THE PROTEIN MOIETY OF NUCLEAR RIBONUCLEOPROTEIN PARTICLES CONTAINING DNA-LIKE RNA: PRESENCE OF HETEROGENEOUS AND HIGH MOLECULAR-WEIGHT POLYPEPTIDE CHAINS

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1. Introduction

In the cell nucleus probably all DNA-like RNA is complexed to proteins thus forming ribonucleoprotein particles (RNP particles) of different size [1]. Their protein moiety has been characterized by polyacrylamide gel electrophoresis in several laboratories [1–9]. If subjected to polyacrylamide gel electrophoresis at pH 4.5 in the presence of 6 M urea, the protein component of the RNP particles migrates as a slightly basic protein fraction showing a quite different pattern of band distribution as compared to ribosomal proteins, histones or proteins from the nuclear sap [1, 2, 5, 8]. The occurrence of only one main band [6, 7] or three main bands [1–5, 8] in disc gels suggested a simple structural organization of the protein moiety with only a few proteins involved.

Recent experiments from Krichevskaya and Georgiev [10] have indicated that the different electrophoretic mobilities of the three main bands observed in polyacrylamide gels are due to the formation of disulphide bonds between identical protein subunits. Upon treatment with β-mercaptoethanol the conversion into one main band is observed. From this and additional experimental evidence it was concluded by these authors that the protein component of one 30 S RNP particle consists of 15–20 identical protein subunits with a molecular weight of about 40,000–45,000.

Here we wish to present evidence that the protein moiety of the RNP particles is composed of at least ten different polypeptide chains with molecular weights ranging from 32,000 to 130,000. The same proteins, however, migrate as a single band if analysed on polyacrylamide gels at pH 4.5 in the presence of 6 M urea.

2. Methods

30 S RNP particles were isolated from purified rat liver nuclei according to Samarina et al. [1] as previously described [6]. The RNP particles were sedimented at 360,000 *g* for 4 hr and dissolved in 2 M LiCl–6 M urea. The RNA precipitated was removed by centrifugation and the supernatant fraction dialysed against the appropriate buffers for electrophoresis. Alternatively the RNP particles were collected from the sucrose gradient and concentrated in 60% dextrane 500 T.

Gel electrophoresis of proteins was carried out at pH 4.5–6 M urea (10% acrylamide) according to Reisfeld et al. [11] and Leboy et al. [12] with the modification that the proteins were dialysed against 6 M urea containing additional 0.14 M β-mercaptoethanol [10]. For electrophoresis at pH 11.6 in the presence of 4 M urea (10% acrylamide) the technique of Benjamin and Gellhorn [13] was employed. Molecular weight determinations of the polypeptide chains of the RNP particles by sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis was carried out according to Shapiro et al. [14] on gels containing 10% acrylamide and half the normal amount of cross-linker as described by Weber and Osborn [15]. Prior to electrophoresis the proteins were incubated at 37° for 4 hr in 0.01 M sodium phosphate pH 7.0 containing 1% SDS and 1% β-mercaptoethanol. Samples of 100–200 μl (100–200 μg

protein) were applied per gel. The gels were stained with Coomassie brilliant blue and destained with a solution containing 875 ml of water, 50 ml methanol and 75 ml of acetic acid. Proteins were determined according to Lowry et al. [16].

3. Results

Polyacrylamide gel electrophoresis of the protein moiety of the 30 S RNP particles at pH 4.5 – 6 M urea yields one band (fig. 1a). This is in accordance

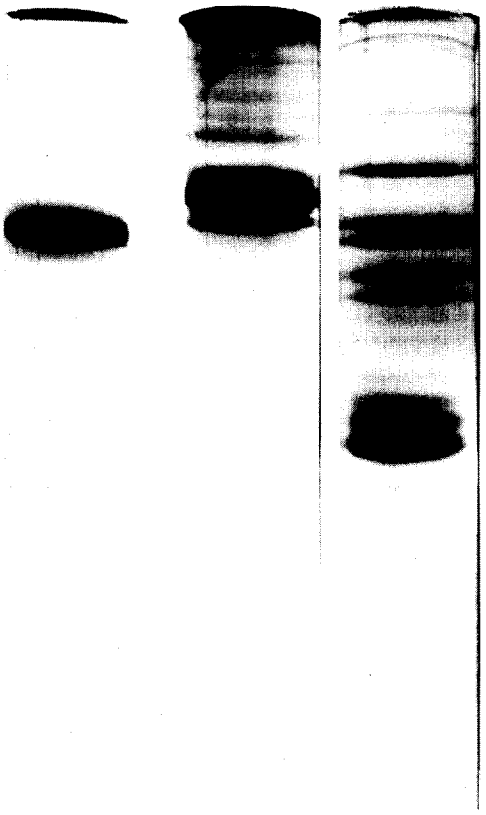

Fig. 1. Polyacrylamide gel electrophoresis of the protein moiety of 30 S RNP particles. (a) gel electrophoresis at pH 4.5 – 6 M urea, (b) gel electrophoresis at pH 11.6 – 4 M urea and (c) gel electrophoresis at pH 7.0 in the presence of 0.1% SDS. Electrophoresis was carried out for 3.5 hr at 6 mA per gel (a), for 2 hr at 6 mA per gel (b) and for 12 hr at 8 mA per gel (c).

