Published in final edited form as:

J Struct Funct Genomics. 2009 September ; 10(3): 249–253. doi:10.1007/s10969-009-9059-x.

X-Ray crystal structure of GarR—tartronate semialdehyde reductase from *Salmonella typhimurium*

J. Osipiuk,

Biosciences Division, Midwest Center for Structural Genomics, Argonne National Laboratory, 9700 South Cass Ave., Bldg 202, Argonne, IL 60439, USA

M. Zhou,

Biosciences Division, Midwest Center for Structural Genomics, Argonne National Laboratory, 9700 South Cass Ave., Bldg 202, Argonne, IL 60439, USA

S. Moy,

Biosciences Division, Midwest Center for Structural Genomics, Argonne National Laboratory, 9700 South Cass Ave., Bldg 202, Argonne, IL 60439, USA

F. Collart, and

Biosciences Division, Midwest Center for Structural Genomics, Argonne National Laboratory, 9700 South Cass Ave., Bldg 202, Argonne, IL 60439, USA

A. Joachimiak

Biosciences Division, Midwest Center for Structural Genomics, Argonne National Laboratory, 9700 South Cass Ave., Bldg 202, Argonne, IL 60439, USA. Biosciences Division, Structural Biology Center, Argonne National Laboratory, 9700 South Cass Ave., Bldg 202, Argonne, IL 60439, USA. andrzej@anl.gov

Abstract

Tartronate semialdehyde reductases (TSRs), also known as 2-hydroxy-3-oxopropionate reductases, catalyze the reduction of tartronate semialdehyde using NAD as cofactor in the final stage of D-glycerate biosynthesis. These enzymes belong to family of structurally and mechanically related β -hydroxyacid dehydrogenases which differ in substrate specificity and catalyze reactions in specific metabolic pathways. Here, we present the crystal structure of GarR a TSR from *Salmonella typhimurium* determined by the single-wavelength anomalous diffraction method and refined to 1.65 Å resolution. The active site of the enzyme contains L-tartrate which most likely mimics a position of a glycerate which is a product of the enzyme reaction. The analysis of the TSR structure shows also a putative NADPH binding site in the enzyme.

Keywords

2-Hydroxy-3-oxopropionate reductases; Tartronate semialdehyde; D-Glycerate; Biosynthesis; X-Ray crystal structure

Correspondence to: A. Joachimiak.

The submitted manuscript has been created by UChicago Argonne, LLC, Operator of Argonne National Laboratory ("Argonne"). Argonne, a US Department of Energy Office of Science laboratory, is operated under Contract No. DE-AC02-06CH11357. The US Government retains for itself, and others acting on its behalf, a paid-up nonexclusive, irrevocable worldwide license in said article to reproduce, prepare derivative works, distribute copies to the public, and perform publicly and display publicly, by or on behalf of the Government.

Introduction

Tartronate semialdehyde reductases (TSRs) catalyze the reduction of tartronate semialdehyde using NAD as cofactor in the final stage of D-glycerate biosynthesis (Fig. 1a) [1]. The enzymes are widely distributed in Eu-bacteria and have some sequence homologs in Archaea and Eukaryota [2]. They belong to COG2084 (Clusters of Orthologous Groups, <http://www.ncbi.nlm.nih.gov/COG/>) currently consisting of 79 proteins which are described as 3-hydroxyisobutyrate dehydrogenase and related β -hydroxyacid dehydrogenases. TSRs belong to family of structurally and mechanically related β -hydroxyacid dehydrogenases which differ in substrate specificity and catalyze reactions in specific metabolic pathways (for review, see [3]). Sequence and mutational analysis identified four distinct functional sequence motifs [3]. The most conserved is the N-terminal cofactor binding motif consisting of highly conserved glycine and several hydrophobic residues. This motif was predicted to specifically bind NAD cofactor. The substrate-binding motif is the most distinctive feature of β -hydroxyacid dehydrogenases. A highly conserved catalytic motif consists several glycines and catalytic lysine which was found essential for enzyme activity [4]. The C-terminal cofactor binding motif is strongly conserved in bacteria but only 2 residues, lysine and glycine, are conserved in all β -hydroxyacid dehydrogenases family members.

