SOLUBLE AND PARTICULATE GLYCOLYSIS IN DEVELOPING CASTOR BEAN ENDOSPERM

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Summary: Proplastids from developing castor bean endosperm have been isolated in a discontinuous sucrose density gradient. There was little contamination of the proplastids by mitochondria. Pyruvate kinase activity and phosphofructokinase activity closely correlated with triose phosphate isomerase activity, a proplastid marker, suggesting these two enzymes were contained in the proplastid. Aldolase was also found in the proplastids. The presence of these enzymes indicates that a glycolytic pathway operates in the proplastid.

Long chain fatty acid biosynthesis is associated with the proplastid fraction from developing castor bean endosperm (1-5). It has been shown that acetyl-CoA for fatty acid biosynthesis is provided by a pyruvate dehydrogenase complex which is located within the proplastid (6). This complex appears to have properties different from the complex isolated from pea mitochondria (7). A 10,000 x g pellet will incorporate radioactivity from sucrose into fatty acids (5) and it was suggested that the entire pathway from sucrose to fatty acids is located in this pellet (5). The 10,000 x g pellet contains both proplastids and mitochondria so that the exact location of the pathway was not identified. Also the incorporation of radioactivity into fatty acids was small. In this report phosphofructokinase and pyruvate kinase are shown to be present in a proplastid fraction free from mitochondria indicating that the entire pathway from hexoses to fatty acids may be located within this organelle.

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Materials and Methods:

Developing castor bean seeds were harvested from plants growing in the Botanical Garden at UCLA. Endosperm tissue (8 g) was homogenized in 12 ml of extraction medium as described previously (1). The 5,000 x g pellet was taken up in 2 ml of extraction medium, centrifuged at 500 x g for 2 min, layered on a discontinuous sucrose gradient and centrifuged as described by Reid et al. (6). The gradients were collected dropwise from the bottom in approximately 1.2 ml fractions and numbered in the order they were collected.

Enzyme activities were measured in either a Gilford modified Beckman DU or Varian recording spectrophotometer. Succinic dehydrogenase was determined by measuring the reduction of 2,6-dichlorophenol-indophenol (DCIP) as described by Reid (6) except 0.3 mM DCIP was used. The assay for triose phosphate isomerase contained: 0.23 mM R,S-glyceraldehyde-3-phosphate, 0.1 mM NADH, 7 units of α-glycerophosphate dehydrogenase, and 50 mM triethanolamine buffer, pH 7.9 in a total volume of 1 ml. The assay for pyruvate kinase contained: 0.1 mM ADP, 0.5 mM phosphoenolpyruvate, 10 mM $MgCl_2$, 45 mM KCl, 0.1 mM NADH, 20 units of lactate dehydrogenase, 0.1% Triton X-100, and 50 mM TES[N-tris-(hydroxymethyl)-methyl-2 amino sulfonic acid] buffer pH 7.5 in a total volume of 1 ml. The assay for phosphofructokinase contained: 2 mM ATP, 0.1 mM NADH, 2 mM F6P, 1 mM dithiothreitol, 5 mM $MgCl_2$, 0.1% Triton X-100, 0.7 units α-glycerophosphate dehydrogenase, 9 units triose phosphate isomerase, 1.6 units aldolase and 50 mM Tris buffer, pH 8.0 in a total volume of 1 ml. The last three enzymes were assayed by measuring the decrease in absorbance at 340 nm. Protein was determined by the absorbance of 280 nm in a 0.1% solution of Triton X-100 or by the method of Lowry et al. (8) after precipitating the protein with 10% trichloracetic acid.

Biochemicals and enzymes were purchased from the Sigma Chemical Company.

