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Properties of Phosphoenolpyruvate Carboxylase Isolated from Maize Leaves

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Phosphoenolpyruvate carboxylase (EC 4.1.1.31) has been purified 100-fold from maize leaves in 25% yield. The purification method differs from that of Lowe & Slack (1971) and involves isoelectric focusing as the final step (pI 5.2–5.3). Specific activities of better than 40 U/mg of protein were obtained.

The enzyme was most stable in 1 mM-dithiothreitol at 15°C at pH 7.0. When glycerol was added to the enzyme solution to a final concentration of 50% (v/v) it increased its specific activity and helped to maintain its activity.

If purified enzyme was fractionated on an agarose column (Bio-Gel A1.5), two protein bands emerged: first a small band of low-activity protein (A) followed by a large band of lighter high-activity protein (B). If samples of band B material were re-run on the same column after 24 h storage at 15°C, two band were eluted at the same positions as bands A and B; after 5 days' storage the proportion of band A material had increased at the expense of band B material.

Band B material sedimented as an homogeneous protein in the analytical ultracentrifuge with an estimated $s_{20,w}^0$ value of $13.5 \pm 0.3S$ (possible variation in the slope of the extrapolation to zero concentration). The diffusion coefficient was estimated to be $3.77 \times 10^{-7} \text{ cm}^2 \cdot \text{s}^{-1}$ and the partial specific volume as $0.74 \text{ cm}^3 \cdot \text{g}^{-1}$. From these data a molecular weight of 340000 ± 8000 was calculated.

When a solution of band B material was diluted from 7 mg of protein/ml to 5.5 mg/ml, a second component was seen with a sedimentation rate corresponding to approximately half the molecular weight of the main component.

Treatment of band A material and band B material with sodium dodecyl sulphate followed by polyacrylamide-gel electrophoresis in the presence of this detergent showed a single component with similar mobility in each case. Comparison with the mobilities of protein standards indicated a molecular weight of approx. 160000 for the detergent-treated enzyme.

Phosphoenolpyruvate carboxylase from maize leaves appears to exist in several forms. The active

enzyme (B) has a molecular weight of approx. 340000. In concentrated solution (10–40 mg of protein/ml) this B form can aggregate to a larger, less active, form (A). On dilution or on detergent treatment the B form dissociates into probably two similar subunits.

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Carbonic Anhydrase: Multiple Forms in Plants

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Carbonic anhydrase (EC 4.2.1.1) catalyses the hydration of carbon dioxide to give HCO_3^- and H^+ ions:

It is present in the green parts of plants, and appears to be important for the efficient fixation of carbon dioxide (Graham & Reed, 1971). Iso-enzymes of carbonic anhydrase from animals can often be located after starch-gel electrophoresis by using their relatively weak esterase activity (Tashian, 1965). We could not detect any esterase activity associated with the carbonic anhydrase from the leaves of the angiosperm *Tradescantia albiflora* Kunth. We therefore devised a method to locate the enzyme after electrophoresis on polyacrylamide gels. The gels are surface-stained with Bromocresol Purple and exposed to carbon dioxide. H^+ ions produced by the enzyme-catalysed hydration of carbon dioxide change the colour of the indicator. The differential staining of the enzyme bands is a transient phenomenon, but further hydration is stopped by freezing the gels on solid carbon dioxide. At low temperatures solid solutions of Bromocresol Purple fluoresce brightly under u.v. light, the acid form yellow and the base form pink, and the position of carbonic anhydrase is therefore shown on the frozen gels as yellow bands against a pink background.

Extracts of leaves from higher plants were separated by electrophoresis through gradients of polyacrylamide (Margolis & Kenrick, 1968). All the extracts examined gave at least two major bands of carbonic anhydrase activity. Although it is

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possible that these different forms of carbonic anhydrase are artifacts of the preparation procedure, extracts from more than 12 plants in diverse Families all showed multiple forms of carbonic anhydrase. In a number of dicotyledons the size of both the major isoenzymes is close to that of the enzyme from parsley, which has a molecular weight of 180 000 (Tobin, 1970). The isoenzymes from the monocotyledons examined had molecular weights of less than 40 000. Like erythrocyte and parsley carbonic anhydrases, the enzymes from *T. albiflora* contained zinc.

C. A. A. is indebted to Rothmans Ltd. for a Postdoctorate Fellowship.

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Histochemical Localization of Enzymes in Heart Muscle

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Histochemical localization by formazan deposition, in an agarose gel-film medium, by using phenazine methosulphate to reduce interference from tetrazolium reductases, has been used for extensive studies on the localization of mitochondrial and cytoplasmic enzymes in rat liver (Pette & Brandau, 1966), rabbit skeletal muscle (Sigel & Pette, 1969) and insect flight muscle (Brandau & Pette, 1966). This variant of the tetrazolium localization method has not been used to study the distribution of soluble enzymes in heart muscle.

We now report the results of studies on the distribution of succinate dehydrogenase (EC 1.3.99.1), glutamate dehydrogenase (EC 1.4.1.2), glutamate-oxaloacetate transaminase (EC 2.6.1.1) and lactate dehydrogenase (EC 1.1.1.27) in rat heart muscle. The muscle fibres were caused to relax by soaking the organ in iso-osmotic potassium chloride solution before freezing and sectioning. This pretreatment made it possible to detect the striations of the myofibrils, normally invisible in freshly excised heart tissue. All the enzymes showed a banded distribution, but that for the purely mitochondrial enzymes succinate dehydrogenase and glutamate

dehydrogenase was coarser and more irregular, in keeping with the expected localization of these enzymes in mitochondria, which are concentrated, but not exclusively located, opposite the I-bands of the fibrils (Klingenberg, 1964). The picture for glutamate-oxaloacetate transaminase, which is known to occur in both cytoplasm and mitochondria in this tissue, was similar to that for the purely cytoplasmic enzyme lactate dehydrogenase.

It cannot at present be proved conclusively that the 'soluble' enzymes are located within the myofibrils, but the localization pattern appears to be in accord with the hypothesis of a concentrated distribution of many glycolytic and related enzymes around the isotropic zone of skeletal-muscle fibres, which has been put forward by Brandau & Pette (1966) and Sigel & Pette (1969).

This work was carried out in Konstanz as the result of an invitation from D. P., and financial support was provided by the Science Research Council.

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Isoenzymes of Glutamine Synthetase in Rat Tissues

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It was recently shown (Iqbal & Ottaway, 1970) that glutamine synthetase from rat muscle has different kinetic properties from that from kidney. In particular, product inhibition by P_i is competitive with respect to ATP. This is incompatible with the reaction mechanism proposed by Meister and co-workers (see Meister, 1968), but is compatible with a mechanism involving binding of P_i to the enzyme and random release of P_i and ADP as products. This latter mechanism predicts that P_i will be an uncompetitive inhibitor of the muscle enzyme with respect to hydroxylamine. It has been possible to show that this is indeed the case, and a semi-random mechanism is now regarded as established for rat muscle glutamine synthetase.

The inhibition of kidney glutamine synthetase by P_i was also studied. P_i was found to inhibit uncompetitively with both hydroxylamine and glutamate as variable substrates; the K_i is about 5 mM in each case. These observations are not in accord with the Meister mechanism, which predicts