Here, we report the crystal structure of GarR a TSR from *Salmonella typhimurium*. The structure has been determined by the single-wavelength anomalous diffraction (SAD) method and refined to 1.65 Å resolution. The selenomethionine (SeMet) derivative of TSR crystallized in the I222 space group with unit cell dimensions of $a = 55.28$ Å, $b = 105.43$ Å, $c = 155.04$ Å, $\alpha = \beta = \gamma = 90.00^\circ$. The active site of the enzyme includes L-tartrate (Fig. 1b) which was present in crystallization solution.

Methods

Protein cloning expression and purification

The open reading frame of *garR* gene, coding TSR protein, was amplified by PCR from genomic *S. typhimurium* LT2 DNA. The gene was cloned using the ligation independent cloning method into the pMCSG7 cloning vector [5] containing a TEV protease cleavage site. The fusion protein was overexpressed in *E. coli* BL21-Gold (DE3) (Stratagene) harboring an extra plasmid encoding three rare tRNAs (AGG and AGA for Arg, ATA for Ile). The cells were grown in minimal medium M9 at 37°C to an OD₆₀₀ of ~0.8 and protein expression induced with 0.1 mM IPTG in the presence of six inhibitory amino acids and SeMet [6]. After induction, the cells were incubated overnight with shaking at 20°C. The harvested cells were re-suspended in binding buffer (500 mM NaCl, 5% glycerol, 50 mM HEPES pH 8.0, 10 mM imidazole, 10 mM 2-mercaptoethanol), flash-frozen in liquid N₂ and stored at -70°C. Thawed cells were lysed by sonication after the addition of protease inhibitor cocktail (Sigma). The lysate was clarified by centrifugation (30 min at 27000g) and applied to a metal chelate affinity-column charged with Ni²⁺. The hexa-histidine fusion protein was eluted from the column in elution buffer (500 mM NaCl, 5% glycerol, 50 mM HEPES pH 8.0, 250 mM imidazole, 10 mM 2-mercaptoethanol) and the tag then cleaved from the protein by treatment with recombinant His-tagged TEV protease. The cleaved protein was then resolved from the cleaved His-tag and the His-tagged protease by flowing the mixture through a second Ni²⁺-column. The TSR protein was dialyzed in 10 mM HEPES pH 8.0, 200 mM NaCl, and concentrated using a BioMax concentrator (Millipore).

Protein crystallization

The protein was crystallized by vapor diffusion in 96-well sitting-drop plates by mixing 0.5 µl of the protein solution (107 mg/ml) with 0.5 µl of 0.1 M imidazole buffer pH 8.0, 1 M

potassium/sodium L-tartrate and 0.2 M sodium chloride, and equilibrated at 16°C over 135 μ l of this solution. Crystals, which appeared overnight, were flash-frozen in liquid nitrogen with crystallization buffer plus 20% glycerol as cryoprotectant prior to data collection.

Data collection

Diffraction data were collected at 100 K at the 19BM beamline of the Structural Biology Center at the Advanced Photon Source, Argonne National Laboratory. The single-wavelength inverse-beam SAD data set (at peak wavelength, 0.97957 Å) was collected from a SeMet-labeled protein crystal using SBC3 CCD detector. The crystal diffracted up to 1.65 Å resolution and belonged to I222 space group with a cell dimension of $a = 55.28$ Å, $b = 105.43$ Å, $c = 155.04$ Å, and $\alpha = \beta = \gamma = 90^\circ$. Because of size and square detector geometry the collected data are complete only to 1.9 Å (edge of the detector). However, high signal-to-noise data were recorded at detector corners (although not complete). Therefore we included all collected data in data processing and refinement. All data were processed and scaled with HKL2000 [7] to an R-merge of 10.4% (Tables 1, 2).

Structure determination and refinement

Heavy atom sites were found by SHELXD program [8] and structure was phased using SOLVE [9]. The initial model was built by RESOLVE program [10] and then model was extended with the wARP program [11]. After one round of refinement with CNS suite [12] model was manually adjusted using the program O [13]. The refinement of the structure was completed using the REFMAC 5 [14] of the CCP4 program suite [15]. The final model was analyzed and validated with PROCHECK [16] and WHATCHECK [17]. Structure similarity analysis was performed using DALI server [18].

