Petr Kolenko,^{a,b,*} Birgit Koch,^c
Jens-Ulrich Rahfeld,^c Stephan
Schilling,^c Hans-Ulrich Demuth^c
and Milton T. Stubbs^a

^aDepartment of Physical Biochemistry,
Institute of Biochemistry and Biotechnology,
MLU, Kurt-Mothes-Strasse 3, 06120 Halle,
Germany, ^bDepartment of Structural
Analysis of Biomacromolecules, Institute of
Macromolecular Chemistry AS CR, Heyrovskeho
nam. 2/1888, 162 06 Prague 6, Czech Republic,
and ^cProbiobdrug AG, Weinbergweg 22,
06120 Halle, Germany

Correspondence e-mail:
petr.kolenko@gmail.com

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Structure of glutaminyl cyclase from *Drosophila melanogaster* in space group *I*4

The structure of ligand-free glutaminyl cyclase (QC) from *Drosophila melanogaster* (*DmQC*) has been determined in a novel crystal form. The protein crystallized in space group *I*4, with unit-cell parameters $a = b = 122.3$, $c = 72.7$ Å. The crystal diffracted to a resolution of 2 Å at the home source. The structure was solved by molecular replacement and was refined to an *R* factor of 0.169. *DmQC* exhibits a typical α/β -hydrolase fold. The electron density of three monosaccharides could be localized. The accessibility of the active site will facilitate structural studies of novel inhibitor-binding modes.

1. Introduction

Glutaminyl cyclases (QCs; EC 2.3.2.5) are enzymes that catalyze the conversion of N-terminal glutamine or glutamate into pyroglutamate and have been identified in many forms of life (>100 entries in the UniProt database; The UniProt Consortium, 2012). Whereas plant and bacterial QCs adopt a five-bladed β -propeller fold (Wintjens *et al.*, 2006; Ruiz-Carrillo *et al.*, 2010), mammalian (human and mouse; hQC and mQC, respectively) and insect QCs are zinc-dependent enzymes (Schilling *et al.*, 2003) with an α/β -hydrolase topology (Huang *et al.*, 2005, 2008, 2011; Ruiz-Carrillo *et al.*, 2011; Koch *et al.*, 2012). The main core of the tertiary structure is formed by a central six-stranded β -sheet surrounded by several α -helices (Huang *et al.*, 2005). The zinc cation at the active site is coordinated by the side chains of aspartate, histidine and glutamate residues.

We have recently published the crystal structures of wild-type *Drosophila melanogaster* QC (*DmQC*) and a cysteine-lacking variant (Koch *et al.*, 2012) in complex with the highly potent competitive inhibitor PBD150 (Buchholz *et al.*, 2006, 2009), which are the first crystal structures of a secreted invertebrate QC. All known crystal structures of mammalian glutaminyl cyclases in complex with PBD150 show the influence of crystal contacts on the binding mode of the inhibitor (Ruiz-Carrillo *et al.*, 2011). In our previous study, inhibited *DmQC* crystallized in space group *P*2₁, with the active site exposed to the solvent, and in space group *P*6₅, in which the crystal contacts significantly affected the binding mode of PBD150 (Koch *et al.*, 2012). In this study, we searched for a new crystal form for the purpose of inhibitor-soaking experiments.

2. Materials and methods

2.1. Macromolecule production

The protein (UniProtKB code Q9VRQ9; residues 29–340) was prepared according to the previously published protocol (Schilling *et al.*, 2007). Briefly, the protein was heterologously expressed in the yeast *Pichia pastoris*, purified by cation-exchange chromatography (Streamline SP XL column) and hydrophobic interaction chromatography (Butyl Sepharose FF column) and concentrated for crystallization (Table 1).

2.2. Crystallization

We performed extensive screening for novel crystallization conditions (roughly 900 conditions). Prism-like protein crystals of a novel crystal form (Fig. 1) appeared within two weeks. Further details

Table 1

Details of protein production.

DNA source	<i>D. melanogaster</i>
Forward primer	ATATATCTCGAGAAAAGAAACATTGGATCCCGAGTGGCGC
Reverse primer	ATATATGCGGCCGCCTATGTTTCGGTAAAAACGCAAATT
Expression vector	pPICαB
Expression host, strain	<i>P. pastoris</i> , X33 (AOX1, AOX2)

Table 2

Crystallization details.