Results:

The separation of the resuspended 5,000 x g pellet on the discontinuous gradient was the same as described previously (6). Three protein bands corresponding to the soluble, mitochondrial and proplastid fractions were found on top of the gradient, at the 35-45% sucrose interface and at the 45-60% sucrose interface, respectively. There was little contamination of the proplastid fraction with mitochondria as judged by the succinic dehydrogenase activity, but there were proplastids in the mitochondrial fraction as judged by the triose phosphate isomerase activity (Figs. 1 and 2). There was also a significant and variable amount of triose phosphate isomerase activity associated with the soluble fraction which probably represents proplastids broken during resuspension as well as soluble enzyme trapped in the 5,000 x g pellet.

Pyruvate kinase activity was found predominantly in the proplastid fraction and closely paralleled the triose phosphate isomerase activity except in the

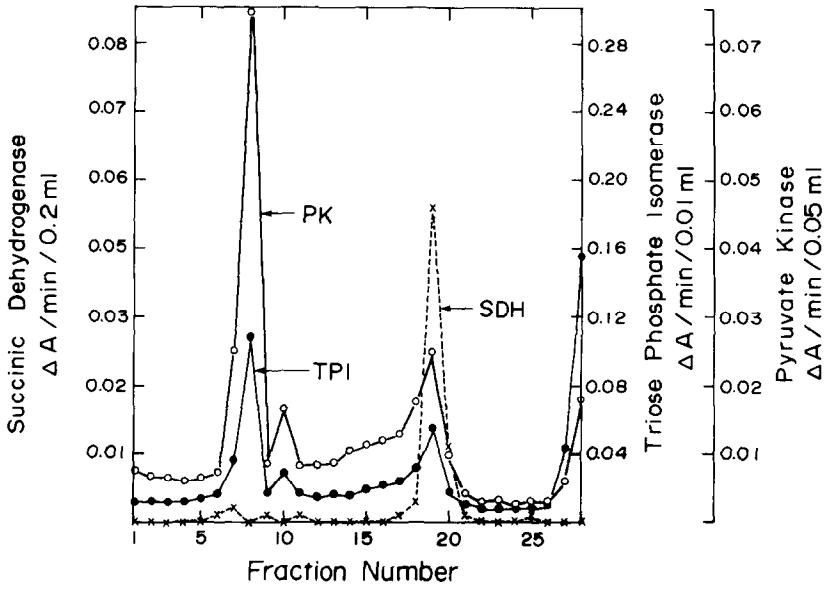

Figure 1: Distribution of succinic dehydrogenase (SDH), triose phosphate isomerase (TPI) and pyruvate kinase (PK) on a discontinuous sucrose density gradient.

soluble fraction (Fig. 1). The lack of correspondence in the soluble fraction may be due to instability of the pyruvate kinase after release from the organelle. There was no activity with the proplastid enzyme in the absence of ADP, phosphoenol pyruvate, $MgCl_2$ or lactate dehydrogenase. Triton X-100 was not necessary to demonstrate pyruvate kinase activity in the proplastids, but its inclusion removed an initial absorbance change in the absence of substrate which is probably due to a change in light scattering of the particles on dilution. Pyruvate kinase was also detected in a 37,500 x g supernatant. In this case there was a considerable absorbance change in the absence of ADP due possibly to a phosphatase activity or, more probably, to a combination of phosphoenolpyruvate carboxylase and malate dehydrogenase.

Phosphofructokinase activity closely followed triose phosphate isomerase activity suggesting that it is also a component of the proplastids (Fig. 2). In this particular gradient there was a large contamination of the mitochondria

