An isolated ribosome preparation from cardiac muscle which retains effects of insulin pretreatment

KEITH D. THOMAS and JOHN MOWBRAY
Department of Biochemistry, University College London, Gower Street, London WC1E 6BT, U.K.

Protein synthesis is very active in cardiac muscle, and the isolated perfused rat heart has been shown to be sensitive to hormone administration (Morgan et al., 1971; Mowbray et al., 1973). We describe here a preparation of purified ribosomes in improved yield and in which the effects of insulin pretreatment have been preserved.

Preparations of polyribosomes from muscle generally involve attempts to solubilize membrane-bound polyribosomes by addition of detergents to postmitochondrial supernatants (Earl & Morgan, 1968; Manchester, 1974). However, there is evidence that up to 20% of rat liver cellular RNA may be lost in the nuclear pellet (Blobel & Potter, 1967; Lewis & Tata, 1973). Hearts were therefore minced with scissors, then homogenized in 9ml of ice-cold buffer A [Tris/HCl, pH7.4, 20mM; KCl, 100mM; MgCl₂, 2mM; dithiothreitol, 1mM; Triton X-100, 1% (w/v)] in a Virtis 45 instrument. Hearts were therefore minced with scissors, then homogenized in 9ml of ice-cold buffer A [Tris/HCl, pH7.4, 20mM; KCl, 100mM; MgCl₂, 2mM; dithiothreitol, 1mM; Triton X-100, 1% (w/v)] in a Virtis 45 instrument. Each pellet was carefully resuspended in 150 µl of buffer B and the ribosome solutions were pooled. Non-ionic detergents such as Triton X-100 are generally preferable to ionic detergents such as sodium deoxycholate, as deoxycholate may cause disaggregation of polyribosomes (Olanes et al., 1972). A K⁺ concentration of 250mM is recommended (Heywood et al., 1967) to avoid co-precipitation of polyribosomes with myosin.

The total RNA content of these hearts, measured after extraction (Munro & Fleck, 1969), was 1.39±0.061 mg/g wet wt. of tissue (C. M. Mackie, unpublished work) and the yield of ribosomes, measured by an ethidium bromide fluorescence assay (Mackie et al., 1979), ranged between 32.2 and 63.4% (mean 45±4%; n = 8). This appears to be a substantial improvement on the preparation of Earl & Morgan (1968). When buffer A (without Triton X-100, but with 5mM-MgCl₂) was used, and the postmitochondrial supernatant made 1% with respect to the detergent Lubrol WX before the incubation on ice, the mean yield was 29±7% (n = 5). Homogenization in the presence of Triton X-100 gave a significantly better yield (P<0.005). In addition, although density-gradient profiles of the ribosomes were indistinguishable, Triton-prepared ribosomes were more active at amino acid incorporation in vitro than Lubrol-prepared ones (results not shown).

It may be that this preparation does not provide a representative sample of cell polyribosomes. However, perfusion in the absence of hormones increased the proportion of subunits, monomers and dimers within 30min (results not shown). Moreover, perfusion with insulin (4 µg/ml) reversed this effect, decreasing the percentage of these 'free' particles (see Fig. 1) by 18.5±2.6% (paired t test, P<0.005; n = 4:

![Fig. 1. Sucrose-gradient analysis of polyribosomes from rat heart](image-url)

Approx. 150 µg of RNA on 12.5ml exponential 15–68% (w/v) sucrose gradients in 50mM-Tris/HCl (pH7.4)/100mM-KCl/2mM-MgCl₂ (Noll, 1967) was centrifuged (3h; 181000gₑᵥₑ ; 4°C). Typical profiles show polyribosomes prepared from hearts perfused for 60min in the absence (a) and presence (b) of insulin (4 µg/ml). Percentages of RNA present as dimers, monomers and subunits are shown.

Vol. 8
polyribosome profiles were modelled by computer simulation by assuming each species to be normally distributed, and hence the percentage of ribosomes present as dimers and smaller species was calculated. Just such results were to be expected, from investigations of heart postmitochondrial supernatants (Morgan et al., 1971). These latter preparations are, however, extremely poor at cell-free amino acid incorporation, probably because of the presence of powerful endogenous inhibitors (Mackie & Mowbray, 1979). By contrast, this preparation not only shows an increased incorporation, in a heterologous support medium, after insulin perfusion, of about 30%, but also a greater sensitivity to inhibitors of initiation (Buxton & Mowbray, 1980).

We thank the Medical Research Council for a studentship for K. D. T.


A salt-washed ribosome preparation from rat cardiac muscle with enhanced initiation activity

DENIS BUXTON and JOHN MOWBRAY
Department of Biochemistry, University College London, Gower Street, London WC1E 6BT, U.K.

Preparations of ribosomes from muscle and other complex mammalian tissues initiate poorly compared with reticulocyte lysates, which readily go through many rounds of protein synthesis. The addition of 250mm-NH₄Cl to a post-mitochondrial supernatant derived from rat heart has been shown to lead to enhanced stability of the subsequent preparation of cardiac polyribosomes (Earl & Morgan, 1968). We show here that the presence of NH₄Cl during preparation increases the incorporation of radioactive amino acid into protein by the ribosome preparation, assayed in a cell-free translational system, and that part of this increased incorporation is due to an increase in initiation.

Ribosomes were prepared as described previously (Mackie et al., 1979), except that the supernatant from the 8000 g spin was divided into two portions, to one of which was added 250mm-NH₄Cl. The two ribosomal preparations were then assayed for incorporation of radioactive leucine into trichloroacetic acid-insoluble material, by using as support system a high-speed-supernatant preparation from rabbit brain cortex (Metafora et al., 1977) in the presence and absence of edeine (Calbiochem), an inhibitor of protein-synthesis initiation.

Fig. 1 shows that the NH₄Cl-prepared ribosomes are considerably more active than the control ribosomes. This increased activity may be attributed to three factors. Firstly, in agreement with other work (Earl & Morgan, 1968), we find that NH₄Cl-prepared ribosomes are more stable to preincubation than are controls (results not shown). Secondly, when characterized on 15–68% (w/v) exponential sucrose gradients (Noll, 1967), the NH₄Cl-prepared ribosomes show an increased proportion of polyribosomes to dimers, monomers and subunits (control 61.8% polyribosomes, NH₄Cl-prepared 70.4% polyribosomes; mean of two experiments). Thus, in the absence of re-initiation, the polyribosomes when run off will incorporate more radioactive leucine per mg of RNA. These findings imply that some endogenous inhibitor (Mackie & Mowbray, 1979) or ribonuclease has been removed by the NH₄Cl treatment. The increased initial activity of NH₄Cl-prepared ribosomes is in contrast with the results of other workers (Earl & Morgan, 1968), who found the initial activity to be the same as that of control ribosomes. The reason for this difference is unclear.

Thirdly, Fig. 1 shows that the NH₄Cl-prepared ribosomes show an increased sensitivity to edeine compared with control ribosomes. With four different ribosome preparations the incorporation of ~[3,4-³H]leucine (50Ci/mmol) into trichloroacetic acid-insoluble fraction (Mackie & Mowbray, 1979) by a preparation of control ribosomes (O, △) and NH₄Cl-prepared ribosomes (□, ▲) in the presence (●, ●) or absence (○, ▼) of edeine. Total volume was 300 ml, and sample volume was 20 ml. For initiation inhibition in this system 0.1 mm-edeine is required.