ISOLATION OF LIVER AND MUSCLE POLYRIBOSOMES IN HIGH YIELD AFTER CELL DISRUPTION BY NITROGEN CAVITATION *

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1. Introduction

It is desireable to prepare pyloribosomes in high yields from large quantities of tissues for the study of specific messenger RNA's, for the study of polyribosomal systems synthesizing a specific protein, and for the synthesis *in vitro* of large quantities of individual proteins by polyribosomal systems. Using the technique of nitrogen cavitation [1], that is, by suddenly decompressing a suspension of grossly minced tissue in buffer that has been equilibrated with an inert gas like nitrogen at approximately 40 atm pressure for 15 min, we have obtained large quantities of undegraded polyribosomes from liver and muscle.

The vigorous homogenization of tissue using tissue grinders leads to mechanical disintegration of polyribosomes and perhaps to damage of the protein synthesizing system due to local heating. On the other hand, many cells are not disrupted if the homogenization is very gentle, which also results in low yields of polyribosomes. Nitrogen cavitation results in almost complete disruption of all cells in tissue fragments with minimal damage to the polyribosomes. Furthermore, if the conditions are carefully chosen, there is minimal damage to intracellular organelles and the liberation of hydrolases into the cytoplasmic supernatant fraction is circumvented to a large extent. In these experiments, a modified high pressure hydrogenation bomb (fabricated from nonmagnetic stainless steel) with a capacity of 500 ml was used, and bombs with much larger capacity are possible.

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2. Experimental

Liver from exsanguinated 75 g Wistar rats, or muscle from 14 day old chick embryos was suspended in two volumes of "H" buffer (0.25 M KCl, 0.01 M $MgCl_2$, 0.1 M RNAse-free sucrose and 0.02 M tris, pH 7.4) and cut into pieces not larger than 6 mm in any dimension. For *in vivo* studies of amino acid incorporation into nascent polypeptides, the animals were injected with 10–15 μC U-^{14}C-amino acid mixture 3 min prior to sacrificing. The tissue suspension was placed in the high pressure chamber and allowed to equilibrate with nitrogen at 40 atm pressure for 15 min stirring gently with a magnetic stirring bar. The tissue suspension then was allowed to flow out through a large exit tube (6.5 mm I.D.) into an erlenmeyer flask. With the sudden fall in pressure, the cells were broken.

The homogenate obtained in this manner was centrifuged at 12,000 × *g* for 11 min to sediment nuclei, mitochondria, other intracellular organelles, and debri. The cytoplasmic supernatant corresponding to 0.4 g tissue was layered on 27 ml of a 12–30% (w/w) linear sucrose gradient (12–40% for muscle) in "H" buffer. After centrifugation for 2 hr at 25,000 rpm in a Spinco No. 25.1 rotor, fractions were collected from the bottom of the tube for measurement of absorbance at 260 mμ and assay of radioactivity.

The polyribosome profiles obtained by this procedure from liver and muscle are depicted in fig. 1. For purposes of comparison, profiles obtained after

