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Expression, crystallization and preliminary crystallographic study of GluB from *Corynebacterium glutamicum*

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GluB is a substrate-binding protein (SBP) which participates in the uptake of glutamic acid in *Corynebacterium glutamicum*, a Gram-positive bacterium. It is part of an ATP-binding cassette (ABC) transporter system. Together with the transmembrane proteins GluC and GluD and the cytoplasmic protein GluA, which couples the hydrolysis of ATP to the translocation of glutamate, they form a highly active glutamate-uptake system. As part of efforts to study the amino-acid metabolism, especially the metabolism of glutamic acid by *C. glutamicum*, a bacterium that is widely used in the industrial production of glutamic acid, the GluB protein was expressed, purified and crystallized, an X-ray diffraction data set was collected to a resolution of 1.9 Å and preliminary crystallographic analysis was performed. The crystal belonged to space group $P3_121$ or $P3_221$, with unit-cell parameters $a = b = 82.50$, $c = 72.69$ Å.

1. Introduction

Substrate-binding proteins (SBPs) are part of the ATP-binding cassette transporters (ABC transporters) that couple ATP hydrolysis with signal transduction or active transmembrane transport of a diverse array of substrates (Berntsson *et al.*, 2010). SBPs usually reside in the periplasmic space of Gram-negative bacteria, where they bind their specific substrates and hand them over to their cognate transmembrane transporters. Gram-positive bacteria, on the other hand, lack the outer membrane and consequently have no periplasmic space where the SBPs could be retained. As a result, SBPs in Gram-positive bacteria are either attached to the cytoplasmic membrane or fused to membrane-anchored domains of ABC importers (Berntsson *et al.*, 2010).

Previous studies reveal that SBPs share low sequence similarities with each other, yet their structural folds are highly conserved. They usually contain two similarly folded domains connected by a two- or three-stranded β-sheet hinge (Quiocho & Ledvina, 1996). The substrate-binding sites are located between these two domains. Among SBPs with known structures, the *Campylobacter jejuni* PEB1a (PDB entry 2v25; Müller *et al.*, 2007), an aspartate and glutamate receptor, is the most closely related to GluB, with 30% sequence identity.

GluB contains a signal sequence followed by a putative target sequence of signal peptidase II; it is thus likely to be anchored to the membrane as lipoprotein (Kronemeyer *et al.*, 1995).

The *gluB* gene shares an operon with *gluA*, *gluC* and *gluD* which encode the ABC transporter. GluA was characterized as the nucleotide-binding protein, whereas GluC and GluD are integral membrane proteins (Kronemeyer *et al.*, 1995). Based on the organization of the *gluABCD* operon, it should belong to the class 3 ABC transporter system which contains most SBP-dependent importers with integral membrane and ABC domains as separate polypeptide chains (Davidson *et al.*, 2008). *GluABCD* is part of the amino-acid metabolism of *C. glutamicum*. It is induced when the cells use glutamate as sole carbon source and is down-regulated in media containing salts and glucose or sucrose as carbon source (Kronemeyer *et al.*, 1995; Trötschel *et al.*, 2003).

The bacterium *Corynebacterium glutamicum* is nonpathogenic, fast growing and has few growth requirements. It thus has seen widespread applications in biotechnology, especially in the production of

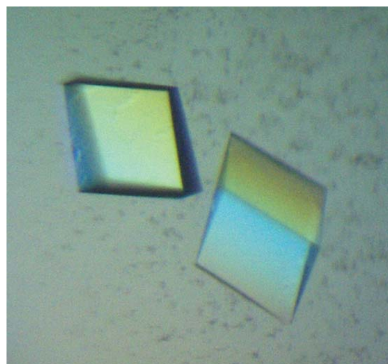

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Table 1
Data-collection statistics of the GluB crystal.

Values in parentheses are for the outermost resolution shell.

