Structure of dihydrodipicolinate synthase from the commensal bacterium *Bacteroides thetaiotaomicron* at 2.1 Å resolution

Nicholas Mank, Amy Arnette, Vince Klapper, Lesa Offermann and Maksymilian Chruszcz*

Received 12 January 2015

Accepted 5 March 2015

Edited by T. C. Terwilliger, Los Alamos National Laboratory, USA

Keywords: dihydrodipicolinate synthase; *Bacteroides thetaiotaomicron*.

PDB reference: dihydrodipicolinate synthase, 4xky

Supporting information: this article has supporting information at journals.iucr.org/f

Department of Chemistry and Biochemistry, University of South Carolina, JM Palms Center for Graduate Science Research, 631 Sumter Street, Columbia, SC 29208, USA. *Correspondence e-mail: chruszcz@mailbox.sc.edu

Dihydrodipicolinate synthase (DapA) catalyzes the first committed step of the diaminopimelate biosynthetic pathway of lysine. It has been shown to be an essential enzyme in many bacteria and has been the subject of research to generate novel antibiotics. However, this pathway is present in both pathogenic and commensal bacteria, and antibiotics targeting DapA may interfere with normal gut colonization. *Bacteroides thetaiotaomicron* is a Gram-negative commensal bacterium that makes up a large proportion of the normal microbiota of the human gut. The structure of DapA from *B. thetaiotaomicron* (BtDapA) has been determined. This structure will help to guide the generation of selectively active antibiotic compounds targeting DapA.

1. Introduction

Dihydrodipicolinate synthase (EC 4.3.3.7, transferred from EC 4.2.1.52; DapA) catalyzes the first committed step in the diaminopimelate biosynthetic pathway of lysine. It catalyzes the condensation of pyruvate and aspartate β -semialdehyde to form 2,3-dihydrodipicolinate or 4-hydroxy-2,3,4,5-tetrahydrodipicolinate (Karsten, 1997; Devenish *et al.*, 2010). This is performed in a multistep process beginning with Schiff-base formation between pyruvate and the ϵ -nitrogen of lysine followed by nucleophilic attack by the enamine form of the Schiff base on aspartate β -semialdehyde (Boughton *et al.*, 2012; Blickling *et al.*, 1997). The conversion ends with cyclization by transamination and the release of 4-hydroxy-2,3,4,5-tetrahydrodipicolinate. It is unclear whether 4-hydroxy-2,3,4,5-tetrahydrodipicolinate is spontaneously converted to 2,3-dihydrodipicolinate or whether the conversion is performed by dihydrodipicolinate reductase (Devenish *et al.*, 2010). DapA has been shown to be essential in many bacteria owing to the need for lysine or *meso*-diaminopimelate in the cross-linking of cell-wall peptidoglycan (Hutton *et al.*, 2007). The absence of this pathway in animals and its essentiality in many pathogenic bacteria make it a very attractive target for the generation of novel antibiotics.

Colonization of the gut by commensal bacteria is critical for human health (Maier *et al.*, 2015). *Bacteroides thetaiotaomicron* is a Gram-negative commensal bacterium commonly found in the human gut microbiota. It has undergone extensive evolution to adapt to conditions in its environment and is known to be involved in the breakdown of otherwise indigestible plant polysaccharides (Hooper *et al.*, 2002). It has also

Table 1

Data collection and processing.

Values in parentheses are for the outer shell.

Diffraction source	SER-CAT 22-ID, APS
Wavelength (Å)	1.000
Temperature (K)	100
Detector	Rayonix MX300HS
Crystal-to-detector distance (mm)	280
Rotation range per image (°)	1
Total rotation range (°)	200
Exposure time per image (s)	1
Space group	$P2_12_12_1$
a, b, c (Å)	67.90, 91.99, 208.56
α, β, γ (°)	90, 90, 90
Mosaicity (°)	0.3
Resolution range (Å)	50.00–2.10 (2.14–2.10)
σ Cutoff	–3
Total No. of reflections	616664
No. of unique reflections	76582 (3759)
Completeness (%)	99.8 (100)
Multiplicity	8.1 (8.3)
$\langle I/\sigma(I) \rangle$	25.4 (2.4)
$R_{\text{r.i.m.}}$	0.097 (0.765)
$R_{\text{p.i.m.}}$	0.034 (0.264)
Overall B factor from Wilson plot (Å ²)	35.2

been implicated in generating innate immune responses in the human gut (Hooper *et al.*, 2003) and is involved in gut angiogenesis (Stappenbeck *et al.*, 2002). The structure of BtDapA (PDB entry 4xky) could help to guide the generation of novel antibiotics that do not interfere with normal *B. thetaiotaomicron* colonization.

