STUDIES ON THE FUNCTION OF INTRACELLULAR RIBONUCLEASES

IV. Some Observations on the Properties of Ribosomes and Polysomes from Rat Liver and Hepatomas

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ABSTRACT

Polysome and ribosome preparations from normal rat liver and from a series of transplantable rat hepatomas of different growth rates were compared. All the hepatomas had a significantly higher percentage of RNA in a polysome preparation than did the normal liver, and the polysome preparations from the tumors, with the exception of the Dunning hepatoma which has a high lipid content, gave a greater yield of RNA and protein per gram of wet tissue than the liver did. Heavier polysomes were considerably less prevalent in the tumors than in the liver, and the tumors contained a larger proportion of monomer and dimer ribosomes than the liver did. Evidence is presented that the increased monomer and dimer ribosome population of the hepatomas studied is not an artifact of preparation, but represents the true intracellular distribution. Ribosomes from normal liver and Morris 5123-D hepatoma were readily dissociated by 20 min' treatment with 1.0 mM EDTA, but ribosomes from the Dunning, Novikoff ascites, and McCoy MDAB hepatomas were little affected by such treatment. With higher concentrations of EDTA, the ribosomes from the Novikoff ascites and McCoy MDAB hepatomas broke down and did not form specific subunits as did ribosomes from liver and the Morris 5123-D hepatoma but rather gave rise to a variety of small degradation products. This behavior is ascribed to a higher RNase content of the Novikoff and McCoy MDAB hepatomas. Dunning hepatoma ribosomes were resistant to 4 mM EDTA.

INTRODUCTION

In a previous report (1) of studies on the RNase activity of rat liver ribosomes and microsomes, the tentative conclusion was drawn that alkaline RNase is a normal constituent of rat liver ribosomes and plays a role in the biological activity of these particles. To establish or negate this conclusion, additional investigations have been carried out on ribosome and polysome preparations obtained from normal rat liver and several hepatomas. These investigations have suggested that the above conclusion is correct except for the word "normal." In recently published reports, Neu and Heppel (2–4), using *Escherichia coli* ribosomes, have reached similar conclusions. Since a review has already been made of the studies of Neu and Heppel, as well as of a relatively large number of papers concerned with RNase activity both in mammalian liver ribosomes and polysomes and in

ribosomes and polysomes prepared from a variety of different forms (5), these publications will be considered only briefly.

This paper describes some of the properties of ribosome and polysome preparations isolated from normal rat liver and from several hepatomas. The accompanying paper considers the RNase activity of particulate fractions in these preparations and the factors affecting this activity and attempts to present a unified concept of the relationship of the RNase activity to the biological activity of ribosomes and polysomes.

MATERIALS AND METHODS

Preparation of Ribosomes and Polysomes

The normal livers of young adult male albino rats of the Holtzman strain weighing about 300 g were used. In each experiment, several rats bearing one kind of hepatoma were used. The methods of transplanting tumors and some properties of the tumors have been described (6). The hepatomas or livers were removed, pooled in a beaker containing ice cold Tris-potassium-Mg^{++} buffer (TKM) containing 0.25 M sucrose, rinsed in this medium, blotted dry, and weighed. About 2.5 ml of TKM-sucrose buffer per 1 g of the original wet weight of tissue were used for homogenization which was done in a Ten Broeck all glass homogenizer in the cold. Only 3 or 4 strokes of the plunger were used, since severe damage to the tissues causes poor yield of polysomes. Cell debris, nuclei, and mitochondria were removed by centrifuging for 15 min at 15,000 *g*. All operations were conducted between 0° and 4°C. For each fractionation, 1 volume of a 10% solution of sodium deoxycholate (DOC) dissolved in cold water was added to 9 volumes of the supernatant fraction from the above centrifugation, which gave a final detergent concentration of 1%. The procedures used with this DOC-treated postmitochondrial preparation were:

Ribosome Preparation

The solution was centrifuged at 105,000 *g* for 2 hr. The sediment was suspended in TKM buffer of one-half the original volume and centrifuged again at 105,000 *g* for 90 min. The resulting pellet was suspended in appropriate buffer or glass-distilled water. The suspension was accomplished in a loosely fitting, 1 ml capacity Ten Broeck homogenizer. The suspension obtained was centrifuged at 1000 *g* for 10 min so that any large aggregates of particles were removed.

