Minireview

Malate Dehydrogenase: Distribution, Function and Properties

R. A. MUSRATI, M. KOLLÁROVÁ, N. MERNIK AND D. MIKULÁŠOVÁ

Department of Biochemistry, Faculty of Natural Sciences, Comenius University, Mlynská dolina CH-1, 842 15 Bratislava, Slovak Republic

Abstract. Malate dehydrogenase (MDH) (EC 1.1.1.37) catalyzes the conversion of oxaloacetate and malate. This reaction is important in cellular metabolism, and it is coupled with easily detectable cofactor oxidation/reduction. It is a rather ubiquitous enzyme, for which several isoforms have been identified, differing in their subcellular localization and their specifity for the cofactor NAD or NADP. The nucleotide binding characteristics can be altered by a single amino acid change. Multiple amino acid sequence alignments of MDH show that there is a low degree of primary structural similarity, apart from several positions crucial for catalysis, cofactor binding and the subunit interface. Despite the low amino acids sequence identity their 3-dimensional structures are very similar. MDH is a group of multimeric enzymes consisting of identical subunits usually organized as either dimer or tetramers with subunit molecular weights of 30–35 kDa. MDH has been isolated from different sources including archaea, eubacteria, fungi, plant and mammals.

Key words: Malate dehydrogenase — Isoenzymes — Protein structure

Malate dehydrogenase is a widely distributed enzyme. The preferred substrate for MDH is oxaloacetate (Nicholls et al. 1992). It plays a crucial role in many important metabolic pathway including the tricarboxylic acid cycle, glyoxylate bypass, amino acid synthesis, gluconeogenesis and facilitation of exchange of metabolites between cytoplasm and subcellular organelles.

MDH is a multimeric enzyme consisting of identical subunits usually organized as either dimers or tetramers with subunit molecular weights of 30–35 kDa. MDH from *Nitzschia alba* is composed of eight identical subunits. It is the only octameric MDH so far reported (Yueh et al. 1989). Each subunit of MDH contains a dinucleotide-binding domain which is similar in sequence and structure to that of

Correspondence to: Marta Kollárová, Department of Biochemistry, Faculty of Natural Sciences, Comenius University, Mlynská dolina CH-1, 842 15 Bratislava, Slovakia
E-mail: kollarm@fns.uniba.sk

other nicotinamide-nucleotide dependent enzymes. X-ray structures of MDH show the bound cofactor to be in an extended conformation (Hill et al. 1972; Birktoft et al. 1989b), and similar to the conformation of cofactor bound to lactate dehydrogenase (LDH) (Piontek et al. 1990).

Malate dehydrogenase isoenzymes can serve as a model system for studying the evolution, protein sorting to different cell compartments, and for comparison of MDH isoenzymes.

Eukaryotic tissue contains multiple forms of MDH involved in different metabolic pathways and located in different subcellular compartments. MDH in eukaryotic organisms is found in microbodies such as glyoxysomes and peroxisomes, in the mitochondria, in the cytoplasm and in chloroplasts. All MDHs are NAD-dependent except the chloroplastic enzyme which requires NADP as a cofactor.

Escherichia coli has only one form of MDH (Hall et al. 1992). Its MDH is most similar to eukaryotic mitochondrial MDH which has 59% sequence identity and a similar tertiary structure. Two isoenzymes of MDH, mitochondrial and cytoplasmic, are present in mammalian cells. In higher plants, there are three NAD-dependent forms located in the cytoplasm, the mitochondria and the microbodies, and one NADP-dependent form present in chloroplasts (Poeydomenge et al. 1995). The different isoenzymes are encoded in genes of the nucleus, and they are synthesized on cytoplasmic ribosomes and imported to their respective organelles.

In principle, different isoenzymes can be derived from unrelated genes, from genes in the same family sharing a common ancestral gene or by alternative RNA splicing and posttranslational modification from a single gene.

The MDH amino acid sequences show divergence into two main phylogenetic groups of closely related enzymes – cytoplasmic and mitochondrial MDHs. Some of the most distanly related isoenzymes of MDH are found compartmentalized in different subcellular organelles of the same cell types (Goward and Nicholls 1994). The archaebacterial sequence of *Haloarcula morismortui* (Cendrin et al. 1993) suggests a link between the evolution of MDHs and lactate dehydrogenases (LDHs). An explanation of the difference between MDHs from organelles and those from the cytosol is that a common ancestral *mdh* gene may have been duplicated before invasion of primordial eukaryotes by bacteria to produce mitochondria according to the probable endosymbiotic origin of these organelles (McAlister-Henn et al. 1987).

