Profiles of Total Ribonucleoprotein Particles from Normal Rat Liver, Primary Liver Tumors, and Novikoff Hepatoma

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SUMMARY

The total ribonucleoprotein particles were isolated from normal rat liver, regenerating rat liver (24 and 36 hours after partial hepatectomy), primary liver tumors, and Novikoff hepatoma. The profiles were established by two consecutive centrifugations on linear 5—30% and 30—80% sucrose gradients.

The results show that the profiles of total ribonucleoprotein particles differ from the normal profile in the conditions mentioned above. There is a decrease in the monomer and dimer proportions accompanied by a shift towards a heavier poly-some population. It would thus seem that active cell proliferation, whether normal or abnormal, is associated with an alteration of the ribosomal population of the tissue.

INTRODUCTION

Previous results on the autodegradation capacity of ribonucleoprotein particle (RNP) preparations have shown that this capacity disappeared in regenerating rat liver (2) as well as in the liver of rats fed 4-dimethylaminoazobenzene (DAB) (3). Furthermore, no autodegradation could be detected in primary liver tumors or in Novikoff hepatoma (3). These changes prompted us to study the total RNP or ribosome profiles of these tissues in comparison with normal liver.

Differences in the ribosome profiles of normal liver and liver tumors have been reported (11, 15, 16, 18). A loss of the heavier polysomes was commonly observed, this change being accompanied by increases in the proportions of monomers, dimers, and trimers. In regenerating liver, on the contrary, it has been reported that the removal of two-thirds of the liver resulted in a relative decrease in the monomer concentration and a marked shift of the mean distribution of the polysomes to the heavier species (17). These results have been obtained with polyribosomes prepared according to the method of Wettstein et al. (20) or modifications of it. This method, although effective for the preparation of polyribosomes, entails the loss of a large proportion of monomers and dimers and smaller losses of trimers and tetramers as shown by Webb et al. (16).

Since this method apparently gives only a partial picture of the RNP of the tissue, an effort has been made to obtain a more complete image using another method of isolation (14). Normal and regenerating livers, primary liver tumors, and Novikoff hepatoma have been studied. The results show that a decrease in the proportions of monomers and dimers and a shift towards heavier polysomes occurs in liver tumors as well as in regenerating liver.

MATERIALS AND METHODS

Wistar white rats of an average weight of 200 gm were used. Partial hepatectomy (66%) was carried out according to the method of Higgins and Anderson (9). The primary liver tumors were induced by feeding the rats for a period of six to seven months a low protein diet [Diet 3 of Miller et al. (12)] containing DAB at a concentration of 0.06%. The solid Novikoff hepatoma were seven-day-old transplants from the inguinal region of Sprague-Dawley rats. For both the primary and transplanted tumors, only small masses of less than 1 cm in diameter were used to eliminate necrotic areas as much as possible. The rats were fasted for 18 hours before sacrifice.

Preparation of Homogenates

The animals were killed by a blow on the head, decapitated, and bled freely. The livers were removed, blotted on filter paper, chilled on ice, and finally minced with a plastic squeezer to give a liver pulp. The primary tumors, after the removal of adjacent liver, and the transplanted hepatoma, after cleaning attached tissue, were cut with scissors in very fine pieces. The liver pulp or the tumor pieces were homogenized with the aid of a Potter-Elvehjem homogenizer at 0—4°C to give a 25% homogenate. The suspending medium was a 0.25 M sucrose solution containing 0.005 M MgCl₂, 0.05 M KCl, and 0.025 M Tris maleate, pH 7.6.

Preparation of Ribosomes

The ribosomes were prepared according to the method of Tashiro and Siekevitz (14) with minor modifications (2). The purified ribosomes suspended in 0.001 M Tris-maleate buffer, pH 7.6, containing 0.001 M MgCl₂ were stored at 0—4°C. Under these conditions the ribosome preparations were found...
RESULTS AND DISCUSSION

Recovery of Ribosomal RNA

The RNA content of the ribosomes was estimated from the absorbancy at 260 μM using the relation 20 absorbancy units equal 1 mg of RNA (20). An average value, from seven separate experiments, of 2.19 ± 0.16 mg per gm of liver was obtained. This value indicates a better recuperation than that reported by Wettstein et al. (20). These authors obtained 1.85, 1.43, and 1.16 mg per gm of liver, depending upon their conditions of ribosome preparation.