Polysome Preparation

The technique for isolation of polysomes was based on the method of Wettstein et al. (7). The DOC-treated postmitochondrial solution was centrifuged through a double layer of sucrose solutions (4 ml of 0.5 M sucrose layered over 3 ml of 2 M sucrose, both made up in TKM buffer) at 105,000 *g* for 4 hr in a No. 40 rotor of a Spinco Model L ultracentrifuge. The pellet was often loosely packed at the bottom of the tube. Most of the supernatant fraction was removed with a syringe, and the pellet, together with 0.5 ml of concentrated sucrose solution, was left at the bottom of the tube. The pellet was suspended in TKM buffer of one-half the volume of the original postmitochondrial solution. This suspension was centrifuged at 105,000 *g* for 90 min, and the sediment was again suspended in TKM buffer so that 0.5 ml of the suspension contained the particulate material of 1 g of tissue. The final solution was always centrifuged once at 1000 x *g* for 10 min so that any large unsuspended aggregates were removed.

Throughout this paper and the following one, the terms ribosome preparation and polyribosome preparation refer specifically to the particulate material obtained by the respective procedures described. It is recognized, and indeed evident from the data presented, that a polysome preparation may contain some monomeric ribosomes and even smaller particulates and that, conversely, a ribosome preparation may contain some polysomes.

Protein was determined by the method of Lowry et al. (8), using bovine serum albumin (Armour Pharmaceutical Company, Chicago, Illinois) as a standard. RNA was determined by Schneider's method (9).

The technique for forming a linear sucrose density gradient was similar to that suggested by Britten and Roberts (10). The gradient was equilibrated in the cold for 1 to 6 hr. A sample of 0.5 to 1.5 ml was generally used over 25 ml of gradient, and 1 ml fractions were collected. The fractions were diluted to 3.0 ml with water, and the absorbance at 260 mμ was determined. Correction for ferritin which might be present was not made.

The sedimentation rates of various particles were analyzed by Schlierin optics in a Spinco Model E analytical ultracentrifuge. A standard 12 mm centerpiece was used in all centrifugations. The temperature of the rotor, adjusted to 20°C before the run, was maintained at all times. The first photograph was taken 13 min after the centrifugation started, and 4 pictures were taken every 4 min thereafter at bar angle 50°C.

TKM-SUCROSE BUFFER: This buffer contained 0.05 M Tris, 0.025 M KCl, 0.005 M $MgCl_2$, and 0.25 M sucrose. The pH was adjusted to 7.6 with HCl. All tissues were homogenized in this buffer. Buffer without the sucrose (TKM) and buffer without the sucrose and Mg^{++} (TK) were also employed for special purposes.

TABLE I

Protein and RNA Content of Polysome Preparations from Normal Liver and Several Hepatomas

Tissue	mg protein/ gm tissue	mg RNA/gm tissue	mg RNA/mg protein	% RNA	Absorbancy ratio $\frac{260}{280}$
Normal rat liver	0.8 (7)*	0.6 (7)	0.75 (7)	43 (7)	1.60 (5)
Morris 5123-D hepatoma	1.0 (2)	1.2 (3)	1.2 (3)	54.5 (3)	1.78 (3)
Novikoff ascites hepatoma	1.1 (2)	—	—	—	1.82 (2)
Dunning hepatoma	0.7 (2)	1.0 (2)	1.3 (2)	56.5 (2)	1.80 (2)
Novikoff hepatoma	0.9 (2)	1.2 (2)	1.4 (2)	58.0 (2)	1.78 (2)
McCoy MDAB hepatoma	1.1 (3)	1.4 (2)	1.2 (3)	54.5 (3)	1.78 (3)

* The figures in parentheses represent the number of separate experiments. Tabulated values are averages.

