
Fructose injection into galactosamine-treated rats resulted in a marked increase in blood lactate concentration (Table 1), an increase in blood glucose concentration (Table 2) and a dramatic decrease in blood ketone-body concentrations (Table 2) when compared with galactosamine-treated control rats. These results are similar to those reported by Rawat & Menahan (1975), who injected fructose into normal rats. Dichloroacetate lowered the initial fructose-induced peak in blood lactate concentration (Table 1) and this rapidly decreased towards normal values. The hyperglycaemia and hypoketonaemia (Table 2) that resulted from fructose injection were also reversed. These findings are consistent with our earlier observation that dichloroacetate decreases extrahepatic lactate production and increases ketogenesis from fructose in the isolated perfused rat liver (Johnson & Man, 1975).

The results show that dichloroacetate brings about metabolic changes in galactosamine-treated rats that resemble those reported in normal rats deprived of food for 24 h. Dichloroacetate reverses the hyperlactataemia that results from galactosamine administration to the rat, and also counteracts the effects of fructose infusion. The effects may be explained by activation of pyruvate dehydrogenase both extrahepatically and in the liver. Dichloroacetate could well have a role as a therapeutic agent in clinical hyperlactataemic states.


Isolation and Partial Characterization of Polyribosomal Messenger Ribonucleoproteins from Embryonic-Chick Tendon Cells

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In eukaryotic cells messenger ribonucleic acids (mRNA) exist in the form of messenger ribonucleoprotein particles (for a review see Williamson, 1973). Among the polypeptides associated with mRNA isolated from a wide variety of cell types, two species are predominant, having mol.wts. of approx. 52000 and 78000 (Blobel, 1972, 1973). The functional role of the proteins associated with mRNA is as yet undetermined, though there is strong evidence that the 78000-mol.wt. species is bound to the poly(A) tract at the 3'-terminus of the molecule (Blobel, 1973; Gander et al., 1975).

It has been demonstrated previously that embryonic-chick tendon cells devote a considerable proportion of their protein-synthesis activity to the production and secretion of procollagen (Dehm & Prockop, 1971). We have therefore undertaken a study using these cells ultimately aimed at the characterization of those messenger ribonucleoprotein particles directing the synthesis of procollagen. The results reported here represent our preliminary data on the total population of messenger ribonucleoprotein particles present in these cells.

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Fibroblasts were obtained from 17-day chick embryos by enzymic digestion of leg tendons (Dehm & Prockop, 1971) and incubated in vitro with [5-3H]uridine for 3 h at 37°C in modified Krebs medium containing 10% (v/v) foetal calf serum to label RNA. Total polyribosomes were isolated by layering the detergent-treated postmitochondrial supernatant [0.5% Triton X-100 and 0.2% sodium deoxycholate in 50 mm-Tris/HCl (pH 7.5)/200 mm-KCl/5 mm-MgCl₂] from the cells over a sucrose bilayer in the above buffer followed by centrifugation as described previously (Blobel & Potter, 1967; Harwood et al., 1975). Heparin (final concn. 250 µg/ml) and rat liver ribonuclease inhibitor (Gribnau et al., 1969; final concn. 5 units/ml) were included in the homogenization buffer. Precautions taken against ribonuclease activity also included the autoclaving of all buffers and sucrose solutions with 0.02% diethyl pyrocarbonate at a pressure of 100 kPa (151 lb/in²) for 20 min (Craig et al., 1976) and sterilization of glassware.

The isolated polyribosomes were washed with 0.5 M-KCl, as described by Blobel (1972), dissociated in 30 mm-EDTA-containing buffer and applied to a column of oligo(dT)-cellulose (Lindberg & Sundquist, 1974). Fractions containing unbound ribonucleoprotein components (peak I) and those containing bound species eluted with 50% (v/v) formamide (peak II) were dialysed against 10 mm-Tris/HCl (pH 7.5)/10 mm-NaCl/10 mm-EDTA for subsequent analysis.

