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Crystal structure of bifunctional 5,10-methylenetetrahydrofolate dehydrogenase/cyclohydrolase from *Thermoplasma acidophilum*

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ABSTRACT

Folate co-enzymes play a pivotal role in one-carbon transfer cellular processes. Many eukaryotes encode the tri-functional tetrahydrofolate dehydrogenase/cyclohydrolase/synthetase (deh/cyc/syn) enzyme, which consists of a N-terminal bifunctional domain (deh/cyc) and a C-terminal monofunctional domain (syn). Here, we report the first analogous archeal enzyme structures, for the bifunctional methylenetetrahydrofolate dehydrogenase/cyclohydrolase from *Thermoplasma acidophilum* (TaMTHFDC) as the native protein and also as its NADP complex. The TaMTHFDC structure is a dimer with a polar interface, as well as a NADP binding site that shows minor conformational change. The orientations of the residues in the NADP binding site do not change on ligand binding, incorporating three water molecules which are hydrogen bonded with phosphate groups of NADP in the structure of the complex. Our structural information will contribute to an improved understanding of the basis of THF and one-carbon metabolism.

1. Introduction

Reactions involving one-carbon units are vital to all organisms. One-carbon transfers mediated by folate co-enzymes play essential roles in several major cellular processes, including nucleic acid biosynthesis, mitochondrial and chloroplast protein biosynthesis, amino acid metabolism, methyl group biogenesis and vitamin metabolism. This protein is also involved in the biogenesis of many products such as choline, lignin and chlorophyll. The synthesis of numerous compounds and the regulation of many metabolic processes require the addition or removal of one-carbon units (one-carbon metabolism) [1–3].

In tetrahydrofolate (THF) metabolism, single carbon units in the oxidation states of formyl-, methenyl- and methylene-THF are interconverted by a series of three enzymes: 5,10-methylene-THF dehydrogenase (deh), 5,10-methenyl-THF cyclohydrolase (cyc) and 10-formyl THF synthetase (syn) [4]. In this metabolic pathway, many eukaryotes encode the tri-functional deh/cyc/syn enzyme, which consists of a N-terminal bifunctional domain (deh/cyc) and a C-terminal monofunctional domain (syn). Conversely, bacteria and mitochondria encode mono- or bifunctional (deh/cyc) enzymes [4–7]. Methylene-THF dehydrogenase and methenyl-THF cyclohydrolase contain enzymatic activities that interconvert 5,10-methylene-THF and 10-formyl-THF, which are utilized in oxidation and hydrolysis reactions, respectively [4,5].

Bacterial bifunctional methylene-THF dehydrogenase–cyclohydrolase is a NADP-dependent enzyme that catalyzes two sequential reactions involved in the interconversion of THF derivatives, and which is similar to the human bifunctional methylene-THF dehydrogenase–cyclohydrolase [4,7]. It has recently been reported that folate deficiency has a significant impact on the methyl cycle and is associated with an increased risk of cancer [9]. Thus, several enzymes in THF metabolism are attractive targets for the development of mechanism-based inhibitors [8,9].

Here, we report the first archeal structures of bifunctional methylenetetrahydrofolate dehydrogenase/cyclohydrolase from *Thermoplasma acidophilum* (TaMTHFDC) both in the native form and as a NADP complex. The TaMTHFDC structure is a dimer with a polar interface, as well as a NADP binding site that exhibits only a minor conformational change when associated with the ligand. The structural study presented here will contribute to an improved understanding of the basis of THF and one-carbon metabolism.

2. Materials and methods

2.1. Cloning and protein preparation

The cloning and purification of TaMTHFDC (Accession No. Q05213) was conducted as previously reported [10]. Briefly, N-terminal 6× His-tagged TaMTHFDC was cloned into the pET28a vector and expressed in *Escherichia coli* BL21(DE3). The protein was then purified via a three-step protocol involving heat-treatment and Ni-affinity chromatography, followed by size-exclusion chromatography on a HiLoad 26/60 Superdex-200 prep-grade

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Table 1
Data collection and refinement statistics.

	Native	Complex
Data collection		
Space group	$P2_1$	$P2_1$
Cell parameter *a*, *b*, *c* (Å)	66.42, 52.92, 86.04	66.33, 52.87, 86.10
β (°)	97.77	97.57
Resolution (Å)	50–2.2	50–2.4
Total reflections	83,770	96,104
Unique reflections	28,641	21,571
Completeness	94.2(78.4)	91.6(59.0)
Redundancy	2.9(1.8)	4.5(2.5)
$I/\sigma(I)$	14.24(2.38)	16.16(2.79)
[a]R_{merge} (%)	6.9(33.7)	8.0(29.3)
Refinement		
Resolution (Å)	30–2.3	30–2.4
[b]R_{work}/R_{free} (%)	19.0/25.6	18.8/24.6
Bond lengths (Å)	0.019	0.016
Bond angles (°)	1.9	1.8
[c]*Ramachandran plot* (%)		
Most favored	89.5	89.3
Additionally allowed	9.2	9.5
Generously allowed	1.1	1.3
Disallowed	0.2	

The highest resolution shell is shown in parentheses.

[a] $R_{merge} = \sum h \sum i |I(h,ii) - \langle I(h)\rangle| / \sum h \sum i I(h,ii)$, where $I(h,i)$ is the intensity of the *i*th measurement of reflection *h* and $\langle I(h)\rangle$ is the mean value of $I(h,i)$ for all *i* measurements.

