# PROTEIN SYNTHESIS BY RABBIT RETICULOCYTE RIBOSOMES AFTER TREATMENT WITH POTASSIUM CHLORIDE

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## 1. Introduction

The treatment of rabbit reticulocyte ribosomes with high concentrations of potassium chloride has been shown to cause dissociation into sub-units [1, 2]. It was also shown that in high concentrations of potassium chloride an equilibrium exists among polyribosomes, monoribosomes and ribosomal sub-units and that the equilibrium was affected by the ribosomal concentrations during the treatment, the lower the concentrations the greater the dissociation into sub-units. The removal of the potassium chloride and adjustment of the electrolytes to near physiological concentrations reversed the process of breakdown and re-associated the sub-units into monosomes and polysomes. The extract removed from the ribosomes by the potassium chloride treatment also associated sub-units into monoribosomes and polyribosomes [1]. An extract prepared by a similar method has been shown to convert monoribosomes into polyribosomes during cell-free protein synthesis [3]. This paper describes experiments that determine which of the components of the potassium chloride treated ribosomes can participate in cell-free protein synthesis in the presence of the extract, and the results obtained indicate that it is predominantly the polyribosomes which are capable of synthesis.

## 2. Materials and methods

Polyuridylic acid (poly U) was obtained from Miles Chemical Corp. (Eikhart, Ind., USA).

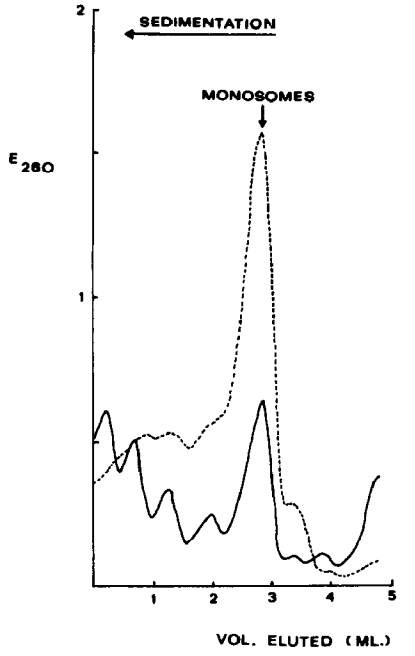


Fig. 1. Sedimentation of untreated and KCl-washed ribosomes through sucrose density gradients. Untreated and KCl-washed ribosomes were adjusted to 0.07M-KCl, 3.5mM-$MgCl_2$ and 35mM-tris-HCl buffer, pH 7.5 at 22° and a ribosomal concentration of 10 mg/ml. 0.05ml of each was placed on a 4.8ml 15–35% (w/v) sucrose gradient adjusted to the same salts concentration. The gradients were centrifuged at 125000$g_{av.}$ for 1.5 hr in the 3 X 5ml swinging bucket rotor of the MSE Superspeed 65 centrifuge. After centrifugation 70% (w/v) sucrose containing haemoglobin as a visual aid was pumped at a constant rate through the bottom of the tube so that the lightest fractions were collected first. The gradient was monitored at 260m$\mu$ in a Unicam SP.800 spectrophotometer fitted with an LKB 4711A-3 flowcell. ——, untreated ribosomes; -----, KCl-washed ribosomes.

