Crystal structure of a putative short-chain dehydrogenase/reductase from Paraburkholderia xenovorans

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Paraburkholderia xenovorans degrades organic wastes, including polychlorinated biphenyls. The atomic structure of a putative dehydrogenase/reductase (SDR) from P. xenovorans (PxSDR) was determined in space group P21 at a resolution of 1.45 Å. PxSDR shares less than 37% sequence identity with any known structure and assembles as a prototypical SDR tetramer. As expected, there is some conformational flexibility and difference in the substrate-binding cavity, which explains the substrate specificity. Uniquely, the cofactor-binding cavity of PxSDR is not well conserved and differs from those of other SDRs. PxSDR has an additional seven amino acids that form an additional unique loop within the cofactor-binding cavity. Further studies are required to determine how these differences affect the enzymatic functions of the SDR.

1. Introduction

Paraburkholderia xenovorans is a model bacterium used to study unique pathways, notably the ability of this genus of bacteria to degrade polychlorinated biphenyls and other organic waste products (Francova et al., 2004; Tehrani et al., 2014). The genome of P. xenovorans has been sequenced and is one of the largest bacterial genomes studied to date. P. xenovorans has diverse functions, including nitrogen fixation, the catabolism of aromatic compounds and the degradation of various organic wastes (Francova et al., 2004; Tehrani et al., 2014). However, the underlying mechanisms of many of these processes are poorly understood. Additionally, unlike some other Paraburkholderia species, P. xenovorans is not pathogenic to humans. The Seattle Structural Genomics Center for Infectious Disease (SSGCID) selected targets from P. xenovorans for high-throughput structural studies to increase the breadth of drug-target structures to aid drug development against human pathogenic Burkholderia species such as B. mallei and B. pseudomallei. Here, we present the structure of one of these target proteins, a short-chain dehydrogenase/reductase (SDR) which shares less than 37% sequence identity with any published structure. P. xenovorans SDR (PxSDR) is predicted to be a type II fatty-acid synthetase and NAD(P)(H)-dependent oxidoreductase involved in the metabolism of diverse molecules, including lipids, amino acids, carbohydrates, cofactors, hormones and xenobiotics, or other compounds. The structure reported here may offer insights into the metabolism of small molecules by P. xenovorans.
1m TCEP, 0.025% sodium azide. The column was washed with 20 column volumes (CV) of loading buffer and was eluted with loading buffer plus 250 m M imidazole in a linear gradient over 7 CV. Peak fractions, as determined by UV absorption at 280 nm, were pooled and concentrated to 5 ml. A SEC column (Superdex 75, GE) was equilibrated with running buffer composed of 25 m M HEPES pH 7.0, 500 m M NaCl, 5% glycerol, 2 m M DTT, 0.025% azide. The peak fractions were collected and analyzed for the protein of interest using SDS-PAGE. The SEC peak fractions eluted as a single large peak at a molecular mass of ~76 kDa, suggesting a dimeric enzyme. The peak fractions were pooled and concentrated to 45 m M−1 using an Amicon purification system (Millipore). Aliquots of 200 µl were flash-frozen in liquid nitrogen and stored at −193 K until use for crystallization.

2.2. Crystallization

PxsDR was crystallized by the sitting-drop vapor-diffusion method using the JCSG+ commercial crystallization screen (Rigaku Reagents). Crystals were obtained by mixing 0.4 µl protein solution at 22.5 mg ml−1 with 0.4 µl precipitant (Rigaku Reagents JCSG+ screen condition H11: 0.2 M magnesium chloride, 0.1 M bis-Tris pH 5.5, 25% PEG 3350), equilibrating against a reservoir consisting of 80 µl precipitant and incubating at 287 K (Table 2). A single crystal was transferred into a cryoprotectant (reservoir solution supplemented with 20% ethylene glycol) and vitrified by plunging into liquid nitrogen before data collection.

2.3. Data collection and processing

X-ray diffraction data were collected on LS-CAT beamline 21-ID-F at the Advanced Photon Source. Data were integrated using XDS and reduced with XSCALE (Kabash, 2010). Additional data-collection information is provided in Table 3. The raw images and detailed data-collection information are available for download (https://proteindiffraction.org/project/5jc8/).

2.4. Structure solution and refinement

The structure of PxsDR was solved by molecular replacement with MOLREP (Vagin & Teplyakov, 2010; Lebedev et al., 2008) using PDB entry 4ni5 (36% sequence identity), an unpublished structure from the SSGCID, as a search model.
The structure was refined in Phenix (Liebschner et al., 2010) with manual model building in Coot (Emsley & Cowtan, 2004; Emsley et al., 2010). The quality of the model was assessed using MolProbity (Headd et al., 2009) and structure-refinement statistics are provided in Table 4. The structure was deposited in the Protein Data Bank as entry 5jc8. All structure figures were made using PyMOL (DeLano, 2002).

