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Coenzyme F_{420}-dependent methylenetetrahydromethanopterin dehydrogenase from *Methanopyrus kandleri*: the selenomethionine-labelled and non-labelled enzyme crystallized in two different forms

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Coenzyme F_{420}-dependent methylenetetrahydromethanopterin dehydrogenase (Mtd) is an enzyme involved in methanogenic energy metabolism which reversibly catalyzes the reduction of methenyltetrahydromethanopterin (methenyl-H_4MPT$^+$) to methylenetetrahydromethanopterin (methylene-H_4MPT). The enzyme from the hyperthermophilic methanoarchaeon *Methanopyrus kandleri* could be crystallized: the non-labelled enzyme had unit-cell parameters a = 119.1, b = 151.0, c = 219.4 Å and space group $C222_1$, while the selenomethionine-labelled enzyme had unit-cell parameters a = 119.6, b = 151.0, c = 109.9 Å and also belonged to space group $C222_1$, indicating a surprising bisection of the c axis. The crystals grown from the non-labelled and labelled enzyme contained six and three monomers in the asymmetric unit and diffracted to about 1.9 and 1.5 Å, respectively. The crystal packing of the two crystal forms seems to be similar. In particular, the crystals of the selenomethionine-labelled enzyme are highly suitable for X-ray structure determination.

1. Introduction

Methanogenic archaea perform the final process in anaerobic microbial degradation of biomass to methane and are mostly found in anaerobic freshwater habitats. However, a special group of methanogenic archaea live in the vicinity of geothermal springs or vents and use the hydrothermal fluid as their nutrient. The protein used in this study is from *Methanopyrus kandleri*, which is a representative of this group of hyperthermophilic archaea. This organism was isolated at the Guaymas hot vents and grows optimally at 371 K on CO_2 and H_2 (Huber *et al.*, 1989; Kurr *et al.*, 1991).

Coenzyme F_{420}-dependent methylenetetrahydromethanopterin dehydrogenase (Mtd) is an essential enzyme of the methanogenic C_1-unit metabolism because it is involved in vital processes such as carbon fixation and energy conservation (Thauer, 1998). Mtd catalyzes the reversible reduction of methenyl-H_4MPT$^+$ with $F_{420}H_2$ to methylene-H_4MPT and F_{420} (Fig. 1), whereby a hydride is transferred from the *Si*-face of $F_{420}H_2$ to the *Re*-face of methenyl-H_4MPT$^+$ (Klein & Thauer, 1995).

The coenzyme H_4MPT is a pterin derivative which is structurally similar to tetrahydrofolate (van Beelen *et al.*, 1984; Maden, 2000). The electron donor, coenzyme F_{420}, is a 5′-deazaflavin (Eirich *et al.*, 1978). Mtd has been isolated and characterized from the methanogenic archaea *Methanothermobacter marburgensis* (Mukhopadhyay & Daniels, 1989), *Methanosarcina barkeri* (Enssle *et al.*, 1991; te Brömmelstroet *et al.*, 1991), *Methanopyrus kandleri* (Klein & Thauer, 1997) and the sulfate-reducing archaeon *Archaeoglobus fulgidus* (Schwörer *et al.*, 1993). It consists of one type of subunit with an apparent molecular

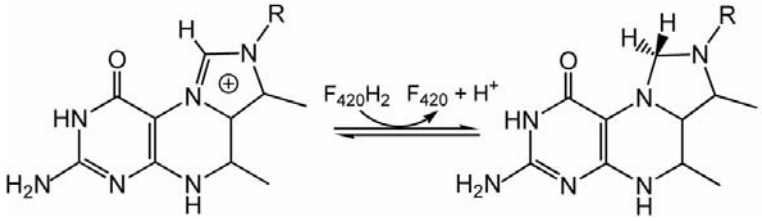

Figure 1
Reaction of coenzyme F_{420}-dependent methylene-H_4MPT dehydrogenase. Methenyl-H_4MPT$^+$ is reduced to methylene-H_4MPT at expense of coenzyme $F_{420}H_2$. For the complete structure of tetrahydromethanopterin see Vorholt *et al.* (1998). The structure of F_{420} is shown in Warkentin *et al.* (2001).

mass of 30–35 kDa and lacks a prosthetic group. Primary structures are available for Mtd isolated from the above-mentioned organisms as well as for three other organisms. A three-dimensional structure of Mtd has not been reported so far and the primary structure does not reveal any significant sequence identity to already structurally characterized proteins.

