Plant NADP-dependent isocitrate dehydrogenases are predominantly localized in the cytosol

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Abstract. The isoenzyme patterns of NADP-isocitrate dehydrogenase (NADP-IDH; EC 1.1.1.42) have been investigated in 15 species of higher plants using dietylaminoethyl ion-exchange chromatography and immunological techniques. The obtained results unambiguously demonstrate that the cytosolic enzyme is the predominant form in leaf extracts of all the surveyed plant species. The chloroplastic isoenzyme, previously reported in pea \( \text{(Pisum sativum L.)} \) leaves (R.D. Chen et al., 1989, Planta 178; 157–163), is a minor form in ferns and dicotyledonous angiosperms and is undetectable in gymnosperms and monocotyledonous angiosperms. Comparison of immunological relatedness suggests that the proteins of cytosolic isoenzymes have been highly conserved in the course of plant evolution. The data support the previously proposed idea that the cytosol is the major site for \( \alpha \)-ketoglutarate production to be used for nitrogen assimilation.

Key words: Chloroplast – Cytosol – Isocitrate dehydrogenase – Isoenzyme pattern – \( \alpha \)-Ketoglutarate

Introduction

In higher plants, \( \alpha \)-ketoglutarate (\( \alpha \)-KG) provides the primary carbon skeleton for the assimilation of reduced inorganic nitrogen into amino acids through the glutamine synthetase/glutamate synthase cycle (Lea and Miflin 1974), and thus represents a key point in the linking of carbon and nitrogen metabolism (Chen and Gadal 1990a). Despite its fundamental importance, it remains uncertain as to which subcellular compartment the \( \alpha \)-KG used for nitrogen assimilation is synthesized. It is generally believed that this keto-acid is derived from the Krebs Cycle where it is produced through the action of mitochondrial NAD-isocitrate dehydrogenase (NAD-IDH; EC 1.1.1.41) (Miflin and Lea 1982; Douce 1985). According to this model the pathway is initiated by the oxidation of citrate and the derived \( \alpha \)-KG then traverses mitochondrial membranes and the chloroplast envelope (Fig. 1). Recently, Chen and Gadal (1990a) proposed an alternative pathway whereby citrate synthesized inside the mitochondria is exported into the cytosol and converted to \( \alpha \)-KG via the enzymes cytosolic aconitase and NADP-isocitrate dehydrogenase (NADP-IDH; EC 1.1.1.42) (Fig. 1). New indirect evidence has accumulated supporting the hypothesis that cytosolic NADP-IDH is the major catalyst for the production of \( \alpha \)-KG required for nitrogen assimilation (Chen and Gadal 1990b; Hanning and Heldt 1993; Fieuw et al. 1995; Scheible et al. 1997).

Isoenzyme patterns and their intracellular localization are crucial clues to understanding isoenzyme function in cellular metabolism. In a previous study combining chromatographic and immunological techniques along with subcellular localization studies, we demonstrated the presence of two different isoforms in pea leaves (Chen et al. 1989). One isoform, NADP-IDH\(_1\), is the predominant form and is localized in the cytosol; the other isoform, NADP-IDH\(_2\), is found in the chloroplast. The two isoforms have been characterized by their differential immunological reaction to antibodies raised against the cytosolic isoenzyme. Also, the two isoforms exhibit differences in their \( K_m \) values for the substrates NADP and isocitrate, as well as differences in pH optimum and heat stability (Chen et al. 1989). Similar results have been reported for tobacco (Galvez et al. 1994). In this paper the experiments were extended to study a wide range of higher plants. The relative quantitative importance of the two isoenzymes is established in a range of species, including ferns, gymnosperms, monocotyledonous and dicotyledonous angiosperms. In addition, the degree of relatedness between NADP-IDH proteins from different plant
species is compared by using an immunological approach.

Materials and methods


Isolation and purification of chloroplasts. Young leaves of oak (Q. pedunculata) and the fern A. filix-femina were harvested in the springtime and analyzed immediately after collection. The chloroplasts were isolated from the leaves and purified on a linear Percoll gradient as described previously (Chen et al. 1989). Chlorophyll content was determined according to Bruinsma (1963).

Enzyme assays. The activity of NADP-IDH was measured spectrophotometrically by monitoring the reduction of NADP at 340 nm, as described previously (Chen et al. 1988). The enzyme activity was determined in 100 mM K-phosphate buffer (pH 7.5) which contained 2-mercaptoethanol. After centrifugation for 30 min at 20 000 g, the soluble proteins were extracted and purified as described above. The resulting supernatant was the chloroplast extract.

Immunological assays. Immunoprecipitation was carried out using the antibodies raised against the cytosolic NADP-IDH from pea roots (Chen et al. 1988). Enzyme samples with the same activities were incubated overnight at 4 °C with various concentrations of antibodies. Enzyme activity was measured in the supernatant after removing the immune complexes by centrifugation at 10 000 g for 5 min (Chen et al. 1988). Immunoprecipitates were then rinsed three times with 50 mM sodium phosphate buffer (pH 7.5), containing 0.18 M NaCl, 1% Triton X-100, and redissolved in Laemmli electrophoresis sample buffer which contained 1% SDS and 10% 2-mercaptoethanol. Electrophoresis was carried out on a 10% SDS/polyacrylamide gel (Laemmli 1970).

Ouchterlony double immunodiffusion was performed on 1.2% agarose plates according to Chen et al. (1988). The plates were incubated at 20 °C overnight. Immunoprecipitated proteins were visualized with Coomassie brilliant blue R-250.

Results

Enzyme activities in plants. The enzyme NADP-IDH was found to be present in the green leaves of all of the 24 plant species studied, including ferns, gymnosperms and angiosperms (Tables 1, 2). Enzyme activity was detectable in all plant tissues or organs: dry seeds, cotyledons, endosperms, roots, stems, host parts of nodules, flowers, etiolated seedlings and cultured cells (results not shown). The NADP-IDH activity varied from 10 to 300 nmol NADPH produced (mg protein)-1 min-1, and was dependent on the organ and species of plant. In general, the activity in roots was higher than that in green leaves with levels as high as 150–250 and 30–90 nmol (mg protein)-1 min-1, respectively.