PREPARATION AND SOME PROPERTIES OF ACTIVE SUBUNITS FROM RAT LIVER RIBOSOMES

K. Terao and K. Ogata

Department of Biochemistry, Niigata University
School of Medicine, Niigata, Japan.

Received October 28, 1969

SUMMARY

Rat liver ribosomes were dissociated to 60S and 40S fractions by incubation with 1 M KCl at 37° for 2 h, and separated by subsequent density-gradient centrifugation. While the 40S fraction contained 40S subunits only, the 60S fraction consisted of 60S subunits as a major fraction and dimers of 40S subunits, which could be removed by a 2nd sucrose gradient centrifugation in Medium IV containing 3 mM Mg^{++}. The pure 60S and 40S subunits thus prepared could be reassociated to 80S particles which were active in poly U-dependent polyphenylalanine synthesis. ^{3}H-poly U interacted only with the smaller subunits.

It has been demonstrated that E. coli ribosomes are readily dissociated to 50S and 30S subunits by decreasing Mg^{++} concentration in the suspending medium and that these subunits can be reassociated at higher Mg^{++} concentration to active 70S ribosomes. On the other hand, on decreasing the Mg^{++} concentration by dialysis, rat liver ribosomes were gradually dissociated to large and small subunits, through 63S and 50S intermediate components (Hamilton and Peterman, 1959; Peterman, 1964). The complete removal of Mg^{++} ion by the addition of excess EDTA results in rapid dissociation of ribosomes to 47S and 32S subunits (Tashiro et al, 1965), which were reported, however, to be biologically inactive even after dialysis against solutions containing a suitable concentration of Mg^{++}. Thus it has been difficult to obtain active subunits from liver ribosomes.

Recently, Martin and Wool (1968) reported that active 60S and 40S subunits could be prepared from rat muscle ribosomes by brief incubation with 1 M KCl at 37°, followed by density gradient centrifugation at room temperature. Modification of the method of Martin and Wool has made it possible to obtain pure preparations of active subunits from rat liver

ribosomes. This communication describes the preparative method and some properties of the active subunits of rat liver ribosomes.

MATERIALS AND METHODS

Albino rats of the Wistar strain, weighing about 150 g and fasted overnight, were employed. Ribosomes were prepared from rat liver by the method of Rendi and Hultin (1960), except that only desoxycholate was used as a detergent and the ribosomes were suspended in Medium I (0.25 M sucrose, 10 mM $MgCl_2$, 25 mM KCl, 10 mM $KHCO_3$, 10 mM β-mercaptoethanol and 50 mM Tris-HCl buffer, pH 7.6).

RESULTS AND DISCUSSION

Preliminary experiments showed that liver ribosomes were more difficult to dissociate into subunits than muscle ribosomes and that it was necessary to incubate them with 1 M KCl at 37° for 2 h instead of 3 to 5 min. The pattern of density gradient centrifugation of KCl-treated ribosomes at 22-24° is shown in Fig. 1, in which a 15 to 30 % linear sucrose gradient in Medium II (850 mM KCl, 10 mM $MgCl_2$, 10 mM β-mercaptoethanol and 50 mM Tris-HCl buffer, pH 7.6) was employed. Rat liver ribosomes were dissociated into two major fractions, the S values of which were about 60S and 40S, in addition to some particles heavier than 60S (Fig. 1).

When those fractions were treated with EDTA (10 mM excess over Mg^{++} concentration in the suspending medium), the 40S fraction was converted to a single component of 32S. On the contrary, the 60S fraction yielded two particles, 47S subunits as the major component and 32S subunits as the minor component. When the 40S fraction was suspended in Medium III (50 mM KCl, 5 mM $MgCl_2$, 10 mM β-mercaptoethanol and 50 mM Tris-HCl buffer, pH 7.6) and subjected to sucrose density gradient centrifugation in the same medium, particles with S value of about 60S were formed, as indicated by Tashiro et al (1965, 1966). From these results it may be concluded that the 60S fraction consists of both 60S subunits and dimers of 40S subunits, while the 40S fraction is a single ribosomal subunit.

