Plant Physiol. (1995) 107: 1009–1010

Plant Gene Register

Cloning of a Full-Length cDNA for Malic Enzyme (EC 1.1.1.40) from Grape Berries

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Malic enzyme (EC 1.1.1.40, $NADP^+$, decarboxylating) catalyzes the oxidative decarboxylation of malic acid to give pyruvate and CO_2. The enzyme utilizes $NADP^+$ as its electron acceptor and requires a divalent cation such as Mg^{2+} or Mn^{2+}. Other forms of malic enzyme (EC 1.1.1.38 and EC 1.1.1.39) are distinguished by their distribution, specificity for NAD^+ or $NADP^+$, and ability to decarboxylate oxaloacetate (Edwards and Andero, 1992). Malic enzyme plays important roles in respiration and photosynthesis in plants. It is found in leaves, seeds, roots, etiolated tissue, and fruit (Edwards and Andero, 1992). In fruit tissue, malic enzyme is a key participant in respiration during ripening (Ruffner et al., 1984; Goodenough et al., 1985).

Malic enzyme purified from grape (*Vitis vinifera*) berries is similar to those purified from other sources. It has a subunit molecular mass of 63 kD and is thought to be located in the cytosol, where it provides pyruvate for respiration during ripening (Franke and Adams, 1992). cDNA sequence information is available for malic enzyme from several plant sources including bean, poplar, maize, *Flaveria trinerva*, and tomato. Interestingly, the deduced amino acid sequences show a high degree of similarity except for the N termini of the *Flaveria* and maize sequences, which are both plastidic and contain transit peptides (Rothermel and Nelson, 1989; Börsch and Westhoff, 1990).

As part of a study of malic acid metabolism during grape ripening, we cloned and sequenced a full-length grape malic enzyme cDNA. A library in λZAP II was constructed from mRNA isolated from Thompson Seedless berries (7.8° Brix) and screened with a partial, heterologous tomato cDNA previously isolated in our laboratory (K.E. Franke and D.O. Adams, unpublished data). One of seven putative clones was found to be a full-length clone for grape malic enzyme by sequencing and comparing the deduced amino acid sequence with the sequence DIRDGASVLDLDP-KATVGGGVEDLYGED, which was obtained by N-terminal sequencing of purified grape malic enzyme (Table I). The deduced amino acid sequence was also compared with the other published malic enzyme sequences and found to be highly homologous.

The full-length cDNA is 2211 nucleotides long, including 37 bp of 5′ untranslated sequence and 397 bp of 3′ flanking sequence. An open reading frame of 1775 bases begins at position 38 and encodes a polypeptide of 591 amino acids with an estimated molecular mass of 64.3 kD. The deduced sequence from the cDNA has six residues at the N terminus that do not appear in the amino acid sequence obtained from the purified protein. This may be the result of proteolytic cleavage during protein purification.

When the deduced amino acid sequence was compared to the other reported plant malic enzyme sequences, we found it to be 92.7% similar to the poplar (*Populus deltoides*) enzyme, 90.3% similar to bean (*Phaseolus vulgaris*), 89.7% similar to *F. trinerva*, and 85.4% similar to maize. The similarity scores are lower for corn and *Flaveria* because of the presence of signal peptides in these proteins.

Table I. *Characteristics of a cDNA encoding malic enzyme from grape*

Organism:	
	Grape (*Vitis vinifera* cv Thompson seedless 2A).
Enzyme; Function:	
	Malate dehydrogenase (decarboxylating) ($NADP^+$); EC 1.1.1.40; respiration of stored malate during ripening.
Source of Clone:	
	cDNA library from pre-veraison fruit in λZAP II (Stratagene).
Method of Identification:	
	Library was screened with a partial tomato malic enzyme cDNA.
Sequence Confirmation:	
	Putative malic enzyme clones were identified by comparing the deduced amino acid sequence with the sequence from the N terminus of purified grape malic enzyme, DIRDGASVLDLDP-KATVGGGVEDLYGED, and by comparing with the deduced amino acid sequence with reported plant malic enzyme sequences.
Sequence Features:	
	2211 nucleotides, including 397 bp of 5′ flanking sequence and 37 bp of the 3′ untranslated region. An open reading frame of 1775 bp starting at position 38.
Protein Structural Features:	
	Deduced amino acid sequence of 591 residues; molecular mass 64.3 kD.

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Received August 15, 1994; accepted August 29, 1994.
Copyright Clearance Center: 0032–0889/95/107/1009/02.
The GenBank accession number for the sequence reported in this article is L34836.

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