Space group	$P3_121$ or $P3_221$
Unit-cell parameters (Å)	$a = b = 82.50$, $c = 72.69$
Wavelength (Å)	1.0090
Resolution (Å)	19.92–1.90 (2.00–1.90)
No. of reflections	22911
$R_{\rm merge}$† (%)	8.0 (51.4)
Multiplicity	21.7 (21.7)
Mean $I/\sigma(I)$	28.0 (6.3)
Completeness (%)	99.9 (100)

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $\langle I(hkl)\rangle$ is the mean of the observations $I_i(hkl)$ of reflection *hkl*.

glutamate and other amino acids. In order to better understand the glutamate metabolism of *C. glutamicum*, we have purified and crystallized *C. glutamicum* GluB and performed preliminary crystallographic studies of this protein.

2. Materials and methods

2.1. Cloning, expression and purification of GluB

The genes encoding full-length and the N-truncation fragment of GluB were amplified by PCR cloning from the chromosome DNA of *C. glutamicum* res 167 strain using the following primers: 5′-AGCTGACATATGTCTGCAAAGCGTACTTTTACCCGTATCG-3′ and 5′-GACAGTGCGGCCGCGCTTGCGTCGAGGAAGGAGAGGTCACC-3′ for the full-length gene and 5′-AGCTGACATATGGACGGATTCCTCGCAGCCATTGAAAATG-3′ and 5′-GACAGTGCGGCCGCGCTTGCGTCGAGGAAGGAGAGGTCACC-3′ for the N-truncation. The N-terminal truncation fragment of GluB, which has the N-terminal 34 residues removed, was cloned into expression vector pET-22b between the *Nde*I and *Not*I restriction sites. A six-histidine tag was engineered at the C-terminus. The recombinant GluB contains residues 35–295 of the full-length sequence preceded by M and followed by AAALEHHHHHH. The recombinant plasmid was transformed into *Escherichia coli* strain BL21 (DE3). The bacteria were grown in LB medium and induced with 0.2 m*M* isopropyl β-D-1-thiogalactopyranoside for 12 h at 295 K on reaching an OD_{600} of 0.6. The cells were harvested by centrifugation at 4670*g* for 30 min at 277 K. After resuspension in lysis buffer (50 m*M* NaH_2PO_4/Na_2HPO_4 pH 7.5, 300 m*M* NaCl, 10 m*M* imidazole), cells were lysed on ice using a sonicator (3 s pulse, 9 s pause; repeated 99 times). The lysate was centrifuged at 21 130*g* for 30 min at 277 K to remove cell debris. The supernatant was loaded onto Ni–NTA agarose beads (Novagen, Darmstadt, Germany) and washed with washing buffer (50 m*M* NaH_2PO_4/Na_2HPO_4 pH 7.5, 300 m*M* NaCl, 10 m*M* imidazole). The GluB protein was eluted with elution buffer (50 m*M* NaH_2PO_4/Na_2HPO_4 pH 7.5, 300 m*M* NaCl, 250 m*M* imidazole). The protein was then further purified by gel-filtration chromatography using a HiLoad 16/60 Superdex 75 column (GE Healthcare) in a buffer consisting of 20 m*M* Tris–HCl, 150 m*M* NaCl. The purified GluB protein was concentrated to 20 mg ml^{-1} by ultrafiltration (Amicon Ultra-15 Centrifugal Filter, Millipore) prior to crystallization. Protein concentration was determined using Bio-Rad Protein Assay Dye Reagent with a BSA standard curve.

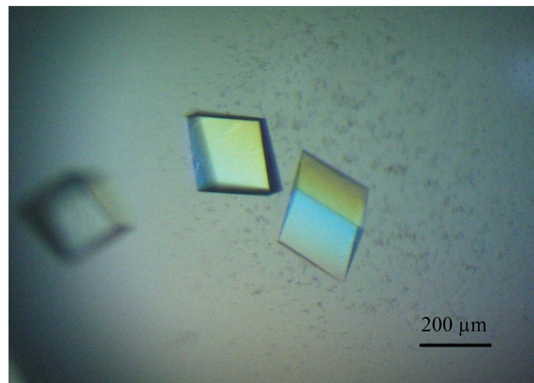

Figure 1
Crystals of GluB with typical dimensions of 0.20 × 0.20 × 0.05 mm.

