J. Mol. Biol. (1988) **200**, 609–610

Crystallization of Halophilic Malate Dehydrogenase from *Halobacterium marismortui*

Malate dehydrogenase from the extreme halophile *Halobacterium marismortui* crystallizes in highly concentrated phosphate solution in space group *I*2 with cell dimensions $a = 113{\cdot}8$ Å, $b = 122{\cdot}8$ Å, $c = 126{\cdot}7$ Å, $\beta = 98{\cdot}1°$. The halophilic enzyme was found to be unstable at lower concentrations of phosphate. It associates with unusually large amounts of water and salt, and the combined particle volume shows a tight fit in the unit cell.

Halobacterium marismortui lives in the Dead Sea, the saltiest body of water on earth. The intracellular components of this organism are exposed to near-saturated salt concentrations. Proteins from halophilic sources have evolved a special adaptation mechanism that enables them to function at high salinity. Work in this area has recently been reviewed (Werber *et al.*, 1986; Eisenberg & Wachtel, 1986). Knowledge of the three-dimensional structure of malate dehydrogenase from *Halobacterium marismortui* (hMDH)† and its comparison to structures of non-halophilic malate dehydrogenases might shed light on haloadaptation. Structural studies of several other halophilic macromolecules from the same organism have been carried out by us: the ribosomal 50 S particle (Makowski *et al.*, 1987); several ribosomal proteins (Shoham *et al.*, 1986); and 2Fe–2S ferredoxin (Sussman *et al.*, 1986).

hMDH can be kept stable and active for several years in 4 to 4·3 M-NaCl at neutral pH at room temperature. It is, however, degraded in low salt, a process that is accompanied by the dissociation of the dimeric enzyme into subunits as well as the loss of all α-helical conformation (Mevarech *et al.*, 1977; Mevarech & Neumann, 1977; Pundak & Eisenberg, 1981).

As in the case of other halophilic proteins, hMDH has a marked excess of acidic over basic amino acid residues, 19·9 mol % (Mevarech *et al.*, 1977), as compared to e.g. beef heart cytoplasmic malate dehydrogenase (6 mol %: Siegel & Englard, 1962). Although hMDH is an enzyme from an archaebacterium it has a higher molar mass (87,000) (Pundak & Eisenberg, 1981) than its eukaryotic counterparts, i.e. beef heart mitochondrial MDH (65,000: Siegel & Englard, 1962), porcine heart cytoplasmic MDH (72,000) and porcine heart mitochondrial MDH (68,000: Tsernoglou *et al.*, 1972). All these enzymes are composed of two chemically identical subunits. The refined crystal structure of porcine heart cytoplasmic MDH showed the two subunits to be structurally nearly identical (Birktoft & Banaszak, 1983).

† Abbreviation used: hMDH, malate dehydrogenase from *H. marismortui*.

Analysis of ultracentrifugation and light-scattering data obtained in concentrated NaCl solutions disclosed that hMDH associates with an unusually large amount of water and salt (Pundak & Eisenberg, 1981). This observation was confirmed by a recent combined analysis of neutron and small angle X-ray scattering with ultracentrifugation data (Zaccai *et al.*, 1986*a*) yielding 0·87(±0·07) g water/g protein and 0·35(±0·08) g NaCl/g protein. For comparison, the corresponding values for a typical non-halophilic protein, bovine serum albumin, are 0·23 g water/g protein and 0·012 g NaCl/g protein. The radius of gyration of the hMDH particle was found to be about 32 Å (1 Å = 0·1 nm) (Reich *et al.*, 1982), whereas that calculated for the protein moiety is about 28 Å and that of the associated water and salt distribution about 40 Å (Zaccai *et al.*, 1986*a*). Although acidic residues are known to bind about seven molecules of water, whereas other residues bind two to four water molecules (Kuntz, 1971), the excess of negatively charged residues in hMDH cannot solely be responsible for the ability of hMDH to retain such large amounts of water and salt. This ability is lost upon denaturation in low salt (Pundak *et al.*, 1981; Zaccai *et al.*, 1986*b*).

In this work, we report the crystallization of hMDH. The enzyme was dialysed against 2 M-phosphate buffer (pH 7·0) and crystallized by vapour diffusion in hanging drops against a reservoir solution of 2·6 M-phosphate at room temperature. Large crystals, up to 1 mm in their longest dimension, developed within several weeks. Preliminary X-ray studies on a Rigaku AFC5-R rotating anode diffractometer indicate the crystals to be monoclinic, space group *I*2, with $a = 113{\cdot}8(\pm 0{\cdot}3)$ Å, $b = 122{\cdot}8(\pm 0{\cdot}3)$ Å, $c = 126{\cdot}7(\pm 0{\cdot}8)$ Å, $\beta = 98{\cdot}1(\pm 0{\cdot}4)°$. (This is equivalent to space group *C*2 with $a = 158{\cdot}0$ Å and $\beta = 134{\cdot}5°$.) The crystals diffract to 2·5 Å and survive for about 24 hours in the X-ray beam using a Rigaku rotating anode generator operated at 15 kW when cooled to ~0°C. They last about half as long at room temperature.

