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Characterization and Crystallisation of the Malate Dehydrogenase from *Streptomyces aureofaciens*

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Malate dehydrogenase (EC 1 1 1 37) is an enzyme that catalyzes conversion of malate to oxaloacetate reversibly using NAD (resp NADP) as a cofactor In eukaryotic cells several isoforms MDHs have been identified, differing in their subcellular localisation One form is a principal enzyme of the citric acid cycle operating within the mitochondria The other is found in the cytosol where it participates in the malate/aspartate shuttle This shuttle exchanges reducing equivalents across the mitochondrial membranes in the form of malate/oxaloacetate rather than as NAD/NADH (Hall *et al* 1992) A third isoenzyme can be found in the glyoxysomes of yeast and plants, where it function to convert malate that is produced from glyoxylate in the glyoxylate cycle (Minard and McAlister-Henn 1991) In prokaryotes there is only one form of the enzyme The enzymes share a common catalytic mechanism and their kinetic properties are similar although they are distinguishable by certain properties including differential inhibition by high concentrations of oxaloacetate (Banaszak and Bradshaw 1975)

Malate dehydrogenases are part of a well-studied family of NAD-dependent dehydrogenases (Branaszak and Bradshaw 1975) This family includes the enzymes lactate dehydrogenase (LDH), alcohol dehydrogenase, and glyceraldehyde-3-phosphate dehydrogrenase (Rossmann *et al* 1975) Characterization of these enzymes has included isolation from a large number of source, sequence determination, and functional characterization The two most closely related enzymes in this family are the MDHs and the well-studied

LDHs because the substrate specifities of MDH and LDH both involve converting 2-hydroxy acids to the corresponding 2-keto acids and because of extensive structural simi larities these two enzymes together form the 2-hydroxy acid dehydrogenases family (Birktoft *et al* 1982) As is true with the other enzymes of the NAD dependent dehydrogenases the NAD-binding domains of MDH and LDH are very similar (Kelly *et al* 1993)

Three-dimensional X-ray crystal structures have been reported from four MDHs these include two eukaryotic forms of MDH from porcine heart, mitochondrial (mMDH) (Roderick and Banaszak 1986) and cytoplasmic (cMDH) (Birktoft *et al* 1989) Information on bacterial MDHs are more scares with the exception of MDHs from *E coli* and *T flavus* which have been the subject of numerous investigations including X-ray crys tallography and site directed mutagenesis (Goward and Nicholls 1994 Hall and Banaszak 1993)

The data concerning from *Streptomyces* species are very limited a consensus amino-terminal sequence has been identified from MDH *S atratum* (Rominel *et al* 1989) but no further biochemical characterization of this enzyme has been done

The malate dehydrogenase from *Streptomyces aureofaciens* was purified 352 fold to electrophoretic homogeneity with a 33 % recovery and its physical and biochemical properties were studied (Mikulasova *et al* 1998) The enzyme was purified by a three-step proce dure comprising anion-exchange chromatography on DEAE cellulose DE-52 hydrophobic interaction on Phenyl Sepharose CL 4B and affinity chromatography on Matrex red A The molecular weight of the purified enzyme was determined by size-exclusion chromatography on Superose 12 HR it was 70 000 Da for the native enzyme When the enzyme was subjected to SDS PAGE it appeared as a single band of 38 000 Da indicating a dimeric structure for the native enzyme similar with this respect to the majority of MDHs studied so far This observation was confirmed by the determination of the amino-terminal sequence perfectly matched the amino-terminal sequence of the MDH from *Streptomyces atratus* In particular the typical MDH signature GAAGXXG a sequence considered to be involved in the binding of the ADP portion of NAD (Charnok *et al* 1992) could be iden tified in the amino terminal sequence (Mikulašova *et al* 1998) MDH from *S aureofaciens* showed a strong specificity for NADH and was much more efficient for the reduction of oxaloacetate than for the oxidation of malate with a pH optimum of 8 Unlike MDHs from other sources it was not inhibited by excess oxaloacetate This first complete functional characterization of an MDH from *Streptomyces* shows that the enzyme is very similar in many respects to other bacterial MDHs with the notable exception of lack of inhibition by excess substrate (Mikulasova *et al* 1998)

The crystallographic study MDH from *S aureofaciens* was done MDH crystals were grown in various crystallographic solutions from HAMPTON RESEARCH SCREEN and UCLA FACTORIAL SCREEN **A:** 30 % PEG 4000 0 1M sodium-citrate pH 5 6 0 2M ammonium acetate **B:** 30 % PEG 8000 0 1M sodium-cacodylate pH 6 5 0 2M ammonium sulfate **C:** 20 % PEG 8000 0 2M $MgCl_2$ 0 1M sodium-citrate pH5 6 and **D.** 20 % PEG 8000 0 2M NaK phosphate 0 1M Tris-HCl pH 8 5 The main problem in the crystallisation of our MDH was to obtain an ordered and crystalline entity Since order is the essence of a crystal, crystallisation of MDH is promoted with the coenzyme NADH which helped the enzyme to crystallise to well ordered crystals Orthorhombic $C222_1$ crystals were grown by vapour diffusion method (Ducruix and Giege 1992) in hanging drops in 0 1-0 3M $MgCl_2$ in 0 1M sodium citrate buffer (pH 5 6) and 10 % polyethylene glycol 8000 with coenzyme NADH Data measurement and processing are in progress

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