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CERTAIN CHARACTERISTICS OF MONOSOMES PRODUCED BY CARBON TETRACHLORIDE

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Abstract—A single injection of CCl_4 causes the disaggregation of hepatic polysomes into monosomes which are dissociable into subunits in buffer containing 0·3 M KCl. These monosomes are free of peptidyl-tRNA. Similarly, starvation produces monosomes which are dissociable into subunits and lack peptidyl-tRNA. We, therefore, conclude that the monosomes produced by CCl_4 resemble runoff ribosomes.

Carbon tetrachloride (CCl_4) is a hepatotoxic agent which rapidly inhibits liver protein synthesis and disaggregates liver polysomes. Three mechanisms of polysome disaggregation by hepatotoxic agents have been proposed: fragmentation of messenger RNA (mRNA) producing complexed ribosomes, inhibition of initiation producing runoff ribosomes [1], and premature falloff of ribosomes from mRNA producing falloff ribosomes [2]. Gravela *et al.* [3] have shown that ribosomes produced by CCl_4 are dissociated into subunits in solutions containing low magnesium concentrations, suggesting they are not complexed ribosomes. Runoff ribosomes and falloff ribosomes can be distinguished on the basis of tRNA and peptidyl-tRNA content [2]. Therefore, we investigated the tRNA content of monosomes produced by CCl_4 to distinguish between the latter two mechanisms of polysome disaggregation.

MATERIAL AND METHODS

Male Swiss Webster albino mice (25–30 g) were injected intraperitoneally with 0·5 ml/100 g body wt of a 1:1 CCl_4–mineral oil mixture. Control animals received mineral oil alone. Animals were sacrificed 30 min after injection and livers were homogenized in 3 vol. of TKMS buffer (25 mM Tris–HCl, pH 7·5, 25 mM KCl, 3 mM $MgSO_4$ and 250 mM sucrose or as indicated in the figures).

Ribosome sedimentation profiles were produced by layering 0·25 mg ribosomes on 17–41 per cent linear sucrose gradients in TKM as indicated in each figure. Gradients were centrifuged in a Beckman Spinco L ultracentrifuge equipped with an SW 50·1 rotor for varying lengths of time. The gradients were scanned at 260 nm by a Gilford spectrophotometer equipped with flow cell and recorder [2]. Ribosome particles were precipitated from sucrose gradients by the method of Dessev and Grancharov [4]. Isolation of ribosomes, extraction of RNA and polyacrylamide gel electrophoresis were performed as previously described [2].

RESULTS

As shown in Fig. 1A, a single dose of CCl_4 (0·5 ml/100 g body wt) completely disaggregated liver polysomes into monosomes within 30 min after injection. Mineral oil alone had no effect on the polysome profile (Fig. 1C and Ref. 5). When monosomes produced by CCl_4 were centrifuged through sucrose gradients containing 0·3 M KCl (Fig. 1B), monosomes dissociated into 40S and 60S ribosomal subunits in agreement with the previous findings of Gravela *et al.* [3].

The number of tRNA molecules per ribosome was determined by extraction of RNA from isolated ribosomes and electrophoresis in 4% polyacrylamide gels (Fig. 2, Ref. 2). Since each ribosome contains 1 molecule of 5S RNA, the number of tRNA molecules attached to each ribosome was determined from the

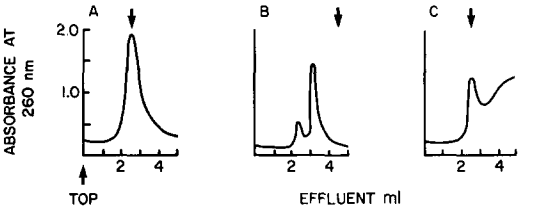

Fig. 1. Sedimentation profile of normal and CCl_4-produced ribosomes. Sedimentation profile of ribosomes 30 min after injection with 0·5 ml/100 g body wt (1:1) CCl_4–mineral oil mixture. Ribosomes were obtained by centrifuging deoxycholate-treated postmitochondrial supernatant for 90 min at 35,000 rev/min in a Beckman type 50 rotor at 5°. Ribosomes were resuspended in TKMS buffer and 0·25 mg ribosomes was layered on a 17–41 per cent linear sucrose gradient containing either (A) 25 mM Tris–HCl (pH 7·5), 25 mM KCl and 3 mM $MgSO_4$ or (B) 25 mM Tris–HCl (pH 7·5), 300 mM KCl, and 3 mM $MgSO_4$. Panel C represents the mineral oil control, 25 mM Tris–HCl (pH 7·5), 25 mM KCl and 3 mM $MgSO_4$. Gradients A and C were centrifuged for 90 min at 35,000 rev/min, while gradient B was centrifuged for 180 min at 35,000 rev/min. The gradients were scanned as described in Methods. The arrows mark the position of the 80S monosome.