In this report, we present the crystallization of coenzyme F_{420}-dependent methylene-H_4MPT dehydrogenase from *M. kandleri* and focus on the observation that under the same crystallization conditions similar but not identical crystals were grown.

2. Methods and results

2.1. Expression and purification of methylene-H_4MPT dehydrogenase

Mtd from *M. kandleri* was overproduced in *Escherichia coli* BL21 carrying plasmid pET17b which contains an inserted *mtd* gene (Klein & Thauer, 1997). Cultures in M9 medium were induced with 1 m*M* isopropyl β-D-thiogalactopyranoside after they had reached an OD_{600} of 0.5. After 4 h induction, cells were harvested by centrifugation. Selenomethionine-labelled protein was produced using the method of metabolic inhibition as described in Doublié (1997) and Van Duyne *et al.* (1993). Non-labelled Mtd as well as selenomethionine-labelled protein was purified as described previously (Klein & Thauer, 1997). The yield of pure Mtd was about 12 mg per litre of culture for the non-labelled enzyme and 4 mg per litre for selenomethionine-labelled protein. Incorporated selenomethionine was verified *via* MALDI–TOF mass spectroscopy. After purification only one band was visible on the SDS–PAGE gel.

2.2. Crystallization

Non-labelled and selenomethionine-labelled Mtd were prepared for crystallization by concentrating the enzyme solution to 12 mg ml^{-1} in 10 m*M* MOPS/KOH buffer pH 7.0. Crystallization was performed with the hanging-drop vapour-diffusion method at a temperature of 277 K using Hampton Research Crystal Screens 1 and 2 and Jena Bioscience Crystal Screen kits 1–10. Crystals could be grown with 2-methyl 2,4-pentanediol (MPD) and PEG 400 as precipitant.

The best crystals of non-labelled Mtd were obtained by mixing 1 µl enzyme solution and 1 µl of a reservoir solution consisting of 13% MPD, 0.1 *M* sodium phosphate buffer pH 8.0 and 0.2 *M* magnesium acetate. The drop was equilibrated against 1 ml of the reservoir solution. After around one week the drop contained separated pyramidal and agglomerated plate-shaped crystals. The morphologically different crystals adopted the same space group $C222_1$, with unit-cell parameters a = 119.1, b = 151.0, c = 219.3 Å. The corresponding crystal volume-to-mass ratio V_M of 2.6 Å^3 Da^{-1} (Matthews, 1968) and the solvent content of 53% are best compatible with six monomers in the asymmetric unit. The pyramidal crystals diffracted to around 1.9 Å and the plate-shaped crystals to around 3.0 Å resolution.

Selenomethionine-labelled enzyme was crystallized under the same conditions. Pyramidal shaped crystals appeared within two weeks. Their space group was $C222_1$ and their unit-cell parameters were a = 120.1, b = 151.2, c = 110.0 Å. Surprisingly, the c axis was only about half that of the non-labelled enzyme crystals. Assuming three monomers per asymmetric unit, the V_M value is 2.6 Å^3 Da^{-1} and the solvent content is 53% (Matthews, 1968). The result of a self-rotation calculation using *POLARRFN* from the *CCP*4 program package (Collaborative Computational Project, Number 4, 1994) confirmed the presence of three monomers per asymmetric unit. The diffraction limit of the selenomethionine-labelled enzyme crystals is about 1.5 Å resolution.

2.3. Data collection and analysis

Prior to X-ray diffraction experiments, the crystals were soaked in a cryoprotectant solution containing 20% MPD, 0.1 *M* sodium phosphate buffer pH 8.0 and 0.2 *M* magnesium acetate and subsequently flash-cooled to 100 K in a nitrogen-gas cold stream. Data were measured in-house using an R-AXIS IV image-plate detector with Cu $K\alpha$ radiation from a Rigaku rotating-anode generator and at beamlines BW6 and ID14-4 at DESY and ESRF, respectively. Reflections were processed using the *HKL* suite (Otwinowski & Minor, 1997); other calculations were performed with the *CCP*4 program package (Collaborative Computational Project, Number 4, 1994). The statistics of five data sets are listed in Table 1.

