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Crystal Structure of a Predicted Precorrin-8x Methylmutase From *Thermoplasma acidophilum*

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Introduction

The biosynthesis of vitamin B_{12} involves many enzymatic steps and two evolutionarily divergent pathways. Although the pathways differ in their requirement for molecular oxygen and the timing of cobalt insertion, the precorrin-8x methylmutase genes cobH and cbiC share greater than 30% identity, and are designated as the CobH family.[1] These enzymes catalyze the methyl isomerization of metal-free (CobH) and cobalt bound (CbiC) precorrin-8x to hydrogenobyrinic acid (HBA). We have determined the 2.1-Å crystal structure of precorrin-8x methylmutase from the facultative anaerobe *Thermoplasma acidophilum* (Ta0654). Although cobalamin synthesis has not been investigated in *T. acidophilum*, the structure bears a striking resemblance to CobH from *Pseudomonas denifitricans* (Pa2905), to which it shares 33% sequence identity. The structure–function relationships for Pa2905 have been characterized, including the role of catalytic residues and crystallographic observation of the product HBA in the active site formed by dimerization.[2–4]

Methods and Results

Growth, expression, and purification of *T. acidophilum* precorrin-8x methylmutase (gene designation ta0654) were performed essentially as described by Korolev et al.[5] Seleno-L-methionine (SeMet)-substituted protein crystallized at room temperature in 14% polyethylene glycol (PEG) 3350, 0.5 *M* di-ammonium tartrate, and 4% glycerol (pH ~6.6). The crystal belongs to the orthorhombic space group $P2_12_12_1$. Multiple-wavelength anomalous dispersion (MAD) data were measured at the Structural Biology Center 191D beam-line of the Advanced Photon Source (APS), Argonne National Laboratory (ANL), and were integrated and scaled using the HKL2000 suite.[6] The asymmetric unit contained 4 protein polypeptides, with 9 SeMet per polypeptide. Twenty-four of the 36 Se atoms in the asymmetric unit were identified with SOLVE-2.02.[7] The MAD map was then subjected to density modification using the RESOLVE subroutine in SOLVE-2.02, which allowed autotracing of most main-chain atoms for polypeptide B. Polypeptides A, C, and D were modeled using monomer B prior to refinement

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using the Crystallography & NMR System (CNS-1.1).[8] After iterative rounds of model building in O[9] and refinement with CNS (simulated annealing, positional minimization, water molecule identification, individual isotropic B refinement), the final 2.1-Å model contains 765 protein residues and 627 waters. No noncrystallographic symmetry restraints were applied. Due to poor electron density, N-terminal residues A1–A16, B1–B13, C1–C15, D1–D15, and C-terminal residue 207 for all polypeptides were not included in the final model. According to PROCHECK,[10] 90.3% of residues were in the most favored Ramachandran plot region, 9.3% were in the additionally allowed region, and 0.4% were in the generously allowed region. The final R_{work} and R_{free} were 20.2% and 23.5%, respectively. A summary of data collection and refinement statistics is given in Table I.

Secondary structure

A ribbon drawing of Ta0654 is illustrated in Figure 1. The 4 polypeptides comprising the asymmetric unit form 2 dimers. Although there are slight conformational differences among monomers, the secondary structure elements are the same. Starting with residue 14 (the first modeled residue), there are 3 packed α-helices (αA, αB, αC), followed by β1 and αD. α-Helix D spans the width of the protein, packing against helices A, B, and C, and β-strands 2–6 with α-Helices G–J assume an α/β flavodoxin-like fold. The spatial order of the parallel β-strands is 3-2-4-5-6-1. α-Helices E and F (prior to β2 and β3, respectively) interact with the C-terminal helix (αK), which also packs alongside α-helix B, bridging helix bundle A, B, C with helices E and F, and thus stabilizing the protein fold.

A DALI[11] search for similar protein structures in the Protein Data Bank (PDB) returned a *Z* score of 25.2 for *P. denitrificans* precorrin-8x methyl mutase (PDB ID: 1f2v-A). Their primary sequences are 34% identical over the length of the proteins. The root-mean-square deviation (RMSD) for Cα atoms between these two proteins was 1.8 Å. The next most similar proteins, aspartate-semialdehyde dehydrogenase and corrin binding proteins, had *Z* scores below 4.6.