2. Materials and methods

2.1. BtDapA production and crystallization

DapA from *B. thetaiotaomicron* VPI-5482 was purchased from DNASU Plasmid Repository (Tempe, Arizona, USA). The *dapA* gene (codons 6–305 of 309) was cloned into the bacterial expression vector pSGX3, which contains an inducible *lac* operon and a C-terminal polyhistidine tag. The plasmid was transformed into *Escherichia coli* strain BL21(DE3). Cultures were grown in LB broth containing kanamycin to an OD₆₀₀ of 0.6–0.8 and were induced with 0.5 mM isopropyl β -D-1-thiogalactopyranoside (IPTG). The cultures were grown at 16°C overnight and the bacteria were collected by centrifugation at 10 000g for 20 min. The bacterial pellet was resuspended in 50 mM Tris, 500 mM NaCl, 10 mM imidazole pH 8.0 containing protease-inhibitor cocktail (Pierce, Rockford, Illinois, USA) and lysed by sonication. Cell debris was then pelleted by centrifugation at 4000g for 20 min and Ni-NTA agarose beads (Qiagen, Chatsworth, California, USA) were added to the supernatant. The slurry was mixed overnight at 4°C. The beads were then collected, washed with 50 mM Tris, 500 mM NaCl, 30 mM imidazole pH 8.0 and DapA was eluted using 50 mM Tris, 500 mM NaCl, 300 mM imidazole pH 8.0. The fractions containing protein were then dialyzed against 10 mM Tris, 150 mM NaCl, 10 mM pyruvate pH 7.5.

Crystallization experiments were performed at room temperature using the sitting-drop vapor-diffusion method in

Table 2

Structure solution and refinement.

Values in parentheses are for the outer shell.

Resolution range (Å)	48.62–2.10 (2.15–2.10)
Completeness (%)	99.1 (90.8)
σ Cutoff	None
No. of reflections, working set	72649 (4877)
No. of reflections, test set	3846 (245)
Final R_{cryst}	0.184 (0.213)
Final R_{free}	0.220 (0.256)
Cruickshank DPI	0.2109
No. of non-H atoms	
Protein	9178
Ligand	5
Solvent	515
Total	9698
R.m.s. deviations	
Bonds (Å)	0.010
Angles (°)	1.528
Average B factors (Å ²)	
Protein	27.71
Ligand	47.05
Ramachandran plot	
Most favored (%)	93.6
Allowed (%)	6.4
Visible residues	
Chain A	1–299
Chain B	1–298
Chain C	1–299
Chain D	1–300

96-well sitting-drop plates (Molecular Dimensions, Alamonte Springs, Florida, USA). A solution of recombinant BtDapA was mixed with well solution from a custom carboxylic acid screen (Offermann *et al.*, 2014). Crystals were grown in 0.1 M succinate pH 7.0, 0.1 M acetate, 0.8 M NaCl, 10 mM 5-aminoisophthalic acid, 15% (w/v) PEG 3350.

2.2. Data collection and processing

Crystals were cryoprotected with well solution and cooled in liquid N₂. Data were collected from a single crystal at 100 K on the SER-CAT 22-ID beamline at the Advanced Photon Source (APS), Argonne National Laboratory (ANL), USA. Data were processed using *HKL-2000* (Otwinowski & Minor, 1997). The data-collection and processing statistics are summarized in Table 1.

