Chloroplast and Cytoplasmic Enzymes

THREE DISTINCT ISOENZYMES ASSOCIATED WITH THE REDUCTIVE PENTOSE PHOSPHATE CYCLE

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ABSTRACT

Three pea (Pisum sativum) leaf chloroplast enzymes—triose phosphate isomerase, glyceraldehyde 3-phosphate kinase, and fructose 1,6-diphosphate aldolase—have been separated from the corresponding cytoplasmic enzymes by isoelectric focusing. These three enzymes of the reductive pentose phosphate cycle are therefore distinct proteins, not identical with the analogous enzymes of the Embden-Meyerhof-Parnas pathway.

In green plants the enzymes of the reductive pentose phosphate cycle are located in the chloroplast (8) separated from the enzymes of the cytoplasm by the chloroplast membrane. Three of these enzymes—ribulose-1,5-diP carboxylase, ribulose-5-P kinase and TPN-linked glyceraldehyde-3-P dehydrogenase—are found only in the chloroplast in higher plants (6, 11, 12), and alkaline fructose-1,6-diP phosphatase is a chloroplast enzyme in Euglena (12). Amino acid compositions of spinach chloroplast (1) and whole leaf (presumably cytoplasmic) fructose-1,6-diP aldolases (4) are disparate. No previously reported studies have shown that the remaining reductive pentose phosphate cycle enzymes differ from the corresponding enzymes of the cytoplasm.

Using the technique of isoelectric focusing we have now been able to demonstrate that pea leaf chloroplast fructose-1,6-diP aldolase (ketone 1-phosphate aldehyde, EC 4.1.2.7) is not identical with the cytoplasmic enzyme, as had been previously indicated, and, in addition, that two other chloroplast enzymes—3-P-glyceric acid kinase (ATP-d-3-phosphoglycerate 1-phosphotransferase, EC 2.7.2.3) and triose-P isomerase (d-glyceraldehyde 3-phosphate ketol-isomerase, EC 5.3.1.1)—have apparent isoelectric points which distinguish them from the corresponding cytoplasmic enzymes.

METHODS

Leaves from 10- to 14-day-old pea plants (Pisum sativum, var. Little Marvel) were used in these experiments. Whole leaf extracts were prepared by homogenizing 1 g of leaves in 10 ml of 0.1%, pH 7, glycine in a glass tissue grinder. Chloroplasts, prepared by the method of Cockburn et al. (without isoascorbate) (3), were suspended in 0.1%, pH 7, glycine and released from a

French pressure cell at 10,000 p.s.i. Cytoplasmic extract consisted of the supernatant from the chloroplast preparation after removal of chloroplasts by low speed centrifugation. All extracts were centrifuged for 20 min at 40,000g.

Extracts, containing 5 mg of protein (10 mg in the case of the cytoplasmic aldolase and isomerase) were subjected to electrophoresis in 3-6 amphotolyte for 1 1/2 days at 450 v, 10 C, in a 110-ml LKB isoelectric focusing column set up according to the manufacturer's directions. The cathode was at the bottom of the column. Sorbitol was substituted for sucrose. Seventeen-drop (1-ml) or 25-drop fractions were collected and analyzed for enzyme activity. The pH of individual fractions was measured at 0 C with a Radiometer pH meter.

Protein was precipitated with 80% aqueous acetone and measured with biuret reagent (9). Triose-P isomerase activity was measured as described by Gibbs and Turner (5); 3-P-glyceric acid kinase activity by the method of Bucher except that glycine was omitted (2); and fructose-1,6-diP aldolase activity by the method of Wu and Racker (13). A Gilford 2400 recording spectrophotometer was used to follow optical density change.

Data were plotted for best fit to pH values. No units or divisions are given on the abscissa in the figures. The units used (drops) were consistent within runs but not between runs.

RESULTS AND DISCUSSION

Chloroplast and cytoplasmic aldolases are resolved in the whole leaf extract (Fig. 1). In the experiment shown here chloroplast fragments were removed by 2 hr of centrifugation at 100,000g. Chloroplast extract subjected to only 20 min of centrifugation at 40,000g contained two additional peaks. Following isoelectric focusing the aldolase activity in these peaks could be removed by low speed centrifugation; the aldolase activity remained in the dark green precipitate. Activity was also found in once-washed chloroplast fragments. Apparently under the conditions used in these experiments (breaking chloroplasts in 0.1% glycine) some of the chloroplast aldolase remains bound. It is clear that the soluble chloroplast aldolase is not identical with the soluble cytoplasmic enzyme.

The major 3-P-glyceric acid kinase peak in the whole leaf extract corresponds to the peak in the cytoplasmic extract (Fig. 2). If the chloroplast extract is subjected to high speed centrifugation (2 hr, 100,000g) prior to electrophoresis only the minor peak 57 peak is found. After electrophoresis the activity in the three main chloroplast peaks can be removed by low speed centrifugation. The dark green precipitate has kinase activity.

Likewise, once-washed chloroplast fragments prepared in 0.1% glycine have kinase activity. When extracts are treated with Triton X-100 (3.3%), the chloroplast kinase is solubilized and can be distinguished from Triton-treated cytoplasmic enzyme.
It is apparent that the 3-P-glycerate kinase of the chloroplast is not identical with the cytoplasmic kinase.

The cationic triose-P isomerase peak in the whole leaf extract corresponds to the chloroplast enzyme (Fig. 3), the anionic peak to the cytoplasmic enzyme. Clearly the chloroplast triose-P isomerase is distinct from the cytoplasmic isomerase.

These experiments indicate that triose-P isomerase, 3-P-glyceric acid kinase, and fructose-1,6-diP aldolase of the chloroplast are distinct from cytoplasmic isomerase, kinase, and aldolase. Mitochondria (10) and peroxisomes (14) also contain organelle-specific enzymes not identical with those of the cytoplasm. It is possible that these three chloroplast enzymes occur in multienzyme complexes which have apparent isoelectric points different from those of the single enzyme components. However, one might then expect the chloroplast aldolase, isomerase, and kinase to have the same pl values since, because they catalyze sequential reactions, they might be expected to occur in the same complexes. The observed pl values are quite different. These enzymes are clearly not associated in one multi-enzyme complex following isoelectric focusing. It also seems unlikely that proteolytic enzyme action on cytoplasmic or chloroplast enzymes during extract preparation could account for the differences in the apparent isoelectric points since both cytoplasmic and chloroplast isoenzymes were found in whole leaf extracts.

The differences in the isoelectric points of the chloroplast and cytoplasmic fructose-1,6-diP aldolases, P-glyceric acid kinases, and triose-P isomases probably reflect differences in the amino acid sequences or differences in the conformations of the isoenzymes. It seems remarkable that the corresponding isoenzymes have such similar isoelectric points. Apparently these isoenzymes are very closely related, possibly being either different members of one isoenzyme set or proteins modified in vivo after biosynthesis (or both). Such modification has been suggested by Koida et al. (7) to account for the heterogeneity in subunit structure of rabbit muscle aldolase. Experiments are in progress to determine whether these chloroplast and cytoplasmic enzymes differ significantly in properties related to their function in the green plant.

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LITERATURE CITED