We found (Zaccai *et al.*, unpublished results) the enzyme to be active in phosphate buffer, with a maximum of activity around 0·5 M, decreasing with

0022-2836/88/070609-02 $03.00/0

increasing phosphate concentration. The enzyme is unstable at low buffer concentration and becomes stable only around 2·0 to 2·5 M-phosphate. From diffusion and neutron scattering experiments in phosphate buffer (Zaccai *et al.*, unpublished results) the volume of the enzyme dimer was found to be similar to its value in $MgCl_2$ solutions but slightly lower than $2{\cdot}52 \times 10^5$ Å^3 in NaCl (Zaccai *et al.*, 1986*b*) and KCl (Calmettes *et al.*, 1987) solutions.

For the ratio, V_m, of the volume $V = 4{\cdot}38 \times 10^5$ Å^3 of the asymmetric unit to the molar mass ($8{\cdot}7 \times 10^4$ g/mol) we calculate $(5{\cdot}04/n)$ Å^3/dalton, where n is the number of molecules per asymmetric unit. From optical density measurements in phosphate buffer on dissolved crystals of known volume, $n = 2$ was established. This yields $V_m = 2{\cdot}52$, falling in the range outlined by Matthews (1968). Consideration of the volume of the enzyme particle complexed with both water and salt in solution, reported above, as compared to the volume of the unit cell, points to a very tight fit in the confines of the crystal unit cell. The density of the crystal was measured by flotation in various concentrations of phosphate buffer. On this basis, the solvent weight fraction in the crystals was calculated according to Banaszak *et al.* (1971) to be about 45%.

The crystal structure of porcine heart cytoplasmic MDH was solved and refined at 2·5 Å (Birktoft & Banaszak, 1983). Although the molar mass of hMDH is about 10% larger, and it probably has different solvent-interacting regions, molecular replacement methods may be useful in solving its crystal structure.

This work was supported in part by a grant from the U.S. Army Research Office (through its European Research Office) and by the Minerva Foundation, Munich, Germany.

M. Harel[1]
M. Shoham[1]
F. Frolow[1]
H. Eisenberg[2]
M. Mevarech[3]
A. Yonath[1]
J. L. Sussman[1]

[1] Department of Structural Chemistry

[2] Department of Polymer Research
The Weizmann Institute of Science
Rehovot, Israel

[3] Department of Microbiology
Tel-Aviv University, Israel

Received 12 October 1987

Edited by R. Huber

References

Banaszak, L. J., Tsernoglou, D. & Wade, M. (1971). In *Probes of Structure and Function of Macromolecules and Membranes* (Chance, B., Yonetani, T. & Mildvan, A. S., eds), vol. 2, pp. 71–78, Academic Press, New York and London.

Birktoft, J. J. & Banaszak, L. J. (1983). *J. Biol. Chem.* **258**, 472–482.

Calmettes, P., Eisenberg, H. & Zaccai, G. (1987). *Biophys. Chem.* **26**, 279–290.

Eisenberg, H. & Wachtel, E. J. (1986). *Annu. Rev. Biophys. Chem.* **16**, 69–92.

Kuntz, I. D. (1971). *J. Amer. Chem. Soc.* **93**, 514–516.

Makowski, I., Frolow, F., Saper, M. A., Shoham, M., Wittmann, H. G. & Yonath, A. (1987). *J. Mol. Biol.* **193**, 819–822.

Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.

Mevarech, M. & Neumann, E. (1977). *Biochemistry*, **16**, 3786–3792.

Mevarech, M., Eisenberg, H. & Neumann, E. (1977). *Biochemistry*, **16**, 3781–3785.

Pundak, S. & Eisenberg, H. (1981). *Eur. J. Biochem.* **118**, 463–470.

Pundak, S., Aloni, H. & Eisenberg, H. (1981). *Eur. J. Biochem.* **118**, 471–477.

Reich, M. H., Kam, Z. & Eisenberg, H. (1982). *Biochemistry*, **21**, 5189–5195.

Shoham, M., Dijk, J., Reinhardt, R. & Wittmann-Liebold, B. (1986). *FEBS Letters*, **204**, 323–330.

Siegel, L. & Englard, S. (1962). *Biochim. Biophys. Acta*, **64**, 101–110.

Sussman, J. L., Brown, J. H. & Shoham, M. (1986). In *Frontiers of Iron–Sulfur Protein Research* (Matsubara, H., Katsube, Y. & Wada, K., eds), pp. 69–82, Japan Scientic Societies Press, Tokyo/Springer-Verlag, Berlin.

Tsernoglou, D., Hill, E. & Banaszak, L. J. (1972). *J. Mol. Biol.* **69**, 75–87.

Werber, M. M., Sussman, J. L. & Eisenberg, H. (1986). *FEMS Microbiol. Rev.* **39**, 129–135.

Zaccai, G., Wachtel, E. & Eisenberg, H. (1986*a*). *J. Mol. Biol.* **190**, 97–106.

Zaccai, G., Bunick, G. J. & Eisenberg, H. (1986*b*). *J. Mol. Biol.* **192**, 155–157.