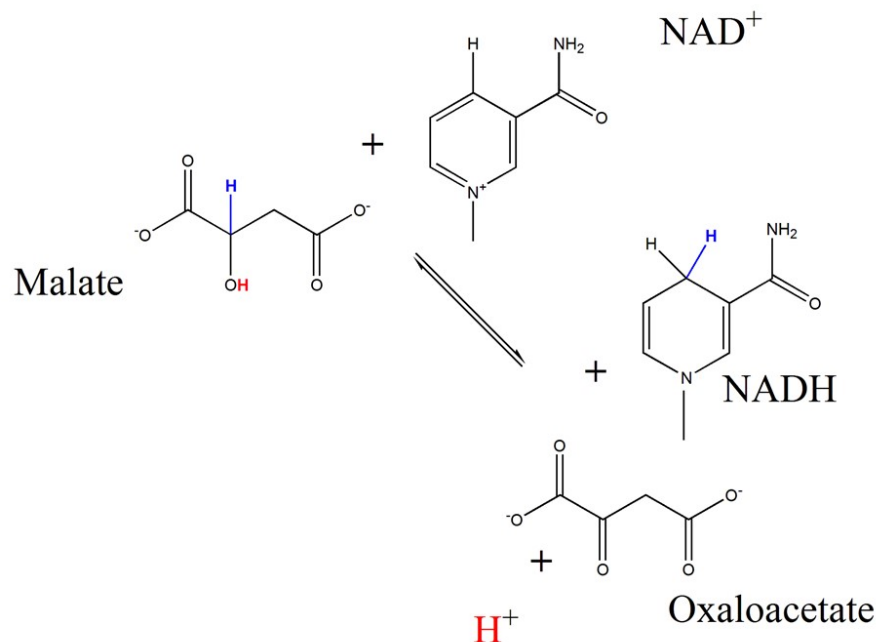
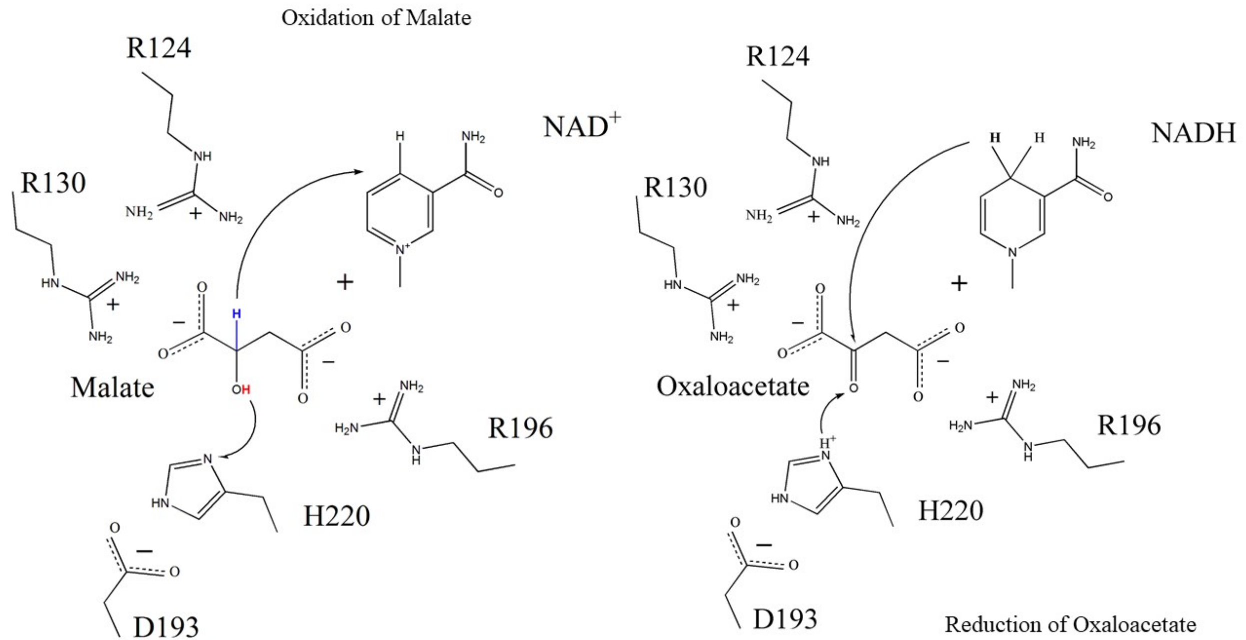
Introduction to Malate Dehydrogenase

Malate Dehydrogenases catalyze the reaction:

involving a simple hydride transfer from the 2 position of Malate to NAD^+ to form the reduced cofactor NADH. The equilibrium constant for the reaction favors the NAD^+/Malate side of the reaction (1).