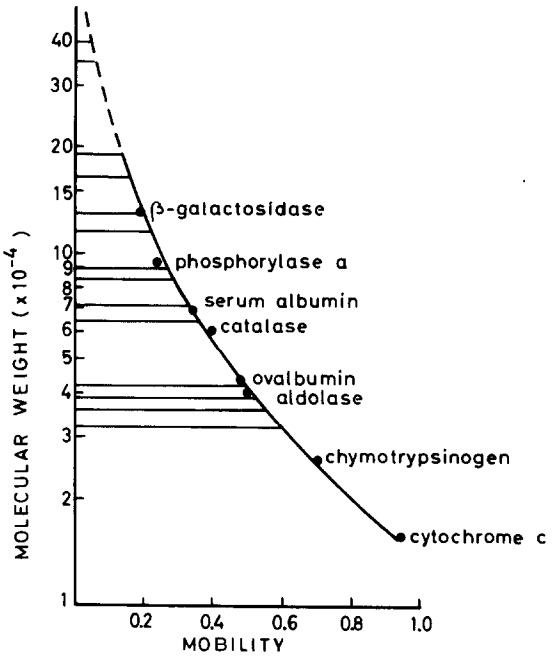

Fig.2. Determination of the molecular weights of the 30 S RNP particle polypeptide chains. The marker proteins used were β-galactosidase (130,000), phosphorylase a (94,000), serum albumin (68,000), catalase (60,000), ovalbumin (43,000), aldolase (40,000), chymotrypsinogen (25,700) and cytochrome *c* (11,700). The horizontal lines indicate the position of the RNP particle polypeptide chains relative to the marker proteins.

with the results obtained by Krichevskaya and Georgiev [10]. Upon gel electrophoresis at pH 11.6 – 4 M urea some additional fine bands with lower electrophoretic mobilities as compared to the main band can be discerned (fig. 1b). The presence of only one main electrophoretic band at acid or alkaline pH favours the view that the protein component of the nuclear RNP particles consists of one protein or a few proteins with similar properties. However, electrophoresis of the particle protein on SDS-gels, where resolution strongly depends on the molecular size, results in a large increase in the number of bands (fig. 1c). More than ten polypeptide chains of different molecular sizes are observed after SDS-treatment, reflecting a considerable heterogeneity of protein subunits present in nuclear RNP particles.

The molecular weights of the main polypeptide chains shown in fig. 1c were estimated as described by

