Crystal structure of the RNA 2',3'-cyclic phosphodiesterase from *Deinococcus radiodurans*

Wanchun Han, Jiahui Cheng, Congli Zhou, Yuejin Hua* and Ye Zhao*

Key Laboratory of Chinese Ministry of Agriculture for Nuclear-Agricultural Sciences, Institute of Nuclear-Agricultural Sciences, Zhejiang University, People's Republic of China. *Correspondence e-mail: yjhua@zju.edu.cn, yezhao@zju.edu.cn

Received 28 February 2017

Accepted 30 March 2017

Edited by I. Tanaka, Hokkaido University, Japan

Keywords: RNA repair; CPDase; RNA 2',3'-cyclic phosphodiesterase; *Deinococcus radiodurans*.

PDB reference: native drCPDase, 5h7e

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

2',3'-Cyclic phosphodiesterase (CPDase) homologues have been found in all domains of life and are involved in diverse RNA and nucleotide metabolisms. The CPDase from *Deinococcus radiodurans* was crystallized and the crystals diffracted to 1.6 Å resolution, which is the highest resolution currently known for a CPDase structure. Structural comparisons revealed that the enzyme is in an open conformation in the absence of substrate. Nevertheless, the active site is well formed, and the representative motifs interact with sulfate ion, which suggests a conserved catalytic mechanism.

1. Introduction

Deinococcus radiodurans is well known for its extreme resistance to DNA-damaging treatments such as ionizing radiation (Ishino & Narumi, 2015; Munteanu *et al.*, 2015; Krisko & Radman, 2013). It encodes a predicted 2'–5' RNA ligase (DR2339, LigT), which might be involved in RNA repair and metabolism (Makarova *et al.*, 2001; Omelchenko *et al.*, 2005). Comparative transcriptomics research suggests that the gene encoding this enzyme is highly upregulated *in vivo* following exposure to radiation (Chen *et al.*, 2008; Liu *et al.*, 2003). The LigT protein is conserved within the *Deinococcus*–*Thermus* phylum and is homologous to the *Escherichia coli* enzyme (Fig. 1). A recent proposal suggests that instead of performing a ligase reaction, *E. coli* LigT is actually a CPDase enzyme that converts an RNA 2',3'-cyclic phosphodiester into a 2'-PO₄ terminus (Omelchenko *et al.*, 2005).

CPDase (EC 6.5.1) is found in almost all organisms from bacteria to mammals and can exist as a 'standalone' protein in addition to forming part of tRNA ligase (Kato *et al.*, 2003; Hofmann *et al.*, 2000; Myllykoski *et al.*, 2013; Remus *et al.*, 2014; Mazumder *et al.*, 2002). Based on domain arrangements and sequence alignments, CPDase is classified into the 2H phosphoesterase superfamily, which can be divided into four subfamilies: the archaeobacterial LigT-like, eukaryotic viral LigT-like, YjcG-like and mlr3352 groups (Mazumder *et al.*, 2002). These subfamilies share two conserved histidines in a pair of HXT/SX motifs (where X is a hydrophobic residue), hence the name of this protein family. Superimpositions of these 2H phosphoesterase structures, which have been solved independently by nuclear magnetic resonance or X-ray crystallography in recent years, revealed that they share similar overall structures (Remus *et al.*, 2014; Myllykoski *et al.*, 2013, 2016; Sakamoto *et al.*, 2005). The active site is located between two lobes formed by antiparallel β-strands with α-helices wrapping around them. The two signature histidines serve as a general acid and a general base, respectively, which are critical for catalysis.

Table 1

Macromolecule-production information.