2.2. Crystallization

Crystallization screening was performed using the hanging-drop vapour-diffusion method with Hampton Research Crystal Screen kits. 1 µl GluB solution (20 mg ml^{-1}) was mixed with 1 µl reservoir solution and the drop was equilibrated against 450 µl reservoir solution at 293 K. The initial condition for crystal growth was further optimized by altering the pH and the concentration of the precipitant. A heavy-atom screen was performed using the Hampton Research Heavy Atom Screen kit. The heavy-atom stock solutions were diluted to 50 or 100 m*M* and added directly to the hanging drops to a final concentration of 5 or 10 m*M*, followed by 3 d of soaking.

2.3. Data collection and processing

The X-ray diffraction data set of the mercury derivative was collected on beamline 3W1A at the Beijing Synchrotron Radiation Facility (BSRF; Beijing, People's Republic of China) at a wavelength of 1.0090 Å. The crystal was soaked in reservoir solution supplemented with saturated Li_2SO_4 for 15 s before flash-cooling in liquid nitrogen on a nylon loop. The cryoprotectant was prepared by adding a saturating amount of Li_2SO_4 powder to the reservoir solution and

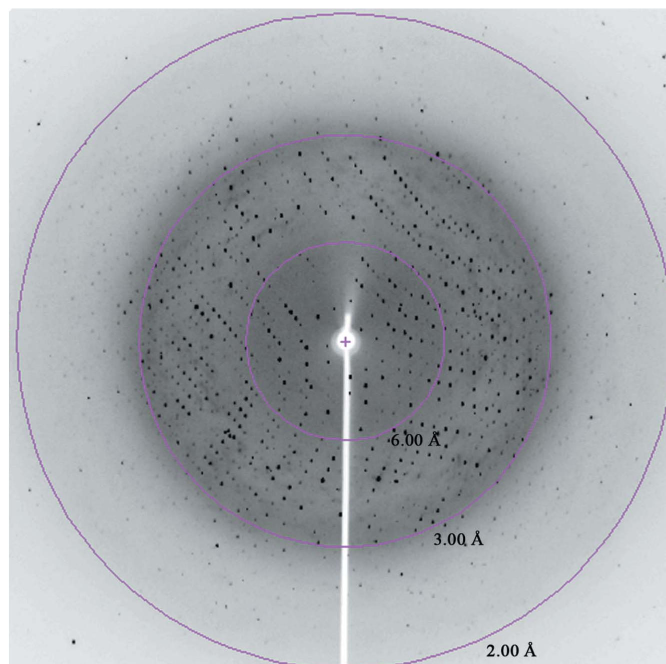

Figure 2
Diffraction pattern of *C. glutamicum* GluB crystal.

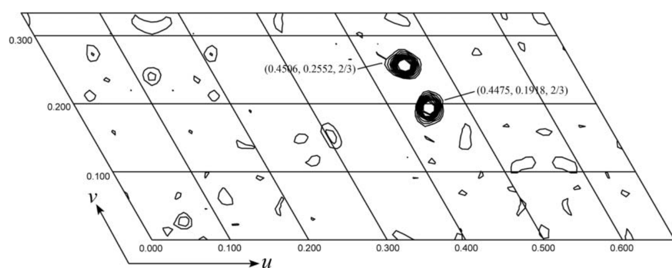

Figure 3
Anomalous difference Patterson map of GluB crystal on Harker section w = 2/3. The two peaks are at 15σ levels.

removing the extra powder by centrifugation. A total of 360 images were collected with an oscillation of 1° per image. The diffraction data were indexed and integrated with the program *MOSFLM* (Leslie & Powell, 2007) and scaled with *SCALA* (Evans, 2006) from the *CCP*4 program suite (Winn *et al.*, 2011).

