Journal of Chromatography, 545 (1991) 196–200
Elsevier Science Publishers B.V., Amsterdam

CHROM. 23 191

Short Communication

Separation of ribosomal subunits on Trisacryl GF 2000

DEENA BHOOLIA and KEITH L. MANCHESTER*
Department of Biochemistry, University of the Witwatersrand, Johannesburg (South Africa)
(Received December 6th, 1990)

ABSTRACT

The separation of rat liver and *E. coli* ribosomal subunits was attempted on Trisacryl GF 2000. Contrary to experiments with Sepharose 4B and Bio-Gel A-15 the 60S mammalian subunit did not bind to the resin at 4°C but eluted within the column volume ahead of the 40S subunit. Puromycin, however, used to prepare the subunits, which on the agarose gels had eluted at the total column volume, exhibited anomalous retardation on the Trisacryl resin. Trisacryl therefore behaves as the more non-polar resin, and the binding of 60S subunits to agarose gels is a result of hydrophilic interaction.

INTRODUCTION

Ribosomal subunits, so-called 40S and 60S for eukaryotes, of relative molecular mass (M_r) $1.5 \cdot 10^6$ and $3.0 \cdot 10^6$ [1], are normally separated from each other during preparation by centrifugation on sucrose gradients and subsequent fractionation of the gradient [2]. In a previous study [3], it was shown that rat liver 40S and 60S subunits could be separated from each other by column chromatography on Sepharose 4B or Bio-Gel A-15. This separation, however, was not the consequence of gel exclusion chromatography, but because at 4°C the 60S subunit, although not the 40S, bound to the gel matrix. After elution of the 40S peak, and of puromycin used in the preparation of the subunits at around the total column volume, the 60S subunit could be eluted if the temperature was raised to 25°C or above. At this temperature in other experiments the individual subunits eluted in the order to be expected based on their respective M_r, but too close to be easily resolved.

The explanation for the binding of the 60S to the gel at 4°C is not known. It is not critically dependent on gel pore size, as a similar phenomenon is observed with Sepharose 2B and 6B (unpublished observations) and the large RNA of the 60S subunit, 28S RNA, also behaves in an anomalous manner on Sepharose gels [4–6]. In an attempt to explore this phenomenon further, we examined the behaviour of the subunits on the synthetic matrix Trisacryl GF 2000, which is formed by copolymerization of a hydroxylated acrylic monomer and N-acryloyl-2-amino-2-hydroxy-

0021-9673/91/$03.50

methyl-1,3-propanediol. The makers (Reactifs IBF) describe it as possessing a separation range of $1.2 \cdot 10^5$–$1.5 \cdot 10^7$ dalton.

EXPERIMENTAL

The behaviour of 40S and 60S ribosomal subunits was studied on columns of Trisacryl GF 2000 of length 30 and 135 cm (1.2 cm I.D.), which were loaded and equilibrated with buffer A [500 m*M* KCl–2 m*M* $MgCl_2$–0.2 m*M* EDTA–20 m*M* 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid (HEPES), pH 7.8, supplemented with 5 m*M* melanocyte-stimulating hormone (MSH) when in use with ribosomal components]. Elution was monitored at 254 nm and the flow-rate was normally 3 ml/h. Experiments were carried out at either 4 or 20°C. The void volume was determined with glycogen and the volume of the stationary phase with either acetone or ^3H_2O.

Ribosomes were prepared from rat liver as described previously [7] and for dissociation into subunits were incubated at 37°C for 15 min with 1 m*M* puromycin in buffer A. Separation and collection of subunits by centrifugation were as described previously [8].

RESULTS

Ribosomal subunits aggregate readily, particularly at low temperatures [2,8]. This aggregation is in part prevented by a high concentration of monovalent cations and a low concentration of Mg^{2+}. The use of 500 m*M* KCl and 2 m*M* $MgCl_2$ is a compromise between seeking to maximize dissociation without producing conditions so stringent that the subunits are stripped of proteins and/or unfold and become denatured.

The first striking feature about chromatography of the rat liver subunits on Trisacryl 2000 is that, unlike the behaviour of the 60S subunit on Sepharose or Bio-Gel, both subunits elute at 4°C within a column volume and show no signs of anomalous retardation. Initial experiments with the 30-cm column showed only a slight shift in the elution position for the two subunits at either 4 or 20°C, although at 20°C the peaks were sharper than at 4°C. This would be consistent with less dimerization of 40S to 55S at that temperature and of 60S to 90S [8]. All subsequent experiments were performed with the longer column.

The elution profile of the individual subunits prepared from rat liver and of a mixture of subunits at 4 and 20°C is shown in Fig. 1. The individual subunits eluted with peaks at both 4 and 20°C with $K_d = 0.19$ and 0.13 for the 40S and 60S, respectively (Fig. 1a), the 60S showing a clear shoulder at 4°C which at 20°C resolved into a distinct peak with $K_d = 0.22$ (Fig. 1b). The solution representing this peak is brown and is believed to be due to ferritin, which is a normal contaminant of rat liver ribosomes as conventionally prepared. Preparations of rabbit reticulocyte ribosomal subunits behaved virtually identically with the rat liver subunits except that no ferritin peak was observed (not shown).

