An Assay for L-Alanine-Glyoxylate Amino-transferase

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We have adopted an assay method for L-alanine-glyoxylate aminotransferase that measures pyruvate formation colorimetrically by the salicylaldehyde method of Berntsson (1955). Incubations contain 0.02 M-L-alanine, 0.02 M-glyoxylate, 0.05 M-phosphate buffer, pH 7.4, and tissue homogenate prepared in phosphate buffer. Samples are taken into an equal volume of 10% (w/v) trichloroacetic acid and pyruvate is measured after centrifugation. The other materials present in the denatured extract do not interfere.

For the same assay pyruvate concentrations are equivalent whether measured by the salicylaldehyde method or by a modification of the lactate dehydrogenase method of Thompson & Richardson (1968).

Glycine formation measured by separation on paper is equivalent to pyruvate for incubations with rat and rabbit liver. For these experiments assay samples were inactivated in perchloric acid. After neutralization with potassium hydroxide, supernatant samples were incubated at 25° for 30 min. with 1% (w/v) hydrogen peroxide to remove glyoxylate before paper chromatography; otherwise, non-enzymic transamination occurred, apparently on the paper.

The salicylaldehyde method is not very sensitive; 1 μ mole of pyruvate gives an extinction of 0.15 and the reagent blank is high (extinction 0.3). Nevertheless, we obtain linear progress curves and proportionality between initial rate and tissue concentration, and the method is simple.

If it were easy to dispose of glyoxylate specifically in extracts one could take advantage of the greater sensitivity of the dinitrophenylhydrazine method for pyruvate determination. A procedure based on this principle, reported by Rao & Ramakrishnan (1962), removes glyoxylate by reaction with cysteine before pyruvate analysis. In our experience much pyruvate is also removed by cysteine.

From assays with salicylaldehyde we previously reported especially high activities for the enzyme in rabbit and pig livers (Rowsell, Carnie & Taktak, 1966). We suggested this might signify a large requirement for glycine synthesis for glycocholate formation. However, this cannot be the only factor involved. Extension of this work has reproducibly revealed high activities in cat liver (1550 μ moles of pyruvate/g./hr.), and in frog and toad liver (4690–7120), and glycine conjugates do not occur in the bile of these species (Haslewood, 1967).

We find this enzyme does not occur in brain, heart, skeletal muscle or spleen of mouse, rat, guinea pig or rabbit. Kidney activity varies with species, but in every case the total kidney contribution is a small fraction of the total liver enzyme.

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The Separation of Rat Liver Mitochondria into Two Morphologically Different Fractions by Density-Gradient Centrifugation

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Apparent separation of rat liver mitochondria into different populations by centrifugation has been ascribed either to abnormalities caused by high sucrose concentration or to contamination of mitochondrial fractions by other cell organelles such as lysosomes or microbodies (Beaufay *et al.* 1964).

Hackenbrock (1966, 1968) showed that mitochondria in different metabolic states differ in their ultrastructure.

Mitochondria prepared as described by McMurray & Dawson (1969) were placed on a continuous sucrose gradient ranging from 0.9 M to 1.9 M, containing 0.001 M-EDTA, and centrifuged at 130 000 g_{av} for 90 min. in the 59589 MSE swing-out rotor. Three distinct bands were formed: the top band consisted mainly of damaged mitochondria, but the middle band (sp.gr. 1.184) and the lowest band (sp.gr. 1.216) consisted of intact mitochondria. The 1.184-sp.gr. mitochondria resembled the 'condensed' type described by Hackenbrock (1968), and the mitochondria in equilibrium with the 1.216-sp.gr. medium resembled 'orthodox' mitochondria. Both types had identical protein:phospholipid ratios and showed no significant qualitative or quantitative differences in the fatty acid composition of their neutral lipids.

Both types had similar specific activities for cytochrome oxidase, glutamate dehydrogenase, malate dehydrogenase and monoamine oxidase, but glucose 6-phosphatase and acid phosphatase were virtually absent.

In normal rat liver the lower-density mitochondria predominated, but in starved rats both

types were present in approximately equal proportions. On the other hand mitochondrial preparation from embryonic rat livers (-6 days) consisted predominantly of the high-density type (10:1); as development proceeded this ratio changed, until at 1 day after birth the low-density mitochondria were the predominant type.

The low-density mitochondria can be transformed into high-density mitochondria by incubating the former with succinate and P_i and subsequently placing the mixture on a sucrose gradient containing these substrates.

Similarly, if mitochondria from normal liver are isolated in the presence of succinate and P_i , virtually all the mitochondria are of the high-density type. On the other hand, high-density mitochondria can be partially converted into low-density mitochondria by incubation with either antimycin or ADP.

These results show that ultrastructurally different mitochondria, reflecting different respiratory states, can be separated from one another on a density gradient; yet their membranes do not differ in density.

On investigating the total water and sucrose-inaccessible space of these two types of mitochondria it was found that the change in the total mitochondrial volume and the sucrose-inaccessible space could account for an increase in density of the 'orthodox' mitochondria due to the equilibration of the internal sucrose content with the high sucrose content present in the gradient.

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The Permeability of Rat Heart Mitochondria to Citrate

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It has been postulated that in perfused rat heart the rate of glycolysis may be controlled in some circumstances by the concentration of citrate regulating the activity of phosphofructokinase (Randle, Newsholme & Garland, 1964; Randle, Denton & England, 1968). In rat heart, phosphofructokinase is cytoplasmic, whereas citrate synthesis is intramitochondrial. Translocation of citrate across the mitochondrial membrane must therefore occur if the above hypothesis is to be

tenable. Liver mitochondria are readily permeable to citrate in the presence of malate, etc., whereas heart mitochondria were shown to be impermeable to citrate under the same conditions (Chappell & Robinson, 1968). The work reported here attempted to resolve this discrepancy.

The technique used was to label the intra-mitochondrial citrate and isocitrate specifically, and then to follow the efflux of the label under various conditions. (In what follows the term citrate is taken to mean both citrate and isocitrate.) Citrate was initially labelled by incubating heart mitochondria with $KH^{14}CO_3$, rotenone and 2-oxoglutarate; this process specifically labels citrate by a reversal of isocitrate dehydrogenase and aconitase (Bowman, 1966). The mitochondria were washed, incubated at 30° for times up to 3 min. in 120 mM-KCl-20 mM-tris, pH 7.4, and rapidly spun down at 0°. The medium was assayed for $[^{14}C]$ citrate by liquid-scintillation counting after removal of $^{14}CO_2$ either by acidification and degassing or on Dowex-1 formate resin. Control experiments showed that these separation procedures were identical, and that the remaining radioactivity was probably present in citrate only.

The results showed that there was no citrate efflux when heart mitochondria were incubated in tris-KCl alone, but the addition of malate, citrate, tartronate or aspartate to the medium caused a rapid efflux of radioactivity. Malonate, butylmalonate and glutamate did not cause an efflux. Equilibration of citrate between intra- and extra-mitochondrial spaces was complete in 90 sec. in the presence of malate. The concentration of malate required for half-maximal rate of efflux ($K_{act.}$) was 0.2 mM; in the presence of 2 mM- P_i it was 0.1 mM. $K_{act.}$ for externally added citrate was 0.5 mM.

These results show that the permeability to citrate of heart mitochondria is qualitatively similar to that of liver mitochondria, but is quantitatively very much less. The maximum citrate efflux found (10 mμmoles/min./mg. of protein) is sufficient to account for the rate required in perfused heart to explain the inhibition of glycolysis by citrate reported by Randle *et al.* (1968).

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