Proportions of Monomers and of Monomers Plus Dimers in Normal Liver

The proportions of monomers and of monomers plus dimers in the ribosomal preparations obtained from a microsomal fraction and from a postmitochondrial supernatant are shown in Table 1. These values were calculated from the optical densities of the fractions corresponding to the peaks of monomers and dimers. There are slightly more monomers and

Profile Determination

An amount of ribosome preparation equivalent to 20 O.D. was placed over 29 ml of a linear 5—30% sucrose gradient containing 0.001 M MgCl₂, and was spun at 25,000 rpm for 2 hours in a swinging bucket rotor SW 25; 1-ml fractions were collected. The sediment left in the centrifugation tube corresponding to 30—50% of the original O.D. and giving an absorption curve similar to the original ribosomes was resuspended and placed over 29 ml of a linear 30—80% sucrose gradient. The sample was centrifuged, and 1-ml fractions were collected exactly as above. The 1-ml portions from the gradients were diluted with 2 ml of the suspending medium, and the O.D. was determined spectrophotometrically.

Ribonuclease Treatment and Amino Acid Incorporation

The material sedimenting through the 5—30% sucrose gradient was treated with 0.3 μg of pancreatic ribonuclease (5 X crystallized from Sigma Chemical Company) during 15 minutes at room temperature. This preparation was then centrifuged over gradients as described above, and the profiles were determined.

Both the material retained in the 5—30% sucrose gradient and the material sedimenting through it were tested for leucine (New England Nuclear Corporation) incorporation in vitro according to the method of Hoagland et al. (10), using an equal O.D. as a measure of an equal amount of ribosomal material put into the reaction mixture.

Table 1

<table>
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<tr>
<th>Ribosomal preparation from</th>
<th>Monomers</th>
<th>Monomers plus dimers</th>
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</thead>
<tbody>
<tr>
<td>Microsomal fraction</td>
<td>22.8 ± 2.4</td>
<td>30.1 ± 4.1</td>
</tr>
<tr>
<td>Postmitochondrial supernatant</td>
<td>17.9 ± 2.7</td>
<td>26.7 ± 4.1</td>
</tr>
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Proportions of monomers and of monomers plus dimers in ribosomal preparations obtained from a microsomal fraction than those obtained from a postmitochondrial supernatant, but the difference is not statistically significant. Even though there was a slight difference between the two preparations, the preparation of ribosomes from microsomal fraction was adopted because the ratio RNA/proteins was much higher (1.3) than the one obtained for preparations from a postmitochondrial supernatant (0.7).

Pancreatic RNase Treatment and Leucine Incorporation in Vitro

The curve in white dots in the right part of Chart 1 shows the profile on a 30—80% sucrose gradient of the material sedimenting through a 5—30% sucrose gradient. There are at least three distinct peaks. In addition, the bar corresponding to 9.2 O.D. shows that some larger material was passing through the gradient. After treatment with 0.3 μg of pancreatic RNase (curve in black dots), it is clear that the bulk of the material retained on the 30—80% sucrose gradient, as well as the material sedimenting through it, has disappeared and is now found mostly in the form of monomers on a 5—30% sucrose gradient (left part of the Chart). Since the material passing through the 5—30% sucrose gradient is transformed into monomers by a very mild treatment with pancreatic RNase, it would seem that this material consists of very heavy polysomes.

The material sedimenting through the 5—30% sucrose gradient was compared with dimers and heavier polysomes in its capacity to incorporate leucine in vitro (Table 2). It is clear that the sediment incorporates leucine as well, if not better, than the polysomes retained on the gradient.

Two of the most characteristic properties of polysomes are their transformation into monomer by mild treatment with pancreatic RNase and their capacity of incorporating amino acids into protein; the material sedimenting through the 5—30% sucrose gradient possesses both properties. It would thus seem that this material consists of very heavy polysomes. Consequently this material was studied in the present work. These results favor the conclusion that the sedimented material is of polysomal nature.

The presence of very large polysomes has been shown, either in tissue sections or in ribosomal preparations, by electron microscopic studies. The presence of polysomes containing 10, 12, 15, and even more monomers has been established in neurons (8), muscle fiber (1, 6), and calf lens (13), as well as in liver (4, 5, 7). Furthermore, these authors have shown that some large ribosomal aggregates sensitive to the action of RNase had a helical structure. These observations are in line...
with our conclusion that the large aggregates found in ribosomal preparations are of polysomal nature.