[b] $R_{work} = \sum ||F_o| - |F_c|| / \sum |F_o|$, where F_o and F_c are the observed and calculated structure-factor amplitudes, respectively. R_{free} was calculated as R_{work} using a randomly selected subset (10%) of unique reflections not used for structure refinement.

[c] Categories were defined using PROCHECK.

column (GE Healthcare) using an elution buffer composed of 10 mM Tris–HCl, 50 mM KCl, and 2 mM DTT.

2.2. Crystallization, data collection and structural determination

The purified TaMTHFDC was concentrated to 1.8 mg/ml in 10 mM Tris–HCl, 50 mM KCl, and 2 mM DTT. Crystallization of the native form of TaMTHFDC (TaMTHFDC-native) was then conducted using the sitting-drop vapor-diffusion method at 22 °C in a solution of 18% PEG 4000, 400 mM NaCl, and 100 mM Tris–HCl (pH 8.0). Crystallization of the TaMTHFDC–NADP complex was conducted as described above; however, 5 mM NADP was added during crystallization, as in the previous paper [10]. For both forms, X-ray diffraction data were collected from the cooled crystal in 30% (v/v) sucrose as a cryo-protectant using beamline 4A MXW at the Pohang Light Source (PLS, South Korea). The raw data were then indexed and scaled using the HKL2000 program [11], after which an initial model was obtained using molecular replacement. The MOLREP program in the CCP4 program suite was used with a model of *E. coli* 5,10-methylene-tetrahydrofolate dehydrogenase/cyclohydrolase protein (PDB ID 1B0A) [4,12]. Refinement resolution of the final structure was re-designed against the experimental data using the SFCHECK Program [13]. The structure was then refined using the CNS and REFMAC programs [14,15]. Editing and adjustment of the model were conducted using a sigma A weighted $2F_o - F_c$, $F_o - F_c$ with the Coot program [16]. Figures were generated using PyMOL [18] The statistics for data processing and refinement of native and complex forms are listed in Table 1. The same

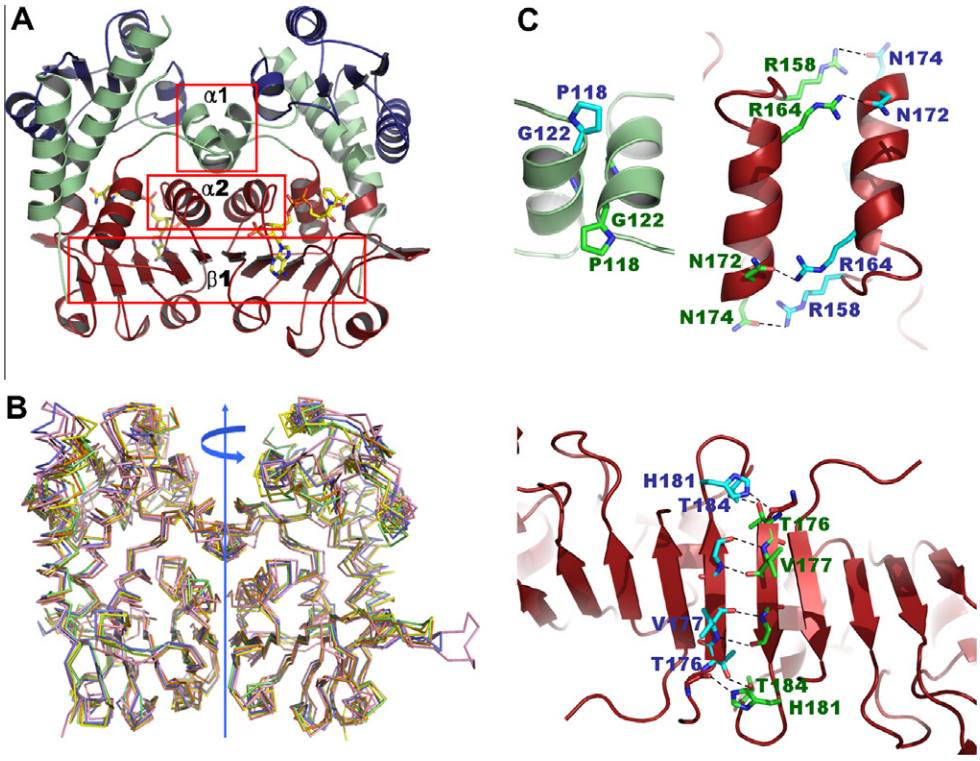

Fig. 1. (A) Overall structure of the TaMTHFDC–NADP complex dimer. The N-terminal domain (blue) predicted to have a MTHF biding site is colored in blue and the C-terminal domain (red) of the Rossmann fold with NADP binding is colored in red. Red boxes indicate regions of interface depicted in column (C). (B) Superimposition of methylenetetrahydrofolate dehydrogenase/cyclohydrolase dimers from *Human* (pink), *Escherichia coli* (blue), *Francisella* (yellow), and *Mycobacterium* (red) (RMSD of 1.85, 1.60, 1.56 and 2.03 Å, respectively with TaMTHFDC. TaMTHFDC (native: green, complex: orange) forms a dimer by non-crystallographic twofold pseudo-symmetry with a perpendicular axis. (C) α1-helixes interact via hydrophobic force contributed by conserved Pro118 and Gly122. The α2-helix is structurally and sequentially conserved and interacts via a hydrogen bond between non-conserved loop residues of Arg158 and Asn174, and Arg164 and Asn172. β-Sheets form anti-parallel β-sheet interaction and include the conserved residues, Thr176 and Val177. At the end of the sheet, conserved His181 bolsters this interaction through hydrogen bonding with the backbone oxygen on the other chain. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)