PHYSICOCHEMICAL CHARACTERISTICS OF ISOLATED 55-S MITOCHONDRIAL RIBOSOMES FROM RAT-LIVER

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SUMMARY

A procedure for the preparative isolation of rat-liver mitochondrial ribosomes free of cytoplasmic contamination is described. The 55-S ribosome was characterized by electrophoretic analysis on acrylamide and agarose gels. It is concluded that its charge/mass ratio is very low and that its volume is larger than that of *E.coli* ribosomes and smaller than that of rat-liver cytoplasmic ribosomes. Therefore, the 55-S ribosome is not adequately described by the term "miniribosome".

INTRODUCTION

During the last years, it has become generally accepted that animal mitochondria contain small ribosomes (sometimes called "miniribosomes") as the native protein-synthesizing structures. The small size was deduced from the sedimentation coefficient, 55 S, and from the small RNAs they contain (for reviews, see refs. 1 and 2). Electronmicroscopically the 55-S particle appears somewhat smaller than its cytoplasmic counterpart[3].

The experiments presented in this paper were meant to get a better insight into the real dimensions and physicochemical properties of the 55-S ribosome.

METHODS

a. *Buffers:* KMEDT buffer: 100 mM KCl, 10 mM $MgCl_2$, 0.1 mM EDTA, 1 mM dithiothreitol, 10 mM Tris-HCl, pH 7.6; Gel buffer: 60 mM NH_4 acetate, 4 mM Mg acetate, 6 mM β-mercaptoethanol, 10 mM Tris-acetic acid, pH 7.6; ST buffer: 0.25 M sucrose, 1 mM Tris-HCl, pH 7.4.

b. *Isolation and washing of mitochondria:* The method has been adapted

from Loewenstein *et al.*[4]. For each experiment the livers of 40 male Wistar rats of 80-120 g, starved overnight, were used. Sterile ST buffer was used throughout. After homogenization, cells and nuclei were removed (1 min at 3,200 x *g*) and the mitochondria were pelleted (2 min at 17,000 x *g*), resuspended and spun down at 19,000 x *g* for 5 min. Then the mitochondria were suspended in 200 ml, and 200 ml buffer containing 1.75 mg of digitonin per ml were added (giving a ratio of 0.12 - 0.16 mg digitonin/mg protein). After 2 min at 0° C, the suspension was diluted to 1000 ml and the mitochondria were spun down at 19,000 x *g* for 5 min, resuspended and spun down again. The final pellet was suspended and lysed as described under c.

c. *Isolation of ribosomal particles from mitochondria:* washed mitochondria were suspended in 140 ml KMEDT buffer and lysed by the addition of 10 ml 20 % Triton X-100. After stirring for 5 min at 0° C and clarification at 9,5000 x *g* for 10 min, the supernatant was layered onto 10 ml of 1 M sucrose, 1 % Triton X-100 in KMEDT buffer. After 3 h at 60,000 rpm in a Beckman Ti60 rotor, the pellets, containing mitochondrial ribosomes were resuspended in KMEDT buffer, layered on 35 ml isokinetic sucrose gradients in the same buffer (sucrose concentration at the top 15.0 % (w/v)) and spun for 15 h at 22,000 rpm in a Beckman SW27 rotor at 4° C. The gradients were pumped through a flow-cell of a LKB Uvicord (wavelength 254 nm), to which a recorder was connected, and the peak fractions were collected. The ribosomal fractions were diluted twice with KMEDT buffer and spun for 4 h at 50,000 rpm in a Beckman Ti50 rotor. The pellets of ribosomal particles were resuspended in KMEDT buffer.

d. *Other ribosomes:* Total rat-liver cytoplasmic ribosomes were prepared according to Blobel & Potter[5]; 80-S ribosomes were isolated from these ribosomes by sucrose-gradient separation.

Total *E.coli* ribosomes and S-100 were prepared according to Nirenberg[6]. 70-S ribosomes were purified on sucrose gradients.

RESULTS AND DISCUSSION

Sedimentation characteristics of mitochondria ribosomes.