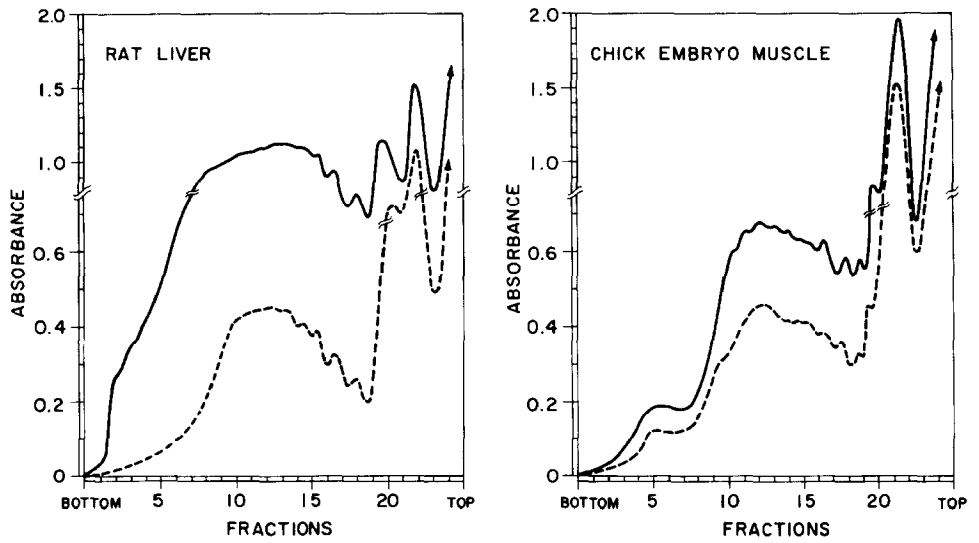

Fig. 1. Sucrose gradient analyses of cytoplasmic supernatants of rat liver (left panel) and chick embryo muscle (right panel) suspended in 0.25 M KCl, 0.01 M $MgCl_2$, 0.1 M sucrose and 0.02 M tris, pH 7.4, and homogenized by nitrogen cavitation. The sucrose were 15–30% (w/w) left panel, and 15–40% right panel. The dashed lines show comparable profiles obtained after homogenization with a Dounce tissue grinder.

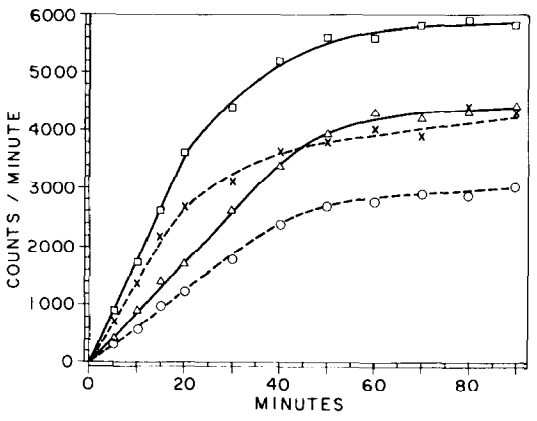

Fig. 2. The time course of U-^{14}C-amino acid incorporation by liver polyribosomes isolated after homogenization by nitrogen cavitation (□——□), by liver polyribosomes isolated after homogenization with a Dounce tissue grinder (x——x), by muscle polyribosomes isolated after homogenization by nitrogen cavitation (△——△), and by muscle polyribosomes isolated after homogenization with a Dounce tissue grinder (○——○). The incubation mixture (1 ml) contained 0.15 M KCl, 0.01 M $MgCl_2$, 0.01 M tris (pH 7.4), 6 mM β-mercaptoethanol, 2 mM ATP, 0.5 mM GTP, 10 mM phospho*enol*pyruvate, 0.3 μmole of each amino acid containing 0.5 μC ^{14}C, 50 μg pyruvate kinase, 0.2 mg homologous tRNA, 0.4 mg homologous pH 5 enzyme fraction, and 0.5 mg polyribosomes.

homogenization with a Dounce tissue grinder also are shown. Cell disruption by nitrogen cavitation gave higher yields of polyribosomes and better defined and more reproducable profiles. After preincubation with labelled amino acids, most of the radioactivity was found in the polyribosome region of the gradient while little was found associated with the single ribosomes; these findings indicate the incorporation of labelled amino acids into nascent polypeptides associated with the polyribosomes. Treatment of the cytoplasmic supernatant with 2 μg/ml of bovine pancreatic ribonuclease at 12° for 20 min resulted in complete degradation of the polyribosomes and their conversion into single ribosomes. This was accompanied by a shift in the radioactivity from the polyribosome region of the gradients to the single ribosome region.

The capacity of polyribosomes prepared after nitrogen cavitation to carry on polypeptide synthesis was evaluated by *in vitro* amino acid incorporation studies using conditions described previously [2]. Typical results are summarized in fig. 2. For comparison, amino acid incorporation after homogenization with a Dounce tissue grinder also is shown. Amino acid incorporation was somewhat greater, and the period of linear incorporation was longer when polyribosomes

prepared after cell disruption by nitrogen cavitation were used.

References

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