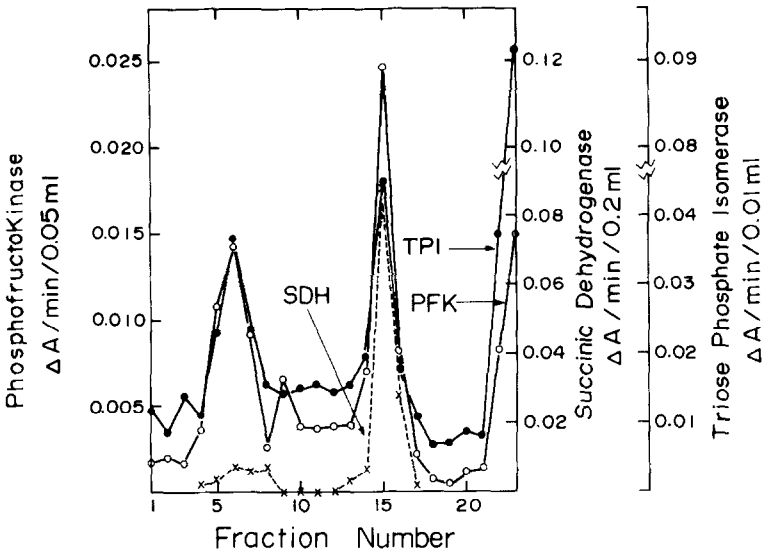

Figure 2: Distribution of succinic dehydrogenase (SDH), triose phosphate isomerase (TPI) and phosphofructokinase (PFK) on a discontinuous sucrose density gradient.

with proplastids and a large amount of broken proplastids. Triton X-100 was added to the assays to reduce the absorbance change brought about by light scattering of the particles. It appeared to stimulate the phosphofructokinase rate at low concentrations but was inhibitory at higher concentrations. This effect was dependent on the Triton X-100 to protein ratio. ATP, F6P and $MgCl_2$ were required for activity. There was also no rate in the absence of the coupling enzymes, but when α-glycerophosphate dehydrogenase was added alone a significant but not maximal rate was observed. This indicates that there is some aldolase in addition to the triose phosphate isomerase in the proplastids. The phosphofructokinase activity in the proplastids was unstable and had to be assayed shortly after the proplastids were isolated from the gradient.

In many of the gradients a small peak of proplastid activity was found which sedimented behind the main proplastid peak (Fig. 1). This may be an artefact of the preparation or there may be two types of proplastid. Electron micrographs of the total proplastid fraction (7) show mitochondria to be

absent, but the proplastids are of two types. Both are of proplastid size but one is more dense staining (7). It is possible that the dense staining organelles are proplastids which are differentiated for fatty acid biosynthesis and should be regarded as differentiated organelles. The less staining organelles are typical proplastids.

Discussion:

Phosphofructokinase and pyruvate kinase are regulatory enzymes in plants and have a key role in glycolysis (9-12). The demonstration of these enzymes and of aldolase and triose phosphate isomerase in the proplastid indicates that the glycolytic pathway operates in this organelle. Since the optimum conditions for assay of the proplastid enzymes have not been determined, it is impossible to indicate their activities relative to those in the cytosol. Also both enzymes appear to be unstable after release from the proplastid. These data, however, indicate that the proplastid may be capable of conversion of sucrose into fatty acids as has been suggested (5,6). The presence of the glycolytic pathway along with the pyruvate dehydrogenase complex would supply the carbon substrates and sufficient ATP and reducing power for fatty biosynthesis. This pathway would also produce cofactors and carbon substrates for terpene biosynthesis which also appears to be a capacity of this organelle (13).

Phosphofructokinase and pyruvate kinase activities also can be demonstrated in the 37,500 x g supernatant indicating that the glycolytic pathway may operate both within the proplastid and in the soluble phase of the cell. Until a complete kinetic analysis has been performed on these enzymes it is impossible to determine if there are isozymes of phosphofructokinase or pyruvate kinase in plants, although the fact that they are located in different parts of the cell would make it a possibility. The possibility exists that glycolysis is regulated in an entirely different manner in two parts of the cell and the flux through the particulate pathway may be different from the flux through the soluble pathway. Pool sizes of the intermediates may also be quite different

in the two locations in the cell. An important aspect of the control of these pathways may be the rate at which substrate and intermediate can cross the proplastid membrane.

This report demonstrates the importance of compartmentation in cellular regulation. It also indicates that the proplastid from the developing castor bean may be independent of the rest of the cell in converting hexoses into fatty acids. A comparison of the kinetics of the soluble and particulate forms of these enzymes is at present under investigation and will be reported elsewhere.

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