ABB

Malate dehydrogenase in plants: Its genetics, structure, localization and use as a marker

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ABSTRACT

This article is an overview of literature data on the structure, properties, functions and genetic control of the enzyme malate dehydrogenase (MDH) in plants. In most of the plant entities studied, this enzyme is highly polymorphic, which means that malate dehydrogenase has multiple molecular forms. It has been found that MDH polymorphism in each species is genetically determined by several loci with multiple alleles. A readily identifiable phenotypic manifestation and a high activity of malate dehydrogenase in diverse organs and tissues make this enzyme a reliable and convenient genetic marker, which can effectively be used in special, ecological and population genetics.
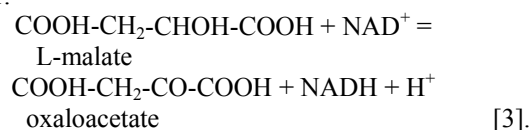
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1. INTRODUCTION

The discovery of isozymes [1,2] has provided researchers with simple and reliable marker traits that can be used for studying the widest class of biological phenomena, and so all-new possibilities for marking genes and genetic systems have been opened up. An advantage of isozyme markers is that they themselves perform functions in metabolism, and so these functions can now be identified using not only genes or genomic loci, but also using isozyme markers. Isozymes can be used both as markers for phylogenetic analysis and identification of introgressions and as markers for detection of species differentiation, cultivar identification and analysis of populations. The most prominent advantages of isozymes as genetic markers are their codominant inheritance, readily identifiable phenotypic manifestations and that isozymes themselves are easily identifiable. One of the most extensively studied isozyme systems are dehydrogenases, including malate dehydrogenase.

NAD-dependent malate dehydrogenase (MDH, L-malate: NAD oxidoreductase; EC 1.1.1.37) is an enzyme very commonly occurring in animals, plants and microorganisms. MDH catalyzes the oxidation (dehydration) of L-malate to oxaloacetate in the presence of NAD+ as a cofactor:

$$COOH\text{-}CH_2\text{-}CHOH\text{-}COOH + NAD^+ =$$
L-malate
$$COOH\text{-}CH_2\text{-}CO\text{-}COOH + NADH + H^+$$
oxaloacetate [3].

MDH is a well-studied enzyme. In the cell, malate dehydrogenase exists in various molecular forms. Two MDH forms have been found in most of the plant objects studied: mitochondrial MDH (m-MDH) and cytoplasmic MDH (s-MDH) [4-8]. Mitochondrial MDH functions as part of the Krebs cycle, while soluble (cytoplasmic) MDH can be involved in acid metabolism in plant tissues, in autotrophic carbon dioxide fixation in higher plants and in more other metabolic pathways [9-11]. In plant tissues, MDH has additionally been found in glyoxysomes [6,7, 12,13], peroxisomes [14,15] and microsomes [16-18]. The microbody form of MDH is involved in photorespiration and the glyoxylate cycle [19].

2. THE STRUCTURE AND PROPERTIES

Studies of the structure of the malate dehydrogenase molecule in maize, celery, rice, avocado and other plants have demonstrated that each MDH molecule consists of two subunits, that is, based on its quaternary structure, MDH is a dimer [6,20-23].

Malate dehydrogenase is best studied in maize [5,11, 24-31]. In that plant, MDH is located in chloroplasts [11], microbodies, mitochondria and the cytoplasm [10]. Much bigger differences in primary structure are found between cytoplasmic and mitochondrial form in the same plant than between mitochondrial forms of MDH in different genera [10,31,32]. This conclusion was reached by comparing the reactions of different MDH isozymes to antisera each raised against the product of each homozygous *Mdh* locus. The antiserum raised against purified mitochondrial MDH was cross-reactive with microbody MDH; however, it was not reactive with soluble MDH [10, 31,32].

Studies of the biochemical properties of mitochondrial and cytoplasmic MDH in maize have demonstrated that these two forms have different thermal stability, different optimum pH values, different kinetic properties and other differences [7,8]. Isoelectric focusing has identified differences between cytoplasmic and mitochondrial MDH isoforms in grape. The cytoplasmic forms have pH values ranging from 4.0 to 5.5, while the mitochondrial forms are less acidic (pH 5.5 - 7.5) and have a molecular weight of 80000 Da [33]. Differences have also been found between the respective molecular properties of glyoxysomal and mitochondrial MDH in watermelon cotyledons [13,34]. In that plant, the molecular weight of glyoxysomal MDH is 67,000 Da, while that of mitochondrial MDH is 74,000 Da. At high MDH concentrations, it has been observed that the glyoxysomal enzyme aggregates, while the mitochondrial enzyme does not. These enzymes differ in isoelectric points, thermal stability, optimum pH values and other characteristics [13,34].

