CIRCULAR DICHROISM OF RIBONUCLEOPROTEIN COMPLEXES FROM RAT LIVER NUCLEI

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SUMMARY

Circular dichroism measurements were performed with 38 S ribonucleoprotein (nRNP) particles from rat liver nuclei. The positive CD-band around 264 nm increased 1.5-fold in the presence of 2 M NaCl consistent with the breakage of ionic interactions between RNA and proteins. Several distinct low molecular weight RNA species ranging from 5 to 8 S were detected in the 38 S nRNP particles by means of acrylamide gel electrophoresis in formamide.

INTRODUCTION

The formation of mRNA in eukaryotes is a complex process of several individual steps such as hnRNA synthesis, complex formation with proteins, dissociation from chromatin, polyadenylylation, "cap" formation, methylation, endo- and exonucleolytic cleavages and translocation of ribonucleoprotein complexes from the nucleus to the cytosol. The primary transcription products of chromatin can be isolated from nuclei as complexes of hnRNA and protein, designated as nuclear ribonucleoprotein (nRNP) particles (1). Depending on the conditions of preparation particles of either high (polyparticles) or low molecular weight (monoparticles) can be isolated (2, 3). Recent studies (4) have shown that the 30-40 S particles

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isolated from rat liver nuclei contain mRNA sequences. Further investigations on the structure and function of the nRNP particles are desirable for the understanding of the mechanisms of mRNA formation in eukaryotes.

In this paper we report the results of our studies using circular dichroism to study the conformation of RNA of nRNP particles and its interaction with proteins. The presence of low molecular weight RNA species in nRNP particles is also demonstrated.

MATERIALS AND METHODS

Monoparticles (38 S) and polyparticles (up to 200 S) were isolated as described previously (2,3). The particles were characterized by a buoyant density of 1.39 g/ml in CsCl, and a protein to RNA ratio of 7-8. RNA was extracted from the nRNP particles as described by Brawerman (5).

Acrylamide gel (6 %) electrophoresis of RNA was performed in formamide according to the method of Pinder et al. (6). The gels were stained with 0.02 % methylene blue for 60 min and destained in distilled H_2O.

Circular dichroism measurements were carried out with a Cary 60 spectropolarimeter with a 6003 CD attachment. Cylindrical cuvettes of 0.5 cm path length kept at 4°C in nitrogen atmosphere were used. Results are expressed as molar ellipticity, $|\theta|$, in deg x cm^2 x $dmole^{-1}$ of RNA assuming a mean molecular weight of 330 for RNA nucleotide residues. The CD spectra in the presence of NaCl were obtained by addition of solid NaCl to the nRNP particle solution.

RESULTS

Fig. 1 shows the CD-spectrum of 38 S nRNP particles in the wavelength range of 240-350 nm. A positive Cotton effect with a maximum at 264 nm was observed. Addition of NaCl to a final concentration of 2 M resulted in an increase in intensity of the positive ellipticity band at 264 nm. A marked decrease of the positive CD-band at 264 nm was observed after incubation with pancreatic RNAase. There was very