Catalysis

The active site of MDH consists of a predominantly "hydrophobic vacuole", which contains binding sites for the substrate and the nicotinamide ring of the coenzyme. Upon formation of the enzyme:coenzyme:substrate ternary complex there is a protein conformational change in which an external loop closes over the active-site vacuole. In addition, other functionally important residues are brought into close

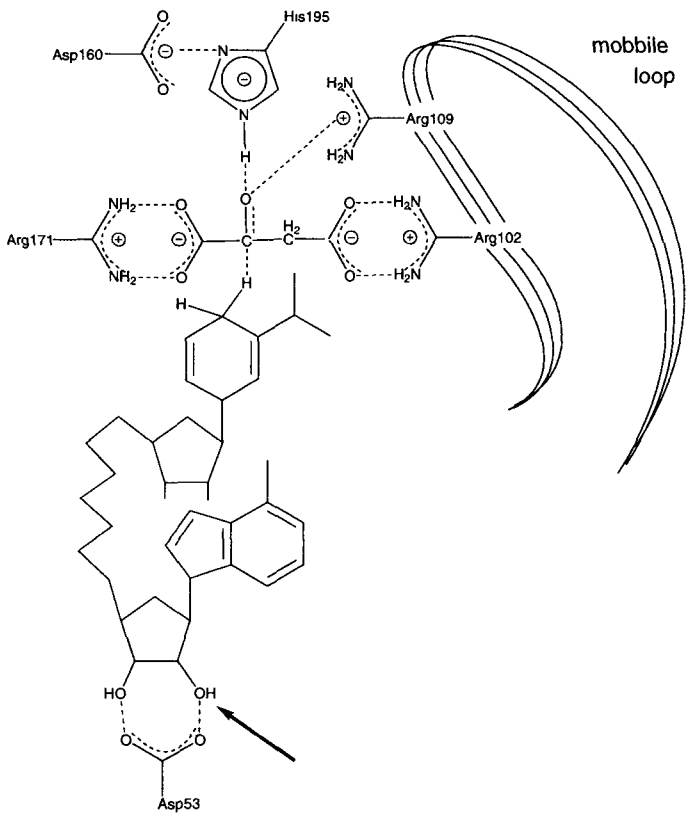

Figure 1. Active-site vacuole of MDH. The schematic drawing summarizes the function of active-site residues with the substrate oxaloacetate and coenzyme NADH. The arrow indicates the position of phosphate in the case of $NADP^+$ (Goward and Nicholls 1994).

proximity of the substrate (Grau et al. 1981; Clarke et al. 1986; Wigley et al. 1992). The loop is highly conserved among MDHs (residues 98–110), reflecting its crucial role in catalysis (Fig. 1). X-ray structures of MDHs crystallized in the presence and absence of substrate analogues, which bind to the active site, have identified 2 conformationally distinct forms of the enzyme with external loop in either the up or down position. The active site of this enzyme contains invariant and interacting H195 and D168 residues. His-Asp pair linked by a hydrogen bond may function as a proton relay system during catalysis and allow the imidazole ring of the histidine to act as both an acid and a base. This pair could also provide an explanation for the relatively stronger binding by cytoplasmic MDH of NADH versus NAD (Birktoft and Banaszak 1983).

The side chain of residue D53 is important for coenzyme binding and specificity by hydrogen bonding with the adenosine ribose hydroxyl groups. It is chemically conserved with an acidic side chain in all NAD-MDHs. G53 was found in the chloroplastic NADP-MDH of maize and sorghum (Birktoft et al. 1989a; Hall et al. 1992; Wigley et al. 1992; Kelly et al. 1993). There are 3 arginine residues (R102, R109 and R171) which are important for substrate binding and catalysis. R102 and R109 are on the underside of the mobile loop and interact with substrate in ternary complex. The quanidium side chains of R102 and R171 form counterions for the substrate carboxylate groups, which contribute to binding and orientation of the substrate in the active site (Clarke et al. 1986; Goward and Nicholls 1994).