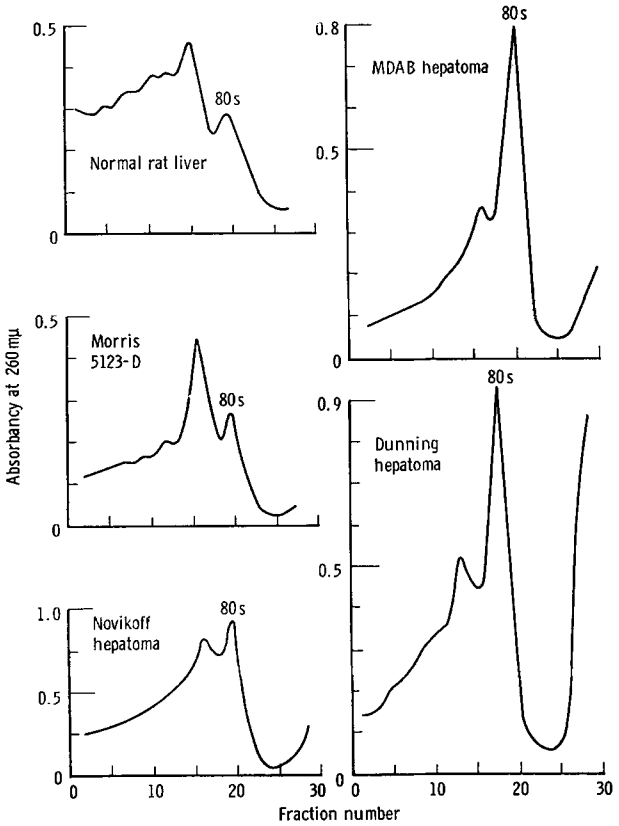

FIGURE 1 The distribution of particulate material in a polysome preparation from normal rat liver and some hepatomas in a linear sucrose density gradient (5 to 20%) in TKM-buffer pH 7.6. Centrifuged at 25,000 RPM for 3 hr in a Spinco SW-25 rotor. Ribosomes of known sedimentation coefficient were used as markers, under identical conditions of centrifugation, to identify the absorption peak of monomeric ribosomes of the polysome preparations.

SODIUM DEOXYCHOLATE: A 10% solution by weight was prepared in cold, glass-distilled water about 30 min before use.

RESULTS AND DISCUSSION

Protein and RNA Content of Polysome Preparations

Average values for a number of samples are shown in Table I. It is interesting to note that the polysome content of rat liver was less than that o any tumor, with the exception of the Dunning hepatoma. The large amounts of lipid materials associated with this tumor may account for the relatively low amounts of RNA or protein per gram of wet weight. All the tumors, however, had a significantly higher ratio of RNA to protein than did normal liver, which is reflected in the optical density ratios. The nature of this extra RNA is not clear.

The Distribution of Particulate Material of a Polysome Preparation in a Sucrose Density Gradient

Typical patterns for normal rat liver and some hepatomas are shown in Fig. 1. In accordance with findings of Webb, Blobel, and Potter (11), polysome preparations from both rapidly and slowly growing tumors exhibited distribution patterns that were distinctly different from those of polysome preparations from normal rat liver, and the heavier polysomes were relatively less prevalent in tumors. A large amount of dimers and trimers were observed in Novikoff ascites, McCoy MDAB, Novikoff, Dunning, and Morris 5123-D hepatomas. The distributions of polysomes from normal rat liver and from Morris 5123-D hepatoma in the density gradient were similar, in that the amount of monomer ribosomes was less than the amount of dimer ribosomes in both tissues. It is possible that some dimerization of 80S ribosomes takes place under the conditions used. On the other hand, relatively large amounts of monomer ribosomes, in comparison with the dimer ribosomes, were observed in polysome preparations from McCoy MDAB, Dunning and Novikoff hepatomas. Morris 5123-D hepatoma resembled the other tumors as it had a smaller proportion of heavier polysomes than did normal rat liver. This common feature of tumors could be due to an increased output of ribosomes, a decreased output of messenger RNA, or a marked decrease in the stability of a fraction of the RNA as suggested by Webb and his co-workers (11). The relationship of these patterns to RNase activity is discussed in the following paper, but it should be noted that the scarcity of heavy polysomes in the tumors examined might be due either to high RNase activity in the microsome and ribosome fraction or to a relatively small amount of RNase inhibitor or both (12). The profile of polysome distribution for liver was not altered by a change of pH of the suspension medium from 7.6 to 8.5; the latter is the optimum pH of ribosomal RNase.

It appears likely that the tumor polysome distribution patterns obtained are the ones that exist in the cell and are not an artifact of the preparative procedure which exposes the polysomes to RNase activity. Evidence for this conclusion was obtained by homogenizing a portion of McCoy MDAB hepatoma in the supernatant fraction prepared from normal rat liver and by homogenizing another portion in the supernatant fraction prepared from Dunning hepatoma. The latter two supernatant fractions contain high amounts of RNase inhibitor activity (12), which should help to prevent any breakdown of McCoy MDAB hepatoma polysomes; the McCoy MDAB hepatoma contains a low amount of RNase inhibitor. The polysome patterns for the control and the two experimental samples were identical, a fact which leads to the conclusion stated above.[1]

Effect of EDTA on Ribosomes

It has been clearly established that Mg^{++} ions play an important role in stabilizing intact ribosomal particles from all sources (for a complete review, see reference 13). Removal of Mg^{++} ions by treatment with EDTA (or many other means) results in dissociation of these particles into subunits. In the presence of a high concentration of EDTA (5 to 10 mM), ribosomes from normal rat liver and from the hepatomas utilized were dissociated into 4 S units, and, to a considerable extent, formed aggregates which gave a visible precipitate. Since dissociation into distinct subunits was desired for studies of the location of RNase, attempts were made to determine the optimum concentrations of EDTA which would give a controlled dissociation.