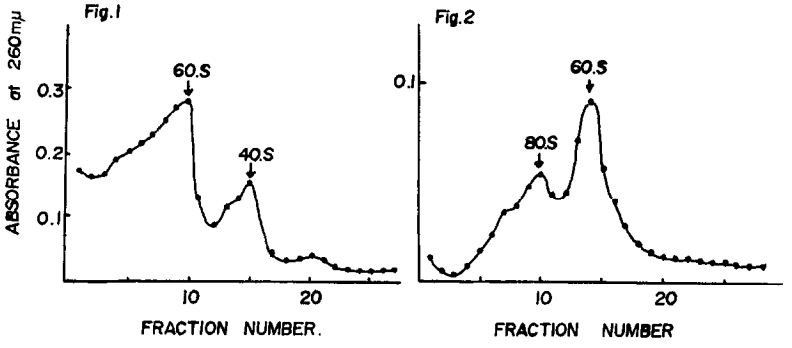

Fig. 1. Sucrose gradient centrifugation of rat liver ribosomes incubated with 1 M KCl. About 10 mg of protein of ribosomes were employed. Centrifugation was carried out at 23,000 rpm for 7 h in a Hitachi RPS 25 rotor at 24-26°. Fractions of about 1 ml each were collected and the absorbance was measured at 260 mμ after 50 times dilution with water. The fractions indicated by arrows were used as 60S and 40S fractions, respectively.

Fig. 2. Sucrose gradient centrifugation of the 60S fraction. The 60S fraction (about 1 mg of protein), which was dialyzed against Medium IV at 0° overnight, was layered on 15-30 % linear sucrose gradient in Medium IV and centrifuged in a Hitachi RPS 25 rotor at 23,000 rpm for 5 h at 24-26°. The 60S subunits indicated by the arrow were used in the following experiments.

In order to prepare pure 60S subunits, the 60S fraction was dialyzed against Medium IV overnight at 0°; this had the same composition as Medium III, except that the Mg^{++} concentration was 3 mM. The suspension was centrifuged in a sucrose density gradient in Medium IV. As shown in Fig. 2, 40S subunits in the 60S fraction associated with the 60S subunits to form 80S particles. Thus pure 60S subunits could be prepared which were used in the following experiments.

Sixty S subunits and 40S subunits, which had been dialyzed against Medium III at 0° overnight, were mixed in a ratio of 2 to 1 by weight, kept at 30° for 5 min, and then subjected to sucrose density gradient centrifugation at 22-24°. It was found from the sedimentation profile

Table 1. Incorporation of ^{14}C-phenylalanine. The basic reaction mixture contained in the total volume of 0.5 ml; 1 mM ATP, 0.25 mM GTP, 10 mM glutathione, 10 mM phosphocreatine, 25 µg creatine kinase, 0.25 M sucrose, 10 mM $MgCl_2$, 0.05 M Tris-HCl, pH 7.6, 0.1 µC ^{14}C-phenylalanine (459 mC/mmole, Radiochemical centre, Amersham, England), 25 µg of poly U (Sigma Co.), 2 mg of protein of cell sap and 30 µg of various ribosomes. Incorporation was carried out at 30° for 30 min. After incubation the radioactivity of hot trichloroacetic acid insoluble materials retained on Millipore filter was determined by a gas flow counter.

	Total Radioactivity (c.p.m.)					
Exp. No.	1		2		3	
	poly U		poly U		poly U	
	-	+	-	+	-	+
original ribosomes* (30 µg)			13	618	31	759
KCl treated ribosomes# (30 µg)	3	466	3	512	13	501
40S subunit (10 µg)		2		40		10
60S subunit (20 µg)		7		36		26
reassociated particles (30 µg)	20	431	0	435	5	376

*store at 0° for the same time periods as in the cases of KCl treated ribosomes and their subunits.

The ribosomal suspension was incubated with 1 M KCl, allowed to stand at a room temperature for 5 h and subjected to dialysis against Medium III containing 10 mM Mg^{++} at 0° until use.

that a greater part of the added particles reassociated to form 80S particles. On the contrary, 47S and 32S subunits prepared from ribosomes by EDTA treatment, did not form 80S particles by the same procedures.