Sequence conservation

The position-specific iterated basic local alignment search tool (PSI-BLAST)[12] identified 74 homologs in 65 species, 14 in archaea and 60 in bacteria. Of these homologs, approximately half share greater than 30% identity with Ta0654.

Discussion

CobH family members convert precorrin-8x into HBA by methyl migration en route to the eventual biosynthesis of cobalamin. CobH family members from both aerobic (CbiC) and anaerobic organisms (CobH) share a high degree of sequence identity. These proteins are found exclusively in prokaryotes, family members belong to the cluster of orthologous group (COG) 2082.[13] Biochemical studies of this enzyme family (EC 5.4.1.2) have been limited to CobH from *P. denitrificans* (Pa2905). The 2.1-Å crystal structure of Pa2905 is also known.[4] Although the 33% identical homolog from the facultative anaeorobe *T. acidophilum* has not been functionally characterized, the high degree of structural similarity of Ta0654 reported here to the CobH structure is noteworthy and suggests functional conservation.

Ta0654 aligns with an RMSD of 1.8 Å with the apo form, and 1.9 Å with the HBA-bound form of CobH. The Cα superposition of the 2.6 Å HBA–CobH crystal complex with Ta0654 is shown in Figure 2. The CobH active site is formed by the dimer interface, and the activity of the dimer in solution has been demonstrated.[4] Ta0654 is also expected to be active as a dimer, and the 4 polypeptides in the asymmetric unit can best be described as a dimer of dimers. For clarity, only the A/D dimer is shown in Figure 2. The dimer is formed by hydrophobic or Van der Waals interactions primarily between helices αJ and αA (F165, I166, N167-S17; N167-R16)

and αJ and αC (F165-F47). In addition, αE residues interact with their symmetry related identity (M79-M79, A82-A82) or αK (M79-S192, M79-P193). Similar contact surfaces are observed in Pa2905. The N-terminal residues, although not critical for dimer formation, are responsible for approximately half of the total buried surface area in Pa2905 (3346 $Å^2$). These residues are missing in the Ta0654 structure. However, the removal of the equivalent N-terminal stretch from Pa2905 results in similar buried surface area to that of Ta0654 (1600 $Å^2$ vs 1500 $Å^2$).

The active site of Ta0654 is formed on the interface between subunits. Conserved residue methionine 79 forms the boundary between identical shared ligand-binding cavities. Several other conserved Ta0654 residues, which structurally align with Pa2905 and are expected to bind HBA, include S17, H42, A43, S86, N101, T114, R115, S116, N137, T139, S192, A196, and N200, and conservative substitutions K39, S86, and N100. Histidine 42 is conserved among all family members, and is thought to play a critical catalytic role. Interestingly, Shipman et al.[4] postulate that arginine 40 and arginine 116 of CobH Pa2905 favor nonmetal corrin binding by charge destabilization of a cobalt-bound substrate. Ta0654 has structurally equivalent residues lysine 39 and arginine 115. Additionally, tyrosine 14 of Pa2905 protrudes into the HBA ring C, perhaps sterically prohibiting a metal-bound corrin from binding. In the structure of Ta0654, there is a glutamate at this position, which does not appear to limit binding to metal-free corrins. A similar substitution is also found in the anaerobe *Salmonella typhimurium* (33 % identical to Ta0654). Whether *T. acidophilum* synthesizes vitamin B_{12} under both aerobic and anaerobic conditions has yet to be investigated.

(Note: The atomic coordinates and structure factors have been deposited in the Research Collaboratory for Structural Bioinformatics (RCSB) PDB with accession code 1OU0, RCSB018668.)