Ribosome preparations isolated from rat liver and hepatomas by the procedure described in Materials and Methods were suspended in cold glass-distilled water. After addition of appropriate amounts of buffer containing EDTA (details are given in the figure legends), the samples were centrifuged in the Spinco Model E analytical ultracentrifuge. The effect of different concentrations of EDTA on ribosomes from normal rat liver and Morris 5123-D hepatoma is shown in Fig. 2. The components *a* through *e* are named according to the suggestions of Hamilton and Petermann (14). Component *b* (monomer ribosome) was converted to component *d* in the presence of 2 mM EDTA in the ribosomal preparations from both normal rat liver and Morris 5123-D hepatoma. It is of interest to note that ribosomes from normal rat liver were much more sensitive to EDTA than those from the Morris 5123-D hepatoma. In

[1] *Note Added in Proof*: However, in a report to be published soon, Blobel and Potter have observed a striking increase in the proportions of larger polysomes in a rat liver polysome preparation centrifuged through a discontinuous sucrose gradient containing some high speed supernatant fraction.

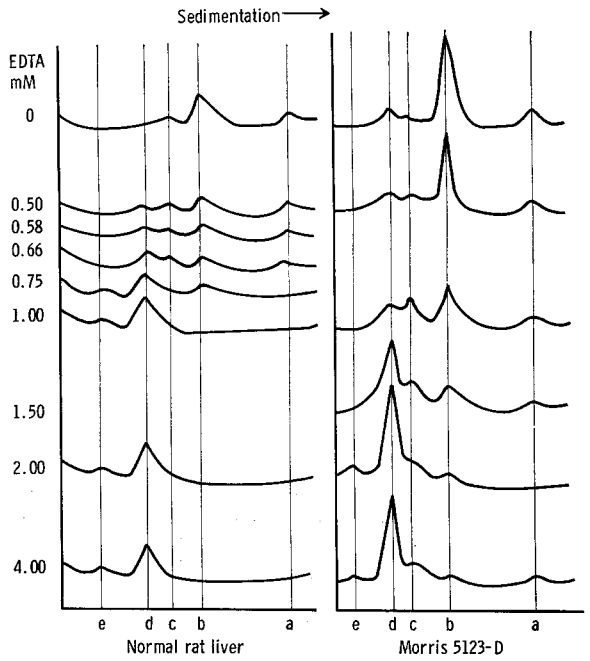

FIGURE 2 The effect of EDTA on a ribosome preparation obtained from normal rat liver and Morris 5123-D hepatoma. An aliquot of 0.7 ml of sample containing 3 mg (dry weight) of ribosomes per milliliter in Tris buffer pH 7.6, 0.1 M KCl, and EDTA of the final concentration indicated was centrifuged at 37,000 RPM at 20°C, bar angle 50°. Photographs were taken 7 min after reaching speed. *a*, 105S; *b*, 80S; *c*, 57S; *d*, 46S; and *e*, 27S (apparent sedimentation coefficients not corrected for infinite dilution). The particles were treated with EDTA for 20 min before the run.

agreement with the findings of Hamilton and Petermann (14), monomer ribosomes from rat liver were completely dissociated into 46S and smaller units at an EDTA concentration of 4 mM.

The results with Dunning, Novikoff ascites and McCoy MDAB hepatomas are shown in Fig. 3. Unlike the ribosomes from normal rat liver and Morris 5123-D hepatoma, the ribosomes from these hepatomas were insensitive to the EDTA treatment under these conditions, and no appreciable degradation of monomer ribosomes occurred at concentrations of EDTA from 0 to 1 mM. At a concentration of 4 mM, however, component *b* of McCoy MDAB ribosomes was largely dissociated into subunits but not into clearly distinguishable peaks. Many of the subunits were further degraded to material which accumulated at the top of the cell and produced a greater refractive gradient in that region.

