Crystallization and preliminary crystallographic analysis of dUTPase from the helper phage Φ11 of Staphylococcus aureus

Ibolya Leveles, Gergely Róna, Imre Zagyva, Ábris Bendes, Veronika Harmat and Beáta G. Vértessy*

CONFIDENTIAL – NOT TO BE REPRODUCED, QUOTED NOR SHOWN TO OTHERS

SCIENTIFIC MANUSCRIPT
For review only.
Sunday 21 August 2011

Category: crystallization communications

Co-editor:
Professor L.J. Beamer
117 Schweitzer Hall, Department Biochemistry, University of Missouri, Columbia, MO 65211, USA
Telephone: 573 882 6072
Fax: 573 884 4812
Email: beamerl@missouri.edu

Contact author:
Beata G. Vertessy
Karolina, Budapest, Hungary
Telephone: ?
Fax: ?
Email: vertessy@enzim.hu
Crystallization and preliminary crystallographic analysis of dUTPase from the helper phage Φ11 of Staphylococcus aureus

Ibolya Leveles*a, Gergely Rónaa, Imre Zagyvaa, Ábris Bendesa, Veronika Harmatb and Beáta G. Vértessya,b,c,*

aInstitute of Enzymology, Hungarian Academy of Sciences, Budapest, Hungary, bHungarian Academy of Sciences-Eötvös Loránd University, Protein Modeling Research Group, and Eötvös Loránd University, Institute of Chemistry, and cDepartment of Applied Biotechnology, Budapest University of Technology and Economics, Budapest, Hungary
correspondence e-mail: vertessy@enzim.hu, leveles@enzim.hu

Abstract:

Staphylococcus aureus superantigen-carrying pathogenicity islands (SaPIs) have a determinant role in spreading virulence genes among bacterial populations that constitute a major health hazard. Repressor (Stl) proteins are responsible for transcriptional regulation of pathogenicity island genes. Recently, a derepressing interaction between the repressor Stl SaPIbov1 with dUTPase from the Φ11 helper phage was suggested [Tormo-Mas et al. (2010). Nature 465, 779-782]. Towards elucidating the molecular mechanism of this interaction, this study reports expression, purification, and X-ray analysis of Φ11 dUTPase that contains a phage-specific polypeptide segment not present in other dUTPases. Crystals were obtained using the hanging-drop vapor-diffusion method at room temperature. Data were collected from one type of crystal to 2.98 Å resolution. The crystal of Φ11 dUTPase belonged to the cubic space group I23, with unit-cell parameters a=98.16 Å, α=β=γ= 90.00°. 

Synopsis:

The cloning, purification, crystallization and preliminary X-ray diffraction analysis of a novel Staphylococcal phage dUTPase is reported. This protein contains a specific polypeptide insertion potentially responsible for modulation of expression of superantigenicity island genes.
1. Introduction

*Staphylococcus aureus* is a major human bacterial pathogen responsible for frequent infections causing severe diseases. It constitutes a serious health care problem especially due to the fast appearance of resistant strains, most notably the methicillin-resistant *Staphylococcus aureus* (MRSA) (van Belkum, 2011). Bacterial virulence in *Staphylococcus aureus* has multiple major factors, including an intriguing network of communication between pathogenicity islands and helper phages (Chen & Novick, 2009). Recently, it was proposed that transcriptional regulation of superantigen-carrying pathogenicity islands (SaPIs) relies on helper phage proteins with multiple functions (Tormo-Mas et al., 2010). Importantly, binding of the transcription-related repressor factor Stls encoded within the SaPI genomic regions to their specific promoter element was suggested to be modulated by interaction with moonlighting proteins. In one of such interactions, the binding of the Stl repressor of the pathogenicity island SaPIbov1 to the Φ11 helper phage dUTPase protein suppressed the repressor function of SaPIbov1. This interaction was suggested to rely on a protein segment of Φ11 dUTPase not involved in catalytic activity (Tormo-Mas et al., 2010, Vertessy & Toth, 2009).

To get insight into the molecular details of this intriguing interaction, we aim to examine the complex formation between the Stl repressor SaPIbov1 and Φ11 dUTPase by determination of the three dimensional structure of the interacting proteins. As a first step in this process, we hereby report cloning, purification and crystallization of Φ11 dUTPase.
2. Materials and Methods

2.1. Cloning

The cDNA of the dUTPase protein (protein GenBank ID: AAL82253.1) from the helper phage Φ11 was synthesized as a codon-optimized (EnCor Biotechnology Inc.) construct. The codon-optimized construct was cloned into the vector pETDuet-1 from Novagen with NdeI and XhoI restriction sites using the services of Eurofins MWG Operon. No affinity tag was attached to the protein sequence. The recombinant plasmid DUET-ΦDUT was verified by DNA sequencing on both strands using DuetUP2 'TTGTACACGGCCGATAATC' and T7 terminator ‘GCTAGTTATTGCTCAGCGG’ primers.

