SEPARATION OF RIBOSOMAL SUBUNITS ON AGAROSE GELS

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1. Introduction

This communication describes conditions under which the 60 S subunit of rat liver ribosomes is adsorbed by Sepharose and Biogel agarose gels at 4°C whereas the 40 S subunit elutes in the internal volume. At 35°C the 60 S subunit is not retarded and can be eluted from gel to which it has adsorbed at 4°C. This phenomenon provides a novel method for the separation and preparation of ribosomal subunits.

2. Methods

Rat liver ribosomes were prepared as in [1]. To form subunits that were used to calibrate the columns the ribosomes were suspended in buffer A (20 mM Tris (pH 7.6), 500 mM KCl, 2 mM $MgCl_2$, 0.1 mM EDTA, 5 mM MSH). Puromycin was added to 1 mM and the mixture allowed to stand for 1 h at room temperature. After heating for 10 min at 37°C the mixture was layered over 10–30% linear sucrose gradients prepared for the SW 27 Spinco rotor and centrifuged at 4°C for 16 h. Gradients were analysed and the separated subunits collected by centrifugation. They were resuspended in buffer A with [KCl] reduced to 100 mM and containing 30% glycerol. Subunits so prepared retain activity in polyphenylalanine synthesis for many months when stored at −20°C. Acrylamide gel electrophoresis of RNA from the subunit fractions was performed as in [2].

Sepharose 4B (Sigma) and Biogel A-15m (Biorad Labs.) were packed in a column 1 × 30 cm which was initially eluted at 4°C with buffer A except where otherwise indicated at a flow rate of ~8 ml/h. Void volume was determined as described by the makers with DNA.

3. Results

Figure 1 shows the elution profile of a sample (18 A_{260} units) of 40 S subunits on Sepharose 4B. The subunit shows a clear peak at a volume significantly greater than the void volume, but less than the total column volume which for Sepharose 4B is virtually equal to $V_i + V_o$. The peak trailed a little. It was also preceded by a very small peak emerging at about the void volume. All the applied A_{260} units were recovered. Application of a similar amount of 60 S subunit resulted in no detectable profile at all. From this it was concluded that the 60 S subunit was adsorbed. The alternative possibility that the subunit precipitated was excluded by the observation that on

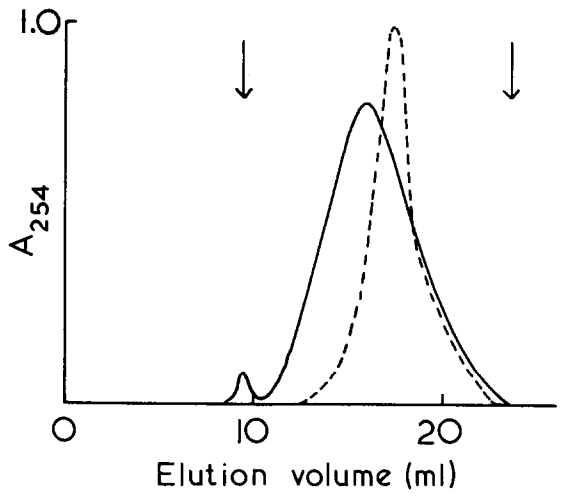

Fig.1. Analysis of rat liver 40 S ribosomal subunits on Sepharose 4B and Biogel A-15m. About 18 A_{260} units of 40 S subunits were applied to each column and eluted as in section 2. (——) Sepharose 4B; (– – –) Biogel A-15m. The arrows indicate the approximate void and total column volumes.

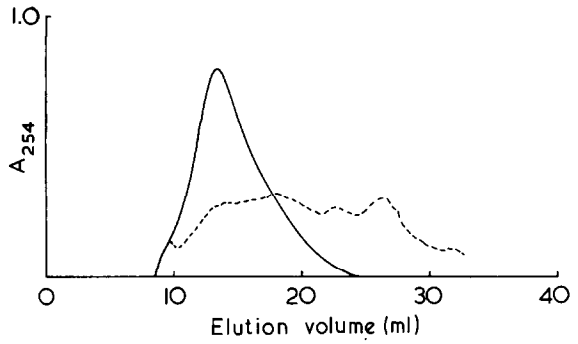

Fig.2. Analysis or rat liver 40 S and 60 S subunits on Sepharose 4B in buffer A with KCl reduced to 10 mM. About 18 A_{260} units of each subunit were applied. (——) 40 S subunit; (– – – –) 60 S subunit.

an equivalent Sephadex G-25 column the 60 S subunit was completely recovered in the void volume. A similar adsorption was seen with Sepharose 6 B (not shown).