3. Results

The crystals of GluB appear in several conditions of the Hampton Research Index kit (Nos. 3–6) that use 2.0 *M* ammonium sulfate as precipitant. After optimization, the best crystal was obtained by mixing 1 µl GluB solution (20 mg ml^{-1}) with 1 µl reservoir solution consisting of 0.1 *M* Tris–HCl pH 7.5, 2.9 *M* ammonium sulfate. Within 2–3 d, the crystal grew to a size of 0.20 × 0.20 × 0.05 mm (Fig. 1).

Of the known structures of glutamate-binding proteins such as *E. coli* DEBP (PDB entry 2vha; Hu *et al.*, 2008) and *Campylobacter jejuni* PEB1a (PDB entry 2v25; Müller *et al.*, 2007), the proteins always acquire their cognate ligands from the environment and are purified as complexes even in the absence of added ligands (Müller *et al.*, 2007; Hu *et al.*, 2008). The same is true for the SBPs of other amino acids. It is therefore very likely that the GluB samples used in our studies are in the form of complexes with glutamate.

The structures of several glutamate-binding proteins have been solved, but all of them have sequence identities with GluB of 30% or lower. Attempts to solve the structure of GluB by the molecular-replacement method failed, probably owing to the low sequence identity between GluB and the search model. We then carried out a heavy-atom screen. The best derivatized crystals of GluB were obtained using 10 m*M* mercury acetate solution and were found to be suitable for data collection. Data-collection statistics are listed in Table 1.

The mercury acetate derivatized crystal diffracted to a resolution of 1.9 Å (Fig. 2) and the space group is $P3_121$ or $P3_221$ with unit-cell parameters of $a = b = 82.50$, $c = 72.69$ Å. The asymmetric unit contains one GluB molecule with a calculated Matthews coefficient of 2.38 Å^3 Da^{-1}, corresponding to a solvent content of 48.36% (Matthews, 1968). The anomalous difference Patterson map calculated with *FFT* from the *CCP*4 program suite shows prominent Harker peaks at 15σ (Fig. 3), indicating that the data set from the mercury-derivatized crystal contains strong anomalous scattering signals and could be used for phase calculations using SAD (single-wavelength anomalous dispersion) or SIRAS (single isomorphous replacement with anomalous signal) techniques. The research reported here provides us with a good foundation for structure determination and further functional studies of GluB.

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References

Berntsson, R. P., Smits, S. H., Schmitt, L., Slotboom, D. J. & Poolman, B. (2010). *FEBS Lett.* **584**, 2606–2617.
Davidson, A. L., Dassa, E., Orelle, C. & Chen, J. (2008). *Microbiol. Mol. Biol. Rev.* **72**, 317–364.
Evans, P. (2006). *Acta Cryst.* D**62**, 72–82.
Hu, Y., Fan, C.-P., Fu, G., Zhu, D., Jin, Q. & Wang, D.-C. (2008). *J. Mol. Biol.* **382**, 99–111.
Kronemeyer, W., Peekhaus, N., Krämer, R., Sahm, H. & Eggeling, L. (1995). *J. Bacteriol.* **177**, 1152–1158.
Leslie, A. G. W. & Powell, H. (2007). *Evolving Methods for Macromolecular Crystallography*, edited by R. J. Read & J. L. Sussman, pp. 41–51. Dordrecht: Springer.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Müller, A., León-Kempis, M. R., Dodson, E., Wilson, K. S., Wilkinson, A. J. & Kelly, D. J. (2007). *J. Mol. Biol.* **372**, 160–171.
Quiocho, F. A. & Ledvina, P. S. (1996). *Mol. Microbiol.* **20**, 17–25.
Trötschel, C., Kandirali, S., Diaz-Achirica, P., Meinhardt, A., Morbach, S., Krämer, R. & Burkovski, A. (2003). *Appl. Microbiol. Biotechnol.* **60**, 738–742.
Winn, M. D. *et al.* (2011). *Acta Cryst.* D**67**, 235–242.