protein was retained, 3–4% could be detected with the flow through fraction. The binding of protein to the gel was remarkably stable against washing at 1 M NaCl as this treatment did not remove more than 5–8% of the total protein. Elution of the major portion of it was only accomplished using urea or formamide as eluants. Fig.1 and fig. 2A illustrate typical experiments in which proteins were detached with a 0–50% formamide gradient (fig.1) or in a stepwise manner with 25% and 50% formamide (fig.2A). Gradient elution led to the partial separation of two peaks at formamide concentrations of about 15% and 30% which were also observed in the stepwise procedure. Peak 1 could be obtained in a similar way using 6 M urea, the elution of peak 2 was restricted to the presence of formamide. Most of the labeled RNA has been detected in the flow through, in the 25% formamide peak and in a fraction eluted at 50% formamide, 0.5 M NaCl. It should be noted that with the use of 50% formamide as the final eluant recoveries of protein were consistently close to 100%.

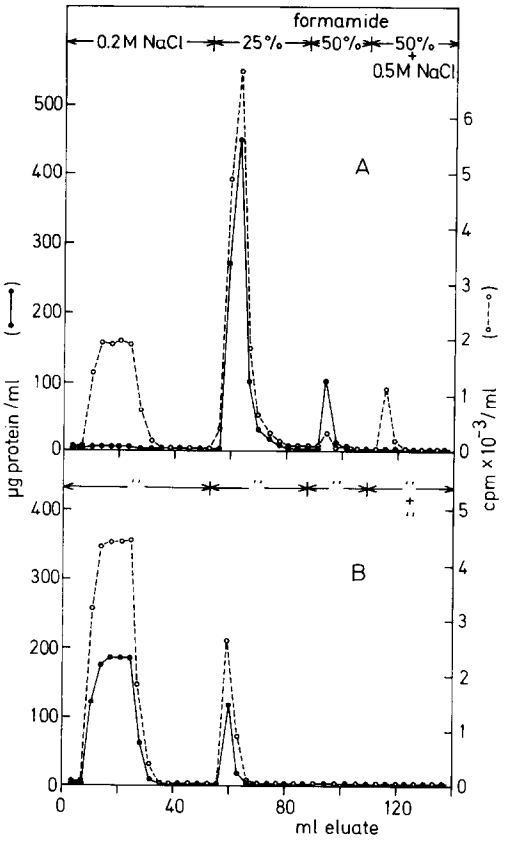

Fig.2. Affinity chromatography on Sepharose 4B-polyuridylate (A) and -polyadenylate (B) of rat liver 30S-particles. Elution was performed in a stepwise manner as indicated.

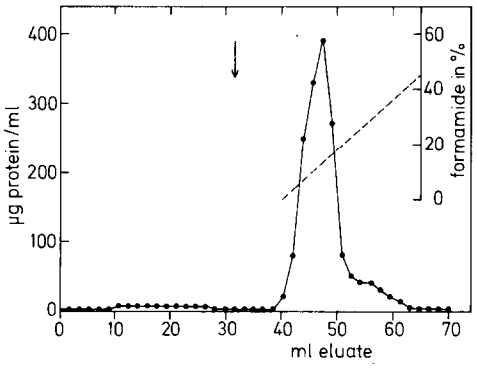

Fig.1. Affinity chromatography on Sepharose 4B-polyuridylate of rat liver 30S-particles. Proteins were eluted with the use of a 0–50% formamide gradient. Material not retained by the gel was recovered in fractions between 10 and 28 ml eluate. The arrow indicates beginning of the gradient elution.

To evaluate the specificity of the protein–polynucleotide interaction the affinity of 30S-particles or their proteins to Sepharose-poly(A) was studied under identical conditions as in the poly(U) experiment. Fig.2B shows that less than 15% of the protein bound to the gel; this fraction was completely eluted with 25% formamide (or 6 M urea).