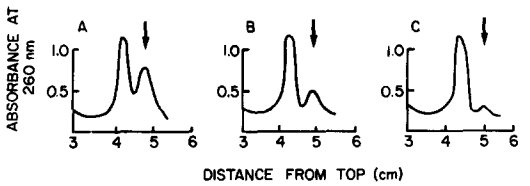

Fig. 2. Electrophoretic profile of 5S and 4S RNA. RNA from ribosomes was extracted and electrophoresed as previously described.[2] Gels were scanned at 260 nm with a Gilford spectrophotometer equipped with a linear transporter. The area under each peak was weighed. The value of the 4S peak was multiplied by 1·5 and divided by the value of the 5S peak. (A) normal polysomes extracted after incubation with Pronase, (B) monosomes produced by CCl_4 intoxication extracted without Pronase, and (C) 4S and 5S RNA from the 60 S subunit from CCl_4-produced monosomes. The 60 S subunits were isolated by centrifuging either CCl_4 or starvation-produced monosomes on linear sucrose gradients containing 0·3 M KCl for 3 hr at 35,000 rev/min in a Beckman type SW 50.1 rotor. Gradients were scanned as described in Methods, the 60 S subunit peaks were collected, and RNA was extracted. The arrows mark the position of the 4S peak.

molar ratio of 4S RNA to 5S RNA. Table 1, line 1, and Fig. 2A demonstrate the tRNA content of normal polysomes. When polysomes were incubated with Pronase prior to extraction of RNA, the 4S to 5S RNA ratio was 1·7. When extraction was done without prior incubation with Pronase the 4S:5S RNA ratio was decreased to 0·9. It has been shown previously [2, 6] that incubation with Pronase before extraction of RNA allows all types of tRNA (tRNA, aminoacyl-tRNA and peptidyl-tRNA) to be extracted, since nascent peptides are hydrolyzed from peptidyl-tRNA. However, if extraction is done without prior incubation with Pronase, only tRNA and aminoacyl-tRNA are extracted. Therefore, the difference in the values of the 4S:5S RNA ratios in samples treated with and without Pronase indicated the amount of peptidyl-tRNA. Thus, normal ribosomes contain 0·9 molecule of free and/or aminoacyl-tRNA and 0·8 molecule of peptidyl-tRNA.

In contrast, monosomes produced by CCl_4 have a 4S:5S RNA ratio of 0·9 and 0·8 when incubated with and without Pronase, respectively (Table 1, line 2). This indicates the lack of peptidyl-tRNA on CCl_4-produced monosomes. Monosomes produced by 48 hr starvation (Table 1, line 3) contained 0·7 and 0·7 tRNA molecule when incubated with and without Pronase, indicating that they also lack peptidyl-tRNA [1, 2]. To exclude the possibility that tRNA was nonspecifically adsorbed to the monosomes produced by CCl_4, ribosomes were isolated in TKM buffer containing 1·5 mM $MgSO_4$ (Table 1, line 4, Ref. 7). The CCl_4-produced monosomes still contained 0·5 tRNA molecule.

To further characterize the monosomes produced by CCl_4 and starvation, isolated monosomes were centrifuged in sucrose gradients containing 0·3 M KCl which caused their dissociation into 40S and 60S subunits (Fig. 1B). When RNA was extracted from samples of isolated 60S ribosomal subunits, a 4S:5S RNA ratio of 0·1 was obtained from livers of both CCl_4-treated and starved animals (Fig. 2C, Table 1, lines 5 and 6). In addition, a tRNA peak was observed in polyacrylamide gels containing samples of RNA extracted from 40S subunits of both CCl_4-treated and starved animals. However, the amount of tRNA could not be quantitated due to the lack of 5S RNA in the 40S subunit. These results indicate a small amount of tRNA was present on each ribosomal subunit.

DISCUSSION

We have shown in this paper, in agreement with others [5, 8–10] that a single dose of CCl_4 rapidly disaggregated liver polysomes into monosomes. We have also shown in agreement with Gravela *et al.* that these monosomes were dissociable into subunits under appropriate ionic conditions. However, we further characterized the monosomes produced by CCl_4 by determining their tRNA content, since we have previously demonstrated that the mechanism by which hepatotoxic agents disaggregate liver polysomes cannot be ascertained by merely determining the dissociability of the monosomes produced [2]. It was found

Table 1. tRNA Content of ribosomes*

Type of ribosome	4S:5S Ratio with Pronase	4S:5S Ratio without Pronase
1. Normal polysomal ribosomes	1·7	0·9
2. CCl_4 monosomes	0·9	0·8
3. Starvation monosomes	0·7	0·7
4. CCl_4 monosomes (1·5 mM Mg^{2+})†	0·7	0·5
5. CCl_4 60S subunit‡		0·1
6. Starvation 60S subunit‡		0·1

* tRNA content of ribosomes was determined as previously described [2, 6].

† Livers from CCl_4-intoxicated mice were homogenized in 50 mM Tris–HCl (pH 7·5), 100 mM KCl, 1·5 mM $MgSO_4$ and 250 mM sucrose. Deoxycholate-treated postmitochondrial supernatant was centrifuged for 4 hr at 35,000 rev/min in a Beckman type 50 rotor through 1·0 ml of a 1 M sucrose cushion in TKM buffer. Ribosomal subunits were resuspended and processed as above.

‡ Isolated CCl_4 and starvation 60S subunits were obtained as described in Fig. 2.

that the monosomes produced by CCl_4 contained less than 1 tRNA molecule (0·8) of free and/or aminoacyl-tRNA, but no peptidyl-tRNA. These monosomes showed a striking similarity in dissociability and tRNA content to the monosomes produced by starvation. Monosomes produced by CCl_4 and starvation also contained a similar amount of tRNA on the isolated 60S subunit.

We, therefore, conclude that hepatic monosomes produced by CCl_4 intoxication are of the runoff type because of their dissociability into subunits [1] and the lack of peptidyl-tRNA [1, 2]. Furthermore, we conclude that the polysome disaggregation produced by various hepatotoxic agents can occur by different mechanisms. We have previously shown that monosomes produced by dimethylnitrosamine are probably the result of premature falloff of ribosomes from messenger RNA, since they are dissociable and still contain peptidyl-tRNA [2].

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