LIVER POLYRIBOSOMES AND PHOSPHOLIPASE A

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Partial hepatectomy has been shown to cause changes in liver polyribosomes that can be measured as an enhanced rate of incorporation of amino acids into protein and as a greatly increased stability of the aggregates at 37° and in the presence of phospholipase A (1).

The ability of the phospholipase to disaggregate polyribosomes from normal liver led to the suggestion that phospholipids, in addition to mRNA, play a role in maintaining polyribosomal structure. To explain the resistant aggregates, it was suggested that removal of part of the liver caused the appearance on the ribosome of molecules that prevented the lipase from attacking the phospholipids (1).

Some effort has now been made to understand the nature of the stability differences of liver polyribosomes from normal and partially hepatectomized rats. The purpose of this report is to show that: 1) polyribosomes from normal and regenerating liver contain similar amounts of total RNase (active + latent); 2) some of the RNase of the normal aggregates, but almost none of the RNase of the regenerating polyribosomes, is present in the active form; and 3) phospholipase A activates the latent RNase of normal polyribosomes but has little or no effect on the latent activity of aggregates from partially hepatectomized animals.

These results are consistent with the presence of additional molecules on the regenerating polyribosome. They are not consistent, however, with the

idea that phospholipids assist mRNA to form the ribosomal aggregate.

MATERIALS AND METHODS

Male albino rats, obtained locally, were used when they weighed about 80 g. Partial hepatectomy refers to the removal of 67% of the liver (2). Phospholipase A (venom of Naja naja), the generous gift of Dr. C. R. Hackenbrock, was heated for 30 min at 100° and pH 5.9. Crotalus adamanteus venom, used as the source of phosphodiesterase, was from Ross Allen's Reptile Institute, pancreatic RNase A was from the Worthington Biochemical Corporation, and twice-crystallized, salt-free trypsin was from Mann Research Laboratories, Inc.

Liver polyribosomes were prepared without detergent by the method of Bloemendal, Bont, and Benedetti (3). Sedimentation profiles were obtained by gradient centrifugation in sucrose (1).

The estimation of RNase was based on the procedures of Roth and Milstein (4), Shortman (5), and Blobel and Potter (6). The test mixtures contained 0.2 ml of a 2% solution of yeast RNA (Schwarz BioResearch Inc., treated with acid-ethanol as described by Blobel and Potter (6)) and 0.5 ml of the enzyme preparation in medium A (7). After incubation at 37° for 30 minutes, 0.5 ml of ice-cold acid-ethanol was added, 0.5 ml of the supernatant solution obtained by centrifugation was diluted with 2.5 ml of water, and adsorbance was measured at 260 mμ. Under the assay conditions used, the rate of appearance of ultraviolet-absorbing, acid-ethanol soluble material was linear with time and proportional to the concentration of pancreatic RNase or ribosomal protein.

Phosphodiesterase was assayed according to Boman and Kaletta (8) as modified by Saito and Hanahan (9) and protease was measured as described by Kunitz (10). The estimation of phospholipase A was by the lysis of human red blood cells (11). Protein was determined by the procedure of Lowry et al. (12).

RESULTS

Absence of Nuclease and Protease Activities in the Phospholipase A Preparation

Although it had not been excluded, the possibility that nucleases in the phospholipase A preparation were responsible for the disaggregation of normal polyribosomes had been shown to be weak (1). For one thing, unlike pancreatic RNase (0.005-0.02 μg/ml) which disaggregated in an indistinguishable manner the polyribosomes of normal and partially hepatectomized animals, the venom preparation (4-20 μg/ml) attacked almost exclusively the normal aggregates. The phospholipase A preparation has now been found to be largely or completely free of RNase and phosphodiesterase (Table I). As the table shows, protease activity was also absent.

Table I

Enzyme Activities in the Phospholipase A Preparation

The activities were estimated as described in "Materials and Methods". The adsorbancies of the reaction products were measured at 260 mμ (RNase), 420 mμ (phosphodiesterase), or 280 mμ (protease).