Mitochondrial MDH

The majority of mitochondrial proteins are encoded in the nucleus, synthesized in the cytoplasm and imported to one of four locations in the mitochondria: the matrix, the intermembrane space, the outer membrane or the inner membrane. Many mitochondrial proteins are synthesized as precursors, which are imported post-translationally with the aid of nucleotide triphosphate hydrolysis and the electrochemical potential across the inner mebrane. Many precursors of matrix proteins such as malate dehydrogenase contain N-terminal presequences (transit sequences) of 10–70 residues in length, which are cleaved off by specific peptidases after import to the mitochondria. Import is dependent on the precursor protein being in an unfolded state, and it requires membrane potential across the inner membrane (Hartl et al. 1989). There is evidence that import is mediated by proteins in the cytosol, receptors in the membrane and chaperons in the matrix (Schatz 1993). Transit sequences are relatively rich in positively charged residues (mainly arginine), lack acidic side-chain and have a high content of serine and threonine and small clusters of adjacent hydrophobic groups (MacLachlan et al. 1994).

Comparison of watermelon mMDH with mammalian, yeast and *E. coli* MDH gives 55–60% overall identity of residues (McAlister-Henn et al. 1987). On the other hand, the amino acid sequences of mouse mMDH and cytosolic MDH (cMDH) show only 23% overall identity (Joh et al. 1987). Surprisingly, comparisons of the amino acid sequences among the eukaryotic and bacterial MDHs revealed that the similarity between the mMDHs from plants, yeast, *E. coli* and the thermophilic bacterium *Thermus flavus* exceeds the intraspecies sequence similarity between mitochondrial and cytoplasmic MDH (Nishiyama et al. 1986).

The structure of mitochondrial MDH is well defined. In most organisms, it is a dimer composed of identical subunits with approximate molecular weights of 34 kDa (Banaszak and Bradshow 1975), and the known amino acid sequence contains 314 residues. The gene encoding the mitochondrial isoenzyme of MDH is present in a single copy in genomic DNA of *S. cerevisiae*. The specific activity of

mMDH was elevated approximately eight-times in yeast cells transformed with a multicopy plasmid containing the entire encoding region for the corresponding gene (McAlister-Henn et al. 1987). Although several collections of yeast mutants with defects in mitochondrial functions and oxidative energy metabolism have been isolated, mutants with specific defects in mitochondrial MDH have not been described (Parker and Matoon 1969; Tzagoloff et al. 1975; Ciriacy 1977).

Mitochondrial MDH is involved in three different pathways: 1. tricarboxylic acid cycle, 2. conversion of glycine to serine to provide reducing equivalents, and 3. supply of CO_2 for fixation in bundle sheath chloroplasts of higher plants.

In the tricarboxylic acid cycle mitochondrial MDH provides, together with isocitrate dehydrogenase and α-ketoglutarate dehydrogenase, for NADH to be oxidised in the respiratory chain, while succinate dehydrogenase yields $FADH_2$. Plant mitochondria preferentially oxidise NADH produced in the matrix space as a result of substrate oxidation. The latter two pathways suggests cooperation of the mitochondrion with the peroxisome in photorespiration and with the chloroplast in the concentration mechanism of CO_2 for C_4 photosynthesis, respectively (Gietl 1992).

In mammalian and yeast cells mitochondrial and cytosolic MDHs there are components of the malate/aspartate shuttle; they represent an important mechanism for exchange of substrates and reducing equivalents between metabolic pathways separated by the mitochondrial membrane. NADH is formed in the cytoplasm during glycolysis and is required for respiration and a variety of metabolic processes. From the cytoplasm to the mitochondria it is transported across the mitochondrial membrane by the malate/aspartate shuttle because intact mitochondria are impermeable for NADH (Setoyama et al. 1990).

Cytosolic MDH

When the mitochondrial isoenzyme is synthesized as higher molecular weight precursor, the in vitro translation of the cytosolic MDH yields a product which has the same molecular weight as the native isoenzyme.

Eukaryotic cells contain two forms of MDH enzymes – one in the mitochondria and the other one in the cytoplasm. Both forms of MDH are synthesized in the cytoplasm, where cMDH remains after acetylation of the N-terminal residue. mMDH is synthesized with an N-terminal extension residue and is subsequently imported into the mitochondrial matrix (Chien and Freeman 1984). Cytosolic MDH is a homodimer, each subunit having a molecular mass of 35 kDa and containing 332 amino acids of known sequence (Birktoft et al. 1987; Joh et al. 1987).

