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Dissociation of ribosomes produced by dimethylnitrosamine and lasiocarpine in mouse liver

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HEPATOTOXIC agents such as dimethylnitrosamine and pyrrolizidine alkaloids are activated in liver into alkylating agents which cause rapid disaggregation of liver polysomes,[1–4] though the mechanism of disaggregation is unknown. One possible mechanism is the alkylation of messenger RNA in polysomes with polysome fragmentation occurring either *in vivo* or during experimental procedure. In this event, the ribosomes would consist of monosomes analogous to those produced by the action of ribonuclease on polysomes.[5] As recently reviewed by Davis,[5] the 70 S ribosome fraction in bacterial cell lysates was found to contain two kinds of ribosomes with different dissociabilities. In view of this, it seemed of interest to determine the dissociability of the ribosomes produced by these alkylating agents in comparison to the dissociability of monosomes produced by ribonuclease and ribosomes produced by the action of puromycin *in vivo*. We found that ribosomes produced by these hepatotoxic agents and puromycin, unlike those produced by ribonuclease, are dissociated into ribosome subunits by potassium treatment, suggesting that the fragmentation of messenger RNA is an unlikely mechanism to explain polysome disaggregation by these agents.

Swiss albino female mice were given a single intraperitoneal injection of 50 mg/kg body weight of either dimethylnitrosamine or lasiocarpine dissolved in 0·15 M NaCl. One hr later the animals were sacrificed. Livers were perfused with ice-cold physiological saline, removed and weighed. The post-mitochondrial supernatant was prepared as previously described.[4] A 10 per cent aqueous solution of sodium deoxycholate was added to the post-mitochondrial supernatant to a final concentration of 1 per cent, and it was centrifuged at 35,000 rev/min for 80 min at 5° in a Spinco L centrifuge with a type 50 rotor. The pellet was rinsed twice with buffer containing 10 mM Tris–HCl (pH 7·6), 5m M $MgSO_4$, 0·25 M sucrose and either 25 mM KCl or 0·3 M KCl and resuspended in 1 ml of the same buffer per g of original liver weight. The buffer containing 0·3 M KCl had been used by Mechler and Mach[6] to dissociate puromycin-produced ribosomes.

Two-tenths ml of suspension was layered onto 4·6 ml of 0·5 M to 1·2 M linear sucrose gradient containing 70 mM Tris–phosphoric acid (pH 7·2), 5 mM $MgSO_4$, either 25 mM KCl or 0·3 M KCl, and acrylamide gel components as described by Cole and Brooks.[7] The gradients were centrifuged at 35,000 rev/min in a Spinco L centrifuge with a SW 50·1 rotor for 90 min at 5°. The gradients were layered with 30 μl of water to flatten the meniscus. Photopolymerization with a fluorescent lamp was completed within 30 min. The gels were removed from the tubes, sliced longitudinally into four slabs,[8] fixed in 1 N acetic acid, and stained with methylene blue (0·2%) in acetate buffer, pH 4·7.[9] This method was chosen for its good resolution of the ribosomal particles and subunits in a crude ribosomal preparation and its simplicity.

The 80 S monosomes were produced by adding 2 μg of bovine pancreatic ribonuclease to 1 ml of deoxycholate-treated post-mitochondrial supernatant. The mixture was incubated at room temperature for 15 min. Run-off ribosomes were produced by intraperitoneal injection of 350 mg/kg body weight of puromycin hydrochloride dissolved in 0·15 M NaCl 3 hr before sacrifice. These ribosomes were centrifuged as described above.

For each treatment three to four mice were used. Figure 1a presents a normal mouse liver ribosome–polysome profile showing distinct 60 S subunits, 80 S ribosomes, oligosomes and increasingly larger polysomes. Ribonuclease treatment of polysomes produced monosomes (Fig. 1b) which were not dissociated in 0·3 M KCl (Fig. 1c). In contrast, puromycin-produced ribosomes (Fig. 1d) dissociated into 40 S and 60 S subunits in 0·3 M KCl solution (Fig. 1e), consistent with their being run-off ribosomes.[6]

The high-potassium treatment caused only slight dissociation of 80 S ribosomes seen in the sample from normal liver (Fig. 2a, b) leaving a distinct band of 80 S ribosomes which may be monosomes (Fig. 2b). The 80 S ribosomes produced by dimethylnitrosamine and lasiocarpine, which are present in low potassium (Fig. 2c, e), were dissociated into 40 S and 60 S subunits in the presence of 0·3 M KCl as shown by the large increase in subunit bands and little remaining 80 S bands (Fig. 2d, f). Thus these agents caused an accumulation of 80 S ribosomes which have dissociability in 0·3 M KCl similar to that of the 80 S ribosomes produced by treatment with puromycin but dissimilar to that of ribonuclease-produced monosomes.

Extraction of RNA from the small and large ribosome subunits and electrophoresis in 3% acrylamide gels[10] indicated that these subunits contained 18 S and 28 S RNA, respectively, as compared to purified 16 S and 23 S *Escherichia coli* ribosomal RNAs. This further suggests that the

ribosomes produced by these hepatotoxic agents are 80 S ribosomes, which upon high-potassium treatment are dissociated into 40 S and 60 S subunits.

The present study on the dissociability of the ribosomes suggests that the fragmentation of messenger RNA is an unlikely mechanism of rapid polysome disaggregation by dimethylnitrosamine and lasiocarpine. It also excludes the possible activation of tissue ribonuclease as the mechanism of polysome disaggregation by these agents, which is consistent with the finding of Mizrahi and Emmelot[11] that liver ribonuclease activity is not elevated after dimethylnitrosamine treatment.

The small amount of ribosomes observed in the normal liver was not entirely dissociated in 0·3 M KCl. This suggests that the experimental procedure artificially fragmented the polysomes present in the normal liver, producing monosomes. The particular sample of lasiocarpine treatment had some polysomes remaining (Fig. 2e) and this may have similarly led to the formation of monosomes through artificial fragmentation, resulting in the presence of residual monosomes in the high-potassium treatment while the majority of ribosomes were converted into ribosome subunits (Fig. 2f).

As shown in the present study, the evaluation of dissociability of ribosomes in mammalian tissue may be helpful in exploring the mechanism of polysome disaggregation as to whether or not it is due to fragmentation of polysomes.

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Effect of GABA and 3-aminopropane sulphonic acid on the labelling of brain amino acids from [U-^{14}C] glucose in the conscious mouse

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IN A recent publication[1] the effects of intraventricularly injected excitatory and inhibitory amino acids on the cerebral metabolism of [U-^{14}C]glucose in mice anaesthetized with pentobarbitone sodium (Nembutal) were reported. In these experiments the metabolic effects of the amino acids were superimposed upon a cerebral metabolism which was already profoundly modified by the anaesthetic. In addition, the depression of nervous activity produced by the anaesthetic limited the dose of the depressant amino acids which could be injected without severe hypoxic consequences. Nevertheless, the conclusion was reached that the inhibitory amino acids had metabolic effects which were consistent with a depression of oxidative processes, and that the effects were similar in this respect to those produced by the barbiturate itself.

To substantiate these conclusions, a limited series of similar experiments has now been conducted on conscious mice. The labelled glucose and the inhibitory amino acids were injected intraventricularly, and all methods were the same as previously described[1] except that the anaesthetic was omitted and