As has been demonstrated by exposing MDH in maize to various inhibitors, mitochondrial MDH isozymes are synthesized on cytoplasmic ribosomes [24]. It has been reported that the mitochondrial and glyoxysomal forms of MDH are synthesized as higher molecular weight of predecessors, which, as they are transported to organelles, lose parts of their polypeptide chains: a 8000-Da fragment is cleaved off each glyoxysomal predecessor and a 3300-Da fragment is cleaved off each mitochondrial predecessor, to lead to the observed functional subunits. Cytoplasmic MDH has not been observed to be synthesized as a predecessor [35].

Malate dehydrogenase has also been studied in other plant entities: amaranth [36], avocado [22,37], barley [38-40], beans [41,42], cedar [43], celery [21,23], cereus [44], citrus [45,46], *Corydalis soli da* [47], *Cynosurus cristatus* [48], eucalyptus [49], oats [50], peach [51], pecan [52], pepper [53], petunia [54], pine [55-58], prickly pear [59], rice [20], soybean [60], wheat [38,61,62].

Not only do the multiple molecular forms of MDH in plants have differences as indicated, but they also have things in common. In most of the entities studied, the molecular weight of MDH ranges from 60,000 Da to 70,000 Da. In starch gel electrophoresis, the malate dehydrogenase forms that are contained in organelles move from anode to cathode in an ordered manner: the closest to the cathode end is microbody MDH, the second closest is mitochondrial MDH and the farthest away is cytoplasmic MDH [63]. Another point in common has been revealed by immunological studies: in most eukaryotes, mitochondrial MDH has similar structural properties, which suggests a high degree of evolutionary preservation [31]. Based on the above considerations and considering the fact that different and highly specific forms of this en-

zyme are present in almost each main subcellular component, it may be concluded that MDH is a unique model enzyme, which can be used in studying crosstalk between organelles [11].

3. THE GENETIC CONTROL OF MALATE DEHYDROGENASE

The polymorphism and specificity of multiple molecular forms of NAD-MDH are typical of both different tissues of a single plant and different compartments of a single cell. Additionally, the polymorphism of molecular forms has been revealed for each compartment. Seven mitochondrial and six cytoplasmic MDH isoforms have been identified in maize (all resolved electrophoretically) [27, 28,30,32,64] three soluble and three mitochondrial MDH isoforms, in soybean [60]; four soluble and five mitochondrial MDH isoforms, in Arabidopsis [65]; two soluble and two mitochondrial MDH isoforms, in pine [57]; four mitochondrial and three microsomal MDH isoforms, in sugar beet [16-18] and so on. As is known, the molecular heterogeneity of enzyme forms either is determined genetically or is the result of epigenetic changes.

The first evidence of the genetic origin of MDH polymorphism has been found in maize by Longo and Scandalios [6]. Reciprocal hybrids were obtained by crosses between two inbred maize lines, 59 and Oh 51A, which carry different mitochondrial forms of MDH. The isozyme spectra of these hybrids were identical and each contained both parental and hybrid forms of MDH. Segregation of F_2 plants into two parental and one hybrid phenotype was Mendelian.

To date, malate dehydrogenase isozymes have been better studied in maize than in any other plant. There are two models for describing the genetic control of MDH in maize. According to Goodman's and the associates' model, mitochondrial MDH is encoded by three structural genes (*Mdh*1, *Mdh*2 and *Mdh*3), while cytoplasmic MDH is encoded by two genes (*Mdh*4 and *Mdh*5). When mitochondrial forms of MDH interact, they form intergenic and interallelic heterodimers. The isoforms that are located in different subcellular organelles do not form hybrid dimers [31,32]. The structural genes for MDH are in a variety of linkage groups: *Mdh*1 is localized to chromosome 8; *Mdh*2, to the distal end of the long arm of chromosome 6; and *Mdh*3, to the distal end of the long arm of chromosome 3 [31,64]. McMillin and Scandalios suggest a different interpretation [27,28]. These models use different nomenclatures for and different substantiations of separate MDH variants (**Table 1**). One of the reasons the isozyme spectra are interpreted differently is the use of different buffer systems in electrophoresis. The use of Tris-citrate buffer (pH 7.0) (which is McMillin's

and Scandalios' choice) results in a relatively weak separation of isozymes, which, in Goodman's and Stuber's opinion [30], may lead to misinterpretation.