Several measurements using crystals of selenomethionine-labelled and non-labelled enzyme were obtained in order to clarify whether the difference in the c-axis length was really a consequence of the selenomethinonine labelling. This was indeed the case. The crystals of the non-labelled enzyme showed an additional set of generally much weaker reflections along the c^* axis in addition to the stronger reflections corresponding to the small unit cell ($c \simeq 110$ Å) of the selenomethionine-labelled enzyme (Fig. 2). This set of weaker reflections causing the doubling of the c axis was mostly overlooked in the autoindexing

Table 1
Data-processing statistics.

Crystal	X-ray source	Space group	Unit-cell parameters (Å)	Resolution (Å)	R_{sym}† (%)	Completeness (%)	Multiplicity
kMtd-nat1‡	BW6	$C222_1$	a = 119.1, b = 151.0, c = 219.3	2.4	6.1	98.3	7.2
kMtd-nat2‡§	ID14-4	$C222_1$	a = 119.6, b = 151.3, c = 221.1	2.4	6.0	84.3	1.6
kMtd-nat3¶	In-house	$C222_1$	a = 119.0, b = 151.3, c = 219.9	3.0	9.4	93.5	3.4
kMtd-se1	BW6	$C222_1$	a = 119.6, b = 151.0, c = 109.9	2.4	6.4	98.3	7.2
kMtd-se2	ID14-4	$C222_1$	a = 120.1, b = 151.2, c = 110.0	1.5	7.5	99.2	3.5

† $R_{sym} = \sum |I_i - \langle I \rangle| / \sum I_i$, where I_i is the observed intensity and $\langle I \rangle$ is the average intensity obtained from multiple observations of symmetry-related reflections. ‡ Pyramidal-shaped. § Crystal kMtd-nat2 diffracted to 1.9 Å, but could only be processed to 2.4 Å resolution because of severe spot overlapping at high resolution. ¶ Plate-shaped.

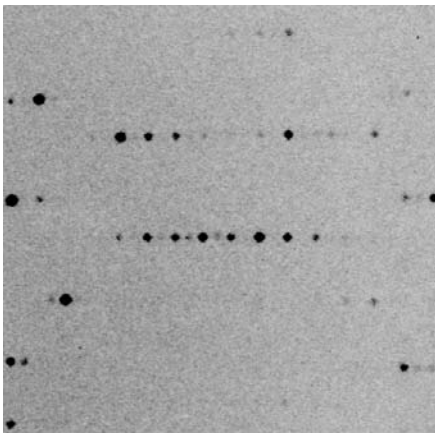

Figure 2
Section of an oscillation photograph obtained from crystals of non-labelled Mtd indicating the diffraction pattern along the c axis. The alternating 'weak–strong' intensity profile suggests a packing analogy to a unit cell where the length of the c axis is bisected.

procedure. The close relationship between the two unit cells might indicate very similar packing of the molecules in both crystal forms. This assumption was confirmed by a comparison between a subset of the structure factors of the pyramidal non-labelled crystals corresponding to the small *c* axis (kMtd-nat1, kMtd-nat2) and the selenomethionine-labelled crystals (kMtd-Se1, kMtd-Se2). The *R* factor of around 15% in the resolution range 5–10 Å is significantly higher than the internal *R* factor between the two selenomethionine and the two non-labelled data sets of around 8%, but is sufficiently low to assume a related crystal packing. Interestingly, the analysis of data collected from pyramidal non-labelled crystals (kMtd-nat1, kMtd-nat2) and plate-shaped non-labelled crystals (kMtd-nat3) revealed a somehow different protein structure or packing. The *R* factor between them is around 28%, compared with internal $R_{\rm sym}$ values of below 4% in the resolution range 5–10 Å (Table 1). This observation is consistent with the different diffraction limits of the crystals. The structure determination must be awaited in order to clarify whether subdivision of these crystals into two forms is appropriate.

The analysis of these data indicates that the most attractive approach for phase determination is a MAD experiment at the selenium edge of a selenomethionine-labelled protein crystal. This measurement has been performed and structure determination is in progress.

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