Method	Hanging-drop vapour diffusion
Plate type	24-well NeXtal crystallization plate (Qiagen)
Temperature (K)	293
Protein concentration (g l ⁻¹)	15
Buffer composition of protein solution	0.1 M NaCl, 25 mM bis-tris pH 6.8
Composition of reservoir solution	1.6 M ammonium sulfate, 10% (v/v) 1,4-dioxane, 0.1 M HEPES pH 7.5
Volume of drop (μl)	2
Protein:reservoir ratio in drop	1:1
Volume of reservoir (μl)	1000

of the crystallization conditions are summarized in Table 2. Prior to the diffraction experiments, the crystal was flash-cooled in a nitrogen stream at 100 K with 20% ethylene glycol as a cryoprotectant.

2.3. Data collection and processing

The diffraction data set was collected using a Rigaku RU-300 rotating-anode generator equipped with focusing mirrors (MSC, USA) and a Saturn 940+ CCD detector. Integration of the images was performed using the *XDS* program package (Kabsch, 2010). Scaling was performed using *SCALA* from the *CCP4* program package (Winn *et al.*, 2011). Statistics are given in Table 3.

2.4. Structure solution and refinement

The structure was solved by molecular replacement with the program *MOLREP* (Vagin & Teplyakov, 2010) using the *DmQC* monomer in space group *P*₂₁ (PDB entry 4f9u; Koch *et al.*, 2012) excluding water molecules and inhibitor as a search model. The process resulted in the localization of one monomer in the asymmetric unit (contrast = 30.6; score = 0.71; *R*_{work}/*R*_{free} = 0.225/0.264). Refinement was carried out with *REFMAC5* (Murshudov *et al.*, 2011) and manual corrections of the model were performed using *Coot* (Emsley & Cowtan, 2004). Initially, water molecules were built with the help of *ARP/wARP* (Langer *et al.*, 2008). Refinement was

Figure 1

Crystal of *DmQC* (~400 μm in one direction) in space group *I*₄ after four weeks of growth. The photograph was taken shortly before cryoprotection and the diffraction experiment.

Table 3

Data-collection and processing statistics.

Values in parentheses are for the outer shell.

Wavelength (Å)	1.541
Temperature (K)	100
Crystal-to-detector distance (mm)	50
Rotation range per image (°)	1
Total rotation range (°)	180
Exposure time per image (s)	15
Space group	<i>I</i> ₄
Unit-cell parameters (Å, °)	<i>a</i> = <i>b</i> = 122.3, <i>c</i> = 72.7, α = β = γ = 90
Mosaicity (°)	0.2
Resolution range (Å)	45.0–2.0 (2.11–2.00)
Total No. of reflections	125968 (11796)
No. of unique reflections	35645 (4846)
Completeness (%)	98 (93)
Multiplicity	3.5 (2.4)
<i>I</i> / <i>σ</i> (<i>I</i>)	11.9 (2.0)
<i>R</i> _{merge}	0.075 (0.460)
Overall <i>B</i> factor from Wilson plot (Å ²)	24

Table 4

Structure-refinement statistics.

Values in parentheses are for the outer shell (2.05–2.00 Å).

No. of reflections, working set	32085
No. of reflections, test set	1778 [5%]
Final <i>R</i> _{work}	0.166 (0.276)
Final <i>R</i> _{free}	0.208 (0.318)
Final <i>R</i> _{all}	0.169
R.m.s. deviations	
Bonds (Å)	0.015
Angles (°)	1.448
No. of non-H atoms	
Protein	2427
Zinc ion	1
Saccharides	39
Ethylene glycol	12
Solvent	381
Total	2860
Average <i>B</i> factors (Å ²)	
Protein	23
Zinc ion	16
Saccharides	45
Ethylene glycol	31
Solvent	38
Total	25
Ramachandran plot	
Residues in favoured region	285
Residues in additionally allowed regions	10
Outliers	0

continuously monitored using the *R*_{free} statistic (which was calculated using 5% of the reflections). The last refinement cycle was performed using all measured reflections. Statistics are given in Table 4.

Structure validation was carried out with *MolProbity* (Lovell *et al.*, 2003; Chen *et al.*, 2010) and *SFCHECK* (Vaguine *et al.*, 1999). The coordinates and structure factors have been deposited in the PDB (Berman *et al.*, 2000) with accession code 4fwu.

