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 P21, with the following unit cell parameters: $a = 66.333$ Å, $b = 52.868$ Å, $c = 86.099$ Å, and $\beta = 97.570$, and diffracts to a resolution of 2.3 Å at the synchrotron. Assuming a dimer in the crystallographic asymmetric unit, the calculated Matthews constant ($V_M$) was 2.44 Å³/Da and the solvent content was 49.7 %. The coordinate 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 $\alpha/\beta$ 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.