2.3. Structure solution and refinement

The BtDapA structure was solved by molecular replacement. Several different DapA models were used for molecular replacement and the structure of *Rhodopseudomonas palustris* DapA (PDB entry 3eb2; New York SGX Research Center for Structural Genomics, unpublished work) resulted in the best initial BtDapA model, as judged by R and R_{free} values. Molecular replacement was performed using *HKL-3000* (Minor *et al.*, 2006) integrated with *MOLREP* (Vagin & Teplyakov, 2010) and the *CCP4* program suite (Winn *et al.*, 2011). The initial model was rebuilt using *Buccaneer* (Cowtan, 2006), subsequent rebuilding was completed using *Coot* (Emsley & Cowtan, 2004) and the model was refined with *REFMAC5* (Murshudov *et al.*, 2011). The structure was validated using *MolProbity* (Chen *et al.*, 2010), *ADIT* (Yang *et al.*,

2004) and *CheckMyMetal* (Zheng *et al.*, 2014). Structure-resolution and refinement results are summarized in Table 2.

3. Results and discussion

The structure of BtDapA belonged to space group $P2_12_12_1$ and the structure was determined to a resolution of 2.1 Å. Successive rounds of manual building and restrained refinement resulted in an R_{cryst} of 0.184 and an R_{free} of 0.220. The asymmetric unit of BtDapA contains four polypeptide chains that form the functional tetramer observed for homologs of DapA from other species and is shown in Fig. 1(b) (Boughton *et al.*, 2012; Kefala *et al.*, 2008; Conly *et al.*, 2014). Sequence alignment of BtDapA with DapA sequences from *R. palustris* (RpDapA), *Mycobacterium tuberculosis* (MtDapA), *Streptococcus pneumoniae* (SpDapA), *Campylobacter jejuni* (CjDapA) and *E. coli* (EcDapA) is shown in Fig. 2 along with the percentage identity between sequences. Despite relatively low sequence identities, all of the analyzed proteins share the same overall fold, with root-mean-square deviation (r.m.s.d.)

values ranging from 1.8 to 2.2 Å after alignment of their C $^{\alpha}$ atoms.

There are two distinct interfaces in BtDapA; the first is a tight interface forming the active sites and the second is the tetramerization interface (Fig. 1b; red and green arrows, respectively). Fig. 1(a) is colored to indicate the DapA (β/α) $_8$ -barrel, or TIM barrel, characterized by alternating α/β secondary structures. The polyhistidine tags are completely absent from the model as there is no electron density that corresponds to these parts of the polypeptide chains. The S atom of the N-terminal methionine of chain A appears to be oxidized, while it is reduced in all other chains. The structure also contains an octahedrally coordinated sodium ion and a single acetate molecule at the interface of chain A. Identification of the Na $^{+}$ ion was not only based on the octahedral coordination of this atom, but also on the basis of observed metal–oxygen distances. The choice of the metal ion was validated with the *CheckMyMetal* server, which uses several different criteria to evaluate the placement of the metal (Zheng *et al.*, 2014).

Figure 1

(a) Ribbon representation of a BtDapA monomer colored according to secondary structure (blue, α -helices; magenta, β -strands). The N- and C-termini are colored red and yellow, respectively. The (β/α) $_8$ -barrel of the overall fold is clearly visible in the monomer. (b) Arrangement of the BtDapA tetramer. (c) Location of the flexible loop (Pro139–Lys143) that restricts access to the active site in the closed conformation. (d) Active-site conformation of BtDapA. Residues from an adjacent chain are shown in orange. (e) Active-site conformation of EcDapA with covalently bound pyruvate and succinic semialdehyde. Residues from an adjacent chain are in green.

Unexplained density is observed adjacent to Lys164 for all chains, as shown in Figs. 3(a)–3(d). In homologous structures, this residue was found to form a Schiff base with pyruvate and is in agreement with the experimentally determined mechanism (Soares da Costa *et al.*, 2010). This density could be attributed to a chloride ion or water molecule in chains A and D; however, chains B and C have density suggestive of a lysine covalent adduct, although the molecule bound could not be unambiguously determined. Other residues implicated in the mechanism of DapA are Tyr136, Tyr110' from an adjacent chain and Thr47. These residues were identified by sequence homology with EcDapA (PDB entry 4eou; Boughton *et al.*, 2012) and are proposed to form a catalytic triad involved in proton shuffling (Dobson *et al.*, 2004). The positions of Lys164, Tyr136 and Thr47 agree well with substrate-bound *E. coli* DapA, but Tyr110' is shifted drastically. This shift is likely to be owing to the lack of a second substrate, resulting in the enzyme adopting an open conformation.

