Intracellular Distribution of Nicotinamide—Adenine Dinucleotide Phosphate-Linked Isocitrate Dehydrogenase, Fumarase and Citrate Synthase in Bovine Heart Muscle

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Pette (1966) reported that 14% of the total NADP-linked isocitrate dehydrogenase activity in the rat heart-muscle cell is in the cytoplasm and the remainder in mitochondria. Uhr et al. (1974) concluded that in pig heart muscle all the enzyme is in mitochondria. The main aim of the present work was to determine the relative activities of this enzyme in the cytoplasm and mitochondria of the bovine heart-muscle cell.

The techniques of fractional extraction of minced tissue with iso-osmotic sucrose and phosphate buffer solutions, developed for such studies by Delbrück et al. (1959) and Pette (1966), proved to be unsatisfactory for NADP-linked isocitrate dehydrogenase in bovine heart muscle because of the low cytoplasmic activity and sensitivity of the assay method. The method did afford an accurate estimate of the maximum proportion of citrate synthase activity in the cytoplasm. Minced tissue (10g) was successively extracted, by stirring for 20-30min at 0°C, twice with 0.25M-sucrose solution (200ml) and once with 0.1M-sodium phosphate buffer, pH7.5 (200ml). The residual tissue was finally homogenized with 0.25M-sucrose containing 0.1% (v/v) Triton X-100 to release the remaining enzymes. Supernatants from each extract obtained after centrifugation at 27600g were assayed for citrate synthase, lactate dehydrogenase and glyceraldehyde 3-phosphate dehydrogenase; 80% of the total lactate dehydrogenase and glyceraldehyde 3-phosphate dehydrogenase activities and 2-4% of the total citrate synthase activity extracted were in the sucrose and phosphate-buffer extracts. This result is identical with those reported by Zebe (1960) for locust flight muscle and Pette (1966) for rat heart muscle, and shows that not more than 4% of the total citrate synthase activity is present in the cytoplasm. Most probably the enzyme is exclusively mitochondrial and this small proportion is released from mitochondria during the extractions.
The intracellular distributions of NADP-linked isocitrate dehydrogenase and fumarase were studied by fractionation of 750g supernatants from homogenates in 0.25M-sucrose, pH 7.5, containing 10mM-EDTA. Citrate synthase was used as a marker enzyme to measure the proportion of mitochondria from which enzymes were released into high-speed supernatants. Homogenates were prepared for some experiments by sand-grinding (MacFarlane et al., 1977) and for others in a Polytron, and centrifuged at 750g for 15min. The distributions of enzymic activities between the soluble fraction and mitochondria were measured in two ways. First, portions of supernatants were assayed with and without addition of 0.1% (v/v) Triton X-100. The difference between the two assays was taken as a measure of the activity in mitochondria, and the assay without detergent as a measure of activity in the soluble fraction. In the second method, the mitochondria were separated by centrifugation at 27600g for 10min. Control experiments showed that there was no loss of activity from the supernatants when they were centrifuged again at 100000g for 30min. In some experiments the activities in mitochondrial pellets were assayed after sonication (MacFarlane et al., 1977) and in others after homogenization of the pellets in 0.25M-sucrose containing 0.1% Triton X-100. The difference between the results obtained by the two methods was no greater than anticipated from the reproducibility of individual assays (±2%).

In six experiments, the citrate synthase activities in the soluble fractions were in the range 31 to 63% of the total activity, reflecting the different proportions of mitochondria from which enzyme was released during the homogenizations. In each experiment, the proportions of isocitrate dehydrogenase and fumarase activities in the soluble fraction were significantly greater than that of citrate synthase, showing that they are present in the cytoplasm or in a compartment of the mitochondrion from which they can be released into solution without concomitant release of citrate synthase. There is good evidence that these three enzymes are located in the mitochondrial matrix and are not present in the intermembrane space or the outer membrane (Addink et al., 1972). On this basis, and with the assumption that citrate synthase in the soluble fraction has come from broken mitochondria, the proportion of the soluble isocitrate dehydrogenase and fumarase activities originating from mitochondria can be calculated and the remainder identified as true cytoplasmic activity. The results of the six experiments show that 9.8 (s.d. ±2.6)% of the total isocitrate dehydrogenase activity and 10.5 ±1.9% of the total fumarase activity is cytoplasmic and the remainder is in mitochondria.

These results for NADP-linked isocitrate dehydrogenase agree closely with those obtained by Pette (1966) for rat heart muscle by the method of fractional extraction of minced tissue, when the latter are corrected for enzyme released from mitochondria (4%) on the basis of citrate synthase activity measurements. Nakashima et al. (1976) concluded that 40% of the total fumarase activity in rat liver homogenates, found in high-speed supernatants, was derived from cytoplasm, but made no allowance for broken mitochondria. Tolley & Craig (1975) showed that fumarase is present in the cytoplasm of human and mouse cultured cells and that it differs from the mitochondrial enzyme in electrophoretic mobility.

Zebe, E. (1960) Biochem. Z. 332, 328–332