

Minireview

NADP-malic enzyme from plants: a ubiquitous enzyme involved in different metabolic pathways

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Abstract NADP-malic enzyme (NADP-ME) is a widely distributed enzyme that catalyzes the oxidative decarboxylation of L-malate. Photosynthetic NADP-MEs are found in C₄ bundle sheath chloroplasts and in the cytosol of CAM plants, while non-photosynthetic NADP-MEs are either plastidic or cytosolic in various plants. We propose a classification of plant NADP-MEs based on their physiological function and localization and we describe recent advances in the characterization of each isoform. Based on the alignment of amino acid sequences of plant NADP-MEs, we identify putative binding sites for the substrates and analyze the phylogenetic origin of each isoform, revealing several features of the molecular evolution of this ubiquitous enzyme. © 2001 Federation of European Biochemical Societies. Published by Elsevier Science B.V. All rights reserved.

Key words: NADP-malic enzyme; C₄ plant; CAM plant; C₃ plant; Molecular evolution

1. Introduction

NADP-malic enzyme (NADP-ME; L-malate:NADP oxidoreductase [oxaloacetate decarboxylating], EC 1.1.1.40) is a widely distributed enzyme involved in different metabolic pathways in prokaryotic and eukaryotic microorganisms. It catalyzes the oxidative decarboxylation of L-malate to yield pyruvate, CO₂ and NADPH in the presence of a bivalent cation. Since the last (and only) review of this enzyme in plants [1], which mostly focused on the catalytic function of the enzyme, a remarkable advance has been made in the characterization of NADP-ME isoforms from a wide variety of sources, and a considerable number of nucleotide sequences of cDNAs or genomic structures are now available. Thus, the purpose of the present review is to classify NADP-ME isoforms from different plant sources and to analyze information on the evolution of this enzyme in plants. The comparison of C₄, C₃ and CAM NADP-MEs made in the present review helps to determine the mechanisms that might have been involved in the evolution of C₄ and CAM photosynthesis.

The best studied isoform of NADP-ME is the one involved in C₄ photosynthesis. In these plants, the enzyme plays a specialized role in bundle sheath chloroplasts, where it pro-

vides CO₂ for fixation by RuBisCO. Another photosynthetic isoform of NADP-ME is found in certain CAM plants, where it performs an analogous role. Nevertheless, both isoforms differ in the subcellular localization, as the C₄-type enzyme is chloroplastic and the CAM-type enzyme is cytosolic. Apart from this specialized role, the enzyme has been found in varied tissues of C₃ plants, where it plays non-photosynthetic roles, and has also different subcellular localizations depending on the species. Non-photosynthetic isoforms of NADP-ME have also been found in tissues of C₄ and CAM plants. In order to classify the NADP-MEs that have been characterized to date, we refer to the isoforms of NADP-ME as follows: C₄₍₁₎-NADP-ME: photosynthetic isoform found in bundle sheath chloroplasts of some C₄ plants; C₄₍₂₎-NADP-ME: non-photosynthetic isoform of NADP-ME found in plastids of C₄ plants; C₄₍₃₎-NADP-ME: cytosolic NADP-ME found in C₄ plants; CAM₍₁₎-NADP-ME: photosynthetic isoform of NADP-ME found in the cytosol of some CAM plants; CAM₍₂₎-NADP-ME: non-photosynthetic isoform of NADP-ME found in the cytosol of CAM plants; C₃₍₁₎-NADP-ME: non-photosynthetic isoform of NADP-ME found in the cytosol of some C₃ plants; and C₃₍₂₎-NADP-ME: non-photosynthetic isoform of NADP-ME present in plastids of some C₃ plants.

2. C₄₍₁₎-NADP-ME

This isoform is found in some C₄ plants such as maize, sugar cane, sorghum and C₄ *Flaveria* species, where it is used for the decarboxylation of malate in bundle sheath chloroplasts; the CO₂ produced is then fixed by RuBisCO.

The best studied C₄₍₁₎-NADP-ME is the enzyme from maize leaves. This enzyme has been purified and its kinetic parameters (Table 1), molecular properties and amino acid residues essential for catalysis have been determined. The corresponding cDNA of this enzyme was the first to be determined among plant NADP-ME [2], and the N-terminal sequence of the mature protein was also determined [3]. Immunolocalization studies have shown that this protein is specifically located in bundle sheath chloroplasts [4] and its expression is regulated by light [3]. As is the case for other bundle sheath-specific proteins, compartmentalization of this enzyme in this type of cells seems to be regulated at both the transcriptional and posttranscriptional levels in maize [5]. Recent studies have also shown a novel physiological function of this enzyme in repairing UV-induced damage [6,7].

