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Extraction and study of enzymes linked to malate metabolism in tree leaves

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Introduction

It is well established that malate plays a central role in plant cellular metabolism (Lance and Rustin, 1984). Malate is the organic acid which can be accumulated to the highest level in plant cells and its internal concentration can display normal daily fluctuations and can also present permanent or long-term changes with environmental conditions (Lance and Rustin, 1984). Enzymes implicated in synthesis and catabolism of this substrate are widely distributed in the cellular compartments. If these enzymes have become well known in herbaceous plants (Macrae, 1971; Davis and Patil, 1975; Wiskich and Dry, 1985; Artus and Edwards, 1985), very few studies have been made on woody plants (Pitel and Cheliak, 1985, 1986; Weimar and Rothe, 1987). In this study, the extraction of enzymes linked to malate metabolism, particularly those located in mitochondria, was investigated in coniferous and deciduous leaves. Particular attention was devoted to the variations in enzyme capacities during the growing season. Finally, the first steps of purification of these enzymes are presented and discussed.

Materials and Methods

Oak leaves (*Quercus pedonculata*) were collected in a local forest and spruce needles (*Picea abies*) in Donon (Vosges mountains). Twigs were cut and kept at 4°C until use. Based on previous studies, the extraction buffer was sufficiently protective against deleterious compounds, such as phenolic compounds, tannins and terpenoids (Gerant *et al.,* 1988).

Fresh tissues were homogenized in a potter grinder at 4°C in the presence of the extraction buffer and centrifuged at 50 000 x *g* for 30 min. The supernatant was collected and desalted on a Sephadex G-25 column (Pharmacia PD10).

Enzymatic activities were measured according to Hatch (1978) for fumarase (EC 4.2.1.2), Davis and Patil (1975) for NAD-malic enzyme (EC 1.1.1.39), Queiroz (1968) for NADP-malic enzyme (EC 1.1.1.40).

Results

Two of the studied enzymes are considered as mitochondrial markers: fumarase implicated in the tricarboxylic acid cycle and NAD-malic enzyme which generates pyruvate internally. By contrast, NADP-malic enzyme catalyzing the same reaction as NAD-malic enzyme is located in the cytosol.

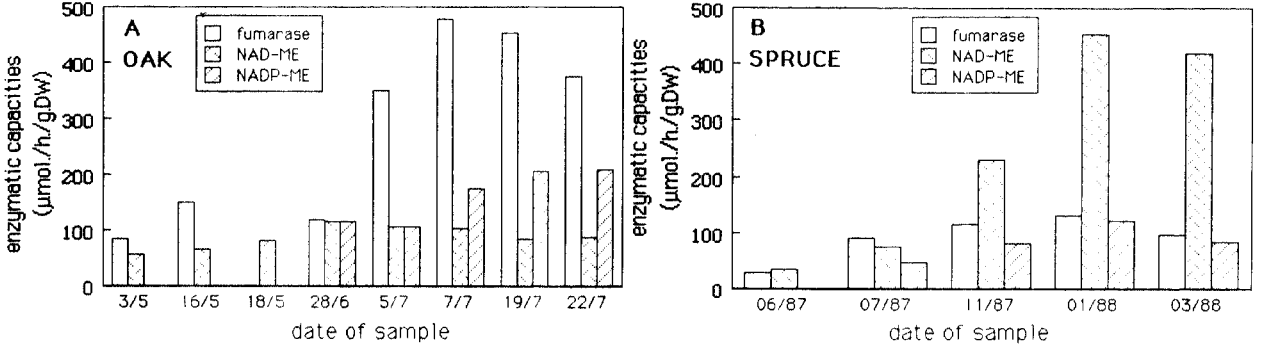

Fig. 1. Seasonal variations in the capacities of enzymes linked to malate metabolism during the growing season in oak leaves (**A**) and spruce needles (**B**).