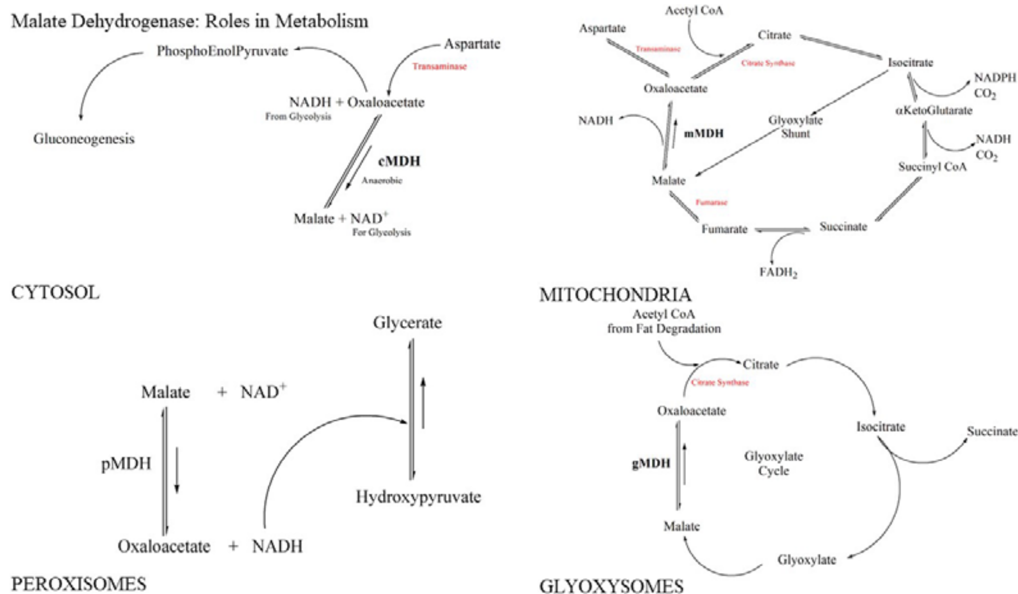


The reaction is thought to involve a base catalyzed abstraction of the proton from the Malate O-H group involving a conserved histidine in the active site of the enzyme. In all known MDHs there is a conserved Aspartate adjacent to the histidine which is thought to increase the basicity of the N: of the histidine ring, enhancing its ability to abstract the proton from the Malate O-H.(2) The malate/oxaloacetate substrate is held in place by the presence of three conserved arginine residues, whose positive charges interact with the negative charges of the malate/oxaloacetate. One of them, (R153 in e Coli) has been the subject of mutagenesis and rescue using chemical biology to probe its involvement in binding and catalysis (3).