2.2. Protein expression and purification

The plasmid DUET-ΦDUT was transformed into E. coli strain BL21 Rosetta (DE3). Cells were cultured at 310 K in LB medium. Cultures were induced using 1 mM isopropyl β-d-1-thiogalactopyranoside (IPTG) at the logarithmic growth phase. After induction, cell cultures were grown for a further four hours followed by centrifugation at 277 K. All subsequent procedures were carried out on ice, except when noted differently.

Cell pellet was resuspended in lysis buffer (10 mM HEPES pH 7.5, 10 mM KCl, 10 mM β-mercaptoethanol, 1 mM PMSF, 10 μg/mL DNase I, 10 μg/mL RNase and 1 tablet of EDTA-free Complete ULTRA protease inhibitor preparation (Roche, Switzerland) per 50 mL solution), sonicated, then centrifuged at 16 000 g. Supernatant solution was applied on anion exchange using Q-Sepharose (GE HealthCare) column chromatography in 10 mM HEPES, 10 mM KCl, 10 mM β-mercaptoethanol, 0.1 mM phenyl-methyl sulfonyl fluoride (PMSF), pH 8.0 (buffer A). Elution was followed at 280 nm wavelength. Column was washed with buffer A till no further protein elution was observed. Φ11 dUTPase protein was eluted in a linear gradient of 45 mL buffer A and buffer B (10 mM HEPES, 10 mM KCl, 1 M NaCl, 10 mM β-mercaptoethanol, 0.1 mM PMSF, pH 8.0). Elution of Φ11 dUTPase was observed at 0.35 M NaCl.

Ion exchange chromatography was followed by gel filtration on a Superdex 75 column (GE Healthcare) using ÄKTA purifier instrument, in buffer A. Elution of Φ11 dUTPase was observed at an elution volume corresponding to a native molecular mass of 51.8 kDa.
Considering that the molecular mass of the protein, calculated from the primary sequence, is 18.35 kDa, gel filtration data indicate that Φ11 dUTPase most probably adopts the trimeric oligomer structure, characteristic of dUTPases.

Protein fractions were analyzed by SDS-PAGE, which indicates that after the second chromatography step, the protein purity was > 90%. Protein concentration was determined using $A_{280}^{\text{nm} 0.1\%} = 0.786$, estimated from the amino acid composition. Protein solution was concentrated up to 10 mg/mL.

2.3. Crystallization

For crystallization, protein samples were used right after purification. Initial crystallization trial was performed using JCSG-\textit{plus} screen (Molecular Dimensions) by vapor diffusion method at room temperature. Hanging drop plates were set up using 1 μL protein solution to equal amount of reservoir solution. The protein solution content was 5 mg/mL Φ11 dUTPase, 2.3 mM α,β-imido-dUTP (slowly hydrolysable dUTP substrate analogue), 5 mM MgCl$_2$ (metal cofactor). Crystals could be observed in several conditions from the first screen. Crystals up to 0.2 mm in size were grown using the well solution of 0.1 M ammonium acetate, 0.1 M bis-TRIS pH 5.5, 17% w/v PEG 10000 (condition A). Smaller crystals were grown using the well solution of 0.2 M ammonium nitrate pH 6.3, 20%w/v PEG 3350 (condition B). Cryoprotection of the reservoir solution was tested in liquid nitrogen stream at 100 K (Oxford Cryosystem), samples were flash frozen and prepared for X-ray testing.

2.4. X-ray diffraction, data collection and processing

Pre-experimental home source testing was performed on a rotating anode Rigaku instrument (RU-200 generator, confocal optics, R-AXIS IV++ detector, Cu K$_\alpha$ radiation) and on SuperNova sealed tube system, equipped with Eos CCD detector (Agilent).