The profile for the mixture of subunits obtained by treatment with puromycin shows three peaks at essentially the positions expected for 40S, 60S and ferritin (Fig. 1c and d). Not shown in the elution profiles is the unexpected observation that puromycin did not elute as expected near the total column volume, but at a position corresponding

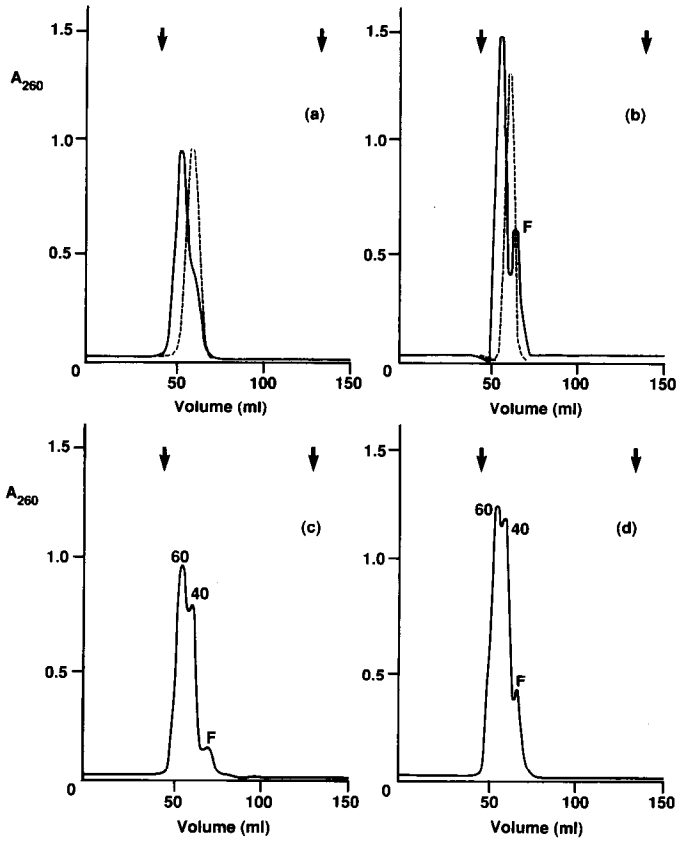

Fig. 1. Elution profiles of rat liver ribosomal subunits on a 135-cm column of Trisacryl GF 2000 equilibrated and eluted with buffer A at (a) and (c) 4°C and (b) and (d) 20°C as described under Experimental: (a) and (b) *ca.* 20 A_{260} units of isolated subunits; (c) and (d) *ca.* 20 A_{260} units of ribosomes treated with puromycin. Arrows on the left-hand side indicate V_0 and on the right V_t.

to a $K_d \approx 1.6$. Krauss and Schmidt [9] observed retardation of nucleotides on Trisacryl GF 05. The separated subunits showed activities in poly-U directed protein synthesis comparable to subunits isolated on Sepharose 4B or by sucrose gradient centrifugation.

Fig. 2 shows the behaviour of ribosomal subunits from *Escherichia coli* on Trisacryl GF 2000. At 4°C both subunits eluted in the order expected (Fig. 2a), but despite the lower molecular masses than for mammalian subunits their K_d values were smaller (0.11 and 0.15) than for the liver and reticulocyte subunits. The 30S subunit shows a small peak at V_0, which could be reassociated 70S if the 30S fraction were contaminated with a small amount of 50S during preparation. Under the conditions used, a mixture of the 30S and 50S subunits appeared to elute as 70S couples and excess 30S (Fig. 2b).

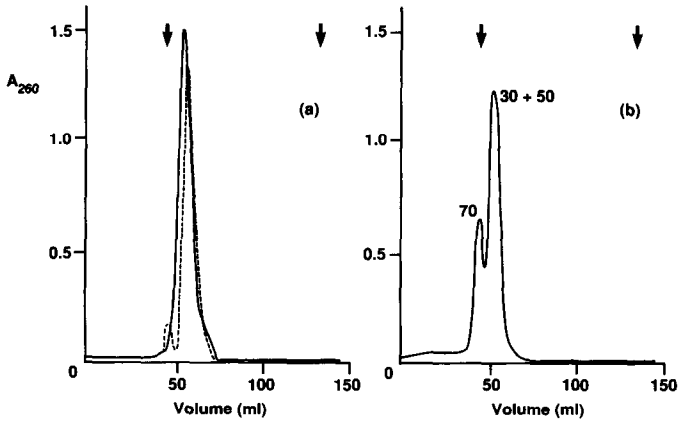

Fig. 2. Elution profiles of *E. coli* ribosomal subunits at 4°C on a 135-cm column of Trisacryl GF 2000 equilibrated and eluted with buffer containing 6 m*M* KCl, 0.2 m*M* $MgCl_2$ and 25 m*M* Tris–HCl (pH 7.6). (a) *Ca.* 20 A_{260} units of 30S (– – –) and 50S (———) subunits prepared from *E. coli* ribosomes by density gradient centrifugation; (b) *ca.* 20 A_{260} units of *E. coli* ribosomes. Arrows on the left-hand side indicate V_0 and on the right V_t.