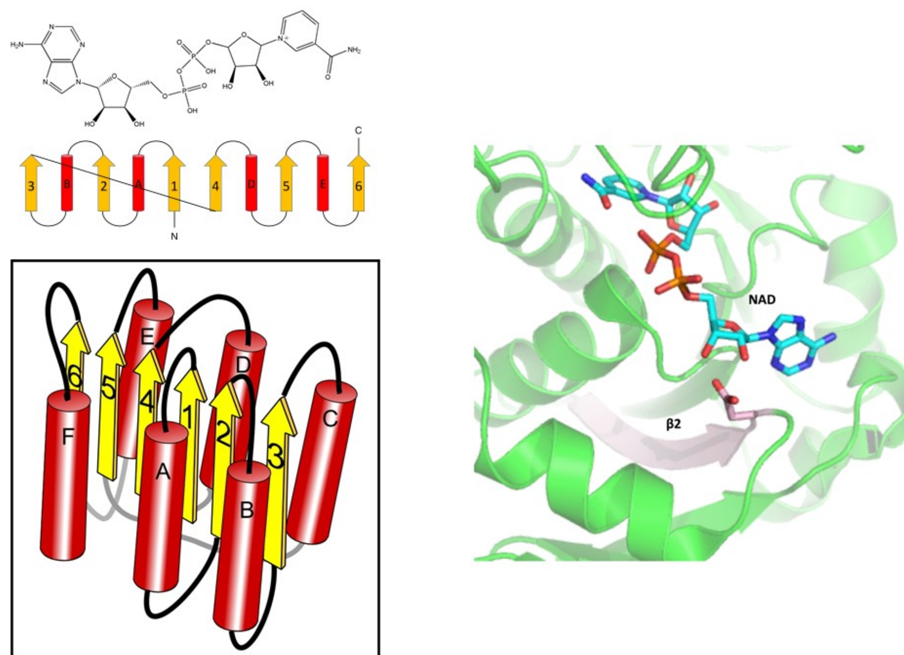
The Hydrogen on the C2 carbon of Malate is transferred as a hydride ion (H-) to the 4-Carbon of the nicotinamide ring of the cofactor NAD^+ to give NADH. During the process a proton is also released to the solvent.

This reaction plays a number of important roles in metabolism, illustrated by a reaction in the mitochondrion in the Tricarboxylic acid cycle, a reaction playing a role in the shuttling of reducing equivalents from the cytosol to the mitochondria, in peroxisomes, and in plants a reaction in the Glyoxysome:(4)

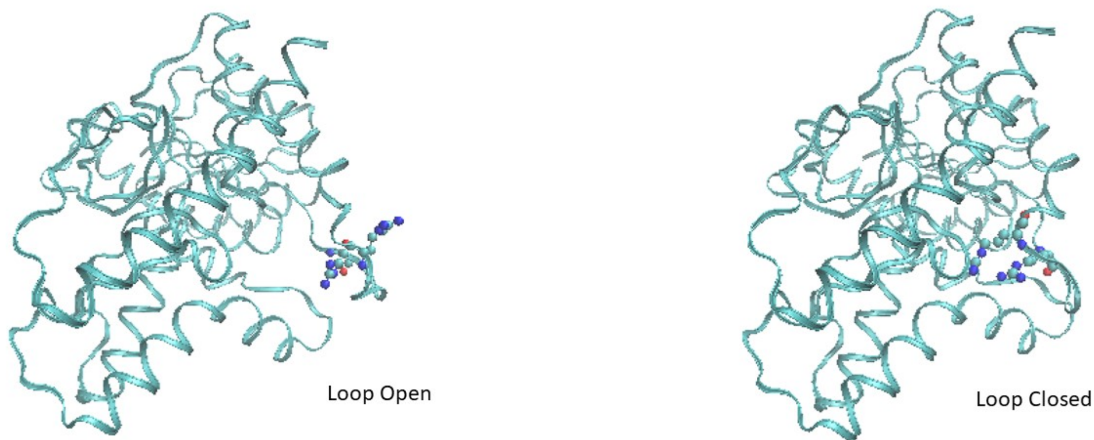


It is clear that there must exist Malate Dehydrogenase in at least two different locations within the cell and in fact there are distinct cytoplasmic MDH [cMDH] (5) and mitochondrial MDH [mMDH] (6) isoenzymes in higher eukaryotes which have different amino acid sequences and slightly different three dimensional structures. Peroxisomes [pMDH] (7) and in plants, glyoxysomes [gMDH] (8), also have distinct isoenzymes. The organelle forms (mitochondria, glyoxysomes) are synthesized as precursors coded for by nuclear genes, synthesized in the cytosol and transported to the appropriate organelle, guided by a "pre-sequence" of about 40 amino acids that is removed upon import to the organelle (9).

Pig cytoplasmic and mitochondrial malate dehydrogenases were among the first enzyme structures to be determined by X ray crystallography (10,11). Since then the structure of Malate Dehydrogenase from a number of sources have also been determined by X Ray crystallography. In addition to the details of the amino acids that play a role in substrate binding and catalysis, indicated in figure 2, the structure has several notable features. As with many enzymes that bind NADH there is a clear "Rossman fold" (12) associated with cofactor binding consisting of a β−α−β−α−β−β−α−β−α−β secondary structure motif that in enzymes with specificity for NAD(H) has an aspartate at the C-terminal end of the β2 strand that interacts with the Adenine ribose of the cofactor, figure 3.