Table 1 provides a listing of plant objects, for which the genetic control of malate dehydrogenase has been studied. The inheritance of MDH in maize, eucalypt, sugar beet was determined by a classical method of genetic analysis including hybridization of contrasting forms and analysis of segregation in F_2 and backcross plants. In wheat, the inheritance of the isozymes was determined by comparing their spectra in normal and aneuploid forms. For some plants (pine, cedar, peach, to mention a few), analyses were performed for the correspondence between the frequencies of phenotypic classes in panmictic populations and the theoretical frequencies calculated by the Hardy-Weinberg equation and isozyme spectra in somatic tissue and pollen were compared. It was found that, in addition to the structural loci that control MDH synthesis, maize carries the *Mmm* gene, pine carries the *Mdhm* gene, and, cedar carries the *Mdh*1*m* gene, each modifying both electrophoretic mobility and enzymatic activity [30,43, 55]. In addition to maize, the chromosomal localization of *Mdh* loci has been determined in barley, wheat and petunia [30,32,38-39,54,62,66].

Table 1. Genetic control of NAD-dependent malate dehydrogenase in plants.

Crop	Locus	Alleles	Chromo some	Method of study	Buffer	Tissue	References
Amaranth (*Amaranthus* L.)	*Mdh*1 *Mdh*2	*Mdh*2-*F* *Mdh*2-*N* *Mdh*2-*S*	- -	starch gel	triscitrate, pH = 7.0	leaves, seedlings	Yudina *et al.*, 2005 [36]
Avena barbata	*Mdh*1	*Mdn*1	-	starch gel	histidine, triscitrate, pH = 7.0	leaves, seedlings	Price and Kahler, 1983 [50]
Avocado (*Persea americana*)	*Mdh*1	*F* *S*	-	starch gel	triscitrate, pH = 6.9	fruit, leaves, pollen	Torres, 1983 [22], Torres, 1984 [37]
Beta vulgaris L.	*Mor*1 *Mor*2 *Mor*3	*Mor*1-*N* *Mor*1-*P* *Mor*2-*F* *Mor*2-*S* -	- - -	starch gel	triscitrate, pH = 7.0	leaves, seedlings	Tarasova, 1988 [16]
Calocedrus deccurens	*Mdh*1 *Mdh*2 *Mmdh*1	*Mdh*1-0.60 *Mdh*1-1.00 *Mdh*1-1.07 *Mdh*1-1.39 *Mdh*1-1.46 *Mdh*1-1.54 *Mdh*1-1.77 *Mdh*2-0.11 *Mdh*2-1.00 *Mdh*2-2.22	- - -	starch gel	morpholincitrate, pH = 6.1	makrogametofit	Harry, 1986 [43]
Caria illinoinen	*Mdh*1	-	-	starch gel	morpholincitrate, pH = 6.1	leaves, pollen, stems	Marquard and Scorpenske, 1989 [52]
Celery (*Apium graveolens*)	*Mdh*3	*MDH*1 *MDH*2 -*F* -*S*	- - - -	starch gel	histidine, triscitrate, pH = 7.0	seedlings	Orton, 1983 [21], Arus and Orton, 1984 [23]
Cereus peruvianus		*sMDH*1 *sMDH*2 *sMDH*3 *sMDH*4 *mMDH*1 *mMDH*2 *mMDH*3 *mMDH*4 *mMDH*5 *gMDH*1	- - - - - - - - - -	starch gel	triscitrate	callus, seedlings	Machado *et al.*, 1993 [44]
Citrus and Pancirus trifoliata	*Mdh*1 *Mdh*2 *Mdh*3 *Mdh*4	*F* *S* *F* *S*	- - - -	starch gel	-	nucellus	Torres *et al.*, 1978 [45], Torres *et al.*, 1982 [46]

