J. Mol. Biol. (1989) **208**, 507–508

Preliminary X-ray Crystallographic Study of Malate Dehydrogenases from the Thermoacidophilic Archaebacteria *Thermoplasma acidophilum* and *Sulfolobus acidocaldarius*

Malate dehydrogenases from the thermoacidophilic Archaebacteria *Thermoplasma acidophilum* and *Sulfolobus acidocaldarius* have been crystallized and characterized by X-ray diffraction measurements. Crystals of the enzyme from *T. acidophilum* display space-group symmetry $P2_1$, $a = 63$ Å, $b = 135$ Å, $c = 83$ Å and $\beta = 105°$; they scattered to approximately 4 Å resolution. Two crystal modifications of malate dehydrogenase from *S. acidocaldarius* were characterized; one displayed trigonal symmetry corresponding to space groups $P321$, $P3_121$ or $P3_221$ with lattice parameters $a = 151$ Å and $c = 248$ Å and with resolution approximately to 5 Å, whereas the other modification displayed space group symmetry $I23$ or $I2_13$ with lattice parameters $a = 129$ Å and approximately 4·5 Å resolution.

Malate dehydrogenases catalyze the reversible oxidation of malate to oxaloacetate. Most examples from eukaryotic and prokaryotic cells studied so far use NAD^+ as the coenzyme (EC 1.1.1.37); however, a malate dehydrogenase from maize leaf is known to use $NADP^+$ (EC 1.1.1.82). In nearly all living systems, malate dehydrogenase occurs as a dimer (Murphey *et al.*, 1967); however, it is a tetramer in *Bacillus* species and in a few other eubacteria. The enzymes isolated from the archaebacteria *Thermoplasma acidophilum* (Grossebüter *et al.*, 1986) and *Sulfolobus acidocaldarius* (Hartl *et al.*, 1987) are both tetramers and use both NAD^+ and $NADP^+$ as coenzymes to oxidize malate; both enzymes were obtained in crystalline form. The enzyme from *T. acidophilum* exhibits maximum activity at about 65 °C and that from *S. acidocaldarius* at about 80 °C.

Single crystals of both enzymes have been grown at room temperature and preliminary characterization of their crystallographic properties has been carried out. For both enzymes, crystals of distinctly different morphologies were obtained, often in the same drop, either appearing simultaneously or one after the other. Crystallographic characterization was carried out with crystals and mother liquor (mixed with solution from the respective reservoirs) enclosed in glass capillaries. Diffraction intensities were recorded with an image plate system under development at the EMBL Outstation Hamburg.

A solution of 5 mg ml^{-1} malate dehydrogenase from *T. acidophilum* (3 µl) was mixed with an equal volume of a solution of 13% polyethylene glycol 4000, 0·1 M-NaCl, 0·1 M-Tris·HCl (pH 7 to 8) and containing a trace of NaN_3; crystals were prepared by the hanging-drop method. In most cases, apparently hexagonal prismatic crystals elongated along the unique axis (0·9 mm × 0·15 mm × 0·15 mm) were obtained. Attempts to characterize them with X-rays were not successful. Two, more nearly equidimensioned crystals of 0·25 mm × 0·25 mm × 0·3 mm were obtained at pH 7·1 and 7·3, and were found to display space group symmetry $P2_1$, $a = 63$ Å, $b = 135$ Å, $c = 83$ Å (1 Å = 0·1 nm) and $\beta = 105°$; they scattered to approximately 4 Å resolution. Assuming a tetramer (144,000 daltons) per asymmetric unit, the volume per unit molecular weight is 2·3 $Å^3$ $dalton^{-1}$.

Crystals of malate dehydrogenase from *S. acidocaldarius* were obtained by both hanging-drop and sandwich-drop techniques; crystals displaying very similar morphology were obtained with polyethylene glycol 3000 and 4000. In many cases, elongated prismatic crystals with typical dimensions of 0·4 mm × 0·2 mm × 0·05 mm, which nearly always displayed defects describable as conical channels parallel to the long dimension of the crystal, were obtained. In other drops, cubes measuring up to 0·4 mm on an edge were obtained, either as the first crystals to grow in a given drop or at a later (from days to several weeks) time. In the latter case, the more "needle-like" crystals were typically observed to dissolve as the cubes formed. Two long needles, approximately 2 mm × 0·15 mm × 0·15 mm, that did not contain the channel-like defects were obtained in sandwich drops from a solution of 4 mg ml^{-1} malate dehydrogenase from *S. acidocaldarius* mixed with an equal volume of a solution of 12% polyethylene glycol 4000, 0·1 M-NaCl, 0·1 M-Tris·HCl (pH 8) and a trace of NaN_3. They were found to display trigonal symmetry corresponding to space groups $P321$, $P3_121$ or $P3_221$ (the latter are thought to be most likely because of the small number of $00l$ reflections observed) with resolution approximately to 5 Å. The lattice parameters are

0022–2836/89/150507–02 $03.00/0

$a = 151$ Å and $c = 248$ Å; assuming a monomer molecular weight of 34,000 daltons and two tetramers per asymmetric unit (space group $P3_121$ or $P3_221$) the volume per unit weight is 3·0 Å^3 dalton^{-1}. In another sandwich drop prepared with 15% polyethylene glycol 4000 (pH 7·6), a prismatic crystal measuring up to 0·4 mm on an edge was obtained. It displayed space group symmetry $I23$ or $I2_13$ with lattice parameters $a = 129$ Å and approximately 4·5 Å resolution. Assuming a relative molecular weight of 34,000 daltons gives 2·6 Å^3 dalton^{-1}, which we take to indicate that the correct space group is $I23$ with a tetramer generated from one monomer per asymmetric unit by the 222 crystallographic symmetry.

Banaszak and colleagues have reported crystal structure determinations for two malate dehydrogenases; cytoplasmic malate dehydrogenase (Birktoft & Banaszak, 1983) and mitochondrial malate dehydrogenase (Roderick & Banaszak, 1986), and have discussed similarities of the enzymes with lactate dehydrogenase. Although formally dimers, both of these mesophilic malate dehydrogenases crystallize as "dimers of dimers". Comparison of the structures of the thermophilic, tetrameric malate dehydrogenases reported here with those described by Banaszak and with tetrameric lactate dehydrogenase (Adams *et al.*, 1970) may be expected to shed additional light on conformational stability of proteins and enzyme activity.

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Received 25 January 1989

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Edited by R. Huber