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References

1. Roper JM, Raux E, Brindley AA, Schubert HL, Gharbia SE, Shah HN, Warren MJ. The enigma of cobalamin (vitamin B_{12}) biosynthesis in *Porphyromonas gingivalis*. J Biol Chem 2000;275:40316–40323. [PubMed: 11007789]
2. Thibaut D, Couder M, Famechon A, Debussche L, Cameron B, Crouzet J, Blanche F. The final step in the biosynthesis of hydrogenobyrinic acid is catalyzed by the cobH gene product with precorrin-8x as the substrate. J Bacteriol 1992;174:1043–1049. [PubMed: 1732194]
3. Roessner CA, Spencer JB, Stolowich NJ, Wang J, Nayar GP, Santander PJ, Pichon C, Min C, Holderman MT, Scott AI. Genetically engineered synthesis of precorrin-6-x and the complete corrinoid, hydrogenobyrinic acid. Chem Biol 1994;1:119–124. [PubMed: 9383380]
4. Shipman LW, Li D, Roessner CA, Scott AI, Sacchettini JC. Crystal structure of precorrin-8x methyl mutase. Structure 2001;9:587–596. [PubMed: 11470433]
5. Korolev S, Ikeguchi Y, Skarina T, Beasley S, Edwards A, Joachimiak A, Pegg AE, Savchenko A. The crystal structure of spermidine synthase with a multisubunit adduct inhibitor. Nat Struct Biol 2001;9:27–31. [PubMed: 11731804]
6. Otwinowski Z, Minor W. Processing of X-ray diffraction data collected inoscillation mode. Meth Enzymol 1997;276:307–326.
7. Terwilliger TC, Berendzen J. Automated MAD and MIR structure solution. Acta Crystallogr D Biol Crystallogr 1999;55:849–861. [PubMed: 10089316]

8. Brünger AT, Adams PD, Clore GM, Delano WL, Gros P, Grosse-Kunstleve RW, Jiang JS, Kuszewski J, Nilges M, Pannu NS, Read RJ, Rice LM, Simonson T, Warren GL. Crystallography & NMR System: a new software suite for macromolecular structure determination. Acta Crystallogr D Biol Crystallogr 1998;54:905–921. [PubMed: 9757107]

9. Jones TA, Zou JY, Cowan SW, Kjeldgaard M. Improved methods for building protein models in electron density maps and the location of errors in these models. Acta Crystallogr A 1991;47:110–119. [PubMed: 2025413]

10. Laskowski RA, MacArthur MW, Moss DS, Thornton JM. PROCHECK: a program to check the stereochemical quality of protein structures. J Appl Crystallogr 1993;26:283–291.

11. Holm L, Sander C. Protein structure comparison by alignment of distance matrices. J Mol Biol 1993;233:123–138. [PubMed: 8377180]

12. Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 1997;25:3389–3402. [PubMed: 9254694]

13. Tatusov RL, Natale DA, Garkavtsev IV, Tatusova TA, Shankavaram UT, Rao BS, Kiryutin B, Galperin MY, Fedorova ND, Koonin EV. The COG database: new developments in phylogenetic classification of proteins from complete genomes. Nucleic Acids Res 1991;29:22–28. [PubMed: 11125040]

14. Kraulis PJ. MOLSCRIPT—a program to produce both detailed and schematic plots of protein structures. J Appl Crystallogr 1991;24:946–950.

15. Merritt EA, Bacon DJ. Raster3D photorealistic molecular graphics. Methods Enzymol 1993;277:505–524. [PubMed: 18488322]

Fig. 1.
Ribbon drawing of the *T. acidophilum* precorrin-8x methylmutase. Secondary structure elements are colored from N- to C-terminus, blue to red. Figure generated by MOLSCRIPT[14] and Raster3D.[15]