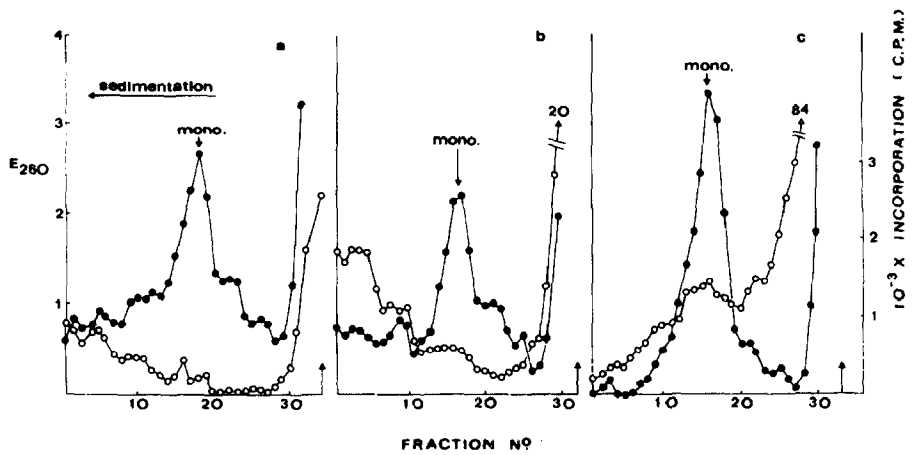


Fig. 2. Sedimentation through sucrose gradients of KCl-washed ribosomes after incubation with G25 ribosomal extract in the amino acid incorporation system. Five 1.4 ml samples of the standard incorporation mixture were incubated at 37°. In addition the mixture contained L-$^3$H-leucine of specific radioactivity 2500 Ci/mole, 2.3 mg of KCl-washed ribosomes and 0.5 ml of G25 ribosomal extract. After 2 min (a), 8 min (b), 40 min (c), the tubes were chilled and 1 ml of each was applied to a 29 ml 5–20% (w/v) sucrose gradient adjusted to 0.07M-KCl, 3.5mM-$MgCl_2$, 14.3mM-GSH and 35mM-tris-HCl buffer pH 7.5 at 22°. The gradients were centrifuged at 63500$g_{av}$. for 3.5 hr in the SW25 rotor of the Spinco preparative ultracentrifuge. After centrifugation 16 drop fractions were collected through the bottom of the tubes. The two median drops were collected on to filter paper discs and the amino acid incorporation measured. The counting effiency was 3.5%. 2.5 ml of distilled water was added to the rest of the samples and the extinction at 260mμ determined. •, $E_{260}$; ○, radioactivity.

### 2.1. *Preparation of ribosomes, KCl-washed ribosomes and ribosomal extract*

These were prepared as previously described [3]. Ribosomal concentrations were estimated by taking $E^{1\%}_{1cm}$ 120 at 260 mμ.

In some experiments the KCl concentration of the ribosomal extracts was reduced by passing a sample (0.5–1 ml) through a Sephadex G25 column (1 × 12 cm) equilibrated and eluted with 0.1M-KCl, 2mM tris-HCl buffer, pH 7.5 at 22° (designated "G25 ribosomal extract"). This procedure resulted in a 2–3 fold dilution of the extract.

### 2.2. *Amino acid incorporation*

The reaction mixture used was that described previously [3]. When poly U was added to the reaction mixture the concentrations of soluble RNA and $MgCl_2$ were doubled.

## 3. Results

### 3.1. *The nature and synthetic activity of KCl-washed ribosomes*

KCl-washed and untreated ribosomes were analysed by sucrose gradient sedimentation (fig. 1). Although the proportion of polysomes has decreased after treatment with KCl, it is still approximately 50% of the total ribosomes.

Amino acid incorporation by KCl-washed ribosomes requires the presence of the ribosomal extract. Incorporation is complete after 30 min of incubation.

To determine which of the ribosomal components were active in amino acid incorporation in the presence of the ribosomal extract, samples of a reaction mixture were removed at different times during the course of the incubation, and analysed by sucrose gradient centrifugation (fig. 2). After 2 and 8 min the incorporation was mainly into polyribosomes and released polypeptide chains, there being little incorporation into the monosome region. After longer times of incubation when synthesis is practically over incorporation also appears in the monoribosomes. This was possibly due

to the breakdown of polyribosomes without the release of the polypeptide chain.

Although incorporation was shown to be into the polyribosomes, it is possible that the ribosomal extract was active in converting smaller ribosomal components into polyribosomes during the incubation. To determine which components in KCl-washed ribosomes could be stimulated in the cell-free system by the ribosomal extract, the ribosomes were fractionated on a sucrose gradient and each fraction assayed in the cell-free system in the presence and absence of the ribosomal extract and also in the presence of poly U (fig. 3). The specific incorporations (incorporation/$E_{260}$) of fractions 2, 3, 4 (polysomes) and 12, 13, 14 (monosomes) when assayed alone were 36, 34, 33 and 3, 3, 3.5 respectively; in the presence of ribosomal extract 200, 215, 207 and 66, 55, 24; and in the presence of poly U 205, 191, 195 and 181, 188, 153. Thus in the presence of poly U all of the ribosomes have approximately equal activities, but in the presence of the ribosomal extract the polysomes are at least 4 times as active as the monosomes. Since the monosome fractions probably contained polysomes it is possible that much of the incorporation by the monosome fractions in the presence of extract was by contaminating polysomes, and the decrease in specific activity across the monosome fractions is consistent with this.