Normal and Regenerating Liver

The ribosomal profile of normal liver is shown in Chart 2 (curve in white dots). The left part of the figure shows the profile of the ribosomes retained on the 5–30% sucrose gradient; the right part illustrates the profile of the polysomes passing through the above gradient but retained on the 30–80% sucrose gradient. Two main peaks corresponding to the monomers and dimers and various shoulders corresponding to larger polysomes can be seen on the 5–30% sucrose gradient. In addition, three well-defined peaks and some shoulders can be seen on the 30–80% sucrose gradient indicating the presence of various classes of very heavy polysomes. The profiles obtained with the RNP preparations from laparatomized rats were also determined and were found to be similar to the normal profile. For this reason they have not been included.

The profile of 24-hour regenerating liver (curve in black dots) shows an important decrease in the monomers and dimers regions with a corresponding increase in the polysome region of the 5–30% sucrose gradient. There is also an increase of the polysomes retained in the first half of the 30–80% gradient. However, there seems to be a loss of the heavier polysomes retained in the second half of the gradient.

Chart 3 shows the ribosomal profile of 36-hour regenerating liver. The decrease in monomers and dimers is still present as well as the increase in the polysomes retained in the 5–30%
crease in the heavier polysome region.

The ribosomal profile of Novikoff hepatoma is shown in Chart 5. The decrease in monomer proportion is still more pronounced than in primary liver tumor. The increase in the polysomes retained in the 5–30% gradient is also more marked. The increase in the polysomes retained in the top half of the 30–80% gradient is similar to that observed in primary liver tumors, but there is a definite increase in the polysome region corresponding to the bottom half of the gradient.

The present results do not agree with those of the various authors (11, 15, 16, 18) who found an increase in the dimer proportion of the RNP preparations obtained from tumors. However, Webb and Potter have shown that these dimers do not seem to be held together by functional messenger RNA, but by some labile component necessary for their integrity. Furthermore, they have shown that part of the dimer peak consists of a particle with a sedimentation constant of 93 s, which may be formed by the combination of a monomer and a ribosomal subunit (19). The formation of this complex might explain the increased dimer proportion observed by these authors.

The ribosomal profile on 5–30% and 30–80% sucrose gradients of normal rat liver (o–o–o) and of regenerating rat liver, 36 hours after hepatectomy (e–e–e).

These results are in agreement with those of Webb et al. (17), who found a decrease in the monomer proportion and a shift of the distribution towards heavier polysomes 14 hours after partial hepatectomy. These authors also stated that the changes were still more pronounced after 21 hours. It has been reported that no autodegradation could be detected in ribosomal preparations from regenerating liver 24 and 36 hours after hepatectomy (2). It would thus seem that normal cell proliferation is accompanied not only by the loss of autodegradation capacity, but also by decreases in monomer and dimer proportions and a redistribution of the heavier polysomes.

**Primary Tumors and Novikoff Hepatoma**

The ribosomal profile of DAB-induced primary liver tumors is shown in Chart 4. There is a decrease in the monomer and dimer proportions and a small increase in the polysomes retained in the 5–30% sucrose gradient. However, a greater difference is the large increase in the polysomes retained in the top half of the 30–80% sucrose gradient. There is also a de-
It has been reported recently that ribosome preparations from primary tumors as well as from solid Novikoff hepatoma did not show autodegradation capacity (3). The situation for the tumors is then similar to that mentioned above for regenerating rat liver. It would thus seem that abnormal cell proliferation, like normal cell proliferation, is accompanied by the absence of ribosomal autodegradation capacity and by a decrease in the monomer and dimer proportions with a shift toward heavier polysomes.

If it is assumed that the messenger RNA which holds together the polysome is the point of attack during autodegradation, the absence of autodegradation would favor the stability of the polysomes. In fact, in two instances in which this capacity could not be determined, such as liver regeneration (2) and tumor formation (3), the proportions of polysomes were found to be increased and concomitant smaller proportions of monomers and dimers were observed. Even though this coincidence does not constitute a proof of the relation between the two phenomena, it indicates that such a relation may exist.

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