

**Crystallization and Preliminary X-ray Diffraction Analysis of
5,10-methylenetetrahydrofolate dehydrogenase/cyclohydrolase
from *Thermoplasma acidophilum* DSM 1728**

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The methylene-tetrahydrofolate dehydrogenase/cyclohydrolase (MTHFDC) from the thermoacidophilic archaeon, *Thermoplasma acidophilum*, is a 30.6 kDa molecular-weight enzyme which sequentially catalyzes the conversion of formyltetrahydrofolate to methylene-tetrahydrofolate, with a preference for NADP as a cofactor, rather than NAD. In order to elucidate the functional and structural features of MTHFDC from archaeons at a molecular level, it was overexpressed in *Escherichia coli* and crystallized in the presence of its cofactor, NADP, at 295 K using polyethylene glycol (PEG) 4000 as a precipitant. The crystal is a member of the monoclinic space group *P*21, with the following unit cell parameters: $a = 66.333 \text{ \AA}$, $b = 52.868 \text{ \AA}$, $c = 86.099 \text{ \AA}$, and $\beta = 97.570^\circ$, and diffracts to a resolution of 2.3 \AA at the synchrotron. Assuming a dimer in the crystallographic asymmetric unit, the calculated Matthews constant (V_M) was $2.44 \text{ \AA}^3/\text{Da}$ and the solvent content was 49.7 %. The coordinates of MTHFDC have R and R_{free} values of 22.1 and 29.0 % including NADP and solvents and we refine it continuously. The overall structure is similar to reported MTHFDC and its subunit is composed of two α/β domains that assemble to form a wide cleft. We expected that the cleft walls are lined with highly conserved residues and NADP is bound along one wall. The NADP-binding domain has a Rossmann folds.