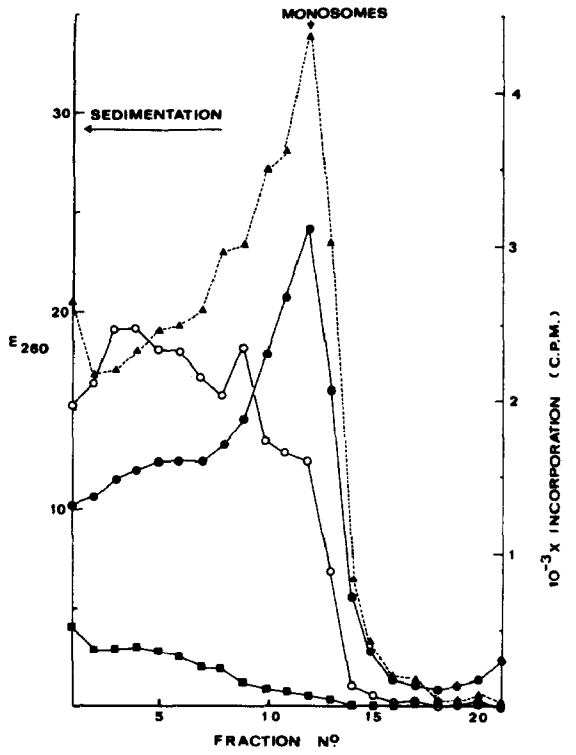


Fig. 3. Amino acid incorporation by KCl-washed ribosomes after sedimentation through a sucrose density gradient. KCl-washed ribosomes were adjusted to 0.07M-KCl, 3.5mM-$MgCl_2$, 35mM-tris-HCl buffer pH 7.5 at 22°, and a ribosomal concentration of 40mg/ml. 1 ml was placed on a 29 ml 10–23% (w/v) sucrose gradient of the same salts concentrations. The gradient of the same salts concentration. The gradient was centrifuged at 63500$g_{av.}$ for 4 hr in the SW25 rotor of the Spinco preparative ultracentrifuge. After centrifugation 21 samples (25 drops each) were collected. Samples of each fraction were diluted and the extinction at 260mμ determined. Portions (0.1 ml) of each sample were added to the cell-free incubation mixture to give a final volume of 0.35 ml. In addition the reaction mixture contained L-$^{14}$C-phenylalanine of specific radioactivity 15Ci/mole. Ribosomal extract (0.03 ml) and poly U (30μg) were added as indicated. Control tubes with ribosomes alone were also assayed. The tubes were incubated for 37° for 60 min, samples (0.1 ml) removed and incorporation measured. The highest point in the figure corresponds to an incorporation of 3.5mμmole phenylalanine/mg of ribosomes. ■—■ amino acid incorporation by fractions alone; ○—○, incorporation in the presence of ribosomal extract; ▲—▲, incorporation in the presence of poly U; ●—●, $E_{260}$.

## 4. Discussion

In the experiments described in this paper rabbit reticulocyte ribosomes washed with KCl are inactive in cell-free protein synthesis. Analysis of the ribosomes after this treatment showed that a high proportion of polyribosomes were still present and that it is predominantly these polyribosomes which are capable of amino acid incorporation in the presence of added ribosomal extract. Thus the results presented in this paper may be interpreted as follows: in 0.6M-KCl the ribosomal stimulatory extract is detached from the polyribosomes leaving them largely intact but inactive. The active components in the ribosomal extract are protein in nature [3, 4], thus it follows that only those ribosomes already containing an active native messenger-RNA will be capable of amino acid incorporation, which in this case are the inactive polyribosomes and possibly to a small extent monosomes. The results described here are in agreement with those presented previously describing the treatment of reticulocyte

ribosomes with high concentrations of KCl [1, 2]. However, although these workers reported that the ribosomal extract acted by converting ribosomal sub-units into active ribosomes, they were unable to obtain an active system by mixing the two types of sub-units together in the presence of the ribosomal extract in the cell-free system. These workers and others [5, 6] have shown that ribosomal sub-units are active in polypeptide synthesis but only when added messenger RNA (poly U or plant viral RNA) is present in the system. Exogenous messenger of a non-specific type added in large amounts would be expected to show less stringency for ribosomal binding and for the initiation of polypeptide chain [7]. This may explain the results obtained using poly U and those described here using natural endogenous messenger, where predominantly only the polysome fraction was capable of amino acid incorporation.

## References

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