

## Expression, purification, crystallization and preliminary X-ray diffraction data of methylmalonate-semialdehyde dehydrogenase from Bacillus subtilis

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© 2004 International Union of Crystallography Printed in Denmark – all rights reserved Methylmalonate-semialdehyde dehydrogenase from *Bacillus subtilis* was cloned and overexpressed in *Escherichia coli*. Suitable crystals for X-ray diffraction experiments were obtained by the hanging-drop vapour-diffusion method using ammonium sulfate as precipitant. The crystals belong to space group  $P2_12_12_1$ , with unit-cell parameters a = 195.2, b = 192.5, c = 83.5 Å, and contain one tetramer per asymmetric unit. X-ray diffraction data were collected to 2.5 Å resolution using a synchrotron-radiation source. The crystal structure was solved by the molecular-replacement method.

#### 1. Introduction

The members of the aldehyde dehydrogenase (ALDH) superfamily oxidize a wide variety of aldehydes to non-activated or CoA-activated acids via a two-step chemical mechanism. Mechanistic and structural aspects have been studied extensively for homotetrameric and homodimeric CoA-independent ALDHs (see, for example, Liu et al., 1997; Steinmetz et al., 1997; Marchal & Branlant, 1999; Marchal et al., 2000; Cobessi et al., 2000). In contrast, little information about the mechanistic and/or structural aspects of CoA-dependent ALDHs is available so far, except for the recent structure of a bifunctional aldolase-dehydrogenase (Manjasetty et al., 2003). Methylmalonatesemialdehyde dehydrogenase (MSDH), which is one of the few ALDHs belonging to this family (Steele et al., 1992; Leal et al., 2003), has been found in a wide variety of organisms ranging from bacteria and archaea to mammals. Pseudomonas aeruginosa (Bannerjee et al., 1970; Steele et al., 1992) and Streptomyces coelicolor (Zhang et al., 1996) MSDHs are involved in valine catabolism. Investigation of the myo-inositol catabolism of Bacillus subtilis (Yoshida et al., 1997) and Rhizobium leguminosarium bv. Viciae (Fry et al., 2001) revealed the putative implication of MSDH in this pathway. In mammals, MSDH is a mitochondrial enzyme that is involved in the distal portions of the valine and pyrimidine catabolic pathways (Goodwin et al., 1989). Over the two last decades, a possible correlation of organic acidaemia with MSDH deficiency has been explored (Gray et al., 1987; Chambliss et al., 2000). Roe et al. (1998) proposed that a psychomotor delay associated with methylmalonyl aciduria, without any increase of propionylcarnitine in the blood or urine, could be a direct consequence of MSDH deficiency.

This NAD-dependent ALDH catalyzes the oxidation of methylmalonate semialdehyde (MMSA) and malonate semialdehyde (MSA) to propionyl-CoA and acetyl-CoA, respectively. Therefore, MSDHs show another difference with respect to the chemical mechanism of the CoA-independent ALDH family because the catalyzed reaction also includes a  $\beta$ -decarboxylation step. Here, we report the cloning, overexpression, purification, crystallization and preliminary X-ray diffraction analysis of the MSDH from *B. subtilis* (*Bs\_MSDH*).

#### 2. Material and methods

## 2.1. Cloning procedure, expression and purification

The iolA ORF was amplified by PCR using the B. subtilis 168 genomic DNA as a template and the forward and reverse primers 5'-GAAGGAGGCAATACATATGGCAGA-AATCAG-3' and 5'-CTTACGCAACAAA-GAGCTCATAGAGAAGC-3', respectively. The forward primer contains an NdeI restriction site (in bold) and the reverse primer contains a SacI restriction site (in bold). These restriction sites were used to clone iolA into a plasmid derived from pBluescriptSK+, yielding pSKmsdbsub for overexpression under control of the lac promoter. The cloned iolA ORF was sequenced in order to confirm that no mutations had been introduced in the amplification reaction.

*Escherichia coli* DH5 $\alpha$  transformants containing the pSKmsdbsub plasmid were grown at 310 K for 24 h in Luria–Bertani medium supplemented with ampicillin (200 µg ml<sup>-1</sup>). Cells were harvested by centrifugation (20 min at 3000g) at 277 K and the pellet was suspended in 50 mM potassium

phosphate pH 8.2 (buffer *A*) containing 10 m*M*  $\beta$ -mercaptoethanol. Cells were disrupted by sonication, after which unbroken cells and debris were removed by centrifugation (45 min at 20 000*g*). MSDH was purified by ammonium sulfate fractionation (40–80%) and gel filtration on ACA 34 resin equilibrated in buffer *A*. Fractions containing MSDH activity were then applied to a Q-Sepharose column using a FPLC system (Amersham Biosciences) previously equilibrated with buffer *A*. MSDH was eluted at 330 m*M* KCl with a linear gradient of 0–1 *M* KCl in buffer *A* at 5 ml min<sup>-1</sup>.

