Journal of Structural Biology 193 (2016) 157–161

Contents lists available at ScienceDirect

Journal of Structural Biology

journal homepage: www.elsevier.com/locate/yjsbi

Crystal structure of Cdc11, a septin subunit from *Saccharomyces cerevisiae*

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ARTICLE INFO

Article history:
Received 24 September 2015
Received in revised form 21 December 2015
Accepted 8 January 2016
Available online 9 January 2016

Keywords:
Septins
Yeast
Cdc11
Crystal structure
Nucleotide binding
Binding interface

ABSTRACT

Septins are a conserved family of GTP-binding proteins that assemble into a highly ordered array of filaments at the mother bud neck in *Saccharomyces cerevisiae* cells.

Many molecular functions and mechanisms of the septins in *S. cerevisiae* were already uncovered. However, structural information is only available from modeling the crystallized subunits of the human septins into the EM cryomicroscopy data of the yeast hetero-octameric septin rod. Octameric rods are the building block of septin filaments in yeast. We present here the first crystal structure of Cdc11, the terminal subunit of the octameric rod and discuss its structure in relation to its human homologues. Size exclusion chromatography analysis revealed that Cdc11 forms homodimers through its C-terminal coiled coil tail.

1. Introduction

Septins are highly conserved GTP binding proteins that belong to the superclass of P-loop NTPases (Weirich et al., 2008; Pringle, 2008). They are present in all mammalian and fungal cells.

Crystal structures of the human septins Sept2, Sept3, Sept7 and the human septin complex consisting of Sept2, Sept7 and Sept6 became available in the past few years (Sirajuddin et al., 2007, 2009; Zent et al., 2011; Macedo et al., 2013). All septins share a central GTP binding domain (or short G domain) that is flanked by variable C- and N-terminal extensions. The G domain is conserved (30–40% pairwise identity) among the different species and is either loaded with GTP or GDP. Septins assemble into non-polar filaments through interactions between their G domains (G interface) or between their N- and C-termini (NC interface), respectively. The baker's yeast *Saccharomyces cerevisiae* expresses five septins during vegetative growth, namely Cdc3, Cdc10, Cdc11, Cdc12 and Shs1. They assemble into hetero-oligomeric rods with the order Cdc11–Cdc12–Cdc3–Cdc10–Cdc10–Cdc3–Cdc12–Cdc11 with Shs1 sometimes replacing Cdc11 (Bertin et al., 2008). The rods polymerize further into filaments. While biological functions of septins in yeast have been extensively studied, structural information is limited to the physical properties of the rods (Bertin et al., 2012; Kaplan et al., 2015). No structural data are available for the individual septin subunits. In this study we present the crystal structure of Cdc11, the terminal subunit of the octameric rod.

2. Protein preparation

The ORFs of $Cdc11_{1\text{-}415}$, $Cdc11_{19\text{-}415}$ and $Cdc11_{19\text{-}415}$(W251A) were PCR amplified from a septin rod expression plasmid (Renz et al., 2013) and cloned in a pET15b derived plasmid for expression in *Escherichia coli* as N-terminally 6xhis tagged protein (Iffland et al., 2000). The mutation W251A was introduced by overlap extension PCR. In the resulting proteins, the Cdc11 ORF is preceded by the amino acid sequence MSSHHHHHHSSAMASTA. $Cdc11_{20\text{-}298}$ was used for the crystallization experiments. This construct contains an additional TEV site between the 6xhis tag and residue 20 of Cdc11. The preceding amino acid sequence thus reads MSSHHHHHHSSAMASTAENLYFQG.

Protein expression was carried out in the *E. coli* strain BL21 (DE3) in SB medium at 18 °C over night. The cell pellet was resuspended in IMAC buffer A (50 mM KH_2PO_4, 300 mM NaCl, 20 mM Imidazole, pH 7.5) and purified via IMAC on an Äkta Purifier chromatography system (GE Healthcare) using a HisTrap HP 5 ml

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http://dx.doi.org/10.1016/j.jsb.2016.01.004
1047-8477/

column (GE Healthcare). The protein was eluted by applying a step gradient of 25%, 40% and 100% IMAC buffer B (50 mM KH_2PO_4, 300 mM NaCl, 200 mM Imidazole, pH 7.5). Protein containing fractions (as judged by SDS PAGE and Coomassie staining) were pooled and subjected to size exclusion chromatography (SEC) against HBSEP buffer (10 mM HEPES, 150 mM NaCl, 3 mM EDTA, 0.05% Tween 20, pH 7.4) using a Superdex 200 16/60 column (GE Healthcare). The purified protein was concentrated and subsequently used for crystallography (only $Cdc11_{20\text{-}298}$) or analytical SEC. The dimerization state of $Cdc11_{20\text{-}298}$, $Cdc11_{1\text{-}415}$, $Cdc11_{19\text{-}415}$ and $Cdc11_{19\text{-}415}$(W251A) was determined by analytical SEC using a Superose 6 10/300 GL column (GE Healthcare), PBS buffer and a gel filtration MW standard (BioRad). The average retention volumes of the MW standard proteins were: Thyroglobulin (670 kD): 12.5 ml; γ-globulin (158 kD): 15.6 ml; Ovalbumin (44 kD): 17.2 ml; Myoglobin (17 kD): 18.3 ml; Vitamin B12 (1.4 kD): 21.0 ml.