X-ray data was collected at ESRF beamlineID14-1 at 0.9334 Å wavelength at 100 K. Diffraction data were collected to a resolution of 2.98 Å. Molecular replacement was employed using the structure of \textit{Mycobacterium tuberculosis} dUTPase (PDB ID: 3HZA) that shows 32% sequence identity to Φ11 dUTPase. Crystallographic data was processed using
iMosflm (Battye et al., 2011) and Scala (Evans, 2006) from CCP4 software package (CCP4, 1994).
3. Results and Discussion

The Φ11 dUTPase was successfully expressed using the *E. coli* expression host and the T7 – pET vector system, in accordance with our previous results on dUTPase proteins from other sources (Varga *et al.*, 2008, Varga *et al.*, 2007, Nemeth-Pongracz *et al.*, 2007, Kovari *et al.*, 2004, Barabas *et al.*, 2004, Mustafi *et al.*, 2003). Purification using ion exchange and size exclusion chromatography steps resulted in protein preparations suitable for crystallization. Denaturing sodium-dodecyl sulfate-polyacrylamide gel analysis indicated that the purified protein has an apparent molecular mass of 18 kDa, corresponding to the monomer mass of the Φ11 dUTPase that includes a phage-specific polypeptide segment of approximately 40 residues (Tormo-Mas *et al.*, 2010). The oligomerization status of Φ11 dUTPase in solution was assessed by analytical gel filtration and indicated trimeric organization, also observed for most dUTPases (Persson *et al.*, 2001, Cedergren-Zeppezauer *et al.*, 1992) (Vertessy & Toth, 2009, Fiser & Vertessy, 2000).

Using the JCSG-*plus* screen, many conditions provided crystals, however, only two conditions led to diffracting protein crystal specimens (Figure 1). Crystals were pre-tested on home source. A full data set was collected on a crystal segment broken away from a specimen similar to that shown on Figure 1A on the ESRF_ID14-1 beamline and the results are summarized in Table 1. No evidence for twinning was found. X-ray data analysis showed that the asymmetric unit contains one molecule. Matthews coefficient and solvent-content estimations were performed using the CCP4 software (CCP4, 1994). Calculated Matthews coefficient (Matthews, 1968) and the solvent content are 2.19 and 43.85% respectively. Considering the high homology of the Φ11 and *Mycobacterium tuberculosis* dUTPase proteins (32% sequence identity), the phase problem is planned to be solved by molecular replacement using the structure of the monomer of the *Mycobacterium tuberculosis* dUTPase (PDB ID 3HZA) as search model.
Acknowledgements

This work was supported by OTKA K68229; OTKA-A08 CK78646, OTKA NK-84008, OTKA K72973, OTKA NK67800; Howard Hughes Medical Institutes #55000342, Alexander von Humboldt-Stiftung, Germany; the New Hungary Development Plan (Project ID: TAMOP-4.2.1/B-09/1/KMR-2010-0002, 0003); the Baross program of the New Hungary Development Plan (Project ID: 3DSTRUCT, OMFB-00266/2010 REG-KM-09-1-2009-0050).

We acknowledge the European Synchrotron Radiation Facility for provision of synchrotron radiation facilities and we thank Dr. Stéphanie Monaco for assistance in using beamline ID14-1.

References


Table 1. X-ray data collection statistics

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Φ11 dUTPase</strong></td>
<td>I23</td>
</tr>
<tr>
<td>Resolution (Å)</td>
<td>40.07-2.98 (3.14-2.98)</td>
</tr>
<tr>
<td>Unit-cell parameters</td>
<td>a=98.16 Å, α=β=γ= 90.00°</td>
</tr>
<tr>
<td>Total reflections</td>
<td>18394 (2571)</td>
</tr>
<tr>
<td>Unique reflections</td>
<td>3337 (490)</td>
</tr>
<tr>
<td>Completeness (%)</td>
<td>99.7 (99.6)</td>
</tr>
<tr>
<td>( R_{merge}^a )</td>
<td>0.096 (0.474)</td>
</tr>
<tr>
<td>I/σI</td>
<td>11.2 (3.5)</td>
</tr>
</tbody>
</table>

\( R_{merge} = \frac{\sum_{hkl} \sum_i | I_i(hkl) - <I(hkl)> |}{\sum_{hkl} \sum_i I_i(hkl)} \), where \( I_i(hkl) \) is the intensity of the \( i \)th observation of reflection \( hkl \) and \( <I(hkl)> \) is the average intensity over symmetry-related observations of reflection \( hkl \).

Figure legends

Figure 1. Crystals of native Φ11 dUTPase. Panel A: crystals grown from condition A and pretested on home source and used for data collection. Panel B. Crystals from condition B, pretested on home source. Scale bars are shown.

Figure 2. Diffraction image collected on synchrotron beamline. Black circle corresponds to the resolution limit of 2.98 Å.