Separated dRNP proteins were analysed by SDS polyacrylamide gel electrophoresis and the distribution of bands compared with the typical pattern obtained with total proteins from 30S-particles as shown in fig.3a. This pattern corresponds with the composition

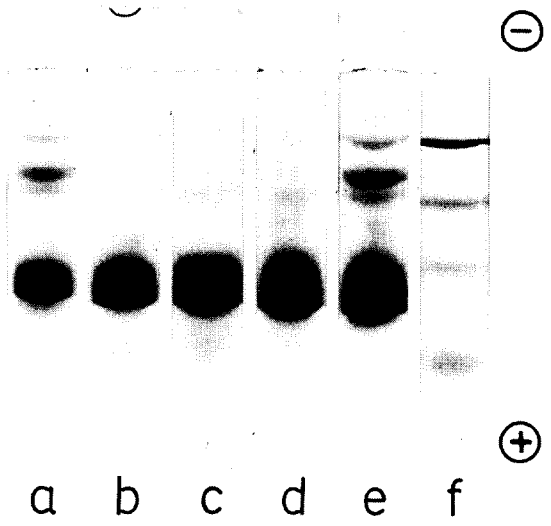

Fig.3. Sodium dodecylsulfate gel electrophoresis of total protein of rat liver 30S-particles. Pelleted particles were suspended in 0.5 ml of the gradient buffer, pH 8.0. To 0.1 ml aliquots were added 2 ml each of the gradient buffer (a); 6 M urea (b), 25% formamide (c), 50% formamide (d) all in equilibration buffer; and 50% formamide in equilibration buffer, 0.5 M NaCl (e). Protein was then precipitated by the addition of TCA to a final concentration of 15% and, after washing it with 80% ethanol, incubated with the SDS buffer. On gel 3f a mixture of 10 μg each of chymotrypsinogen, ovalbumin, bovine serum albumin and β-galactosidase was separated.

described earlier for proteins from the same source [5]. In order to exclude possible errors due to the influence of formamide on the mobilities of individual components in gel electrophoresis, aliquots of a particle sediment were also dissolved, prior to electrophoresis, in the solutions used as eluants. Figure 3, b–e illustrate that these treatments were virtually of no effect on the mobility of main protein bands. Protein from the flow through fractions of the poly(U) column was identified as 4–6 very faintly staining components with mol. wt between 22 000 and 130 000 (fig.4a). Analysis of protein from fractions of increasing affinities for poly(U) revealed that the species separated had also slightly differing mol. wt from about 40 000 (fig.4, b–c) to 50 000 (fig.4d). These differences were even more conspicuous when poly(U)-bound proteins were eluted with 6 M urea prior to the 25% and 50% formamide steps (distribution of protein not shown).

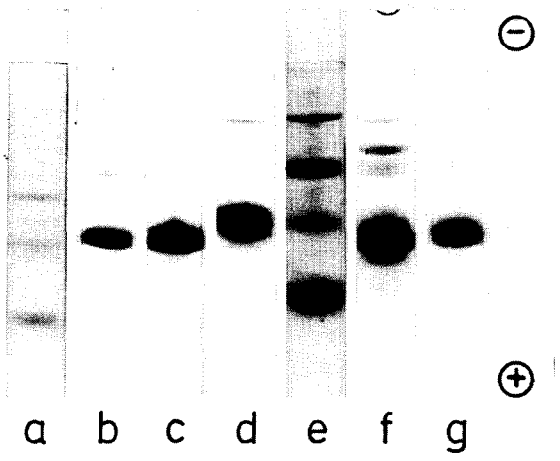

Fig.4. Sodium dodecylsulfate gel electrophoresis of particle proteins after affinity chromatography. a–d, protein eluted from Sepharose-poly(U) in the experiment 2A: a, flow through; b–c, peak fractions of 25% and d, 50% formamide eluates. f–g, protein from Sepharose-poly(A) in experiment 2B: f, flow through; g, 25% formamide eluate; e, marker proteins.

Figure 5, a–d give the SDS pattern of subsequent fractions obtained in this experiment. On the other hand, there was no such separation of main protein

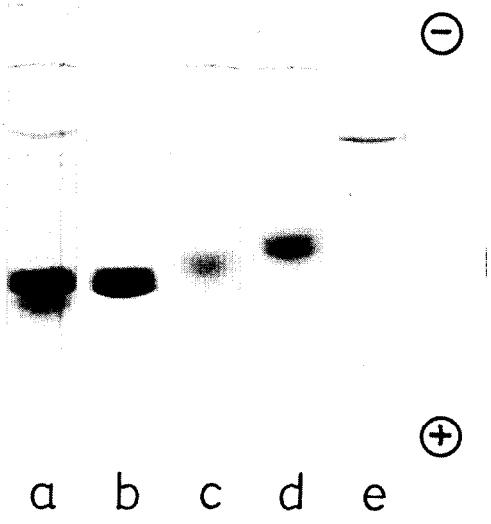

Fig.5. Sodium dodecylsulfate gel electrophoresis of particle proteins after affinity chromatography on Sepharose-poly(U). a–b, protein eluted at 6 M urea; c, 25% and d, 50% formamide eluates. e, marker proteins.