To determine the buoyant densities of the two classes of ribonucleoproteins separated by oligo(dT)-cellulose, samples were fixed overnight with 6% (v/v) glutaraldehyde and layered over pre-formed CsCl gradients (1.35-1.67 g/cm³) containing 0.8% Brij-35. After centrifugation at 20°C analysis of the distribution of radioactivity in the gradients showed that peak-I material sedimented as a single peak of density 1.39 g/cm³, which is in close agreement with values obtained for messenger ribonucleoprotein particles from other cell types (Lindberg & Sundquist, 1974). The ribonucleoprotein species present in peak I had a buoyant density of 1.6 g/cm³, which corresponds to the density of salt-washed ribosomal subunits (Clegg & Arnstein, 1970).

For protein analysis, the dialysed fractions were centrifuged (Kumar & Pederson, 1975) and the pellets resuspended in sample buffer before electrophoresis on 10% (w/v) polyacrylamide gels in the presence of sodium dodecyl sulphate (Laemmli, 1970). The protein profile of peak I was characteristic of ribosomal subunit proteins with most of the bands lying in the mol.wt. range 15000-45000. Analysis of the proteins present in the peak-II fractions revealed the presence of two polypeptides of mol.wt. 79000 and 50000 respectively, together with two to three minor components of mol.wts. between 62000 and 68000. In addition to these polypeptides, proteins in the mol.wt. range 15000-45000 similar to those found in peak-I material were observed. Comparison of the ribosomal proteins present in peak-II fractions with those from the small and the large subunits of embryonic-chick liver ribosomes indicated that these fractions containing messenger ribonucleoprotein are contaminated predominantly with small ribosomal subunits.

Polyribosomal messenger ribonucleoprotein particles from tendon cells were also examined after dissociation with 1 mm-puromycin in the presence of high-salt buffer for 30 min at 37°C (Blobel, 1972; Gander et al., 1975) rather than 30 mm-EDTA. Oligo(dT)-cellulose chromatography of puromycin-dissociated polyribosomes again yielded a peak of [3H]uridine-labelled material which was retained by the column. Analysis of the protein content of the peak-I and -II fractions revealed patterns similar to those obtained in the EDTA-dissociated case. The finding that the two methods of dissociation yield very similar results suggests that the non-ribosomal proteins associated with the mRNA species are not a result of binding artifacts.

Previous work has shown that procollagen synthesis takes place predominantly on membrane-bound polyribosomes (Harwood et al., 1973, 1975), which can be obtained separate from free polyribosomes by omitting the detergent treatment of the postmitochondrial supernatant (Blobel & Potter, 1967; Harwood et al., 1975). Messenger ribonucleoprotein particles obtained from membrane-bound polyribosomes were examined and compared with those from the free polyribosomal population. In this case dissociation was achieved by using puromycin, since the earlier experiments suggested
there was less contamination of the messenger ribonucleoprotein species prepared by this method than by EDTA dissociation. No major qualitative differences were observed between the proteins present in the particles obtained from free and membrane-bound polyribosomes.

The results described here demonstrate the presence in tendon cells of polyribosomal messenger ribonucleoprotein particles whose buoyant densities and protein composition are similar to those described in other cell systems (Blobel, 1973; Lindberg & Sundquist, 1974; Irwin et al., 1975; Kumar & Pederson, 1975). Preliminary studies on messenger ribonucleoprotein species derived from free and membrane-bound polyribosomes indicated that the 79000- and 50000-mol.wt. proteins were associated with both classes.

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A Region of the Troponic C Molecule Involved in Interaction with Troponin I

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Troponin C is the component of the troponin complex that binds Ca$^{2+}$ specifically and thus initiates the biochemical events in the myofibril leading to contraction. The primary sequence of troponin C from rabbit fast white muscle has been determined and four Ca$^{2+}$-binding sites have been presumptively identified (Collins et al., 1973). These sites are present on four of the eight peptides obtained when troponin C from fast white skeletal muscle of the rabbit is digested with CNBr.

The whole CNBr digest from which partially digested material had been removed by gel filtration possessed a number of the biological properties of troponin C itself. These included the neutralization of the inhibition of Mg$^{2+}$-dependent ATPase* of desensitized actomyosin produced by troponin I, inhibition of the phosphorylation of troponin I catalysed by 3':5'-cyclic AMP-dependent protein kinase and by phosphorylase kinase, and Ca$^{2+}$-dependent complex-formation.

These properties were preserved in CNBr digests of [1-14C]carbamoylmethylated troponin C. The only 1-14C-labelled peptide in these digests, which consists of residues

* Abbreviation: ATPase, adenosine triphosphatase.