Accession numbers

Atomic coordinates and structure factors have been deposited into the Protein Data Bank (PDB) as 1TEA (recently updated and replaced by 1VPD).

Results and discussion

The overall structure of *S. typhimurium* TSR consists of two structural domains and four previously identified functional motifs can now be mapped to these domains (Fig. 2a). N-terminal domain (residues 3–162) is formed by a 9-strand β -sheet surrounded by nine α -helices and resembles FAD/NAD(P)-binding Rossmann fold superfamily. This domain includes N-terminal cofactor binding motif (residues 6–25) and substrate binding motif (residues 119–132). The C-terminal domain (residues 164–296) is formed exclusively by seven α -helices with one of them (helix 166–199) extruding outside the main body of the domain and reaching toward the N-terminal domain with a possible hinge at Gly163. This domain contains a catalytic motif (residues 163–176) and the C-terminal part of the cofactor binding motif (residues 240–247). The L-tartrate molecule was found bound between two structural domains. It interacts with Ser123-Gly124-Gly125, a part of the substrate-binding domain, and Gln176 and Lys172, part of the catalysis domain (Fig. 2b). There is also indirect van der Waals contact through water molecule with Met13 from the N-terminal cofactor-binding motif, and water mediated hydrogen bonds with Ser 97 and Asp 241 from C-terminal cofactor-binding motif. The orientation of the L-tartrate molecule suggests that C4 carboxylate of L-tartrate in our structure provides the majority of contacts with the protein.

Structure similarity search using the program DALI [18] showed that the *S. typhimurium* TSR structure closely resembles two other structures in Protein Data Bank (PDB): tartronic semialdehyde reductase from *S. typhimurium* (PDB id: 1YB4) and hydroxyisobutyrate dehydrogenase from *Thermus thermophilus* HB8 (PDB id: 1J3 V). The first structural homolog

is an enzyme catalyzing the same reaction in *S. typhimurium* as the GarR. Two enzymes share 41% sequence identity. The structural homology of two structures is very high with a Z-score of 38.3 (the strength of structural similarity in standard deviations above expected) and RMSD (of superimposed atoms) equal to 2.8 Å for all residues. Tartronic semialdehyde reductase structure contains a protein dimer in asymmetric unit which likely represents a putative biological unit of the enzyme based on PISA [19] results (Protein Interfaces, Surfaces and Assemblies service at European Bioinformatics Institute (http://www.ebi.ac.uk/msd-srv/prot_int/pistart.html)). Very similar dimer is formed by the GarR, two monomers are related by two fold crystallographic symmetry. Interface between two GarR molecules in the dimer has buried area of 6130 Å² based on PISA analysis [19].

The second structural homolog, hydroxyisobutyrate dehydrogenase, has structural homology to our structure with a Z-score of 27.2 and RMSD equal to 1.5 Å for all residues. These two proteins share 34.7% sequence identity. The hydroxyisobutyrate dehydrogenase structure contains NADPH molecule (dihydro-nicotinamide-adenine-dinucleotide phosphate) bound to protein. The NADPH interacts with cofactor-binding domain of *T. thermophilus* protein. The superposition of two structures places reactive site of nicotinamide ring directly next to L-tartrate in the TSR structure (Fig. 3). Tartrate terminal oxygen O11 at carbon C1 is located only 1.37 Å from reactive carbon C2 of nicotinamide ring. The real TSR substrate is tartronate semialdehyde which is shorter by carboxyl group. The oxygen O2 at carbon C2 in our structure may mimic the real terminal oxygen of tartronate. This oxygen is only 2.64 Å apart from NADPH reactive site in superposed structures. This observation strongly suggests that L-tartrate binding in our structure serves as a good model for binding tartronate to the enzyme and proper positioning substrate for enzymatic reaction. Nevertheless, experiments with an exact substrate for TSR protein, tartronate semialdehyde, should be performed for more precise results.