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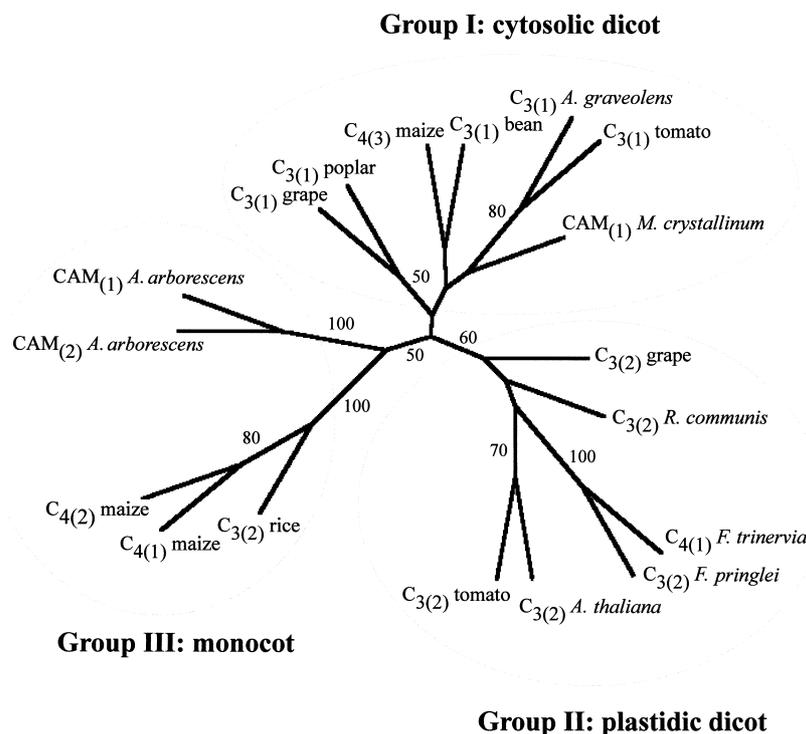


Fig. 1. Phylogenetic tree of plant NADP-ME. The existence of transit peptides in each NADP-ME sequence (listed in Table 2) was predicted by ChloroP1.1 software. Mature proteins were aligned using ClustalW (1.81) and the alignment obtained was modified by visual inspection to exclude the sites containing gaps. The phylogenetic tree was constructed by the neighbor-joining (NJ) method. Statistical significance of each branch of the tree was evaluated by bootstrap analysis by 100 iterations of bootstrap samplings and reconstruction of trees by the NJ method. The topology obtained by this method is shown, along with statistical significance higher than 50%.

NADP-ME, which is constitutively expressed in all organs at low and nearly equal level, was isolated [21]. Although the physiological function of this isoform has not yet been determined, it is probably involved in non-photosynthetic functions.

7. $C_{3(1)}$ -NADP-ME

Different cytosolic NADP-MEs have been characterized in C_3 plants by the isolation of their respective cDNA clones lacking transit peptides. These include the NADP-ME from bean [22], poplar [23], grape berries [24], tomato (GenBank AF001270) and *Apium graveolens* (GenBank AJ132257).

The expression of bean NADP-ME gene has been extensively studied. The promoter of this gene presented *cis*-regulatory elements possibly involved in the activation of the gene by fungal elicitors and UV [25]. Later, fusions of this promoter to the β -glucuronidase reporter gene were analyzed in transgenic tobacco plants, indicating that the promoter was activated by different effectors related to plant defense responses and agents that produce redox perturbations [26]. Direct evidence of the induction of bean NADP-ME by UV-B radiation was also obtained [27]. From these studies, it was concluded that this cytosolic enzyme is involved in plant defense responses, possibly by providing NADPH for the biosynthesis of lignin and flavonoids.

NADP-ME in fruit tissues of tomato and grape berries was implicated in respiration during ripening, providing pyruvate and/or NADPH as a substrate for respiration [1,28]. In earlier work, the enzyme from tomato fruit was found in the non-

mitochondrial fraction and suggested to be cytosolic, although the plastidic localization was not ruled out [29]. Later, both cytosolic and plastidic NADP-ME cDNA clones were identified in both tomato (GenBank AF001270 and AF001269) and grape berries ([24] and GenBank U67426), indicating that more than one NADP-ME is expressed in these species although the specific role of each isoform is not known.

8. $C_{3(2)}$ -NADP-ME

A plastidic isoform of NADP-ME has been identified in C_3 plants by either cloning of the corresponding cDNAs having putative plastidic transit peptides (rice [30], *Flaveria pringlei* [31], tomato (GenBank AF001269), grape berries (GenBank U67426), *Arabidopsis thaliana* (GenBank AC010793) and *Ricinus communis* (GenBank AF262997)), detecting the enzyme in isolated chloroplasts (*Cucurbita pepo* and *Glycine max* [32], *Hydrilla verticillata* [33] and *Egeria densa* [34]) or by in situ immunolocalization studies (wheat [4] and C_3 *Flaveria* species [12]).

The plastidic NADP-ME cDNA clone from the C_3 *F. pringlei* was expressed with similar levels in leaves, stems and roots [31]. Analogous results in C_3 *Flaveria* species were obtained when the same immunoreactive band of NADP-ME was found in stems, roots and leaves [12]. In this way, this isoform seems to be rather constitutive, at least in *Flaveria* C_3 plants.

NADP-ME from wheat stems was purified and its kinetic parameters determined (Table 1) [35]. The enzyme was induced by different effectors which cause lignification of

