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Crystallization and Preliminary X-Ray Diffraction Studies of Methyl-Coenzyme M Reductase from *Methanobacterium thermoautotrophicum*

Seigo Shima,* Marcel Goubeaud,* Daniela Vinzenz,† Rudolf K. Thauer,* and Ulrich Ermler†,1

**Max-Planck-Institut für Terrestrische Mikrobiologie and Laboratorium für Mikrobiologie der Philipps-Universität, D-35043 Marburg, Germany; and* †*Max-Planck-Institut für Biophysik, D-60528 Frankfurt, Germany*

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Methyl-coenzyme M reductase isoenzyme I from the methanogenic Archaeon, *Methanobacterium thermoautotrophicum* (strain Marburg), was crystallized by vapor diffusion methods. Crystal form M obtained with 2-methyl-2,4-pentanediol as the precipitant displayed space group $P2_1$, with unit cell parameters of $a=83.2$ Å, $b=117.4$ Å, $c=125.1$ Å, and $\beta=92.6°$, and diffracted at better than 2.8 Å resolution. Crystal form P grown from polyethylene glycol 400 belonged to space group $P2_1$, and had unit cell parameters of $a=83.1$ Å, $b=120.2$ Å, $c=123.1$ Å, and $\beta=91.7°$, diffracting at least to 1.7 Å resolution. Both crystal forms have one molecule per asymmetric unit and are suitable for X-ray structure analysis.

Key words: coenzyme F_{430}, methanogenic Archaea, methyl-coenzyme M reductase, protein crystallization, X-ray crystallography.

Methyl-coenzyme M reductase (Mcr) catalyzes the reduction of methyl-coenzyme M (CH_3-S-CoM) by coenzyme B (H-S-CoB) to yield methane and CoM-S-S-CoB, the heterodisulfide of coenzyme M (H-S-CoM) and coenzyme B. This methane-forming reaction is the final step in the energy metabolism of all methanogenic Archaea (*1, 2*).

Mcr has a molecular mass of approximately 300 kDa. It is composed of three different subunits in an $\alpha_2\beta_2\gamma_2$ configuration and contains two molecules of coenzyme F_{430} as chromophoric prosthetic group (*1, 2*). Coenzyme F_{430} is a yellow nickel porphinoid of unique structure and properties (*3, 4*).

Methanobacterium thermoautotrophicum contains two methyl-coenzyme M reductase isoenzymes designated as Mcr I and Mcr II (*5*). The two isoenzymes resemble each other in biochemical features and primary structures (*6, 7*). Here, we report on the crystallization and preliminary X-ray analysis of Mcr I.

Mcr I from *M. thermoautotrophicum* was anaerobically purified as described previously (*8*). The purified enzyme exhibited the EPR signal MCR-ox1 (*9, 10*). The enzyme was washed by dilution and concentration in 10 mM Tris/HCl, pH 8.0. All the steps after enzyme purification were performed under aerobic conditions.

Crystallization trials were performed with the hanging drop vapor diffusion method. Initial screening using a sparse matrix crystallization screening kit (Hampton Research, Laguna Hills, CA) yielded thin needle-like crystals in drops with 2-methyl-2,4-pentandiol (MPD) as the precipitant (*11*). The crystallization conditions could be subsequently improved by means of a systematic search. Two forms of crystals, forms M and P, were obtained. The X-ray diffraction patterns were analyzed in house with a MAR-Research image-plate detector system mounted on a Rigaku RU-200 X-ray generator using double-mirror focused CuK_α radiation. The reflection intensities were evaluated with the program package, MOSFLM (*12*), and the CCP4 program suite (*13*).

Suitable crystals of form M were obtained by mixing 2 μl of the enzyme solution (12 mg/ml) and 2 μl of a reservoir solution consisting of 28% MPD, 0.15 M magnesium acetate, and 0.1 M sodium cacodylate/HCl, pH 6.0. The drop was equilibrated against 1 ml of the reservoir solution at 4°C. A yellow-coloured crystal agglomerate of Mcr grew within two months. However, single crystals with a maximum size of $0.1\times0.3\times0.5$ mm^3 could be separated from the bulk crystals with a microneedle. Form M crystallizes in space group $P2_1$, with unit cell dimensions of $a=83.2$ Å, $b=117.4$ Å, $c=125.1$ Å, and $\beta=92.6°$, as determined by autoindexing oscillation photographs with the program, REFIX (*14*). The diffraction power of the crystals was beyond a resolution of 2.8 Å. Assuming one molecule of Mcr per asymmetric unit, the crystal volume to protein mass ratio, V_M, is 2.2 Å^3/Da, and the derived solvent content is 45% (*15*). Although both values fall with in the normal ranges for water-soluble proteins, the packing density is surprisingly high for this large multisubunit protein complex.