Initially, rat-liver mitochondrial ribosomes showed very high blanks without puromycin in the peptidyl transferase reaction[7]. Since contamination with lysosomal enzymes, causing splitting off of acetyl-leucine or other small ethylacetate-soluble molecules from acetyl-leucyl-tRNA, might explain this, the digitonin treatment of mitochondria (*cf.* METHODS) was introduced: this method effectively removes lysosomes from mitochondria without affecting oxidative phosphorylation[4]. Fig. 1 shows the profile that was obtained

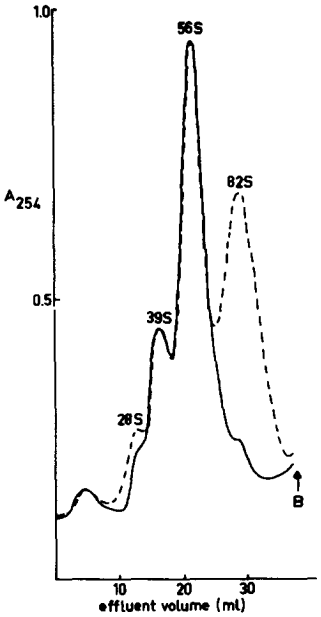

Fig. 1. *Separation of mitochondrial ribosomes on isokinetic sucrose gradients.* The procedure is described under METHODS. Sedimentation coefficients were relative to *N. crassa* cytoplasmic 77-S ribosomes run in a parallel tube. ——: ribosomes from mitochondria washed with digitonin; — — —: ribosomes from mitochondria washed twice with 0.25 M sucrose, 1 mM EDTA, pH 7.4.

when crude mitochondrial ribosomes were separated on isokinetic sucrose gradients. Mitochondria treated with digitonin yielded a major fraction of 56-S ribosomes (about 30 A_{260} units from 40 rat livers) and subunit peaks of 39 S and 28 S. A clear difference was seen with ribosomes from

mitochondria washed twice with 0.25 M sucrose, 1 mM EDTA instead of digitonin: with this method a high peak of 82-S ribosomes was obtained. Obviously, the digitonin treatment does not only remove lysosomes from the mitochondria but also adhering 80-S ribosomes. The latter finding is consistent with observations by Malkin[8]. In the peptidyl transferase reaction the blanks without puromycin were indeed brought back to normal values if 55-S ribosomes from mitochondria treated in this way were used. This makes the assumption that lysosomal hydrolases interfere with the peptidyl transferase assay quite plausible.

Electrophoresis of mitochondrial ribosomes.

Fig. 2a shows a comparison of the migration velocities of 55-S ribosomes

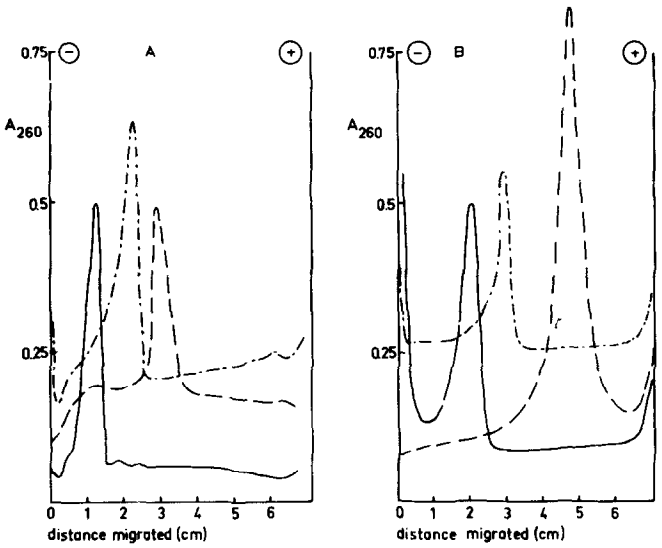

Fig. 2. *Gel electrophoresis of ribosomes.*
A: 2.2 % polyacrylamide gels. The electrophoresis was performed according to Talens *et al.*[9], except that pre-swelling was omitted. The buffer was 74 mM NH_4 acetate, 5 mM $MgCl_2$, 50 mM Tris-acetate, pH 7.8. Electrophoresis was for 3 h at 12 mA/gel at 4° C.
B: 0.4 % agarose gels. The electrophoresis was carried out according to Talens *et al.*[10], in gel buffer (METHODS). Electrophoresis was for 2.5 h at 9 mA/gel at 4° C. Gels were scanned at 260 nm on a Gilford 2400-S spectrophotometer with a linear transport system. ——: 55S; —·—·—: rat-liver 80S; — — —: *E.coli* 70S.

