The Separation of Microsomal Membranes and Free Polyribosomes by Gel Filtration

By NEIL MCCOLE, DAVID N. PALMER and DAVID J. WILLIAMS

Department of Biochemistry, University College London, Gower Street, London WC1E 6BT, U.K.

(Received 12 February 1979)

The integrity of polyribosome-membrane complexes in microsomal preparations is dependent on the centrifugal conditions used for their preparation. This paper describes techniques based on gel filtration that enable the rapid and gentle separation of microsomal membranes from both free polyribosomes and soluble protein.

The characteristics of subcellular fractions are invariably affected by the methods used for their isolation. Currently most subcellular-fractionation procedures are based on various forms of centrifugation, involving inevitably the subjection of particles to high hydrostatic pressures. We have previously reported (Palmer *et al.*, 1978) the loss of a fraction of membrane-bound ribosomes from endoplasmic-membrane preparations caused by centrifugation at high g values (lower g values, however, than those regularly used to separate membrane-bound and free polyribosomes).

Alternatives to centrifugation, at least for some subcellular separations, have been described. Tangen *et al.* (1973), for example, described a method for separating particulate microsomal material from soluble proteins by gel filtration, and, using the same gel (Sephacrose 2B, with an exclusion limit of 4×10^7 daltons) Ibbetson & Freedman (1975) reported the separation of microsomal vesicles from ribosomes after degranulation of rough microsomal vesicles by EDTA treatment. Attempts have similarly been made to fractionate polyribosomal preparations by gel filtration (García-Patrone *et al.*, 1976).

In the present paper we describe a gel-filtration technique that is able to separate microsomal membranes from unattached ribosomes. Such membrane fractions have the same RNA content as those made by flotation in 2M-sucrose at relatively low g values, but greater than those recovered after separation in high centrifugal fields.

Fig. 1 shows the dramatic effect of flotation at high g on the RNA content of recovered membrane. Centrifugally labile ribosomes are lost from the membrane when exposed to $g_{\max}$ values of, or greater than, 40000g. Membranes were recovered from rat liver postmitochondrial supernatant by flotation in 2M-sucrose for 16h as described previously (Palmer *et al.*, 1978). All operations were carried out in a buffer containing 50mM-Tris/HCl, 25mM-KCl, 5mM-MgCl₂ and 1mM-EDTA, at pH7.5 (TKME), containing various concentrations of

Fig. 1. RNA contents of membranes recovered from a postmitochondrial supernatant by flotation in 2M-sucrose in various centrifugal fields

Postmitochondrial supernatant was diluted 1:3 (v/v) with 2.6M-sucrose/TKME and centrifuged at various speeds in an MSE SW40 Ti rotor at 4°C for 16h, exactly as described previously (Palmer *et al.*, 1978). Membrane floating over this layer at the end of centrifugation was aspirated, diluted, and harvested for RNA and protein determinations (Palmer *et al.*, 1978). Results are plotted as RNA (mg/mg of protein), $\pm$ S.E.M., as a percentage of the value for membrane prepared at the lowest g value.

sucrose. It is clearly important to establish the RNA content of membranes separated by even gentler procedures. However, the measurement of the RNA content (mg/mg of protein) of membranes recovered at lower g , or after shorter times of centrifugation, is precluded by the poor recovery of membrane under these conditions. In order to overcome this limitation, the possibility of performing equivalent separation by gel filtration was investigated. Mono-ribosomes show a particle weight of approx. 4×10^6

daltons, so that even fairly large polyribosomes should be included in the nominal fractionation range of Sepharose CL-2B (Pharmacia, Uppsala, Sweden), i.e. 7×10^4 – 4×10^7 daltons. Membrane vesicles will be totally excluded from such a gel. Fig. 2 shows the distribution of RNA (mg/mg of protein) throughout the turbid membrane peak, which contains 85–90% of the applied postmitochondrial phospholipid. This membrane peak appears to be heterogeneous with respect to RNA/protein values, the RNA content per mg of protein rising sharply toward the end of the peak. Subsequent flotation of these membrane fractions, however, at $40000g_{\max}$ for 16h to remove any contaminating free polyribosomes enables the recovery of membrane fractions with essentially the same RNA contents (mg/mg of protein) as each other and the same as those of the first column fractions (leading edge of peak) before flotation. These findings strongly suggest that the 'membrane' peak eluted from the columns of Sepharose CL-2B is overlapped by a closely following peak of ribosomal material, of presumably very high particle weight. Attempts to increase the resolution of these peaks by using the same gel were unsuccessful.