One of the more noticeable differences between cMDH and mMDH is the difference in the overall polarity of the two enzymes. cMDH is more polar as well as more acidic than mMDH, and this difference is due solely to the difference in the

number of charged residues. There are 41 basis residues (31 lysines + 10 arginines) and 43 acidic residues (25 aspartates + 18 glutamates) in cMDH. In mMDH there are 25 lysines and 8 arginines for total of 33 basic residues, and 13 aspartates and 16 glutamates for a total of 29 acidic groups (Birktoft et al. 1989a).

The sequence identity between cMDH and mMDH is relatively low, being of the order of about 20–25%. The best fit of the molecular structure of cMDH to that of lactate dehydrogenase has been obtained by the least square method. This similarity between the dimeric cMDH and the tetrameric LDH reported earlier by Rao and Rossmann (1973), particularly in the nucleotide binding domain, has been confirmed. The active sites of these two enzymes contain similarly oriented His-Asp pairs linked by a hydrogen bond which may function as a proton relay system during catalysis. This pair could also provide an explanation for the relatively stronger binding by cMDH and LDH of NADH versus NAD.

Peroxisomal and glyoxysomal MDH

Peroxisomes are organelles present in almost all eukaryotic cells. Like the mitochondrion, the peroxisome is a major site of oxygen utilization. In fact, the peroxisome is thought by some to represent the vestige of an ancient organelle that carried out all of the oxygen metabolism of primitive preeukaryotic cells when oxygen entered the atmosphere. Most peroxisomes catalyze the breakdown of fatty acids to acetyl-CoA using a special H_2O_2-producing enzyme. The acetyl-CoA produced can be transported via the cytosol to the mitochondria to feed the citric acid cycle, or it can be used for biosynthetic reactions elsewhere.

Two very different types of peroxisomes have been extensively studied in plants. One type is present in leaves, where it catalyzes the oxidation of a side product of the reaction that fixes CO_2 in carbohydrate (photorespiration). A very different type of peroxisome is present in germinating seeds, where it serves to convert the fatty acids stored in seed lipids into sugars needed for the production of the materials of the young plant. Because this is accomplished by a series of reactions known as the glyoxylate cycle, these peroxisomes are also called glyoxysomes.

Metabolism of glycolate carbon occurs sequentially in three organelles, the peroxisomes, the mitochondria and the chloroplasts (Lorimer and Andrews 1980). In the peroxisome glycolate is oxidised to glyoxylate and then transaminated to glycine with either glutamate or serine (Rehfeld and Tolbert 1972). Glycine then stays in the peroxisome and is oxidised to ammonia, CO_2 and serine in the mitochondrion. Serine is converted to glycerate by serine:glyoxylate aminotransferase and hydroxypyruvate reductase in the peroxisome, and in this form photorespiratory carbon returns to the chloroplast. Glycerate is phosphorylated to phosphoglycerate by glycerate kinase, and can reenter the photosynthetic cycle.

Photorespiration is an example for the tight cooperation of metabolic pathways

located in different cell compartments. A malate/oxaloacetate/aspartate shuttle has been proposed for plant microbodies similar to that for mitochondria (Dry et al. 1987). Leaf peroxisomes as well as seed glyoxysomes contain a large amount of activity of an isoenzyme of NAD-malate dehydrogenase (Yamazaki and Tolbert 1969; Curry and Ting 1973). Besides catalase (an enzyme that constitutes up to 40 percent of the total peroxisomal protein), malate dehydrogenase is the most active enzyme in plant microbodies. It is therefore not surprising that enzymes present in both glyoxysomes and peroxisomes are identical. Peroxisomal and glyoxysomal MDHs are serologically indistinguishable and have the same isoelectric points and function as dimers (Hock and Gietl 1982).

The transport of glyoxysomal MDH from the site of synthesis in the cytoplasm to the site of function in the organelle involves translocation of the protein across the single membrane of the organelle. Glyoxysomal MDH is very similar to mitochondrial and cytoplasmic MDHs and also lactate dehydrogenases. Especially the amino acids Arg-87, Gly-185 and Gly-228 which are crucial for selecting malate as substrate, are rigorously conserved in MDH enzymes, while in LDH enzymes an equally strong conservation is observed for Gln, Asp and Thr in the same positions (Wilks et al. 1988).