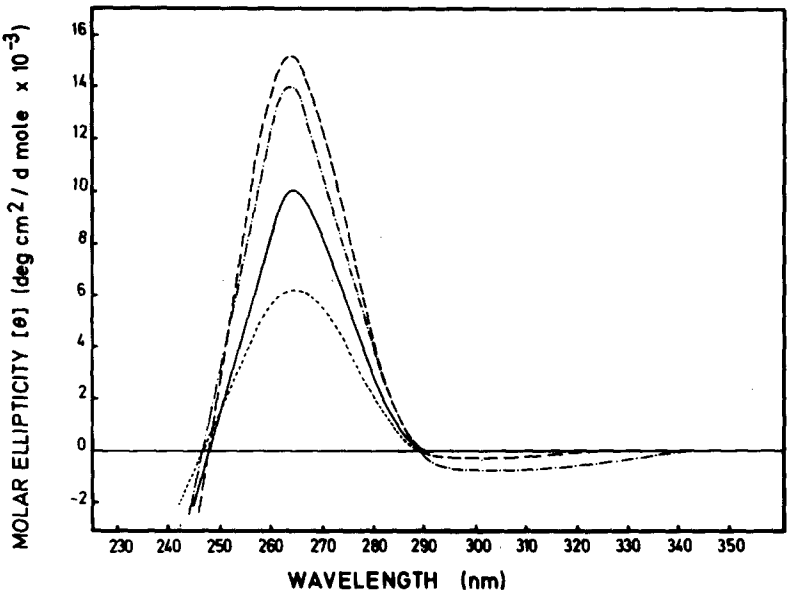

Fig. 1

CD spectra of 38 S nRNP particles

———, nRNP particles in 0.01 M Tris/HCl buffer, pH 8.0
-----, nRNP particles in 0.01 M Tris/HCl, 2 M NaCl, pH 8.0 after 30 min at 0°C.
....., nRNP particles in 0.01 M Tris/HCl, pH 8.0 plus 1 µg/ml pancreatic RNAase after 30 min at 0°C.
-.-.-, nRNP particles in 0.01 M Tris/HCl, pH 8.0, 2 M NaCl plus 1 µg/ml pancreatic RNAase after 30 min at 0°C.

little alteration when RNAase was added to the nRNP particle solution containing 2 M NaCl. The resistence of the 38 S nRNP particle RNA in 2 M NaCl to the action of pancreatic RNAase was due to an inhibition of RNAase activity. It is shown in Fig. 2 that increasing concentrations of NaCl inhibited degradation of particle RNA.

The increase in the ellipticity of particle RNA at 264 nm after addition of NaCl was very probably due to the removal of proteins from the nRNP particle complex, since recentrifugation of the nRNP particles containing 2 M NaCl resulted

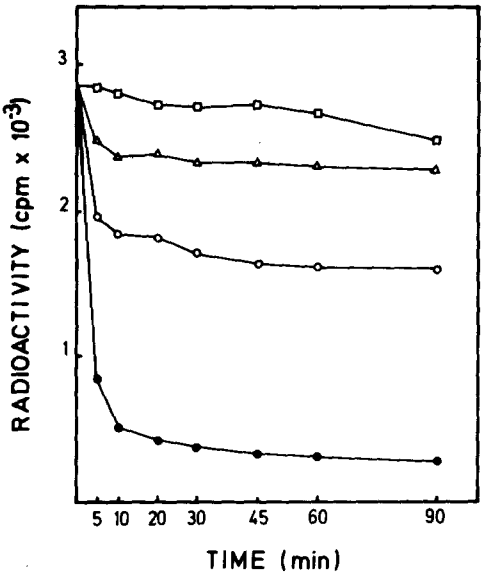

Fig. 2

Effect of sodium chloride on the degradation of nRNP particles by pancreatic RNAase

The preparation of 38 S nRNP particles from rat liver nuclei after injection of 1.0 mCi/kg [5-^{3}H]orotic acid 30 min before killing is described in Materials and Methods. In a total volume of 1.0 ml of 0.01 M Tris/HCl buffer, pH 8.0, 38 S nRNP particles (25-30 µg RNA) were incubated at 0°C in the presence of different NaCl concentrations and 1.0 µg of pancreatic RNAase. At the times indicated samples of 100 µl were taken for the determination of TCA precipitable radioactivity.

no NaCl (●), 0.3 M NaCl (o), 1.0 M NaCl (Δ), 2.0 M NaCl (□).

in a particle preparation characterized by a protein to RNA ratio of approximately 2 as compared to 7-8 in the 38 S particles.

Since only the RNA of the nRNP particles contributes to the circular dichroism in the 245-290 nm region (7,8), it was of interest to analyze the RNA components of the nRNP particles. When phenol-extracts of 38 S nRNP particles or polyparticles - prepared in the presence of RNAase inhibitor - were analyzed by acrylamide gel electrophoresis in formamide,