and of rat-liver cytoplasmic 80-S and *E.coli* 70-S ribosomes on 2.2 % polyacrylamide gels. As expected[9], the larger 80-S ribosomes move slower than the smaller *E.coli* ribosomes, but 55-S mitochondrial ribosomes, instead of running in front of 70 S, migrate considerably slower than 80 S. It is not allowed, of course, to conclude by extrapolation that the volume of the 55-S ribosome is much larger than that of the 80-S ribosome. Another reason for this unexpected behaviour could be that the charge/mass ratio of the mitochondrial ribosome is lower than of other ribosomes. This can be tested by investigating the electrophoretic mobility in 0.4 % agarose gels (fig. 2b). Under these circumstances practically no sieving effect occurs, migration velocity is only determined by the charge/mass ratio. Fig. 2b shows that under these conditions 70-S ribosomes travel fastest, 80-S ribosomes slower and 55-S ribosomes slowest. This indicates that 55-S ribosomes have a very low negative charge as compared to its mass, which is probably due to a high amount of ribosomal protein relative to its RNA.

In gels with a pore size gradient (*i.e.* with a gradient in the acrylamide concentration) it is possible to electrophorese ribosomes to a point that no further penetration is possible[10,11]. The distance migrated to reach equilibrium therefore depends on the volume of a ribosome, although factors like rigidity and flexibility may also slightly influence penetration. Fig. 3 shows the migration of *E.coli* 70-S, rat-liver cytoplasmic 80-S and rat-liver mitochondrial 55-S ribosomes in 3-8 % linear acrylamide gradients. It is evident that the 55-S ribosomes get stuck in a position intermediate between 70-S and 80-S ribosomes (that *E.coli* 70-S ribosomes split up into two populations has also been found by Talens *et al.*[9,10]). This shows that the volume of 55-S ribosomes is much higher than its sedimentation coefficient suggests.

These experiments were done with highly purified 55-S ribosomes showing an $A_{260\ nm}/A_{230\ nm}$ ratio of 1.2 or higher. Contamination with membrane fragments[1], causing a slow migration velocity in gels, could be excluded with

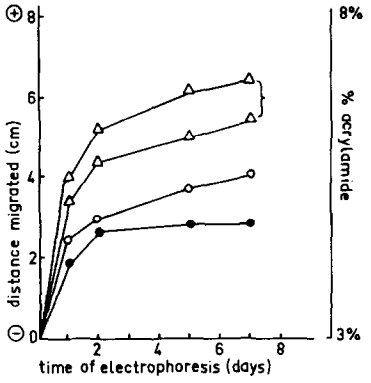

Fig. 3. *Electrophoresis of ribosomes on a pore size gradient of polyacrylamide.* Linear 3-8 % polyacrylamide gradients were made as described by Talens *et al.*[10]. Electrophoresis was in "gel buffer", at 3.6 mA/gel at 4° C, with continuous circulation of the buffer via a peristaltic pump. Runs were in triplicate, and 55-S ribosomes were from two separate experiments. At the times indicated gels were scanned (see fig. 2), and then electrophoresis was continued. o—o: 55S; ●—●: rat-liver 80S; Δ—Δ: *E.coli* 70S.

certainty: the ribosomes from mitochondria labelled *in vivo* in their phospholipids by [^{14}C]-ethanolamine were the only fractions definitely devoid of radioactivity.

It is possible to make a rough estimate of the ribosomal volumes: we have determined the buoyant density in CsCl of the 55-S ribosomes to be 1.43. This accords to 37 % RNA and 63 % protein[12] (*cf.* also fig. 2b). A similar low density has also been found by Attardi and Ojala[13]. Taking the RNA content per ribosome as about 1.0 x 10^6 D (*cf.* ref. 14), the molecular weight of the 55-S ribosome is then about 2.7 x 10^6, equal to that of *E.coli* ribosomes[15]. Assuming that in the gel buffer about the same ratios between the buoyant densities of different ribosomes exist as in CsCl (a very rough assumption, of course), we again arrive at a volume of the 55-S ribosome intermediate between those of 70-S and 80-S ribosomes.

In conclusion, it is evident that the ribosome of rat-liver mitochondria can be isolated as a 55-S particle. We now interpret the previously reported [16] chloramphenicol-sensitivity of the peptidyltransferase reaction of 80-S

particles to be due to contamination of this fraction with 55-S particles.

Our results further show that notwithstanding the "mini-RNAs" present in and the low sedimentation coefficient of the 55-S ribosome, the rat-liver mitochondrial ribosome is not "mini" with respect to volume and molecular weight. Its physicochemical properties are determined by its relatively low charge and its high protein content.

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