3. Results and discussion

We have found a new crystallization condition for *DmQC*, leading to crystals that belonged to space group *I*₄ and diffracted to 2.0 Å resolution. The structure could be solved by molecular replacement, and the enzyme adopts the α/β-hydrolase fold common to all known mammalian and insect glutaminyl cyclases (hQC, hisoQC, mQC, *DmQC* and *DmisoQC*; Koch *et al.*, 2012). The asymmetric unit contains one protein chain (Gln33–Phe337); localization of amino acids Leu195–Gln200 was not possible owing to the low quality of the electron-density map in this region.

The C-terminal region of *DmQC* shows high flexibility and can cover distinct regions of the protein depending on the crystal contacts. Two possible conformations of the ten terminal residues have been observed (Koch *et al.*, 2012). The C-terminal conformation of the structure presented here is similar to that of chain *B* of wild-type *DmQC* in space group $P2_1$ (PDB entry 4f9u), in which the

C-terminal residues are directed away from the N-terminus of the protein.

In previously determined structures of *DmQC* (PDB entries 4f9u and 4f9v), the crystal packing stabilized the conformation of the oligosaccharides attached to Asn42, allowing the localization of seven

Figure 2
The structure of unliganded glutaminyl cyclase from *D. melanogaster* represented by secondary-structure elements (N-terminus in blue; C-terminus in red), and detail of the glycosylation site. (a) The oligosaccharides, sulfate anions and ethylene glycol molecules are represented as sticks. The zinc cation at the active site is represented as magenta sphere. (b) The $2F_o - F_c$ map around the oligosaccharide contoured at the 1σ level (blue).

Figure 3
The active site and its solvent accessibility. (a) The two ethylene glycol molecules near the *DmQC* active site. Amino acids are represented by lines, the zinc ion is shown as a sphere and ethylene glycol molecules are represented by sticks; hydrogen bonds are depicted by black dashes. The $2F_o - F_c$ map around the ethylene glycol molecules is contoured at 1σ (blue). (b) The *DmQC* active site is accessible in the new *I4* crystal form. *DmQC* (green) and its active site (red) are represented by a surface; a symmetry-related molecule (cyan) is represented by a transparent surface and secondary-structure elements.

Table 5Available crystallization conditions for glutaminyl cyclase from *D. melanogaster*.

Reservoir composition	pH	Protein concentration (g l ⁻¹)	Space group	Radiation source	Resolution (Å)	PDB code
10% PEG 35 000, 0.1 M Tris, 2 mM PBD150	8.0	7.5	<i>P</i> 2 ₁	BESSY II	1.8	4f9u
12% PEG 8000, 0.1 M MES, 2 mM PBD150	6.5	10	<i>P</i> 6 ₅	BESSY II	2.1	4f9v
1.6 M ammonium sulfate, 10% 1,4-dioxane, 40 mM MEGA-9, 0.1 M HEPES	7.5	15	<i>I</i> 4	Rigaku RU-300	2.0	4fwu

monosaccharides in the electron density (Koch *et al.*, 2012). In the present structure (PDB entry 4fwu) only three solvent-exposed monosaccharide units could be detected (see Fig. 2); the sugars do not participate in crystal contacts.

Three ethylene glycol molecules, one chloride anion and five sulfate anions originating from the reservoir solution and cryoprotection could be localized on the surface of the molecule (see Fig. 2). One molecule of ethylene glycol binds directly to the active site and a second molecule binds close to the active site. Superposition of all five individual monomers of *DmQC* deposited in the PDB (PDB entries 4f9u, 4f9v and 4fwu) revealed a high level of structural conservation, which included all of the residues in the active site: the r.m.s.d. between C α atoms of pairwise compared monomers (~298 atoms) does not exceed 0.45 Å (McNicholas *et al.*, 2011; Krissinel & Henrick, 2007). There are no significant conformational changes upon ligand binding.

The symmetry-related molecule in space group *I*4 interacts closely with the region around Phe292, preventing binding of the inhibitor PBD150 or similar derivatives in the active site. Our inhibition studies have previously shown that the potency of PBD150 is significantly lower for *DmQC* than for hQC or mQC (Koch *et al.*, 2012). However, *DmQC* remains a useful tool for the design and testing of new inhibitors with novel scaffolds and different binding modes. Soaking of these inhibitors would not be restricted by the crystal contacts observed in space group *I*4 (Fig. 3b).

4. Conclusion

We have succeeded in finding a novel crystallization condition for *DmQC* that led to the growth of crystals in the new space group *I*4. This is the third space group found for crystals of *DmQC* (a brief summary is given in Table 5). The new crystals will facilitate soaking experiments for novel inhibitors with alternative scaffolds and binding modes.

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