Table 1
Amino acid composition of proteins separated from rat liver nuclear particles by affinity chromatography on Sepharose-poly(U) and free-flow electrophoresis in the presence of 5 M urea [2,15]

Amino acid	proteins eluted from poly(U)				proteins after free flow electrophoresis
	25% formamide			50% formamide	
	1	2	3	4	
Lys	7.1	8.3	8.1	13.0	5.6
His	3.2	3.3	3.4	2.6	2.1
Arg	5.7	5.9	5.3	3.5	5.7
Asp	9.8	10.1	11.5	7.4	11.6
Thr	3.4	3.2	3.1	4.1	3.6
Ser	6.2	6.4	6.3	6.3	6.7
Glu	11.6	9.7	10.5	15.0	9.2
Pro	4.7	4.1	4.0	2.4	4.0
Gly	21.9	22.7	22.5	15.2	26.1
Ala	4.6	4.4	3.7	7.5	4.0
Val	4.4	3.7	4.4	4.2	4.0
Met	–	1.2	1.4	1.7	–
Ile	2.8	2.5	2.2	3.6	2.9
Leu	4.2	3.2	3.1	4.4	2.9
Tyr	4.9	5.4	4.2	4.6	6.5
Phe	5.3	5.8	5.9	4.3	5.5

200 μg protein from each of 3 subsequent peak fractions eluted by 25% formamide and the fraction eluted by 50% formamide in the experiment illustrated in fig.2A was analysed. Amino acid compositions are expressed in terms of moles per 100 mol of recovered amino acids, uncorrected for hydrolytic loss, or for amides of aspartic or glutamic acids.

species in poly(A) chromatography as can be seen from fig.4, f and g.

Amino acid compositions of proteins separated on the poly(U) gel are shown in table 1. The first three columns (1–3) have been obtained when subsequent fractions eluted at 25% formamide were analysed; the last column (4) gives the analysis of protein detached with 50% formamide. Obviously, there are significant differences between fractions 1–3 on the one hand and fraction 4 concerning mainly values of basic and acidic amino acids. The figure for glycine which is relatively high with all preparations of dRNP protein [3,14] is lower in fraction 4.

4. Discussion

The present report shows that the major part of the proteins of a 30S nuclear fraction binds to poly(U) on the basis of non-ionogenic forces. Most likely, the proteins become associated directly with the nucleic acid. It is, however, not possible at present to completely rule out the binding of particle proteins to poly(U) via base-paired poly(A)-containing RNA though the probability for this seems to be low when recent data on the poly(A) content of 30S-particles from rat liver [16, own unpublished results] and ascites carcinoma cells [16,17] are considered.

The large amount of 'main protein' species in the mol. wt range of 40 000–50 000 was detached at a 100% recovery using formamide and fractionated into components of increasing affinities to poly(U). Apparently, the fractions eluted with 25% and 50% formamide, 0.2 M NaCl contained proteins or groups of proteins which were distinguishable from the affinities to poly(U), the SDS mol. wts and their amino acid com-

position. The remarkably tight binding to poly(U) of the 50% formamide eluted protein and its specificity as concluded from the poly(A) experiment suggest that this protein may have a similar affinity in vivo, possibly binding to oligo(U) stretches of HnRNA [18]. Whether it is related to the mRNA-associated protein with a mol. wt close to 50 000 [19] remains to be investigated.

The treatment of sedimented or poly(U)-associated nuclear particles with 6 M urea and, more efficiently, 25% or 50% formamide resulted in the decrease of high molecular weight bands which were normally detected on SDS gels of total particle proteins. Calculations showed that this effect was not due to merely separating high molecular weight proteins in the flow through of the poly(U) column as the 3–4% of total protein detected in these fractions could definitely not account for the amount localized in bands with mol. wts higher than 50 000 which constituted at least 10–15% of the stained material on a gel (fig.3a). It is difficult at present to explain the phenomenon but one may assume that substances as formamide at low or moderate ionic strength can dissociate for example highly resistant residual complexes (oligomers) between certain nucleotides and low mol. wt proteins.

Our results indicate that the protein moiety of rat liver nuclear particles is composed of a limited number of species and thus are in favour of a relatively simple structure of the particles as initially proposed by Georgiev [3], and recently by Martin et al. [8].

Acknowledgements

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