Chloroplast Aldolase is Controlled by a Nuclear Gene

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ABSTRACT

Variant chloroplast fructose 1,6-diphosphate aldolases were found in Pisum sativum when 10 commercial varieties were examined for electrophoretically distinct species of chloroplast triose phosphate isomerase, phosphoglyceric acid kinase, glyceraldehyde 3-phosphate dehydrogenase, and aldolase. When reciprocal crosses are made, both aldolases appear in individuals in the F1 generation. Backcrossing gives offspring havingaldolases characteristic of the homozygous or of the heterozygous parent; the inheritance is therefore not maternal but Mendelian. Clearly this chloroplast reductive pentose phosphate cycle enzyme is under nuclear gene control in P. sativum.

The nucleus, chloroplast, and mitochondrion of the green leaf contain DNA (6). Since the inheritance of chloroplast characteristics may be either uniparental or Mendelian in higher plants (2), both nuclear and extranuclear DNA must contain genetic information (but not necessarily structural genes) for chloroplast proteins. Recently we demonstrated that three chloroplast reductive pentose phosphate cycle enzymes had isoelectric points which distinguished them from the corresponding cytoplasmic enzymes (1). The experiments reported here were designed to determine whether one of these chloroplast enzymes is under nuclear or extranuclear gene control. The results indicate then in Pisum sativum the isoelectric properties of the chloroplasm enzyme fructose-1,6-diP aldolase (ketose 1-phosphate aldehyde-lyase, EC 4.1.2.7) are inherited in Mendelian fashion and therefore this chloroplast protein is probably coded by nuclear DNA.

Chloroplasts were isolated as previously described (1), lysed by suspension in 3.3% Triton X-100, 44 mM (pH 7) glycine (K+), and centrifuged for 30 min at 20,000g. The supernatant fraction was subjected to electrophoresis in 3–6 ampholyte in an LKB isoelectric focusing column, 25-drop (about 1 ml) fractions were collected, and enzymic activity and pH of each were determined (1).

When 10 inbred strains of P. sativum (Vaughn’s Seed Co., Downers Grove, Ill.) were examined for electrophoretically distinct forms of chloroplast aldolase, triose-P isomerase, 3-P-glyceric acid kinase, and TPN-glyceraldehyde-3-P dehydrogenase, only aldolase variants were found. The apparent isoelectric points of the chloroplast aldolases from varieties Little Marvel and Laxtons Progress differed by 0.15 pH unit (Fig. 1a). When equal quantities of leaves from these two strains were mixed, both

Fig. 1. a: Patterns obtained when chloroplast extracts from Little Marvel and Laxtons Progress varieties are subjected to isoelectric focusing. Data are plotted for best fit to pH values. No units are given on the abscissa. The units used (25-drop fractions) were consistent within runs but not between runs. Open symbols: pH; filled symbols: aldolase activity: ▲: Laxtons Progress; ■: Little Marvel. b: Isoelectric

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chloroplast aldolases could be distinguished. The isoelectric points of the cytoplasmic aldolases from these two strains were not measurably different.

Reciprocal crosses were made. Chloroplast extracts were prepared from approximately 1 g of leaf material from individual F1 plants. The isoelectric focusing patterns obtained (Fig. 1b) were similar to those obtained when leaves from the two varieties were mixed, indicating that both types of chloroplast aldolase occur in the chloroplasts of the heterozygotes. The direction of the cross had no effect on the pattern obtained. These results indicate that chloroplast aldolase is controlled by a Mendelian gene.

To eliminate the possibility that the inheritance of the chloroplast aldolase might be uniparental but not strictly maternal, backcrosses were made to the original paternal parent. The progeny contain either both types of aldolase (Fig. 1c) or the type characteristic of the homozygous parent (Fig. 1d). Of five plants from the cross Little Marvel X (Laxtons Progress/Little Marvel) two contained aldolase typical of Little Marvel and three contained aldolases typical of both varieties. Identical results were obtained with five plants from the cross Laxtons Progress X (Little Marvel/Laxtons Progress) except that the homozygous aldolase type was Laxtons Progress. Clearly in the garden pea chloroplast aldolase is inherited according to Mendelian rules.

The simplest explanation of these results is that the structural gene for the chloroplast enzyme aldolase is in the nucleus and changes in the nucleotide sequence are reflected in changes in the apparent isoelectric point. Peptide mapping of the chloroplast aldolases from these two varieties should allow us to determine whether or not the two aldolases do have different amino acid sequences and are therefore the products of different structural genes.

It is interesting to note that three mitochondrial carbon metabolism enzymes—malic dehydrogenase in maize (3), and isocitric dehydrogenase and β-hydroxybutyric dehydrogenase in Paramecium aurelia (8, 9)—are also under nuclear gene control. The primary structure of yeast cytochrome c is coded by a chromosomal gene (5).

At present no specific proteins are known to be under chloroplast gene control, but chloroplast DNA apparently codes for some of the chloroplast rRNA (4, 7, 10). Experiments of Smillie and Scott (6) indicate that in the alga Euglena, chloroplast aldolase is translated on chloroplast ribosomes. If this is true in higher plants and if chloroplast aldolase is actually coded in the nucleus, then either mRNA or a replicate of nuclear DNA coding for chloroplast aldolase must be transferred to the proplastid during chloroplast formation.

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LITERATURE CITED