Elution of poly(A) containing RNA from bonding to oligo(dT) cellulose is achieved by lowering of ionic strength [3]. When the above experiments were repeated with the [KCl] in buffer A reduced to 10 mM the profile of the 40 S subunit tended to broaden such that it merged with material eluting in the void volume, but trailed throughout a whole column volume (fig.2). The existence of faster running components suggests larger aggregates being formed, e.g., 55 S particles [4]. The 60 S subunit under these conditions emerged as an irregular profile from the void volume onwards, extending well past the total column volume. When the [KCl] was 100 mM the 40 S peak was closer to the appearance at 500 mM KCl and the 60 S profile was lower, though detectable (not shown).

Although Biogel is, like Sepharose, an agarose gel, slightly different results were obtained when Biogel A-15m replaced Sepharose 4B. With a [KCl] of 500 mM the 40 S peak was sharper and more symmetrical and also slightly more retarded (fig.1). The 60 S unit however, as with Sepharose 4B, was strongly adsorbed and only eluted very slowly over a large volume. Figure 3 shows the elution profile of the 40 S and 60 S subunits on the same Biogel column maintained at 35°C. Flow rate at 4°C was maintained by gravity and at 35°C the same gravity head resulted in a nearly 2-fold increase in flow. The 60 S peak now eluted ahead of the 40 S peak.

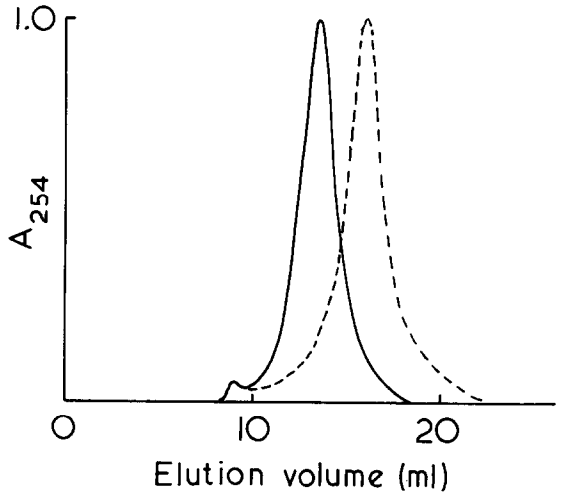

Fig.3. Analysis of rat liver 40 S and 60 S subunits on Biogel A-15m maintained at 35°C. About 18 A_{260} units of each subunit were applied. (——) 60 S subunit; (– – – –) 40 S subunit.

Figure 4 shows the elution profile at 4°C of a mixture of ribosomal subunits obtained by incubating a sample of ribosomes at 37°C with puromycin as in section 2. Suspensions of hepatic ribosomes and subunits prior to separation always have a slightly opalescent appearance. The material responsible for this appears to be of high molecular weight and elutes in the void volume (peak A). Its $A_{230-290}$ shows a progressive decline with no clear peak at A_{260}. The second peak is of 40 S subunits in a yield, after allowing for

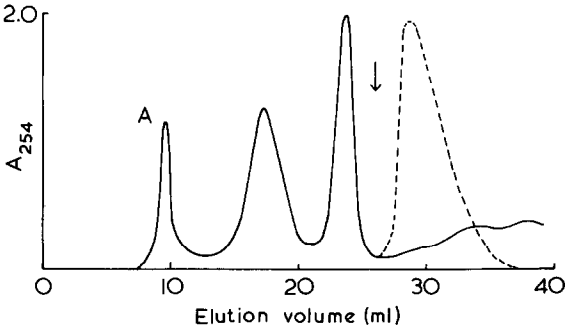

Fig.4. Analysis of a mixture of liver ribosomal subunits incubated with puromycin. About 135 A_{260} units of material were applied to the column of which 22 units were recovered in the void volume, 35 units in the 40 S peak and 88 in the 60 S fraction. (——) Elution at 4°C; (– – – –) elution profile when the temperature was raised to 35°C at point indicated by arrow.

Table 1
Activity of rat liver ribosomal subunits prepared on Biogel A-15m in poly(U)-promoted synthesis of polyphenylalanine

	Incorporation of [^{14}C]phenylalanine (cpm/tube)
40 S alone [a]	2513
60 S alone [a]	8156
40 S + 60 S [a]	18 930
40 S + 60 S [b]	18 840

Each tube contained 10 μg 40 S subunits and 25 μg 60 S subunits as indicated. Incubation was for 10 min at 37°C in the presence of liver cytosol, ATP, GTP, phosphocreatine and [^{14}C]phenylalanine as in [1]. [Mg] was 8 mM. (a) Subunits concentrated by centrifugation; (b) subunits concentrated by dialysis against polyethylene glycol

material eluting in the void volume, consistent with quantitative conversion of the original ribosomes to subunits. The solution is clear and shows a good ultraviolet profile. The third peak, eluting at about the total column volume is recognisable from its spectrum as puromycin. Thereafter there is a long irregular peak of 60 S subunits. On Sepharose 4B a similar pattern was observed. The peaks however were not quite as sharp as on Biogel and the 60 S subunit emerged more slowly. Figure 4 also shows the effect of raising the temperature of the column to 35°C after the puromycin has eluted. The appearance of the 60 S subunit fraction is much accelerated.