The extent of ribosome dissociation in the presence of EDTA could be a function of time. To test this possibility, a sample of ribosomes from Morris 5123-D hepatoma was examined by ultracentrifugation after 20 min' treatment with 2 mM EDTA, and another sample was similarly examined after 18 hr of treatment. Only small differences were noted, which indicated that the effect of 2 mM EDTA on monomer ribosomes from Morris 5123-D hepatoma is immediate and that the particles are converted from 80 to 46S within 20 min after addition of EDTA to the ribosomal preparation. No appreciable further degradation occurred, at least within 18 hr. The ribosomes from normal rat liver, Novikoff, and MDAB hepatomas were also quite stable in the absence of added Mg^{++} ions in the cold for several days; only 10 to 20% of these ribosomes were dissociated during that time. Similar findings were reported by Tashiro and Siekevitz (15), who carried out a careful study of the effect of EDTA and other factors on guinea pig liver ribosomes.

The results of the above experiments have established that ribosomes isolated from normal rat liver and Morris 5123-D hepatoma may be converted to distinct smaller subunits with use of a 2 mM concentration of EDTA. Further dissociation did not occur, probably because of the very low ribosomal RNase activity in the samples. On the other hand, ribosomes from McCoy MDAB hepatoma were largely dissociated at the same concentration of EDTA used above to form a variety of small nonspecific components. This suggests that, as soon as the ribosomes were dissociated by the EDTA, the subunits were further degraded by the high RNase activity shown to be present (see following paper). The absence of

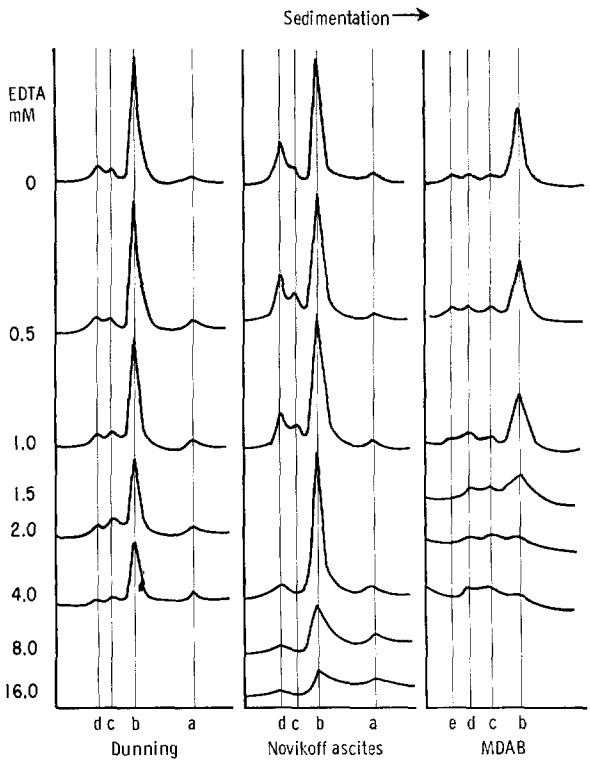

FIGURE 3 The effect of EDTA on a ribosome preparation obtained from Dunning, Novikoff ascites, and McCoy MDAB hepatomas. Conditions are the same as described for Fig. 2. Protein contents in the ribosomal preparations were 1.5 mg (Dunning), 2.0 mg (Novikoff ascites), and 1.5 mg (McCoy MDAB) per ml. For Novikoff ascites, curve 4 from the top is 4.0 mM EDTA, curve 5 is 8.0 mM EDTA, and curve 6 is 16.0 mM EDTA. For MDAB, curve 4 from the top is 1.5 mM EDTA.

distinct ribosomal subunits of Dunning hepatoma after treatment with 4 mM EDTA may be explained similarly, since the RNase activity of ribosomes from the Dunning hepatoma has also been shown to be high. It is not clear, however, why the Dunning hepatoma ribosomes are so resistant to EDTA.

Additional effects of other reagents on intact and dissociated ribosomes from tumors in conjunction with the RNase activity are considered in the accompanying paper.

From the work described in this report, it is clear that with respect to their physical and chemical properties, hepatoma ribosomes may differ considerably from those of the tissue of origin and among themselves. Apparently, these differences are not a disadvantage to the tumor and may, in fact, be advantageous for rapid protein synthesis which is necessary for rapid proliferation.

This paper was supported by grants from the National Cancer Institute, National Institutes of Health, CA-07824, and the American Cancer Society, P303. For paper III, see reference 1. Dr. Roth was supported by Research Career Award, RC 31-63, from the National Cancer Institute, National Institutes of Health.

This paper was abstracted from Dr. Utsunomiya's thesis presented in partial fulfillment of the requirements for the Ph.D. degree, University of Connecticut, 1965.

Received for publication 28 October 1965.

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