A flexible loop region in BtDapA, which extends from Pro139 to Lys143, is in the open conformation, allowing access to the active site of the enzyme as shown in yellow in Figs. 1(e) and 2. This region undergoes a conformational shift upon

binding of the second substrate, aspartate β -semialdehyde. This loop contains Arg138 in EcDapA, which is proposed to bind to the carboxyl group of aspartate β -semialdehyde. Arg138 in EcDapA corresponds to His141 in BtDapA. A mutational study of EcDapA showed that an arginine-to-histidine mutation at this residue results in drastically reduced catalytic efficiency (Dobson, Devenish *et al.*, 2005). The majority of the other bacterial homologs of DapA have an arginine at this position. It is unclear how this substitution will affect the function of BtDapA, but it may result in reduced catalytic efficiency.

Several enzymatic and structural studies of DapA indicate lysine to be an allosteric inhibitor (Devenish *et al.*, 2009; Skovpen & Palmer, 2013; Muscroft-Taylor *et al.*, 2010). The *E. coli* DapA structure was determined with lysine present in the allosteric site. The allosteric site is made up by residues Ser48, Ala49, His56, Asn80, Glu84 and Tyr106 (Dobson, Griffin *et al.*, 2005). These residues correspond to positions Ser51, Gln52, Arg59, Thr83, Glu87 and Tyr109 in BtDapA. A recent study suggested that mutation of residue Tyr110 of *C. jejuni* DapA, which corresponds to Tyr109 in BtDapA, to a phenylalanine eliminates the lysine sensitivity of DapA

Figure 2 Sequence alignment of DapA from *B. thetaiotaomicron* (BtDapA), *R. palustris* (RpDapA), *M. tuberculosis* (MtDapA), *S. pneumoniae* (SpDapA), *C. jejuni* (CjDapA) and *E. coli* (EcDapA). Secondary-structure annotations correspond to BtDapA (top) and EcDapA (bottom). Highlighted residues correspond to the active site (black), the allosteric site (green), contributions from a second chain (orange) and the flexible loop which encloses the active site (yellow). The table indicates the percentage identity between the sequences.

Figure 3

Lys164 of chains *A*, *B*, *C* and *D* with a $2F_o - F_c$ map (blue) contoured at 1σ and a $F_o - F_c$ map contoured at 3σ (green) overlaid. The continuous $2F_o - F_c$ electron density in chains *B* and *C* is suggestive of a covalent adduct, although the molecule could not be unambiguously determined. (e) A putative allosteric site of BtDapA. (f) Allosteric site of EcDapA with two lysines bound.

(Conly *et al.*, 2014). This tyrosine is conserved in all of the bacterial species in the alignment except for RpDapA. The remainder of the allosteric site is poorly conserved between the bacterial species. Of the sequences in the alignment, EcDapA and CjDapA have been shown to be inhibited by lysine, while MtDapA is not inhibited by lysine (Kefala *et al.*, 2008; Conly *et al.*, 2014; Dobson, Griffin *et al.*, 2005). This makes it unclear whether BtDapA will be inhibited by lysine.

The structure of DapA from the commensal bacterium *B. thetaiotaomicron* closely resembles the structures of other bacterial homologs. Structural homology suggests that it may be allosterically regulated by lysine and have reduced catalytic efficiency. This structure will aid in the design of antibiotics which do not interfere with normal gut colonization by *B. thetaiotaomicron*.

Acknowledgements

Data were collected at the Southeast Regional Collaborative Access Team (SER-CAT) 22-ID beamline at the Advanced Photon Source, Argonne National Laboratory. Supporting

institutions may be found at <http://www.ser-cat.org/members.html>. Use of the Advanced Photon Source was supported by the US Department of Energy, Office of Science, Office of Basic Energy Sciences under Contract No. W-31-109-Eng-38. This work was partially supported by an ASPIRE grant from the Office of the Vice President of Research at the University of South Carolina.

