Heparin–Sephacrose 4B at Low Temperatures Retains Ribosomes

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Ribosomes were separated by zonal ultracentrifugation from the fraction of reticulocyte postmitochondrial supernatants retained on heparin bound to Sepharose 4B at 4°C. No ribosomes were retained at 22°C. Ribosomes were also adsorbed on Sepharose 4B and 6B at low temperatures and could be eluted after increasing the column temperature.

Peptide-initiation factors are specifically retained on heparin bound to Sepharose 4B and may be eluted by increased salt concentrations (Waldman *et al.*, 1975). van der Mast *et al.* (1977) have demonstrated initiation-factor activities in the fraction of Krebs II ascites-cell lysates retained on this affinity material. They were able to purify one of these factors to homogeneity and concluded that proteins retained on heparin–Sepharose are useful materials for large-scale isolations of peptide-initiation factors. Slobin (1976) has provided evidence that not only peptide-initiation factors but also peptide-elongation factor 1 is adsorbed on heparin–Sepharose. Hradec & Dušek (1978) have reported that essentially all factors required for cell-free protein synthesis are retained on this affinity material and that an effective translation of natural message takes place in systems containing this fraction.

Our present paper provides evidence that, in addition to protein-synthesis factors, ribosomes are retained on heparin–Sepharose if chromatography is performed at low temperatures.

Materials and Methods

Sources of animals, chemicals and radiochemicals have been described by Hradec & Dušek (1978). The S-30 fraction of rabbit reticulocyte lysates was subjected to affinity chromatography on heparin–Sepharose 4B as described in the same paper. The purification of peptide-elongation factors has been described by Hradec *et al.* (1978).

Results and Discussion

The material retained at 4°C on heparin–Sepharose 4B columns was separated into at least five major fractions by zonal ultracentrifugation. The fraction retained on affinity columns at 22°C

showed a dissimilar pattern after zonal centrifugation (Fig. 1).

As revealed by sucrose-density-gradient analysis, fraction I obtained by zonal ultracentrifugation was apparently identical with fraction C described by Hradec & Dušek (1978) (results not shown). Fraction II contained, in addition to considerable quantities of lighter materials, two main components sedimenting at 40S and at 15S (Fig. 2a). In fraction III, peaks of 40S and 60S were found (Fig. 2b), whereas in fractions IV and V the 80S component

Fig. 1. Zonal centrifugation of the material retained on affinity columns at 4°C (—) and 22°C (----)

Conditions used for the separation were as follows: cushion, 165 ml of 50% (w/w) sucrose; 7.4–38% (w/w) hyperbolic gradient of sucrose, 350 ml; sample, 20 ml (750–900 mg of protein); overlay, 130 ml of buffer. All solutions were buffered as described by Hradec & Dušek (1978). Centrifugation in a Beckman 14 Ti zonal rotor was at 45 000 rev./min and 4°C for 180 min. Contents of the rotor were displaced by 50% sucrose and recorded continuously at 254 nm. Fractions (14 ml each) were pooled as follows: I, tubes no. 7–18; II, no. 21–27; III, no. 29–33; IV, no. 34–41; V, no. 42–50. Pooled fractions were concentrated by ultrafiltration through Amicon PM 30 membranes.

Fig. 2. Sucrose-gradient analysis of fractions obtained by zonal ultracentrifugation. Fractions (II) (a), III (b), IV (c) and V (d) were centrifuged on linear gradients (10–30%, w/w) of sucrose buffered with 20 mM-Tris/HCl buffer, pH 7.5, containing 5 mM-MgCl₂ and 120 mM-KCl (—) or with 50 mM-Tris/HCl buffer, pH 7.5, containing 2.5 mM-MgCl₂, 500 mM-KCl and 7 mM-2-mercaptoethanol (----) at 38 000 rev./min and 20°C for 150 min as described by Hradec & Dušek (1978).

predominated. In fraction V, in addition to the 80S fraction, a smaller peak of 105S was found (Figs. 2c and 2d). The 80S and 105S components of fractions IV and V were readily dissociated into 40S and 60S particles by increasing the K⁺/Mg²⁺ ratio in the medium (Figs. 2c and 2d). If RNA was extracted from fractions II–V with a phenol/chloroform mixture (Hradec & Dušek, 1978) and this material was centrifuged in linear sucrose density gradients, fraction II contained 4S, 12S and 18S RNA as main components, whereas in all other fractions the 4S, 18S and 28S species were present (results not shown).