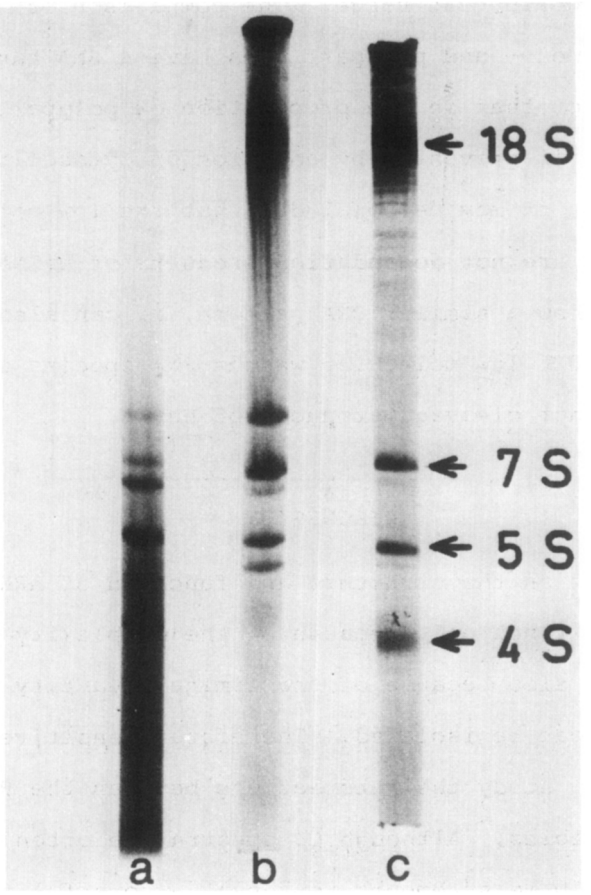

Fig. 3

Acrylamide gel electrophoresis in formamide of RNA from mono- and polyparticles

The preparation of mono- and polyparticles and the methods of RNA extraction and separation are described in Materials and Methods.

Approximately 10-20 µg of RNA was applied to the gels. RNA extracted from polysomes of rabbit reticulocytes was used as standard. 38 S nRNP particles (a), polyparticles (b) polysomal RNA (c).

several low molecular weight RNA species could be identified (Fig. 3). 38 S nRNP particles show 4 distinct RNA bands in the 5-8 S range and a zone of polydisperse low molecular weight material (a), whereas polyparticles exhibit 5 distinct

RNA bands (b) in that range. The comparison of (a) and (b) shows that mono- and polyparticles have 4 RNA bands in common. From the fact that in the preparation of polyparticles RNA degradation is prevented by addition of cytosolic RNAase inhibitor (1), it may be concluded that the low molecular weight RNA species are not degradation products of hnRNA. Since 38 S particles show a similar RNA pattern, it can also be assumed that the 5-8 S low molecular weight RNA species of monoparticles are not cleavage products of hnRNA.

DISCUSSION

Studies on the structure and function of nRNP particles are difficult not only because of the complexity of the particles, but also because of the limited quantity of the material which can be isolated. Therefore, sensitive methods are necessary to study the interactions between the RNA and proteins of the particles. Although CD spectra are often difficult to interpret, CD measurements represent a powerful tool in the study of RNA-protein interactions. RNA shows a strong positive CD-band around 265 nm due to the asymmetry of the bases linked to the pentose residues and to the conformation of the polynucleotide chain (7,8). To learn more about the topography of RNA and protein in nRNP particles, the CD-band at 264 nm was used as a parameter for the RNA-protein interactions. It was shown (Fig. 1) that the positive ellipticity of RNA of the 38 S nRNP particles increased in the presence of 2 M NaCl. This alteration is probably caused by release of conformational restraint on particle RNA. The increased ionic strength weakens ionic interactions between RNA and proteins. Similar observations were made recently in the case of nucleolar RNA-

protein complexes, where nucleoli were first treated with DNAase before NaCl was added to the remaining RNA-protein complex (9).

Although relatively few CD-studies on RNA-protein complexes have been reported, it may be stated that in all cases studied thus far, the effects of proteins on the RNA conformation (10,11) were not as pronounced as in the case of DNA-protein complexes, such as chromatin, where extensive CD studies have been conducted (13). In our experiments a drastic decrease in the intensity of the CD-band at 264 nm was observed after RNAase digestion. This finding is in agreement with the RNAase digestion experiment shown in Fig. 2, where approximately 15 % of the particle RNA was not degraded by RNAase under comparable conditions.

Studies on the structure and function of nRNP particles must ultimately be based on the detailed knowledge of the RNA and protein components. The analysis of mono- and polyparticles in respect to its RNA resulted in the detection of several low molecular weight components (Fig. 3). Low molecular weight RNAs have been described in nuclei by various authors (14,15). As yet no physiological function could be attributed to them. Whether the low molecular weight RNAs found in the nRNP particles play a structural role as postulated by Sekeris and Niessing (16) remains to be demonstrated.

During the preparation of this manuscript a publication of Deimel et al. (17) appeared describing the presence of low molecular weight RNAs in nRNP particles from rat liver.

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