Furthermore, when 60S and 40S subunits prepared as described above were incubated with ^{14}C-phenylalanine in the presence of poly U, the reconstructed 80S particles showed activity in polyuridylic acid (poly U)-dependent polyphenylalanine synthesis (Table 1). The inactivity of 60S or 40S subunits alone indicated that these subunits were free from contamination by the other subunits and that the 40S subunit dimers had been

completely removed from 60S subunits by the treatment described above. The fact that reassociated 80S particles had no ability to incorporate ^{14}C-phenylalanine in the absence of poly U, may indicate that natural messenger RNA is not attached to the reconstructed 80S particles.

Forty S or 60S subunits were incubated with ^{3}H-poly U in Medium III at 30° for 5 min and the mixtures were subjected to sucrose density gradient centrifugation at 24-26°. The sedimentation patterns of ultraviolet absorption at 260 mμ and radioactivity of ^{3}H-poly U are shown in Fig. 3. It is clearly demonstrated that 40S subunits of liver ribosomes interact with poly U, while 60S subunits do not. It must be added that 40S subunits form dimers in Medium III, and ^{3}H-poly U attached to these dimers. These

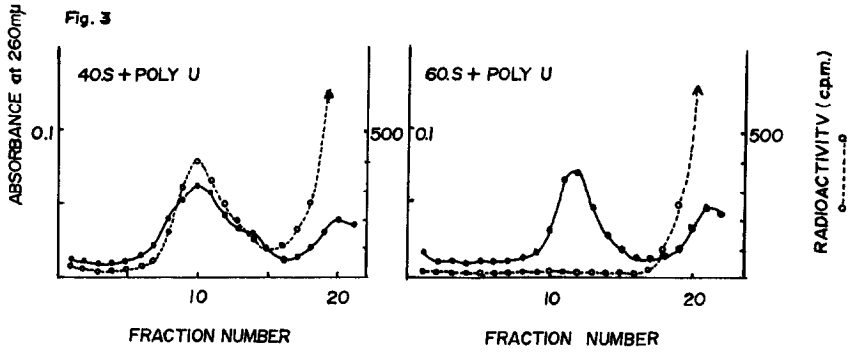

Fig. 3. Interaction of both subunits with poly U. About 60 μg of 40S subunits or 60S subunits was incubated with 0.1 μC ^{3}H-poly U (7.76 mC/mmole of polynucleotide phosphorous, Schwarz BioResearch. Inc.), diluted with 6 μg and 3 μg of unlabeled poly U (Sigma Co.) in the cases of 60S and 40S subunits respectively, in Medium III at 30° for 5 min and the mixtures were subjected to centrifugation at 24-26° in a 15-30 % linear sucrose gradient in Medium III. Centrifugation was carried out at 36,000 rpm for 100 min in a Hitachi RPS 40 rotor. The gradients were fractionated in 4 drops each and subjected to the determination of absorbance at 260 mμ after 10 times dilution with water. Radioactivity of ^{3}H-poly U was measured as follows: An aliquot of each fraction was precipitated by cold 5 % trichloroacetic acid together with 100 μg of bovine serum albumin (Armour Co.) as a carrier and, the radioactivity of the precipitate retained on Millipore filter was determined with a Beckman LS 150 liquid scintillation counter after the addition of toluene-scintillator.

observations suggest that the messenger RNA attaches to the small subunit of rat liver ribosomes as in the case of E. coli ribosomes (Okamoto and Takanami, 1963) and that the dimerization site of the 40S subunits differs from the attachment site for poly U.

It was recently found that 32S subunits, prepared from rat liver ribosomes treated with EDTA, had also the ability to bind ^{3}H-poly U. Furthermore, when 32S subunits and 60S subunits prepared as described above, were incubated at 37°, resulting particles were active in the poly U-dependent ^{14}C-phenylalanine incorporation, although the activity was about one third as that of 40S subunits. These results indicate that 32S subunits retain some activity although 47S subunits prepared by EDTA treatment were shown to be inactive.

We wish to thank Dr. K. Tsurugi for his skillful technical assistance. This work was supported by a grant-in-aid for special project on biophysics from the Ministry of Education.

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