At this stage, wild-type MSDH was pure as checked by electrophoresis on 10% SDS-polyacrylamide gel (Laemmli, 1970) followed by Coomassie Brilliant Blue R-250 staining and electrospray mass-spectrometry analysis. The yield was estimated to be  $\sim$ 200 mg per litre of culture. The observed monomeric weight was 53 319 Da (the calculated weight without the N-terminal Met is 53 316 Da). The relative molecular weight of native wild-type MSDH was estimated by gel-filtration chromatography on a Superose 12 HR column as previously described (Roitel et al., 1999). The obtained value of  $223 \pm 11$  kDa is in good agreement with the theoretical value expected for a tetrameric enzyme, i.e. 213 kDa. Purified enzyme was stored at 253 K in the presence of 10 mM  $\beta$ -mercaptoethanol. MSDH was stable under these conditions for several weeks. The concentration was determined spectrophotometrically using an extinction coefficient of 280 nm of 51 000  $M^{-1}$  cm<sup>-1</sup> deduced by the method of Scopes (1974), Mach et al. (1992) and Gill & von Hippel (1989).

#### 2.2. Crystallization

The purified enzyme was dialyzed against potassium phosphate buffer pH 7.8, 1 mM DTT, 1 mM EDTA and finally concentrated using a Centricon (Amicon/Millipore) with 30 kDa molecular-weight cutoff at 4500g and 277 K. Prior to crystallization experiments, the enzyme was incubated at 303 K for 30 min in phosphate buffer pH 7.8 containing 20 mM NAD. Crystallization experiments were performed using the hanging-drop vapour-diffusion method (Ducruix & Giegé, 1999) at room temperature. Drops were prepared by mixing 2 µl protein solution with an equal volume of reservoir solution and were suspended over 500 µl reservoir solution. The protein concentration used for the first screenings was 10 mg ml<sup>-1</sup>. Initial crystallization trials were carried out at 277 and 293 K using

#### Table 1

Statistics of X-ray data measurement for *B. subtilis* MSDH crystals.

Values in parentheses refer to the outermost resolution shell (2.50–2.59 Å).

Space group	$P2_{1}2_{1}2_{1}$
Unit-cell parameters (Å)	a = 195.2, b = 192.5,
	c = 83.5
Resolution range (Å)	20.0-2.5
No. reflections measured	786249
No. unique reflections	108345
Completeness (%)	99.5 (91.9)
$\langle I/\sigma(I) \rangle$	9.39 (3.58)
$R_{\text{merge}}$ † (%)	13.8 (37.2)

†  $R_{\text{merge}} = \sum_{hkl} \sum_{j} |I_{hklj} - \langle I_{hkl} \rangle| / \sum_{hkl} \sum_{j} I_{hkl}$ , where  $I_{hklj}$  is the intensity of observation *j* of Bragg reflection *hkl*.

commercially available sparse-matrix screens and a home-made ammonium sulfate kit (pH range 5.0-8.0 and ammonium sulfate concentration range 1.0-3.0 M in steps of 0.1). The commercially screens used were Crystal Screens I and II from Hampton Research and Clear Strategy Screens I and II from Molecular Dimensions Ltd. The crystallization experiment was repeated by increasing the protein concentration until crystals were obtained.

# 2.3. Data collection and diffraction measurements

cryoprotectant conditions for The Bs\_MSDH crystals were optimized using an in-house X-ray facility composed of an area detector (DIP2030b) with a  $\varphi$  goniometer using Cu  $K\alpha$  radiation from a rotatinganode generator (Bruker-Nonius FR591 Model) and an Oxford Cryosystems Cryostream. The X-ray diffraction data set was collected using synchrotron radiation from EMBL beamline X11 located at the DORIS storage ring, DESY, Hamburg. The wavelength used was 0.8126 Å and the data were collected at 100 K on a MAR CCD165 detector. 360 images were collected with a crystal-to-detector distance of 240 mm and 0.5° rotation per image. A 2.5 Å data set was processed and merged with the XDS suite (Kabsch, 1993).

#### 2.4. Results and discussion

The first crystals were only obtained at a very high protein concentration  $(>150 \text{ mg ml}^{-1})$  using ammonium sulfate as precipitant. These initial crystallization conditions were further refined by changing the pH and the protein and precipitant concentrations. Finally, thin plates grew within 3–7 d to approximate dimensions of  $0.3 \times 0.3 \times 0.05$  mm under the following conditions: 2.0–2.3 *M* ammonium sulfate without buffer and a protein concentration of 200 mg ml<sup>-1</sup>.

Prior to X-ray diffraction experiments, crystals were soaked briefly in reservoir solution supplemented with 18%(v/v)glycerol for flash-freezing. The diffraction pattern obtained using the home rotatinganode generator was too weak to determine the unit cell of the MSDH crystals. The diffraction pattern obtained using the synchrotron X-rays was consistent with the primitive orthorhombic Bravais lattice, with unit-cell parameters a = 195.2, b = 192.5,c = 83.5 Å. A total of 108 345 unique reflections (average multiplicity 8) were collected in the resolution range 20-2.5 Å. Detailed data-collection statistics are given in Table 1. The systematic absences unambiguously revealed screw twofold axes parallel to a, b and c, leading to the  $P2_12_12_1$ space-group assignment. A reasonable Matthews coefficient of  $3.6 \text{ Å}^3 \text{ Da}^{-1}$ (Matthews, 1968) was obtained for four monomers in the asymmetric unit. The resulting calculated solvent content is 65%.