References

- Blickling, S., Renner, C., Laber, B., Pohlentz, H.-D., Holak, T. A. & Huber, R. (1997). *Biochemistry*, **36**, 24–33.
- Boughton, B. A., Dobson, R. C. J. & Hutton, C. A. (2012). *Proteins*, **80**, 2117–2122.
- Chen, V. B., Arendall, W. B., Headd, J. J., Keedy, D. A., Immormino, R. M., Kapral, G. J., Murray, L. W., Richardson, J. S. & Richardson, D. C. (2010). *Acta Cryst.* **D66**, 12–21.
- Conly, C. J. T., Skovpen, Y. V., Li, S., Palmer, D. R. J. & Sanders, D. A. R. (2014). *Biochemistry*, **53**, 7396–7406.
- Cowan, K. (2006). *Acta Cryst.* **D62**, 1002–1011.
- Devenish, S. R. A., Blunt, J. W. & Gerrard, J. A. (2010). *J. Med. Chem.* **53**, 4808–4812.

- Devenish, S. R. A., Huisman, F. H. A., Parker, E. J., Hadfield, A. T. & Gerrard, J. A. (2009). *Biochim. Biophys. Acta*, **1794**, 1168–1174.
- Dobson, R. C. J., Devenish, S. R. A., Turner, L. A., Clifford, V. R., Pearce, F. G., Jameson, G. B. & Gerrard, J. A. (2005). *Biochemistry*, **44**, 13007–13013.
- Dobson, R. C. J., Griffin, M. D. W., Jameson, G. B. & Gerrard, J. A. (2005). *Acta Cryst. D***61**, 1116–1124.
- Dobson, R. C. J., Valegård, K. & Gerrard, J. A. (2004). *J. Mol. Biol.* **338**, 329–339.
- Emsley, P. & Cowtan, K. (2004). *Acta Cryst. D***60**, 2126–2132.
- Hooper, L. V., Midtvedt, T. & Gordon, J. I. (2002). *Annu. Rev. Nutr.* **22**, 283–307.
- Hooper, L. V., Stappenbeck, T. S., Hong, C. V. & Gordon, J. I. (2003). *Nature Immunol.* **4**, 269–273.
- Hutton, C. A., Perugini, M. A. & Gerrard, J. A. (2007). *Mol. Biosyst.* **3**, 458–465.
- Karsten, W. E. (1997). *Biochemistry*, **36**, 1730–1739.
- Kefala, G., Evans, G. L., Griffin, M. D. W., Devenish, S. R. A., Pearce, F. G., Perugini, M. A., Gerrard, J. A., Weiss, M. S. & Dobson, R. C. J. (2008). *Biochem. J.* **411**, 351–360.
- Maier, E., Anderson, R. C. & Roy, N. C. (2015). *Nutrients*, **2015**, 45–73.
- Minor, W., Cymborowski, M., Otwinowski, Z. & Chruszcz, M. (2006). *Acta Cryst. D***62**, 859–866.
- Murshudov, G. N., Skubák, P., Lebedev, A. A., Pannu, N. S., Steiner, R. A., Nicholls, R. A., Winn, M. D., Long, F. & Vagin, A. A. (2011). *Acta Cryst. D***67**, 355–367.
- Muscroft-Taylor, A. C., Soares da Costa, T. P. & Gerrard, J. A. (2010). *Biochimie*, **92**, 254–262.
- Offermann, L., He, J., Mank, N., Booth, W. II & Chruszcz, M. (2014). *J. Struct. Funct. Genomics*, **15**, 13–24.
- Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
- Skovpen, Y. V. & Palmer, D. R. J. (2013). *Biochemistry*, **52**, 5454–5462.
- Soares da Costa, T. P., Muscroft-Taylor, A. C., Dobson, R. C. J., Devenish, S. R. A., Jameson, G. B. & Gerrard, J. A. (2010). *Biochimie*, **92**, 837–845.
- Stappenbeck, T. S., Hooper, L. V. & Gordon, J. I. (2002). *Proc. Natl Acad. Sci. USA*, **99**, 15451–15455.
- Vagin, A. & Teplyakov, A. (2010). *Acta Cryst. D***66**, 22–25.
- Winn, M. D. *et al.* (2011). *Acta Cryst. D***67**, 235–242.
- Yang, H., Guranovic, V., Dutta, S., Feng, Z., Berman, H. M. & Westbrook, J. D. (2004). *Acta Cryst. D***60**, 1833–1839.
- Zheng, H., Chordia, M. D., Cooper, D. R., Chruszcz, M., Müller, P., Sheldrick, G. M. & Minor, W. (2014). *Nature Protoc.* **9**, 156–170.