3. Results and discussion

The reported apo structure of PxSDR was determined in the monoclinic space group $P2_1$ as a prototypical SDR tetramer (Fig. 1). Each monomer has the prototypical NADPH Rossmann topology as observed in the architecture of PFAM domain PF00106 or the short-chain dehydrogenases. Specifically, PxSDR has the enoyl-(acyl carrier protein) reductase or 3-oxoacyl-ACP reductase domain architecture otherwise referred to as adh_short_C2. The most similar structures to PxSDR were identified by PDBeFold (http://www.ebi.ac.uk/msd-srv/ssm) analysis using the default threshold cutoffs of 70% for the percentage of secondary structure of the target chain identified in the query protein and of the secondary structure of the query chain (Krissinel & Henrick, 2004). The closest structure is that of the ketone reductase ChKRED20 from the genome of Chryseobacterium (Li et al., 2019). This enzyme shares a sequence identity of <36%, with an r.m.s.d. of 1.24 Å for 89% of the matched sequence identity. Wild-type ChKRED20 is an NADH-dependent ketoreductase that reduces over 100 g l$^{-1}$ ketones for some pharmaceutically relevant substrates and can use 2-propanol as the ultimate reducing agent. All of the closest structures have less than 36% sequence similarity to PxSDR (Fig. 2). These structures are PDB entry 1iy8, the crystal structure of levodione reductase from Leifsonia aquatica (Sogabe et al., 2003), PDB entry 3ftp, a 3-ketoacyl-(acyl-carrier-protein) reductase from Burkholderia pseudomallei (Baugh et al., 2013), PDB entry 6t6n, Klebsiella pneumoniae FabG2(NADH-dependent) in complex with NADH (Vella et al., 2021), and PDB entry 6ixm, ketone reductase ChKRED20 from the genome of Chryseobacterium (Li et al., 2019). Interestingly, while all of the other structures have well conserved cofactor-binding domains, an extended loop connecting the first strand in the N-terminus to...
The first helix creates a larger cofactor-binding cavity in \( P_x \) SDR (Figs. 2 and 3). This additional structural difference between \( P_x \) SDR and the other structures in the cofactor-binding domain is unique. This is an unexpected difference between \( P_x \) SDR and the other proteins beyond the expected flexibility in proximity to the substrate-binding cavity. While the flexibility in the substrate-binding cavity explains the specificity of each protein, it is unknown why \( P_x \) SDR has this unique insertion in the cofactor loop (Figs. 2 and 3).

4. Conclusions

While having a prototypical SDR topology, the apo structure of \( P_x \) SDR reveals conformational flexibility in both the

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**Figure 2**

Structural and primary-sequence alignment of \( P_x \) SDR with the closest structures identified by PDBFold. Also shown is the percent identity matrix generated with Clustal2.1. The structures are PDB entry 5jc8 (the apo structure of \( P_x \) SDR), PDB entry 1iy8 (the crystal structure of levodione reductase from Leifsonia aquatica), PDB entry 3ftp [3-ketoacyl-(acyl-carrier-protein) reductase from Burkholderia pseudomallei], PDB entry 6t6n [Klebsiella pneumoniae FabG2(NADH-dependent) in complex with NADH] and PDB entry 6ixm (ketone reductase ChKRED20 from the genome of Chryseobacterium). The secondary-structure elements shown are \( \alpha \)-helices (\( \alpha \)), \( 3_{10} \)-helices (\( 3_{10} \)), \( \beta \)-strands (\( \beta \)) and \( \beta \)-turns (TT). Identical residues are shown in white on a red background and conserved residues are shown in red. This figure was generated using ESPript (Gouet et al., 1999, 2003).
Figure 3

PxSDR superposed on its closest structural orthologues reveals differences in the substrate- and cofactor-binding cavities. (a) The substrate-binding cavities of the proteins, indicated in the blue circle, have differences that are indicative of different substrate specificities. (b) PxSDR in black has a unique loop insertion in the cofactor-binding cavity, while the other proteins have a well conserved cofactor-binding cavity and loops. The superposed structures are PDB entry 5jc8 (apo structure of PxSDR, black), PDB entry 1iy8 (crystal structure of levdione reductase from Leifsonia aquatica, red), PDB entry 3ftp [3-ketoacyl-(acyl-carrier-protein) reductase from Burkholderia pseudomallei, yellow], PDB entry 6t6n [Klebsiella pneumoniae FabG2(NADH-dependent) in complex with NADH, wheat] and PDB entry 6ixm (ketone reductase ChKRED20 from the genome of Chryseobacterium aquamarine). The cofactor, NADH and substrate, (4R)-2-methylpentane-2,4-diol, are from PDB entry 1iy8. Structures were superposed with PyMOL.

cofactor- and substrate-binding cavities that needs to be further investigated in order to determine the roles of this enzyme in the degradation of organic wastes by P. xenovorans.

