# THE REASSOCIATION OF FRACTIONATED RABBIT RETICULOCYTE RIBOSOMAL SUBUNITS INTO PARTICLES ACTIVE IN POLYPHENYLALANINE SYNTHESIS

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## 1. Introduction

The biological activity of reticulocyte ribosomal subunits, derived by treating ribosomes with high salt, has been shown [1–4]. Attempts to fractionate the subunits on sucrose gradients, however, resulted in particles which failed to reassociate into active ribosomes [4], and it was not possible, therefore, to demonstrate unequivocally that the originally observed activity was entirely due to subunits.

Recently, fractionation of rat muscle ribosomes on gradients containing 0.85 M KCl – 0.015 M $MgCl_2$ at room temperature was reported to produce subunits which reassociated into particles active in polyphenylalanine synthesis [5]. This communication shows that this method of fractionation produces subunits which reassociate into active particles also in the reticulocyte system.

## 2. Experimental

### 2.1. *Materials*

ATP, GTP, potassium phosphoenolpyruvate (PEP), pyruvate kinase (PK) and polyuridylic acid (poly U) were obtained from C.F.Boehringer and Soehne, GmbH, Mannheim, Germany. Glutathione was obtained from Sigma Chem. Co., St. Louis, Missouri, 6318, U.S.A.; β-mercaptoethanol, and sodium dodecyl sulphate (SDS) from Koch-Light Laboratories Ltd., Colnbrook, Bucks.; L-[u-$^{14}$C] phenylalanine of high specific activity from the Radiochemical Centre, Amersham, Bucks.

### 2.2. *Isolation of rabbit reticulocyte polysomes and ph 5 enzymes*

The standard method of Arnstein et al. [6] was followed. The pellet obtained from the postmitochondrial supernatant by centrifugation at 105,000 g for 60 min was used without further washing.

### 2.3. *Preparation of ribosomal subunits*

The polysomes were suspended at 0° in medium A (0.25 M sucrose, 20 mM β-mercaptoethanol, 80 mM KCl, 10 mM $MgCl_2$, 50 mM Tris-HCl buffer, pH 7.6 at 20°) at a concentration of 50–100 $OD_{260}$ units/ml and a sample (control polysomes) was retained at 0°. The concentration of potassium was adjusted to 1 M in the rest of the solution by the addition of a stock solution of 2.5 M KCl – 0.01 M $MgCl_2$ and the mixture was warmed at 37° for 4 min. Another sample (KCl-shocked polysomes) was removed, diluted fivefold with 20% (w/v) sucrose in medium B (20 mM mercaptoethanol, 850 mM KCl, 15 mM $MgCl_2$, 50 mM Tris-HCl, pH 7.6 at 20°) and kept at room temperature throughout the following centrifugation and fractionation of subunits. The remaining shocked suspension was layered (30–60 $OD_{260}$) on a 16 ml linear 10–30% (w/v) sucrose gradient in medium B, over a 2 ml cushion of 50% (w/v) sucrose in medium B, and centrifuged in the MSE 3 × 20 swing out rotor at 30,000 r.p.m. for 3 hr 20 min at 20° (± 4°). The tubes were emptied by pumping out the contents from the bottom through a narrow tube, the solution was analysed with a Uvicord, type 4701A optical unit connected to a Speedomax W recorder and fractions were collected using an UltraRac type 700 fraction



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collector. The fractions containing the large and small ribosomal subunits were combined in a ratio of 2 or 2.5 to 1.0 and dialysed overnight versus a 200 fold excess of buffer D (3% [w/v] sucrose, 10 mM β-mercaptoethanol, 50 mM KCl, 5 mM $MgCl_2$, 50 mM Tris-HCl buffer, pH 7.6 at 20°) at 4°. The control and shocked polysomes and any other fractions to be tested in the cell-free system were similarly dialysed.

### 2.4. *The cell-free system*

Poly U-directed incorporation of $^{14}$C-phenylalanine into acid-insoluble protein was assayed as previously described [4,6]. Protein was collected on 2.5 cm diam Whatman glass fibre GF/C filters in a Millipore filtering apparatus, washed twice with cold 5% TCA, three times with ether-ethanol (3:1 v/v) and twice with ether. The samples were counted in a Packard scintillation counter.

## 3. Results

### 3.1. *Dissociation of reticulocyte polysomes into subunits*

Exposure of polysomes to 1.0 M KCl and subsequent centrifugation through medium B at room temperature dissociated them into subunits (see fig. 1). The subunits accounted for 50–70% of the $OD_{260}$ put on the gradient, although occasionally up to 80% dissociation was observed. Ribonucleoprotein sedimenting faster than the larger subunit was always present in regions of the gradient corresponding to monosomes and polysomes. The degree of dissociation was not apparently affected by varying the ratio of $K^+$ to $Mg^{2+}$ between 68 and 145 during the exposure of polysomes to 1.0 M KCl, nor by varying the concentration of polysomes to be shocked between 20 and 120 $OD_{260}$/ml.