Since these results indicated that fractions II–V

may contain ribosomal subunits, these fractions were tested in further experiments for activities characteristic for these particles. Fraction II showed only negligible peptidyl-transfer activity, whereas the activity of fractions IV and V, and in particular that of fraction III, was comparable with that of authentic 60S subunits (Fig. 3a).

Poly(U)-dependent peptide elongation was assayed in systems containing both purified peptide-elongation factors and fractions II–V instead of ribosomes. In this respect systems containing fractions IV and V were very active, whereas fraction III showed an intermediate activity. Fraction II could not, however,

Fig. 3. Peptidyl-transfer (a) and poly(U)-dependent peptide elongation (b) in the presence of fractions separated by zonal ultracentrifugation

Mixtures for the assay of peptidyltransferase were composed and incubated as described by Hradec *et al.* (1974); poly(U)-dependent phenylalanine polymerization in the presence of partially purified elongation factors from rat liver was assayed as described by Hradec *et al.* (1978). Mixtures contained fractions II (▲), III (●), IV (△) or V (○) instead of ribosomes.

replace ribosomes in these systems (Fig. 3b), and unlike 40S ribosomal subunits was incapable of binding $[^3\text{H}]$ phenylalanyl-tRNA in the presence of purified elongation factor 1. Its ability to associate with added 60S subunits to form 80S ribosomes was also very limited (results not shown).

Fraction II has an S value similar to that of the smaller ribosomal subunit, and in its high activity in inducing the translation of globin message (J. Hradec, unpublished work) it resembles fraction B described by Hradec & Dušek (1978). It contains 12S RNA, but unlike the original fraction B it contains 18S RNA, which is a characteristic component of the smaller ribosomal subunit. It remains to be elucidated whether this fraction contains inactive 40S ribosomal subunits or not.

It can be concluded that particles present in fractions III–V have some functional properties characteristic of native ribosomes. However, although they can replace ribosomes in subcellular systems, they are unable to promote the translation of natural mRNA (J. Hradec, unpublished work). It is possible that before their passage through the affinity column these particles may have been native subunits and/or 80S initiation complexes carrying bound initiation factors.

Our results provide good evidence that ribosomes are retained on heparin–Sephacrose 4B at 4°C. To elucidate whether the adsorption of ribosomes at low temperature is specific for this affinity carrier,

the fraction retained on heparin–Sephacrose 4B at 4°C was subjected to gel filtration on Sepharose 4B or 6B. The major portion of this material was eluted from Sepharose 6B columns at 4°C. However, if the column temperature was raised to 30°C and maintained at this temperature, a further u.v.-absorbing fraction was eluted (results not shown). If this latter fraction was centrifuged at 300000g and 4°C for 4h (Beckman 70 Ti rotor) and the resulting pellets were resuspended and subjected to density-gradient analysis, a pattern typical for polyribosomes and both ribosomal subunits was found. Moreover, this fraction was capable of all the ribosomal functions shown for fractions obtained by zonal centrifugation (results not shown).

It is not clear why only a small portion (approx. 11%) of ribosomes is retained on affinity columns at 4°C. These ribosomes differ in neither their sedimentation nor their activity in protein synthesis from particles passing freely through heparin–Sephacrose. In agreement with van der Mast *et al.* (1977), we have found that ribosomes are not damaged by passage through the affinity column, and subunits prepared from these particles are very active in protein synthesis and essentially free of initiation factors. Since polyribosomes retained on heparin–Sephacrose are readily dissociated into subunits by a mere increase in the $\text{K}^+/\text{Mg}^{2+}$ ratio, it seems likely that they are free of peptidyl-tRNA usually removed by either preincubation (Schreier

& Staehelin, 1973) or by treatment with puromycin (Blobel & Sabatini, 1971) before dissociation into subunits takes place. In contrast, ribosomes not retained on heparin-Sepharose must be treated with puromycin to induce complete dissociation.

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