Continued

Corydalis solida	MDH	*ff ss*	-	PAGE	-	seeds, shoots	Nagy *et al.*, 1980 [47]
Cynosurus cristatus	Mdh1 Mdh2	F S F S	- -	DE	-	shoots	Ennos,1986 [48]
Eucalyptus L'Herit	Mdh1 Mdh2	1 2 1 2 3 4	- -	starch gel	histidincitrate, pH = 8.0	seedlings	Moran and Bell, 1983 [49]
Glycine max	Mdh1 Mdh2	1 2 3 4 5 6	- -	PAGE	histidine, pH = 6.0	cotyledons, young leaves	Kiang and Gorman, 1983 [60]
Hordeum vulgare	Mdh1 Mdh2	$-1 -2$ $-1 -2$	5 3	starch gel	histidincitrate, pH = 8.0	embryos, shoots	Brown and Munday, 1982 [39], Powling *et al.*, 1981 [38]
Pepper (*Capsicum* L.)	Mdh1	-	-	starch gel	lithium	leaves	McLeod *et al.*, 1983 [53]
Persica vulgaris	Mdh1 Mdh2	- -	- -	starch gel	histidine, triscitrate, pH = 7.0	leaves	Arulsekar *et al.*, 1986 [51]
Petunia hybrida	Mdh1	1 2	7	starch gel	triscitrate, pH = 7.0	flowers, leaves, roots	Wijsman, 1983 [54]
Pinus attenuata	Mdh1 Mdh2 Mdh3 Mdh4 Mdhm	- - - - -	-	starch gel	morpholincitrate, pH = 6.1	female gametophyte	Strauss and Conkle, 1986 [55]
Pinus banksa	Mdh3 Mdh4	1 2 1 2	- -	starch gel	morpholincitrate, pH = 6.1	female gametophyte, pH = 6.1	Cheliak *et al.*, 1984 [56]
Pinus rigida	Mdh1 Mdh2	-F -S -F -S	- -	starch gel	morpholincitrate, pH = 6.1	female gametophyte	Guries and Ledic, 1978 [57]; O'Malley *et al.*, 1986 [58]
Phaseolus lunatus	Mdh1 Mdh2 Mdh3	$Mdh1^{84}$ $Mdh1^{100}$ $Mdh2^{100}$ $Mdh2^{140}$ $Mdh3^{100}$ $Mdh3^{104}$	- - -	starch gel	0.1 M trismalate, pH = 7.2	cotyledons, leaves, roots	Zoro *et al.*, 1999 [42]
Rice (*Oryza sativa*)	Mdh-A B C	-1 -2 -3	-	starch gel	borate	coleop tile, leaves, roots	Endo and Morishma, 1983 [20]
Triticum aestivum	Mdh	A B	1B	PAGE	-	seedlings	Powling *et al.*, 1981[38]
Triticum aestivum	MDH1 MDH2	1 3 1 3	- -	PAGE, starch gel	histidine, triscitrate, pH = 7.0	seedlings	Benito and Salinas, 1983 [62]
Zea mays	Mdh1 Mdh2 Mdh3 Mdh4 Mdh5 Mmm	-A1 -A6 $-$A10.5 -A null -B3 -B6 -B null -C16 -C18 -D8 -D12 -D14.5 -D null -E12 -E15 -E null -E null	8 6L 3L 1L 5S 1L	starch gel	histidine, pH = 5.7; pH = 5.0	endosperm, roots, scutella, seedlings	Goodman and Stuber, 1983 [30]; Goodman *et al.*, 1980 [30], Newton and Schwartz, 1980 [64]
Zea mays	mMdh1 mMdh2 mMdh3 mMdh4 sMdh1 sMdh2	-m8 -m10 -m5 -m3 -m0 -m8 -m7 -m3 -s5 -s1 -s4a	- - - - - -	starch gel	triscitrate, pH = 7.0	endosperm, pollen, scutella, seedlings	McMillin and Scandalios, 1981 [28]

Note: PAGE (polyacrylamide gel electrophoresis), DE (disc electrophoresis), (-) (data absent).

4. THE EXPRESSION OF LOCI CONTROLLING MDH IN PLANTS

Changes in isozyme spectra that occur as tissue undergoes growth and differentiation have been described in many plants and for different enzyme systems. The same is true of malate dehydrogenase spectra. In maize, cytoplasmic and mitochondrial MDH forms are active in roots, seedlings, endosperm, corymbs, hypocotyls, coleoptiles, leaves and pollen [6,31,33,64], while microbody MDH is active in endosperm and scutellum [6,10,24,25,31]. An increase in the number of MDH isozymes during caryopsis maturation has been demonstrated [11]. A *de novo* synthesis of MDH isozymes has been detected in developing maize seedlings [67]. The disappearance and emergence of separate MDH forms have been observed in developing wheat plants [66] and *Arabidopsis thaliana* [65].