We were able to resolve these two peaks, however, by using Bio-Gel A150m (Bio-Rad Laboratories, Richmond, CA, U.S.A.) with an operating range of 1×10^6 – 1.5×10^8 daltons. The membrane eluted

Fig. 2. RNA/protein values of fractions from the membrane peak eluted from Sepharose CL-2B after loading with postmitochondrial supernatant

Postmitochondrial supernatant (2.5 ml) was applied to a column of Sepharose CL-2B (2.5 cm $\times$ 30 cm) equilibrated with and eluted with 0.25 M-sucrose/TKME. All turbid fractions were collected, and RNA/protein values established for those with a protein concentration in excess of 0.30 mg/ml. Columns were run at 1–4°C at 20–30 ml/h. The profile of eluted membrane protein (●) is shown, as are the RNA/protein values throughout the peak (○). Samples were also subjected to flotation in 2 M-sucrose at $40000g_{\max}$ (Palmer *et al.*, 1978) and RNA/protein values were redetermined (▲).

Fig. 3. Elution of RNA and protein from Bio-Gel A150m loaded with postmitochondrial supernatant

Postmitochondrial supernatant (1 ml) was loaded on to a column of Bio-Gel A150m (1 cm $\times$ 22 cm) equilibrated with 0.25 M-sucrose/TKME and eluted in the same buffer. Columns were run at 1–4°C at 5 ml/h. The elution of protein (●) and RNA (○) is shown for a typical separation. The RNA/protein values of the turbid membrane fractions from two separate experiments are also shown (Δ, ▲). The membrane fractions collected contained 85–90% of the applied postmitochondrial phospholipid.

from this gel is homogeneous with respect to RNA/protein values (Fig. 3). Free RNA is clearly separated from the initial membrane peak, being eluted as a broad peak before the last fraction of soluble protein.

Membranes prepared by gel filtration on Bio-Gel A150m or by flotation show remarkably constant RNA/protein ratios, provided that the centrifugal field does not exceed $40000g_{\max.}$. The RNA/protein ratio of membranes prepared by flotation at $40000g_{\max.}$ is 0.105 ± 0.0046 (16) and for that eluted from the Bio-Gel A150m is 0.102 ± 0.0013 (17). Values are means $\pm$ S.E.M., with the total numbers of fractions analysed in parentheses.

The data presented here show that the membranes and ribosomal components of a microsomal fraction may be separated by gel filtration. Furthermore, the membranes recovered from postmitochondrial supernatants, having experienced a centrifugal field of only $12000g_{\text{av.}}$ for 15 min, have the same RNA content as those prepared by flotation at $40000g_{\max.}$

for 16 h. We conclude that exposure to such centrifugal conditions does not remove centrifugally labile ribosomes; these ribosomes are lost only when centrifugal forces in excess of $40000g_{\max.}$ are used. We further consider that gel filtration will prove to be an extremely useful technique for the rapid and gentle preparation of microsomal subfractions.

D.N.P. thanks the Cancer Research Campaign for financial support during the course of this work.

References

- García-Patrone, M., Perazzolo, C. A. & Algranati, I. D. (1976) *Acta Physiol. Lat. Am.* **26**, 330–336
- Ibbetson, A. L. & Freedman, R. B. (1975) *Biochem. Soc. Trans.* **3**, 197–198
- Palmer, D. N., Rabin, B. R. & Williams, D. J. (1978) *Biochem. J.* **176**, 9–14
- Tangen, O., Jonsson, J. & Orrenius, S. (1973) *Anal. Biochem.* **54**, 597–603