Native data were collected for two crystals at 10°C, the R_{sym} value and completeness being 8.8 and 78%, respectively, within the resolution range between 30.0 and 2.8 Å.

The best crystals of form P were grown using 2 μl the

[1] To whom correspondence should be addressed: Ulrich Ermler, Max-Planck-Institut für Biophysik, Heinrich-Hoffmann-Straße 7, D-60528 Frankfurt, Germany. Tel: +49-69-967-69384, Fax: +49-69-967-69423, e-mail: ermler@mpibp-frankfurt.mpg.de

Abbreviations: CH_3-S-CoM, methyl-coenzyme M [2-(methylthio)-ethanesulfonate]; H-S-CoB, coenzyme B (*N*-7-mercaptoheptanoyl-threoninephosphate); Mcr, methyl-coenzyme M reductase; PEG, polyethyleneglycol; MPD, 2-methyl-2,4-pentanediol.

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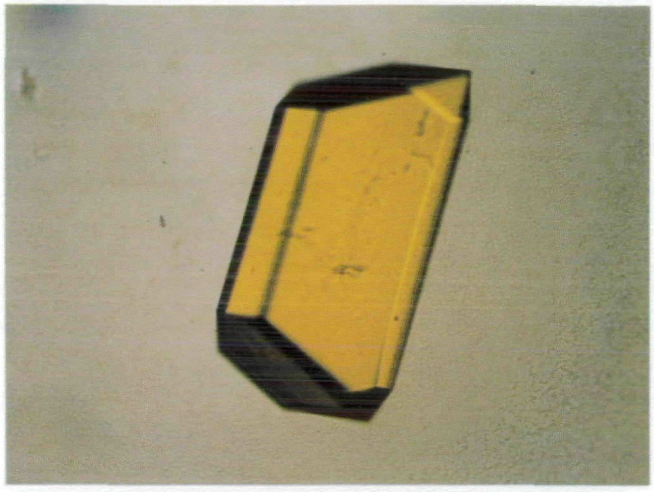

Fig. 1. **A form P crystal of methyl-CoM reductase from *M. thermoautotrophicum*.** The approximate size of the crystal is 0.3×0.4×0.8 mm³.

enzyme solution (12 mg/ml) and 2 μl a reservoir solution which contained 20% polyethylene glycol 400, 0.15 M magnesium acetate, and 0.1 M Hepes/NaOH, pH 7.5. Yellow-coloured crystals appeared within two weeks at 4°C. Again the majority of the crystals did not grow singly, but individual crystals could be manually detached. The yield and size of the crystals of form P could be improved by microseeding techniques using finely crushed crystals. The largest crystals of form P reached 0.3×0.4×0.8 mm³ in size and belonged to space group $P2_1$, with unit cell parameters of $a=83.1$ Å, $b=120.2$ Å, $c=123.1$ Å, and $\beta=91.7°$. The packing density, $V_M=2.2$ Å³/Da, is again consistent with one molecule of Mcr per asymmetric unit, resulting in a solvent content of 45%. The crystals diffracted beyond 1.7 Å resolution, which is rather unusual for a protein with a molecular mass of about 300 kDa. The identical space group and similar unit cell dimensions suggest related packing of crystal forms M and P. For crystal structure determination, form P appears to be more suitable than form M since the crystallization procedure is more reproducible and the crystals are more stable under the X-ray beam.

Native data for crystal form P were collected for 6 crystals using synchrotron radiation ($\lambda=1.1$ Å) of the Max-Planck Beamline at DESY (Deutsches Elektronensynchrotron, Hamburg). The measured 520,749 reflections were reduced to 235,557 independent reflections. The R_{sym} value of the data has been determined to be 6.7%, and the completeness to be 90.4% in the resolution range, 1.7-30.0 Å.

Structure determination by means of the isomorphous replacement method is in progress.

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