The purity of the subunits was checked by looking at their RNA content. After overnight dialysis versus D, selected gradient fractions, indicated in fig. 1, and control polysomes were made 1% in SDS, warmed to 37° for 3 min and centrifuged through a 17 ml 10–25% (w/v) linear sucrose gradient in medium C (0.1 M LiCl, 0.01 M Tris-HCl buffer, pH 7.6 at 20°) at 25,000 r.p.m. in the Spinco SW 25.3 rotor for 14 hr at 5°. About 90% of the RNA of the larger subunit was 29S and about 75% of the RNA of the smaller subunit was 17S. The gradient "polysomes" contained 29S and 17S RNA in the same proportion as the control polysomes.

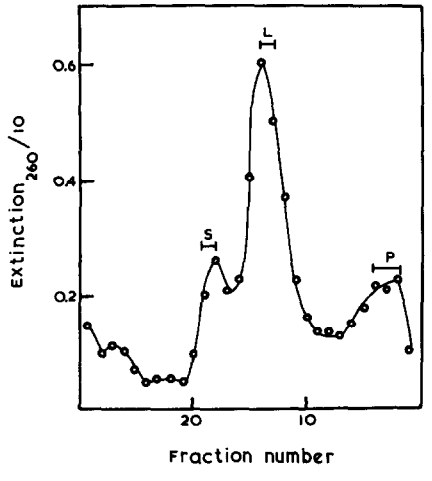


Fig. 1. Preparation of ribosomal subunits. Polysomes in medium A (120 $OD_{260}$/ml) were made 1.0 M in KCl and centrifuged (60 $OD_{260}$/tube) as described in Experimental. Tubes 2–4 ("polysomes"), 13–14 (large subunit) and 18–19 (small subunit) were pooled and dialysed versus buffer D. Direction of sedimentation from left to right.

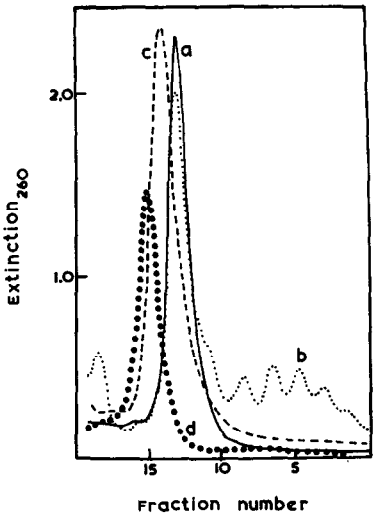


Fig. 2. Sedimentation profile of reassociated particles. Dialysed reassociated particles (a), control polysomes (b), gradient large subunit (c), and gradient small subunit (d) were centrifuged through a sucrose gradient in buffer D as described in the text. Direction of sedimentation from left to right.

Table 1
Activity of reassociated particles.

| Sample | mμmoles $^{14}$C-phenylalanine incorporated/mg ribosomes |
|---|---|
| Control polysomes | 0.278 |
| Shocked polysomes | 0.500 |
| Reassociated particles | 0.685 |
| Large subunit | 0.022 |
| Small subunit | 0.023 |

The samples were dialysed versus buffer D for 3 hr at room temperature. Each tube (0.5 ml) contained 0.35 $OD_{260}$ units of ribosomes, 20 μg poly U, 0.45 mg pH 5 enzymes and the following components (μmoles): KCl (25), $MgCl_2$ (3.5), Tris-HCl buffer, pH 7.6 at 20° (15), glutathione and β-mercaptoethanol (5), PEP (2.5), ATP (0.125), GTP (0.0135), PK (0.025 mg), 20 unlabelled L-amino acids (0.125 each) and 50 mμC $^{14}$C-phenylalanine diluted to a specific activity of 50.

### 3.2. *Reassociation of ribosomal subunits*

Samples containing dialysed polysomes, the large subunit, the small subunit or both subunits together were layered on 17 ml linear 15–30% (w/v) sucrose gradients in buffer D and centrifuged at 25,000 r.p.m. in the Spinco SW 25.3 rotor for 3 hr 40 min at 5°. Fig. 2 shows that when the two subunits were dialysed together they reassociated into particles sedimenting as monoribosomes. Negligible reassociation was observed when either subunit was dialysed on its own.

### 3.3. *Activity of reassociated particles*

The ribosome and energy dependent response of the dialysed samples to saturating amounts of poly U is shown in table 1. In this experiment the specific acitivity of the reassociated particles was 250% that of the control polysomes, while either subunit alone had 3% of the activity of the reassociated particles.

Usually the activity of the reassociated particles was 50–150% that of the control. We have not determined the cause of this variability. Activity is not impaired by dialysis at room temperature, omitting β-mercaptoethanol from the dialysis medium or varying the ratio of the two subunits from 1.0 to 3.0. This would imply that possibly only a fraction of the subunits is able to reassociate, due to degradation or functional heterogeneity. At any rate the activity of either subunit alone was not more than 10–20% that of the reassociated particles, depending on the quality of the gradient separation.

## 4. Discussion

In contrast to the muscle system [5] the treatment, described in 2.3, did not dissociate reticulocyte polysomes entirely into subunits. The nature of these resistant polysomes and stuck ribosomes is being investigated.

The reassociated particles, formed only when the two subunits were dialysed together, were shown to be largely monosomes and to be at least as active as the control polysomes in polyphenylalanine synthesis. We are now trying to determine whether the reassociated particles have endogenous activity, where mRNA travels in the gradient, what propotion of the subunits are active in poly U binding and translation and whether the subunits respond to haemoglobin messenger [7,8,9].

## Acknowledgements

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