Subunit fractions eluting from the column were concentrated either by centrifugation or by dialysis against buffer A, with [KCl] reduced to 100 mM, containing polyethylene glycol. Subunits collected in either fashion have similar activity in polyphenylalanine synthesis (table 1) and also activity quite comparable with subunits prepared on sucrose gradients [5]. The 40 S subunit alone showed low activity but somewhat surprisingly the 60 S subunit alone exhibited substantial activity. Gel electrophoresis of RNA extracted from the two subunit fractions showed only trace contamination with the opposite subunit and sucrose gradient analysis of the separate subunits did not indicate appreciable cross contamination.

4. Discussion

The use of agarose gels together with concentration of the fractions against polyethylene glycol or by pressure filtration allows the preparation of subunits without the need for a centrifuge. Rat liver ribosomes are often contaminated with ferritin, recognisable by its brown colour. When subunits are prepared by zonal centrifugation the ferritin co-sediments with the 60 S fraction. On Biogel the ferritin elutes slightly retarded with respect to the 40 S fraction whereas the 60 S fraction is free of ferritin.

With a column of the size used in the present work binding sites begin to show saturation with quantities of subunits in excess of ~150 A_{260} units as judged by the fact that optical density does not return to base line after elution of the puromycin and before the temperature is raised to 35°C. However even with this load the bulk of the 60 S material is extensively retarded and elutes rapidly when the column is warmed. Since in the experiments with the subunit mixture the 60 S subunit appears at 4°C to elute over a large volume it would not be surprising if the 40 S fraction were to show some contamination with 60 S subunits. However this was found to be minimal as judged by sucrose gradients, electrophoresis of RNA and activity in polyphenylalanine synthesis. Conversely there is no reason to expect 40 S contamination of the 60 S fraction and indeed sucrose gradients and electrophoresis of RNA confirmed this. The high activity of the 60 S fraction on its own is therefore rather surprising. It has been noted [6,7] that 60 S subunits prepared by sucrose gradient sedimentation contain variable amounts of 40 S subunit despite apparent good resolution on the gradients. This contamination has been attributed to dimerisation of the 40 S subunit to 55 S which co-sediments with the 60 S. There is no reason to suppose that on agarose gels 55 S particles would be retarded. We believe that the 60 S subunit may have an intrinsic capability for polyphenylalanine synthesis because the [Mg] optimum is slightly higher for the 60 S subunit alone than for the 40 S + 60 S mixture [5].

When total cell nucleic acid was chromatographed on Sepharose 4B in 0.1 M acetate buffer the order of elution of the main classes of nucleic acid was DNA, 18 S RNA, 5 S RNA and 28 S RNA [8]. It was concluded that separation involved factors other than gel filtration [8]. Although not explicitly stated their data appear to show that the DNA eluted at about V_o, tRNA with a K_d of ~1 and 18 S somewhere in the region expected if the principles of gel filtration had

operated. 28 S RNA had a $K_d > 1$, implying significant adsorption. Similar observations have also been made in [9].

The behaviour of the ribosomal subunits seen here suggest that 40 S subunits behave similarly to their 18 S RNA and that there is no significant adsorption of either to agarose gels. The behaviour of the 60 S subunit on the other hand mirrors that of the 28 S RNA. Thus its binding to agarose gels, particularly at high salt concentrations, is probably the result of interaction between the gel and its 28 S RNA. If correct this must imply that an appreciable amount of the 28 S RNA in the 60 S subunit is exposed, or readily exposable by conformational changes that do not prevent the subunit subsequently participating in polyphenylalanine synthesis. Ribosomes under some conditions also bound to Sepharose in [10,11]. This observation suggests that even in ribosomes the 28 S is sufficiently exposed to interact with the gel. A differential binding of 40 S and 60 S subunits to Sepharose 4B was also observed [11] but, unlike in the present work, appreciable adsorption of 40 S units occurred even at 0.2 M NaCl concentration, despite the fact that 18 S RNA elutes unretarded at 0.6 M NaCl [9].

The nature of the bonding between RNA and agarose gels is uncertain but presumably involves hydrophobic as opposed to ionic interactions since it is favoured by high salt concentration. High concentrations of antichaotropic salts promote the binding of tRNA to Sepharose 4B from which it elutes when the salt concentration decreases [12]. The phenomena with agarose gels are thus analogous to the binding of poly(A) containing RNA to oligo(dT) cellulose [3], but the participating groups of the agarose remain to be identified.

Acknowledgements

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