Acknowledgments

We wish to thank all members of the Structural Biology Center at Argonne National Laboratory for their help in conducting experiments. This work was supported by National Institutes of Health Grant GM62414, GM074942 and by the US Department of Energy, Office of Biological and Environmental Research, under contract DE-AC02-06CH11357.

References

1. Gotto AM, Kornberg HL. The metabolism of C2 compounds in micro-organisms. 7. Preparation and properties of crystalline tartronic semialdehyde reductase. *Biochem J* 1961;81:273–284. [PubMed: 13900766]
2. Njau RK, Herndon CA, Hawes JW. Novel beta-hydroxyacid dehydrogenases in *Escherichia coli* and *Haemophilus influenzae*. *J Biol Chem* 2000;275(49):38780–38786.10.1074/jbc.M007432200 [PubMed: 10978349]
3. Njau RK, Herndon CA, Hawes JW. New developments in our understanding of the beta-hydroxyacid dehydrogenases. *Chem Biol Interact* 2001;130–132(1–3):785–791.10.1016/S0009-2797(00)00234-9
4. Adams MJ, Ellis GH, Gover S, Naylor CE, Phillips C. Crystallographic study of coenzyme, coenzyme analogue and substrate binding in 6-phosphogluconate dehydrogenase: implications for NADP specificity and the enzyme mechanism. *Structure* 1994;2(7):651–668.10.1016/S0969-2126(00)00666-6 [PubMed: 7922042]
5. Stols L, Gu M, Dieckman L, Raffin R, Collart FR, Donnelly MI. A new vector for high-throughput, ligation-independent cloning encoding a tobacco etch virus protease cleavage site. *Protein Expr Purif* 2002;25(1):8–15.10.1006/prep.2001.1603 [PubMed: 12071693]
6. Walsh MA, Dementieva I, Evans G, Sanishvili R, Joachimiak A. Taking MAD to the extreme: ultrafast protein structure determination. *Acta Crystallogr D Biol Crystallogr* 1999;55(Pt 6):1168–1173.10.1107/S0907444999003698 [PubMed: 10329779]

7. Otwinowski Z, Minor W. Processing of X-ray diffraction data collected in oscillation mode. *Macromolecular Crystallography A* 1997;276:307–326.10.1016/S0076-6879(97)76066-X
8. Schneider TR, Sheldrick GM. Substructure solution with SHELXD. *Acta Crystallogr D Biol Crystallogr* 2002;58(Pt 2 Pt 10):1772–1779. [PubMed: 12351820]
9. Terwilliger TC, Berendzen J. Automated MAD and MIR structure solution. *Acta Crystallogr D Biol Crystallogr* 1999;55(Pt 4):849–861.10.1107/S0907444999000839 [PubMed: 10089316]
10. Terwilliger TC. Automated structure solution, density modification and model building. *Acta Crystallogr D Biol Crystallogr* 2002;58(Pt 11):1937–1940.10.1107/S0907444902016438 [PubMed: 12393925]
11. Perrakis A, Harkiolaki M, Wilson KS, Lamzin VS. ARP/wARP and molecular replacement. *Acta Crystallogr D Biol Crystallogr* 2001;57(Pt 10):1445–1450.10.1107/S0907444901014007 [PubMed: 11567158]
12. Brunger 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(Pt 5):905–921.10.1107/S0907444998003254 [PubMed: 9757107]
13. Jones TA, Zou JY, Cowan SW, Kjeldgaard. Improved methods for building protein models in electron density maps and the location of errors in these models. *Acta Crystallogr A* 1991;47(Pt 2):110–119.10.1107/S0108767390010224 [PubMed: 2025413]
14. Murshudov GN, Vagin AA, Lebedev A, Wilson KS, Dodson EJ. Efficient anisotropic refinement of macromolecular structures using FFT. *Acta Crystallogr D Biol Crystallogr* 1999;55(Pt 1):247–255.10.1107/S090744499801405X [PubMed: 10089417]
15. Collaborative Computational Project N. The CCP4 suite: programs for protein crystallography. *Acta Crystallogr D Biol Crystallogr* 1994;50(Pt 5):760–763.10.1107/S0907444994003112 [PubMed: 15299374]
16. Laskowski RA, MacArthur MW, Thornton JM. Validation of protein models derived from experiment. *Curr Opin Struct Biol* 1998;8(5):631–639.10.1016/S0959-440X(98)80156-5 [PubMed: 9818269]
17. Network E-DV. Who checks the checkers? Four validation tools applied to eight atomic resolution structures. *J Mol Biol* 1998;276(2):313–525.10.1006/jmbi.1997.1540 [PubMed: 9512703]
18. Holm L, Sander C. Protein structure comparison by alignment of distance matrices. *J Mol Biol* 1993;233(1):123–138.10.1006/jmbi.1993.1489 [PubMed: 8377180]
19. Krissinel E, Henrick K. Inference of macromolecular assemblies from crystalline state. *J Mol Biol* 2007;372:774–797.10.1016/j.jmb.2007.05.022 [PubMed: 17681537]
20. Kraulis PJ. Similarity of protein G and ubiquitin. *Science* 1991;254(5031):581–582.10.1126/science.1658931 [PubMed: 1658931]