Source organism	<i>D. radiodurans</i> (strain ATCC13939)
DNA source	Genomic DNA
Forward primer	5'-GGAATTCCATATGAACAAGCCGAAAAAAGC
Reverse primer	5'-AAGGATCCTCATGGTGCTCCTGGGG
Cloning vector	pET-28a
Expression vector	pET-28a
Expression host	<i>E. coli</i> BL21 (DE3)
Complete amino-acid sequence of the construct produced	MGSSHHHHHSSGLVPRGSHMASMTGGQMGRGS-MNKPEKKPGSRAAQAGARPSRPAAPRKQPGRE-SEHQPSTYRLFYALRVPADITAPLAEQAQKLRLGNWRAVRPQMHVTLSTYLPVPPPERVEDLKRLGTRLTQDLPLHVNLRGTGYFPNEGSPRVWFV-KTEAEGLTELAENLRAGIRELGIGTDDLAFKA-HITLARKKGPAPRLPLIFDQSWTAPGLTYLR-SILRRTGPIYEVQSTFRFRGSASQTSAESAPT-AAPSAPQEHF

Table 2

Crystallization.

Method	Vapour diffusion, sitting drop
Plate type	24-well Linbro cell-culture plates
Temperature (K)	293
Protein concentration (mg ml ⁻¹)	25
Buffer composition of protein solution	20 mM Tris-HCl pH 7.5, 250 mM KCl, 1 mM DTT, 0.5 mM EDTA
Composition of reservoir solution	0.1 M citric acid pH 3.5, 2 M ammonium sulfate
Volume and ratio of drop	1 µl protein solution mixed with 1 µl reservoir solution
Volume of reservoir (µl)	500

In the present study, we determined the 1.6 Å resolution crystal structure of CPDase from *D. radiodurans* (drCPDase), which shows an enlarged active site containing a sulfate ion that mimics the phosphate group of the substrate.

2. Materials and methods

2.1. Macromolecule production

Full-length drCPDase was amplified *via* PCR and cloned into pET-28a expression vector using NdeI and BamHI sites. Transformed *E. coli* BL21 (DE3) clones were grown at 37°C in LB medium containing 50 µg ml⁻¹ kanamycin to an OD₆₀₀ of between 0.6 and 0.8. Protein expression was induced at 37°C for 3 h by adding isopropyl β-D-1-thiogalactopyranoside (IPTG) to a final concentration of 0.3 mM. The cell pellets were harvested and resuspended in buffer A (20 mM Tris-HCl pH 7.8, 9 mM imidazole, 500 mM NaCl, 3 mM β-mercaptoethanol) followed by sonication. After centrifugation at 35 000g for 30 min, the supernatant was loaded onto an Ni-NTA column (5 ml; GE Healthcare Life Sciences) equilibrated with buffer B [20 mM Tris-HCl pH 7.5, 500 mM NaCl, 5% (w/v) glycerol, 3 mM β-mercaptoethanol, 20 mM imidazole], washed with 40 mM imidazole and finally eluted with 300 mM imidazole. Fractions containing drCPDase were collected and were purified using a Superdex 75 10/30 column (GE Healthcare Life Sciences) with buffer C (20 mM Tris-HCl pH 7.5, 250 mM KCl, 1 mM DTT, 0.5 mM EDTA). Macromolecule-production information is summarized in Table 1.

Table 3

Data collection and processing.

Diffraction source	BL17U, SSRF
Wavelength (Å)	0.9792
Temperature (K)	95
Detector	ADSC Quantum 315r CCD
Crystal-to-detector distance (mm)	220
Rotation range per image (°)	0.5
Total rotation range (°)	90
Exposure time per image (s)	1
Space group	<i>H</i> 32
<i>a</i> , <i>b</i> , <i>c</i> (Å)	91.32, 91.32, 169.83
α, β, γ (°)	90, 90, 120
Mosaicity (°)	0.172
Resolution range (Å)	30–1.600 (1.640–1.600)
Total No. of reflections	194852
No. of unique reflections	34987
Completeness (%)	96.7 (97.3)
Multiplicity	5.6 (5.5)
<i>I</i> /σ(<i>I</i>)	19.40 (2.49)
<i>R</i> _{int}	0.049 (0.72)
Overall <i>B</i> factor from Wilson plot (Å ²)	23.30

Table 4

Structure refinement.