For substrate (Malate/Oxaloacetate) binding there is a “flexible loop” (13) that contains two of the three arginine residues involved in malate/oxaloacetate binding, and swings in and out of the active site to complete the active complex of the enzyme (figure 4).



The enzyme has quaternary structure and is in most instances a dimer of two identical (in terms of amino acid sequence) polypeptide chains and displays a clear interface between the two subunits. The quaternary structure undergoes a pH dependent dissociation (14), and monomeric forms have been engineered by mutation of specific amino acids at the subunit interface (15). Monomeric forms show little if any activity.

MDH is thought to form loose multienzyme complexes with several other enzymes sharing substrates, so called “metabolons” (16). In particular Aspartate AminoTransferases (which catalyze the transamination of Glutamate and Oxaloacetate to give Aspartate and 2-Oxoglutarate, a key reaction in the the aspartate-malate shuttle, the Glyoxylate Cycle and Gluconeogenesis) (17), Citrate synthase, (which catalyzes the next step in the Tricarboxylic Acid (Krebs) Cycle) (18), and Fumarase, (which catalyzes the preceding reaction in the Krebs cycle)(19) have been suggested to form metabolons with malate dehydrogenase in certain organisms. Such metabolons are thought to either increase the effectiveness of consecutive reactions by “channeling” products from one enzyme to the next one and or allow for coordinated regulation of a pathway.

Given its central role in a number of metabolic pathways, some forms of the enzyme are subject to allosteric regulation by citrate (20), by post-translational modifications such as by lysine acetylation (21) and by transcriptional control mediated through miR-743a (22).

The malate dehydrogenase structure and lactate dehydrogenase structures have many similarities and lactate dehydrogenases are thought to have evolved from malate dehydrogenases (23), a step requiring alteration of substrate specificity to bind lactate/pyruvate in place of malate/oxaloacetate. While many malate dehydrogenases are mesophilic, psychrophilic and thermophilic forms have also been characterized and are often studied to explore mechanisms of adaptation (24). As discussed below in the bioinformatics section the existence of cytosolic and organelle forms of malate dehydrogenase is thought to have arisen from a symbiotic relationship between bacteria (precursors of the organelle forms) and eukaryotes which contained a cytosolic form of malate dehydrogenase

Malate Dehydrogenase has also been used as a model system to examine a variety of aspects of protein folding including tertiary structure, quaternary structure (25,26) and the impact of a precursor sequence on structure (27).

Finally, as with many essential enzymes, malate dehydrogenase is a potential target for drug design in pathogenic organisms such as Mycobacterium tuberculosis (28) and Plasmodium falciparum (29), or tumor tissues depending upon enhanced metabolism (30, 31). Such drug design depends upon exploiting often subtle differences in structure function relationships or developing so called “allosteric drugs” that target flexible regions of the protein required for activity (32).

There remain many unanswered fundamental questions about MDH to be investigated.

Some examples are:

i) Folding, Stability & Oligomeric Structure: most MDHs are dimeric, some are monomers and others form tetramers. Folding and acquisition of oligomeric structure in α/β proteins such as MDH is also little studied. The role of protein dynamics and stability in biological activity is another aspect ripe for further investigation

ii) Substrate specificity and catalytic mechanism. (there exist MDH isoforms with LDH like activity, or NADPH (vs. NADH) affinity). Although the roles of the active site His-Asp diad, and the flexible loop containing 2 of the three active site arginines are frequently assumed, detailed information about their roles in catalysis and substrate binding is lacking. Little is known about the roles of so-called “second sphere” residues

iii) Allosteric regulation (some forms are regulated by citrate inhibition/activation and/or substrate inhibition) and pH, together with the role of Subunit Interactions and the subunit interface . Work on the roles of interface residues H90, E256, S266 and L269 is underway in the Bell lab and nearing publication)

iv) Metabolon formation: mammalian mitochondrial MDH (but not cytosolic MDH) can interact with other proteins including citrate synthase and fumarase, while both the cytosolic and mitochondrial forms may interact with aspartate amino transferase

and

v) Adaptation (Salinity, Temperature) and evolution (MDH Isoforms, relationship to LDH)

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