Bs\_MSDH displays 18-34% sequence identity to non-phosphorylating ALDHs whose crystal structures are available in the Protein Data Bank. Consequently, the molecular-replacement method was first tested to solve the crystal structure of Bs\_MSDH. Considering the oligomeric state of Bs\_MSDH, trials were carried out using the crystal structures of tetrameric ALDHs only. The holo form of the cod liver betaine aldehyde dehydrogenase search model gave the best solution (Johansson et al., 1998; PDB code 1bpw). In CNS (Brünger et al., 1998), a cross-rotational search followed by a translational search were performed in the resolution range 10-4 Å. The top two solutions seem to be equivalent, exhibiting correlation coefficients of 34.2 and 33.9%, while that of the next solution was 25.1%. Crystallographic refinement and structure analysis will be published elsewhere (coordinates have been deposited in the PDB with code 1t90).

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#### References

Bannerjee, D., Sanders, L. E. & Sokatch, J. R. (1970). J. Biol. Chem. 245, 1828–1835.

- Brünger, A. T., Adams, P. D., Clore, G. M., DeLano, W. L., Gros, P., Grosse-Kunstleve, R. W., Jiang, J.-S., Kuszewski, J., Nilges, N., Pannu, N. S., Read, R. J., Rice, L. M., Simonson, T. & Warren, G. L. (1998). Acta Cryst. D54, 905– 921.
- Chambliss, K. L., Gray, R. G. F., Rylance, G., Pollitt, R. J. & Gibson, K. M. (2000). J. Inherit. Metab. Dis. 23, 497–504.
- Cobessi, D., Tête-Favier, F., Marchal, S., Branlant, G. & Aubry, A. (2000). J. Mol. Biol. 300, 141– 152.
- Ducruix, A. & Giegé, R. (1999). Crystallization of Nucleic Acids and Proteins: A Practical Approach. Oxford: IRL Press.
- Fry, J., Wood, M. & Poole, P. S. (2001). Mol. Plant-Microbe Interact. 14, 1016–1025.
- Gill, S. C. & von Hippel, P. H. (1989). Anal. Biochem. 182, 319–326.
- Goodwin, G. W., Rougraff, P. M., Davis, E. J. & Harris, R. A. (1989). J. Biol. Chem. 264, 14965– 14971.

- Gray, R. G., Pollitt, R. J. & Webley, J. (1987). Biochem. Med. Metab. Biol. 38, 121–124.
- Johansson, K., El-Ahmad, M., Ramaswamy, S., Hjelmqvist, L., Jornvall, H. & Eklund, H. (1998). Protein Sci. 7, 2106–2112.
- Kabsch, W. (1993). J. Appl. Cryst. 26, 795– 800.
- Laemmli, U. K. (1970). Nature (London), 227, 680–687.
- Leal, N. A., Havemann, G. D. & Bobik, T. A. (2003). Arch. Microbiol. 180, 353–361.
- Liu, Z. L., Sun, Y. J., Rose, J., Chung, Y. J., Hsiao, C. D., Chang, W. R., Kuo, I., Perozich, J., Lindahl, R., Hempel, J. & Wang, B.-C. (1997). *Nature Struct. Biol.* 4, 317–326.
- Mach, H., Middaugh, C. R. & Lewis, R. V. (1992). Anal. Biochem. 200, 74–80.
- Manjasetty, B. A., Powlowski, J. & Vrielink, A. (2003) Proc. Natl Acad. Sci. USA, 100, 6992– 6997.
- Marchal, S. & Branlant, G. (1999). Biochemistry, 38, 12950–12958.

- Marchal, S., Rahuel-Clermont, S. & Branlant, G. (2000). *Biochemistry*, **39**, 3327–3335.
- Matthews, B. W. (1968). J. Mol. Biol. 33, 491–497.
- Roe, C. R., Struys, E., Kok, R. M., Roe, D. S., Harris, R. A. & Jakobs, C. (1998). *Mol. Genet. Metab.* 65, 35–43.
- Roitel, O., Sergienko, E. & Branlant, G. (1999). *Biochemistry*, **38**, 16084–16085.
- Scopes, R. K. (1974). Anal. Biochem. 59, 277–282.
- Steele, M. I., Lorenz, D., Hatter, K., Park, A. & Sokatch, J. R. (1992). J. Biol. Chem. 267, 13585– 13592.
- Steinmetz, C. G., Peiguang, X., Weiner, H. & Hurley, T. D. (1997). *Structure*, **5**, 701– 711.
- Yoshida, K. I., Aoyama, D., Ishio, I., Shibayama, T. & Fujita, Y. (1997). J. Bacteriol. 179, 4591– 4598.
- Zhang, Y. X., Tang, L. & Hutchinson, C. R. (1996). J. Bacteriol. 178, 490–495.