Molecular cloning and expression of chloroplast NADP-malate dehydrogenase during Crassulacean acid metabolism induction by salt stress*

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Abstract

A full-length cDNA clone for NADP⁺-dependent malate dehydrogenase (NADP-MDH; EC 1.1.1.82) from the facultative CAM plant, Mesembryanthemum crystallinum has been isolated and characterized. NADP-MDH is responsible for the reduction of oxaloacetate to malate in the chloroplasts of higher plants. The cDNA clone is 1747 bp in size and contains a single open reading frame encoding a 441 amino acid long precursor polypeptide with a predicted molecular weight of 47,949. The predicted, mature NADP-MDH polypeptide sequence from M. crystallinum shares 82.7% to 84% amino acid identity with other known higher plant sequences. Genomic Southern blot analysis of M. crystallinum DNA indicates that MDH is encoded by a small gene family. Steady-state transcript levels for chloroplast NADP-MDH decrease transiently in the leaves after salt stress and then increase to levels greater than two-fold higher than in unstressed plants. Transcript levels in roots are extremely low and are unaffected by salt-stress treatment. In vitro transcription run-on experiments using isolated nuclei from leaf tissue confirm that the accumulation of NADP-MDH transcripts is, at least in part, the result of increased transcription of this gene during salt stress. The salt-stress-induced expression pattern of this enzyme suggests that it may participate in the CO₂ fixation pathway during CAM.

Abbreviations: CAM – Crassulacean acid metabolism; DHAP – dihydroxyacetonephosphate; NADP-MDH – NADP-malate dehydrogenase; NAD-MDH – NAD-malate dehydrogenase; OAA – oxaloacetate; PEPC – phosphoenolpyruvate carboxylase

Introduction

Higher plants contain various NAD- and NADP-dependent isozymes of malate dehydrogenase that catalyze the reduction of oxaloacetate (OAA) to malate in different subcellular locations in the context of several different metabolic pathways. Chloroplasts contain a unique, nuclear encoded, NADP-specific malate dehydrogenase (malate oxidoreductase, EC 1.1.1.82; NADP-MDH) that exhibits light-dependent activation via the thioredoxin/ferredoxin system (Ferte 1986, Scheibe 1987). In C₃ plants, NADP-MDH is essential for balancing reducing equivalents between the chloroplast and the cytoplasm via the malate/oxaloacetate shuttle (Heber 1974). In C₄ plants, an abundant NADP-MDH activity is located exclusively in the mesophyll chloroplasts.

* The nucleotide sequence data reported in this paper will appear in the EMBL, GenBank and DDBJ Nucleotide Sequence Databases under the accession number X63727.
where it functions to reduce oxaloacetate (OAA), the product of primary CO₂ fixation by phosphoenolpyruvate carboxylase (PEPC), to malate which is then transported into adjacent bundle sheath cells. The CO₂ released during the decarboxylation of malate is then refixed by RUBISCO to enter the Calvin–Benson cycle (Hatch and Slack 1969, Ray and Black 1979). The partial amino acid sequence of the C₃ form of NADP-MDH has been determined in pea (Fickenscher et al. 1987, Scheibe et al. 1991). In C₄ plants, NADP-MDH has been extensively studied (Kagawa and Bruno 1988) and complete amino acid sequences have been deduced from cDNA or genomic clones from maize (Metzler et al. 1989) and Sorghum (Cretin et al. 1990, Luchetta et al. 1990, Luchetta et al. 1991).

In Crassulacean acid metabolism (CAM) plants, the exact role of NADP-dependent malate dehydrogenases in photosynthetic CO₂ fixation has not been clearly established. CAM plants separate carboxylation reactions temporally, rather than spatially, as in C₄ plants. The majority of CO₂ uptake and fixation into C₄ acids occurs at night. During the day, plants close their stomata which results in reduced evaporative water loss and draw upon the stored malate as a carbon source for refixation via the Calvin–Benson (C₃) photosynthesis cycle.

In the facultative CAM plant, Mesembryanthemum crystallinum, the ability to perform CAM can be brought about by water stress in the form of high salinity or drought (Winter 1985). During the transition from C₃ to CAM, the activities of both NADP- and NAD-dependent malate dehydrogenase increase (Holturn and Winter 1982, Winter et al. 1982). The chloroplast localized NADP-MDH activity (OAA → MAL) shows a pH optimum of 8.0 and is dependent upon preincubation with dithiothreitol. The extrachloroplastic (mitochondrial and cytosolic) NAD-MDH activity (OAA → MAL) has a pH optimum of 6.5 and does not require dithiothreitol pretreatment. The reduction of OAA to malate during dark CO₂ fixation is thought to be catalyzed primarily by the NAD-dependent form of MDH. Thus, the increased NADP-MDH activity that occurs during CAM induction is difficult to explain because a specific role for this enzyme in CAM has not been described. To better understand the role this important enzyme plays in CAM, a full-length cDNA clone encoding a chloroplast localized form of NADP⁺-dependent malate dehydrogenase (NADP-MDH) from M. crystallinum was isolated and characterized. The expression of this gene increases as a result of transcriptional induction during the transition from C₃ photosynthesis to CAM brought about by salt stress. The elevated expression levels of NADP-dependent MDH in CAM suggest that this enzyme may participate in CO₂ fixation reactions both in the dark and the light.

Materials and methods

Plant material

Common ice plants (Mesembryanthemum crystallinum) were grown from seed in vermiculite irrigated with half-strength Hoagland’s solution in a growth chamber on a 12-h light (23 °C)/12-h dark (18 °C) cycle. Fluorescent lighting provided a photon flux density of 300–350 μE m⁻² s⁻¹. Three-week-old seedlings were transferred to 25 liter black tubs containing Hoagland’s solution and grown hydroponically with constant aeration. Six-week-old plants were stressed by the addition of NaCl to a final concentration of 400 mM. Plant material was harvested at various times after the stress treatment (as indicated in figure legends), frozen in liquid nitrogen and stored at −70 °C until use.

Construction and screening of cDNA libraries

cDNA libraries were constructed from polyA⁺ RNA isolated from leaf tissue from unstressed or plants in λ-Uni-ZAP XR (Stratagene, Inc., La Jolla, CA) according to manufacturer’s instructions. Libraries were prepared from leaf tissue from plants that had been stressed for 30 h and from root tissue from plants that had been stressed for 6 or 30 h. Libraries were screened using a partial cDNA clone for NADP-MDH kindly provided by Dr. T. Nelson (Yale) by three rounds of plaque hybridization (Benton and Davis 1977). Positive λ Uni-ZAP-XR clones