Resolution range (Å)	30–1.600
Completeness (%)	96.82
No. of reflections, working set	33239 (2736)
No. of reflections, test set	1738 (149)
<i>R</i> _{work}	0.1747
<i>R</i> _{free}	0.1963
No. of non-H atoms	
Protein	1405
Glycerol	12
Sulfate	25
Waters	118
Total	1560
R.m.s. deviations	
Bonds (Å)	0.015
Angles (°)	1.780
Average <i>B</i> factors (Å ²)	
Glycerol	70.60
Sulfate	63.63
Ligand	65.89
Waters	39.17
Ramachandran plot	
Most favoured (%)	97.78
Allowed (%)	2.22

2.2. Crystallization

An initial crystallization screen was performed using the sitting-drop vapour-diffusion method at 293 K. drCPDase crystals were obtained using a reservoir consisting of 0.1 M citric acid pH 3.5, 2 M ammonium sulfate. Cryocooling was achieved by soaking the crystals in reservoir solution containing 30% glycerol for 3 min and flash-cooling them in liquid nitrogen. Crystallization information is summarized in Table 2.

2.3. Data collection and processing

Diffraction intensities were recorded on beamline BL17U at Shanghai Synchrotron Radiation Facility (SSRF), Shanghai, People's Republic of China and were integrated and scaled using the XDS suite (Kabsch, 2010). Data-collection and processing statistics are summarized in Table 3.

2.4. Structure solution and refinement

The structure of drCPDase was determined by molecular replacement using the *E. coli* homologue (PDB entry 4qak; Remus *et al.*, 2014) as a search model. The structure was refined using *PHENIX* interspersed with manual model building using *Coot* (Emsley *et al.*, 2010; Adams *et al.*, 2010). All structural figures were rendered in *PyMOL* (Schrödinger), and electrostatic surface potentials were calculated using *APBS* (Baker *et al.*, 2001). The final drCPDase model consisted of residues 35–216, and all of the residues were in the most favourable and allowed regions of the Ramachandran plot. The coordinates and structure factors have been deposited in the Protein Data Bank with accession code 5h7e. The refinement statistics are listed in Table 4.

3. Results and discussion

drCPDase is a 234-amino-acid protein that shares 33% amino-acid identity with the *E. coli* homologue (Fig. 1). A sequence alignment shows that in addition to the two representative 2H

phosphoesterase motifs, CPDases from the *Deinococcus-Thermus* phylum possess additional N- and C-terminal regions outside the core domain (Fig. 1). To determine the crystal structure of drCPDase, the full-length protein was expressed and purified by metal-affinity and size-exclusion chromatography as described in §2. Crystals were grown and diffracted X-rays to 1.6 Å resolution and the structure was determined by molecular replacement. After refinement, the final model consisted of drCPDase residues 35–216 (the core domain). The N-terminal (amino acids 1–34) and C-terminal (amino acids 217–234) regions appear to be disordered in the crystal (Fig. 2a). The structure was validated by the appearance of a well formed active site containing a sulfate ion, which was present at 2 M in the crystallization solution, mimicking the phosphate group of the substrate (Fig. 2a).

The overall structure of the drCPDase core domain comprises two symmetrical lobes with four α -helices and ten β -strands (Fig. 2a). The lobe containing the N- and C-terminal regions (the so-called terminal lobe; Hofmann *et al.*, 2000) consists of four β -strands (β 1, β 3, β 9 and β 10) and two α -helices (α 3 and α 4), and the opposite lobe (the so-called

Figure 1

Sequence alignments of CPDase-family proteins from *D. radiodurans* (Dra), *D. geothermalis* (Dge), *E. coli* (Eco) and *Bacillus subtilis* (Bsu). The structural elements of drCPDase are shown in distinct colours. Conserved amino acids are highlighted in red.