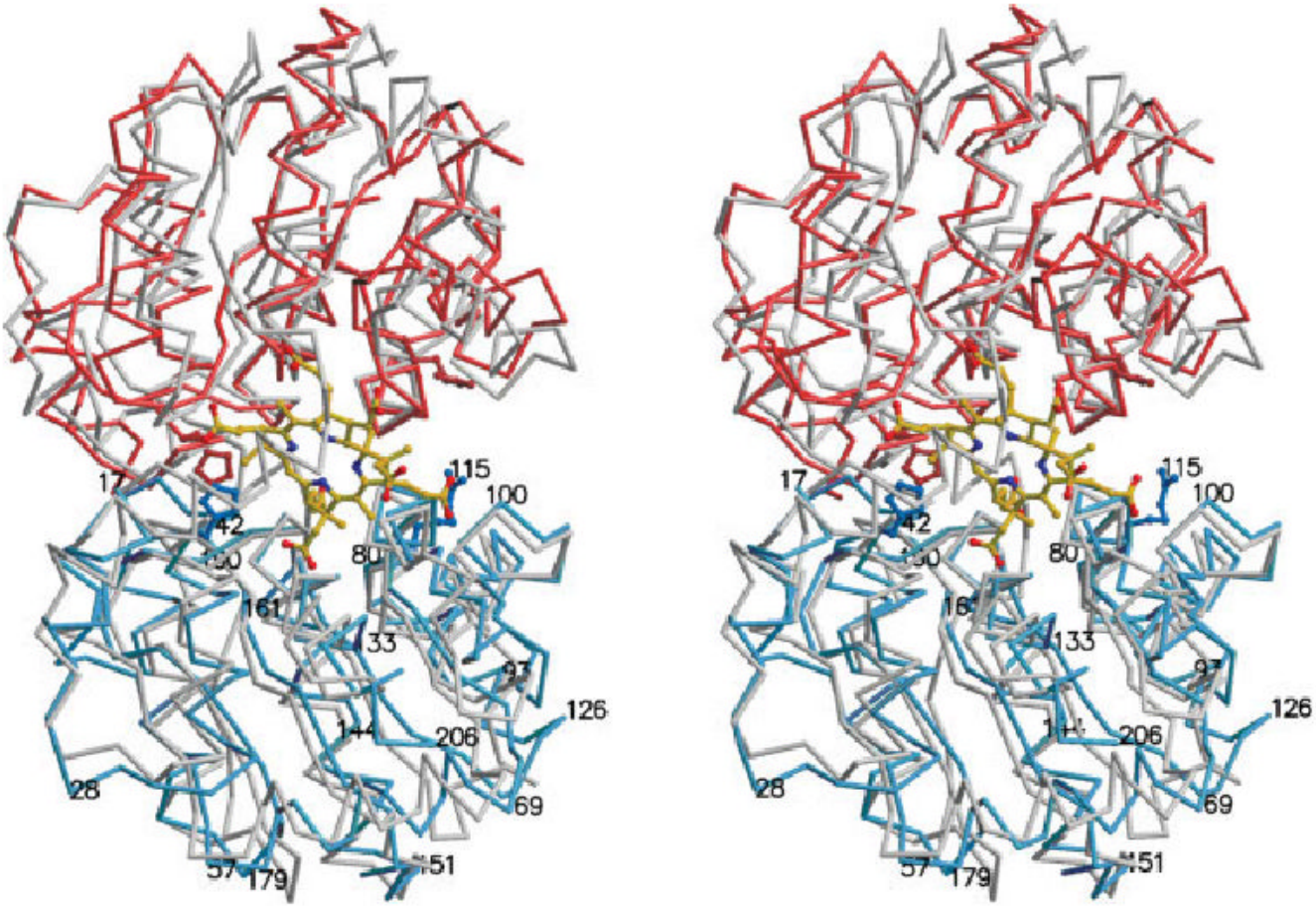

Fig. 2.

Cα superposition of the *T. acidophilum* structure and *P. denitrificans* precorrin-8x methylmutase with product bound. For simplicity, only the A/D dimer is shown, and Cα positions numbered for polypeptide A (colored blue). In the view shown, the 2-fold axis is horizontal. The Pa2905 dimer (the biologically relevant oligomerization state) is colored tan. The active site is formed by the dimer interface, as evidenced by the bound precorrin product hydrogenobyrinic acid (HBA) colored yellow, with nitrogens colored blue, and oxygens red. The Pa2905 dimer was generated by crystallographic 2-fold symmetry. For clarity, only HBA observed in the asymmetric unit is shown. Figure generated by MOLSCRIPT[14] and Raster3D.[15]

TABLE I
Summary of Data Collection and Refinement Statistics

Crystal Details				
Unit cell parameters	a = 72.45 Å, b = 86.82, c = 120.90, α,β,γ = 90.0°			
Space group	$P2_12_12_1$			
Protein MW (207 amino acids, SeMet)	22,819 Daltons			
Protein Molecules per A. U.	4			
SeMet per A. U.	36			
Data Collection and Reduction				
Wavelength (Å)	Edge	Peak	Low	Low high resolution scan (no anomalous)
	0.97891	0.97878	0.99187	0.99187
Resolution limit (Å)	2.35	2.30	2.30	2.00
No. of unique reflections	60710	65235	65722	52396
% completeness	99.8	86.3	99.8	100
I/σ	2.34	1.84	2.47	2.78
% R_{merge} *	11.2	10.0	6.2	7.0
Refinement				
Resolution range (Å)	37.4–2.1			
No. unique reflections	43, 633			
% R_{work} (% R_{free})	20.2 (23.5)			
RMSD	Bonds 0.006 Å, angles 1.2°			
No. of protein residues	765 of 828			
No. of of other molecules	627 waters			
Mean B factor (Å^2)	41.49			

* $R_{merge} = \Sigma(|I - I)/\Sigma I$.

A.U., asymmetric unit.