Activity tested	Enzyme preparation	Product formed
	μg	absorbance
RNase	none	0.02
	0.01 pancreatic RNase	0.70
	0.02	1.45
	500 phospholipase A	0.06
	1000	0.08
	0.02 pancreatic RNase + 1000 phospholipase A	1.47
Phosphodiesterase	100 Crotalus adamanteus venom	0.82
	500 phospholipase A	0.00
	100 Crotalus adamanteus venom + 500 phospholipase A	0.80
Protease	2 trypsin	0.34
	1000 phospholipase A	0.00
	2 trypsin + 1000 phospholipase A	0.35

RNase Activities of Normal and Regenerating Liver Polyribosomes and the Effects of Phospholipase A - Although the total RNase (active + latent) activities (as estimated after treatment with p-chloromercuribenzoate) were similar, two differences were found between normal and regenerating polyribosomes (Table II). First, as the table shows, the normal aggregates contained more of the active enzyme than the regenerating polyribosomes, a difference that may explain the relative instability of the normal aggregates at 37°. Second, phospholipase A

Table II

RNase Activities of Normal and Regenerating Liver Polyribosomes and the Effects of Phospholipase A

Liver samples were taken at 16 hrs after partial hepatectomy and polyribosomes were prepared by the procedure of Bloemendal et al. (3). The freshly prepared polyribosomes (3 mg of protein in 1 ml of medium A) were incubated for 5 min at 37° with no additions or with phospholipase A (20 μg) or p-chloromercuribenzoate (10^{-4}M). The sedimentable fraction was obtained by centrifugation at 105,000 x g for 60 min. RNase was measured in samples corresponding to 0.5 mg of ribosomal protein.

Liver polyribosomes	Addition	RNase activity		
		Sedimentable	Nonsedimentable	Total
		absorbance at 260 mμ		
Normal	none			0.162
	phospholipase A			0.530
	PCMB*			0.485
Regenerating	none			0.020
	phospholipase A			0.018
	PCMB			0.395
Normal	none	0.108	0.050	
	phospholipase A	0.202	0.268	
	PCMB	0.198	0.212	
Regenerating	none	0.028	0.009	
	phospholipase A	0.035	0.020	
	PCMB	0.148	0.298	

* p-chloromercuribenzoate

seemed to activate completely the latent RNase of the normal polyribosomes but had no effect on the latent enzyme of the regenerating aggregates.

As can be seen from the second experiment of Table II, activation of the latent nuclease by the venom enzyme was accompanied by a release of about one-half the RNase activity into the supernatant fluid. Whether the solubilized RNase is more effective in causing polyribosomal disaggregation than the attached enzyme is not known.

On the Relationship Between RNase Activation and Polyribosomal Disaggregation - It seemed likely that a causal relationship exists between the activation of the ribosomal RNase by phospholipase A and the ability of the lipase to disaggregate normal liver polyribosomes. Nevertheless, it was of interest to find that the RNase released by phospholipase A from the normal aggregates caused polyribosomal disaggregation (tested with regenerating polyribosomes) with an effectiveness (based on the hydrolysis of yeast RNA) similar to that of pancreatic RNase. Thus, incubation (5 min, 37°) with ribosomal RNase equivalent in activity to 0.005 and 0.01 μg/ml of pancreatic RNase caused partial and almost complete disaggregation of the polyribosomes, respectively, just as with the pancreatic enzyme.

An additional observation consistent with the role of the phospholipase in disaggregating polyribosomes was obtained with the nonsedimentable (105,000 x g, 1 hr) fraction of liver. This fraction, considered to act as a RNase inhibitor (13, 14, 5, 15, 16, 6), blocked the disaggregation of normal polyribosomes by phospholipase A although the phospholipase activity itself (as measured by the lysis of erythrocytes (11)) was not affected.

DISCUSSION

The disaggregation of the liver polyribosomes of normal rats by preparations of phospholipase A appears to be due to the activation of ribosomal RNase.

Although some other heat stable enzyme cannot be excluded, the activity of the venom preparations probably resides in the phospholipase A itself. If this is so, it would seem to follow that the phospholipids associated with the ribosome are somehow involved in the latency of the RNase.

The resistance of liver polyribosomes from partially hepatectomized animals to disaggregation by phospholipase A may be related to additional ribosomal molecules that make the phospholipids unavailable to the phospholipase. Not only does it remain to learn about the nature of the additional molecules and whether they are simply derived by adsorption from the soluble fraction of regenerating liver but their relationship, if any, to the increased efficiency of the polyribosomes in forming protein must be studied.

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