3. Crystallization

SEC-purified protein was crystallized using vapor diffusion in 96-well sitting drop plates. Initial crystal growth was achieved in several conditions of the Index screen (Hampton Research) that were optimized in polyethylene glycol content and pH. To obtain diffracting crystals, 0.3 µl of protein solution (10 mg/ml) were mixed with 0.3 µl of reservoir solution containing 22% polyethylene glycol 3350 and 0.1 M BIS-TRIS buffer at pH 5.5 using an OryxNano crystallization robot (Douglas Instruments). The protein precipitant drops were equilibrated against 50 µl of reservoir solution at 20 °C. Initial crystals appeared after few hours of incubation and grew to full size after one day. Diffracting crystals had an elongated rod-like appearance with a hexagonal cross-section. For X-ray data collection at 100 K the crystals were mounted in nylon loops, cryoprotected in reservoir solution containing 10% (w/v) 2*R*,3*R*-butane diol and flash-cooled in liquid nitrogen.

4. Data collection and structure determination

X-ray diffraction data were collected on the beamline X06SA at the Swiss Light Source (Paul Scherrer Institute, Villigen, CH) at λ = 1.00002 Å using a Pilatus 6 M detector. Indexing and integration of X-ray diffraction intensities was done using XDS (Kabsch, 2010). For scaling and assessment of data quality the AIMLESS (Evans and Murshudov, 2013) pipeline was used. The crystal structure of *S. cerevisiae* Cdc11 was solved by molecular replacement using MOLREP (Vagin and Teplyakov, 2010) as implemented within the CCP4 suite (Winn et al., 2011) against all chains of PDB entries of 2QA5, 2QAG, 2QNR, 3FTQ, 3SOP, 3T5D, 3TW4 and 4KV9. For all individual molecular replacement solutions an electron density map has been calculated without positional refinement using autoBUSTER (Global Phasing Ltd, version 2.10.1). The resulting reciprocal space correlation (RSCC) coefficients revealed the monomeric chain A from 2QNR as a good search model. After manual model correction by removal of flexible parts a good score of 0.489 was obtained in the subsequent MOLREP run with one molecule per asymmetric unit. The molecular replacement solution was subjected to a first rigid body refinement using autoBUSTER. Iterative model building was carried out with COOT (Emsley et al., 2010) and all rounds of positional refinement were carried out using autoBUSTER with TLS group definitions and automatically determined NCS symmetry restraints. The collection data and refinement statistics are summarized in Table 1.

A composite omit map was generated using *phenix.composite_omit_map* from the PHENIX package (Adams et al., 2010) with default settings (Supplementary Fig. S1).

Table 1
Data collection and refinement statistics.

Data Set		Cdc11
Space group		$P6_322$
Cell constants	*a*, *b*, *c* [Å]	187.0, 187.0, 58.0
	α, β, γ [°]	90.0, 90.0, 120.0
Resolution limits [Å]		162.0 – 2.85 (3.00 – 2.85)
Completeness (%)		99.9 (99.2)
Unique reflections		14,458 (2046)
Multiplicity (%)		34.1 (13.9)
R_{merge} [a]		0.103 (1.05)
$R_{p.i.m.}$		0.024 (0.344)
Mean I/ σ(I)		21.5 (1.0)
$CC_{1/2}$		0.999 (0.43)
Refinement statistics		
R_{cryst} [b]		0.257
R_{free} (%)		0.260
Non-hydrogen atoms		1643
Solvent molecules		–
Cruickshank's DPI		0.335
r.m.s. deviations from ideal values		
Bond lengths (Å)		0.010
Bond angles (°)		1.20
Average *B* values (Å^2)		
Protein main chain atoms		165.8
Protein all atoms		167.5
Wilson plot		95.7

R_{free} is the cross-validation *R* value for a test set of 5% of unique reflections.
[a] $R_{merge} = \Sigma_{hkl}\,[(\Sigma_i\,|I_i - \langle I\rangle|)/\Sigma_i\,I_i]$.
[b] $R_{cryst} = \Sigma_{hkl}\,||F_{obs}| - |F_{calc}||/\Sigma_{hkl}\,|F_{obs}|$.

All molecular images were generated using PyMol (Version 1.7.4, 2015). The atomic coordinates and structure factors were deposited in the Protein Data Bank (PDB-ID: 5AR1).

5. Results and discussion

Our first attempts at crystallizing Cdc11 were focused on the full length protein. $Cdc11_{1\text{-}415}$ was obtained in high amounts and sufficient purity but refused to form crystals in the initial screening attempts (unpublished data).

Human septin structures were only obtained from N- and C-terminally truncated versions and not from the full length proteins (Sirajuddin et al., 2007, 2009; Zent et al., 2011; Macedo et al., 2013). Analogous to the human Sept2 and Sept7 structures, we prepared $Cdc11_{20\text{-}298}$, a truncated protein lacking the predicted N-terminal helix and the complete C-terminal extension. Diffracting crystals were obtained from this protein (Fig. 1A and B). The structure was solved by molecular replacement at a resolution cut-off of 3.97 Å using the structure of Sept2 (PDB-ID: 2QNR) as a model and was subsequently refined to the final resolution of 2.85 Å. Only one monomer was found in the asymmetric unit corresponding to an exceptionally high solvent content of 79% in Cdc11 crystals.

The crystals contained the apo form of Cdc11 without nucleotide (Fig. 1A). Soaking crystals with GDP or adding excess GDP in all buffers used for extract preparation, protein purification and crystallization resulted in crystals that did not diffract beyond 5 Å.

The overall structure of the Cdc11 G-domain resembles the structures of Sept2 and Sept7: they share a canonical Ras-like G-domain consisting of five α-helices and six β-sheets. From the nucleotide-free structure some interesting conclusions about the nucleotide binding properties of Cdc11 can be drawn; especially when aligning the structure with the structure of GDP bound Sept2 (PDB-ID: 2QNR; see Fig. 2A).