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References
Aslanidis, C. & de Jong, P. J. (1990). Nucleic Acids Res. 18, 6069–6074.
Baugh, L., Gallagher, L. A., Patrapuvich, R., Clifton, M. C., Gardberg, A. S., Edwards, T. E., Armour, B., Begley, D. W., Dieterich, S. H., Dranow, D. M., Abendroth, J., Fairman, J. W., Fox, D., Staker, B. L., Phan, I., Gillespie, A., Choi, R., Nakazawa-Hewitt, S., Nguyen, M. T., Napuli, A., Barrett, L., Buchko, G. W., Stacy, R., Myler, P. J., Stewart, L. J., Manoil, C. & Van Voorhis, W. C. (2013). PLoS One, 8, e53851.
Bryan, C. M., Bhandari, J., Napuli, A. J., Leibly, D. J., Choi, R., Kelley, A., Van Voorhis, W. C., Edwards, T. E. & Stewart, L. J. (2011). Acta Cryst. F67, 1010–1014.
Choi, R., Kelley, A., Leibly, D., Nakazawa Hewitt, S., Napuli, A. & Van Voorhis, W. (2011). Acta Cryst. F67, 988–1005.
DeLano, W. L. (2002). http://www.pymol.org
Emsley, P., Cowtan, K. (2004). Acta Cryst. D60, 2256–2268.
Emsley, P., Lohkamp, B., Scott, W. G. & Cowtan, K. (2010). Acta Cryst. D66, 486–501.
Francova, K., Macková, M., Macek, T. & Sylvestre, M. (2004). Environ. Pollut. 127, 41–48.
Gouet, P., Courcelle, E., Stuart, D. I. & Métoz, F. (1999). Bioinformatics, 15, 305–308.
Gouet, P., Robert, X. & Courcelle, E. (2003). Nucleic Acids Res. 31, 3320–3323.
Headd, J. J., Immormino, R. M., Keedy, D. A., Emsley, P., Richardson, D. C. & Richardson, J. S. (2009). J. Struct. Funct. Genomics, 10, 83–93.
Kabsch, W. (2010). Acta Cryst. D66, 125–132.
Krissinel, E. & Henrick, K. (2004). Acta Cryst. D60, 2256–2268.
Lebedev, A. A., Vagin, A. A. & Murshudov, G. N. (2008). Acta Cryst. D64, 33–39.
Li, T. B., Zhao, F. J., Liu, Z., Jin, Y., Liu, Y., Pei, X. Q., Zhang, Z. G., Wang, G. & Wu, Z. L. (2019). *Enzyme Microb. Technol.* **125**, 29–36.

Liebschner, D., Afonine, P. V., Baker, M. L., Bunkóczi, G., Chen, V. B., Croll, T. I., Hintze, B., Hung, L.-W., Jain, S., McCoy, A. J., Moriarty, N. W., Oeffner, R. D., Poon, B. K., Prisant, M. G., Read, R. J., Richardson, J. S., Richardson, D. C., Samuito, M. D., Sobolev, O. V., Stockwell, D. H., Terwilliger, T. C., Urzhumtsev, A. G., Videau, L. L., Williams, C. J. & Adams, P. D. (2019). *Acta Cryst.* **D75**, 861–877.

Myler, P. J., Stacy, R., Stewart, L., Staker, B. L., Van Voorhis, W. C., Varani, G. & Buchko, G. W. (2009). *Infect. Disord. Drug Targets* **9**, 493–506.

Serbzhinskiy, D. A., Clifton, M. C., Sankaran, B., Staker, B. L., Edwards, T. E. & Myler, P. J. (2015). *Acta Cryst.* **F71**, 594–599.

Sogabe, S., Yoshizumi, A., Fukami, T. A., Shiratori, Y., Shimizu, S., Takagi, H., Nakamori, S. & Wada, M. (2003). *J. Biol. Chem.* **278**, 19387–19395.

Stacy, R., Begley, D. W., Phan, I., Staker, B. L., Van Voorhis, W. C., Varani, G., Buchko, G. W., Stewart, L. J. & Myler, P. J. (2011). *Acta Cryst.* **F67**, 979–984.

Studier, F. W. (2005). *Protein Expr. Purif.* **41**, 207–234.

Tehrani, R., Lyv, M. M. & Van Aken, B. (2014). *Environ. Sci. Pollut. Res.* **21**, 6346–6353.

Vagin, A. & Teplyakov, A. (2010). *Acta Cryst.* **D66**, 22–25.

Vella, P., Rudraraju, R. S., Lundbäck, T., Axelsson, H., Almqvist, H., Vallin, M., Schneider, G. & Schnell, R. (2021). *Bioorg. Med. Chem.* **30**, 115898.