Glyoxysomes lack DNA, they are surrounded by a single membrane and share at least two biochemical capabilities with peroxisomes of animals and fungi: O_2 processing (based on the conversion of H_2O_2 by catalase) and fatty acid oxidation. In glyoxysomes they convert the long-chain fatty acids to their CoA esters (Cooper 1971). Their membranes contain an alkaline lipase that hydrolyses triacylglycerols (Muto and Beevers 1974; Huang 1987). They possess a complete set of enzymes for the β-oxidation of fatty acids, and enzymes of the glyoxylate cycle. The β-oxidation of fatty acids ends up with the production of acetyl-CoA. The enzymes of the glyoxylate cycle catalyze the conversion of two molecules of acetyl-CoA to succinate, and comprise citrate synthase, aconitase, isocitrate lyase, malate synthase and malate dehydrogenase enzymes (Beevers 1969). For the fate of NADH synthesized within the glyoxysomes which do not contain an electron transport system linking NADH to O_2, two possibilities are disscused: 1. oxidation of NADH in glyoxysomes by a malate-aspartate shuttle (Mettler and Beevers 1980) would involve transport of malate from the glyoxysomes to mitochondria, and 2. the alternative possibility is coupling of β-oxidation of fatty acids and glyoxylate cycles to NADH:cytochrome c and ferricyanide reductases in glyoxysomes which may allow β-oxidation and the glyoxylate cycle to be partially uncoupled from mitochondrial oxidative phosphorylation (Donaldson and Fang 1987).

Chloroplastic MDH

Besides the NAD-dependent isoforms of malate dehydrogenase located in microbod-

ies, the mitochondria and the cytoplasm, an NADP-dependent form of the enzyme is found in the chloroplasts in higher plants. NADP-dependent MDH (NADP-MDH) (EC.1.1.1.82) is involved in the C_4 dicarboxylic acid cycle responsible for the primary fixation and transfer of CO_2 in C_4 plants. It is also present in C_3 type plants where it is implicated in chloroplast shuttle mechanisms which might help export reducing power.

NADPH-MDH is a model enzyme in that its activity is strictly controlled by light (Johnson and Hatch 1970). Indeed, NADP-MDH is inactive in the dark and gets activated by light via the ferredoxin – thioredoxin system (Wolosiuk et al. 1980).

NADP-MDH resembles the non-regulatory NAD-MDH, except for two sequence additions, one N-terminal and one C-terminal. Due to the presence of both N- and C-terminal extensions chloroplastic MDHs exhibit larger molecular masses, and they represent crucial parts of the protein for redox regulation.

Several cysteine residues are located in the NADP-MDH polypeptide, and all are specific to this chloroplastic redox regulatory isoform. Two of these are located in the N-terminal extension sequence and one in the C-terminal extension. Chemical derivatization followed by sequence analysis in sorghum (Decottignies et al. 1988) has shown that there is a light-dependent reducible disulphide bridge present in the N-terminal extension. Site-directed mutagenesis has indeed confirmed that there is a thioredoxin-reducible disulphide bridge involving these two residues (Issakidis et al. 1992), but has also shown that there is a second disulphide needed for regulation. Additional site-directed mutagenesis experiments have succeeded in creating a redox insensitive NADP-MDH, by mutation of the N-terminal disulphide, together with either or both of the most C-terminal cysteines (C377 and/or C365 in sorghum), indicating that these cysteines could constitute the second regulatory disulphide (Issakidis et al. 1993; 1994). There is experimental evidence that the disulfide bridge involving the C-terminal residues is shielding access to the catalytic residues, and that the N-terminal end is involved in the slow conformational change of the active site needed for activation (Issakidis et al. 1996).

On the basis of the results of mutagenesis experiments and three-dimensional structure modeling of the chloroplastic isoenzyme, Issakidis et al. (1994) proposed a model for the mechanism of activation of NADP-MDH (Fig. 2).

Chloroplast NADP-MDH possesses a His-Asp pair at the active site which probably forms a proton relay system. The involvement of such a His/Asp pair in catalysis has already been described for LDH and NAD-MDH. It is acting as an acid in the reduction of the keto-acid and as a base in the oxidation of the hydroxy-acid (Birktoft and Banaszak 1983). So, His-229 and Asp-201 play a crucial role in the catalytic mechanism of chloroplastic NADP-MDHs (Lemaire et al. 1996). Sequence data of NADP-MDH from pea (Reng et al. 1993), sorghum (Cretin et al. 1990), maize (Metzler et al. 1989) and ice-plant are available.