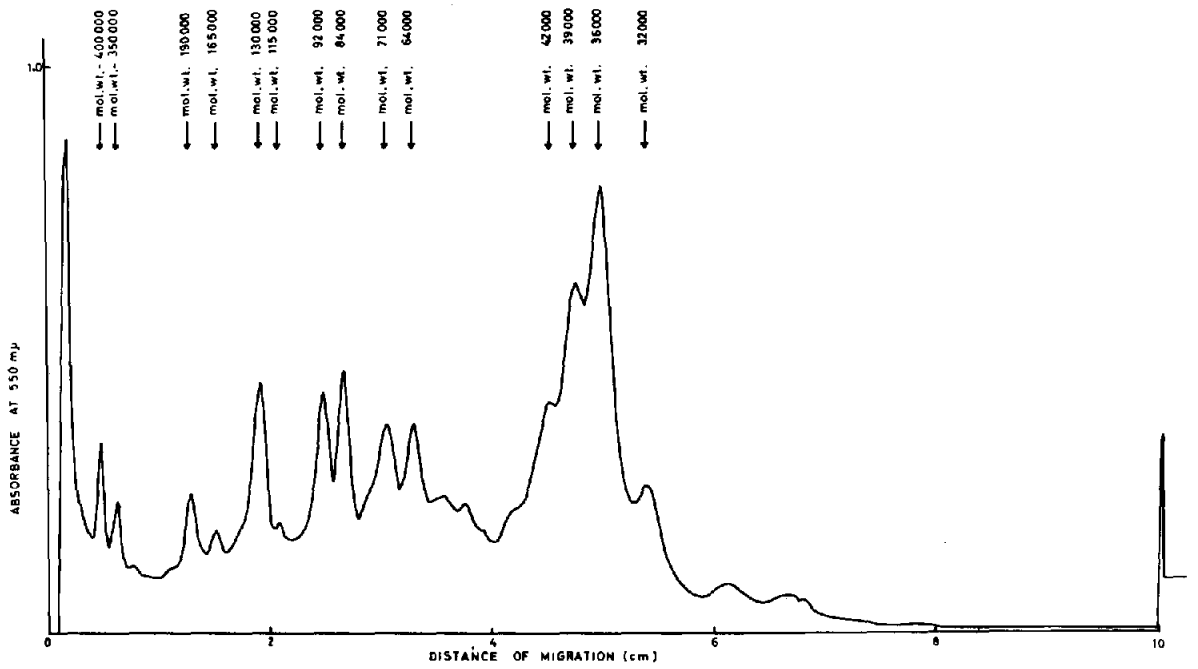

Fig. 3. Absorbance profile of a SDS-gel after electrophoresis of RNP particle protein subunits (see fig. 1c). The proteins were stained with Coomassie brilliant blue and their absorbance profile recorded at 550 nm with an automatic Gilford gel scanner.

Weber and Osborn [15]. The electrophoretic mobilities of 8 marker proteins were determined and plotted against the logarithm of their polypeptide chain molecular weights (fig. 2). The position of the RNP particle polypeptide chains relative to the reference proteins is indicated by horizontal lines, the corresponding molecular weights were taken from the ordinate in fig. 2. Due to the lack of commercially available marker proteins for the high molecular weight region the calculations for the protein subunits over 150,000 daltons must be considered as rough estimations. In fig. 3 the absorbance profile of the SDS-gel from fig. 1c with the molecular weights calculated for each peak is shown. It can be seen that the protein subunits are distributed over the wide range from 32,000 daltons to 400,000 daltons. Until now polypeptide chains with molecular weights exceeding 200,000 have not been described. We therefore suspected that the high molecular weight components (see fig. 1c and fig. 3) either represent proteins which are not denatured by the SDS-treatment in the presence of β-mercaptoethanol or complexes of proteins with DNA, RNA or possibly glyco- or lipoproteins. Therefore several control experiments were conducted including recentrifugation of the 30 S RNP particles on sucrose gradients, treatment with RNase (30 μg/ml at 25° for 4 hr), staining of gels specific for DNA with diphenylamine [17], glycoproteins [18] and lipoproteins [19] as well as heating of small samples of proteins in 1% SDS and 1% β-mercaptoethanol at 95° for 12 min and carboxymethylation of proteins with iodoacetamide [20]. None of these procedures could affect the pattern of band distribution shown in fig. 1c and fig. 3, which in numerous experiments was found to be highly reproducible. Furthermore there was no indication that components other than proteins were present in our preparations.

However, oxidation of proteins with performic acid as described by Hirs [21] caused the complete disappearance of the four bands with the lowest electrophoretic mobility the molecular weights of which had been estimated to be in the range between 165,000 and 400,000 (see fig. 1c and fig. 3). On the other hand, no significant changes were observed as regards the remaining bands located in the molecular weight region between 32,000 and 130,000.

4. Discussion

From the electrophoretic pattern of the constituent proteins of the 30 S RNP particles obtained by gel electrophoresis at pH 4.5 – 6 M urea (fig. 1a) a simple protein structure had been deduced [5] with only a few proteins or a single protein involved [10]. It was the aim of this study to demonstrate a considerable molecular heterogeneity of protein subunits present in 30 S RNP particles. Besides the main components of polypeptide chains located between 32,000 and 42,000 daltons a number of additional prominent peaks with molecular weights between 64,000 and 130,000 can be observed (fig. 3). The high molecular weight component with molecular weights of approximately 165,000, 190,000, 350,000 and 400,000 have shown a remarkable stability against various treatments, they probably represent polypeptide chains linked by disulphide bonds which can be cleaved by oxidation with performic acid.

In recent experiments we have analysed the protein moiety of 30 S–400 S RNP particles carrying 5 S–80 S DNA-like RNA on SDS-polyacrylamide gels [22]: their electrophoretic pattern was identical to that presented here for the 30 S RNP particles. Thus, obviously all size classes of nuclear RNP particles containing DNA-like RNA have the same protein composition which is characterized by an appreciable molecular heterogeneity. This molecular heterogeneity of protein subunits might be a reflection of their functional heterogeneity with respect to the preservation of the particle structure, cleavage of high molecular DNA-like RNA [6] and selection of mRNA to be transported to the cytoplasm.

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