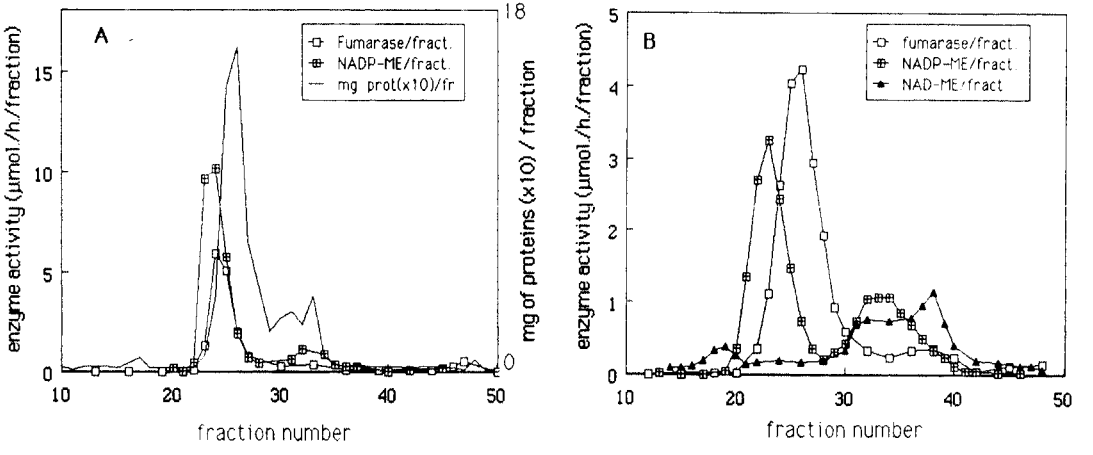

Fig. 2. Elution profiles of fumarase, NAD- and NADP-malic enzymes extracted from oak leaves (**A**) and spruce needles (**B**).

The extraction of these enzymes from oak leaves and spruce needles was performed during the growing season: May 1988–July 1988 for oak (Fig. 1A) and June 1987–March 1988 for spruce (Fig. 1B). Samples were made on the leaves of the first flush for oak leaves (May 1988) and on the needles of 1987 for spruce needles.

The enzymatic capacities increased markedly during the growing season (March) of spruce and during the new flush (June) in oak, except NAD-malic enzyme. Thereafter, they remained constant or slightly decreased during the rest of the time. The highest enzyme capacities were observed for NAD-malic enzyme and fumarase for spruce and oak leaves, respectively.

Actually, no evidence has been presented on the fact that these variations must be related to an increased activity or to the appearance of different isoforms of these enzymes. From these results, it became obvious that the purification of these enzymes was necessary. They were investigated by using an ion–exchange chromatography column. The extracts were applied to a DEAE–Trisacryl column (25 x 1.6 cm) at a flow rate of 1 ml/min. Fifty fractions of 2 ml were collected and tested for enzyme activities.

For oak leaves (Fig. 2A) and spruce needles (Fig. 2B), NADP-malic enzyme was the first to be eluted (fractions 22–24), then fumarase activity was recovered in fractions 24–27. By contrast, NAD-malic enzyme was weakly (spruce) or not detectable (oak); NaCl inhibited this activity.

Discussion

Spruce needles and oak leaves possess potential NAD- and NADP-malic enzyme activities. NAD-malic enzyme has an absolute requirement for Mn^{2+}, whereas NADP-malic enzyme can work without Mg^{2+} (data not shown). These enzymes are eluted in different fractions from an ion–exchange chromatography column. When NAD-malic enzyme is strongly inhibited by NaCl, NADP-malic enzyme is not. From these results, it is evident that the elution buffer used in ion–exchange chromatography was not appropriate for a good separation of the two malic enzymes and to detect the presence of isoforms. The purification study must continue to evaluate the elution buffer and the purification of samples from different periods of tree growth. Seasonal variations of the enzymatic capacities have been shown for both spruce needles and oak leaves. For each species, the specific behavior of the enzymes could reveal differences in energy production in deciduous (oak) and evergreen (spruce) trees. Moreover, it would be of interest to correlate the physiological seasonal changes known to occur in gaseous exchanges (Gerant *et al.,* 1988) with the behavior of the enzymes.

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