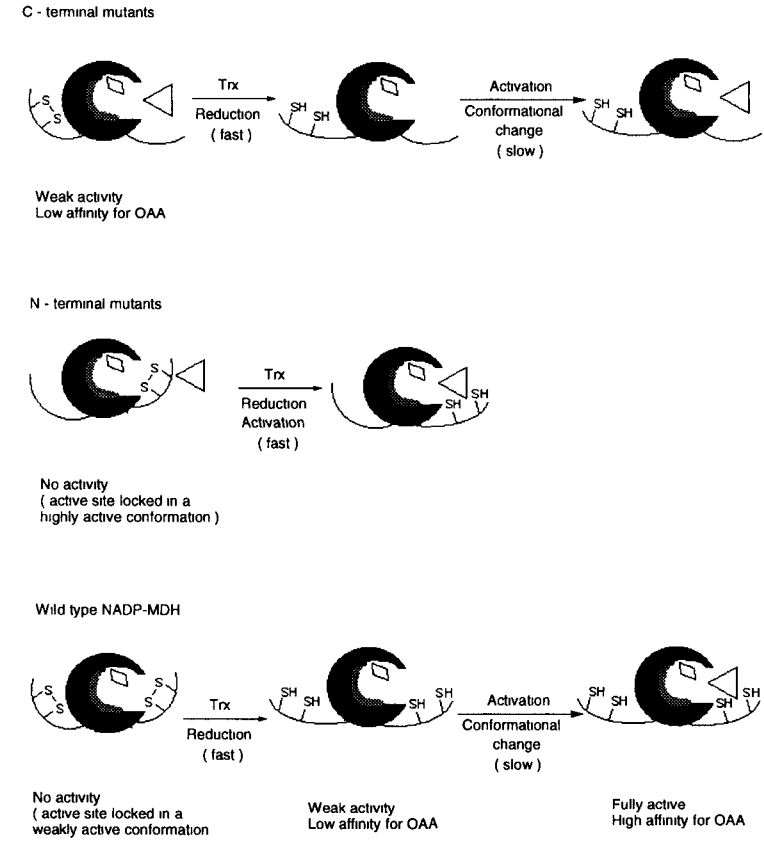

Figure 2. Schematic model of activation mechanism of NADP-MDH (either wild type or mutant). Only one subunit is shown. The coenzyme binding site is symbolized by the *diamond*, as it is accessible from the surface. The *triangles* represent oxaloacetate. The N-terminal extention is to the *left* of the subunit and the C-terminal is to the *right*, close to the active site. *Trx*, thioredoxin (Issakidis et al. 1994).

NADP-MDH is an essential component of the malate/oxaloacetate shuttle, which balances reducing equivalents between the chloroplast and the cytosol. In C_4 plants, NADP-MDH activity is 10-times higher and acts to convert oxaloacetate to malate in chloroplasts of mesophyll cells for transport to bundle sheat cells (Hatch and Slack 1969). During C_4 photosynthesis, atmospheric CO_2 is fixed by carboxylation of phosphoenolpyruvate in mesophyll cells (by PEP carboxylase), giving C_4 dicarboxylic acids which are decarboxylated in bundle sheath cells by one of three different decarboxylases: NADP-malic enzyme, NAD-malic enzyme or PEP carboxykinase. When malate is transported to bundle sheath cells and decar-

boxylated via NADP-malic enzyme, it acts as a carrier of reducing power as well as CO_2. The NADPH formed is directly utilised for phosphoglycerate reduction.

The encoded amino acid sequence of maize predicts that NADP-MDH is synthesized as a preprotein of 432 amino acids and processed into a mature protein of 375 amino acids with removal of a 57 amino acids long transit peptide. The sorghum enzyme is synthesized as a precursor of 429 amino acids and gets imported into the chloroplast where it is processed to a mature subunit of 389 amino acids. Despite the lack of sequence similarities to other chloroplast transit peptides the extra sequence shows the common features : it is rich in the hydroxylated amino acids serine and threonine (14%), it is also rich in small hydrophobic amino acids such as alanine and valine (28%), it shows net positive charge (8 arginines, 1 lysine), and is generally deficient in acidic amino acids (2 aspartates). The maize enzyme is similar to other MDHs in regions related to enzymatic function (Wilks et al. 1988).

The similarity of the C_3 and C_4 forms of NADP-MDH suggests that genes for C_4 enzymes may have been recruited from existing genes encoding C_3 enzymes (Gietl 1992).

