THE CONTAMINATION OF RAT-LIVER POLYRIBOSOMAL PREPARATIONS BY NON-RIBOSOMAL PROTEINS

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1. Introduction

Murty and Hallinan [1] have recently reported that free polyribosome preparations from rat liver can be contaminated with membraneous vesicles. During investigations in this laboratory evidence has accumulated suggesting that preparations of the total (free and bound) polyribosomes from liver are similarly contaminated.

2. Methods

Male rats (Wistar, 240 g weight) were used after food had been withheld for 18 hr. Isotopes, D-[1-^{14}C]-glucosamine (specific acitivity 3.1 mc/mmole), L-[1-^{14}C]-leucine (specific activity 7.8 mc/mmole) and [^{3}H-methyl]-choline chloride (specific activity 2000 mc/mmole) were injected intraperitoneally dissolved in 0.15 M NaCl. Post-mitochondrial supernatant was prepared from rat liver in 0.25 M buffered sucrose [2] and was adjusted to 1% with respect to sodium deoxycholate by adding the appropriate volume of 10% sodium deoxycholate in 0.25 M sucrose. The suspension, 5 ml, was layered over a discontinuous gradient composed of 2.0 ml of 0.5 M buffered sucrose and 2.0 ml of 2.0 M buffered sucrose; the buffered sucrose contained Tris-hydrochloric acid buffer pH 7.4 (50 mM), KCl (25 mM) and $MgCl_2$ (5 mM). The tubes were centrifuged at 150,000 × g (R_{ave}) for 150 min in a Spinco Model L ultracentrifuge and the layered supernatant was decanted. The resulting polyribosomal pellet was comprised of an upper "loose" layer and an underlying "packed" layer; the "loose" layer was removed by washing the surface of the pellet with 0.25 M buffered sucrose using a Pasteur pipette. The washings were gently homogenised by hand and centrifuged at 13,500 × g (R_{ave}) for 10 min to remove any undispersed material, the suspension was then centrifuged at 150,000 × g (R_{ave}) for 60 min to sediment the "loose" ribosomes. The "loose" and "packed" pellets were precipitated and washed twice with 10% trichloroacetic acid, washed twice with 95% methanol and dissolved in 0.5 N NaOH for determination of protein [3], RNA [4] taking $E^{1\%}_{260m\mu} = 300$ and radioactivity (by scintillation counting).

3. Results and discussion

Analysis of the layers showed that both contained RNA and protein, the RNA/protein ratio of the "packed" layer being higher than that for the "loose" layer (table 1). The "packed" layer was transparent and colourless while the "loose" layer was transparent but slightly brown. The amounts of protein and RNA recovered in each layer were approximately the same.

The polyribosomes were prepared for the purpose of studying the significance of the labelling which had been observed to occur in ribosomes isolated from the livers of rats injected with [^{14}C]-glucosamine [5]. In this previous investigation maximum labelling of the ribosomal protein was found to occur 40 min after injection when the microsocomal membranes were themselves highly labelled and the total radioactivity in the ribosomes was found to be small compared with the total microsomal radioactivity. These results suggested that the low ribosomal labell-

Table 1
Comparison between "loose" and "packed" polyribosomes from rat liver.

Polyribosome preparation	Protein/g liver wet weight (mg)	RNA/g liver wet weight (mg)	RNA/protein ratio	"Contaminating" radioactivity dpm/mg protein	"Incorporated" radioactivity dpm/mg protein	Phospholipid radioactivity dpm/mg protein
"packed"	0.96 ± 0.22	1.12 ± 0.24	1.16	50 ± 30	276 ± 51	400 ± 75
"loose"	1.15 ± 0.23	0.96 ± 0.27	0.85	198 ± 39	331 ± 73	1790 ± 156

"Packed" and "loose" polyribosomes were prepared as described in the text. Contaminating radioactivity is the radioactivity present in the polyribosomes when they were prepared from unlabelled livers in the presence of labelled ribosomal supernatant. Incorporated radioactivity is the activity of polyribosomes prepared from the livers of rats 40 min after injection of 4 μc of [^{14}C]-glucosamine. Phospholipid radioactivity was the radioactivity extracted by chloroform: methanol (1:1 v/v) from polyribosomal fractions prepared from the livers of rats 30 min after injection of [^{3}H]-choline (50 μc). The results are the means of four or more experiments + standard deviation.

ing might be due to small quantities of microsomal membrane protein becoming adsorbed by the ribosomes. This hypothesis was tested as follows: labelled ribosomal supernatant was prepared from the livers of rats killed 40 min after [^{14}C]-glucosamine injection (4 μc) by treating the postmitochondrial supernatant with deoxycholate as described and centrifuging at 150,000 × g (R_{ave}) for 60 min; this was mixed with an equal volume of post-mitochondrial supernatant prepared from the livers of rats that had not received isotope injections; the resulting suspension was adjusted to 1% deoxycholate concentration and the "loose" and "packed" polyribosomes prepared. The packed polyribosomes remained virtually unlabelled while the "loose" polyribosomes were found to be radioactive, indeed they were almost as radioactive as "loose" polyribosomes prepared from the livers of injected animals (table 1). Similar experiments conducted with rats injected with [^{14}C]-leucine also showed contamination of the "loose" polyribosomes. In these experiments it was necessary to prepare the liver ribosomal supernatant 30–60 min after injection, when the liver proteins were highly labelled; ribosomal supernatant prepared at short times (5–10 min) after injection did not cause detectable contamination, presumably because the liver protein was not sufficiently radioactive. When an alternative discontinuous gradient was employed [6] a contaminated "loose" surface layer of polyribosomes was again obtained.

Varying the percentage of sodium deoxycholate (between 0.5% and 1.5%) during the preparation did not reduce the degree of contamination of the "loose" layer, but contamination could be reduced by mixing smaller proportions of labelled ribosomal supernatant with the unlabelled post-mitochondrial supernatant. Studies of the rates of amino acid incorporation *in vivo* into the polyribosomes of both layers did not reveal any significant differences in behaviour at short time intervals (3–20 min) after injection. At longer intervals (30–50 min) the specific activities of the "loose" layer were higher than those of the "packed" layer, presumably a consequence of contamination.

Electron microscopic examination of the polyribosome preparations showed that both layers contained polyribosomes. The "loose" layer contained in addition some ferritin particles and a few small membraneous vesicles very similar in appearance and size to those found by Murty and Hallinan [1]. It seemed that treating the post-mitochondrial supernatant with deoxycholate did not destroy all the membraneous structures of the liver and those that escaped destruction sedimented into the surface of the ribosomal pellet, disturbing the close packing of the surface so giving rise to the "loose" layer. This argument was supported by finding that the "loose" layer contained much more phospholipid radioactivity than the "packed" layer (table 1) after injecting the rats with [^{3}H]-choline to label the membrane phospholipid [7]. It is suggested that these vesicles adsorbed labelled protein during dissolution of the membranes and so caused isotopic contamination of the "loose" polyribosomes. Murty and Hallinan [1] proposed that the contaminating membranes were derived from smooth endoplasmic reticulum after finding that the "free" ribosomal fraction became labelled with glucosamine shortly after injection of the isotope. The findings

reported here indicate that such labelling can result from non-specific adsorption and therefore is not a reliable indication of the identity of the membranes.

The results described are of importance in studies which employ sensitive methods, for example immunochemical or radioisotope techniques, in a study of polyribosomal function. Adequate surface washing of polyribosomal pellets needs to be employed to remove the contaminated surface layer.

References

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