DISCUSSION

According to the manufacturer, the separation range of Trisacryl GF 2000 is $1.2 \cdot 10^5$–$1.5 \cdot 10^7$ dalton. Theoretically, if the separation range were linear, two solutes of M_r $3.0 \cdot 10^6$ and $1.5 \cdot 10^6$ would elute with $K_d = 0.33$ and 0.48, respectively. On Sepharose 4B the 40S subunit had previously been found to possess a K_d of 0.43 [3], consistent with an M_r of $1.3 \cdot 10^6$ based on the supplier's claim of an exclusion range for the gel of $6 \cdot 10^4$–$2 \cdot 10^7$ dalton. A 98% separation should be possible if the plate number (N) is not less than 800 for the peak for the 60S subunit and 600 for that for the 40S subunit; N for the 40S subunit was only 180 [3]. For ^3H_2O on the Trisacryl a value of $N \approx 1300$ was obtained with the 30-cm column and 1800 with the 135-cm column, which indicate that a resolving power in excess of 600–800 is possible (use of acetone as opposed to ^3H_2O to determine the total column volume showed both a slightly smaller volume and a lower value of N).

In the event, the rat liver ribosomal subunits eluted much earlier than the above calculations suggest, at $K_d \approx 0.13$ for the 60S and 0.19 for the 40S at both 4 and 20°C. These values approximate to apparent M_r of $8.0 \cdot 10^6$ for the 60S and $6.0 \cdot 10^6$ for the 40S, which are several times greater than those normally quoted [1]. For the ferritin peak where $K_d = 0.22$ the apparent M_r is $5.1 \cdot 10^6$. It is possible that under the conditions used the ribosomal subunits exhibit anomalous M_r, but the same conclusion would have to be drawn for ferritin with an M_r normally considered to be about $5 \cdot 10^5$ [10]. More likely, the relationship of K_d to log M_r is not linear and the gel pore size is not optimum for the separation of these molecular species.

By the same reasoning as for the eukaryote subunits, the apparent M_r of the *E. coli* subunits would be $8.7 \cdot 10^6$ and $7.3 \cdot 10^6$, but we believe that these results can be discounted. It is apparent, however, that factors other than M_r determine the elution behaviour of the ribosomes, as the smaller bacterial subunits elute ahead of the larger

mammalian subunits. A distinction between the *E. coli* and mammalian ribsomes may be the difference in the protein to RNA ratio, being 0.67 for the former and 1.0 for the latter, but more important in the present instance is the dissociation of the bacterial ribosomes by low Mg^{2+} concentrations under low ionic strength conditions, which is likely to lead to greater unfolding of the structure and therefore higher apparent M_r than in the case of the mammalian ribosomes dissociated and eluted with high ionic strength buffer.

For two solutes with K_d values of 0.13 and 0.19 to achieve a 98% separation, one needs a value of N of about 2700, which appears unrealistic. The N value for the two peaks of liver subunits was in practice about 320 for the 60S and 800 for the 40S. An effective if not 98% separation of ferritin from 60S subunits is possible.

In conclusion, it can be said that, comparing the agarose and Trisacryl resins, the latter did not exhibit the binding phenomenon with the 60S subunit observable with the agarose, although there was interaction with puromycin so as to give retardation, which was not observed on agarose. These observations suggest that the Trisacryl gel is more apolar than the Sepharose and therefore that the 60S subunit binds to agarose at 4°C mainly by hydrogen bonding; its release at higher temperatures bears this out. The compactness of the peaks (theoretical plates) was consistently better on the Trisacryl than on the agarose gels.

ACKNOWLEDGEMENTS

We are grateful to the Foundation for Research Development for support for this work and to Dr. R. M. McGrath for helpful comments.

REFERENCES

1 M. G. Hamilton, A. Pavlovec and M. L. Petermann, *Biochemistry*, 10 (1971) 3424.
2 T. E. Martin, I. G. Wool and J. J. Castles, *Methods Enzymol.*, 20 (1971) 417.
3 K. L. Manchester and J. E. Manchester, *FEBS Lett.*, 110 (1980) 177.
4 S. Petrovic, M. Novakovic and J. Petrovic, *Biochim. Biophys. Acta*, 254 (1971) 493.
5 S. L. Petrovic, J. S. Petrovic and M. B. Novakovic, *Biochim. Biophys. Acta*, 308 (1973) 317.
6 M. Zeichner and R. Stern, *Biochemistry*, 16 (1977) 1378.
7 K. L. Manchester and T. Alford, *Biochim. Biophys. Acta*, 563 (1979) 155.
8 K. L. Manchester, *Biochim. Biophys. Acta*, 781 (1984) 279.
9 F. Krauss and A. Schmidt, *J. Chromatogr.*, 264 (1983) 111.
10 C. F. A. Bryce and R. R. Crichton, *J. Biol. Chem.*, 246 (1971) 4198.