The organ specificity of NAD-MDH in sugar beet has been studied in detail. Isozyme spectra have been observed in dry seeds, seedlings, leaves, stems, roots, bracts, buds, ovaries and pollen [68]. The specificity is characterized both by the number of isozymes and their relative activity in the organs studied (seeds, seedlings, leaves, bracts, buds, ovaries and ovules) and by a total lack of isozymes on the electropherograms (stems, roots). However, it should be noted that the lack of some MDH isozymes on electropherograms does not necessarily imply that the corresponding locus is inactive in those organs: it is possible that the isozymes are present at below the minimum detectable amount. The genetic determination of MDH isozymes in sugar beet allows tissue specificity to be interpreted as the result of the differential activity of the genes that control malate dehydrogenase at different stages of ontogenesis. It is possible that the genetic information coming from two different alleles is not implemented simultaneously. For example, in the leaves of sugar beet line P-83-3 plants, which are all heterozygous for the *Mor*1 locus with the *Mor*1-*N* and *Mor*1-*P* alleles, three MDH isozymes (nn, np and pp) should be revealed, which is as expected of the spectrum of a dimeric enzyme in heterozygotes. However, in young leaves, the *Mor*1-*N* allele is not activated, while later on, in normally developed leaves, all the three isozymes will appear. Asynchrony in the activation of parental alleles in heterozygotes during ontogenesis has also been reported [69].

Thus, malate dehydrogenase represents an example of how useful isozymes can be for studying regulatory systems in eukaryotes. Genetic analysis allows both structural and regulatory genes to be comparatively easily localized. Collectively, all this provides an opportunity to obtain reliable information on the regulation of gene activity during ontogenesis and to gain a better insight into cell differentiation pathways in developing organisms [69-71].

5. THE USE OF MALATE DEHYDROGENASE ISOZYMES IN PLANT POPULATION GENETIC STUDIES

Isozyme spectra can serve the basis for cultivar identification and registration purposes, for documenting the genetic resources of cultivated plants and their wild relatives with a view to their consideration, preservation and use in breeding. The results of such work depend upon how well the genetic control of the enzymes involved is studied.

Thus, due to fundamental efforts by Goodman and the colleagues [33], 25,000 maize plants in 125 races and synthetic cultivars from South and North America have been analyzed; inbred lines and their F_1 and F_2 hybrids, synthetic and various commercial and experimental hybrids have been studied; and a catalog of genotypes containing various combinations of allelic variants of malate dehydrogenase, has been developed. Considerable differentiation has been found in the frequency of different MDH genotypes among the objects studied. For example, the *Mdh*1-*A*6, *Mdh*2-*B*6 and *Mdh*3-*C*16 genotypes have been found in 21 lines; *Mdh*1-*A*6, *Mdh*2-*B*3, *Mdh*3-*C*16 have been found in 12 lines; while there are many MDH genotypes each of which is confined to a single line or hybrid. It is possible that this differentiation is because some lines have common origin, while the others are of unique origin. However, it is not excluded that the commonest genotypes have advantages over the others, and so the former have had selective preferences. This malate dehydrogenase example provides further support to the view that the use of loci with multiple alleles is extremely beneficial for breeding, ecological genetics and population genetics.

NAD-dependent malate dehydrogenase isozymes have also been used for identification of inbred sugar beet lines in the collection maintained in the Laboratory of Plant Population Genetics of the Institute of Cytology and Genetics (Novosibirsk, Russia). A total of 394 lines have been analyzed, and to each line the corresponding genotype has been assigned based on the isozyme spectrum of malate dehydrogenase. The frequencies of nine genotypic classes, normally observed when the character being studied is controlled by two loci with two alleles at each locus, have been determined [16,72]. The respective frequencies of the genotype classes for these two alleles were strongly different. The ratio of genotypic classes was 285*NN*:93*NP*:16*PP* at the *Mor*1 locus and 238*SS*:136*FS*: 20*FF* at the *Mor*2 locus. The structure of wild and cultivated amaranth populations has been studied in terms of the frequencies of the alleles at the loci controlling isozymes, including those at the *Mdh*1 locus [73]. Alongside other isozymes, malate dehydrogenase was used for

identification of 57 apple tree cultivars [74]. One of the polymorphic MDH loci has been used in breeding programs with 290 peach cultivars surveyed [51].The genetic structure of a collection of 570 cedar trees from three California regions has been described in terms of the frequencies of multiple alleles at two loci encoding malate dehydrogenase: *Mdh*1 with seven alleles and *Mdh*2 with three alleles [43].

All the above findings indicate that a high level of malate dehydrogenase activity in diverse organs and tissues, and the presence of loci with multiple alleles, codominant inheritance and a clear phenotypic manifestation of isozyme spectra. Therefore, this enzyme is a reliable and useful genetic marker in population and genetic studies.

6. CONCLUSION

The large number of species with MDH studied and used in experiments aimed at describing and characterizing various processes provides evidence that this enzyme represents a highly efficient genetic and physiological marker. In molecular biology, it is important that enzymes with their isozyme systems and protein cofactors represent extremely important biological and genetic markers with many promising uses for theory and practice.

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