Eubacterial MDH

Escherichia coli malate dehydrogenase (eMDH) is a homodimer and it is comprised of 312 amino acid residues per subunit (Sutherland and McAlister-Henn 1985; McAlister-Henn et al. 1987). The structures of cytosolic and mitochondrial MDHs from porcine heart have been determined (Roderick and Banaszak 1986; Birktoft et al. 1989a) and a comparative study between these enzymes and eMDH demonstrated that eMDH has 58% sequence identity with mMDH, but only about 20% identity with cMDH (Hall et al. 1992; Gleason et al. 1994).

In the crystalline structures of 2 prokaryotic and 2 eukaryotic forms, the subunit interfaces are conformationally homologous. To determine whether or not the quaternary structure of MDH is linked to catalytic activity, mutant forms of the enzyme from *E. coli* have been constructed. Utilizing the high-resolution structure of *E. coli* MDH, the dimer interface was analyzed critically for side chains that were spatially constricted and needed for electrostatic interactions. Two such residues were found, D45 and S226. At the nearest point in the homodimer, they were found in different subunits, hydrogen bonded across the interface, and did not interact with any catalytic residues. Each residue was mutated to a tyrosine, which should disrupt the interface because of its large size. All mutants were cloned and purified to homogeneity. Gel fitration of the mutants showed that D45Y and D45Y/S226Y were both monomers, whereas the S226Y mutant remained a dimer. The monomeric D45Y and D45/S226Y mutants had 14,000 and 17,500-times less activity, respectively, than the native enzyme. The dimeric S226Y had only 1.4-times less specific activity.

Crystallographic studies have shown that the dimer interface consists mainly of interacting α-helices that fit compactly together. The active sites in these dimeric proteins are well separated from each other; they are not in the subunit interface and the bound dicarboxylic acid substrates are about 3 nm apart. The 3-dimensional subunit-subunit relationship is the same in all the known MDH structures and in one of the interfaces present in the tetrameric lactate dehydrogenases.

Table 1. Homology within a portion of the α-helical region in the dimer interface In *E. coli* MDH, D45 and H48 have the most extensive hydrogen bonding pattern Residue numbers correspond to *E. coli* MDH (Hall et al 1992)

E coli MDH	G	V	A	V	D	L	S	H	T	L	S	M	G
Yeast mMDH	G	V	A	T	D	L	S	H	T	L	S	M	A
Pig/mouse mMDH	G	V	A	A	D	L	S	H	T	L	S	M	A
Pig cMDH	G	V	L	M	E	L	Q	D	A	M	S	A	A
tMDH	G	V	V	M	E	L	R	D	A	A	S	A	A
PigLDHA, LDHB	G	E	M	M	D	L	Q	H	G	L	S	V	A
	41					45		48	224		226		228

Some researchers have suggested the dimer structure to be critical for enzymatic activity. Several highly conserved amino acid residues are found in this interface (Table 1), with D45 and H48 having the most extensive hydrogen bonding interactions (Breiter et al. 1994). The stability of the subunit-subunit interface in eMDH is the result of direct hydrogen bonds, water-mediated hydrogen bonds and hydrophobic contacts.

Two schools of thought arose surrounding the structure-function relationship (Harada and Wolfe 1968). The first proposed the reciprocating compulsory order mechanism where each subunit alternates as the "active" and the "helper" subunit, but both are needed for activity. This mechanism predicts an inactive monomer, and was confirmed by studies that showed a dramatic reduction of enzyme activity on dissociation to monomers at low enzyme concentration, at pH 5.0 and in the absence of substrate (Bleile et al. 1977; Wood et al. 1981a,b). The second mechanism introduces an equilibrium between two conformers of MDH, one of which preferentially binds citrate and NAD, whereas the other binds NADH. This would suggest an active monomer (Mullinax et al. 1982).

From termophilic organism *Thermus flavus* MDH (tMDH) has been purified and its enzymatic properties have been analyzed (Iijima et al. 1980). It is a dimer enzyme composed of two identical subunits, each of a molecular weight of 35 kDa (Iijima and Saiki 1984). Nishiyama et al. (1986) have cloned the gene for the *T.*

flavus malate dehydrogenase (tMDH) into *E. coli* and reported the sequence. Interestingly, the prokaryotic tMDH sequence is more identical to the eukaryotic cMDH (55%) than to mMDH (20%). Random mutagenesis of *T. flavus* yielded a mutant strain that possessed 3-times higher MDH activity than the wild type strain. The MDH gene cloned from the mutant strain was found to encode an altered enzyme with a single amino acid exchange of Ile for Thr-190 which caused an increase in the apparent enzyme activity and loss of substrate inhibition in the presence of an excess of oxaloacetate (Nishiyama et al. 1991).