transit lobe) consists of six β -strands ($\beta 2$, $\beta 4$, $\beta 5$, $\beta 6$, $\beta 7$ and $\beta 8$) and two α -helices ($\alpha 1$ and $\alpha 2$). Four of the antiparallel β -strands from each lobe ($\beta 1$ – $\beta 3$ – $\beta 9$ – $\beta 10$ and $\beta 4$ – $\beta 5$ – $\beta 6$ – $\beta 8$)

Figure 2
Structural features of drCPDase. (a) The overall structure of drCPDase is shown with green β -strands, pink α -helices and cyan loop regions. The two β -strands bearing the signature motifs are highlighted in red. The N- and C-termini of the polypeptide are indicated. The catalytic residues, sulfate and glycerol in the active site are depicted as stick models. (b) The distribution of the electrostatic surface of drCPDase. Blue and red represent the positive and negative charge potentials on a +8 and -8 kTe^{-1} scale, respectively. (c) The two symmetry-related drCPDase molecules in the *H32* crystal are coloured grey and yellow. The N-terminal region (residues 35–40) of each molecule is shown as spheres.

constitute the solvent-accessible β -barrel architecture. The two conserved catalytic HXT/SX motifs are located in the $\beta 3$ and $\beta 6$ strands (highlighted in red in Fig. 2a), which form a large, positively charged active-site cleft for substrate binding (Fig. 2b). In addition, a molecule of glycerol (used for cryo-protection) was observed to be hydrogen-bonded to the sulfate ion in the active site. Interestingly, the N-terminal disordered region before the drCPDase core domain points towards the active site of the symmetry-related protein molecule (Fig. 2c), suggesting that the N-terminal region might play a role in protein function. In fact, the N-terminal domain of CNPase, a member of the 2H phosphoesterase family, plays a regulatory role in substrate binding (Myllykoski *et al.*, 2013).

We next compared the drCPDase structure with that of its *E. coli* homologue (ecCPDase), which has been recently solved in complex with 2'-AMP (Remus *et al.*, 2014). The overall structure of drCPDase could be superimposed on that of ecCPDase with an r.m.s.d. of 1.213 Å over 104 C^α atoms (Fig. 3a). Despite the differing numbers of β -strands, drCPDase shares a conserved active-site topology with ecCPDase (Fig. 3a). The central β -barrel superimposes well in the two structures. However, the flanking helices, especially $\alpha 1$ and $\alpha 3$, showed noticeable movements that create a wider substrate-entrance channel (Fig. 3a). Compared with ecCPDase, the active-site cavity of drCPDase is filled with water, glycerol and sulfate ion in the absence of 2'-AMP (Figs 3a and 3b). The sulfate ion occupies a similar position to the phosphate group of 2'-AMP in ecCPDase, which is coordinated by a glycerol molecule and conserved residues (Thr78, His161, Thr163 and Arg166) from the two signature motifs (Fig. 3b). Notably, all of the residues that are likely to be involved in the CPDase reaction can be superimposed in drCPDase and ecCPDase, which would suggest that CPDase has an open substrate-binding site in the catalytic domain. This active-site configuration is likely to be conserved among all CPDases and possibly also all 2H phosphoesterases. In the absence of 2'-AMP, the α -helices in drCPDase show a

Figure 3
Structural basis for substrate recognition by drCPDase. (a) Structural comparison of drCPDase (coloured as in Fig. 2a) and ecCPDase (yellow). The relative domain movements are shown with arrowheads. (b) Close-up of the drCPDase active site between two β -strands (red). The conserved residues and the sulfate ion are shown as stick models.

noticeable movement and result in a more open passage for substrate binding, indicating that these α -helices are also involved in substrate recognition.