Fig. 1.
a Reaction catalyzed by tartronate semialdehyde reductase (TSR). **b** A L-tartrate molecule used in crystallization of TSR protein

Fig. 2.

a Ribbon diagram of *S. typhimurium* TSR protein structure. N- to C-terminal domains are colored red and cyan respectively and N- and C-termini are marked. Functional motifs are mapped on the structure with different colors: orange for N-terminal cofactor binding motif (residues 6–25), dark magenta for substrate binding motif (residues 119–132), light magenta for catalytic motif (residues 163–176) and dark orange for C-terminal part of cofactor binding motif (residues 240–247). The L-tartrate molecule is shown in CPK sticks. Numbers in black correspond to protein residue positions. The diagram was generated using PyMol [20]. **b** Schematic representation of secondary elements in TSR protein structure. TSR residues interacting L-tartrate molecule are marked with red dots. Diagram was generated based on secondary structure analysis by PDBsum server at <http://www.ebi.ac.uk/thornton-srv/databases/pdbsum/>

Fig. 3.

The putative orientation of cofactor and substrate in the active site of *S. typhimurium* TSR protein. The structure of *T. thermophilus* hydroxyisobutyrate dehydrogenase (PDB id: 1J3 V) (yellow and orange) was superposed over *S. typhimurium* TSR structure (grey and CPK colors, residue description in grey). The NADPH molecule from 1J3 V structure is shown in green (the reactive site is marked in magenta). The L-tartrate from TSR structure is shown in blue. The figure was generated by the Accelrys DS ViewerPro 5.0 program

Table 1

Crystal and data collection statistics

X-Ray wavelength	0.97957 Å
Space group	I222
Unit cell dimensions	a = 55.28 Å, b = 105.43 Å, c = 155.04 Å, $\alpha = \beta = \gamma = 90^\circ$
Resolution	40–1.65 Å (1.69–1.65 Å)
No. of unique reflections	48128 (1512)
Completeness	87.2% (44.2%)
$I/\sigma(I)$	34.6 (2.75)
R-Merge	0.104 (0.324)
Molecules per asymmetric unit	1
No. of protein residues	296
SeMet per asymmetric unit	16

Table 2

Structure refinement statistics

Resolution range (Å)	40–1.65 Å
Reflections	48124
σ cutoff	None
R-Value (R-work) (%)	12.0
Free R-value (%)	14.4
R-Value (all) (%)	12.1
Rms deviations from ideal geometry	
Bond length (Å)	0.011
Angle (degrees)	1.322
Chiral (Å)	0.086
No. of atoms	
Protein	2226
L-Tartrate	10
Chloride ion	1
Water	497
Mean B-factor (Å ²)	
All atoms	21.6
Protein atoms	17.9
Protein main chain	16.5
Protein side chain	19.5
L-Tartrate	19.4
Chloride ion	10.4
Water	38.3
Ramachandran plot statistics	
Residues in most favored regions (%)	96.8
Residues in additional allowed regions (%)	3.2
Residues in disallowed region (%)	0.0