The position of the NAD-binding loop relative to the body of the protein in tMDH was compared to that in cMDH and LDH; it was observed that the tMDH-NAD binary complex is more similar to that in LDH than to that in the cMDH-NAD binary complex (Kelly et al. 1993).

The malate dehydrogenase from grampositive bacteria *Streptomyces aureofaciens* has been purified and the molecular and catalytic properties of the enzyme have been studied. The protein is a homodimer, with a 38 kDa subunit molecular mass. The enzyme is very similar in many respects to other bacterial MDHs with the notable exception of a lack of inhibitionn by excess substrate. The enzyme is much more efficient in reducing oxaloacetate than in oxidating of malate (Mernik et al. 1998; Mikulášová et al. 1998).

Archaebacterial MDH

The limits of viability of organisms in their natural habitat are determined by extremes of temperature (−5℃ to 110℃), hydrostatic pressure (≤120 MPa), pH (0–12) and water activity (≤ 5.5 mol/l NaCl and KCl). Adaptation to these extremes during the evolution led to development of thermophiles, barophiles, acidophiles, halophiles, etc. Numerous investigations of the molecular mechanism of adaptation at the genomic, metabolic and cellular level have not indicated a general strategy (Jaenicke 1981, 1988; Hecht et al. 1989). Comparison of homologous enzymes from species adapted to widely different conditions have revealed that ligand affinities seem to be conserved. The kinetic analog of this conservation of "corresponding states" would be compensatory effects via adjustments of activation free energies. To give an example, it has been established that cold and warm-adapted organisms show similar metabolic rates when the comparison is based on the temperature of their natural habitats (Hochachka and Somero 1973; Somero and Low 1976; Jaenicke 1981).

Halophilic eukaryotes and eubacteria overcome the extracellular osmotic pressure by accumulating "osmoprotectants" (Yancey et al. 1982). On the other hand, halophilic archaebacteria accumulate salt (particularly K^+ ions) at concentrations that can reach and exceed saturation (Christian and Waltho 1962). The biochemical machinery of these prokaryotes has, therefore, been adapted in the course of

evolution to be able to function at salt concentrations at which most biochemical systems will cease to function The biochemical and biophysical properties of several halophilic enzymes were studied in great detail (Eisenberg et al 1992)

The enzyme malate dehydrogenase of the extremely halophilic archaebacteria *Haloarcula marismortui* (hMDH) has been studied most extensively using a wide range of biochemical and biophysical methods (Pundak and Eisenberg 1981, Pundak et al 1981, Zaccai et al 1989) It was found that the enzyme is only stable in highly concentrated solutions of certain salts The gene encoding hMDH could be isolated and sequenced The enzyme is composed of 303 amino acids and its molecular mass is 32,638 Da The deduced amino acid sequence of the enzyme was found to be more similar to the sequence of L-lactate dehydrogenase (LDH) from various sources than to the sequence of other MDHs The structural gene was cloned in the *E coli* expression vector pET11a, and large amounts of a soluble but inactive form of the enzyme were produced upon its induction Activation of the enzyme was obtained by increasing the salt concentration to 3 mol/l NaCl (Cendrin et al 1993) Both the halophilic and non-halophilic (mitochondrial) MDH show a close similarity in their Km profiles On other hand, the catalytic efficiency of the two homologous enzymes varies dramatically, halophilic malate dehydrogenase exhibits deactivation at low salt (Hecht et al 1989)

42 residues of the N-terminal amino acid sequence of MDH from thermoacidophilic archaebacterium *Sulfolobus acidocaldarius* have been determined In the archaebacterial enzyme the residue Gly(7) Gly(11) and Asp(33) are also present The data suggest that in the enzyme from *S acidocaldarius* like in the other MDHs the binding domain for NAD(H) is localized at the N-terminal part of the polypeptide chain (Gorisch and Jany 1989)

MDH from the extremely thermophilic methanogen *Methanothermus fervidus* has been isolated and its properties were characterized This enzyme is a homomeric dimer with a molecular mass of 70 kDa displaying low specifity for NAD or NADP (Honka et al 1990)

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