In summary, we have determined the crystal structure of the CPDase homologue from *D. radiodurans* at 1.6 Å resolution. The structure delineates an open active site for substrate binding with a conserved catalytic mechanism. These results will aid in future biochemical analyses of CPDase as well as other members of the 2H phosphoesterase superfamily.

Acknowledgements

We would like to thank the staff at the Shanghai Synchrotron Radiation Facility (SSRF) in China for assistance in data collection.

Funding information

Funding for this research was provided by: National Basic Research Program of China (award No. 2015CB910600); Zhejiang Provincial Natural Science Foundation for Outstanding Young Scientists (award No. LR16C050002); National Natural Science Foundation of China (award No. 31670819, 31670065, 31500656, 31370102, 31210103904).

References

Adams, P. D. *et al.* (2010). *Acta Cryst.* **D66**, 213–221.
Baker, N. A., Sept, D., Joseph, S., Holst, M. J. & McCammon, J. A. (2001). *Proc. Natl Acad. Sci. USA*, **98**, 10037–10041.

Chen, H., Xu, G., Zhao, Y., Tian, B., Lu, H., Yu, X., Xu, Z., Ying, N., Hu, S. & Hua, Y. (2008). *PLoS One*, **3**, e1602.
Emsley, P., Lohkamp, B., Scott, W. G. & Cowtan, K. (2010). *Acta Cryst.* **D66**, 486–501.
Hofmann, A., Zdanov, A., Genschik, P., Ruvinov, S., Filipowicz, W. & Wlodawer, A. (2000). *EMBO J.* **19**, 6207–6217.
Ishino, Y. & Narumi, I. (2015). *Curr. Opin. Microbiol.* **25**, 103–112.
Kabsch, W. (2010). *Acta Cryst.* **D66**, 125–132.
Kato, M., Shirouzu, M., Terada, T., Yamaguchi, H., Murayama, K., Sakai, H., Kuramitsu, S. & Yokoyama, S. (2003). *J. Mol. Biol.* **329**, 903–911.
Krisiko, A. & Radman, M. (2013). *Cold Spring Harb. Perspect. Biol.* **5**, a012765.
Liu, Y., Zhou, J., Omelchenko, M. V., Beliaev, A. S., Venkateswaran, A., Stair, J., Wu, L., Thompson, D. K., Xu, D., Rogozin, I. B., Gaidamakova, E. K., Zhai, M., Makarova, K. S., Koonin, E. V. & Daly, M. J. (2003). *Proc. Natl Acad. Sci. USA*, **100**, 4191–4196.
Makarova, K. S., Aravind, L., Wolf, Y. I., Tatusov, R. L., Minton, K. W., Koonin, E. V. & Daly, M. J. (2001). *Microbiol. Mol. Biol. Rev.* **65**, 44–79.
Mazumder, R., Iyer, L. M., Vasudevan, S. & Aravind, L. (2002). *Nucleic Acids Res.* **30**, 5229–5243.
Munteanu, A., Uivarosi, V. & Andries, A. (2015). *Extremophiles*, **19**, 707–719.
Myllykoski, M., Raasakka, A., Lehtimäki, M., Han, H., Kursula, I. & Kursula, P. (2013). *J. Mol. Biol.* **425**, 4307–4322.
Myllykoski, M., Seidel, L., Muruganandam, G., Raasakka, A., Torda, A. E. & Kursula, P. (2016). *Brain Res.* **1641**, 64–78.
Omelchenko, M. V., Wolf, Y. I., Gaidamakova, E. K., Matrosova, V. Y., Vasilenko, A., Zhai, M., Daly, M. J., Koonin, E. V. & Makarova, K. S. (2005). *BMC Evol. Biol.* **5**, 57.
Remus, B. S., Jacewicz, A. & Shuman, S. (2014). *RNA*, **20**, 1697–1705.
Sakamoto, Y., Tanaka, N., Ichimiya, T., Kurihara, T. & Nakamura, K. T. (2005). *J. Mol. Biol.* **346**, 789–800.